Journal of
Plant Ecology

Research Article

Soil microbe-induced plant volatiles can alert neighboring plants to heavy metal stress

Jieren Jin[1], Tiantian Lin[2,*], Chaonan Wang[1], Xiao Xu[2], Danfeng Liu[2], Yi Wang[2], Yupeng Geng[2], Rui-Ting Ju[1], Ming Nie[1,*] and Bo Li[1,2,*]

[1]Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, National Observations and Research Station for Wetland Ecosystems of the Yangtze Estuary, Institute of Biodiversity Science and Institute of Eco-Chongming, School of Life Sciences, Fudan University, Shanghai 200433, China, [2]State Key Laboratory for Vegetation Structure, Functions and Construction (VegLab), Ministry of Education Key Laboratory for Transboundary Ecosecurity of Southwest China, Yunnan Key Laboratory of Plant Reproductive Adaptation and Evolutionary Ecology, School of Ecology and Environmental Science, and Southwest United Graduate School, Yunnan University, Kunming 650500, China

*Corresponding authors. E-mail: tlin@ynu.edu.cn (T.L.); mnie@fudan.edu.cn (M.N.); bool@fudan.edu.cn (B.L.)

Handling Editor: Jianqing Ding

Received: 26 February 2025, **First Decision:** 3 April 2025, **Accepted:** 26 April 2025, **Online Publication:** 17 May 2025

Citation: Jin Jieren, Lin Tiantian, Wang Chaonan, Xu Xiao, Liu Danfeng, Wang Yi, Geng Yupeng, Ju Rui-Ting, Nie Ming, Li Bo (2025) Soil microbe-induced plant volatiles can alert neighboring plants to heavy metal stress. *J Plant Ecol* **18**:rtaf065. https://doi.org/10.1093/jpe/rtaf065

Abstract

While the alerting effects of microbe-induced plant volatiles (MIPVs) to biotic stressors have been extensively studied, the ecological functions of MIPVs responding to abiotic stressors have received less attention. Using an interplant communication assay setup, we employed *Phytolacca americana* as a study species to investigate whether heavy metal-induced MIPVs released by the emitter plants contribute to metal tolerance in neighboring receiver plants. We found that high levels of manganese (Mn) stress increased the total MIPV emissions of plants cultivated in non-sterilized soil, in contrast to volatile organic compounds emitted by plants in sterilized soil. MIPVs produced by the Mn-stressed plants notably altered the hormonal profiles of the receiver plants, leading to increased similarity in soil microbial assembles and modification of competitive, stress-tolerant, ruderal strategies. Consequently, the receiver plants exhibited enhanced tolerance to subsequent Mn stress, as evidenced by improved growth performance, increased antioxidant enzyme activities and reduced membrane damage. By unraveling the mechanism underlying MIPV-mediated tolerance priming for neighboring plants, we reveal a key signal role of soil microorganisms involved in plant–plant communication. This study represents one of the initial efforts to elucidate the alerting effects of MIPVs induced by heavy metal stress on neighboring plants and its ecological consequences.

Keywords: volatile organic compounds, abiotic stress, alerting effect, heavy metal tolerance, *Phytolacca americana*, CSR strategies

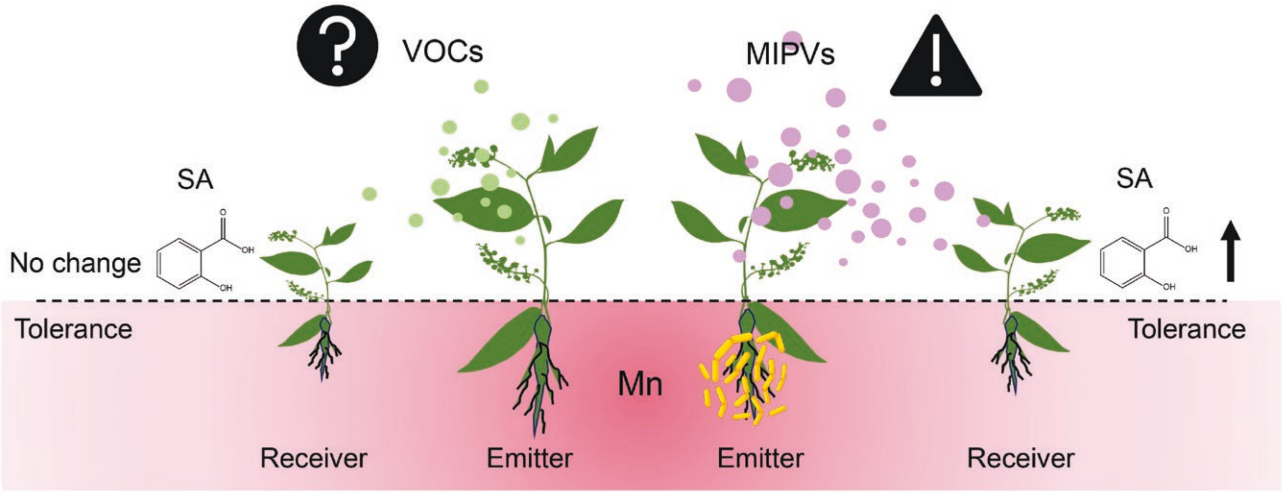
土壤微生物诱导的植物挥发性物质对邻近植物重金属胁迫的预警作用

摘要：当前学术界在微生物产生的植物挥发性物质(microbe-induced plant volatiles, MIPVs)对生物胁迫的预警作用方面已经进行了广泛研究，但对非生物胁迫如何影响MIPVs的生态功能关注较少。为此，本研究以美洲商陆(*Phytolacca americana*)为研究对象，利用植物间通讯实验装置，研究了重金属胁迫诱导

下美洲商陆个体释放的MIPVs是否有助于邻株植物对重金属的耐受作用。结果发现，高强度锰胁迫增加了未灭菌土壤中植株总MIPVs排放量，与之相反，在灭菌土壤中植株排放的总挥发性有机化合物并未显著变化。锰胁迫诱导下植株个体产生的MIPVs显著改变了邻株植物的激素水平，并导致土壤微生物组成的相似性增加和CSR策略的调整。因此，受体植株对后续锰胁迫的耐受性增强，表现为生长性能改善、抗氧化酶活性增加以及膜损伤减少。通过解析MIPVs介导的邻株植物重金属耐受性的启动机制，该研究揭示了土壤微生物在植物间信息交流中的关键信号作用。本研究初步尝试从MIPVs预警作用的角度揭示重金属胁迫下植物间的信息交流及其生态后果，可为将来研究提供重要启示。

关键词：挥发物，非生物胁迫，预警效应，重金属耐受，美洲商陆(*Phytolacca americana*)，CSR策略

Graphical Abstract



INTRODUCTION

Plant volatile organic compounds (VOCs) are defined as carbon-hydrogen-based volatile substances emitted by plant tissues into the atmosphere and soil (excluding carbon dioxide) (Kesselmeier and Staudt 1999; Laothawornkitkul *et al.* 2009). VOCs encompass a vast array of both saturated and unsaturated hydrocarbon derivatives (Simpraga *et al.* 2016; Vlot and Rosenkranz 2022), which are essential chemical signaling employed by plants to facilitate communication with various organisms within their neighborhood, including plants, herbivores, pollinators and microorganisms (Brosset and Blande 2022; Loreto and D'Auria 2022; Simpraga *et al.* 2016; Wenke *et al.* 2010). Generally, VOC emissions of plants can be either constitutive or induced by abiotic and biotic factors (Loreto and D'Auria 2022). Induced plant VOCs are believed to have an "alerting effect" and have been shown to prime defenses and tolerance in neighboring plants (Caparrotta *et al.* 2018; Ninkovic *et al.* 2021). For example, herbivore-induced plant volatiles (HIPVs) altered by stemborers stimulate higher levels of defensive proteinase inhibitors in neighboring HIPV-exposed plants (Yao *et al.* 2022). Maize seedlings primed with cold-induced plant VOCs exhibit increased growth and reduced damage after cold stress (Cofer *et al.* 2018).

As an important component of plant-induced volatiles, microbe-induced plant volatiles (MIPVs) also exert strong alerting effects for neighboring plants through activating hormone-dependent defense responses (Quintana-Rodriguez *et al.* 2015; Sharifi *et al.* 2018). Salicylic acid (SA) is considered to be a key enhanced hormone signal in MIPV-exposed plants (Erb 2018; Rosenkranz *et al.* 2021). SA signaling can directly stimulate defense responses or indirectly regulate the growth and resistance phenotype by antagonizing indole-3-acetic acid (IAA) signaling in receiver plants (Brosset and Blande 2022; Pokotylo *et al.* 2022; Vlot and Rosenkranz 2022). Moreover, Kong *et al.* (2021) have found that MIPVs act as air signaling that can upregulate the SA level in root exudates of neighboring receiver plants, adjusting the composition of rhizosphere bacterial community similar to the MIPV-emitter plants.

Although the responses of plant MIPVs to biotic stresses have been extensively documented (Quintana-Rodriguez *et al.* 2015; Sharifi *et al.* 2018), the effect of abiotic factors on the emission of MIPVs and its ecological consequences still need to be further explored (Loreto and Schnitzler 2010). Soil contamination resulting from anthropogenic activities, particularly heavy metal pollution, has emerged as a significant global concern (Feng *et al.* 2021; Yuan *et al.* (2024). Heavy metals in soil exhibit significant permeability, persistence and toxicity, thereby presenting a global threat to biodiversity (Podar and Maathuis 2022; Wang *et al.* 2023). In a previous review, Boyd (2010) posited that soil heavy metals could "disrupt" interspecific chemical communication among species dependent on VOCs. Indeed, previous studies have documented that heavy metal stress affects the interactions between plants and associated herbivores through disrupting leaf VOC emissions (Lin *et al.* 2022; Winter *et al.* 2012). However, whether such changes in plant VOCs induced by heavy metal stress lead to disruptions in plant–plant communication remains unclear. In addition, microorganisms and heavy metals coexist within the soil environment associated with plant roots, where the toxicity of heavy metal can adversely affect the microbial communities (Montreemuk *et al.* 2024). However, heavy metal stress-induced changes in soil microbial assembles can in turn affect plant VOC emissions (del Carman Sosa *et al.* 2016; Lin *et al.* 2023), which may lead to changes in quality and quantity of MIPV production. Nevertheless, few studies have evaluated the alterations in MIPVs emitted by plants subjected to heavy metal stress and their role as alert signaling among neighboring plants.

