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RESEARCH ARTICLE

Heavy metal pollution enhances pathogen resistance of an invasive plant species over its native congener

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Abstract

1. Many invasive plant species have demonstrated a better ability to tolerate and accumulate heavy metals than their co-occurring native plants. Given the potential biological toxicity of heavy metals to phytopathogens, we postulated that heavy metal contamination could enhance the pathogen resistance of metal-accumulating invasive plant species over their local congeneric competitors.
2. Here, we compared the resistance of the invasive *Alternanthera philoxeroides* and its native congener *Alternanthera sessilis* to infection by a leaf pathogenic fungus under soil cadmium (Cd) contamination.
3. The findings revealed that *A. philoxeroides* exhibited superior growth and accumulated superior amounts of Cd in its leaves compared with *A. sessilis*. Cd exposure decreased leaf lesions in both plant species following artificial infection by the leaf pathogen, with *A. philoxeroides* experiencing fewer leaf lesions than *A. sessilis*. Subsequent analysis of the phyllosphere microbiome and toxicity tests suggested that the increased pathogen resistance of *A. philoxeroides* following Cd exposure could be attributed to both the direct toxic effects of Cd and the Cd-induced alterations in the microbial composition of the epiphytic phyllosphere.
4. We further isolated a bacterial strain with a strong antagonistic effect against the leaf pathogen, potentially elucidating the enhanced pathogen resistance of *A. philoxeroides* under soil Cd pollution. Collectively, these findings indicate that heavy metal contamination may promote plant invasion by altering interactions between plants and pathogens.

KEYWORDS
Alternanthera philoxeroides, antagonistic strain, *Bacillus velezensis*, elemental defence hypothesis, heavy metals, phyllosphere microbiomes, plant invasion, plant-microbe interactions

Tiantian Lin and Abdul Manan contributed equally to this work.

1 | INTRODUCTION

Plant invasion represents a significant global concern, characterised by the introduction and establishment of non-indigenous plant species within new habitats (Kueffer et al., 2013; Roiloa et al., 2020). Plant invasion frequently results in detrimental impacts on indigenous species and local ecosystems through interfering with the growth and development of native plants (Roiloa et al., 2020). Therefore, elucidating the underlying mechanisms of plant invasion is crucial for predicting and controlling the spread of exotic plant species (Feng et al., 2022). It is generally accepted that habitats with high levels of disturbance and low biodiversity are more susceptible to the colonisation of invasive plants (Catford et al., 2012). Heavy metal contamination has emerged as one of the most significant environmental disturbances, exacerbated globally by the rise in anthropogenic activities (Roy et al., 2022). Numerous studies have demonstrated that heavy metal stress can not only disrupt the growth and reproduction of plants (Goyal et al., 2020) but also potentially contribute to the invasiveness of non-indigenous plants (Li et al., 2021). For example, a number of invasive plant species have exhibited superior tolerance to heavy metals and enhanced abilities to accumulate these metals compared to native plant species, which confer them a competitive edge over native co-existing plant species (Gulezian et al., 2012; Lin, He, et al., 2023; Piola & Johnston, 2007; Wang, Chen, et al., 2021; Zhang et al., 2008; Zhao et al., 2023).

Interestingly, research indicates that heavy metals also possess biological toxicity towards herbivores and phytopathogens (Borowska & Pyza, 2011; Hanson et al., 2003; Hörger et al., 2013). As suggested by the elemental defence hypothesis (EDH), the accumulation of heavy metals within plant tissues is regarded as a distinctive defensive strategy against attackers (Martens & Boyd, 1994). Nevertheless, there exists a limited number of studies that have investigated the interaction between heavy metal contamination and plant invasion in terms of EDH, which focuses mainly on herbivorous insects but not on diseases (Lin, He, et al., 2023; Wang et al., 2022; Zhou et al., 2023). On the other hand, a wide range of microorganisms, primarily consisting of bacteria and fungi, are found in abundance within the plant phyllosphere (Vacher et al., 2016). These microorganisms residing in the phyllosphere interact with their host plants, which not only influence plant fitness directly but also can contribute to plant defence against diseases (Stone et al., 2018; Vannier et al., 2019). As an open environment, the phyllosphere and its associated microorganisms are often impacted by abiotic factors, such as nitrogen fertilisation, water availability or ultraviolet radiation (Vorholt, 2012). For example, a number of studies have documented a strong influence of heavy metal stress on the structure and composition of phyllosphere microbiomes (Dobrone Toth et al., 2009; Jia et al., 2018; Lin, Lu, et al., 2023). Therefore, we hypothesise that the accumulation of heavy metals could potentially alter plant resistance to pathogens by influencing the diversity of associated microbial communities. We here studied the changes in the composition and structure of phyllosphere microbiomes to unravel the underlying mechanisms of plant–pathogen interactions mediated by heavy metals.

In accordance with EDH, we propose that invasive plant species may demonstrate increased resistance to pathogen infection in comparison to co-occurring native plants when grown in metal-contaminated soils through direct metal toxicity or through an indirect metal-induced change in microbial composition of the phyllosphere. To test this hypothesis, we utilised the invasive alligator weed *Alternanthera philoxeroides* and its native co-occurring congeneric species *A. sessilis* as a study model. We first assessed the variation in pathogen resistance between the two plant species, both under control and subjected to soil cadmium (Cd) treatment, subsequent to artificial infection with a leaf pathogen. In addition, we analysed the inhibitory effect of a series of Cd concentrations on pathogen growth through in vitro toxicity tests. Additionally, distinctions in the composition of both epiphytic and endophytic fungal and bacterial assemblages associated with the phyllosphere between the two plant species were investigated. Furthermore, we explored the inhibitory effects of cultivable phyllosphere microorganisms from plants exposed to soil Cd treatment on the growth of the leaf pathogen through in vitro bioassays. Finally, we isolated and identified an antagonistic bacterial strain that might contribute to plant resistance to pathogens. To the best of our knowledge, our study represents one of the initial investigations into the mechanisms by which heavy metal pollution influences plant–pathogen interactions within the framework of biological invasion.

