Received: 6 June 2025 | Revised: 24 September 2025 | Accepted: 25 September 2025

DOI: 10.1002/ppp3.70118

RESEARCH ARTICLE

Plants People Planet PPP Open Access

Rhizosphere microbial detoxification and phosphorus solubilization drive ecological remediation of Pb–Zn contaminated soils

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 41967058; Guizhou Provincial Forestry Projects, Grant/Award Number: [2023]10

Societal Impact Statement

Heavy metal contamination of soils poses a serious threat to ecosystem health and food security worldwide. This study investigated how native plants, such as *Artemisia annua* and *Buddleja davidii*, interact with soil microbes in Pb–Zn waste slag areas. We found that these plants stimulate microbes, which improve phosphorus availability and reduce metal toxicity in the soil. These nature-based solutions offer a sustainable and eco-friendly way to restore polluted lands, supporting greener remediation policies and practices for contaminated environments globally.

Summary

- Microorganisms are critical for phosphorus (P) turnover in heavy metal-contaminated soils, with native plants enhancing these processes by shaping rhizosphere microbial communities. In extreme lead (Pb)–zinc (Zn) slag environments characterized by barrenness and high heavy metal stress, adaptive microbial mechanisms remain underexplored.
- This study investigates microbial functional genes related to P turnover in the rhizospheres of native plants *Carex breviculmis*, *Artemisia annua*, and *Buddleja davidii* colonizing Pb–Zn waste slag to elucidate the underlying mechanisms.
- The rhizospheres of *A. annua* and *B. davidii* showed increased abundance of genes involved in inorganic P solubilization, indicating their potential for improving soil health. Key P-cycling genes, including *gcd*, *phnGHKLMN*, and *phnT*, were major predictors of soil available phosphorus (AP), with *gcd* exhibiting a significant positive correlation with AP. Metagenome-assembled genomes (MAGs) revealed a diverse community harboring genes for both P cycling and heavy metal resistance. The co-occurrence of *gcd* and *zntA* in *Pseudomonadota* and *Actinomycetota* suggests a dual role in P solubilization and metal detoxification, facilitating inorganic P release and efflux of Pb, Zn, and Cd. This gene co-occurrence may be driven by horizontal gene transfer (HGT), with evidence for 822 potential HGT events detected at the phylum level, including two MAGs acquiring both *zntA* and *gcd*.

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- These findings highlight how plant–microbe interactions regulate rhizosphere P cycling in Pb–Zn contaminated soils, demonstrating the potential of *A. annua* and *B. davidii* to promote microbial functions that enhance P bioavailability and metal detoxification for ecological remediation.

KEYWORDS
heavy metal resistance, metagenome-assembled genomes, microbial functional genes, native plants, soil phosphorus cycling

1 | INTRODUCTION

Mining activities produce substantial amounts of waste, with lead (Pb)–zinc (Zn) tailings presenting particularly severe challenges owing to their high contents of heavy metals (e.g., Pb, Zn, and Cd) and marked nutrient deficiencies (Li et al., 2023; Luo et al., 2019). Globally, annual mineral waste residues exceed 10 billion tons, posing a significant threat to soil ecosystems worldwide (Adiansyah et al., 2015). In regions such as northwestern Guizhou, China—rich in Pb–Zn ores—long-term smelting operations have led to the accumulation of over 20 million tons of largely unmanaged waste by 2000, resulting in heavily contaminated and nutrient-depleted soils (Sun et al., 2022a; Yang et al., 2010). In these environments, toxic metals compromise soil health and disrupt essential nutrient cycles, including that of phosphorus (P). Phosphorus availability, critical for plant growth, is often limited due to adsorption and precipitation processes, which are further aggravated under metal contamination (Shen et al., 2022). Native plants colonizing such extreme habitats can enhance microbial functions that improve P bioavailability, thereby supporting remediation and ecosystem recovery (Sun et al., 2022a; Zhang et al., 2024).

Rhizosphere microorganisms play a crucial role in mediating P turnover in metal-contaminated soils. Key functional genes regulate processes such as inorganic P solubilization (e.g., *gcd*), organic P mineralization (e.g., *phoD*, *phn*), P uptake (e.g., *pstS*), and P-starvation response (e.g., *phoB*) (Liu et al., 2023; Rodríguez et al., 2006). These genes enable microbes to release organic anions or enzymes that enhance phosphorus bioavailability (Zhi et al., 2023). For example, *gcd* encodes quinoprotein glucose dehydrogenase (PQQGDH), which oxidizes glucose and acidifies the periplasmic space to solubilize inorganic P (Liang et al., 2020). Similarly, genes encoding alkaline phosphatase (*phoD*, *phoA*), phytase (*appA*), and C–P lyases (*phn*) facilitate the mineralization of organic P compounds (Siles et al., 2022; Wu et al., 2022). Meanwhile, genes such as *pst* and *pit* are involved in inorganic P assimilation, and P-starvation response genes regulate phosphatase expression under low-P conditions (Wang et al., 2024). Although these genes are widespread across ecosystems, their expression and regulation under multi-metal stress remain poorly understood (Siles et al., 2022; Zhi et al., 2023). Elucidating the genetic potential of rhizosphere microbes is essential to understand how P cycling is maintained in contaminated and nutrient-deficient environments.

Microbial survival in metal-contaminated soils also relies on heavy metal resistance mechanisms. Genes such as *zntA*, which encodes a Zn^{2+}/Cd^{2+} efflux pump, help maintain cellular metal homeostasis and mitigate toxicity (Cui et al., 2008). The coexistence of metal resistance genes and P cycling genes implies a synergistic microbial adaptation strategy that concurrently supports nutrient acquisition and detoxification. Horizontal gene transfer (HGT) may further promote the dissemination of these traits, enabling microbial communities to rapidly adapt to combined stressors (Liu et al., 2023; Wilson & Metcalf, 2005). For instance, Liang et al. (2020) reported HGT-mediated acquisition of P-solubilizing genes, and Liu et al. (2023) demonstrated that HGT facilitates the transfer of heavy metal resistance genes within *gcd*-harboring MAGs, enhancing microbial resilience and metabolic functionality under dual stress conditions.

Despite these advances, current research has predominantly focused on individual functional genes or microbial taxa. There is a lack of systematic analysis regarding the synergistic mechanisms between P cycling genes and heavy metal resistance genes—especially those potentially co-transferred via HGT—in the rhizospheres of native plants under heavy metal contamination. To address this gap, we selected three native plant species—*Carex breviculmis*, *Artemisia annua*, and *Buddleja davidii*—based on their successful natural colonization and ecological adaptability to Pb–Zn slag heaps. This study aims to (1) identify key microbial taxa and functional genes involved in P cycling and metal resistance, (2) examine co-occurrence patterns between P cycling and metal resistance genes, and (3) assess the role of HGT in the potential co-acquisition of these adaptive traits. We hypothesize that (1) native rhizosphere microbiota harbor overlapping genetic modules for P solubilization and metal detoxification, (2) significant genetic co-occurrence exists between these functional traits, and (3) HGT contributes to the dissemination of metal resistance genes among P-solubilizing microbes. Through metagenomic sequencing and soil biogeochemical analyses of a century-old Pb–Zn waste heap colonized by these native species, this research provides new insights into microbial ecological strategies that underpin phytoremediation and soil restoration.

2 | MATERIALS AND METHODS

2.1 | Study area

This study was conducted at an abandoned Pb-Zn waste heap in northwestern Guizhou Province, China (26°54′N, 104°54′W). The

region experiences a subtropical humid climate, with an average annual precipitation of 800–1000 mm, distributed unevenly throughout the year. The area has a mining history spanning approximately 100 years, during which smelting activities generated significant amounts of waste slag (Sun et al., 2022b). The soil in the Pb–Zn waste heap is mainly composed of fine-grained mineral residues with heavy metal contamination, classified as sandy loam soil (Jain, 2023). The concentrations of Pb (6395 mg kg^{-1}), Zn (49,261 mg kg^{-1}), and Cd (51 mg kg^{-1}) in the waste heap significantly exceed the local geochemical background values (Pb: 18.32 mg kg^{-1}, Zn: 90.46 mg kg^{-1}, Cd: 0.453 mg kg^{-1}; Li et al., 2024), severely degrading soil quality and hindering plant growth (Luo et al., 2019). Over the past century, the site has undergone natural succession, with *C. breviculmis*, *A. annua*, and *B. davidii* emerging as dominant species during the early to mid-late stages of herbaceous succession (Sun et al., 2022a).

