



Trade-offs and adaptation to metalliferous soils: The role of soil microbiome in metal tolerance and uptake in *Arabidopsis halleri* ecotypes from a reciprocal transplant experiment

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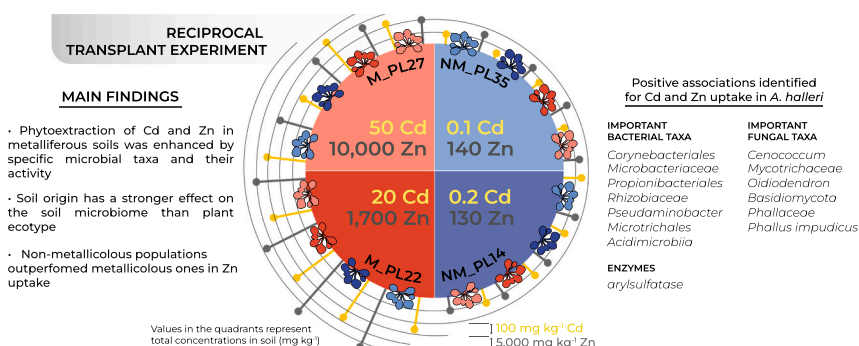
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HIGHLIGHTS

- Metal-uptake does not negatively affect biomass in non-metalliferous plants.
- Soil origin has a stronger effect on the soil microbiome than plant ecotype.
- Different microbial taxa are associated with varying levels of metal uptake.
- Phytoextraction in metalliferous soils is linked to microbial functional activity.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil contamination with trace metal elements is a worldwide issue, prompting research on plant species capable of hyperaccumulating metals to reduce soil toxicity. Previous research suggests that both plant species and their populations can affect soil microbial communities, yet little is known about how different populations of hyperaccumulator species influence these microbial communities to enhance metal-uptake and tolerance. This study evaluated the effect of soil origin, soil microbiome, and plant population on phytoextraction efficiencies of Cd and Zn among four *A. halleri* populations: two each from metalliferous and non-metalliferous sites. In a controlled transplant experiment, clonal replicates of *A. halleri* were grown in native and three non-native soils for six months. Biogeochemical analyses of plants and soils were conducted, alongside sequencing of root-associated soil bacterial/archaeal and fungal DNA. Soil treatments primarily differed in pH, total Cd, Pb, and Zn, as well as acid and alkaline phosphatase enzyme activities. A combined effect of soil origin and population was noted for arylsulfatase and β -glucosidase activities, as well as ammonium and nitrate concentrations. Both non-metalliferous and metalliferous populations accumulated high levels of Cd and Zn in metalliferous soils with the non-metalliferous population NM_PL14 outperforming the metalliferous populations in Zn hyperaccumulation. Interestingly, non-metalliferous populations grown in metalliferous soils exhibited no trade-offs in

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plant performance despite higher Cd and Zn accumulation. Soil origin had a stronger effect on the bacterial/archaeal and fungal community composition than plant ecotype. Partial least square regression models explained 66 % and 79 % of the variability in *A. halleri* Cd and Zn hyperaccumulation. There was a positive association between Zn-uptake and specific microbial taxa (e.g., *Cornebacteriales*, *Microbacteriaceae*, *Propionibacteriales*, *Rhizobiaceae*, *Basidiomycota*, *Oidiodendron*, *Phallaceae*) and functional activity (e.g., arylsulfatase, S oxidation) in metalliferous soils. Taken together, our findings suggest that non-metalliferous *A. halleri* populations may be better suited for Zn phytoextraction applications.

1. Introduction

Soil contamination with trace metal elements is a global concern, particularly in areas where the metal concentrations exceed the recommended safe levels (Su et al., 2014; Carré et al., 2017; Khan et al., 2021). To clean up these sites, environmentally friendly, cost-effective, and energy-efficient methods are needed. One such remediation technique is phytoextraction that uses plants to remove metals from soils (Li et al., 2018; Wan et al., 2020). Efficient phytoextraction is achieved by metal-hyperaccumulating plant species as they specialize in allocating extraordinarily high concentrations of metals in their foliage (Chaney et al., 2007; Sheoran et al., 2010). Moreover, the plants used for phytoextraction are often characterized with the following traits: (i) high tolerance to metals, (ii) rapid growth, (iii) high biomass, and (iv) capacity to uptake metals from soil to roots, as well as transport to and allocate in leaves of the plants (Pirzadah et al., 2015; Yu et al., 2022). As phytoextraction efficiencies can vary among populations of the same species (Deram et al., 2007; Meyer et al., 2010), optimization of this technology is crucial for achieving successful phytoremediation.

The interface between root-surface and soil (rhizosphere) serves as a dynamic system for interactions among plants, microorganisms, and soils, and thereby plays a critical role in the process of phytoremediation (Wenzel et al., 2003). Soil microorganisms that colonize the rhizosphere generally promote nutrient acquisition and plant growth, enhance metal availability and solubility, and increase metal tolerance and accumulation capacity of plants (Chaney et al., 2007; Deng et al., 2020; Muehe et al., 2015). Previous studies have demonstrated a strong association between the soil microbiome and metal hyperaccumulation in various plant species (Martos et al., 2021; Luo et al., 2022; Huang et al., 2023). Additionally, plants can influence the physicochemical properties of the root-zone associated microbial communities, which may in turn impact the plant metal accumulation (Shi et al., 2015; Adedeji and Babalola, 2020). In fact, it has been shown that different accessions or ecotypes within the same species can influence the rhizosphere microbial community in distinct ways (Micallef et al., 2009; Schlemper et al., 2017; Bowsher et al., 2020; Wang et al., 2020; Kushwaha et al., 2022). Thus, a deeper understanding of the microbiome associated with the root-zone of different plant populations under varying environmental conditions is necessary to optimize the phytoextraction performance of metal hyperaccumulators.

Arabidopsis halleri (L.) O'Kane and Al-Shehbaz is a promising candidate to study phytoextraction mechanisms as it shows high metal tolerance and hyperaccumulates cadmium (Cd) and zinc (Zn) (Roosens et al., 2008). *A. halleri* metalliferous (M) and non-metalliferous (NM) populations tolerate high concentrations of metals at metal-contaminated sites and grow at uncontaminated sites, respectively. Previous research has shown that while Cd and Zn hyperaccumulation is higher in metalliferous populations at metal contaminated sites, some non-metalliferous populations can accumulate Cd and Zn more efficiently on uncontaminated soils than metalliferous populations on contaminated soils (Babst-Kostecka et al., 2018; Dietrich et al., 2019; Kushwaha et al., 2022). Moreover, recent work has demonstrated a link between variation in metal hyperaccumulation among *A. halleri* populations and soil biota (Klimek et al., 2023; Kushwaha et al., 2022), but a detailed characterization and comparison of microorganisms associated with the root-zone of *A. halleri* from both contaminated and

uncontaminated sites, as well as the microbiome's association with metal hyperaccumulation, is still lacking. Although some *A. halleri* accessions have recently been evaluated for their phytoextraction efficiency and phytoremediation potential (Dietrich et al., 2021; Klimek et al., 2023), the largely unexplored associations between variability in populations' responses and microbial-metal interactions hinder the full realization of its phytoextraction potential.

To address this knowledge gap, we characterized the effect of soil origin, soil microbiome, and plant population on Cd and Zn hyperaccumulation among four *A. halleri* populations, two each from metalliferous and non-metalliferous sites in Southern Poland (Babst-Kostecka et al., 2018; Dietrich et al., 2021; Kushwaha et al., 2022). The four populations are genetically similar and located in close geographic proximity (Sailer et al., 2018; Babst-Kostecka et al., 2018), yet they display significant quantitative variation in metal tolerance and hyperaccumulation capacity (Meyer et al., 2010; Babst-Kostecka et al., 2018; Dietrich et al., 2021). Additionally, the evolutionary dynamics of metal hyperaccumulation are particularly interesting, as an increase in hyperaccumulation in the lowland non-metalliferous population from the Niepolomice Forest (NM_PL14) might be attributed to the colonization of this site by plants from former metalliferous populations in the Olkusz region (M_PL22, M_PL27). To determine if these patterns are a result of specific interactions with microorganisms associated with the root-zone of *A. halleri* local soils, we have evaluated fitness, interactions with the soil microbiome, and phytoextraction capacity of these *A. halleri* accessions in a transplant experiment, where clonal replicates were grown in both native and non-native soils under controlled conditions. Plant growth, phenotypic properties, and metal accumulation were combined with soil biogeochemistry and microbiome datasets to investigate plant-metal-microbe interactions. Using this setup, we addressed the following questions: (1) Are the metal tolerance and uptake capacities of the non-metalliferous and metalliferous *A. halleri* populations different in non-metalliferous and metalliferous soils? (2) Are the differences in soil microbial diversity and composition associated with plant populations or soil origin? (3) Are there specific microbial phylotypes in non-metalliferous vs. metalliferous soils that associate with different hyperaccumulation patterns in plant populations?

2. Materials and methods

2.1. Plant and soil material

The sampling included two non-metalliferous (NM_PL14 and NM_PL35) and two metalliferous (M_PL22 and M_PL27) locations of *A. halleri* in southern Poland. Organo-mineral soil horizon (topsoil) was collected in each location using sterile instruments, from five 2 × 2 m quadrats with *A. halleri* vegetation (Table 1). Soil was sieved (7 mm), homogenized, and placed into sterile plastic bags. Approximately 100 L of composite soil sample per site was transferred to the laboratory, redistributed to experimental pots, and placed in the greenhouse. The 0.5 L pots were immediately used for rooting *A. halleri* cuttings, while the 2.5 L pots were stored for 2 weeks before starting the reciprocal transplant experiment (Fig. S1; see below). Additionally, five mature *A. halleri* individuals (genotypes) were collected in each location, ensuring a minimum distance of 5 m between plants to avoid potential

clones. Each plant was then asexually propagated *via* cuttings, yielding 20 clones per genotype. Plants were individually rooted in 0.5 L pots filled with four soils collected from the field locations (NM_PL14, NM_PL35, M_PL22, and M_PL27) – 5 clonal replicates per each genotype in each soil – and grown in a greenhouse for 2 weeks prior to the experiment (day/night regime of 20°/18 °C, photoperiod of 13 h/11 h, and a light intensity of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

2.2. Reciprocal transplant experiment under controlled conditions

Four similar-size plants per genotype were selected from the two-week old cuttings, each already rooted in the four soils, and individually placed into corresponding 2.5 L pots (Fig. S1). Pots were filled with topsoil collected directly from the four study sites: NM_PL14, NM_PL35, M_PL22, and M_PL27 (see above). Thus, each plant genotype had one clonal replicate grown in the substrate from its native site and from the three non-native locations (Fig. S1). This approach ensured that all plants were exposed to different soil treatments from the beginning – warranting that any observed differences in performance during the experiment were attributable to the soil treatment, and not pre-existing native soil conditions or acclimatization effects. Further, by establishing initial microbial interactions in each soil, we facilitated microbial colonization and community development, which could influence plant health and interactions with different soils used in the experiment.

A total of 80 pots with plants were included in the experiment. This comprised 4 populations $\times$ 5 genotypes $\times$ 4 clonal replicates, each grown in their native and in three non-native soil treatments (Fig. S2). Additionally, 20 non-planted pots were established as negative controls (5 per soil treatment) to ensure that any variations in metal concentrations and pH were due to plant and microbial activity rather than other variables related to the experimental setup and manipulation (e.g., irrigation; Table S1). The experiment was conducted under controlled conditions with a day/night regime of 20°/18 °C, photoperiod of 13 h/11 h, and a light intensity of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Pots were watered every four days to maintain soil water content close to field capacity (no leaching occurred), and their location was randomized monthly. The experiment ran for 6 months.

2.3. Plant phenotypic traits

The phenotypic variability in response to soil treatment was assessed by scoring at the beginning (day 1), in the second half (day 129), and at the end of the experiment (day 178): maximum quantum efficiency of photosystem II (Fv/Fm), performance index on absorption basis (PI_{abs}), relative growth rate, and leaf percentage showing visible symptoms of metal toxicity. At the end of the experiment, we additionally scored the dry shoot biomass, and Cd and Zn shoot concentrations. The relative growth rate was calculated as $(\ln LA_y - \ln LA_x)/D$, where LA_x and LA_y is the total plant leaf area at times x and y , and D is the time elapsed between y and x (Cambrollé et al., 2013). Plant leaf area (cm^2) was estimated through a non-destructive technique based on digital photographs analyses with Easy Leaf Area software (Easlon and Bloom, 2014). Fv/Fm and PI_{abs} were provided as estimators of photosynthetic performance of each plant and consisted of three measurements of medium-sized leaves per plant using the Handy PEA chlorophyll

fluorometer (Hansatech Instruments, United Kingdom), after 20 min sample dark adaptation with leaf clips. The Fv/Fm parameter was selected as an indicator of plant photosynthetic performance, presented as a ratio of variable fluorescence (Fv) over the maximum fluorescence value obtained for continuous light intensity (Fm); the PI_{abs} reflects the functionality of both photosystems I and II. At the end of the experiment, plants were harvested, their shoots washed twice in deionized water, dried at 80 °C for 48 h, and dry shoot mass (g) was quantified for each plant.

The dry shoots were used to determine the epigeous Zn and Cd concentrations. Approximately 0.3 g of dry-ground shoot tissue samples were mixed with 6 mL of nitric acid (HNO₃, 69–70 %) and HClO₄ (70–72 %) (4:1 v/v), left for 24 h, and boiled on a hot plate (Digester 40 Auto, Foss Tecator, Sweden) at 282–284 °C for 1.5–2 h. Elemental concentration was determined using atomic absorption spectrometers (AAS; AA280FS, Varian; AA280Z, GTA 120, Varian, Australia) and the results were ascertained using certified Standard Reference Material 1570a – Spinach Leaves (National Institute of Standards & Technology). The recovery values ranged between 96 % and 99 %, with RDS $\pm$ 0.5 %.