In this study, therefore, we hypothesized that exposure to heavy metal pollution would lead to changes in the MIPVs released by the emitter plants, which would exert a strong alerting effect on the neighboring receiver plants to prime them for incoming heavy metal stress. We here selected *Phytolacca americana* as our study species to answer the following two specific questions. (i) Does heavy metal stress induce differences in the quality and quantity of MIPVs released by plants? (ii) Do the changes in MIPVs lead to enhanced heavy metal tolerance of neighboring receiver plants? To understand the mechanism underlying such alerting effect, we measured plant traits related to competitive, stress-tolerant, ruderal (CSR) strategies and tolerance as well as explored the changes in rhizosphere microbial assembles using high-throughput microbial community analysis based on the Illumina MiSeq platforms. Then, the mechanism was clarified by measuring the SA levels, hydrogen peroxide and activities of antioxidant enzymes in neighboring receiver plants. To the best of our knowledge, this study is one of the first few attempts that explore the ecological consequences of plant–plant communication mediated by MIPVs in response to heavy metal pollution.

MATERIALS AND METHODS

Plant materials

Phytolacca americana (Phytolaccaceae) is a perennial invasive plant species native to North America, which has been introduced into various countries including China, Japan, Germany and Turkey (Chen *et al.* 2019; Fahmy *et al.* 2024). It is identified as an aggressive weed in its introduced ranges, due to its remarkable capacity for rapid spread and colonization (Chen *et al.* 2019; Zhu *et al.* 2024). *Phytolacca americana* has been demonstrated to have the ability to hyperaccumulate heavy metals such as manganese (Mn) and cadmium (Cd) (Zhao *et al.* 2019), and it also displays a pronounced symbiotic relationship only with soil bacteria (Meng *et al.* 2024). Therefore, *P. americana* serves as an ideal model for investigating the impacts of heavy metal pollution on plant–soil interactions. The seeds of *P. americana* were collected from different mother plants of the same population at a forest park, Kunming, Yunnan Province, China (24.833° N, 102.852° E). The process of seed germination listed in Supplementary Method S1 followed the method described by Liu *et al.* (2022). Upon germination, individual seedlings were transplanted into growing trays (H: 8 cm × L: 5 cm × W: 5 cm) with sterilized soil and grown for 14 days in a climate chamber (20 °C, 60% relative humidity, 16:8-h light/dark).

Experimental design

Plant cultivation

The soil utilized in this study was obtained from a natural habitat of *P. americana* in a forest park in Kunming, Yunnan Province, China (24.833° N, 102.857° E). The top layer (0–5 cm) of soil was removed, and the 5–20 cm layer was collected. The collected soil underwent air-drying for 7 days at room temperature followed by homogenization and screening with 5-mm and 2-mm mesh (Niu *et al.* 2017). In order to improve soil conditions and prevent soil from becoming compacted during subsequent planting, the air-dried soil was mixed with commercial Sphagnum Moss Peat (Pindstrup) in a ratio of 4:1 volume and stored in darkness at 4 °C for later use (Li *et al.* 2021). Soil properties of γ-irradiation (10 kGy h^{-1}, 6 h, at 60 kGy) sterilized and non-sterilized soil are listed in Supplementary Table S1.

Soil Mn treatment of emitter plants

In the preliminary experiment, we set the concentration gradient of 0–50 m mol L^{-1} Mn stress and found that the seedlings of *P. americana* did not show obvious damage symptoms under 10 m mol L^{-1} Mn stress, the plant biomass decreased significantly under 30 m mol L^{-1} Mn stress and the growth inhibition phenomenon was more prominent under 50 m mol L^{-1} Mn stress (Supplementary Fig. S1). Therefore, we chose 0, 10, 30 and 50 m mol L^{-1} as the Mn concentration for subsequent experiments. Healthy 14-day-old seedlings were selected from the growing trays and individually transplanted into plastic pots (H: 15 cm × L: 12 cm × W: 15 cm) filled with 2 kg of sterilized or non-sterilized soil. A series of Mn^{2+} concentrations (0, 10, 30 and 50 m mol L^{-1}) were applied to the pot cultures with $MnCl_2{\cdot}4H_2O$ dissolved in deionized water. Deionized water was served as the control treatment (0 mM). Every 7 days, 200 mL of Mn solutions or water was added to each pot for the corresponding treatments (this treatment regimen was sufficient to induce significant stress phenotypes in the plants within a short duration; see Supplementary Fig. S1). The soil treatment for emitter plants comprised two soil microbe treatments (sterilized/non-sterilized) × 4 levels of Mn treatments × 4 replicates = 32 emitter groups (Supplementary Fig. S2). The plants were cultivated in a greenhouse (day: 30 °C, night: 25 °C, 70% relative humidity, 16:8-h light/dark) for 45 days before being used for stimulating the receiver plants.

Simulation of neighboring receiver plants

We used an interplant communication assay setup modified from Kong *et al.* (2021) to test alerting effect between neighboring plants. A plastic cage (H: 50 cm × L: 92 cm × W: 50 cm) was constructed as a simulated environment to house both the emitter and receiver plants, effectively shielding them from the effect of external air circulation (Supplementary Fig. S2). Each pot was equipped with a bottom watering tray to prevent the transfer of soil microorganisms and leachate between pots via seepage. A plastic divider (5 cm in height) was installed in the center of the greenhouse to separate the emitters and receivers. Eight pots were arranged within the cage, with four designated for the emission area and four for the receiving area, and we ensured that the plants in the receiving area were isolated from those in the emission area to prevent any potential contact. A fan was employed to enhance air circulation from the emission area to the receiving area. The four emitter plants from the same Mn treatment were situated in the emission area. The receiver plants were cultivated in the same non-sterilized soil for 45 days and were placed in the receiving area. For each Mn treatment of the emitter plants, four replicated cages were used for stimulating the receiver plants. In total, the experiment comprised two soil microbe treatments (sterilized/non-sterilized) × 4 Mn treatments × 4 replicates = 32 cages (Supplementary Fig. S2). In the neighbor simulation stage, only the emitter plants were treated with different degrees of Mn stress to induce volatiles release, while the receiver plants were only affected by volatiles. The simulation processing duration was 14 days.

Exposure of receiver plants to soil Mn stress

After 14 days of simulation processing, the receiver plants were further exposed to 50 m mol L^{-1} Mn stress. Every 7 days, 200 mL of Mn solution was added to each pot of receiver plants. The receiver plants were cultivated in a greenhouse (day: 30 °C, night: 25 °C, 70% relative humidity, 16:8-h light/dark) for 45 days before indicating their growth status after experiencing Mn stress. Heavy metal stress can activate plant antioxidant enzymes, elevate reactive oxygen species (ROS) levels and cause damage to membrane lipids (Ghori *et al.* 2019; Podar and Maathuis 2022). Catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and methylglyoxal (MG) are generally selected as indicators to evaluate the heavy metal tolerance of plants (Ghori *et al.* 2019; Guo *et al.* 2022). So the contents of MDA, MG and H_2O_2, along with the activities of CAT, POD and SOD, in the leaves were quantified using reagent kit from Grace Biotechnology (Suzhou, China) according to the manufacturer's instructions. Leaf, stem and root of receiver plants were harvested and then dried at 60 °C for 48 h before dry weight was determined.

Collection and analysis of VOCs released by emitter plants

The volatiles released by emitter plants grown in sterilized soil were defined as VOCs, while the volatiles released by emitter plants grown in non-sterilized soil were defined as MIPVs. After 45 days of growth, volatiles of the four individual plants from each Mn treatment were collected together using an improved method based on Kalske *et al.* (2022) to collect aboveground VOCs released by emitter plants (Supplementary Fig. S3). A small plastic film bag (polyethylene; 10 cm × 20 cm) was used to tightly wrap the belowground parts of the plants, and avoid collecting the volatile compounds released from the plant's pot and soil. The four pots were placed in a sealed polyethylene container (H60 cm × D40 cm). Air was introduced into the container at a flow rate of 1 L min^{-1} for 10 min, followed by sampling at a rate of 0.25 L min^{-1} for 24 h. The VOCs were collected using adsorption columns (Tenax TA 60/80) and subsequently analyzed by gas chromatography–mass spectrometry (GC-MS) as detailed in Supplementary Method S2.

Data processing was performed using the GC-MS solution software (version 4.20; Shimadzu Corporation), enabling the absolute quantification of each identified volatile compound. By using external standards (C7-C40 n-alkanes, Shimadzu) (Supplementary Table S2) and comparing the sample GC-MS data with the NIST14 library, VOCs were identified and compared with previously published VOCs from different plant species (Bibbiani *et al.* 2018; Kong *et al.* 2021). Volatile compounds detected in at least 25% of the samples were selected for Mn treatment levels (Lin *et al.* 2022).

Measurement of SA and IAA contents in receiver plants

MIPVs can elevate SA levels in neighboring plants, either by stimulating SA accumulation in their leaves (Rosenkranz *et al.* 2021) or by altering the SA contents in root exudates (Kong *et al.* 2021). However, SA signaling typically antagonizes IAA signaling in receiver plants, which ultimately influences overall plant growth (Erb 2018). So SA and IAA contents in plants in the receiving area were measured after 14 days simulation processing. Leaf tissues from the third fully expanded leaf of each receiver plant were collected. The contents of SA in receiver plant leaves and root exudates were determined using an ELISA kit (Wuhan Saipei Biotechnology, Wuhan, China), while leaf IAA contents were determined using an ELISA kit (MEIMIAN, Jiangsu, China). The collection of root exudates was performed using a hydroponics-hybrid method described by Williams *et al.* (2021), and the details are described in Supplementary Method S3.