2.2 | Sample collection

Rhizosphere soils from three plant species, *C. breviculmis*, *A. annua*, and *B. davidii*, which predominantly appear on the upper, sun-exposed areas of the Pb–Zn slag heap, were collected during their peak growing season in September 2023. Rhizosphere soil collection followed the protocol described by Cheraghi et al. (2023), focusing on soil tightly adhering to roots after gentle shaking. Briefly, plants were carefully uprooted, and loose soil was gently shaken off. The soil tightly adhering to the root surface was then carefully brushed off with sterilized brushes and collected as the rhizosphere sample. Unvegetated control (CK) soil was sampled from bare patches (0–20 cm depth, corresponding to the main rooting zone of the target plants) within the same waste heap to represent the baseline soil conditions without direct plant influence. Each plant treatment and the CK were replicated eight times, yielding a total of 32 samples (3 plant species $\times$ 8 replicates $+$ 1 control $\times$ 8 replicates). After removing roots, stones, and litter, the soil samples were placed in sterile containers and stored at low temperatures to preserve microbial integrity for subsequent analysis. In the laboratory, each sample was divided into two portions: One was stored at $-80^{\circ}C$ for microbiological analysis, while the other was air-dried for physicochemical and heavy metal analyses.

2.3 | Sample analysis

Total heavy metal concentrations (Cd, Pb, and Zn) were determined following the aqua regia digestion method outlined in the international standard ISO 11466:1995 (Soil quality – Extraction of trace elements soluble in aqua regia). Briefly, 0.1000 g of slag was placed in a Teflon vessel, and 3 ml of a 1:3 (v/v) mixture of HNO_3 and HCl was added. The sealed vessels were heated in an electric oven at 180°C for 12 h (overnight). After heating, the vessels were opened and placed on a heating plate at approximately 105°C to evaporate the acids. Once the solution volume was reduced to about 1 ml, it was allowed to cool, filtered, and diluted to 50 ml with deionized water. To ensure accuracy, duplicates, reagent blanks, and standard reference materials (GBW07404) were included in the analysis. The concentrations of Zn, Cd, and Pb were measured using atomic absorption spectroscopy (atomic absorption spectroscopy; PinAAcle 900H, PerkinElmer, USA).

Soil organic carbon (SOC) was determined by the Walkley–Black method (Nelson & Sommers, 1982), total nitrogen (TN) via the Kjeldahl method (Bremner & Mulvaney, 1982), and total phosphorus (TP) using molybdenum–antimony–scandium colorimetry (Murphy & Riley, 1962). Available phosphorus (AP) was measured using the Olsen method (Olsen & Sommers, 1982). Soil pH was analyzed in a 1:2.5 soil-to-deionized water slurry, and soil moisture content was calculated after drying at 105°C.

2.4 | Metagenome assembly, binning and annotation

DNA was extracted from the rhizosphere soils of the three native plants and the unvegetated control (CK), using 0.5 g of soil per sample, with the FastDNA SPIN Kit for Soil (MP Biomedicals), following the manufacturer's protocol. DNA quality was assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc.). Approximately 1 μg of high-quality DNA per sample was used to construct libraries, which were sequenced on the Illumina NovaSeq PE150 platform (Illumina Inc., San Diego, CA, USA), yielding an average of >15 Gb raw data per sample. A total of 779.00 Gb of metagenomic sequencing data was obtained, identifying ~20 million ORFs, including genes involved in P turnover and heavy metal resistance.

Raw reads were quality-controlled with Fastp v0.23.4 (Chen et al., 2018). Per-sample assembly was performed using MEGAHIT v1.2.9 (Li et al., 2015), and gene prediction was carried out with Prodigal v2.6.3 (Hyatt et al., 2010). Functional annotation was conducted against the KEGG KofamKOALA database (version 2023-03-01) to identify genes involved in microbial phosphorus acquisition and metal resistance based on previously established methods (Buchfink et al., 2021). A total of 56 genes were identified, including 21 related to metal resistance, each assigned a KEGG orthology (KO) number and categorized into P-cycling or heavy metal resistance functions. P-cycling genes were further grouped into three functional categories: inorganic phosphorus solubilization and organic phosphorus mineralization, phosphorus transport and assimilation, and regulation of phosphorus starvation responses. Detailed classifications and functions of these genes are provided in Table S1. The taxonomic origins of key genes were determined by matching predicted nonredundant sequences against the RefSeq database using Kraken2 v2.1.3 (Wood et al., 2019).

Contigs were binned using metaWRAP v1.3.2 (Uritskiy et al., 2018), integrating MaxBin2 v2.2.7 (Wu et al., 2016), metaBAT, and metaBAT2 v2.12.1 (Kang et al., 2015). Nonredundant metagenome-assembled genomes (MAGs) were obtained using dRep v3.4.3 with default parameters (Olm et al., 2017), and their completeness and contamination were evaluated with CheckM2 v1.2.2 (Parks

et al., 2015). MAGs with >90% completeness and <5% contamination were retained for further analysis. Protein-coding open reading frames were predicted in the selected MAGs using Prodigal v2.6.3 (Hyatt et al., 2010), and functional annotation of amino acid sequences was performed against the KEGG KofamKOALA database (Aramaki et al., 2020). MAGs were taxonomically classified using GTDB-Tk v2.4.0, completing the comprehensive analysis of soil microbial genomics (Chaumeil et al., 2019).

2.5 | HGT identification

Contigs derived from MAGs were compared to a customized mobile genetic element (MGE) database established by Pärnänen et al. (2018) using *hmmsearch* with an e-value threshold of $\leq 10^{-5}$. This analysis aimed to identify potential gene transfer events between microbial groups. To investigate the dissemination of the *zntA* gene, HGT events among MAGs were analyzed at various phylogenetic levels using MetaCHIP v1.10.10 (Song et al., 2019) with default configurations. The investigation also incorporated BLASTn v2.12.0+ (Altschul et al., 1990) to perform optimal alignments and phylogenetic analyses, providing insights into gene relationships and potential horizontal transfer mechanisms.

2.6 | Statistical analysis

Gene abundance was normalized using transcripts per million (TPM) to account for variations in gene length and sequencing depth, allowing more accurate cross-sample comparisons. One-way analysis of variance (ANOVA) was applied to evaluate differences in soil properties—including SOC, TN, TP, AP, moisture, and heavy metal (Cd, Pb, Zn) concentrations—across plant species and CK. Normality and homoscedasticity assumptions were verified using the Shapiro–Wilk test and Levene's test, respectively. Post hoc pairwise comparisons were performed using Tukey's honest significant difference (HSD) test at a significance level of $p < .05$. To evaluate microbial community structure, principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarities calculated from KEGG Orthology (KO) profiles was conducted to visualize beta-diversity in microbial functions related to phosphorus cycling and heavy metal resistance. Permutational multivariate analysis of variance (PERMANOVA, or Adonis) was applied to test for statistically significant differences in microbial functional beta-diversity among treatments. Correlation analysis was performed to explore the relationship between phosphorus-cycling gene abundance and soil available phosphorus (AP). Pearson's correlation coefficients were calculated to assess statistical significance ($p < .05$). The relationship between the relative abundance of *gcd* genes and soil AP was further analyzed using linear regression, with model fit evaluated by calculating R^2 values. Partial least squares path modeling (PLS-PM) was employed to explain the variance in soil AP, considering the combined effects of microbial functional genes, soil properties, and heavy metal concentrations. Path coefficients were evaluated for statistical significance ($p < .01$). Random forest (RF) analysis was used to identify key phosphorus-cycling genes associated with soil AP. Genes were ranked based on their mean decrease in accuracy, and the most influential genes were highlighted. All statistical analyses were conducted in R v4.0.5, utilizing packages such as "vegan" for beta-diversity and Adonis analysis (Dixon, 2003), "plspm" for PLS-PM (Bertrand, 2024), "rfPermute" for random forest analysis (Archer, 2023), and "ggplot2" for data visualization (Wickham et al., 2024).

3 | RESULTS

3.1 | Comparison of rhizosphere soil properties of different plants growing on Pb-Zn waste slag

The rhizospheres of *A. annua* and *B. davidii* exhibited significantly higher SOC and TN contents, as well as elevated AP content in the rhizosphere of *B. davidii*, compared to the control (CK). In contrast, no significant differences were observed between *C. breviculmis* and the CK. The Cd content in the rhizosphere of *A. annua* and the Pb content in the rhizosphere of *C. breviculmis* was significantly lower than those in the CK. However, soil pH, TP content, moisture, and Zn content did not differ significantly among the treatments (Table 1).