2 | MATERIALS AND METHODS

2.1 | Study species

2.1.1 | Plant materials

As a clonal perennial herb, *A. philoxeroides* originates from South America and has extensively expanded in Asia, North America and Oceania (Stohlgren et al., 2013). This invasive plant species thrives in both aquatic and terrestrial environments, which has been considered an aggressive weed in its invasive habitats due to its great adaptability and colonising ability. Previous studies have indicated that *A. philoxeroides* not only demonstrates enhanced tolerance to heavy metal pollution but also has the distinct ability to accumulate considerable amounts of heavy metals within its tissues (Lin, He, et al., 2023; Wang, Chen, et al., 2021). *Alternanthera sessilis* is a congener of *A. philoxeroides* and is native to Asia (Liang et al., 2020). These two plant species are frequently found together in wetland environments and are highly similar in morphology and phenotypic plasticity (Geng et al., 2006). Ramets from five distinct populations of each plant species were collected from a metal-contaminated area adjacent to an abandoned mining site located in Meigu County, Southwestern China (28°04′ N, 102°11′ E). The spatial separation between any two populations exceeded 1 kilometre. The concentration

of Cd in the soil at the sampling locations varied between 40 and 80 mg Cd^{2+} kg^{-1} of dry soil.

2.1.2 | Leaf pathogen

In this study, *Fusarium oxysporum* was employed as the representative leaf pathogenic fungus due to its widespread occurrence as a generalist pathogen, causing leaf spot disease in numerous plant species (Wang, Zhang, et al., 2021; Xiao et al., 2023; Xu et al., 2022). Furthermore, previous studies have reported the successful infection of *Fusarium* spp. on both *A. philoxeroides* and *A. sessilis* leaves (Liu et al., 2023; Oladiran & Gana, 1991; Tan et al., 2002). In a tryout experiment, we observed that *F. oxysporum* was capable of successfully infecting the leaves of both species under controlled laboratory conditions, resulting in the manifestation of leaf spot. These findings largely excluded the possibility of a species-specific infection pattern associated with this pathogenic fungus. The specific strain utilised in this study was isolated from field-collected leaves of *A. philoxeroides* and kept as a laboratory culture.

2.2 | Experimental design

2.2.1 | Plant cultivation

For each plant species, ramets with ~5 nodes and 4 cm in height, from each population were individually selected and transplanted into plastic pots with a diameter of 13.5 cm and a height of 11.5 cm. Each pot was filled with a substrate composed of 1 L of potting soil mixed with sand in a ratio of 3:7 (soil characteristics were list in the Supporting Information Table S1) The soil in pots subjected to the Cd treatment was amended with $CdCl_2{\cdot}2.5H_2O$ to achieve a Cd^{2+} concentration of 60 mg $Cd^{2+}{\cdot}kg^{-1}$ dry soil, reflecting the average Cd concentration observed at the site of collection. The experiment comprised four treatment groups: control + *A. philoxeroides*, control + *A. sessilis*, Cd + *A. philoxeroides*, and Cd + *A. sessilis*, with three ramets per population serving as replications. In total, 60 plants were grown, consisting of 3 ramets × 5 populations × 2 treatments × 2 species. All plants were cultivated in a naturally lit greenhouse for 60 days from April to June (average daytime temperature 31.8°C, average night-time temperature 21.5°C, average relative humidity 68.4%). At the end of the soil Cd treatment, all plants from different treatments were subjected to the following experiments, as shown in Figure S1.

2.2.2 | Measurement of host plant traits related to growth and leaf chemistry

After 60 days of soil Cd treatment, leaf number and total leaf area were measured prior to the separate harvesting of the leaves, stem, and root ($N = 15$ plants from 5 populations per treatment). Since leaf water and nitrogen contents have been recorded to involve in plant defence to pathogens (Grimmer et al., 2012; Mur et al., 2016), we calculated the leaf water content of each plant based on the fresh and dry mass, while the Kjeldahl method was used to detect leaf nitrogen content (Čeh-Brežnik & Tajnšek, 2018). The concentration of Cd in the leaves was analysed using an ICP-AES (Avio 550 Max, PerkinElmer). In addition, as leaf defensive chemicals could also protect plants from leaf pathogen infections (Biere et al., 2004; Kaur et al., 2022), we determined the amounts of three defensive chemical compounds including tannins, total flavonoids and total phenolics according to the method as mentioned by Lin et al. (2022).

2.2.3 | Illumina MiSeq sequencing of phyllosphere bacterial and fungal communities

The 5th to 15th fully expanded fresh leaves were harvested from each plant and separated from the leaf stalk, and pooled for each population. In total, 20 leaf samples were collected, comprising 5 populations × 2 treatments × 2 species. We extracted the DNA of epiphytic and endophytic microbes according to the method as detailed in Appendix S1. The amplification of the fungal ITS1 region (Martin & Rygiewicz, 2005) and the bacterial 16S rRNA region (Zhang et al., 2021) was conducted using the primers specified in Table S2. The resulting PCR products of bacterial and fungal microbes were sequenced on a MiSeq platform (Illumina, Inc., United States) by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). In total, 80 PCR products were sequenced (5 populations × 2 treatments × 2 species × 2 spheres (epiphytic and endophytic) × 2 microbial groups (bacteria and fungi)).

2.2.4 | Leaf pathogen infection and in vitro toxicity test

We artificially infected the two plant species with a leaf pathogenic fungus, *Fusarium oxysporum*, to evaluate the influence of Cd stress on their pathogen susceptibility. A total of 15 plants from each treatment group had their leaves infected with a spore suspension of *F. oxysporum* at a concentration of 1×10^6 spores·mL^{-1}. After being inoculated, all plants were cultivated in the same greenhouse for an additional 7 days. At the end, all the remaining leaves of each plant were scanned, and the leaf lesion area was quantified utilising Adobe Photoshop CS6 software (Adobe Systems, San Jose, CA, USA).