2.4. Soil sample collection at the end of the experiment

Upon harvest, root-associated soil samples were collected for each plant. Roots were excised using sterile instruments, placed into a sterile plastic bag, and shaken to separate and collect ~50 g of soil that adhered to the roots for DNA extraction and soil microbiome analyses (Solís-Domínguez et al., 2012; Fig. S1). Additional ~50 g and 100 g of soil were sampled next to the *A. halleri* roots for geochemical and biogeochemical/enzymatic analyses, respectively. All samples for soil microbiome analyses were immediately placed on ice and stored at –80 °C until processing. Samples for analyses of soil respiration rate and enzymatic activity were stored at –20 °C. The samples for geochemical analysis were either air dried at room temperature for about 1 week (pH, NH₄-N, and NO₃-N) or at 105 °C overnight for the measurement of total Cd, Zn, and Pb concentrations (Öhlinger, 1996; PN-ISO 11464, 1999).

2.5. Soil chemical analyses

Soil dry weight was determined after drying overnight at 105 °C. Maximum water holding capacity was assessed according to Öhlinger (1996) with minor modifications, using plastic cylinders with a fine mesh at one end. Soil pH was measured in a 1:5 (w:v) suspension of soil in deionized water (ISO 10390) using HQ40d meter (Hach, USA). For the analyses of pseudo-total (hereafter total) Zn, Cd, and Pb concentrations, 0.5 g of dried (dried at 105 °C for 24 h) and ground soil (Analysette 3 Spartan Pulverisette 0, Fritsch, Germany) was mineralized in 10 mL HClO₄, using a hot plate (Digester 40 Auto, Foss Tecator, Sweden). The extracted elements were analyzed with AAS (AA280FS, Varian; AA280Z, GTA 120, Varian, Australia) and the results were verified using the certified reference material CRM048-050 (RTC). The recovery values ranged between 97 % and 101 %, with RDS $\pm$ 0.5 %. Bioaccumulation factors for Zn and Cd were calculated as the ratio of metal concentration in plant shoots and metal concentration in soil. To analyze water-extractable NH₄-N and NO₃-N concentrations, air-dried soil samples were shaken (Laboratory Shaker type 358S, Elpan, Poland) in water for 1 h (1:10, w:v) and filtered through cellulose

Table 1

Geographic location, GPS coordinates, elevation, pH, and total Cd and Zn concentrations in the soil samples (mean $\pm$ SD, n = 5) from the four field study sites at the start of the transplant experiment. NM = non-metalliferous sites; M = metalliferous sites.

Site	Location	Latitude (°N)	Longitude (°E)	Elevation (m)	pH	Total Cd (mg kg ⁻¹)	Total Zn (mg kg ⁻¹)
NM_PL14	Niepolomice Forest	50.108833	20.367467	188	5.6 $\pm$ 0.1	0.20 $\pm$ 0.04	136 $\pm$ 28
NM_PL35	Kościelisko	49.287056	19.879417	927	4.8 $\pm$ 0.1	0.10 $\pm$ 0.01	141 $\pm$ 107
M_PL22	Bukowno	50.282800	19.478717	339	7.8 $\pm$ 0.1	17 $\pm$ 1	1662 $\pm$ 198
M_PL27	Galman	50.198367	19.538817	471	7.3 $\pm$ 0.1	54 $\pm$ 2	10,075 $\pm$ 30

acetate membrane syringe filters (Huang and Schoenau, 1998, modified). NO₃-N concentration in the extracts was determined with an ion chromatograph ICS-1100 (Dionex, USA), while NH₄-N concentration was determined with an ion chromatograph DX-100 (Dionex, USA).

2.6. Soil biogeochemical and enzymatic analyses

Frozen soil was thawed for a few days in a refrigerator at 4 °C prior to microbiological analyses (Öhlinger, 1996). All soil samples were sieved (2 mm mesh). Soil basal respiration and substrate-induced respiration were determined in samples adjusted to 50 % water holding capacity. Moist soil samples (3 g dw soil) were incubated in small glass ampoules at 22 °C in the dark. The concentration of CO₂ was measured after about 20 h incubation with a gas chromatograph equipped with a methanizer and a flame ionization detector (GC-FID 450; Bruker, USA). After the soil basal respiration measurements, a mixture of talcum and glucose monohydrate (4:1; 10 mg glucose g⁻¹ dw soil) was added and mixed into the soil samples to measure substrate-induced respiration after about 4 h incubation. The concentration of evolved CO₂ was determined as described above. Blank samples were included to estimate ambient CO₂ concentrations (Beck et al., 1996; Rousk and Frey, 2015).

To measure acid and alkaline phosphatase activities, moist soil samples (~0.5 g) were incubated at 37 °C for 1 h after the addition of *p*-nitrophenyl phosphate solution and adequate buffer (pH = 6.5 for acid and pH = 11 for alkaline phosphatase). Only buffer solution was added to control samples prior to the incubation. Released *p*-nitrophenyl was colored with NaOH after the addition of CaCl₂ and determined photometrically at 400 nm (Margesin, 1996) using a colorimeter DR3800 (Hach Lange, USA). To measure arylsulfatase activity, moist soil samples (~0.5 g) were incubated for 1 h at 37 °C after the addition of acetate buffer (pH = 5.8) and *p*-nitrophenylsulfate solution. Only buffer solution was added to control samples prior to the incubation. After incubation, distilled water and NaOH solution were added. The content of *p*-nitrophenol was determined photometrically at 420 nm (Strobl and Traunmüller, 1996a) with a colorimeter DR 3800 (Hach Lange, USA). β -glucosidase activity was measured according to Strobl and Traunmüller (1996b). Moist soil samples (~2.5 g) were incubated for 3 h at 37 °C following the addition of acetate buffer (pH = 6.2) and β -glucosido-saligenin (salicin) as the substrate. Water was added to control samples instead of substrate solution prior to the incubation. Released saligenin was colored with 2,6-dibromochinon-4-chlorimide following the addition of borate buffer (pH = 10) and measured at 578 nm with a DR 3800 colorimeter (Hach Lange, USA).

2.7. Soil microbiome analyses: DNA extraction and amplicon sequencing

DNA extraction and DNA clean-up were performed using the FastDNA Spin Kit for Soil™ (MP Biomedicals, Solon OH, USA) and DNeasy PowerClean Pro Cleanup kit (Qiagen, Hilden, Germany), respectively, as described in Kushwaha et al. (2022). To characterize bacterial/archaeal and fungal communities, V4 region of the 16S ribosomal RNA (rRNA) was amplified using 515F/806R primers and ITS1f-ITS2 primers were used to amplify the fungal internal transcribed spacer (ITS) region, respectively (Walters et al., 2016). A paired-end (read length of 2 × 150-bp) sequencing was performed after pooling purified amplicons from all the samples together in equimolar concentrations. The sequencing run was conducted on the Illumina MiSeq platform (Illumina, Inc., CA, USA) at the Microbiome Core, University of Arizona. The DADA2 pipeline was used to conduct bioinformatics analyses (Callahan et al., 2016) as described in the Supplementary material. Raw bacterial/archaeal and fungal sequencing data were submitted to NCBI's Sequence Read Archive under the BioProjects: PRJNA1092725 and PRJNA1092728, respectively.

2.8. Statistical analyses

Raw datasets for soil enzyme activity, biogeochemical and abiotic parameters as well as plant variables were tested for normality using Shapiro-Wilk test and homogeneity of variance was evaluated using Levene's test. Several parameters departed from normality and were subsequently transformed using the bestNormalize function (Peterson, 2021). While transformed data were used for the statistical analyses, raw data for all variables are presented in tables and figures to enhance interpretability. Analysis of variance (ANOVA) was carried out to test the differences across the 16 treatments (4 soil treatments × 4 plant populations), followed by a Tukey's honestly significant difference (HSD) *post hoc* test, using the package agricolae (de Mendiburu, 2016).

Alpha (richness and Shannon index) and beta diversity of the microbial community were calculated using the vegan package (Oksanen et al., 2008). Community dissimilarity was evaluated using the Bray-Curtis distance and non-metric multidimensional scaling (NMDS) ordination plots were used for visualization. Alpha diversity and community composition differences were determined using ANOVA and nested permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001), respectively. The factor soil origin (NM_PL14_S, NM_PL35_S, M_PL22_S, M_PL27_S) was nested within site type (non-metalliferous vs. metalliferous) for a nested PERMANOVA. The predicted microbial functional proportions were visualized using a heatmap with hierarchical clustering (complete linkage method) and generated via ComplexHeatmap package (Gu et al., 2016).

A linear discriminant effect size (LEfSe; Segata et al., 2011) analysis was conducted to identify microbial taxa that most likely differentiate across soils (S) from the four different locations for each plant population (P), i) population NM_PL14_P grown in NM_PL14_S, NM_PL35_S, M_PL22_S, and M_PL27_S soils, ii) population NM_PL35_P grown in NM_PL14_S, NM_PL35_S, M_PL22_S, and M_PL27_S soils, iii) population M_PL22_P grown in NM_PL14_S, NM_PL35_S, M_PL22_S, and M_PL27_S soils, and iv) population M_PL27_P grown in NM_PL14_S, NM_PL35_S, M_PL22_S, and M_PL27_S soil. The LEfSe analysis uses standard statistical tests to identify these microbial taxa across each group and generates a logarithmic score value. For the linear discriminant analysis (LDA) score, we used the default cutoff value of LDA > 2.0.

Partial Least Square (PLS) regression models were used to investigate the contribution of abiotic and biotic soil variables together with plant metrics (= a matrix of 178 predictor variables) in the variation in Cd and Zn concentrations in *A. halleri*. The microbial taxa used in the PLS analyses were the unique taxa that were identified in LEfSe analyses and had the highest mean among all the soils (> 4.5 logarithmic value; Tables S2 & S3). Detailed methodology for PLS analyses is described in the Supplementary material. All the analyses were performed in R 3.4.2 (R Core Team, Vienna, Austria) except for PLS, that was performed using XLSTAT.

3. Results

3.1. Biogeochemical characteristics of soils

Recall that the clonal replicates of four *A. halleri* populations were grown for 6 months in soils from four locations (two non-metalliferous and metalliferous site types each), including their native soil and the corresponding three non-native soils. The bio-geochemical properties of these soils before the start of the experiment are listed in Table S4. Briefly, both the metalliferous soils had significantly greater Cd (35 ± 19 mg kg⁻¹), Pb (2706 ± 2224 mg kg⁻¹), Zn (5869 ± 4440 mg kg⁻¹), and nitrate concentrations (101 ± 36 mg kg⁻¹) as well as higher pH (7.5 ± 0.3) in comparison to non-metalliferous soils (0.2 ± 0.1 mg kg⁻¹, 23 ± 2.6 mg kg⁻¹, 138 ± 74 mg kg⁻¹, 35 ± 16 mg kg⁻¹, 5.2 ± 0.4 , respectively).

At the end of the experiment, the site type (non-metalliferous vs. metalliferous) and origin of soil (NM_PL14_S, NM_PL35_S, M_PL22_S,

M_PL27_S) had the dominant effect vs. the population effect on the differences in total Cd, Pb, and Zn, and pH as well as acid and alkaline phosphatase enzyme activity (Figs. 1 & 2; Table S5). Total Cd (Fig. 1A), Zn (Fig. 1B), and Pb (Table S5) were significantly higher in metalliferous soils compared to non-metalliferous soils, with the highest values found in M_PL27 soils ($60 \pm 3 \text{ mg kg}^{-1}$, $9758 \pm 630 \text{ mg kg}^{-1}$, $5657 \pm 574 \text{ mg kg}^{-1}$, respectively), followed by M_PL22 ($19 \pm 0.8 \text{ mg kg}^{-1}$, $1634 \pm 94 \text{ mg kg}^{-1}$, $781 \pm 33 \text{ mg kg}^{-1}$, respectively) and non-metalliferous soils ($0.12 \pm 0.03 \text{ mg kg}^{-1}$, $169 \pm 89 \text{ mg kg}^{-1}$, $30 \pm 7 \text{ mg kg}^{-1}$, respectively). On comparison of Zn concentrations at the end of the experiment to the starting values, it was noted that the plants grown in M_PL27 soil were the most effective in removing Zn from the soils (Fig. 1D). Further, an intra-population variation was observed with regards to plants' ability in removing Zn from the root-associated soil. In contrast, there was an increase in Cd in root-zone of metalliferous soils across all populations, with NM_PL35 population having the highest increase (Fig. 1C). Regarding pH, it ranged between 4.4 and 7.7 and increased across the sites in the following order: NM_PL35 < NM_PL14 < M_PL27 < M_PL22 (Table S5). Metalliferous soils had a significantly higher alkaline phosphatase activity than non-metalliferous soils, with the highest values in M_PL27 soils, followed by M_PL22, NM_PL35, and NM_PL14 soils (Fig. 2B; Table S5). In contrast, acid phosphatase activity was significantly higher in non-metalliferous vs. metalliferous soils (Fig. 2A; Table S5).