3.2 | Impact of plants on microbial functional genes involved in phosphorus cycling and heavy metal resistance

PCoA at the KEGG Orthology (KO) level revealed distinct functional beta-diversity patterns for *C. breviculmis* compared to *A. annua* and *B. davidii* along the PCoA1 axis, accounting for 59.5% and 50.7% of the variation, respectively. ADONIS analysis confirmed that plant treatments significantly influenced microbial functional beta-diversity, with all three plant treatments showing functional beta-diversity significantly different from the CK (Figure 1a,b).

The abundance of genes encoding P-uptake and transport was higher than that of the other three P cycle-related gene categories. Compared to the CK, the three plant treatments significantly reduced the total abundance of genes encoding P-regulation, P-uptake, and transport. Conversely, *A. annua* and *B. davidii* significantly increased the abundance of genes encoding inorganic P-solubilization (Figure S1). Approximately 65% of genes related to inorganic P-solubilization (e.g., *gcd*, *ppa*, and *ppk*) and organic P-mineralization (e.g., *phoD*, *phy*, *phnGHIJJKMN*) were more abundant in the rhizospheres of *A. annua* and *B. davidii* compared to the CK (Figure 2a). Plants also influenced genes encoding heavy metal resistance. Genes such as *zntA*, *czcD*, and *mntH* were predominantly more abundant in the CK compared to the three plant treatments (Figure 3).

There was a strong positive relationship between the abundance of P-cycling genes and soil AP. Specifically, genes encoding inorganic P-solubilization and organic P-mineralization exhibited a

TABLE 1 The geochemical parameters in rhizosphere soil of different plants on the Pb–Zn slag.

Parameters	*Artemisia annua*	*Buddleja davidii*	*Carex breviculmis*	CK
SOC (g kg^{-1})	13.89 ± 2.91a	14.48 ± 4.44a	8.58 ± 0.98b	6.76 ± 1.89b
TN(g kg^{-1})	4.84 ± 1.17a	3.11 ± 0.55b	1.72 ± 0.3c	1.07 ± 0.21c
TP(g kg^{-1})	0.89 ± 0.63a	0.81 ± 0.48a	0.76 ± 0.59a	0.54 ± 0.32a
AP (mg kg^{-1})	7.47 ± 2.87ab	8.74 ± 1.99a	7.17 ± 1.62ab	5.7 ± 0.62b
pH	6.38 ± 0.48a	6.52 ± 0.44a	6.45 ± 0.15a	6.1 ± 0.31a
Moisture	0.28 ± 0.14a	0.23 ± 0.1a	0.21 ± 0.08a	0.19 ± 0.07a
Zn (mg kg^{-1})	42504.2 ± 12692.6a	51645.5 ± 5726.44a	48329.66 ± 10893.49a	49261.1 ± 5812.99a
Cd (mg kg^{-1})	34.5 ± 15.05b	43.6 ± 15.96ab	59.61 ± 8.76a	51.43 ± 3.71a
Pb (mg kg^{-1})	7330.81 ± 1572.56a	6117.66 ± 2075.28a	3859.29 ± 788.56b	6395.64 ± 734.88a

Note: Different letters suggest significant differences ($p < .05$) by LSD test. CK: original tailing from the bare land, *C. breviculmis*: tailing from the rhizosphere of *C. breviculmis*, *A. annua*: tailing from the rhizosphere of *A. annua*, *B. davidii*: tailing from the rhizosphere of *B. davidii*.
Abbreviations: AP, available phosphorus; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus.

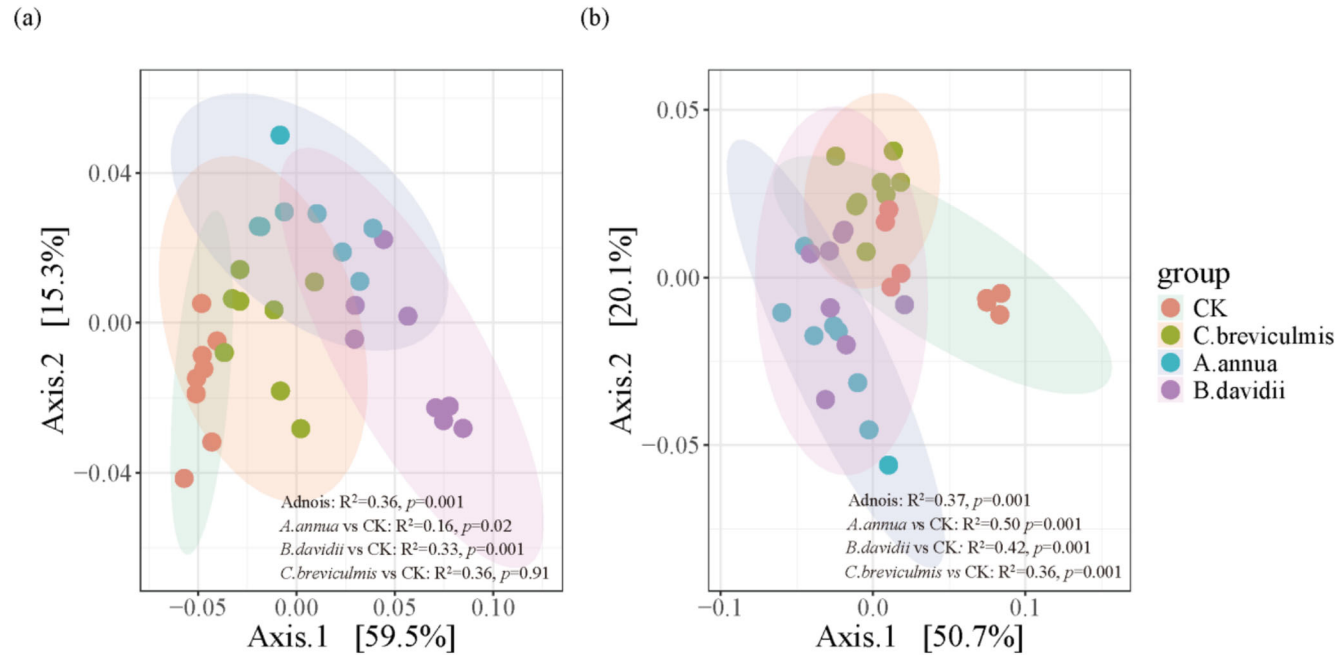


FIGURE 1 Microbial functional beta-diversity involved in soil P cycle (a) and metal resistance (b) identified by principal coordinate analysis (PCoA) based on Bray–Curtis distances for all samples. The ellipses represent 95% confidence intervals. Permutational multivariate analysis of variance using distance matrices (Adonis) was used to examine the differences in microbial functional beta-diversity between plant treatments and CK. CK: original tailing from bare land, *Carex breviculmis*: tailing from the rhizosphere of *C breviculmis*, *Artemisia annua*: tailing from the rhizosphere of *A. annua*, *Buddleja davidii*: tailing from the rhizosphere of *B. davidii*.

significant positive correlation with soil AP (Figure 2b,c), while genes encoding P-regulation, P-uptake, and transport showed no significant negative correlation with soil AP (Figure 2d,e). Notably, the relative abundance of *gcd* genes demonstrated a significant positive correlation with soil AP ($R^2 = 0.545$, $p < .001$; Figure 2b). PLS-PM explained 56% of the total variance in soil AP (Figure 4a). Genes encoding inorganic P-solubilization (path coefficient = .895, $p < .01$) and P-regulation (path coefficient = .599, $p < .01$) had strong positive effects on soil AP. Additionally, soil heavy metals (Pb and Zn), nutrients (SOC and TN), and moisture influenced soil AP by affecting microbial functional genes involved in P cycling (Figure 4a). RF analysis identified key P-cycling genes predicting soil AP, with *gcd*, *phnGHKLMN*, and *phnT* emerging as the dominant factors determining soil AP (Figure 4b).