We also examined the direct impact of Cd on the growth of the fungal mycelium of the leaf pathogen *F. oxysporum*. This was accomplished by growing fungal mycelium on PDA plates that were enriched with varying concentrations of Cd, including 0, 5, 10, 15, 20, and 25 mg·L^{-1-1} Cd^{2+}. A 1 cm agar cutting containing *F. oxysporum* mycelium, sourced from a Cd-free PDA plate, was centrally placed on new PDA plates (9 cm in diameter) that contained varying concentrations of Cd, with five plates allocated for each concentration. After a 7-day cultivation period at 25°C and 60% relative

humidity, the growth diameter of the fungal mycelium was assessed. Furthermore, another in vitro toxicity test was conducted to determine the potential inhibitory effect of cultivable phyllosphere microorganisms on the growth of *F. oxysporum* following the method of Lin, Lu, et al. (2023) as described in Appendix S2.

2.2.5 | Isolation, screening and identification of antagonistic microbial strains

Antagonistic microbial strains were further isolated and screened from the cultivable epiphytic and endophytic microorganisms of *A. philoxeroides* subjected to soil Cd treatment following the method of Lin, Li, et al. (2023). The extracted epiphytic and endophytic microorganisms from *A. philoxeroides* subjected to soil Cd treatment, as previously described, were diluted with sterilised water to four dilutions (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}). Subsequently, 100 μL of the diluted solution from the various dilutions was uniformly distributed onto NA medium for bacterial culture and PDA medium for fungal culture ($N = 5$ plates for replicates). Subsequently, the NA plates were cultured at 37°C for a duration of 24 h, while the PDA plates were maintained at 25°C for a period of 5 days. Individual colonies exhibiting distinct morphological characteristics were chosen and re-cultured on NA or PDA medium for the purpose of purification. We purified four bacterial and two fungal strains only from the epiphytic phyllosphere of *A. philoxeroides*. These strains were subsequently inoculated onto media and screened for antagonistic activity against *F. oxysporum*. The screening of antagonistic microorganisms was carried out using the PDA confrontation assay. In this assay, a disc (8 mm in diameter) of the pathogenic fungus *F. oxysporum* was positioned on one side of the PDA medium, while a disc of the same size of an isolated antagonistic microbe was placed 3 cm away from *F. oxysporum* on the opposite side of the PDA culture medium ($N = 5$ plates as replicates for each candidate strain). PDA plates inoculated exclusively with *F. oxysporum* served as controls. The PDA plates were then cultured at 25°C for a period of 4 days before measuring the diameter of the *F. oxysporum* mycelium. The procedures for DNA extraction and identification of the antagonistic strain were elaborated in Appendix S3. A PCR was carried out with the universal bacterial primers gyrA, gyrB and rpoB (Tables S3 and S4). PCR products of different antagonistic strains were submitted for Sanger sequencing through Chengdu Qingke Biotechnology Co., Ltd. The nucleotide sequences were subsequently deposited in the GenBank database (Table S5).

2.2.6 | In vivo antagonistic effect of the antagonistic strain on *F. oxysporum*

Individual *A. philoxeroides* plants were grown under greenhouse conditions for a period of 60 days. The antagonistic bacterial strain suspension was prepared following Lin, Li, et al. (2023) and the antagonistic effect of the strain was tested as described in Appendix S4. Plants inoculated solely with the spore suspension of *F. oxysporum* were designated as the positive control, whereas those inoculated only with sterilised water were considered the negative control. Fifteen plants were tested as replicates for each treatment, and the leaf lesion area of each plant was assessed using the same method after 7 days.

2.3 | Bioinformatic analysis

The original dataset underwent quality control procedures using QIIME (Version 1.7.0) (Caporaso et al., 2010) to delete low-quality sequences. After undergoing processes of quality filtration, denoising, merging, and chimera screening, we successfully acquired a total of 1,351,996 and 1,810,162 high-quality fungal and bacterial sequence reads, respectively, from the epiphytic samples. The average lengths of these sequences were 245 bp for fungi and 377 bp for bacteria, respectively. Similarly, 1,371,884 and 1,449,254 high-quality reads were acquired for fungi and bacteria, respectively, from the endophytic samples. The average lengths of these sequences were 240 bp for fungi and 376 bp for bacteria, respectively. Sequences aligning with chloroplast and mitochondrial DNA were excluded to prevent potential contamination. UPARSE (version 7.1) was utilised to cluster Operational taxonomic units (OTUs) at a 97% similarity cutoff. Taxonomic classification of fungal and bacterial OTUs was performed using the UNITE database (version 18.11) and USEARCH (version 8.0) consensus taxonomy classifier against the SILVA 16S rRNA database (Version 13.2), respectively (Lin, Tang, et al., 2023). At the end, we deposited the fungal and bacterial sequence data in the National Centre for Biotechnology Information (NCBI), assigning them the Sequence Read Archive (SRA) accession numbers PRJNA1026056 and PRJNA1025945, respectively.

The composition and structure of phyllosphere microbial assemblies were assessed through the Majorbio online platform (www.majorbio.com) (Ren et al., 2022). Alpha diversity was evaluated by the Shannon index, and Beta diversity was analysed through principal coordinates analysis (PCoA) based on Bray–Curtis distances. We further employed a one-way analysis of similarity (ANOSIM) to investigate variations among groups of microbial communities from each treatment derived from the PCoA using Bray–Curtis distance matrices. In addition, we utilised redundancy analysis (RDA) and Spearman correlation to explore the relationships between leaf Cd concentration, leaf lesion area and the taxonomic distribution of microbiomes. Heatmap clustering was conducted to examine variations in the top 30 abundant genera of fungi and bacteria present in both the epiphytic and endophytic phyllosphere of *A. philoxeroides* and *A. sessilis* cultivated in soils with or without Cd amendment.