A combined effect of soil origin with the population that grew in the respective soil was observed for arylsulfatase and β -glucosidase activities (Fig. 2C & D), as well as ammonium and nitrate concentration (Table S5). Arylsulfatase activity was the highest in M_PL22 soils but there was no difference between the populations within this specific soil treatment (Fig. 2C). In both non-metalliferous soils, the highest and the lowest values corresponded to M_PL22 and NM_PL35 populations, respectively, while the activity in soils affected by NM_PL14 and M_PL22 populations was intermediate. Interestingly, the growth of both non-metallicolous populations in M_PL27 soil increased arylsulfatase

activity compared to the growth of metallicolous populations. With regards to β -glucosidase, its activity was the highest in M_PL27 soils (Fig. 2D). As for the population effect, NM_PL35 population had positive effect on β -glucosidase activity in three soils: NM_PL14, NM_PL35, and M_PL27, in comparison to the other three populations (Fig. 2D). For soil nitrate, the lowest and the highest concentrations were found in NM_PL14 and M_PL27 soils, respectively (Table S5). Additionally, in both metalliferous soils, higher nitrate values were associated with NM_PL35 population compared to the remaining three populations growing in the same soils. The positive effect of NM_PL35 population was also reflected in ammonium concentrations across all four soils, with the highest values found in M_PL27 soils (Table S5).

3.2. Differences in Cd and Zn concentrations in shoots

Cadmium and Zn levels in plant shoots differed between site types, with significantly higher values for plants grown in metalliferous compared to non-metalliferous soils (Fig. 3A & B). Further, Cd and Zn concentrations in metallicolous populations were significantly lower in non-metalliferous ($98 \pm 32 \text{ mg kg}^{-1}$, $6590 \pm 1763 \text{ mg kg}^{-1}$, respectively) than in metalliferous soils ($329 \pm 115 \text{ mg kg}^{-1}$, $16,602 \pm 8185 \text{ mg kg}^{-1}$, respectively). All populations grown in metalliferous soils exceeded the hyperaccumulation threshold for Cd (Fig. 3A; 100 mg kg^{-1} ; van der Ent et al., 2013). Regarding plants grown in non-metalliferous soils, Cd hyperaccumulation was found in the majority of non-metallicolous and metallicolous plants (75 %) grown in NM_PL14 soils, while only 1 out of 20 plants grown in NM_PL35 soils reached the hyperaccumulation threshold for this element (Fig. 3A). In M_PL22 soils, shoots of NM_PL14 plants had the highest average Cd levels, followed by M_PL22, M_PL27, and NM_PL35 populations. In M_PL27 soil, the highest and the lowest Cd accumulation was found in M_PL22 and NM_PL14 populations, respectively. Overall, non-metallicolous populations accumulated more Cd in metalliferous vs. non-metalliferous soils (Fig. S3). All non-metallicolous and metallicolous populations had Cd

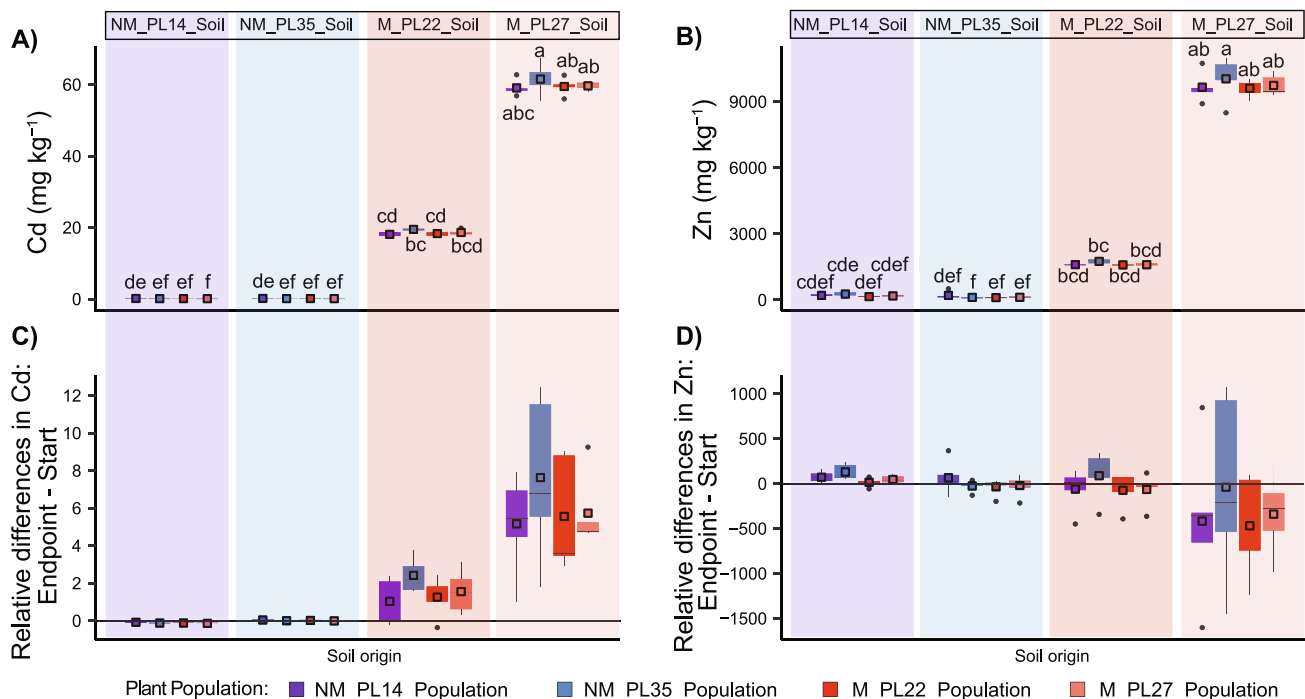


Fig. 1. Soil total Cd (panel A) and Zn (panel B) concentrations after 6 months of growth of two non-metallicolous (NM; blue) and two metallicolous (M; red) *Arabidopsis halleri* populations in the reciprocal transplant experiment under controlled conditions. Panel C and D represent the differences in total Cd and Zn concentrations at the end of the experiment relative to the starting total Cd and Zn concentrations in the soil, respectively. The boxes represent the inter-quartile range of the raw data, with the mean and median indicated by the square and horizontal line within the boxes, respectively. Black circles outside the boxes represent outliers. Statistically significant differences across the 16 treatments are represented by the different letters at $p \leq 0.05$ (ANOVA, Tukey's HSD *post hoc* test).

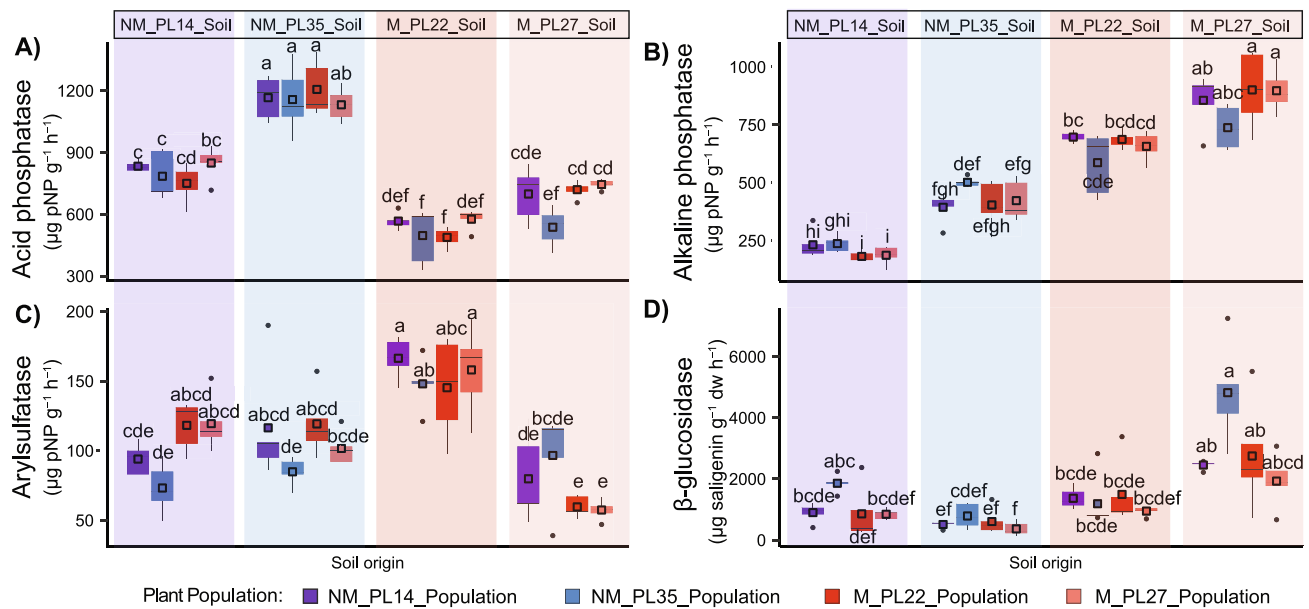


Fig. 2. Soil enzymatic activities in *Arabidopsis halleri* soil samples after 6 months of growth of two non-metallicolous (NM; blue) and two metallicolous (M; red) populations in the reciprocal transplant experiment under controlled conditions. A) Acid phosphatase, B) Alkaline phosphatase, C) Arylsulfatase, and D) β -glucosidase. The boxes represent the inter-quartile range of the raw data, with the mean and median indicated by the square and horizontal line within the boxes, respectively. Black circles outside the boxes represent outliers. Statistically significant differences across the 16 treatments are represented by the different letters at $p \leq 0.05$ (ANOVA, Tukey's HSD *post hoc* test).

bioaccumulation factors that exceeded one (Fig. 3C), and the highest bioaccumulation factor was found in plants grown in NM_PL14 soil.

For all non-metallicolous and metallicolous populations, Zn shoot concentrations (Fig. 3B) across all four soils were above the hyper-accumulation threshold (3000 mg kg⁻¹; van der Ent et al., 2013) and particularly high concentrations were found in plants grown in M_PL22 soils (24,474 $\pm$ 6960 mg kg⁻¹). In metalliferous soils, NM_PL14 population was particularly efficient in Zn hyperaccumulation and out-competed the metallicolous populations. Further, M_PL27 population had the lowest concentration of Zn in its native M_PL27 soil, compared to the other three populations grown in this soil. Like Cd, all populations grown in non-metalliferous soils had the highest bioaccumulation factor for Zn (Fig. 3D). While the M_PL27 population had higher average Zn bioaccumulation factor in non-metalliferous soils, only 1 out of the 5 plants exceeded the bioaccumulation factor of 1 in M_PL27 soil.

3.3. Differences in *A. halleri* plant performance metrics

Although non-metallicolous populations in metalliferous soils accumulated more Cd and Zn (Fig. S3 & Fig. S4), there were no trade-offs in plant biomass (Fig. S5), as both fresh and dry plant biomass did not vary significantly between the populations and across all four soils (Table S5 & Fig. 3E). The maximum quantum efficiency of PSII (Fv/Fm) ranged from 0.78 to 0.85 and photosynthetic performance remained unaffected for all populations in metalliferous soils, indicating their tolerance to elevated metal concentrations (Fig. 3F). The Fv/Fm of metallicolous populations was not affected by any type of soil for most of the experiment (Table S5), yet by the end of the experiment the significant drop in this parameter was observed for M_PL27 population in NM_PL14 soil (Fig. 3F). Overall, similar patterns were reflected by Pl_{abs} values (Table S5).

At harvest, population NM_PL35 had the lowest leaf area, in particular in metalliferous soils, followed by population M_PL22, NM_PL14, and M_PL27 (Fig. S6; Table S5). Temporal trends in leaf surface area were more diverse during the experiment. For example, both non-metallicolous populations in NM_PL14 soil had a significantly larger leaf area at day 129 when compared to the remaining three populations,

but by the end of the experiment the parameter slightly decreased and reached the level of other populations (Fig. S6). Accordingly, both non-metallicolous and metallicolous populations grown in non-metalliferous soils had similar leaf area at the end of the experiment. Leaf area of metallicolous populations in metalliferous soils continued to increase during the entire experiment, with M_PL27 population having the higher value compared to M_PL22 in both soils. Therefore, leaf area of metallicolous populations remained larger in metalliferous vs. non-metalliferous soils at the end of the experiment and this difference was significant for the M_PL22 population. In contrast, the overall relative plant growth rate (between the 1st and last day of the experiment) did not vary significantly across the populations and soil treatments (Fig. S7).