Analysis of CSR strategies of receiver plants

Grime's CSR strategies were employed to assess the overall growth performance of plants (Guo *et al.* 2018; Liao *et al.* 2020). The C-strategy plants generally adapt to resource-rich and low-stress environments, with rapid growth and strong resource acquisition ability. The S-strategy plants were adapted to high-stress environment, with relatively slow growth but strong stress resistance. The R-strategy plants were adapted to high disturbance and low-stress environment (Escobedo *et al.* 2021; Guo *et al.* 2018). We measured the CSR strategies of the receiving plants at two stages: during the 14-day simulation processing stage and after 45 days of Mn stress treatment. For each stage, we measured three leaf traits used for CSR ordination, including leaf area (LA), leaf fresh weight (LFW) and leaf dry weight (LDW). Building upon the three key leaf traits (LA, LFW and LDW) that encapsulate the fundamental functional spectrum of receiver plants, we utilized the "StrateFy" tool developed by Pierce *et al.* (2017) to quantify Grime's CSR strategies for each treatment. The spreadsheet tool was widely used to assess the extent of CSR strategies adoption, which provides one of the 19 tertiary CSR strategy classes (C, S, R, CS, CR, SR, CSR, etc.), as well as the percentage of competitiveness (C%), stress tolerance (S%) and ruderality (R%) (Escobedo *et al.* 2021; Zhang *et al.* 2024b).

Soil bacterial microbiome analysis of emitter and receiver plants

As MIPVs could adjust the composition of rhizosphere bacterial assembles in neighboring plants through SA release in root exudates, we compared the similarity of the composition and structure of the soil bacterial communities between the MIPV-emitter and MIPV-receiver plants after 14-day simulation processing. The rhizosphere soil was collected from each plant, and the soil samples from all four emitter or receiver plants within the same cage were pooled as one composite soil sample. In total, we analyzed four Mn treatments × 2 plant types (emitter or receiver plants) × 4 replicates = 32 soil samples. Microbial DNA was extracted using the HiPure Soil DNA Kits (Magen, Guangzhou, China) according to the manufacturer's protocols, and PCR amplification was performed using primers targeting regions V3 and V4 of the 16S rRNA gene. The primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACHVGGGTATCTAAT-3′) were used for the amplification of the bacterial 16S rRNA region (Kong *et al.* 2021). The details of the sequencing amplification are described in Supplementary Method S4. All raw amplicon reads were included in the NCBI database (accession number: PRJNA1170449).

Statistical analyses

The non-metric multidimensional scaling (NMDS) was employed to assess the variations in the volatile compositions among groups. Clustering heatmaps were employed to assess the quality of VOCs/MIPVs between the treated and control groups.

The above analysis was completed using the online tool platform (OmicShare tools, https://www.omicshare.com/tools). The similarity index (Pearson correlation coefficient) was utilized to calculate the similarity of rhizosphere microbial communities between MIPV-emitter and MIPV-receiver plants (Kong *et al.* 2021). Statistical analyses were conducted using SPSS29 (IBM, New York, USA) for Windows, and generalized linear models (GLMs) were used to compare mean values of total VOC emissions, hormone content, dry weight, hydrogen peroxide and activities of antioxidant enzyme between treatments, followed by Wald pairwise comparisons. Origin Pro 2021 (Origin Lab, Northampton, USA) was used for visualization of the above analyses.

RESULTS

Effects of Mn stress on MIPV and VOC emissions of emitter plants

When grown in sterilized soil, the emitter plants subjected to 10 m mol L^{-1} Mn treatment released 18% less VOCs than the control plants, while plants from the other two Mn treatments (e30 and e50mM) released similar amounts of VOC compared to control plants (Fig. 1a). However, the emitter plants grown in non-sterilized soil treated with 30 and 50 m mol L^{-1} Mn released 21% and 63% more total MIPVs than control plants, respectively, while 10 m mol L^{-1} Mn treatment largely reduced that of MIPVs. As a result, *P. americana* released 44% more total MIPVs than VOCs under 50 m mol L^{-1} Mn treatment. In addition, the NMDS plots (Fig. 1b, c) and clustering heatmaps (Fig. 1d, e) indicated a clear difference in the compositions of both VOCs and MIPVs released by the emitter plants subjected to different Mn treatments. Volcano plots further showed that more individual compounds of MIPVs were induced than those of VOCs released by emitter plants regardless of the concentrations of Mn treatments (Fig. 2).

Performance of the receiver plants exposed to MIPVs and VOCs

There were no significant differences in leaf SA and IAA levels among the VOC-receiver plants under different treatments (Fig. 3a–c). A significantly higher SA level was observed only in the root exudates of the VOC-receiver plants under R30mM treatment compared to other treatments. Furthermore, after 14-day simulation processing, the CSR strategies of the VOC-receiver were categorized as C-strategies (Fig. 3d). However, the SA levels in leaves and root exudates of the MIPV-receiver plants under R30mM and R50mM treatments were largely induced compared to the control receiver plants (Fig. 3a, b). In contrast, the leaf IAA levels in the MIPV-receiver plants under R30mM and R50mM treatments were markedly reduced than those in the control receiver plants (Fig. 3c). In addition, the CSR strategies of MIPV-receiver plants exhibited significant differences among treatments after 14-day simulation processing (Fig. 3e). More specifically, the MIPV-receiver plants from control and R10mM treatment were mainly categorized as pure C-strategies, whereas MIPV-receiver plants from R30mM and R50mM treatments exhibited C/CS-strategies and CS-strategies, respectively.

Further correlation analysis indicated that the total MIPV emissions of the emitter plants grown on non-sterilized soil were positively correlated with the SA contents in the leaves and root exudates but negatively correlated with the leaf IAA contents of the MIPV-receiver plants (Fig. 4a–c). However, no significant correlations were observed for the total VOC emissions of the emitter plant grown in sterilized soil and the three hormone levels in the VOC-receiver plants (Supplementary Fig. S4a–c). In addition, the total MIPV emissions of the emitter plants and root SA contents of the MIPV-receiver plants strongly contributed to the separation of rhizosphere bacterial compositions of the receiver plants that were exposed to MIPVs from emitter plants under low and high Mn treatments (Fig. 4d). As a result, the compositions of rhizosphere bacterial communities between the emitter and corresponding MIPV-receiver plants were more similar at high levels

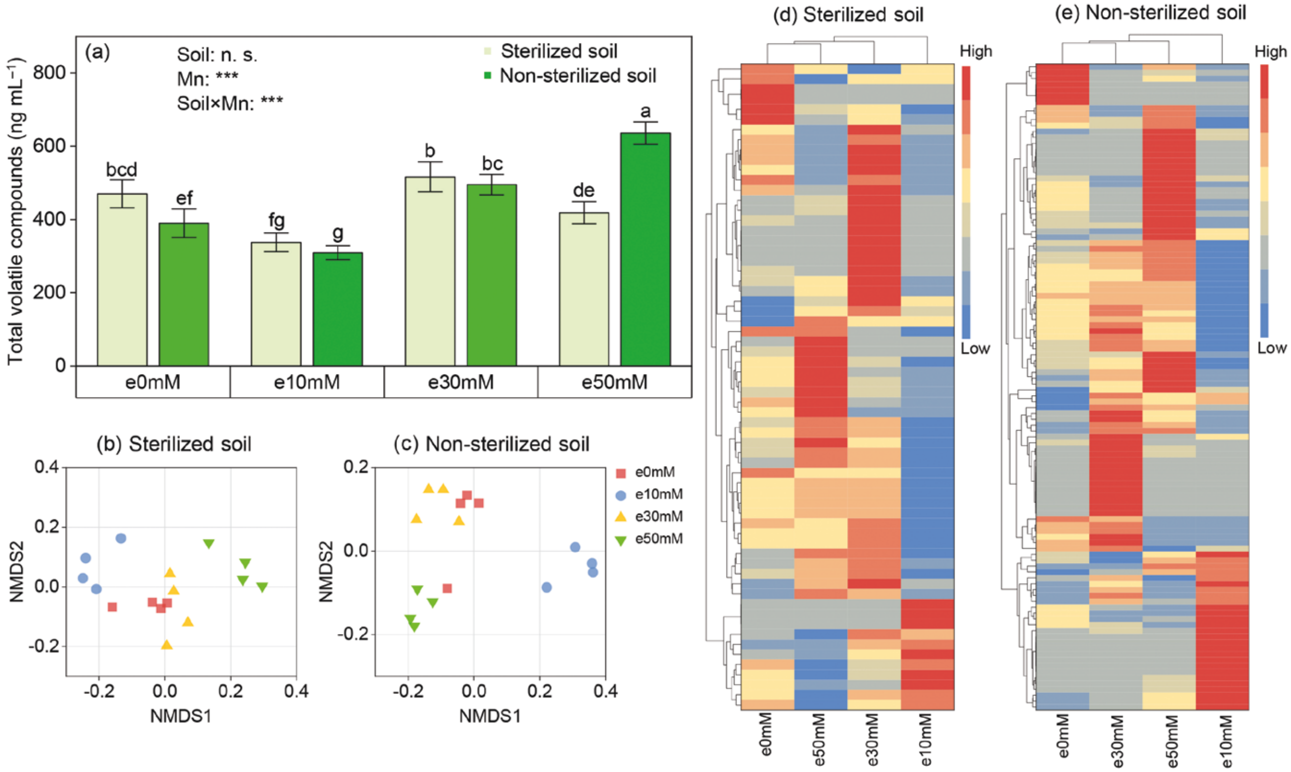


Figure 1: Difference in leaf volatile organic compounds (VOCs) and microbe-induced plant volatiles (MIPVs) released by *Phytolacca americana* emitter plants under different concentrations of Mn treatments. (a) Total amounts of VOCs or MIPVs released by emitter plants grown on sterilized or non-sterilized soils. Values are means ± SE. Different letters indicate significant differences among treatments at $P < 0.05$ according to generalized linear models (GLMs) followed by Wald pairwise comparisons. Significance values of the GLMs are shown as follows: n.s., not significant; *$0.01 < P \le 0.05$, **$0.001 < P \le 0.01$, ***$P \le 0.001$. (b, c) NMDS plot of leaf VOCs or MIPVs released by emitter plants grown on sterilized or non-sterilized soils. (d, e) Clustering heatmap of relative abundance of VOCs or MIPVs released by emitter plants grown on sterilized or non-sterilized soils. e0mM, e10mM, e30mM and e50mM represent MIPV-emitter plants that under different Mn treatments.

of soil Mn treatment (30 and 50 mM) compared to low Mn treatments (0 and 10 mM) (Fig. 4e).