2.4 | Statistical analysis

We employed generalised linear mixed models (GLMMs) to examine the effect of plant species and Cd treatment on plant

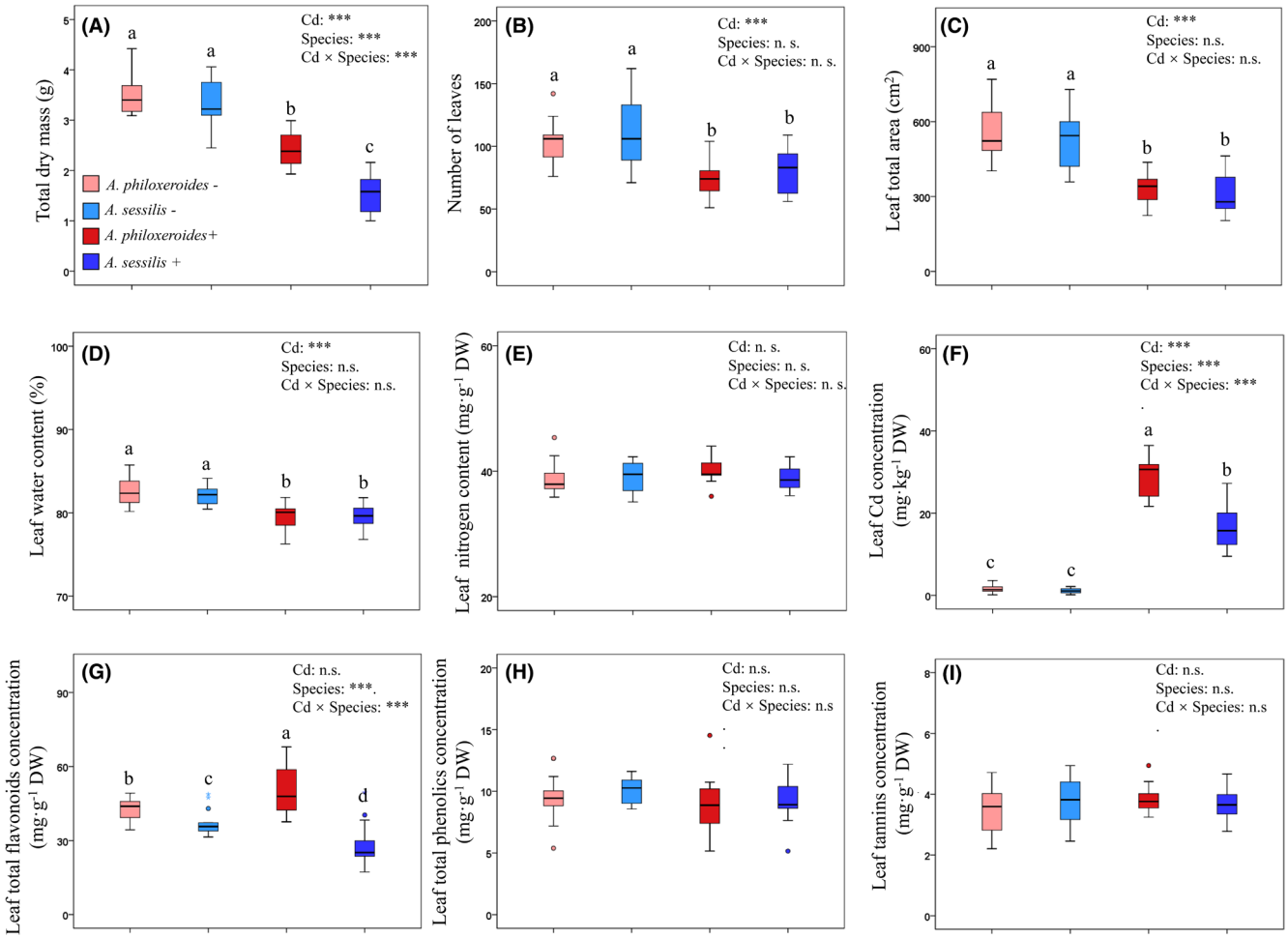


FIGURE 1 Plant growth-related traits and leaf chemicals of *A. philoxeroides* and *A. sessilis* grown on control and Cd-containing soils. (A) total dry mass, (B) number of leaves, (C) leaf total area, (D) leaf water content, (E) leaf nitrogen content, (F) leaf Cd concentration, (G) leaf total flavonoids concentration, (H) leaf total phenolics concentration, (I) leaf tannins concentration. $N = 15$ plants from 5 populations per treatment. Values are means ± SD. Different letters indicate significant differences among treatments at $p < 0.05$ according to GLMMs followed by Wald pairwise comparisons. n.s. = not significant; $^{***}p \leq 0.001$. *A. philoxeroides*−: A. philoxeroides from control soil, *A. sessilis*−: *A. sessilis* from control soil, *A. philoxeroides*+: *A. philoxeroides* from Cd-containing soil, *A. sessilis*+: *A. sessilis* from Cd-containing soil.

characteristics related to growth and leaf chemistry. In this analysis, Cd and species were treated as fixed factors, while population was considered a random factor. Subsequently, a Wald pair-wise comparison was conducted to assess the differences among the treatment groups. The differences in the Shannon index and the relative abundance of the key genera of phyllosphere microorganisms, as well as the relative abundance of OTUs aligned to the antagonistic strain among the four treatments were examined through generalised linear models (GLMs) followed by a Wald pair-wise comparison. Partial correlation was conducted to examine the correlation between leaf water content and leaf lesion area with leaf Cd concentration as a control factor. Moreover, the relationship between the mycelium growth of *F. oxysporum* and Cd concentration amended in the PDA plates was also tested by Spearman rank correlations. All the aforementioned analyses were conducted using SPSS 23.0 (IBM, Chicago, USA).

3 | RESULTS

3.1 | Effect of soil Cd treatment on plant growth-related traits and leaf chemistry

Both plant species exhibited similar growth in the control, whereas the soil Cd treatment notably decreased the dry mass of both plant species (Figure 1A and Figure S2). However, *A. philoxeroides* still demonstrated 50.2% ($p < 0.001$), 63.7% ($p < 0.001$), 56.4% ($p < 0.001$), and 56.3% ($p < 0.001$) higher leaf, stem, root and total dry mass compared with *A. sessilis* following Cd exposure. Moreover, the soil Cd treatment substantially reduced the number of leaves, total leaf area and leaf water content of both plant species, while no large differences were observed between the two species subjected to either control or soil Cd treatment (Figure 1B–D). Conversely, plants from different treatments showed a similar amount of leaf nitrogen

content (Figure 1E). In addition, leaf Cd concentration was highly affected by soil Cd treatment, with *A. philoxeroides* accumulating 69.0% ($p < 0.001$) more Cd in the leaves compared with *A. sessilis*, while in the control treatment, they did not significantly differ (Figure 1F). Regarding the three leaf defensive chemicals, only leaf total flavonoids increased in *A. philoxeroides* but decreased in *A. sessilis* after soil Cd treatment, which led to 1.8 times ($p < 0.001$) more leaf total flavonoids accumulated in *A. philoxeroides* than *A. sessilis* (Figure 1G). Neither Cd treatment nor plant species significantly affected the concentrations of leaf total phenolics and tannins (Figure 1H,I).