3.4. Microbial diversity and community composition

The bacterial/archaeal richness and Shannon index were similar across NM_PL14, M_PL22, and M_PL27 soils and were higher than in NM_PL35 soils (Fig. 4A; Table S5). Contrarily, the fungal richness and Shannon diversity were the highest in M_PL27 soils followed by NM_PL14, NM_PL35, and M_PL22 soils (Fig. 4B; Table S5). Both bacterial/archaeal and fungal metrics had little to no effect from the different *A. halleri* populations. For bacterial/archaeal community composition, site type (metalliferous vs. non-metalliferous) and soil origin (NM_PL14, NM_PL35, M_PL22, M_PL27) explained 48 % and 38 % of the variation, respectively ($p = 0.001$) (Fig. 4C). The strong soil origin effect is also evident from extremely close clustering of samples from each soil regardless of the plant population origin. Additionally, the two non-metalliferous soils were closer to each other in the ordination plot while the metalliferous soils grouped away from each other (Fig. 4C). Regarding fungal community composition, site type and soil origin also had a strong effect and explained 29 % and 53 % of the community variation, respectively ($p = 0.001$) (Fig. 4D). Like bacterial/archaeal ordination, samples within the non-metalliferous soils grouped relatively closer together. Metalliferous soils clustered away from each other but M_PL27 soils grouped closer to NM_PL14 soils (Fig. 4D). Taken together, site type and soil origin explained 86 % and 82 % of the

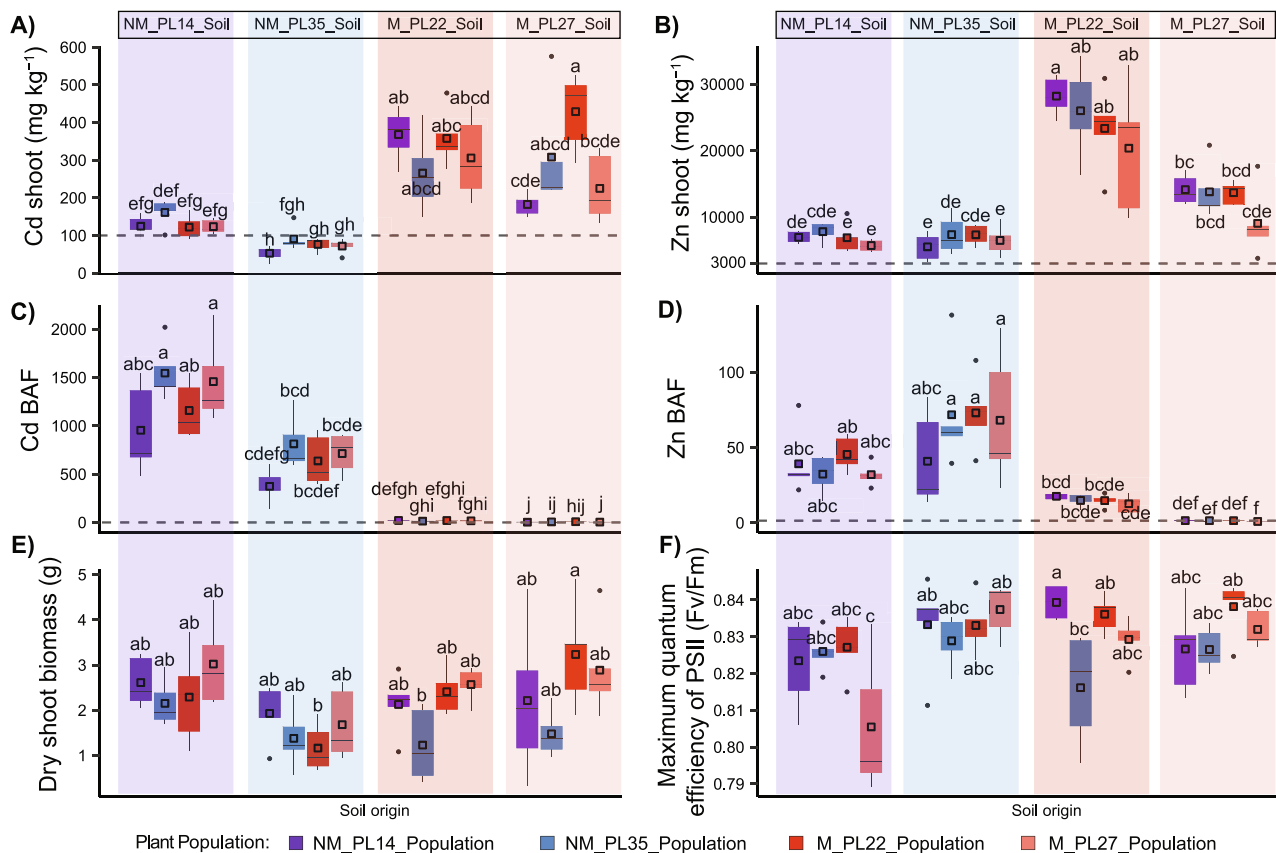


Fig. 3. Plant traits measured in two non-metalliferous (NM; blue) and two metalliferous (M; red) *Arabidopsis halleri* populations after 6 months of growth in the reciprocal transplant experiment under controlled conditions. Shoot concentrations of Cd and Zn (panels A & B, respectively), Bioaccumulation factors (BAFs) for Cd and Zn (panel C & D, respectively), dried shoot biomass (panel E), and maximum quantum efficiency of photosystem (PS) II (Fv/Fm; panel F). The boxes represent the inter-quartile range of the raw data, with the mean and median indicated by the square and horizontal line within the boxes, respectively. Black circles outside the boxes represent outliers. The dotted lines in panels A and B indicate the thresholds for Cd and Zn hyperaccumulation, respectively. The dotted lines in panels C and D indicate a BAF = 1. Statistically significant differences across the 16 treatments are represented by the different letters at $p \leq 0.05$ (ANOVA, Tukey's HSD *post hoc* test).

bacterial/archaeal and fungal community composition, respectively, with site type influencing the bacterial/archaeal community 1.7-fold more than the fungal community.

3.5. Microbial functional predictions

Significant differences for predicted bacterial/archaeal functions were observed at the site type level (metalliferous vs. non-metalliferous) across the 16 treatments *i.e.*, 4 soil origins $\times$ 4 plant populations (Fig. S8). Hierarchical clustering grouped the samples within these 16 treatments according to their site type and identified two main clusters, that reflect their respective metalliferous and non-metalliferous site type origin. Ten bacterial/archaeal functions differed significantly between metalliferous and non-metalliferous sites. Particularly, methanotrophy, methylotrophy, aerobic ammonia oxidation, nitrification, aerobic chemoheterotrophy, chemoheterotrophy, and hydrogen carbon degradation dominated at metalliferous sites, whereas cellulolysis, fermentation, and iron respiration were predominantly present at non-metalliferous sites.

Regarding fungal guilds, samples from the 16 treatments were primarily organized according to the soil origin (Fig. S9). Like bacteria/archaea, the analysis identified two main clusters, yet they were comprised of: i) M_PL22 soil vs. ii) M_PL27, NM_PL14, and NM_PL35 soils, with the latter two sharing a sub-cluster. Unlike bacterial/archaeal functional guilds, fungal guilds did not have distinct differences based on the site type, except ectomycorrhizal fungi and soil saprotrophs that dominated in metalliferous and non-metalliferous sites, respectively.

Alternatively, both arbuscular mycorrhizal fungi and endophytes were significantly more abundant in NM_PL35 and M_PL27 soils. Additionally, M_PL27 soils had the greatest abundance of fungal parasites and orchid mycorrhizal fungi in comparison to other soils. In contrast, NM_PL14 and M_PL22 soils had a greater abundance of wood saprotroph and ericoid mycorrhizal fungi, respectively, when compared to other soils.

3.6. Cd and Zn shoot concentrations associations with abiotic and biotic variables

Two partial least square (PLS) components were identified as optimum for both PLS regression models on Cd and Zn shoot concentrations. As the number of components is the same in both regression models, these two models can be directly compared to each other. Together, the first two PLS components explained 66 % and 79 % of the variations in Cd and Zn shoot concentrations, respectively (Fig. S10). In the Cd model, all explanatory variables were responsible for 62 % and 3 % of the variance for the first and the second PLS component, respectively, and Cd shoot concentrations explained 24 % (PLS 1) and 18 % (PLS 2). On the other hand, all the explanatory variables in the Zn model accounted for 75 % and 4 % of the variance for the first and second PLS components, while the percentage explained by Zn shoot concentration was 21 % on each PLS 1 and PLS 2 components. The significance of each explanatory variable in our two models was assessed based on the Variable Importance in Projection >1.0 criterion (Table S6). Accordingly, 49 and 65 out of 178 variables were considered as important for

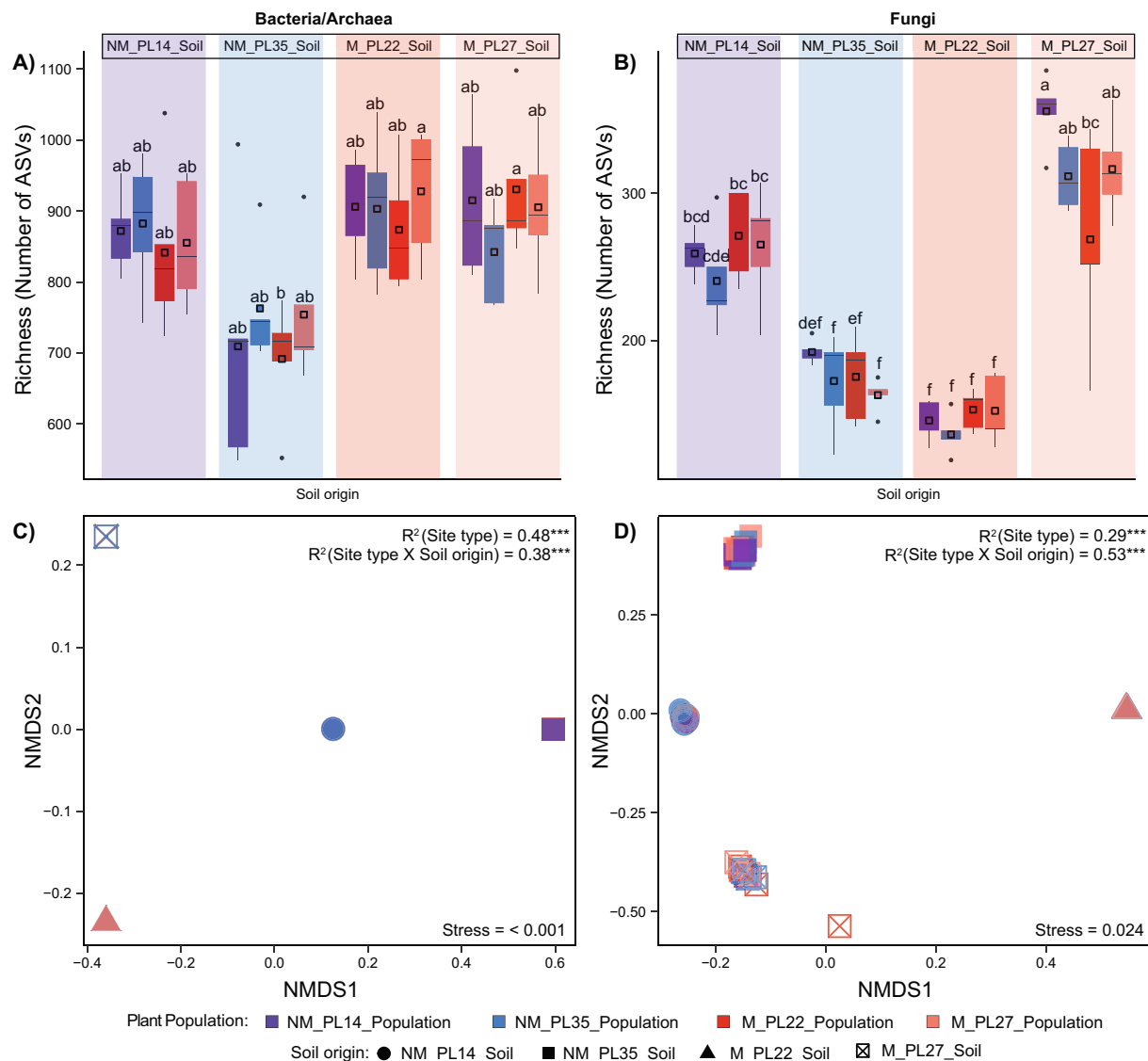


Fig. 4. Microbial diversity in *Arabidopsis halleri* root-zone soil samples, after 6 months of plant growth in the transplant experiment. Number of observed amplicon sequence variants (ASVs) represents the richness for bacteria/archaea (panel A) and fungi (panel B) in the 16 treatments. The boxes represent the inter-quartile range of the data, with the mean and median indicated by the square and horizontal line within the box, respectively. Black circles outside the box represent outliers. Statistically significant differences across the 16 treatments are represented by the different letters at $p \leq 0.05$ (ANOVA, Tukey's HSD *post hoc* test). Bacterial/archaeal (panel C) and fungal (panel D) community composition are represented by Nonmetric multidimensional scaling (NMDS) ordination plots. Site type (non-metalliferous (NM) vs. metalliferous (M)) and soil origin (NM_PL14, NM_PL35, M_PL22, M_PL27) explained the variation in the microbial community composition and are represented by R^2 ($p = 0.001$ represented as ***; PERMANOVA).

our Cd and Zn hyperaccumulation PLS regression models, respectively. The absolute values of the beta regression co-efficients reflect the relative contribution of each variable in explaining the differences in Cd (Fig. 5A) and Zn hyperaccumulation (Fig. 5B).

There was a stark difference in the strongest positive variables identified by PLS analysis for Cd and Zn hyperaccumulation. For Cd hyperaccumulation, Zn shoot concentration was the strongest positive variable, followed by the fungal taxa, *Phallaceae* and *Phallus impudicus*; bacterial taxa, *Microbacteriaceae*; and soil pH (Fig. 5A). In contrast, the top four variables in the Zn hyperaccumulation model included: fungal beta-diversity (NMDS1), M_PL22 soil, Cd shoot concentration, and the bacterial order, *Corynebacteriales* (Fig. 5B). Additionally, 26 out of 30 strong positive predictors detected in the Zn model were also identified in the Cd model. These shared predictors included a combination of bacteria/archaea taxa (15), fungal taxa (5), fungal diversity, metalliferous site type, soil origin, pH, WHC, and alkaline phosphatase enzyme activity. Also, metalliferous and non-metalliferous site types

were identified as important variables that explained the variation in Zn as well as Cd hyperaccumulation traits, with strong positive and negative associations with both traits, respectively.

Five out of the eight variables that had the strongest negative impact on Zn and Cd hyperaccumulation were the same in both models. These five variables included: bacterial NMDS1, non-metalliferous site type, acid phosphatase activity, bacterial taxon, *Acidobacteriaceae* and fungal taxon, *Tremellomycetes*. Interestingly, Zn and Cd bioaccumulation factors were negatively linked to Cd and Zn hyperaccumulation, respectively. This association contrasts with the strong positive relationship of plant shoot metal concentrations in the Zn and Cd models. Notably, both NM_PL14 and NM_PL35 soils negatively impacted Zn uptake, whereas only NM_PL35 soil hampered Cd uptake.