3.3 | Distribution and diversity of *gcd*- and *znta*-harboring bacteria in response to plants growing on Pb-Zn waste slag

The *gcd* gene, identified through RF analysis as a dominant factor enhancing soil P bioavailability (Figure 4b), plays a critical role in

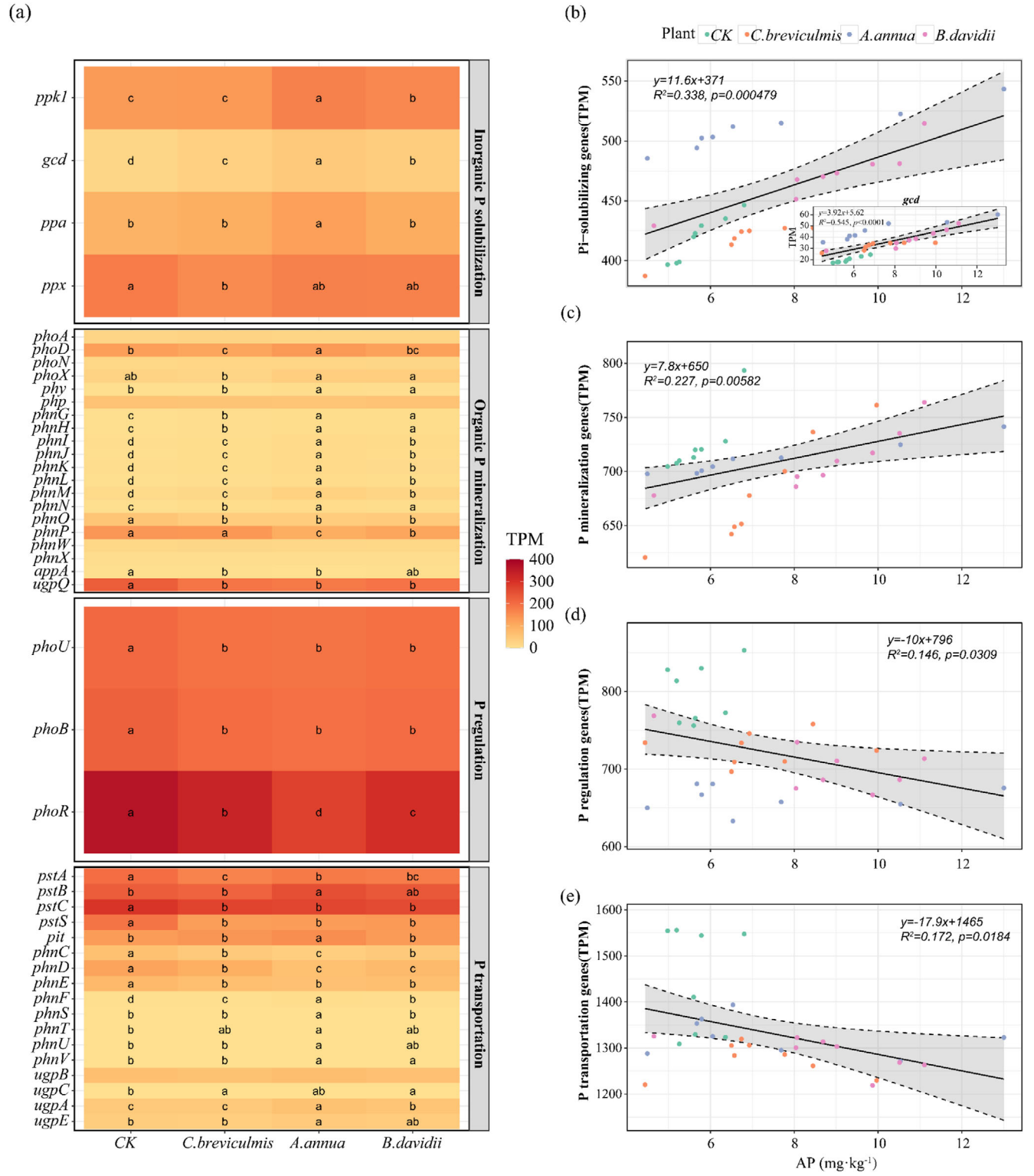


FIGURE 2 Microbial P-cycling potential as depicted by metagenome sequencing of representative samples from rhizosphere of three plants in the Pb–Zn waste slag. (a) Differences in the abundances of the P-cycling microbial genes with plants treatments. Ordinary least squares (OLS) regression was used to investigate the relationship between the abundance of Pi-solubilizing genes and gcd genes (b), organic P mineralization genes (c), P regulation genes (d), P transportation genes (e), and soil available phosphorus (AP). The abundance of related genes was calculated as TPM (transcripts per kilobase of exon model per million mapped reads). Different letters suggest significant differences ($p < .05$) by LSD test. CK: original tailing from bare land, *Carex breviculmis*: tailing from the rhizosphere of *C breviculmis*, *Artemisia annua*: tailing from the rhizosphere of *A. annua*, *Buddleja davidii*: tailing from the rhizosphere of *B. davidii*.

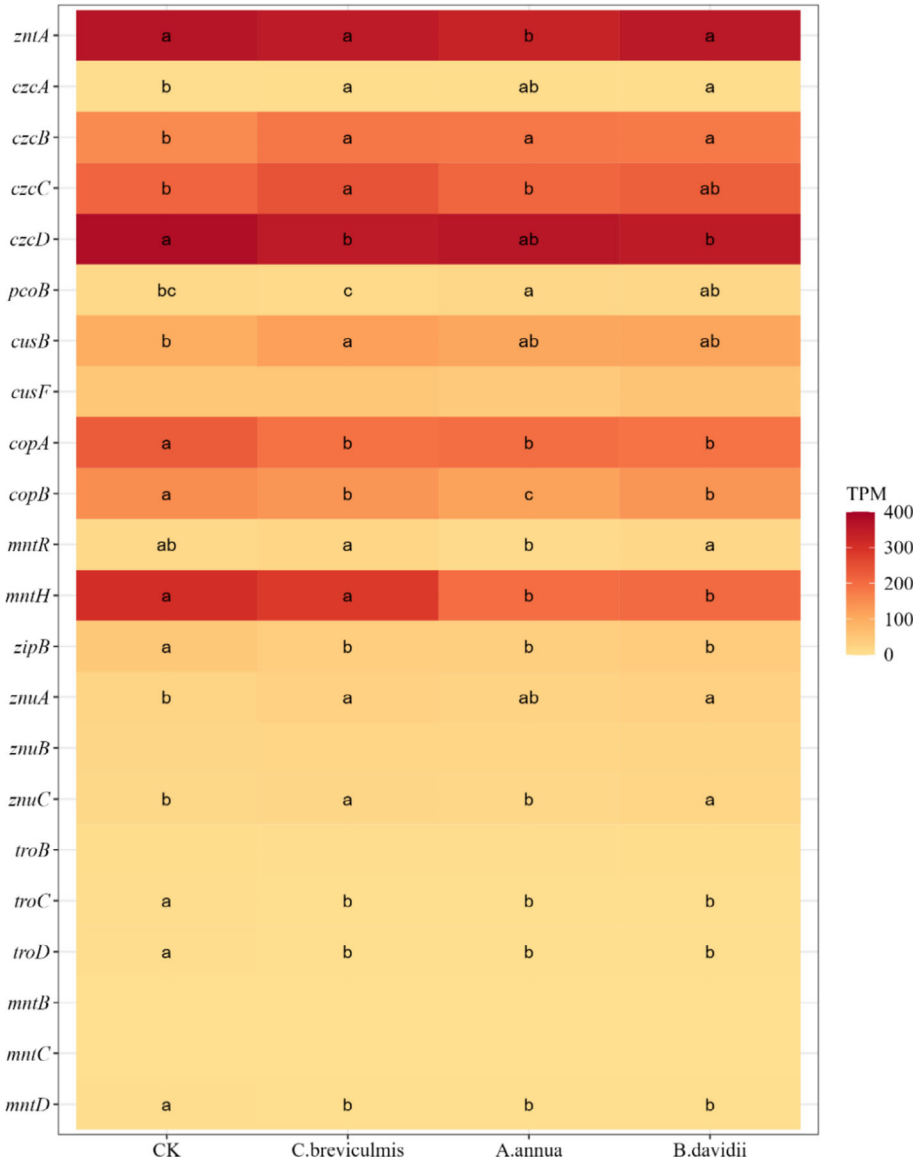


FIGURE 3 Differences in the abundances of the genes associated with metal resistance. The abundance of related genes was calculated as TPM (transcripts per kilobase of exon model per million mapped reads). Different letters suggest significant differences ($p < .05$) by LSD test. CK: original tailing from bare land, *Carex breviculmis*: tailing from the rhizosphere of *C breviculmis*, *Artemisia annua*: tailing from the rhizosphere of *A. annua*, *Buddleja davidii*: tailing from the rhizosphere of *B. davidii*.

phosphorus cycling. The *zntA* gene encodes a cation-translocating ATPase that exports Pb, Zn, and Cd and was found to be highly abundant (Figure 3). To understand bacterial distribution in response to plant treatments, taxonomic classification of *gcd*- and *zntA*-harboring bacteria was conducted. Approximately half of the *gcd*-harboring bacteria were classified as *Pseudomonadota*, followed by *Actinomycetota*, *Gemmatimonadota*, and *Acidobacteriota*. The abundance of *gcd*-harboring *Pseudomonadota* was highest in the *A. annua* treatment, followed by *B. davidii* and *C. breviculmis*, with the lowest abundance observed in the CK (Figure 5a). Similarly, *zntA*-harboring bacteria were predominantly associated with *Pseudomonadota*, followed by *Actinomycetota*.