3.2 | The effect of soil Cd treatment on plant resistance to *F. oxysporum* infection

Seven days following the artificial introduction of the leaf pathogenic fungus *F. oxysporum*, both plant species displayed a similar level of leaf lesion area in the control soil (Figure 2A). However, soil Cd treatment remarkably reduced the leaf lesion area in both plant species, with *A. sessilis* exhibiting 1.85 times ($p < 0.001$) more leaf lesion area than *A. philoxeroides*. Among all plant traits related to leaf chemistry, leaf lesion area declined with leaf Cd concentration but increased with leaf water content for both species, whereas other traits showed no strong correlations (Figure 2B,C, Table S6). Since leaf water content exhibited a negative correlation with leaf Cd concentration for both plant species (*A. philoxeroides*: $r = -0.725$, $p < 0.001$; *A. sessilis*: $r = -0.792$, $p < 0.001$), a partial correlation was conducted and indicated no strong correlation between leaf water content and leaf lesion area (*A. philoxeroides*: $r = 0.133$, $p = 0.491$; *A. sessilis*: $r = 0.274$, $p = 0.150$).

3.3 | In vitro Cd toxicity test of *F. oxysporum*

The in vitro Cd toxicity test showed that the mycelium growth of *F. oxysporum* declined with Cd concentration amended in the PDA plates (Figure 2D). The concentration of Cd necessary to achieve a 50% reduction in mycelium diameter (EC_{50}) was found to be ~18 mg $Cd^{2+} \cdot L^{-1}$. Furthermore, the average leaf Cd concentration of plants exposed to Cd stress was 28.8 mg Cd^{2+} kg^{-1} DW in *A. philoxeroides* and 17.0 mg Cd^{2+} kg^{-1} DW in *A. sessilis*. Such concentrations equated to those of 5.9 mg Cd^{2+} L^{-1} and 3.5 mg Cd^{2+} L^{-1} in the PDA medium, considering an average water content of 79.4% and 79.6% in the leaves of *A. philoxeroides* and *A. sessilis*, respectively. These Cd concentrations in the PDA medium led to a reduction of 16.4% and 9.7% in mycelium growth, respectively.

3.4 | The effect of soil Cd treatment on the taxonomic distribution of phyllosphere-associated microbes

The epiphytic fungal community harboured in the phyllosphere of *A. philoxeroides* and *A. sessilis* plants showed greater diversity, with 2141 operational taxonomic units (OTUs), compared with the endophytic fungal community, which had 525 OTUs. Conversely, the endophytic bacterial community exhibited higher diversity, with 2515 OTUs, compared with the epiphytic bacterial community, which had 2188 OTUs. The taxonomic classification of OTUs was listed in Table S7. A Venn diagram showed that epiphytic and endophytic fungal assemblies that were shared for all plant species and Cd soil treatments were 662 (30.1%) and 47 (9.0%) identical OTUs, respectively. Epiphytic and endophytic bacterial communities that were shared for both plant species and Cd soil treatments were 330 (15.1%) and 256 (10.2%) identical OTUs, respectively (Figure S3).

3.5 | The effect of soil Cd treatment on the diversity of phyllosphere microbial communities

The epiphytic fungal communities for *A. sessilis* from the control treatment showed the highest Shannon index, while Cd-treated *A. philoxeroides* exhibited the lowest (Figure 3A). As a result, the Shannon index of the fungal communities in the epiphytes of control *A. sessilis* was 1.6 times ($p = 0.008$) higher than that of *A. philoxeroides* exposed to Cd treatment. In addition, the Shannon index of fungal taxa harboured in the endophytic phyllosphere of *A. sessilis* was reduced by 48.9% ($p < 0.001$) in response to soil Cd treatment, which led to a 1.6 times ($p = 0.008$) higher Shannon index of fungal taxa in *A. philoxeroides* than in *A. sessilis* (Figure 3B). On the contrary, Cd exposure increased the Shannon index of epiphytic bacterial communities by 14.9% ($p = 0.036$) but reduced that of endophytic bacterial communities by 44.2% ($p < 0.001$) in *A. philoxeroides* (Figure 3C,D).

The PCoA was employed to evaluate the variation in phyllosphere microbial composition of the two plant species under control or soil Cd treatment. Distinct differences between the two plant species and across different treatments were found for the diversity of epiphytic fungal and bacterial communities as confirmed by an ANOSIM analysis (Figure 3E,G, Table S8). No evident separation in the endophytic fungal communities between the plant species or treatments was detected (Figure 3F). However, the diversity of the endophytic bacterial communities from different treatments was clustered together, except for a clear separation observed for *A. philoxeroides* in the control (Figure 3H). Consequently, both the plant species and soil Cd stress showed a significant impact on the diversity of the endophytic bacterial taxa but not on that of the endophytic fungal taxa (Table S8).