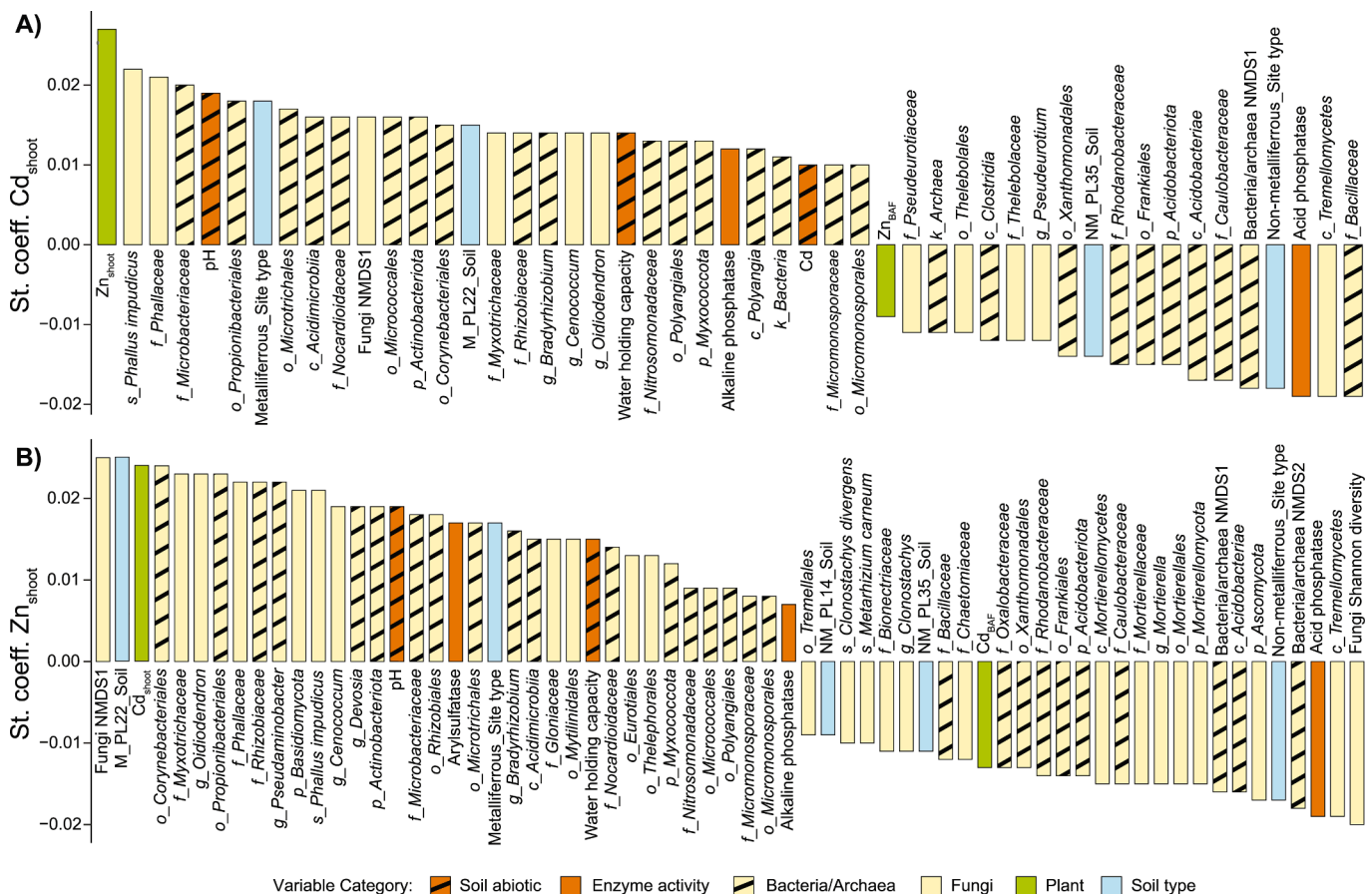


Fig. 5. Abiotic and biotic drivers of the Cd and Zn hyperaccumulation as determined by partial least square regression analysis. The relevant explanatory variables (with Variable Importance in Projection >1.0) for Cd (panel A) and Zn (panel B) hyperaccumulation are arranged in the descending order based on their standardized regression coefficients (St. coeff.). Letters preceding the microbial taxa names indicate the level of taxonomic hierarchy: p, phylum; c, class; o, order; f, family; g, genus; and s, species, and NMDS 1 & 2 refer to the non-metric dimensional scaling axes scores.

3.7. Unique microbial taxa associated with Cd and Zn shoot concentrations

A total of 56 taxa (29 bacterial/archaeal and 27 fungal) were identified by the PLS analysis as significant explanatory variables for Cd and Zn hyperaccumulation in plant shoots, including positive (29) and negative (27) associations (Fig. 6). Notably, most of the taxa (28) associated with both Cd and Zn hyperaccumulation, 21 taxa associated uniquely with Zn, and only seven taxa were unique to Cd hyperaccumulation. Out of the 29 positive associations of taxa, 11 taxa had a higher relative abundance in metalliferous vs. non-metalliferous site type and nine of them associated with both Cd and Zn uptake. These nine taxa belonged to the bacterial groups of *Acidimicrobiia*, *Actinobacteriota*, *Microbacteriaceae*, *Micrococcales*, *Micromonosporaceae*, *Micromonosporales*, *Microtrichales*, *Nocardioidaceae*, and *Polyangiales*. In addition, there were 19 taxa that were abundant exclusively in M_PL22 soils and they associated with either Zn (8) or both Zn and Cd hyperaccumulation (11). Among these 19 taxa, a notable abundance was noted for the following microbial taxa: *Actinobacteriota*, *Bradyrhizobium*, *Corynebacteriales*, *Rhizobiales*, and *Basidiomycota*.

Regarding the negative associations of taxa, 24 out of the 27 taxa were abundant in either of the two non-metalliferous soils. Eight taxa were abundant exclusively in NM_PL14 soil and impacted Zn uptake only; the other nine taxa were abundant only in NM_PL35 soil. Of the latter, six taxa solely associated with Cd uptake, and they included: all archaea; bacterial taxon, *Clostridia*; and fungal taxa, *Pseudeurotiaceae*, *Pseudeurotium*, *Thelebolaceae*, and *Thelebolales*. Interestingly, the four taxa that had similar relative abundances in both non-metalliferous

soils included a fungal species, *Metarhizium carneum*, and three bacterial taxa, *Caulobacteraceae*, *Rhodanobacteraceae*, and *Xanthomonadales*, with the latter three associating with both Zn and Cd hyperaccumulation.

4. Discussion

This study investigated the effect of soil origin, soil microbiome, and plant population on variations in Cd and Zn hyperaccumulation among *A. halleri* populations originating from metalliferous and non-metalliferous sites. Clonal replicates of plants were grown in their corresponding native and non-native soils in a transplant experiment under controlled conditions. Despite the extreme differences in the properties of metalliferous and non-metalliferous soils, all populations tolerated high concentrations of Cd and Zn and exceeded the hyperaccumulation thresholds for these metals when grown in both site types (except Cd in NM_PL35 soil). Previous work has demonstrated that metalicolous populations typically exhibit higher tolerance levels for Zn and Cd, while non-metallicolous populations in uncontaminated soils tend to accumulate metals more efficiently than metalicolous populations in metal contaminated soils (Meyer et al., 2010; Stein et al., 2017; Babst-Kostecka et al., 2018; Dietrich et al., 2021; Kushwaha et al., 2022; Klimmek et al., 2023). Consequently, *A. halleri* shoots often contain extremely high concentrations of these metals, even when present in low concentrations in the soil – suggesting active trace metal foraging beyond what could be explained by pH or bioavailable fractions of these metals (Dietrich et al., 2021). Additionally, Kushwaha et al. (2022) demonstrated a strong association of soil fungal richness and diversity as

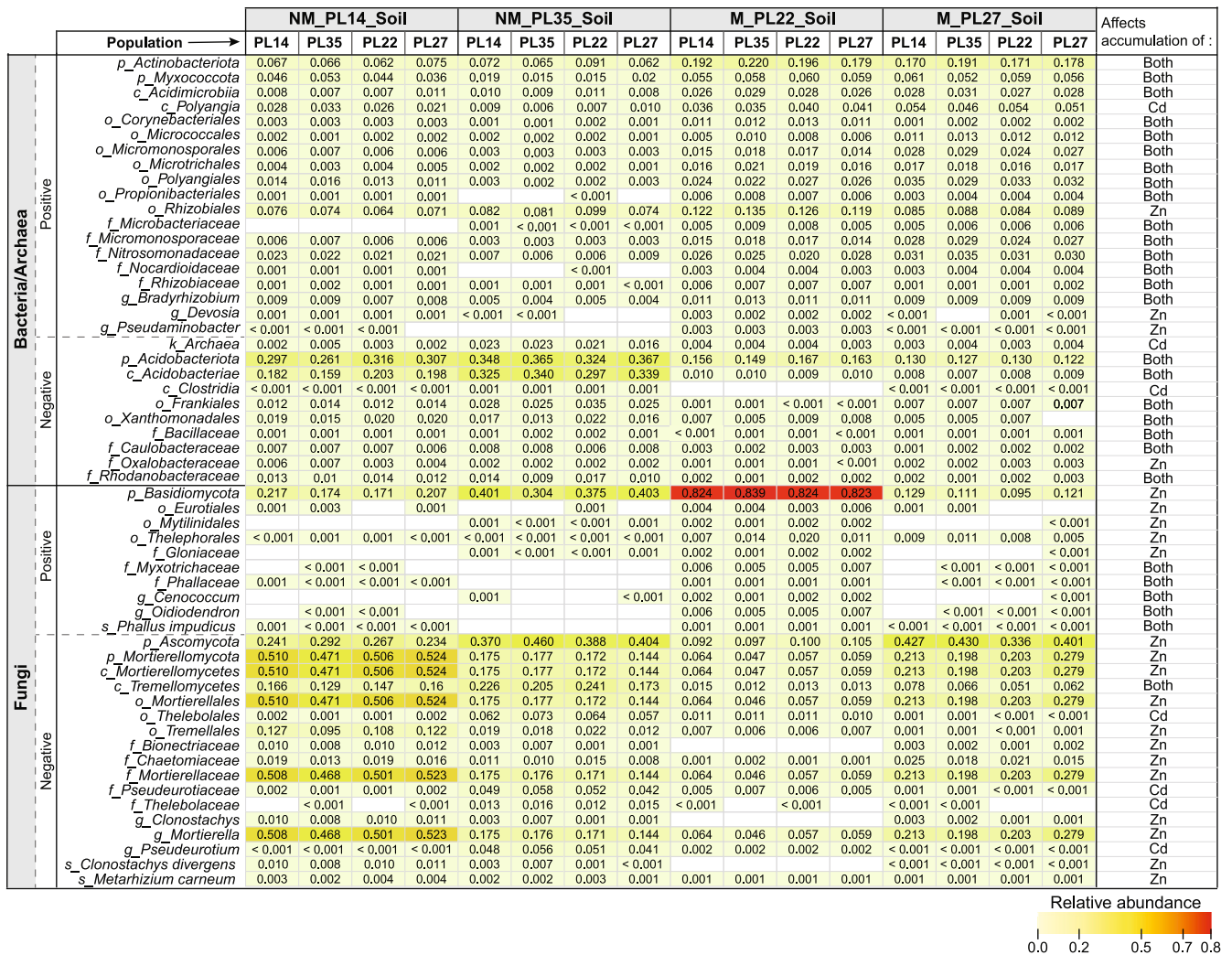


Fig. 6. Heatmap depicts the relative abundance of microbial taxa that were identified as explanatory variables for the Cd and Zn hyperaccumulation in the partial least square (PLS) regression models. Positive and negative categories reflect the direction of the standardized regression coefficients for the microbial taxa in the PLS models. The letters preceding the microbial taxa names indicate the level of taxonomic hierarchy: p, phylum; c, class; o, order; f, family; g, genus; and s, species. The taxa are arranged alphabetically within their taxonomic hierarchy level.

well as ten unique bacterial taxa with Zn hyperaccumulation in both metalicolous and non-metallicolous *A. halleri* populations. Hence, our study's unique reciprocal transplant setup, allowed for the first evaluation of the combined contributions of plant population, soil origin, and plant-microbial interactions on metal uptake patterns by *A. halleri*. These findings and their implications are discussed in detail below.

4.1. Population-specific and soil-driven patterns of Zn and Cd tolerance and uptake in *A. halleri* from a transplant experiment

study. All populations across all soil treatments did not show any toxicity symptoms, indicating that there is no trade-off between higher metal uptake and tolerance vs. maintaining physiological processes and normal plant growth in the four studies *A. halleri* accessions. Nevertheless, reaction norms generally pointed to better adaptation of populations to their native soils. Interestingly, some populations performed better in M_PL27 and NM_PL14 soils, suggesting that the biogeochemical properties of these sites may be particularly favorable. One significant factor distinguishing these two sites in our study is the higher fungal richness, which plays a crucial role in supporting metal hyperaccumulation and plant overall health. Greater fungal diversity improves metal tolerance and uptake by providing plants with essential nutrients and enhancing soil structure (Eisenhauer et al., 2017). In the case of NM_PL35 population, where biomass consistently remained low across all treatments regardless of soil origin, this suggests tighter genotype control over plant response. Additionally, the distinct small size of NM_PL35 plants further underscores its evolutionary separation from the other investigated populations in Southern Poland (Babst-Kostecka et al., 2018).