As the *gcd* gene facilitates inorganic P-solubilization, and both *gcd*- and *zntA*-harboring bacteria primarily belong to *Pseudomonadota* and *Actinomycetota*, the findings suggest that these taxa have a dual role in phosphorus cycling and heavy metal expulsion. This highlights their critical importance in enhancing the P cycle in Pb–Zn waste slag. Additionally, compared to *gcd*-harboring bacteria, *zntA*-harboring bacteria exhibited significantly higher diversity ($p < .001$; Figure 5c,d).

3.4 | Co-occurrence of phosphorus cycling and heavy metal resistance genes in bacterial MAGs from Pb–Zn waste slag

A total of 104 high-quality bacterial MAGs (average completeness 96.10%; contamination 1.30%) spanning 16 phyla were retrieved and classified, with over half belonging to the dominant phyla

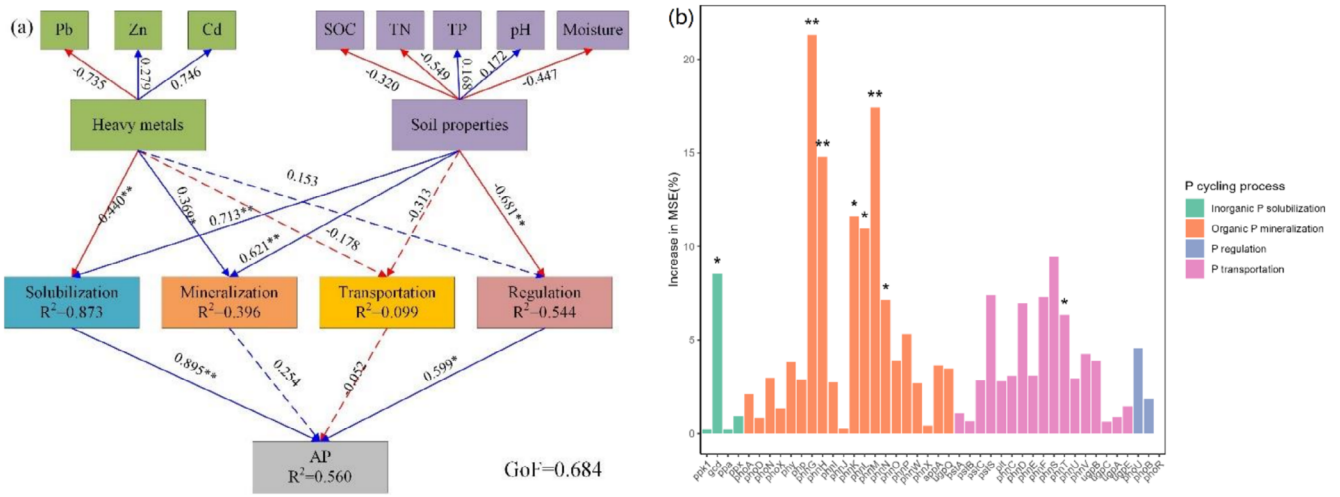


FIGURE 4 Linkages between genes associated with soil microbial P cycling and P bioavailability in the Pb-Zn waste slag. (a) Partial least squares path model analysis (PLSPM) showing the environmental and microbial effects on soil P bioavailability. Path coefficients, significance levels and coefficients of determination (R^2) were calculated after 1000 bootstraps. Blue and red arrows represent positive and negative effects, respectively. (b) Random forest analysis (RF) showing the contribution of microbial P-cycling genes to soil P bioavailability. The variance interpretation rate of the whole model (R^2), the significance levels of the whole model and each variable were calculated after 1000 bootstraps. Available P, available phosphorus, GOF, goodness of fit; MSE, mean squared error; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus. Significance levels were denoted as *, $p < .05$, **, $p < .01$ and ***, $p < .001$.

Pseudomonadota, *Actinomycetota*, *Gemmatimonadota*, and *Acidobacteriota*. Functional annotation revealed the versatile roles of these MAGs in phosphorus cycling and heavy metal resistance. Over half of the MAGs harbored functional genes involved in inorganic P-solubilization (e.g., *gcd*, *ppx*, *ppa*), with most also carrying genes for P uptake and transport (e.g., *pit* and *pstABCS*). Among the 104 MAGs, 74 carried the *mntH* gene, 35 carried the *zipB* gene, and nearly, all of these also possessed the *zntA* and *czcBCD* genes. Additionally, 148 *zntA* genes were identified across 83 MAGs, while 151 *czcD* genes were detected in 91 MAGs, emphasizing the widespread role of *zntA* and *czcD* in bacterial heavy metal efflux in Pb–Zn waste slag (Figure 6a).

Four representative MAGs (*CK6_bin.68*, *CB4_bin.8*, *BD2_bin.13*, and *AA7_bin.7*), abundant in each plant treatment, were further analyzed for their roles in microbial P cycling and heavy metal resistance (Figure 6b). *CK6_bin.68*, affiliated with *Gemmatimonadota*, and *CB4_bin.8*, *BD2_bin.13*, and *AA7_bin.7*, belonging to *Pseudomonadota*, harbored diverse functional genes. Both *CK6_bin.68* and *AA7_bin.7* contained the *gcd*, *ppa*, and *ppx* genes, indicating their involvement in inorganic P solubilization. Additionally, *CB4_bin.8* and *AA7_bin.7* carried the *phoD* gene, which encodes extracellular alkaline phosphatases for organic P mineralization.

All four MAGs carried the *pstABCS* genes for inorganic P uptake, while *CK6_bin.68* and *AA7_bin.7* also contained the *pit* gene. Furthermore, all MAGs harbored the *mntH* and *zipB* genes for Cd, Pb, and Zn uptake. Heavy metal resistance was supported by the presence of *zntA* in *CK6_bin.68*, *CB4_bin.8*, and *BD2_bin.13*, encoding a cation-translocating ATPase for Cd, Pb, and Zn expulsion. The *czcBCD* genes, essential for heavy metal ion efflux, were identified in all four MAGs. These results demonstrate the versatile roles of bacteria in Pb–Zn waste slag, combining phosphorus cycling capabilities with robust heavy metal resistance.

3.5 | Metal efflux pump *zntA* and its potential HGT

An investigation into the co-occurrence of MGEs and metal resistance genes revealed various mechanisms of coexistence, particularly through insertion sequences for transposase (*IS91*), integrases (*int3*), and transposase-encoding insertion sequences (*tnpA*). These findings highlight the potential for rapid dissemination and adaptation of metal resistance elements within bacterial populations (Figure 7a). In a MAG, the *zntA* gene, which plays a critical role in metal efflux, was identified co-located with phosphorus cycling genes, suggesting a possible functional linkage.

At the phylum level, 822 instances of potential HGT were detected, including two MAGs (*AA1_bin.6* and *CK6_bin.6*) that simultaneously acquired the *zntA* and *gcd* genes (Figure 7b–d). These findings suggest that phosphate-solubilizing bacteria can acquire metal resistance through HGT in Pb-Zn mining environments, emphasizing the ecological importance of HGT in shaping bacterial resistance profiles.