Growth and tolerance of receiver plants in response to subsequent Mn Stress

After subsequent Mn stress, VOC-receiver plants under R50mM treatment produced on average 21% and 8% lower leaf and total dry weight, respectively, whereas those under R30mM treatment produced 20% higher steam dry weight, compared to the control receiver plants (Fig. 5a–d), However, when receiver plants were exposed to MIPVs released by emitter plants grown in non-sterilized soil, the biomass of leaf, stem and root of the MIPV-receiver plants under R30mM and R50mM treatments was much greater than the control receiver plants. As a result, the total dry weight of the MIPV-receiver plants from R30mM and R50mM treatments was 10% and 22% higher compared to that of the VOC-receiver plants under the same treatment, respectively (Fig. 5d). In addition, the VOC-receiver plants from different treatments were all categorized as C-strategies in response to subsequent Mn stress (Fig. 5e), while the CSR strategies of MIPV-receiver plants from different treatments exhibited significant divergence (Fig. 5f). Specifically, the MIPV-receiver plants from R0mM and R10mM treatments were categorized as C-strategies, while the receiver plants from R30mM and R50Mm treatments were categorized as CS-strategies.

After subsequent Mn stress, MIPV-receiver plants from different treatments all exhibited greater activities of the antioxidant enzymes, including CAT, POD, SOD and H_2O_2 compared to the VOC-receiver plants (Fig. 6a–c). In contrast, the contents of membrane damage indicators such as MG and MDA in the leaves of MIPV-receiver plants decreased with increasing Mn concentrations applied to the emitter plants. As a result, the MG levels of MIPV-receiver plants from R50mM treatments were reduced by 10% compared to those

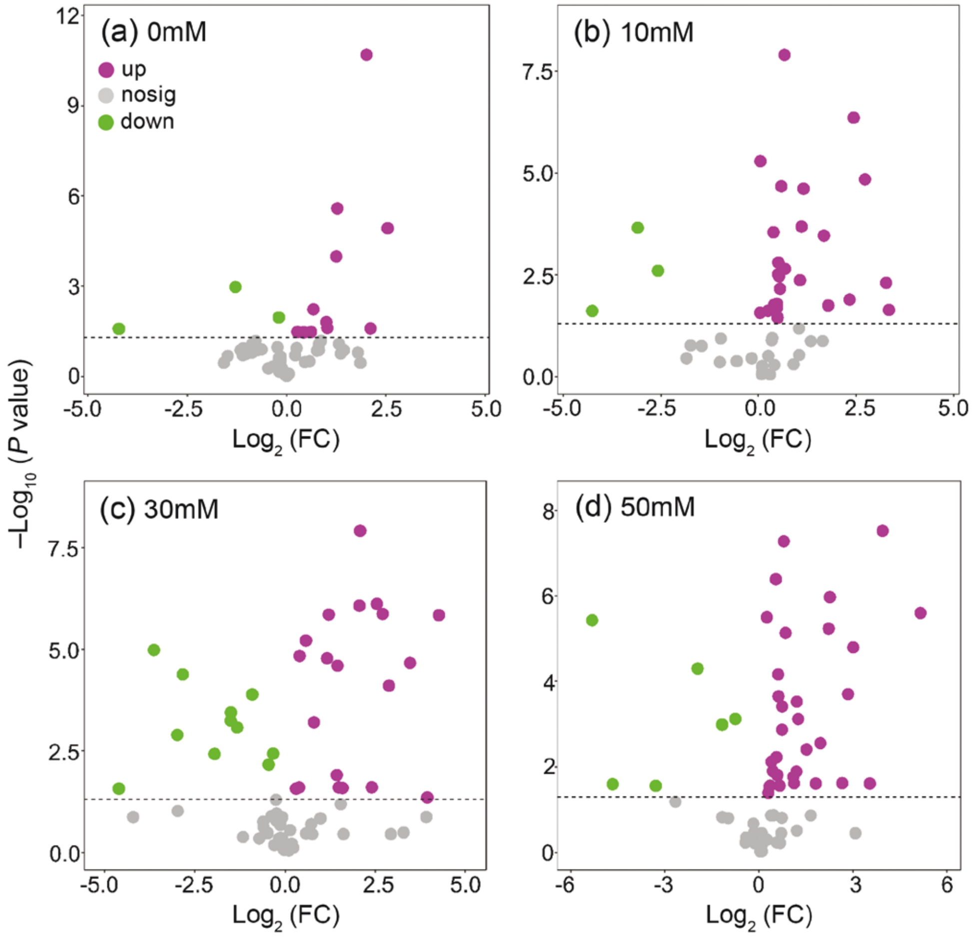


Figure 2: Volcano plots of overall emission between volatile organic compounds (VOCs) *vs.* microbe-induced plant volatiles (MIPVs) released by *Phytolacca americana* emitter plants under different concentrations of Mn treatments: (a) 0mM, 0 m mol L^{-1}; (b) 10mM, 10 m mol L^{-1}; (c) 30mM, 30 m mol L^{-1}; (d) 50mM, 50 m mol L^{-1}. The x-axis shows the log$_2$-fold change (FC) in emission between VOCs and MIPVs samples. The y-axis shows the $-\log_{10}$ (normalized *P*-value) of the difference in emission. Significant differences were determined by Welch's *t*-test ($P < 0.05$). $N = 4$ replicates for each treatment. The further from 0 on the x-axis, the greater the change in emission, and the higher on the y-axis, the greater the significance.

of the VOC-receiver plants (Fig. 6e). The MDA levels of the MIPV-receiver plants from R10mM, R30mM and R50mM treatments decreased respectively by 7%, 19%, and 24% compared to those of the VOC-receiver plants under the same treatment (Fig. 6f).

DISCUSSION

Although the roles of MIPVs involved in plant–plant communication have been extensively studied, the ecological consequences of MIPVs in relation to environmental stresses are still under debate. In this study, we demonstrated that high levels of Mn treatment induced significant alterations in both the total amounts and compositions of leaf MIPVs emitted by *P. americana*, triggering an alerting effect on neighboring receiver plants in response to the subsequent Mn stress. We utilized an interplant communication assay setup to demonstrate that the alerting effect was mediated by leaf MIPVs released from emitter plants grown in non-sterilized soil, rather than being influenced by leaf VOCs released from emitter plants grown in sterilized soil. Moreover, the MIPVs significantly enhanced the tolerance of neighboring receiver plants to the subsequent Mn stress. These findings offered a theoretical framework for advancing our comprehensive understanding of the mechanisms and functions of plant–plant communication facilitated by soil microorganisms under conditions of heavy metal pollution.

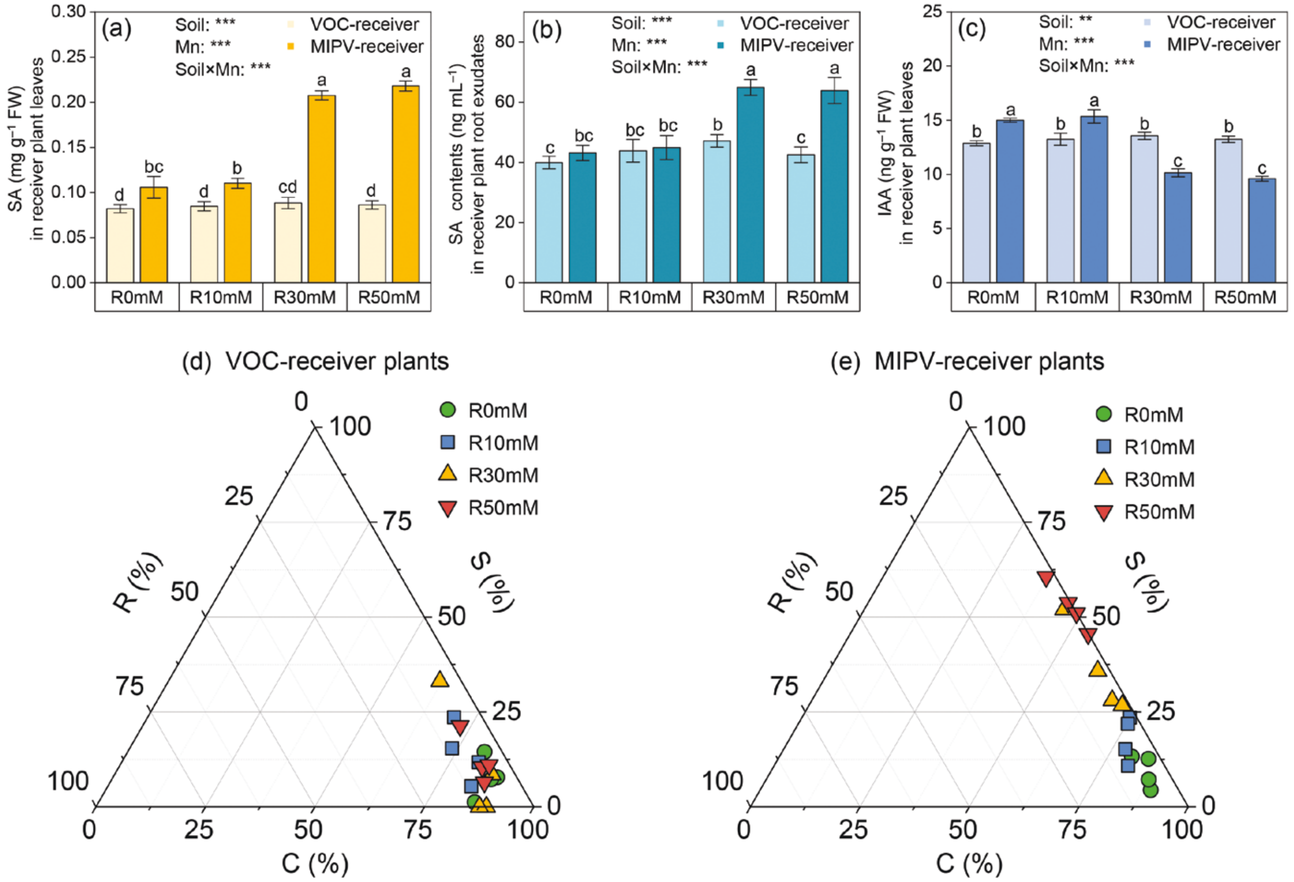


Figure 3: Differences in hormone contents and CSR strategies of receiver plants after exposed to leaf volatile organic compounds (VOCs) or microbe-induced plant volatiles (MIPVs) released by emitter plants subjected to different Mn treatments. (a) Contents of salicylic acid (SA) in leaves. (b) Contents of SA in root exudates. (c) Contents of indole-3-acetic acid (IAA) in leaves. Values are means ± SE. Different letters indicate significant differences among treatments at $P < 0.05$ according to GLMs followed by Wald pairwise comparisons. Significance values of the generalized linear models (GLMs) are shown as follows: n.s., not significant; *$0.01 < P \leq 0.05$, **$0.001 < P \leq 0.01$, ***$P \leq 0.001$. (d, e) CSR strategies. R0mM, R10mM, R30mM and R50mM represent MIPV-receiver plants that under different Mn treatments. $N = 4$ replicates for each treatment.