Our study also demonstrates a large variability in Cd uptake among populations, a characteristic previously observed in *A. halleri* (Bert et al., 2002; Corso et al., 2018). It has been suggested that Cd accumulation is primarily a population-specific trait in *A. halleri* (Stein et al., 2017). However, our transplant experiment, in which plants were grown in soils from their natural metalliferous and non-metalliferous locations, as opposed to hydroponic and soil-spiking methods or field surveys, indicates that Cd uptake is driven more by soil Cd concentrations than by population origin. Supporting this, both non-metalliferous and metalliferous populations exhibited significantly elevated Cd concentrations in their biomass, without any negative impact on their phenotype when grown in contaminated soils (i.e., no reduction in size or photosynthetic efficiency). Thus, we hypothesize that similar to Zn accumulation, Cd accumulation might be a constitutive trait in *A. halleri*, influenced mainly by interactions with soil biogeochemistry rather than being strictly controlled by population genetics (Dietrich et al., 2021; Sánchez et al., 2024). As recently demonstrated by Sánchez et al. (2024), Cd hyperaccumulation might not be triggered in soils with low Cd content, which could explain the low uptake in non-metalliferous soil and extreme concentrations in the same non-metalliferous populations once transplanted to metalliferous soils, as shown in our study. Additionally, we observed an increase in total Cd concentration in the root-affected soil at harvest (after 6 months of plant growth) compared to the concentration in pre-experimental soil when both non-metalliferous and metalliferous *A. halleri* populations were grown in contaminated soils. This suggests increased mobility of Cd in the root-zone compared to bulk sediments. Such patterns could result from the oxidation of metal sulfides and proton-induced dissolution of minerals (Li et al., 2022; Zhang et al., 2023), potentially enhancing metal transfer into aboveground plant tissues (Sánchez et al., 2024).

4.2. Role of soil origin vs. plant population in shaping microbial community composition

In this reciprocal transplant study, microbial diversity for bacteria/archaea and fungi was characterized more by the soil origin than the plant population. Similarly, both bacterial/archaeal and fungal community composition was influenced by the metalliferous and non-metalliferous site type and sampling location. These findings are congruent with earlier studies that reported striking differences in microbial community composition between metalliferous and non-metalliferous sites (Tipayno et al., 2018; Xu et al., 2019; Kushwaha et al., 2022). However, considering i) the rapid response of rhizosphere microbial communities to distinct root exudates produced by different plant species (Sanon et al., 2009; Maul and Drinkwater, 2010; Kulmatiski and Beard, 2011; Steinauer et al., 2023), ii) the natural variations in root exudates among different accessions of the same plant species

(Czarnota et al., 2003; Micallef et al., 2009; Monchgesang et al., 2016), and iii) the quantitative variation in metal tolerance and hyperaccumulation among *A. halleri* populations investigated in our study that is not driven by soil metal concentration (Dietrich et al., 2021), we anticipated observing plant population-driven changes in the *A. halleri* associated root-zone microbial community after 6 months of growth in the transplant experiment. In contrast, our study demonstrates that the growth of *A. halleri* populations in non-native soils did not alter the original native soil microbiome. Nonetheless, Klimek et al. (2023) found that the non-metalliferous and metalliferous *A. halleri* populations had different soil microbial communities when grown in the same metalliferous soil for ~ 1.5 years. Thus, the 6-month duration of our study appears insufficient to capture plant population-specific shifts in soil microbiome composition. Hence, to thoroughly investigate plant legacy effects and elucidate the timeline for the development of a resident soil community, we advocate for longer transplant experiments.

4.3. Zn uptake from M_PL22 soil is potentially associated with specific microbial taxa and functional activity

Despite M_PL27 having the highest metal concentrations in the soil, all *A. halleri* populations accumulated more Zn when grown in M_PL22 soil. Given that previous research showed that Cd and Zn translocation factors in *A. halleri* did not depend on the bioavailable fractions of these elements in soil at this particular location (Dietrich et al., 2021), our study suggests that soil microbiome may play a pivotal role in driving Zn hyperaccumulation in *A. halleri* populations grown in M_PL22 soils. In particular, nine bacterial taxa that were exclusively abundant in M_PL22 soil either belonged to *Actinobacteriota* (phylum) or *Rhizobiales* (order). Members of *Actinobacteriota* have previously been reported in metal-contaminated sites and their positive associations with plant metal uptake have been attributed to several metal-tolerance properties, including: (i) production of siderophores, (ii) precipitation of metals, (iii) DNA repair, and (iv) secretion of metal-binding proteins that reduce metal toxicity and oxidative stress (Bandara et al., 2022). Accordingly, strong associations have been reported between elevated concentrations of metals in soils and the presence of the following *Actinobacteriota* taxa, *Corynebacteriales*, *Microbacteriaceae*, and *Propionibacteriales* (Qian et al., 2022; Wang et al., 2022). Further, *Bradyrhizobium* and *Devosia* (*Rhizobiales*) are well-known N-fixing bacteria, also frequently reported at metal-contaminated sites (Honeker et al., 2019; Salmi and Bouilla, 2021; Geng et al., 2022). *Bradyrhizobium* can produce indole acetic acid and siderophores, and thus has the potential to alleviate metal-stress as well as increase metal solubility (Seneviratne et al., 2016; Asad et al., 2019). Indeed, inoculation with *Bradyrhizobium japonicum* enhanced Cd and Ni uptake and promoted plant growth in *Atriplex lentiformis* (Kumar et al., 2023). Similarly, *Devosia* has been associated with Zn hyperaccumulation in *Noccaea caerulea* (Lodewyckx et al., 2002) and Ni hyperaccumulation in *Alyssum murale* (Lopez et al., 2017).

Not only did we detect bacterial taxa that were exclusively abundant in M_PL22 soils, but we also found associations between microbial functional activity and metal hyperaccumulation in the corresponding plant samples. The levels of arylsulfatase enzyme activity and the abundance of dark oxidation of S compounds (functional prediction), responsible for transforming sulfur into sulfate (the bioavailable S form for plant uptake; Scherer, 2009), were the highest in M_PL22 soil. Two of the bacteria associated with this function, *Thiobacillus* and *Limnobacter*, have been reported in several metal-contaminated sites, including the current study (Xiao et al., 2016; Valentín-Vargas et al., 2018; Lopes et al., 2020; Qian et al., 2023). *Thiobacillus* with the potential to enhance metal bioavailability and mobility in contaminated soils by bioleaching metals via biological and/or indirect chemical oxidation processes is of particular interest (Ye et al., 2020). Moreover, studies by Nocito et al. (2002, 2006) have demonstrated that metals like Cd and Zn stimulate higher absorption of sulfate in plants as it is used in synthesizing sulfur-containing compounds, such as glutathione and phytochelatin, that are

critical to metal tolerance. Such links between sulfur concentrations and Zn uptake have been noted in *Salix purpurea* L. in metal-contaminated peat soils (Sullivan et al., 2013). Thus, we hypothesize that sulfur/sulfate transport systems may contribute to metal accumulation in *A. halleri* in extreme metalliferous soils, highlighting the need for further investigation of this association, along with the measurement of sulfur and sulfate levels in the soils and *A. halleri* plants in future studies.

Finally, the ten fungal taxa that were exclusively abundant in M_PL22 soil, included both *Ascomycota* and *Basidiomycota*. In fact, *Basidiomycota* phylum contributed to the majority of fungal community composition (relative abundance of 82–84 %) in M_PL22 soil and was strongly associated with Zn uptake in *A. halleri*. Previous studies have successfully applied *Basidiomycota* fungi to remove heavy metals from soils via fungal bioaugmentation (Chen et al., 2012; Hassan et al., 2020). Further, a metagenomics study on *Basidiomycota* reported sequences associated with metal-dependent enzymes, including Zn. Thus, use of metals for cellular activity by *Basidiomycota* may be influencing the metal availability for *A. halleri* plants (Passarini et al., 2022).

Unlike *Basidiomycota*, the abundance of other fungal taxa that positively associated with metal uptake was <1 %, characterizing them as rare taxa. Such rare microbial taxa have been reported to play a role in multiple processes, including: (i) degrading pollutants and toxins in activated sludge (Dell'Anno et al., 2012), (ii) facilitating primary plant succession on mine tailings (Li et al., 2024), (iii) bioremediation of Cd contaminated paddy soil (Li et al., 2019), and (iv) other biochemical processes (Jousset et al., 2017). Notably, Gao et al. (2022) highlighted the importance of rare rhizosphere bacterial communities in Cd uptake in *Lolium multiflorum* Lam. Accordingly, it is likely that the rare fungal taxa associated with *A. halleri* in M_PL22 soil are enhancing the root uptake of Cd and Zn along with transport of other essential nutrients. Regarding the fungal functional groups, ericoid mycorrhizal was only abundant in M_PL22 soil. A particular strain of *Oidiodendron maius*, capable of forming ericoid mycorrhiza, has been isolated from heavy metal-contaminated soils and shown to have high tolerance to Zn and Cd (Lacourt et al., 2000; Martino et al., 2000, 2003). Collectively, these results suggest that site-, species-, and/or population-specific microbial taxa and their associated functional activities may greatly influence Cd and Zn uptake in *A. halleri* plants. Therefore, further investigation into this association is needed, particularly through increased replication in future experiments.

5. Conclusions

Our reciprocal transplant experiment showed that both non-metallicolous and metallicolous *A. halleri* ecotypes are tolerant to high levels of metal exposure and possess significant phytoextraction potential. Indeed, most plants recorded bioaccumulation factors >1 for both Cd and Zn, with a more efficient uptake of metals from non-metalliferous soils. Notably, after 6-months of growth, *A. halleri* populations were able to reduce the total Zn in their root zone when grown in metalliferous soils, highlighting their usage in remediating Zn-contaminated soils. Our findings suggest that non-metallicolous vs. metallicolous populations may be better suited for phytoextraction technologies – as they possess traits of high tolerance and extremely efficient metal uptake without incurring any trade-offs to the plant biomass. Further long-term studies will be necessary to confirm their maintained efficacy in prolonged applications. Additionally, a stronger effect of the soil origin vs. plant ecotype was observed on the soil microbiome. Moreover, the positive association of specific microbial taxa and functional activity with extraordinary Zn uptake in M_PL22 soil indicates that the potential cost of metal tolerance associated with plant development in contaminated soils and high internal metal concentrations in plant tissues was counterbalanced by site-specific soil microbiome interactions. Therefore, to have a better understanding of the role of microbial taxa on metal hyperaccumulation across different populations, future studies should: (1) evaluate how plant populations

interact with soil microbiome over time; (2) assess the role of root exudates; and (3) carry out inoculation experiments. Quantification and optimization of the microbial-driven variability in metal-tolerance will ultimately influence the phytoextraction capacity of most promising populations.

CRediT authorship contribution statement

Priyanka Kushwaha: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Kamila Murawska-Włodarczyk:** Writing – original draft, Visualization, Investigation. **Małgorzata Stanek:** Methodology, Investigation, Conceptualization. **Anna M. Stefanowicz:** Conceptualization. **Barbara Seget:** Investigation. **Alicja Babst-Kostecka:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.177470>.

Data availability

Sequencing and metadata are submitted to NCBI

References

- Adediji, A.A., Babalola, O.O., 2020. Secondary metabolites as plant defensive strategy: a large role for small molecules in the near root region. *Planta* 252. <https://doi.org/10.1007/s00425-020-03468-1>.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>.
- Asad, S.A., Farooq, M., Afzal, A., West, H., 2019. Integrated phytobial heavy metal remediation strategies for a sustainable clean environment - a review. *Chemosphere* 217, 925–941. <https://doi.org/10.1016/j.chemosphere.2018.11.021>.
- Babst-Kostecka, A., Schat, H., Saumitou-Laprade, P., Grodzńska, K., Bourceaux, A., Pauwels, M., Frérot, H., 2018. Evolutionary dynamics of quantitative variation in an adaptive trait at the regional scale: the case of zinc hyperaccumulation in *Arabidopsis halleri*. *Mol. Ecol.* 27, 3257–3273. <https://doi.org/10.1111/mec.14800>.
- Bandara, T., Krohn, C., Jin, J., Chathurika, J.B.A.J., Franks, A., Xu, J., Potter, I.D., Tang, C., 2022. The effects of biochar aging on rhizosphere microbial communities in cadmium-contaminated acid soil. *Chemosphere* 303. <https://doi.org/10.1016/j.chemosphere.2022.135153>.
- Beck, T., Öhlinger, R., Baumgarten, A., 1996. Indirect estimation of microbial biomass. In: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (Eds.), *Methods in Soil Biology*. Springer, Berlin, pp. 47–75.
- Bert, V., Macnair, M.R., De Laguerie, P., Saumitou-Laprade, P., Petit, D., 2000. Zinc tolerance and accumulation in metallicolous and nonmetallicolous populations of *Arabidopsis halleri* (Brassicaceae). *New Phytol.* 146 (2), 225–233. <https://doi.org/10.1046/j.1469-8137.2000.00634.x>.
- Bert, V., Bonnin, I., Saumitou-Laprade, P., De Laguerie, P., Petit, D., 2002. Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? *New Phytol.* 155 (1), 47–57. <https://doi.org/10.1046/j.1469-8137.2002.00432.x>.