4 | DISCUSSION

4.1 | Plant species differentially enhance microbial P cycling via rhizosphere processes

Our findings demonstrate that plant species significantly influence microbial functional traits associated with phosphorus (P) cycling in Pb–Zn waste slag, primarily through species-specific root exudation. The rhizospheres of *A. annua* and *B. davidii* exhibited an increased

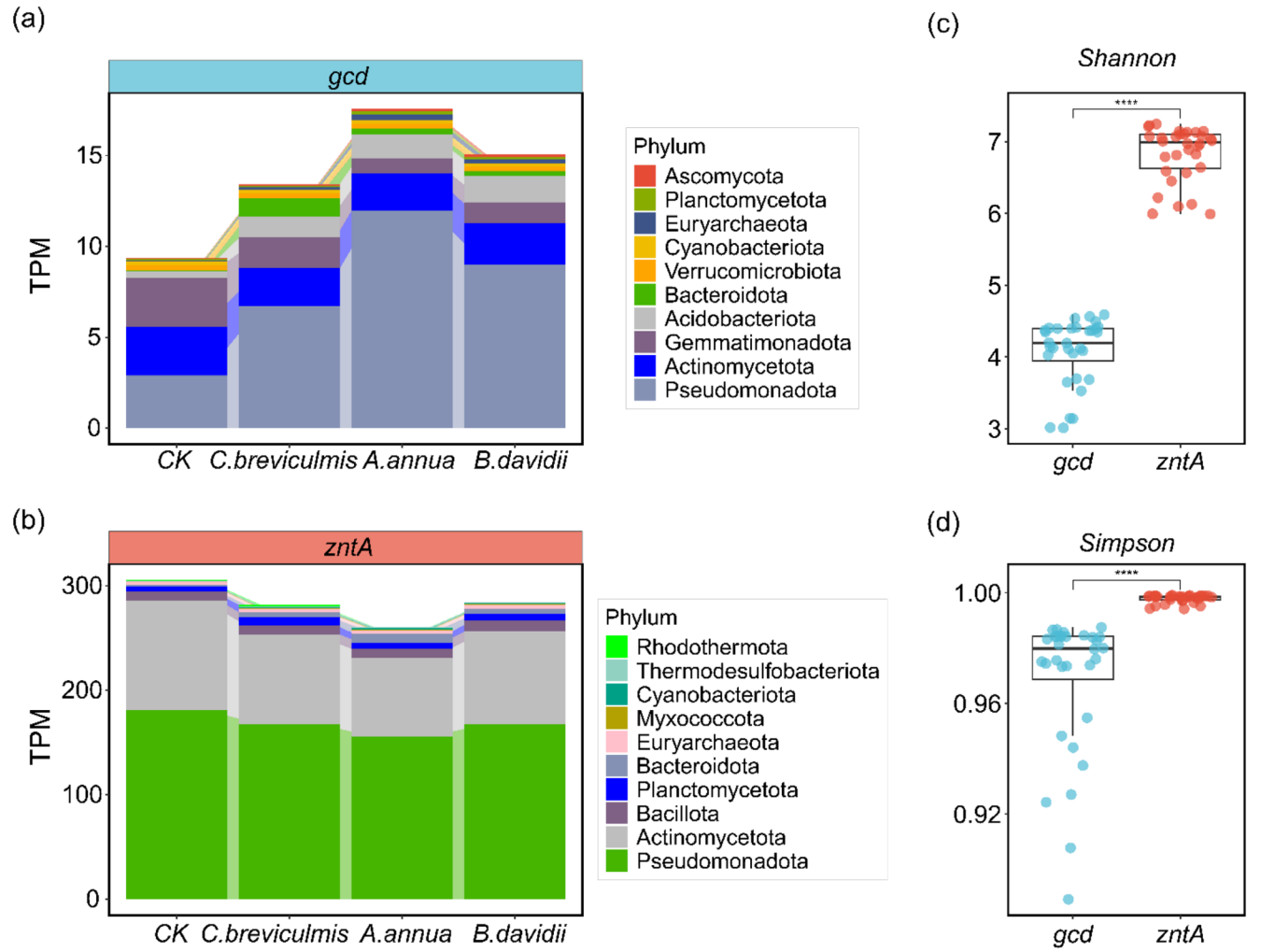


FIGURE 5 Microbial composition and diversity analysis reveals inorganic P solubilizing and heavy metal efflux functional groups in rhizosphere of three plants. Microbial composition of functional groups as depicted by the inorganic P solubilizing gene (gcd, a) and heavy metal efflux gene (zntA, b), respectively. Comparison of the alpha diversity of microbial communities was indicated by Shannon index (c) and Simpson index (d), respectively. The taxonomic assignments were based on the open reading frames of all the assembled contigs against the RefSeq database. Gene abundance was normalized to transcript per million (TPM). Significance levels were calculated by paired Student's *t*-test and denoted as ***, $p < .001$.

abundance of genes involved in inorganic P solubilization (e.g., *gcd*, *ppa*, *ppx*) and organic P mineralization (e.g., *phoD*), which correlated with higher soil available P (AP). We propose that these plants likely secrete specific compounds, such as organic acids (e.g., citrate and malate), phenolics, and sugars, that act as both carbon sources and signaling molecules (Huang et al., 2021; Na et al., 2025), stimulating microbial activity and enriching taxa carrying P-solubilizing genes, particularly Pseudomonadota. The observed reduction in the abundance of P uptake and transport genes (e.g., *pst*, *pit*) under plant treatments may reflect a strategic shift in microbial phosphorus acquisition strategies. Root exudates from plants *A. annua* and *B. davidii* likely provide ample carbon sources, enabling microbes to prioritize energy investment into direct solubilization mechanisms (e.g., via *gcd* and organic acid production) over high-affinity transport systems (Yang, Han, et al., 2022).

In contrast, *C. breviculmis*, an early-successional herbaceous species on Pb–Zn waste slag, showed limited improvement in rhizosphere nutrient conditions and microbial recruitment compared to the CK, with both remaining highly oligotrophic. Its shallow root system and low quantity/quality of root exudates likely constrain the recruitment of robust microbial communities, leading to minimal changes in nutrient availability and microbial genetic potential. This limited influence underscores the importance of plant ecological strategy and successional stage in remediation efficacy. Although root exudates were not directly measured, these observations align with established plant–microbe mechanisms in contaminated environments (Yang, Yang, et al., 2022; Zhi et al., 2023). The rhizosphere effects not only enhance P bioavailability but also contribute to metal immobilization, as indicated by reduced Cd and Pb levels in specific plant treatments.