Previous investigations have primarily focused on the effect of microbes on the release of MIPVs, while relatively few studies have examined the influence of environmental factors on the amount and composition of MIPVs. In this study, we found that the release of MIPVs from *P. americana* showed a biphasic response with low Mn stress inhibiting and high Mn stress promoting. Low heavy metal stress may be regarded as mild stress, with plants preferentially using energy and resources for basal metabolism (e.g. growth, nutrient uptake) rather than volatiles synthesis (Jesus *et al.* 2016). However, when heavy metal stress exceeded a certain threshold, it often triggered obvious rhizosphere effects. Plants recruit rhizosphere microorganisms through root exudates while beneficial microorganisms secrete chelating agents (such as siderophores) and antioxidant substances, which reduce the negative effects of heavy metals on plants (Podar and Maathuis 2022). Microorganisms may stimulate induced systemic resistance (ISR) of plants, activate jasmonic acid (JA)/SA pathway and promote volatiles release (Rodriguez *et al.* 2019; Vlot and Rosenkranz 2022). Similarly, some plants (e.g. *Tagetes minuta* and *Populus yunnanensis*) promote leaf volatile emissions under heavy metal stress in non-sterilized soil (del Carman Sosa *et al.* 2016; Lin *et al.* 2022). Furthermore, various environmental factors such as drought, elevated temperature or cold stress have also been noticed to firmly influence plant VOC emissions (Loreto and Schnitzler 2010; Ninkovic *et al.* 2021). However, we observed that higher levels of Mn stress did not have a strong effect on the total VOC emissions of *P. americana* grown in sterilized soil, suggesting the crucial roles of soil microorganisms

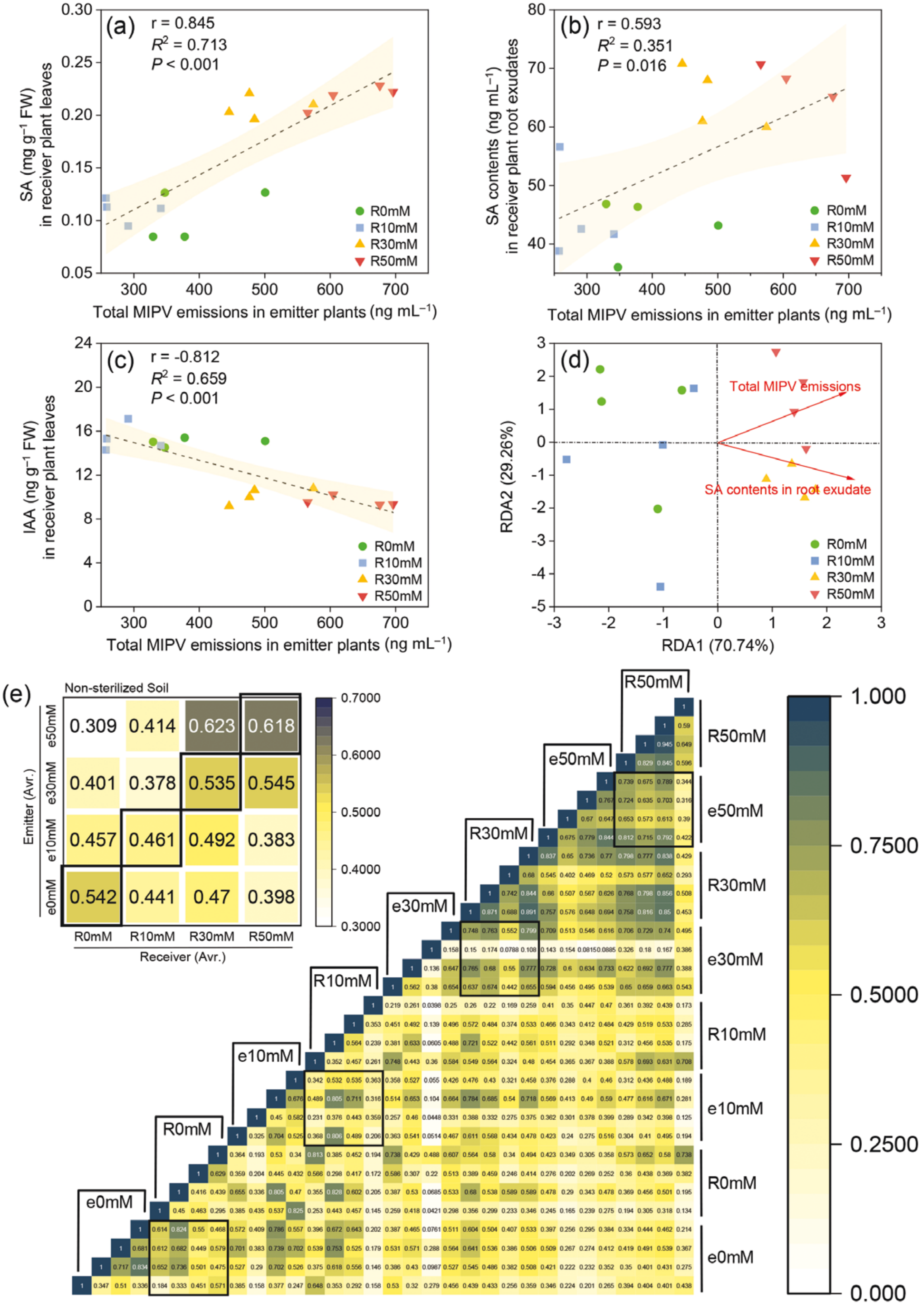


Figure 4: Correlations and redundancy analysis (RDA) for revealing the relationships between microbe-induced plant volatiles (MIPVs) from emitter plants and status of receiver plants. (a) Correlations between MIPVs and salicylic acid (SA) contents in leaves of receivers. (b) Correlations between MIPVs and SA contents in root exudates of receivers. (c) Correlations between MIPVs and indole-3-acetic acid (IAA) contents in leaves of receivers. (d) RDA for revealing the

relationships between MIPVs and the soil bacterial communities of receivers. (e) Composition similarity of soil bacterial communities between MIPV-emitter and MIPV-receiver plants using Pearson's correlation coefficient. The bold box in the right down panel indicates in the raw data of Pearson's correlation coefficient numbers for similar microbiota enrichment between the rhizosphere of MIPV-emitter plants and that of MIPV-receiver plants. The bold box in the left top panel indicated average of Pearson's correlation coefficient numbers in each treatment. e0mM, e10mM, e30mM and e50mM represent MIPV-emitter plants that under different Mn treatments; R0mM, R10mM, R30mM and R50mM represent MIPV-receiver plants. $N = 4$ replicates for each treatment.

involved in heavy metal stress-mediated changes in leaf volatile emissions.

As previous studies have documented that MIPVs could alert neighboring receiver plants and enable them to prepare for subsequent stress by activating their hormone-dependent immune responses (Erb 2018; Sharifi *et al.* 2018), we further examined the differences in hormone levels of the receiver plants after being exposed to the VOCs and MIPVs of the emitter plants under different Mn treatments. We observed that the total amounts of MIPVs released by the emitter plants were positively correlated with the SA levels in both leaves and root exudates but negatively correlated with leaf IAA levels in the neighboring MIPV-receiver plants. However, such relationships disappeared in VOC-receiver plants. This further emphasizes that soil microorganisms played a key role in VOC-mediated communications between neighboring plants. In general, the plant's responses to volatiles are often accompanied by an overall change in hormone signaling (Erb 2018; Hu 2022). SA is the main hormone signaling affected by MIPVs (Sharifi *et al.* 2018) and acts as a negative regulator of IAA signaling to inhibit plant growth (Erb 2018). It may explain the negative correlation we observed between IAA level and MIPV emissions.

We found that MIPV-receiver plants showed clear transformation from C-strategies to CS-strategies compared to C-strategies generally exhibited by VOC-receiver plants. The receiver plants exposed to MIPVs induced by 30 and 50 mM Mn treatments exhibited CS-strategies. Plants are unable to maximize both C- and S-strategies, and stress tolerance is often improved at the expense of competitiveness and fecundity. C-strategies rely on high resource acquisition capacity, but the cost of maintaining rapid growth is too high in stressed environments (Escobedo *et al.* 2021; Guo *et al.* 2018). However, environmental stress shifted plants to S-strategies, allocating more resources to survival rather than rapid growth (Vasseur *et al.* 2018). The increase in the relative proportion of S-strategies in MIPVs-receiver plants might be an adaptive strategy to cope with environmental stress in advance, which improved the tolerance of plants through resource allocation, morphological adjustment and physiological optimization. SA signaling may serve a regulatory function in the plant's overall growth, akin to its balancing role in the trade-off between growth and immunity (Pokotylo *et al.* 2022). In light of this, we propose that volatiles can modulate the overarching ecological strategies of neighboring receiver plants by influencing plant hormone levels (Ren *et al.* 2023). Supportively, previous studies have also noticed negative impacts of volatile signaling on neighboring plant growth (Barney *et al.* 2009; Liu *et al.* 2022; Souza-Alonso *et al.* 2014).

Interestingly, we found that the rhizosphere bacterial communities of the emitter and receiver plants showed increasing similarity as the soil Mn levels applied to the emitter plants increased. Meanwhile, the MIPV emissions of the emitter plants were positively correlated with SA levels in the root exudates of the neighboring receiver plants. The phenomenon suggested that MIPV-induced increases in SA may contribute to the similarity of rhizosphere bacterial communities between the emitter and receiver plants. This aligns with the findings of Kong *et al.* (2021), which have demonstrated that the composition of rhizosphere microbes can be synchronized by elevating SA level in root exudates. SA, a key immune regulator in plants, modulates the composition of the root microbiome, rather than by altering the abundance of a small number of dominant strains within each differential abundance family (Lebeis *et al.* 2015). Consequently, the degree of similarity between the rhizosphere microbiomes of emitter and receiver plants appeared to reflect the strength of MIPVs in acting on the neighboring receiver plants. Furthermore, plants develop tolerance by recruiting beneficial microbes in response to heavy metal stress (Wang *et al.* 2020). The similarity between the emitter and receiver rhizosphere microbes may enhance the capacity of the neighboring receiver plants to withstand Mn stress.