3.6 | Correlation between leaf lesion area, leaf Cd concentration and phyllosphere microbial community composition

The RDA analysis was conducted to examine the impact of leaf lesion area and leaf Cd concentration on the distribution of microbial taxa within the phyllosphere, given the significant correlation observed between leaf Cd concentration and leaf lesion area, as leaf Cd concentration was significantly correlated with leaf lesion area. We found

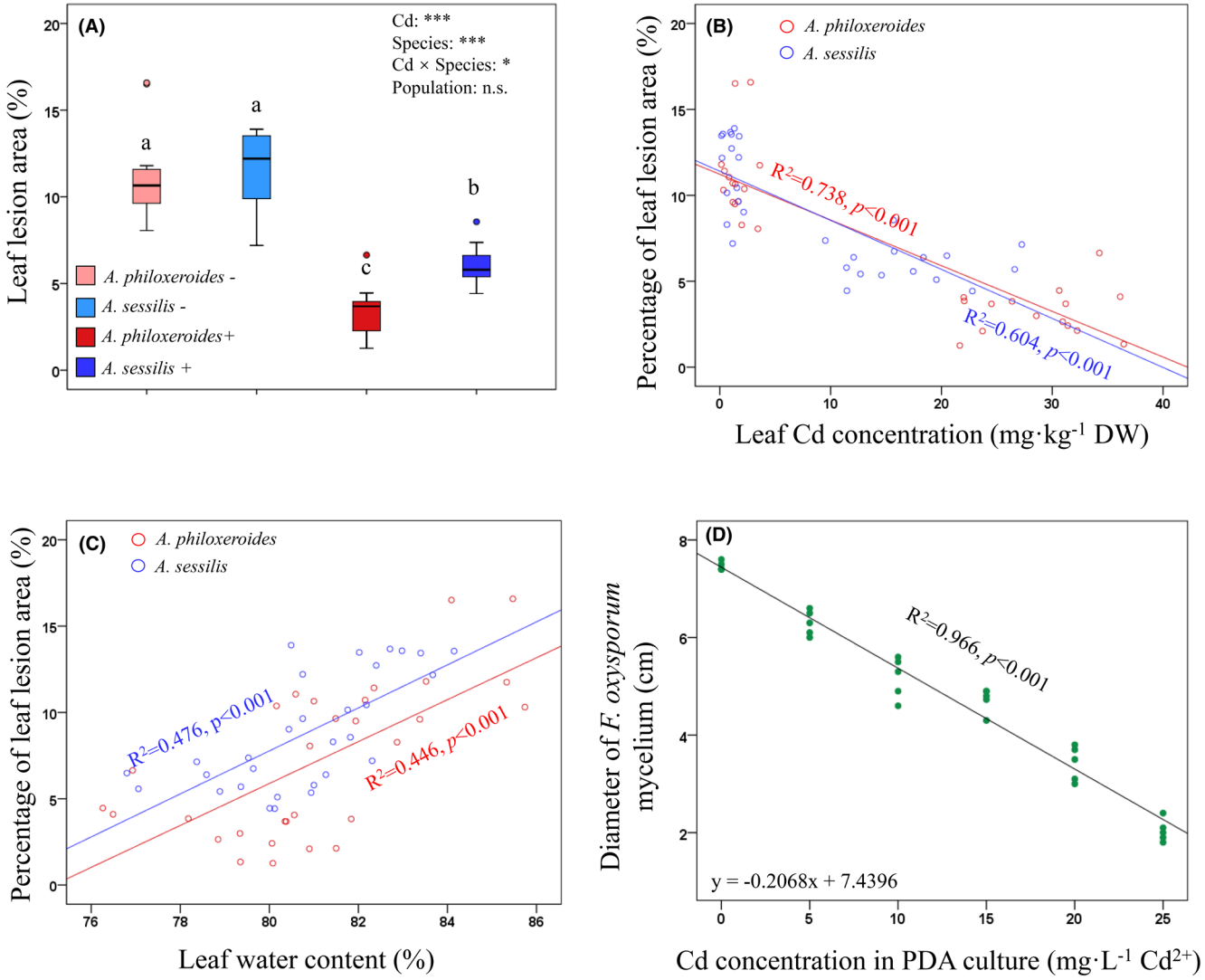


FIGURE 2 The growth of the pathogenic fungus *Fusarium oxysporum* on plant leaves and on PDA medium containing a range of Cd concentrations as well as the correlations between leaf lesion area and leaf Cd or water content. (A) Percentage of leaf lesion area of *A. philoxeroides* and *A. sessilis* grown on control and Cd-containing soils after infection by *F. oxysporum*. $N = 15$ plants from 5 populations per treatment. Values are means ± SE. Different letters indicate significant differences among treatments at $p < 0.05$ according to GLMMs followed by Wald pairwise comparisons. n.s. = not significant; $^{*}p \leq 0.05$; $^{***}p \leq 0.001$. (B, C) Spearman rank correlations between the percentage of leaf lesion area and leaf Cd concentration and leaf water content of *A. philoxeroides* and *A. sessilis* from control and soil Cd treatment. (D) Growth of *F. oxysporum* on PDA plates containing a range of Cd concentrations after 7 days. $N = 5$ replicates for each Cd concentration. Spearman rank correlation two-tailed test: $r = -0.976$, $p < 0.001$. *A. philoxeroides*−: *A. philoxeroides* from control soil, *A. sessilis*−: *A. sessilis* from control soil, *A. philoxeroides*+: *A. philoxeroides* from Cd-containing soil, *A. sessilis*+: *A. sessilis* from Cd-containing soil.

that variations in the relative abundances of epiphytic fungal and bacterial communities were strongly influenced by the two plant traits (Figure 4a,e, Table S9). Furthermore, the analysis indicated that the clustering of both epiphytic fungal and bacterial taxa of control plants was driven by leaf lesion area, whereas those of Cd-treated plants were driven by leaf Cd concentration. The Spearman rank correlation heatmap also revealed that the fungal genera of *Trametes*, *Peniophora*, *Coprinellus*, *Flavodon*, *Nigrospora*, *Gibellulopsis* and *Periconia* from epiphytes were all negatively associated with leaf Cd concentration but had a positive correlation with leaf lesion area (Figure 4b). Conversely, the genera of *Cladosporium* and *Ramularia* showed the opposite pattern. Additionally, the relative abundance of the bacterial genera *Stenotrophomonas*, *Bacillus*, *Acinetobacter*, *Glutamicibacter*, *Delftia* and *Comamonas* from epiphytes all exhibited a positive correlation with leaf Cd concentration but were negatively associated with leaf lesion area, while the genera of *Acidovorax*, *Shinella*, *Microbacterium* and C39 showed reversed correlations (Figure 4f). On the contrary, none of these two plant traits strongly impacted the composition of endophytic fungal and bacterial taxa (Figure 4c,g, Table S9). The Spearman rank correlation heatmap further indicated that the abundance of the *Exophiala* genus of endophytic fungi and *Sphingomonas* genus of endophytic bacteria were both negatively associated with leaf Cd concentration but positively associated with leaf lesion area (Figure 4d,h). Conversely, the *Klebsiella* genus of endophytic bacteria exhibited the opposite correlation.