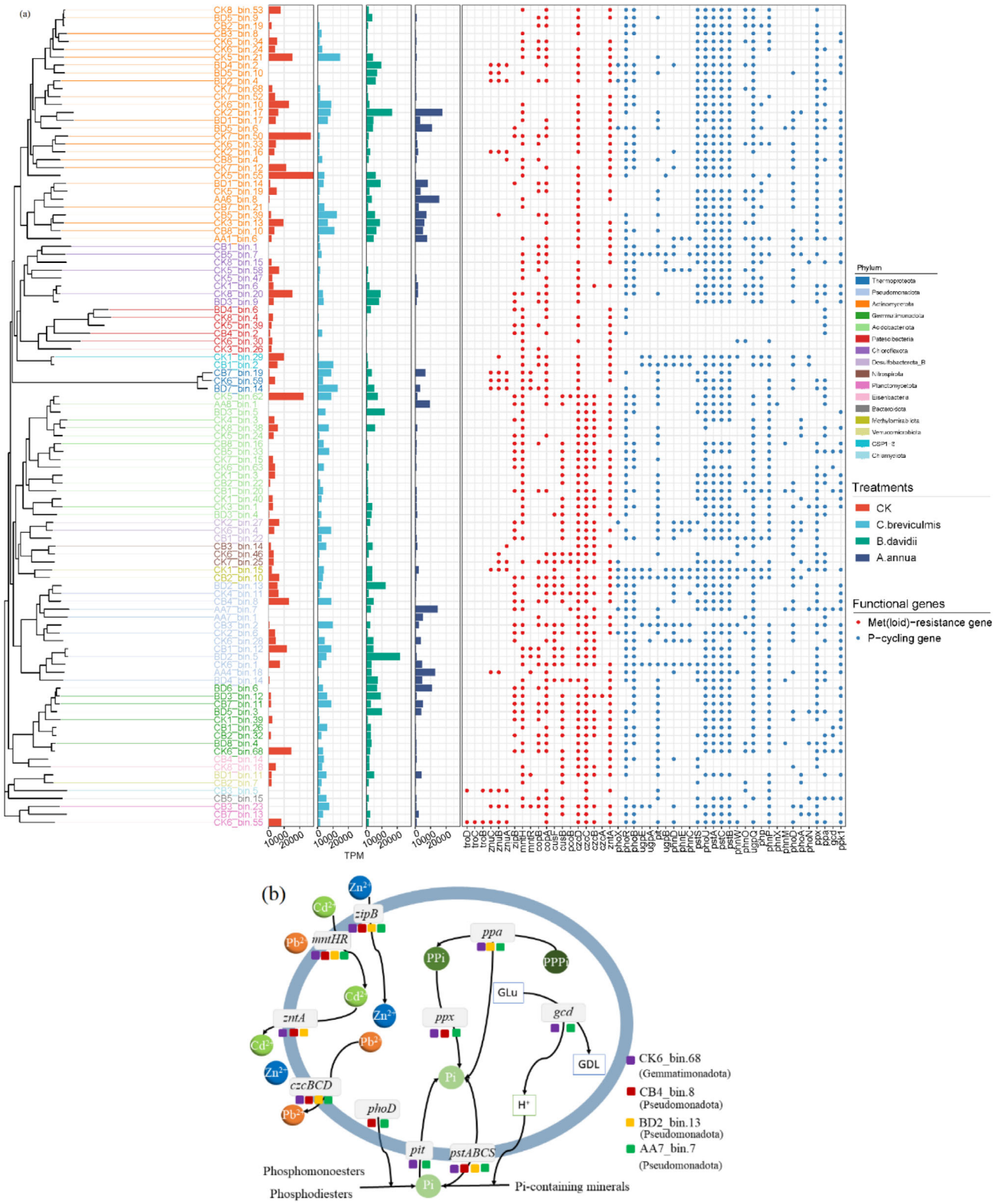


FIGURE 6 Phylogenetic and functional characterization of high-quality bacterial MAGs and related gene associated with P cycle and heavy metal resistance. (a) Phylogenetic and functional characterization of high-quality bacterial MAGs. (b) Mechanisms of P cycle and heavy metal resistance as indicated by four representative MAGs. The construction of the maximum likelihood tree was based on a concatenated alignment of 120 marker genes from GTDB-Tk. The bar plot showed the relative abundance of each MAG at the four types of plant treatments. The presence (colored) and absence (blank) of protein-encoding genes are represented by the heatmap. Squares with different colors represent different MAGs. GDL, glucono-1,5-lactone; GLu, glucose; Pi, inorganic phosphate; PPi, inorganic diphosphate; PPPi, inorganic triphosphate.

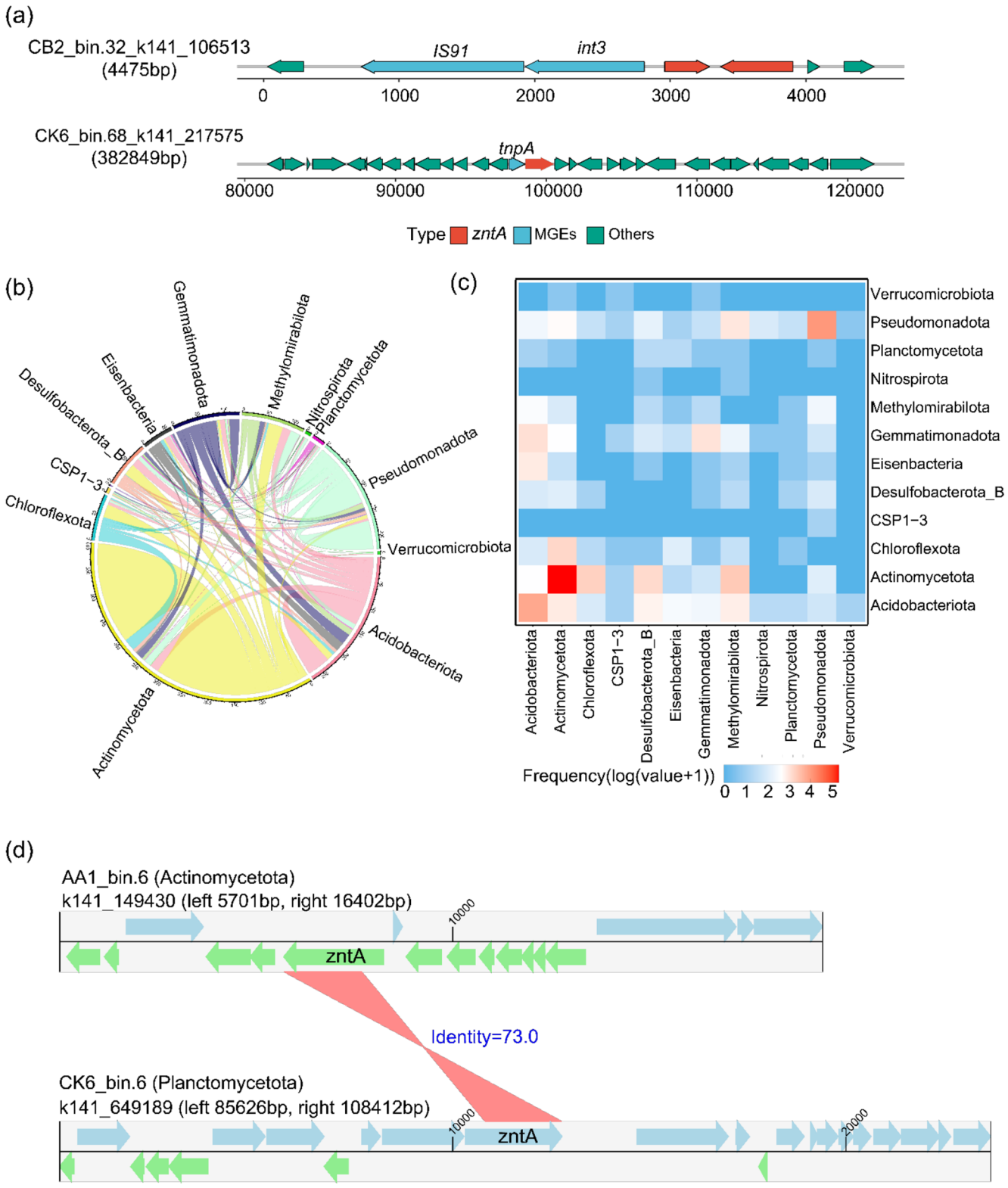


FIGURE 7 Relationship between recovered MAGs at the phylum level and potential horizontal gene transfer (HGT) events of zntA gene. (a) Investigation into the co-occurrence of mobile genetic elements (MGEs) and metal resistance genes. (b) Chord diagram showed the relationship among recovered MAGs at the phylum level. (c) The number of times HGT occur among MAGs at the phylum level. (d) Identification of HGT events of zntA gene among the recovered MAGs at the phylum level.

4.2 | Integrated microbial processes drive phosphorus availability in contaminated soils

Phosphorus is essential for maintaining the compositional and functional stability of soil microbial communities, yet AP remains a limiting factor in mining ecosystems (Liu et al., 2023). Rhizosphere microorganisms play a vital role in regulating AP levels through various P bioconversion processes (Elhaissoufi et al., 2022). In this study, a strong positive correlation was observed between the abundance of the *gcd* gene and soil AP ($R^2 = .545$, $p < .001$), highlighting its critical role in enhancing P bioavailability. The *gcd* gene encodes glucose dehydrogenase, which catalyzes gluconic acid production—a key organic acid that solubilizes inorganic P compounds (Wu et al., 2022). These results corroborate previous studies showing that P-solubilizing bacteria carrying *gcd* significantly enhance P cycling and bioavailability in post-mining soils (Liang et al., 2020).

Using a multi-method approach, we further elucidated how microbial communities regulate P cycling in Pb–Zn contaminated soils. PLS-PM explained 56% of the variance in soil AP, with genes encoding inorganic P solubilization exerting the strongest positive effects (path coefficient = .895, $p < .01$). P-regulation genes also showed significant positive contributions (path coefficient = .599, $p < .01$), underscoring the dominance of these microbial processes in controlling P bioavailability. These findings are consistent with studies by Liang et al. (2020) and Liu et al. (2023), which identified microbial P solubilization and regulation as key mechanisms enhancing P bioavailability in mining soils.

RF analysis identified *gcd*, *phnHKLMN*, *pstS*, and *phnS* as key predictors of soil AP. The presence of *phn* genes, involved in organic P compound degradation (Siles et al., 2022), highlights the potential for bacterial-mediated organic P mineralization in Pb–Zn waste slag. Similarly, *pstS*, associated with P uptake (Gu et al., 2020), emphasizes the role of transport mechanisms in P homeostasis.