In general, studies regarding the alerting effect of MIPVs involved in plant–plant communications mainly focused on biotic stress (Sharifi *et al.* 2018).

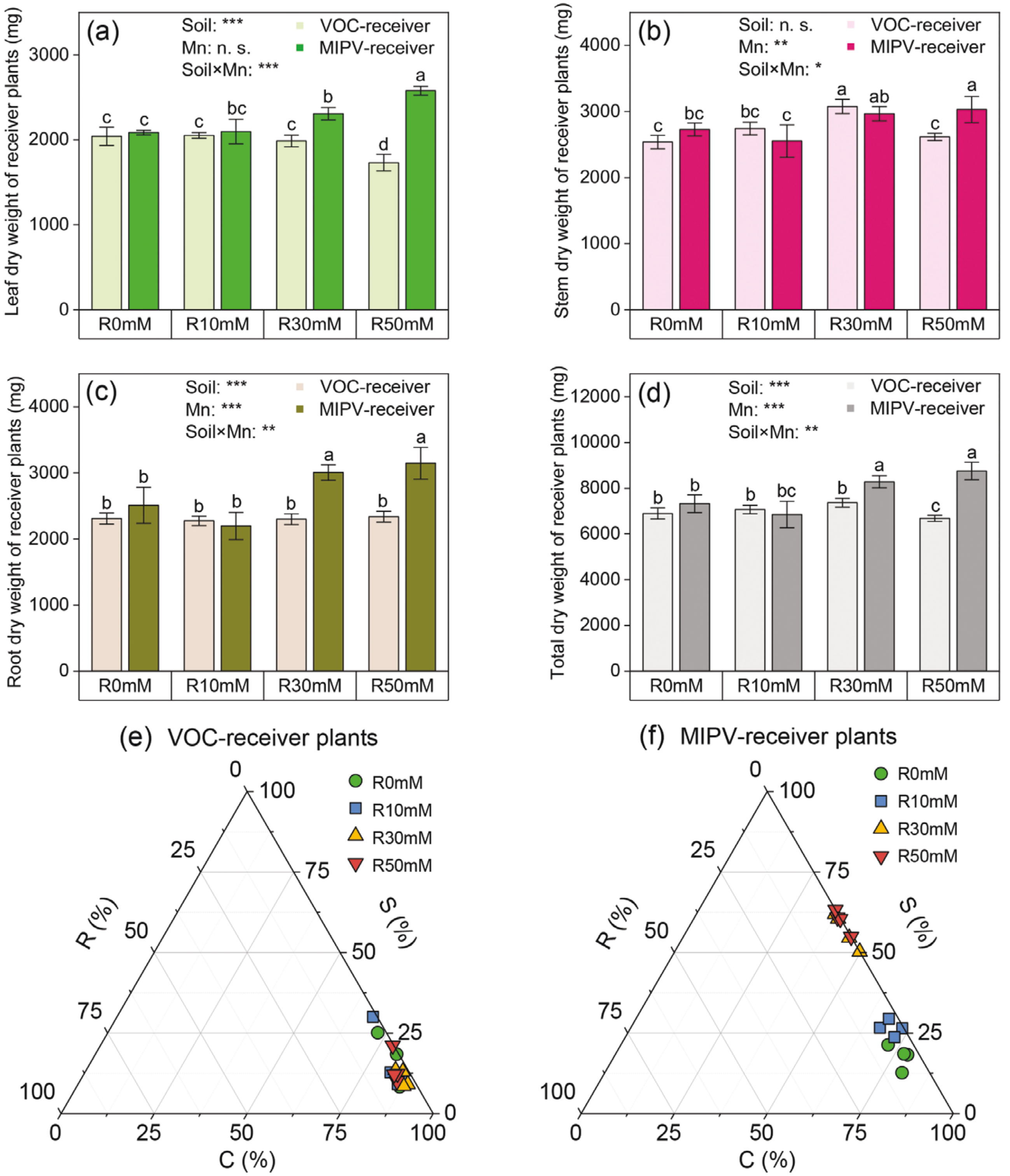


Figure 5: Differences in growth performance and CSR strategies of receiver plants after experiencing Mn stress for 45 days. (a) Leaf dry weight; (b) stem dry weight; (c) root dry weight; (d) total dry weight. Values are means ± SE. Different letters indicate significant differences among treatments at $P < 0.05$ according to generalized linear models (GLMs) followed by Wald pairwise comparisons. Significance values of the GLMs are shown as follows: n.s., not significant; *, $0.01 < P \leq 0.05$; **, $0.001 < P \leq 0.01$; ***, $P \leq 0.001$. (e, f) CSR strategies. R0mM, R10mM, R30mM and R50mM represent MIPV-receiver plants that under different Mn treatments. VOCs = volatile organic compounds, MIPVs = microbe-induced plant volatiles. $N = 4$ replicates for each treatment.

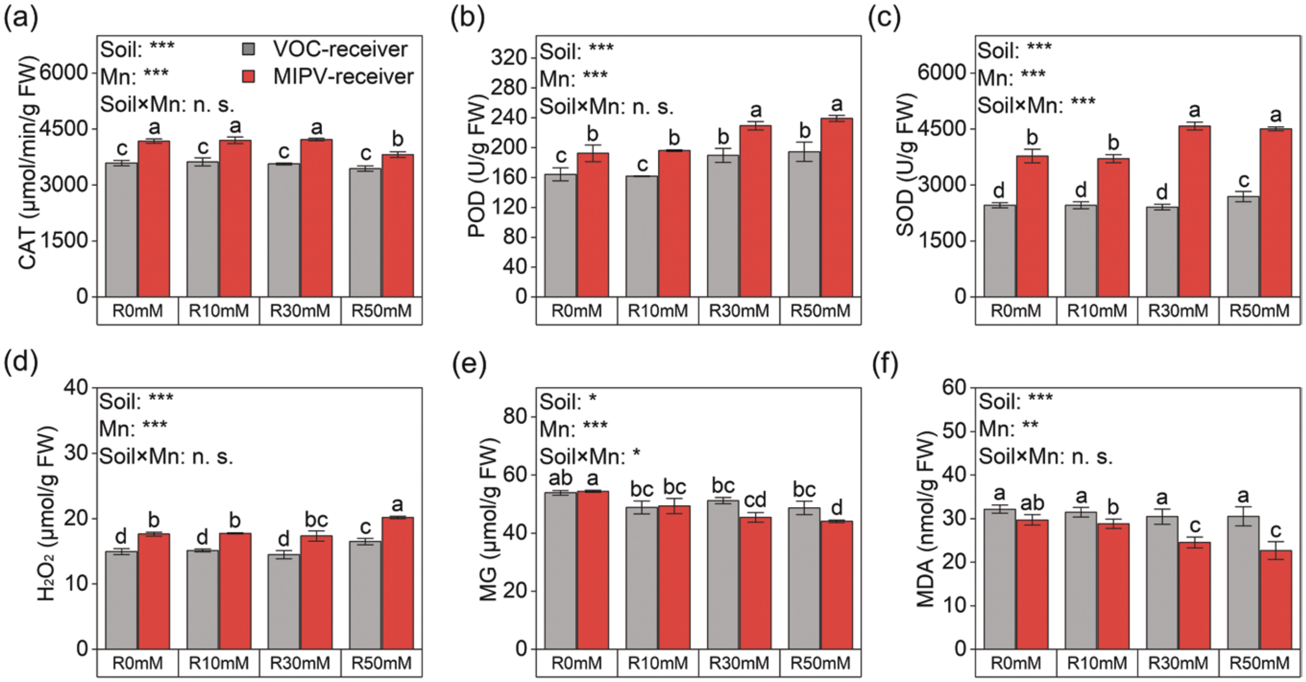


Figure 6: Differences in antioxidant enzyme activities and membrane damage indicators of receiver plants after exposed to leaf volatile organic compounds (VOCs) or microbe-induced plant volatiles (MIPVs) released by emitter plants subjected to different Mn treatments. Receiver plants were tested after experiencing 50 mM Mn stress for 45 days. (a) Activities of Catalase (CAT); (b) activities of peroxidase (POD); (c) activities of superoxide dismutase (SOD); (d) contents of H_2O_2; (e) contents of methylglyoxal (MG); (f) contents of malondialdehyde (MDA). Values are means ± SE. Different letters indicate significant differences among treatments at $P < 0.05$ according to GLMs followed by Wald pairwise comparisons. Significance values of the generalized linear models (GLMs) are shown as follows: n.s., not significant; $*0.01 < P \leq 0.05$, $**0.001 < P \leq 0.01$, $***P \leq 0.001$. R0mM, R10mM, R30mM and R50mM represent MIPV-receiver plants that under different Mn treatments. $N = 4$ replicates for each treatment.

For example, Quintana-Rodriguez *et al.* (2015) showed that the susceptible varieties of *Phaseolus vulgaris* were more resistant to the infection of pathogenic fungi *Colletotrichum lindemuthianum* after being exposed to MIPVs emitted from resistant varieties of *P. vulgaris*. In this study, we found that the receiver plants exposed to MIPVs of emitter plants under high levels of Mn treatments (30 and 50 mM) exhibited better tolerance to subsequent Mn stress, which was indicated by higher growth performance and antioxidant enzyme activities but lower level of membrane damage. Therefore, this study represents the first evidence that plant MIPVs induced by heavy metal stress can serve as alerting signaling to neighboring receiver plants, thereby allowing them to acquire strong tolerance against subsequent heavy metal stress. These findings largely extend our understanding of MIPV-mediated communications between plants in response to abiotic stress.