3.7 | Differences in relative abundance of important microbial genera among treatments

Heatmap clustering was employed to compare the variations in the top 30 abundant fungal and bacterial genera present in the phyllosphere of the two plant species grown in soils with or without Cd amendment. We specifically focused on the key microbial genera that significantly correlated with both leaf lesion area and leaf Cd concentration as described above. In terms of epiphytic fungal taxa, Cd exposure promoted the enrichment of the *Ramularia* genus but reduced that of the genera *Nigrospora*, *Gibellulopsis* and *Periconia* in both plant species (Figure 5a). In comparison to corresponding controls, Cd exposure strongly decreased the accumulation of the genera *Trametes* ($p<0.001$), *Peniophora* ($p<0.001$) and *Flavodon* ($p<0.001$) in the *A. sessilis* phyllosphere but not in *A. philoxeroides*. As a result, the *A. philoxeroides* phyllosphere accumulated 3.0 times ($p=0.006$) higher abundance of the *Ramularia* genus but 1.8 and 2.6 times less abundance of the genera *Flavodon* and *Nigrospora* than *A. sessilis*. Furthermore, the fungal genus *Exophiala* accumulated similar abundance in the endophytic phyllosphere of both species, while it was reduced by 72.2% ($p<0.05$) and 54.2% ($p<0.05$) in the phyllosphere of *A. philoxeroides* and *A. sessilis*, respectively, after exposure to Cd treatment (Figure 5b). Regarding the epiphytic bacterial taxa, Cd stress increased the relative abundance of the three genera *Bacillus*, *Delftia* and *Glutamicibacter* in both plant species, while that harboured in *A. philoxeroides* exhibited 9.2 ($p<0.001$), 1.4 ($p=0.002$) and 20.0 ($p<0.001$) times more abundance than *A. sessilis*, respectively (Figure 5c). Moreover, the relative abundance of the genera *C39*, *Shinella* and *Microbacterium* was decreased by Cd exposure in both plant species, whereas that harboured in *A. philoxeroides* showed 8.0 ($p<0.001$), 5.0 ($p<0.001$) and 5.7 ($p=0.004$) times more abundance, respectively, than in *A. sessilis*. Lastly, soil Cd stress stimulated the accumulation of the bacterial genus *Klebsiella* by 1.9 times ($p=0.028$) in the endophytic phyllosphere of *A. sessilis*, whereas that of the genera *Massilia*, *Sphingomonas* and *Flavobacterium* decreased in both plant species (Figure 5d).

3.8 | Inhibitory effects of cultivable microorganisms on the growth of leaf pathogenic fungus

The mycelium of *F. oxysporum* grew the best on control plates, while it showed a reduced growth when cultivated with epiphytic microorganisms collected from the leaf surfaces of *A. philoxeroides* and *A. sessilis* grown in control soil (Figure 6A). Nevertheless, exposure to epiphytic microorganisms collected from the leaf surfaces from the soil Cd treatment resulted in a further reduction of 54.0% and 34.3% in the mycelium diameter of *F. oxysporum* for *A. philoxeroides* and *A. sessilis* plants, respectively. Notably, the epiphytic microbes

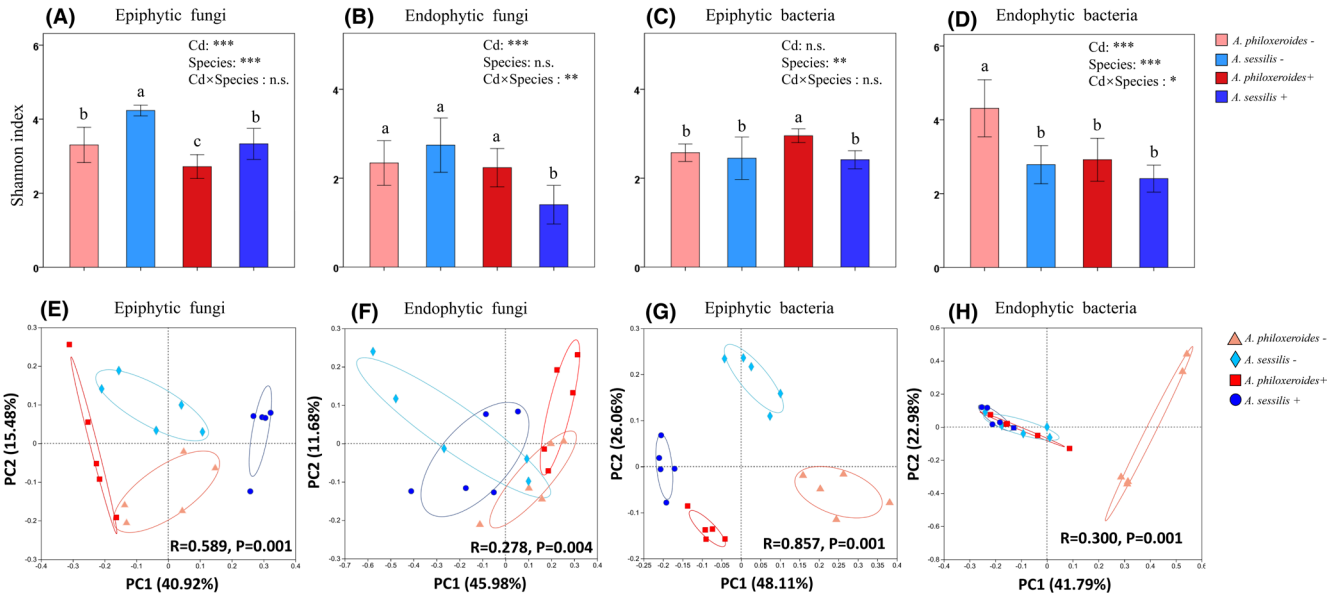
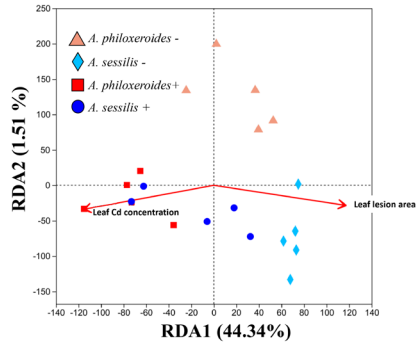
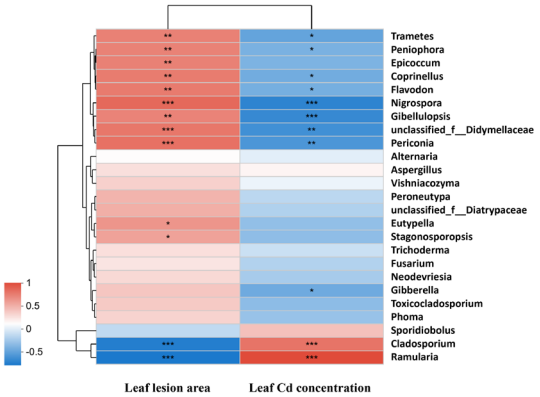