Environmental factors—including heavy metals (Pb and Zn), SOC, TN, and moisture—also modulated soil AP by influencing microbial functional genes, adding complexity to these relationships. Heavy metals exert selective pressure on microbial communities, favoring resistant strains that are often efficient P cyclers (Naz et al., 2022; Zeng et al., 2024). This dual capacity for metal resistance and P cycling underscores the critical role of microbes in maintaining soil health under contamination.

4.3 | Co-occurrence of P cycling and heavy metal resistance genes and its ecological implications

We detected a widespread co-occurrence of genes related to P cycling (e.g., *gcd*, *pstS*, and *phn* series) and heavy metal resistance (e.g., *zntA*, *czcBCD*) within bacterial genomes, particularly in *Pseudomonadota* and *Actinomycetota*. Over half of the recovered MAGs contained genes associated with inorganic P solubilization (e.g., *gcd*, *ppa*, *ppx*), and most of these also carried genes for P uptake and transport (e.g., *pit* and *pstABCS*). These results align with Zhang et al. (2021), who reported substantial genetic potential for both P cycling and heavy metal resistance in soil microbial communities. Genes such as *zntA* and *czcBCD*, which encode efflux pumps for toxic metals, are essential for reducing intracellular metal concentrations and mitigating toxicity (Nesler et al., 2017). This efflux capability supports microbial viability and sustains nutrient cycling under contamination (Mathivanan et al., 2021). The prevalence of *zntA* and *czcD* demonstrates a robust capacity for heavy metal detoxification; notably, *zntA* encodes a cation-translocating ATPase that exports Pb, Zn, and Cd (Cui et al., 2008; Delgado-Baquerizo et al., 2016). This genetic architecture enables microbes to efficiently mobilize and assimilate P while resisting metal stress (Liu et al., 2023).

Taxonomic analysis revealed that nearly half of the *gcd*-harboring bacteria belonged to Pseudomonadota, with additional representatives from *Actinomycetota*, *Gemmatimonadota*, and *Acidobacteriota*. The abundance of *gcd*-harboring *Pseudomonadota* varied significantly among plant treatments, being highest in the rhizosphere of *A. annua*, followed by *B. davidii* and *C. breviculmis*, and lowest in the control. This suggests that plant identity strongly influences the distribution of these beneficial bacteria. Similarly, *zntA*-harboring bacteria were predominantly *Pseudomonadota* and *Actinomycetota*, confirming the co-occurrence of *gcd* and *zntA* in these phyla and underscoring their integral role in metal expulsion and P solubilization. These findings are consistent with Aguilar-Barajas et al. (2010) and Li et al. (2023), emphasizing the adaptive strategies and ecological importance of *Pseudomonadota* in metal-contaminated environments.

Analysis of four representative MAGs (CK6_bin.68, CB4_bin.8, BD2_bin.13, AA7_bin.7) further revealed diverse strategies for P cycling and metal resistance. CK6_bin.68 (*Gemmatimonadota*) and AA7_bin.7 (*Pseudomonadota*) contained genes for inorganic P solubilization (*gcd*, *ppa*, *ppx*) and organic P mineralization (*phoD*). All four MAGs carried genes such as *mntH* and *zipB*, involved in essential micronutrient uptake (e.g., Zn), enhancing microbial resilience. The consistent presence of *mntH*, *zipB*, and *zntA* highlights comprehensive mechanisms for metal uptake and efflux crucial for survival in polluted environments (Li et al., 2024). These results illustrate the fundamental role of heavy metal efflux systems in protecting phosphate-solubilizing bacteria, thereby supporting nutrient cycling and ecosystem restoration in Pb–Zn waste slag.

4.4 | HGT contributed to the acquisition of zntA in P-solubilizing bins

HGT is a key mechanism enabling microorganisms to acquire resistance genes in extreme environments such as metal-contaminated soils (Liu et al., 2023). Our study demonstrates that HGT significantly facilitates the acquisition of *zntA*—essential for metal efflux—in P-solubilizing microbial bins from Pb–Zn polluted environments, supporting our third hypothesis. MGEs promote rapid gene dissemination, allowing bacterial communities to adapt to environmental stressors (Brito, 2021; Zhao et al., 2021). These findings align with prior research indicating that MGEs, including insertion sequences (IS91),

integrases (int3), and transposases (tnpA), mediate the spread of resistance genes in contaminated ecosystems (Pärnänen et al., 2018).

The co-location of *zntA* with P-cycling genes such as *gcd* suggests a functional linkage between metal resistance and P metabolism. This aligns with other studies where HGT connects resistance traits with nutrient cycling, enabling microbes to simultaneously mitigate metal toxicity and address nutrient limitations (Liu et al., 2023; Sun et al., 2021). We identified 822 potential HGT events, including the co-acquisition of *zntA* and *gcd*, highlighting the central role of HGT in microbial adaptation to mining environments. P-solubilizing bacteria, which improve soil fertility by converting insoluble P into bioavailable forms (Shi et al., 2017), benefit from this dual acquisition. By gaining both *zntA* and *gcd*, these bacteria enhance their ability to tolerate metal stress and solubilize P, providing a competitive advantage in contaminated soils.

While this study provides novel insights into plant–microbe–metal interactions, several limitations should be noted. First, root exudate composition was not experimentally characterized, so inferences regarding specific exudates remain speculative. Future work incorporating metabolomics could clarify their role in microbial recruitment and gene regulation. Second, although HGT events were predicted using rigorous bioinformatic criteria, functional validation through culture-based assays or gene expression analysis would strengthen causal inferences. Finally, this study focused on genetic potential rather than activity; transcriptomic or proteomic approaches would help verify the expression of key genes under field conditions. Despite these limitations, our findings highlight the potential of native plants such as *A. annua* and *B. davidii*—with their enhanced exudation and microbial recruitment capabilities—to improve microbial functions supporting phytoremediation. The limited impact of *C. breviculmis* further emphasizes the importance of plant traits and successional stage in remediation strategy design. Future studies should integrate multi-omics and exudate profiling to fully elucidate plant–microbe interactions in heavy metal–contaminated environments.

5 | CONCLUSION

This study highlights the crucial role of native plants in promoting rhizosphere microbial communities and their functional traits associated with P cycling and heavy metal resistance in Pb–Zn waste slag soils. Specifically, the rhizospheres of *A. annua* and *B. davidii* showed an increased abundance of genes involved in inorganic P solubilization, suggesting a strong potential to enhance soil P bioavailability. Among these, genes responsible for inorganic P solubilization had the most significant positive impact on soil AP. Key P-cycling genes, such as *gcd*, *phnGHKLMN* and *phnT*, were identified as major predictors of AP, underscoring their essential roles in nutrient cycling. Notably, the co-occurrence of *gcd* and *zntA* within bacterial taxa—especially *Pseudomonadota* and *Actinomycetota*—points to a dual functionality in these microbes, supporting both P solubilization and heavy metal detoxification. The prevalence of *Pseudomonadota* among *gcd*-harboring bacteria, particularly in the rhizospheres of *A. annua* and *B. davidii*, indicates that these plants foster a microbial environment conducive to beneficial phosphate-solubilizing organisms. MAGs further revealed co-occurrence of key genes involved in P solubilization (e.g., *gcd*, *ppa*, *ppx*) and metal resistance (e.g., *zntA*, *czcBCD*), highlighting the potential for synergistic microbial actions in coupled P cycling and metal detoxification. Evidence of HGT acquiring *zntA* within *gcd*-containing MAGs emphasizes the importance of metal efflux mechanisms in facilitating P cycling under contamination stress. Overall, these findings demonstrate that *A. annua* and *B. davidii* effectively shape microbial communities that enhance both phosphorus availability and heavy metal tolerance, offering promising strategies for ecological restoration of Pb–Zn contaminated sites. Further multi-omics and mechanistic studies are warranted to confirm the functional activity of these genes and clarify plant-level mechanisms.

AUTHOR CONTRIBUTIONS

Caili Sun and Yongwei Liu planned and designed the research. Yajuan Yao, Yongwei Liu, and Xiaoyu Lu performed experiments, conducted fieldwork, and analyzed data. Caili Sun wrote the manuscript.

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (41967058) and Guizhou Provincial Forestry Projects ([2023]10).

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Supporting Information of this article. Further inquiries can be directed to the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sun, C., Yao, Y., Liu, Y., & Lu, X. (2026). Rhizosphere microbial detoxification and phosphorus solubilization drive ecological remediation of Pb–Zn contaminated soils. *Plants, People, Planet*, *8*(2), 639–653. https://doi.org/10.1002/ppp3.70118