- Bowsher, A.W., Kearns, P.J., Popovic, D., Lowry, D.B., Shade, A., 2020. Locally adapted *Mimulus* ecotypes differentially impact rhizosphere bacterial and archaeal communities in an environment-dependent manner. *Phytobiomes J.* 4 (1), 53–63. <https://doi.org/10.1094/PHYBIOMES-05-19-0026-R>.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. <https://doi.org/10.1038/nmeth.3869>.
- Cambrollé, J., García, J.L., Ocete, R., Figueroa, M.E., Cantos, M., 2013. Growth and photosynthetic responses to copper in wild grapevine. *Chemosphere* 93, 294–301. <https://doi.org/10.1016/j.chemosphere.2013.04.080>.
- Carré, F., Caudeville, J., Bonnard, R., Bert, V., Boucard, P., Ramel, M., 2017. Soil contamination and human health: a major challenge for global soil security. In: Field, D., Morgan, C., McBratney, A. (Eds.), *Global Soil Security*. Springer, pp. 275–296 eBook.
- Chaney, R.L., Angle, J.S., Broadhurst, C.L., Peters, C.A., Tappero, R.V., Sparks, D.L., 2007. Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. *J. Environ. Qual.* 36, 1429–1443. <https://doi.org/10.2134/jeq2006.0514>.
- Chen, G., Fan, J., Liu, R., Zeng, G., Chen, A., Zou, Z., 2012. Removal of Cd(II), Cu(II) and Zn(II) from aqueous solutions by live *Phanerochaete chrysosporium*. *Environ. Technol.* 33, 2653–2659. <https://doi.org/10.1080/09593330.2012.673015>.
- Corso, M., Schwartzman, M.S., Guzzo, F., Souard, F., Malkowski, E., Hanikenne, M., Verbruggen, N., 2018. Contrasting cadmium resistance strategies in two metalicolous populations of *Arabidopsis halleri*. *New Phytol.* 218 (1), 283–297. <https://doi.org/10.1111/nph.14948>.
- Czarnota, M.A., Rimando, A.M., Weston, L.A., 2003. Evaluation of root exudates of seven sorghum accessions. *J. Chem. Ecol.* 29, 2073–2083. <https://doi.org/10.1023/A:1025634402071>.
- de Mendiburu, F., 2016. *Agriculture: statistical procedures for agricultural research. R Package Version, 1* (2).
- Dell'Anno, A., Beolchini, F., Rocchetti, L., Luna, G.M., Danovaro, R., 2012. High bacterial biodiversity increases degradation performance of hydrocarbons during bioremediation of contaminated harbor marine sediments. *Environ. Pollut.* 167, 85–92. <https://doi.org/10.1016/j.envpol.2012.03.043>.
- Deng, J., Bai, X., Zhou, Y., Zhu, W., Yin, Y., 2020. Variations of soil microbial communities accompanied by different vegetation restoration in an open-cut iron mining area. *Sci. Total Environ.* 704. <https://doi.org/10.1016/j.scitotenv.2019.135243>.
- Deram, A., Denayer, F.O., Dubourgier, H.C., Douay, F., Petit, D., Van Haluwyn, C., 2007. Zinc and cadmium accumulation among and within populations of the pseudometallophytic species *Arrhenatherum elatius*: implications for phytoextraction. *Sci. Total Environ.* 372, 372–381. <https://doi.org/10.1016/j.scitotenv.2006.09.026>.
- Dietrich, C.C., Bilnicki, K., Korzeniak, U., Briese, C., Nagel, K.A., Babst-Kostecka, A., 2019. Does slow and steady win the race? Root growth dynamics of *Arabidopsis halleri* ecotypes in soils with varying trace metal element contamination. *Environ. Exp. Bot.* 167. <https://doi.org/10.1016/j.envexpbot.2019.103862>.
- Dietrich, C.C., Tandy, S., Murawska-Włodarczyk, K., Banaś, A., Korzeniak, U., Seget, B., Babst-Kostecka, A., 2021. Phytoextraction efficiency of *Arabidopsis halleri* is driven by the plant and not by soil metal concentration. *Chemosphere* 285. <https://doi.org/10.1016/j.chemosphere.2021.131437>.
- Easlon, H.M., Bloom, A.J., 2014. Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area. *Appl. Plant Sci.* 2 (7), 140003. <https://doi.org/10.3732/apps.1400033>.
- Eisenhauer, N., Lanoue, A., Strecker, T., Scheu, S., Steinauer, K., Thakur, M.P., Mommer, L., 2017. Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Sci. Rep.* 7 (1), 1–8. <https://doi.org/10.1038/srep44641>.
- Gao, C., Zhu, Y., Xiao, X., Liu, R., Zhu, X., Zhao, Y., 2022. Rare soil species impact the rhizosphere bacterial communities and cadmium uptake by *Lolium multiflorum* Lam. *Plant Soil* 473, 337–350. <https://doi.org/10.1007/s11104-021-05291-3>.
- Geng, H., Wang, F., Yan, C., Ma, S., Zhang, Y., Qin, Q., Tian, Z., Liu, R., Chen, H., Zhou, B., Yuan, R., 2022. Rhizosphere microbial community composition and survival strategies in oligotrophic and metal(loid) contaminated iron tailings areas. *J. Hazard. Mater.* 436. <https://doi.org/10.1016/j.jhazmat.2022.129045>.
- Gu, Z., Eils, R., Schlesner, M., 2016. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinform.* 32, 2847–2849. <https://doi.org/10.1093/bioinformatics/btw313>.
- Hassan, A., Periatnamby, A., Ahmed, A., Innocent, O., Hamid, F.S., 2020. Effective bioremediation of heavy metal-contaminated landfill soil through bioaugmentation using consortia of fungi. *J. Soils Sediments* 20, 66–80. <https://doi.org/10.1007/s11368-019-02394-4>.
- Honeker, L.K., Gullo, C.F., Neilson, J.W., Chorover, J., Maier, R.M., 2019. Effect of re-calcification on buffalo grass rhizosphere and bulk microbial communities during phytostabilization of metalliferous mine tailings. *Front. Microbiol.* 10. <https://doi.org/10.3389/fmicb.2019.01209>.
- Huang, W.Z., Schoenau, J.J., 1998. Fluxes of water-soluble nitrogen and phosphorus in the forest floor and surface mineral soil of a boreal aspen stand. *Geoderma* 81 (3–4), 251–264. [https://doi.org/10.1016/S0016-7061\(97\)00092-X](https://doi.org/10.1016/S0016-7061(97)00092-X).
- Huang, Y., Huang, Y., Hou, J., Wu, L., Christie, P., Liu, W., 2023. Microbial community assembly of the hyperaccumulator plant *Sedum plumbizincicola* in two contrasting soil types with three levels of cadmium contamination. *STOTEN* 863, 160917. <https://doi.org/10.1016/j.scitotenv.2022.160917>.
- Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., Küsel, K., Rillig, M.C., Rivett, D.W., Salles, J.F., Van Der Heijden, M.G.A., Youssef, N.H., Zhang, X., Wei, Z., Hol, G.W.H., 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. *ISME J.* 11, 853–862. <https://doi.org/10.1038/ismej.2016.174>.
- Khan, S., Naushad, M., Lima, E.C., Zhang, S., Shaheen, S.M., Rinklebe, J., 2021. Global soil pollution by toxic elements: current status and future perspectives on the risk assessment and remediation strategies – a review. *J. Hazard. Mater.* 417. <https://doi.org/10.1016/j.jhazmat.2021.126039>.
- Klimek, B., Stepniewska, K., Seget, B., Pandey, V.C., Babst-Kostecka, A., 2023. Diversity and activity of soil biota at a post-mining site highly contaminated with Zn and Cd are enhanced by metalicolous compared to non-metallicolous *Arabidopsis halleri* ecotypes. *Land Degrad. Dev.* 34, 1538–1548. <https://doi.org/10.1002/ldr.4551>.
- Kulmatiski, A., Beard, K.H., 2011. Long-term plant growth legacies overwhelm short-term plant growth effects on soil microbial community structure. *Soil Biol. Biochem.* 43, 823–830. <https://doi.org/10.1016/j.soilbio.2010.12.018>.
- Kumar, H., Ishtiaq, S., Favas, P.J.C., Varun, M., Paul, M.S., 2023. Effect of metal-resistant PGPB on the metal uptake, antioxidative defense, physiology, and growth of *Atriplex lentiformis* (Torr.) S. Wats. in soil contaminated with cadmium and nickel. *J. Plant Growth Regul.* 42, 3868–3887. <https://doi.org/10.1007/s00344-022-10853-5>.
- Kushwaha, P., Neilson, J.W., Maier, R.M., Babst-Kostecka, A., 2022. Soil microbial community and abiotic soil properties influence Zn and Cd hyperaccumulation differently in *Arabidopsis halleri*. *Sci. Total Environ.* 803. <https://doi.org/10.1016/j.scitotenv.2021.150006>.
- Lacourt, I., D'angelo, S., Girlanda, M., Turnau, K., Bonfante, P., Perotto, S., 2000. Genetic polymorphism and metal sensitivity of *Oidiodendron maius* strains isolated from polluted soil. *Ann. Microbiol.* 50, 157–166.
- Li, J.T., Gurajala, H.K., Wu, L.H., Van Der Ent, A., Qiu, R.L., Baker, A.J.M., Tang, Y.T., Yang, X.E., Shu, W.S., 2018. Hyperaccumulator plants from China: a synthesis of the current state of knowledge. *Environ. Sci. Technol.* 52, 11980–11994. <https://doi.org/10.1021/acs.est.8b01060>.
- Li, L., Lin, Q., Li, X., Li, T., He, X., Li, D., Tao, Y., 2019. Dynamics and potential roles of abundant and rare subcommunities in the bioremediation of cadmium-contaminated paddy soil by *Pseudomonas chenduensis*. *Appl. Microbiol. Biotechnol.* 103, 8203–8214. <https://doi.org/10.1007/s00253-019-10059-y>.
- Li, C., Ding, S., Chen, M., Sun, Q., Zhang, Y., Ma, X., Zhong, Z., Tsang, D.C.W., Wang, Y., 2022. Mechanistic insights into trace metal mobilization at the micro-scale in the rhizosphere of *Vallisneria spiralis*. *STOTEN* 806, 150735. <https://doi.org/10.1016/j.scitotenv.2021.150735>.
- Li, Y., Sun, X., Zhang, M., Khan, A., Sun, W., 2024. Dominant role of rare bacterial taxa rather than abundant taxa in driving the tailing primary succession. *J. Hazard. Mater.* 462. <https://doi.org/10.1016/j.jhazmat.2023.132807>.
- Lodewyck, C., Mergeay, M., Vangronsveld, J., Clijsters, H., Van Der Lelie, D., 2002. Isolation, characterization, and identification of bacteria associated with the zinc hyperaccumulator *Thlaspi caerulescens* subsp. *Calaminaria*. *Int. J. Phytoremediation* 4, 101–115. <https://doi.org/10.1080/15226510208500076>.
- Lopes, A.R., Madureira, D., Diaz, A., Santos, S., Vila, M.C., Nunes, O.C., 2020. Characterisation of bacterial communities from an active mining site and assessment of its potential metal solubilising activity. *J. Environ. Chem. Eng.* 8. <https://doi.org/10.1016/j.jece.2020.104495>.
- Lopez, S., Piutti, S., Vallance, J., Morel, J.L., Echevarria, G., Benizri, E., 2017. Nickel drives bacterial community diversity in the rhizosphere of the hyperaccumulator *Alyssum murale*. *Soil Biol. Biochem.* 114, 121–130. <https://doi.org/10.1016/j.soilbio.2017.07.010>.
- Luo, J., Gu, S., Guo, X., Liu, Y., Tao, Q., Zhao, H.P., Liang, Y., Banerjee, S., Li, T., 2022. Core microbiota in the rhizosphere of heavy metal accumulators and its contribution to plant performance. *Environ. Sci. Technol.* 56 (18), 12975–12987. <https://doi.org/10.1021/acs.est.1c08832>.
- Maestri, E., Marmiroli, M., Visioli, G., Marmiroli, N., 2010. Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. *Environ. Exp. Bot.* 68 (1), 1–13. <https://doi.org/10.1016/j.envexpbot.2009.10.011>.
- Maestri, E., Piromdini, A., Visioli, G., Marmiroli, N., 2013. Trade-off between genetic variation and ecological adaptation of metalicolous and non-metallicolous *Noccaea* and *Thlaspi* species. *Environ. Exp. Bot.* 96, 1–10. <https://doi.org/10.1016/j.envexpbot.2013.08.002>.
- Margesin, R., 1996. Enzymes involved in phosphorus metabolism. In: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (Eds.), *Methods in Soil Biology*. Springer, Berlin, pp. 208–227.
- Martino, E., Turnau, K., Girlanda, M., Bonfante, P., Perotto, S., 2000. Ericoid mycorrhizal fungi from heavy metal polluted soils: their identification and growth in the presence of zinc ions. *Mycol. Res.* 104, 338–344. <https://doi.org/10.1017/S0953756299001252>.
- Martino, E., Perotto, S., Parsons, R., Gadd, G.M., 2003. Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. *Soil Biol. Biochem.* 35, 133–141. [https://doi.org/10.1016/S0038-0717\(02\)00247-X](https://doi.org/10.1016/S0038-0717(02)00247-X).
- Martos, S., Busoms, S., Pérez-Martín, L., Llugany, M., Cabot, C., Poschenrieder, C., 2021. Identifying the specific root microbiome of the hyperaccumulator *Noccaea brachypetala* growing in non-metalliferous soils. *Front. Microbiol.* 12, 639997. <https://doi.org/10.3389/fmicb.2021.639997>.
- Maul, J., Drinkwater, L., 2010. Short-term plant species impact on microbial community structure in soils with long-term agricultural history. *Plant Soil* 330, 369–382. <https://doi.org/10.1007/s11104-009-0211-y>.
- Meyer, C.L., Kostecka, A.A., Saumitou-Laprade, P., Créach, A., Castric, V., Pauwels, M., Férot, H., 2010. Variability of zinc tolerance among and within populations of the pseudometallophyte species *Arabidopsis halleri* and possible role of directional selection. *New Phytol.* 185, 130–142. <https://doi.org/10.1111/j.1469-8137.2009.03062.x>.