Heavy metal stress commonly leads to an explosion of ROS in plant cells, damaging the lipid structures of cell membranes (Ghori *et al.* 2019). The main function of CAT is related to the removal of H_2O_2 during metabolic processes. SOD converts ROS into O_2 and H_2O_2, preventing ROS from causing oxidative damage to plant cells. Meanwhile, the function of POD is similar to CAT, further catalyzing the decomposition of H_2O_2 into H_2O and O_2 (Guo *et al.* 2022; Mittler *et al.* 2022). In *Arabidopsis thaliana*, high SA level leads to the inhibition of CAT2 activity, which in turn leads to the accumulation of H_2O_2 (Pokotylo *et al.* 2022; Yuan *et al.* 2017). Furthermore, SA enhances the accumulation of ROS in plant tissues by downregulating the expression of redox and ROS-scavenging-related genes (Xu *et al.* 2017). While oxidative stress induced by H_2O_2 typically results in membrane damage, higher H_2O_2 content did not trigger an increase in membrane oxidation markers such as MDA and MG (Fig. 6e, f). This phenomenon may arise from the enhanced POD and SOD activities offsetting the diminished CAT activity, with H_2O_2 serving solely as a signaling molecule in response to Mn stress (Mittler *et al.* 2022). Furthermore, SA can sustain the growth stability of plants under heavy metal stress by enhancing cell wall synthesis and facilitating nutrient absorption (Dai *et al.* 2022; Zhang *et al.* 2024a), which may contribute to the increased growth of the neighboring receiver plants observed in this study.

However, it should be pointed out that the emission of VOCs from *P. americana* cultivated in sterilized soil did not elicit any alterations in hormone levels or CSR strategies in the neighboring receiver plants. As a result, the VOC-receiver plants were observed to be less tolerant of subsequent Mn stress than MIPV-receiver plants, which was indicated by decreased growth and minor changes in antioxidant enzyme activities. Considering that previous studies have frequently demonstrated the alerting effect following a substantial increase in VOCs (Cofer *et al.* 2018; Gong *et al.* 2023), we here propose that the lack of significant change in the total VOCs of emitter plants under Mn stress is a critical factor preventing the neighboring receiver plants from exhibiting effective tolerance response. It is worth noting that *P. americana* was more tolerant to Mn stress than other plant species, and its stronger antioxidant capacity may mask the oxidative stress induced by Mn, resulting in a higher VOCs release threshold. Furthermore, different heavy metals (such as Cd, Pb and Zn) can change the composition and release of volatiles through different ways due to their differences in chemical properties, biological activities and plant absorption mechanisms (Bibbiani *et al.* 2018; del Carman Sosa *et al.* 2016; Lin *et al.* 2023). So future research should expand the range of invasive plant species rather than limit it to a single species. It should also be tested for the effects of various heavy metals on plant volatiles. Additionally, as abiotic factors such as ozone, freezing and salinity have been shown to trigger induced VOCs (Caparrotta *et al.* 2018; Huang *et al.* 2023; Velikova *et al.* 2008), whether these induced VOCs are sufficient to serve as alerting effect or any other specific ecological effects in plant–plant communication still need to be specifically studied.

CONCLUSIONS

In this study, we observed that elevated levels of Mn stress significantly enhanced the total emissions and altered the composition of MIPVs from plants grown in non-sterilized soil, in contrast to the VOCs emitted by plants cultivated in sterilized soil. Furthermore, the Mn-induced MIPVs released by the emitter plants had a pronounced alerting effect on neighboring receiver plants, resulting in increased SA levels and decreased IAA levels in the receiver plants. These alterations in hormonal profiles facilitated a shift in the CSR strategies of the receiver plants from a C-strategy to a CS-strategy. Consequently, the receiver plants demonstrated enhanced growth performance, increased antioxidant enzyme activities and diminished membrane damage in response to Mn stress. Notably, the Mn stress-induced leaf VOCs emitted by *P. americana* cultivated in sterilized soil did not produce a similar alerting effect on neighboring receiver plants, thereby underscoring the significant role of soil microorganisms involved in plant–plant communication. This study elucidates the ecological interactions among neighboring plants, which depend on MIPV signals to initiate alerting responses under the condition of heavy metal pollution. Intercropping of heavy metal-sensitive plants and hyperaccumulators to transmit stress signals through MIPVs may effectively activate the repair potential of the latter. However, it is imperative to highlight the necessity for further research that encompasses a broader range of plant species and different types of heavy metal stressors to draw more comprehensive conclusions.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: Chemical properties of the non-sterilized soil and sterilized soil used in this experiment.

Table S2: External standards for GC-MS analysis.

Figure S1: Growth-related traits of *P. americana* under different concentrations of Mn treatment.

Figure S2: Experimental design.

Figure S3: Schematic representation of the experimental design to the collection, elution, and loading process of VOCs.

Figure S4: Correlations for revealing the relationships between VOCs from emitter plants and hormone levels of receiver plants.

Method S1: Seed germination.

Method S2: Leaf volatile collection.

Method S3: Root exudates collection.

Method S4: PCR amplification, Illumina MiSeq sequencing and Bioinformatics analysis.

Authors' Contributions

Jieren Jin (Data curation, Visualization, Writing—original draft), Tiantian Lin (Conceptualization, Methodology, Writing—review & editing), Chaonan Wang (Formal analysis, Resources), Xiao Xu (Investigation, Resources, Supervision), Danfeng Liu (Methodology), Yi Wang (Investigation, Resources), Yupeng Geng (Resources), Rui-Ting Ju (Resources), Ming Nie (Conceptualization, Resources, Writing—review & editing), and Bo Li (Conceptualization, Funding acquisition, Project administration, Resources, Writing—review & editing).

Funding

This work was financially supported by the National Key Research and Development Program of China (grant no. 2022YFC2601100), the National Natural Science Foundation of China (grant nos U2102218, and 32001204), the Science and Technology Department of Shanghai (grant no. 23015810100), the Department of Science and Technology of Yunnan Province (grant no. 202405AS350011) and the Yunnan Fundamental Research Projects (grant no. 202501AS070069).

Conflict of interest statement. The authors declare that they have no conflict of interest.

Data Availability

The data that support the findings of this study are openly available in Dryad at http://datadryad.org/stash/share/c_IsjwtSv43xAFw_WwbbenaKFhDVZunS90swEaroEdQ.

REFERENCES

Barney JN, Sparks JP, Greenberg J, *et al.* (2009) Biogenic volatile organic compounds from an invasive species: impacts on plant-plant interactions. *Plant Ecol* **203**:195–205. https://doi.org/10.1007/s11258-008-9529-4

Bibbiani S, Colzi I, Taiti C, *et al.* (2018) Smelling the metal: volatile organic compound emission under Zn excess in the mint *Tetradenia riparia*. *Plant Sci* **271**:1–8. https://doi.org/10.1016/j.plantsci.2018.03.006

Boyd RS (2010) Heavy metal pollutants and chemical ecology: exploring new frontiers. *J Chem Ecol* **36**:46–58. https://doi.org/10.1007/s10886-009-9730-5

Brosset A, Blande JD (2022) Volatile-mediated plant-plant interactions: volatile organic compounds as modulators of receiver plant defence, growth, and reproduction. *J Exp Bot* **73**:511–528. https://doi.org/10.1093/jxb/erab487

Caparrotta S, Boni S, Taiti C, *et al.* (2018) Induction of priming by salt stress in neighboring plants. *Environ Exp Bot* **147**:261–270. https://doi.org/10.1016/j.envexpbot.2017.12.017

Chen P, Hou Y, Zhuge Y, *et al.* (2019) The effects of soils from different forest types on the growth of the invasive plant *Phytolacca americana*. *Forests* **10**:492–507. https://doi.org/10.3390/f10060492

Cofer TM, Engelberth M, Engelberth J (2018) Green leaf volatiles protect maize (*Zea mays*) seedlings against damage from cold stress. *Plant Cell Environ* **41**:1673–1682. https://doi.org/10.1111/pce.13204

Dai ZH, Guan DX, Bundschuh J, *et al.* (2022) Roles of phytohormones in mitigating abiotic stress in plants induced by metal(loid)s As, Cd, Cr, Hg, and Pb. *Crit Rev Environ Sci Technol* **53**:1310–1330. https://doi.org/10.1080/10643389.2022.2134694

del Carman Sosa M, Salazar MJ, Zygadlo JA, *et al.* (2016) Effects of Pb in *Tagetes minuta* L. (Asteraceae) leaves and its relationship with volatile compounds. *Ind Crop Prod* **82**:37–43. https://doi.org/10.1016/j.indcrop.2015.12.011

Erb M (2018) Volatiles as inducers and suppressors of plant defense and immunity-origins, specificity, perception and signaling. *Curr Opin Plant Biol* **44**:117–121. https://doi.org/10.1016/j.pbi.2018.03.008

Escobedo VM, Rios RS, Gianoli E (2021) Interactive effects of shading and disturbance on plant invasion in an arid shrubland: assembly processes and CSR-strategies. *J Ecol* **109**:2405–2420. https://doi.org/10.1111/1365-2745.13650

Fahmy NM, Fayez S, Zengin G, *et al.* (2024) Chemical exploration of different extracts from *Phytolacca americana* leaves and their potential utilization for global health problems: *in silico* and network pharmacology validation. *J Biomol Struct Dyn* **42**:1–21. https://doi.org/10.1080/07391102.2024.2308770

Feng Z, Ji S, Ping J, *et al.* (2021) Recent advances in metabolomics for studying heavy metal stress in plants. *Trend Anal Chem* **143**:116402. https://doi.org/10.1016/j.trac.2021.116402

Ghori NH, Ghori T, Hayat MQ, *et al.* (2019) Heavy metal stress and responses in plants. *Int J Environ Sci Technol* **16**:1807–1828. https://doi.org/10.1007/s13762-019-02215-8

Gong Q, Wang Y, He L, *et al.* (2023) Molecular basis of methyl-salicylate-mediated plant airborne defence. *Nature* **622**:139–148. https://doi.org/10.1038/s41586-023-06533-3

Guo WY, van Kleunen M, Winter M, *et al.* (2018) The role of adaptive strategies in plant naturalization. *Ecol Lett* **21**:1380–1389. https://doi.org/10.1111/ele.13104

Guo Z, Lv J, Zhang H, *et al.* (2022) Red and blue light function antagonistically to regulate cadmium tolerance by modulating the photosynthesis, antioxidant defense system and Cd uptake in cucumber (*Cucumis sativus* L.). *J Hazard Mater* **429**:128412. https://doi.org/10.1016/j.jhazmat.2022.128412

Hu L (2022) Integration of multiple volatile cues into plant defense responses. *New Phytol* **233**:618–623. https://doi.org/10.1111/nph.17724

Huang R, Zhang T, Ge X, *et al.* (2023) Emission trade-off between isoprene and other BVOC components in *Pinus massoniana* saplings may be regulated by content of chlorophylls, starch and NSCs under drought stress. *Int J Mol Sci* **24**:8946–8965. https://doi.org/10.3390/ijms24108946

Jesus DS, Azevedo BO, Pinelli MS, *et al.* (2016) Growth and volatile compounds of *Martianthus leucocephalus* exposed to heavy metal stress. *Cienc Rural* **46**:2110–2117. https://doi.org/10.1590/0103-8478cr20150576

Kalske A, Blande JD, Ramula S (2022) Soil microbiota explain differences in herbivore resistance between native and invasive populations of a perennial herb. *J Ecol* **110**:2649–2660. https://doi.org/10.1111/1365-2745.13975

Kesselmeier J, Staudt M (1999) Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. *J Atmos Chem* **33**:23–88. https://doi.org/10.1023/A:1006127516791

Kong HG, Song GC, Sim HJ, *et al.* (2021) Achieving similar root microbiota composition in neighbouring plants through airborne signalling. *ISME J* **15**:397–408. https://doi.org/10.1038/s41396-020-00759-z

Laothawornkitkul J, Taylor JE, Paul ND, *et al.* (2009) Biogenic volatile organic compounds in the Earth system. *New Phytol* **183**:27–51. https://doi.org/10.1111/j.1469-8137.2009.02859.x

Lebeis SL, Paredes SH, Lundberg DS, *et al.* (2015) Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* **349**:860–864. https://doi.org/10.1126/science.aaa8764

Li H, La S, Zhang X, *et al.* (2021) Salt-induced recruitment of specific root-associated bacterial consortium capable of enhancing plant adaptability to salt stress. *ISME J* **15**:2865–2882. https://doi.org/10.1038/s41396-021-00974-2

Liao H, Li D, Zhou T, *et al.* (2020) The role of functional strategies in global plant distribution. *Ecography* **44**:493–503. https://doi.org/10.1111/ecog.05476

Lin T, Zhu G, He W, *et al.* (2022) Soil cadmium stress reduced host plant odor selection and oviposition preference of leaf herbivores through the changes in leaf volatile emissions. *Sci Total Environ* **814**:152728. https://doi.org/10.1016/j.scitotenv.2021.152728

Lin T, He W, Yang M, *et al.* (2023) Soil cadmium pollution facilitated the invasion of alligator weed through enhanced herbivore resistance and competitive ability over a congeneric species. *Plant Cell Environ* **47**:585–599. https://doi.org/10.1111/pce.14747

Liu D, Chen L, Chen C, *et al.* (2022) Effect of plant VOCs and light intensity on growth and reproduction performance of an invasive and a native *Phytolacca* species in China. *Ecol Evol* **12**:e8522. https://doi.org/10.1002/ece3.8522

Loreto F, D'Auria S (2022) How do plants sense volatiles sent by other plants? *Trends Plant Sci* **27**:29–38. https://doi.org/10.1016/j.tplants.2021.08.009

Loreto F, Schnitzler JP (2010) Abiotic stresses and induced BVOCs. *Trends Plant Sci* **15**:154–166. https://doi.org/10.1016/j.tplants.2009.12.006

Meng Y, Geng X, Zhu P, *et al.* (2024) Enhanced mutualism: a promotional effect driven by bacteria during the early invasion of *Phytolacca americana*. *Ecol Appl* **34**:e2742. https://doi.org/10.1002/eap.2742

Mittler R, Zandalinas SI, Fichman Y, *et al.* (2022) Reactive oxygen species signalling in plant stress responses. *Nat Rev Mol Cell Biol* **23**:663–679. https://doi.org/10.1038/s41580-022-00499-2

Montreemuk J, Stewart TN, Prapagdee B (2024) Bacterial-assisted phytoremediation of heavy metals: concepts, current knowledge, and future directions. *Environ Technol Innovation* **33**:103488. https://doi.org/10.1016/j.eti.2023.103488

Ninkovic V, Markovic D, Rensing M (2021) Plant volatiles as cues and signals in plant communication. *Plant Cell Environ* **44**:1030–1043. https://doi.org/10.1111/pce.13910

Niu B, Paulson JN, Zheng X, *et al.* (2017) Simplified and representative bacterial community of maize roots. *Proc Natl Acad Sci USA* **114**:E2450–E2459. https://doi.org/10.1073/pnas.1616148114

Pierce S, Negreiros D, Cerabolini BEL, *et al.* (2017) A global method for calculating plant CSR ecological strategies applied across biomes world-wide. *Funct Ecol* **31**:444–457. https://doi.org/10.1111/1365-2435.12722

Podar D, Maathuis FJM (2022) The role of roots and rhizosphere in providing tolerance to toxic metals and metalloids. *Plant Cell Environ* **45**:719–736. https://doi.org/10.1111/pce.14188

Pokotylo I, Hodges M, Kravets V, *et al.* (2022) A ménage à trois: salicylic acid, growth inhibition, and immunity. *Trends Plant Sci* **27**:460–471. https://doi.org/10.1016/j.tplants.2021.11.008

Quintana-Rodriguez E, Morales-Vargas AT, Molina-Torres J, *et al.* (2015) Plant volatiles cause direct, induced and associational resistance in common bean to the fungal pathogen *Colletotrichum lindemuthianum*. *J Ecol* **103**:250–260. https://doi.org/10.1111/1365-2745.12340

Ren J, Chen P, Shen C, *et al.* (2023) Functional and phylogenetic similarities of co-occurring invaders affect the growth of an invasive forb. *J Plant Ecol* **16**:rtad007. https://doi.org/10.1093/jpe/rtad007

Rodriguez PA, Rothballer M, Chowdhury SP, *et al.* (2019) Systems biology of plant-microbiome interactions. *Mol Plant* **12**:804–821. https://doi.org/10.1016/j.molp.2019.05.006

Rosenkranz M, Chen Y, Zhu P, *et al.* (2021) Volatile terpenes—mediators of plant to plant communication. *Plant J* **108**:617–631. https://doi.org/10.1111/tpj.15453

Sharifi R, Lee S, Ryu C (2018) Microbe-induced plant volatiles. *New Phytol* **220**:684–691. https://doi.org/10.1111/nph.14955

Simpraga M, Takabayashi J, Holopainen JK (2016) Language of plants: where is the word? *J Integr Plant Biol* **58**:343–349. https://doi.org/10.1111/jipb.12447

Souza-Alonso P, González L, Cavaleiro C (2014) Ambient has become strained. Identification of *Acacia dealbata* link volatiles interfering with germination and early growth of native species. *J Chem Ecol* **40**:1051–1061. https://doi.org/10.1007/s10886-014-0498-x

Vasseur F, Sartori K, Baron E, *et al.* (2018) Climate as a driver of adaptive variations in ecological strategies in *Arabidopsis thaliana*. *Ann Bot* **122**:935–945. https://doi.org/10.1093/aob/mcy165

Velikova V, Fares S, Loreto F (2008) Isoprene and nitric oxide reduce damages in leaves exposed to oxidative stress. *Plant Cell Environ* **31**:1882–1894. https://doi.org/10.1111/j.1365-3040.2008.01893.x

Vlot AC, Rosenkranz M (2022) Volatile compounds—the language of all kingdoms? *J Exp Bot* **73**:445–448. https://doi.org/10.1093/jxb/erab528

Wang R, Hou D, Chen J, *et al.* (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 X, Dai Z, Lin J, *et al.* (2023) Heavy metal contamination collapses trophic interactions in the soil microbial food web via bottom-up regulation. *Soil Biol Biochem* **184**:109058. https://doi.org/10.1016/j.soilbio.2023.109058

Wenke K, Kai M, Piechulla B (2010) Belowground volatiles facilitate interactions between plant roots and soil organisms. *Plant* **231**:499–506. https://doi.org/10.1007/s00425-009-1076-2

Williams A, Langridge H, Straathof AL, *et al.* (2021) Root functional traits explain root exudation rate and composition across a range of grassland species. *J Ecol* **110**:21–33. https://doi.org/10.1111/1365-2745.13630

Winter TR, Borkowski L, Zeier J, *et al.* (2012) Heavy metal stress can prime for herbivore-induced plant volatile emission. *Plant Cell Environ* **35**:1287–1298. https://doi.org/10.1111/j.1365-3040.2012.02489.x

Xu L, Zhao H, Ruan W, *et al.* (2017) ABNORMAL INFLORESCENCE MERISTEM1 functions in salicylic acid biosynthesis to maintain proper reactive oxygen species levels for root meristem activity in rice. *Plant Cell* **29**:560–574. https://doi.org/10.1105/tpc.16.00665

Yao C, Du L, Liu Q, *et al.* (2022) Stemborer-induced rice plant volatiles boost direct and indirect resistance in neighboring plants. *New Phytol* **237**:2375–2387. https://doi.org/10.1111/nph.18548

Yuan H, Xue W, Roiloa S, *et al.* (2024) Increasing biochar diversity promotes the impacts of plant diversity on remediating cadmium in soil. *J Plant Ecol* **17**:rtae068. https://doi.org/10.1093/jpe/rtae068

Yuan HM, Liu WC, Lu YT (2017) CATALASE2 coordinates SA-mediated repression of both auxin accumulation and JA biosynthesis in plant defenses. *Cell Host Microbe* **21**:143–155. https://doi.org/10.1016/j.chom.2017.01.007

Zhang Y, Yang C, Liu S, *et al.* (2024a) Phytohormones-mediated strategies for mitigation of heavy metals toxicity in plants focused on sustainable production. *Plant Cell Rep* **43**:99–117. https://doi.org/10.1007/s00299-024-03189-9

Zhang YS, Meiners SJ, Meng Y, *et al.* (2024b) Temporal dynamics of Grime's CSR strategies in plant communities during 60 years of succession. *Ecol Lett* **27**:e14446. https://doi.org/10.1111/ele.14446

Zhao H, Wei Y, Wang J, *et al.* (2019) Isolation and expression analysis of cadmium-induced genes from Cd/Mn hyperaccumulator *Phytolacca americana* in response to high Cd exposure. *Plant Biol* **21**:15–24. https://doi.org/10.1111/plb.12908

Zhu C, Dalsgaard B, Li W, *et al.* (2024) Generalist and topologically central avian frugivores promote plant invasion unequally across land-bridge islands. *Ecology* **105**:e4216. https://doi.org/10.1002/ecy.4216