- Micallef, S.A., Shiaris, M.P., Colón-Carmona, A., 2009. Influence of *Arabidopsis thaliana* accessions on rhizobacterial communities and natural variation in root exudates. *J. Exp. Bot.* 60, 1729–1742. <https://doi.org/10.1093/jxb/erp053>.
- Monchegang, S., Strehmel, N., Schmidt, S., Westphal, L., Taruttis, F., Muller, E., Herklotz, S., Neumann, S., Scheel, D., 2016. Natural variation of root exudates in *Arabidopsis thaliana*-linking metabolomic and genomic data. *Sci. Rep.* 6. <https://doi.org/10.1038/srep29033>.
- Muehe, E.M., Weigold, P., Adaktylou, I.J., Planer-Friedrich, B., Kraemer, U., Kappler, A., Behrens, S., 2015. Rhizosphere microbial community composition affects cadmium and zinc uptake by the metal-hyperaccumulating plant *Arabidopsis halleri*. *Appl. Environ. Microbiol.* 81, 2173–2181. <https://doi.org/10.1128/AEM.03359-14>.
- Nocito, F.F., Pirovano, L., Cocucci, M., Sacchi, G.A., 2002. Cadmium-induced sulfate uptake in maize roots. *Plant Physiol.* 129, 1872–1879. <https://doi.org/10.1104/pp.002659>.
- Nocito, F.F., Lancilli, C., Crema, B., Fourcroy, P., Davidian, J.C., Sacchi, G.A., 2006. Heavy metal stress and sulfate uptake in maize roots. *Plant Physiol.* 141, 1138–1148. <https://doi.org/10.1104/pp.105.076240>.
- Öhlinger, R., 1996. Methods in soil physics. In: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (Eds.), *Methods in Soil Biology*. Springer, Berlin, pp. 385–395.
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G., Solymos, P., Stevens, M., Wagner, H., 2008. The vegan package. *Community ecology package*, 10, 631–637. <http://vegan.r-forge.r-project.org/>.
- Passarini, M.R.Z., Ottoni, J.R., Costa, P.E. dos S., Hissa, D.C., Falcão, R.M., Melo, V.M.M., Balbino, V.Q., Mendonça, L.A.R., Lima, M.G. de S., Coutinho, H.D.M., Verde, L.C.L., 2022. Fungal community diversity of heavy metal contaminated soils revealed by metagenomics. *Arch. Microbiol.* 204. <https://doi.org/10.1007/s00203-022-02860-7>.
- Peterson, R., 2021. Finding optimal normalizing transformations via bestNormalize. *The R J.* 13, 310–329.
- Pirzad, T., Malik, B., Tahir, I., Kumar, M., Varma, A., Rehman, R., 2015. Phytoremediation: an eco-friendly green technology for pollution prevention, control and remediation. In: Hakeem, K., Sabir, M., Ozturk, M., Murmet, A. (Eds.), *Soil Remediation and Plants: Prospects and Challenges*. Academic Press, Elsevier, pp. 107–129.
- PN-ISO 11464, 1999. Soil Quality – Pretreatment of Samples for Physico-Chemical Analysis. Polish Standards Committee, Warszawa, Poland (in Polish).
- Qian, F., Huang, X., Su, X., Bao, Y., 2022. Responses of microbial communities and metabolic profiles to the rhizosphere of *Tamarix ramosissima* in soils contaminated by multiple heavy metals. *J. Hazard. Mater.* 438. <https://doi.org/10.1016/j.jhazmat.2022.129469>.
- Qian, L., Lin, H., Li, B., Dong, Y., 2023. Physicochemical characteristics and microbial communities of rhizosphere in complex amendment-assisted soilless revegetation of gold mine tailings. *Chemosphere* 320. <https://doi.org/10.1016/j.chemosphere.2023.138052>.
- Roosens, N.H.C.J., Willems, G., Saumitou-Laprade, P., 2008. Using *Arabidopsis* to explore zinc tolerance and hyperaccumulation. *Trends Plant Sci.* 13, 208–215. <https://doi.org/10.1016/j.tplants.2008.02.006>.
- Rousk, J., Frey, S.D., 2015. Revisiting the hypothesis that fungal-to-bacterial dominance characterizes turnover of soil organic matter and nutrients. *Ecol. Monogr.* 85, 457–472. <https://doi.org/10.1890/14-1796.1>.
- Sailer, C., Babst-Kostecka, A., Fischer, M.C., Zoller, S., Widmer, A., Vollenweider, P., Gugerli, F., Rellstab, C., 2018. Transmembrane transport and stress response genes play an important role in adaptation of *Arabidopsis halleri* to metalliferous soils. *Si. Rep.* 8 (1), 16085. <https://doi.org/10.1038/s41598-018-33938-2>.
- Salmi, A., Boulila, F., 2021. Heavy metals multi-tolerant *Bradyrhizobium* isolated from mercury mining region in Algeria. *J. Environ. Manag.* 289. <https://doi.org/10.1016/j.jenvman.2021.112547>.
- Sánchez, N., Vergara-Cid, C., Drabesch, S., Merbach, I., Tarkka, M., Muehe, E.M., 2024. Climate impact on phytoremediation efficacy by the Cd/Zn hyperaccumulator *Arabidopsis halleri* in heavy metal contaminated agricultural soils. In: EGU General Assembly 2024, Vienna, Austria, 14–19 Apr 2024, EGU24-17422. <https://doi.org/10.5194/egusphere-egu24-17422>.
- Sanon, A., Andrianjaka, Z.N., Prin, Y., Bally, R., Thioulouse, J., Comte, G., Duponnois, R., 2009. Rhizosphere microbiota interferes with plant-plant interactions. *Plant Soil* 321, 259–278. <https://doi.org/10.1007/s11104-009-0010-5>.
- Scherer, H.W., 2009. Sulfur in soils. *J. Plant Nutr. Soil Sc.* 172 (3), 326–335. <https://doi.org/10.1002/jpln.200900037>.
- Schlemper, T.R., Leite, M.F., Lucheta, A.R., Shimels, M., Bouwmeester, H.J., van Veen, J. A., Kuramae, E.E., 2017. Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils. *FEMS Microbiol. Ecol.* 93 (8). <https://doi.org/10.1093/femsec/fix096>.
- Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. *Genome Biol.* 12. <https://doi.org/10.1186/gb-2011-12-6-r60>.
- Seneviratne, M., Gunaratne, S., Bandara, T., Weerasundara, L., Rajakaruna, N., Seneviratne, G., Vithanage, M., 2016. Plant growth promotion by *Bradyrhizobium japonicum* under heavy metal stress. *S. Afr. J. Bot.* 105, 19–24. <https://doi.org/10.1016/j.sajb.2016.02.206>.
- Sheoran, V., Sheoran, A.S., Poonia, P., 2010. Role of hyperaccumulators in phytoremediation of metals from contaminated mining sites: a review. *Crit. Rev. Environ. Sci. Technol.* 41, 168–214. <https://doi.org/10.1080/10643380902718418>.
- Shi, S., Nuccio, E., Herman, D.J., Rijkers, R., Estera, K., Li, J., Da Rocha, U.N., He, Z., Pett-Ridge, J., Brodie, E.L., Zhou, J., Firestone, M., 2015. Successional trajectories of rhizosphere bacterial communities over consecutive seasons. *mBio* 6. <https://doi.org/10.1128/mBio.00746-15> e00746–15.
- Solis-Dominguez, F.A., White, S.A., Hutter, T.B., Amistadi, M.K., Root, R.A., Chorover, J., Maier, R.M., 2012. Response of key soil parameters during compost-assisted phytostabilization in extremely acidic tailings: effect of plant species. *Environ. Sci. Technol.* 46, 1019–1027. <https://doi.org/10.1021/es202846n>.
- Stein, R.J., Höreth, S., de Melo, J.R.F., Syllwasschy, L., Lee, G., Garbin, M.L., Clemens, S., Krämer, U., 2017. Relationships between soil and leaf mineral composition are element-specific, environment-dependent and geographically structured in the emerging model *Arabidopsis halleri*. *New Phytol.* 213, 1274–1286. <https://doi.org/10.1111/nph.14219>.
- Steinauer, K., Thakur, M.P., Emilia Hannula, S., Weinhold, A., Uthe, H., van Dam, N.M., Martijn Bezemer, T., 2023. Root exudates and rhizosphere microbiomes jointly determine temporal shifts in plant-soil feedbacks. *Plant Cell Environ.* 46, 1885–1899. <https://doi.org/10.1111/pce.14570>.
- Strobl, W., Traunmüller, M., 1996a. Enzymes involved in sulfur metabolism. In: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (Eds.), *Methods in Soil Biology*. Springer, Berlin, pp. 228–234.
- Strobl, W., Traunmüller, M., 1996b. Enzymes involved in carbon metabolism. In: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (Eds.), *Methods in Soil Biology*. Springer, Berlin, pp. 185–207.
- Su, C., Jiang, L., Zhang, W., 2014. A review on heavy metal contamination in the soil worldwide: situation, impact and remediation techniques. *Environ. Skep. Crit.* 3, 24–38.
- Sullivan, T.S., McBride, M.B., Thies, J.E., 2013. Rhizosphere microbial community and Zn uptake by willow (*Salix purpurea* L.) depend on soil sulfur concentrations in metalliferous peat soils. *Appl. Soil Ecol.* 67, 53–60. <https://doi.org/10.1016/j.apsoil.2013.02.003>.
- Tipayno, S.C., Truu, J., Samaddar, S., Truu, M., Pream, J.K., Oopkaup, K., Espenberg, M., Chatterjee, P., Kang, Y., Kim, K., Sa, T., 2018. The bacterial community structure and functional profile in the heavy metal contaminated paddy soils, surrounding a nonferrous smelter in South Korea. *Ecol. Evol.* 8, 6157–6168. <https://doi.org/10.1002/ecs3.4170>.
- Valentín-Vargas, A., Neilson, J.W., Root, R.A., Chorover, J., Maier, R.M., 2018. Treatment impacts on temporal microbial community dynamics during phytostabilization of acid-generating mine tailings in semiarid regions. *Sci. Total Environ.* 618, 357–368. <https://doi.org/10.1016/j.scitotenv.2017.11.010>.
- van der Ent, A., Baker, A.J.M., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloids trace elements: facts and fiction. *Plant Soil* 362, 319–334. <https://doi.org/10.1007/s11104-012-1287-3>.
- Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A., Apprill, A., Knight, R., 2016. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *mSystems* 1. <https://doi.org/10.1128/msystems.00009-15> e00009–15.
- Wan, X., Lei, M., Yang, J., Chen, T., 2020. Three-year field experiment on the risk reduction, environmental merit, and cost assessment of four in situ remediation technologies for metal(loid)-contaminated agricultural soil. *Environ. Pollut.* 266. <https://doi.org/10.1016/j.envpol.2020.115193>.
- Wang, R., Hou, D., Chen, J., Li, J., Fu, Y., Wang, S., Zheng, W., Lu, L., Tian, S., 2020. Distinct rhizobacterial functional assemblies assist two *Sedum alfredii* ecotypes to adopt different survival strategies under lead stress. *Environ. Int.* 143, 105912. <https://doi.org/10.1016/j.envint.2020.105912>.
- Wang, J., Huang, J.J., Lynch, I., 2022. Seasonal and short-term variations of bacteria and pathogenic bacteria on road deposited sediments. *Environ. Res.* 204. <https://doi.org/10.1016/j.envres.2021.111903>.
- Wenzel, W.W., Bunkowski, M., Puschenreiter, M., Horak, O., 2003. Rhizosphere characteristics of indigenous growing nickel hyperaccumulator and excluder plants on serpentine soil. *Environ. Pollut.* 131–138. [https://doi.org/10.1016/S0269-7491\(02\)00341-X](https://doi.org/10.1016/S0269-7491(02)00341-X).
- Xiao, E., Krumins, V., Dong, Y., Xiao, T., Ning, Z., Xiao, Q., Sun, W., 2016. Microbial diversity and community structure in an antimony-rich tailings dump. *Appl. Microbiol. Biotechnol.* 100 (17), 7751–7763. <https://doi.org/10.1007/s00253-016-7598-1>.
- Xu, Y., Seshadri, B., Bolan, N., Sarkar, B., Ok, Y.S., Zhang, W., Rumpel, C., Sparks, D., Farrell, M., Hall, T., Dong, Z., 2019. Microbial functional diversity and carbon use feedback in soils as affected by heavy metals. *Environ. Int.* 125, 478–488. <https://doi.org/10.1016/j.envint.2019.01.071>.
- Ye, J., Liao, W., Zhang, P., Li, J., Nabi, M., Wang, S., Cai, Y., Li, F., 2020. Fe1-xS/biochar combined with *thiobacillus* enhancing lead phytoavailability in contaminated soil: preparation of biochar, enrichment of *thiobacillus* and their function on soil lead. *Environ. Pollut.* 267. <https://doi.org/10.1016/j.envpol.2020.115447>.
- Yu, F., Tang, S., Shi, X., Liang, X., Liu, K., Huang, Y., Li, Y., 2022. Phytoremediation of metal(loid)s from contaminated soils by six plant species: a field study. *Sci. Total Environ.* 804. <https://doi.org/10.1016/j.scitotenv.2021.150282>.
- Zhang, X., Zhang, S., Liu, S., Ren, D., Zhang, X., 2023. Study on the migration behaviour of heavy metals at the improved mine soil-plant rhizosphere interface. *Environ. Technol.* 1–13. <https://doi.org/10.1080/09593330.2023.2283061>.