FIGURE 3 The Shannon index and principal co-ordinates analysis (PCoA) of endophytic and epiphytic microbial OTUs of *A. philoxeroides* and *A. sessilis* grown on control and Cd-containing soils. (A) Shannon index for epiphytic fungi, (B) Shannon index for endophytic fungi, (C) Shannon index for epiphytic bacteria, (D) Shannon index for endophytic bacteria. Different letters indicate significant differences among treatments at $p<0.05$ of Wald pairwise comparisons. *$p\leq0.05$; **$p\leq0.01$; ***$p\leq0.001$ of the generalised linear models. (E) PCoA of epiphytic fungi, (F) PCoA of endophytic fungi, (G) PCoA of epiphytic bacteria, (H) PCoA of endophytic bacteria. The differences among groupings of microbial samples from each treatment obtained in the PCoA using Bray–Curtis distance matrices were examined by one-way analysis of similarity (ANOSIM). $N=5$ replicates for each treatment. *A. philoxeroides*-: *A. philoxeroides* from control soil, *A. sessilis*−: *A. sessilis* from control soil, *A. philoxeroides*+: *A. philoxeroides* from Cd-containing soil, *A. sessilis*+: *A. sessilis* from Cd-containing soil.

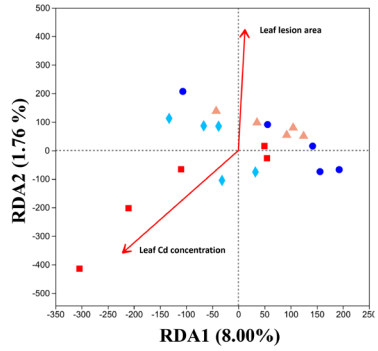
(a) RDA of epiphytic fungal communities on genus level



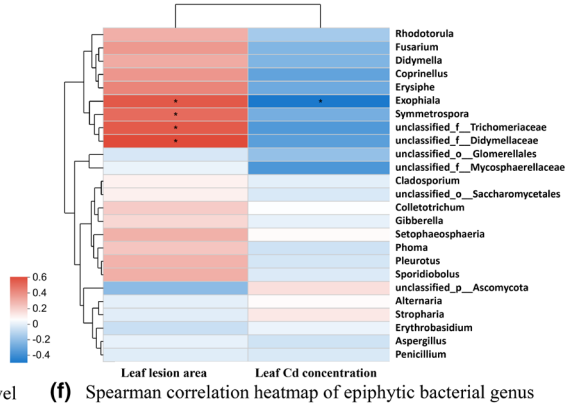
(b) Spearman correlation heatmap of epiphytic fungal genus



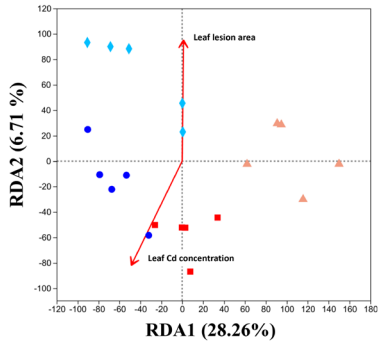
(c) RDA of endophytic fungal communities on genus level



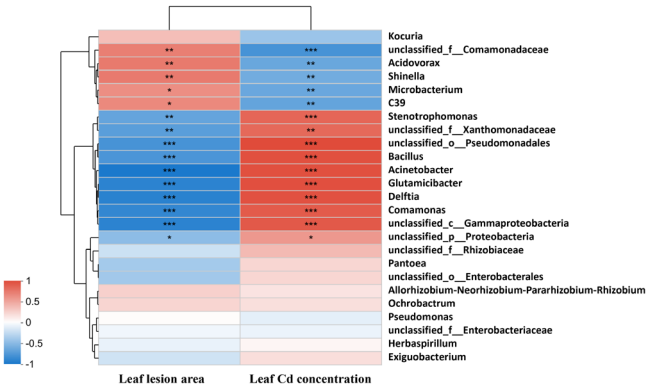
(d) Spearman correlation heatmap of endophytic fungal genus



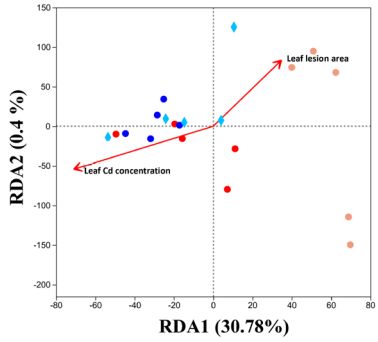
(e) RDA of epiphytic bacterial communities on genus level



(f) Spearman correlation heatmap of epiphytic bacterial genus



(g) RDA of endophytic bacterial communities on genus level



(h) Spearman correlation heatmap of endophytic bacterial genus

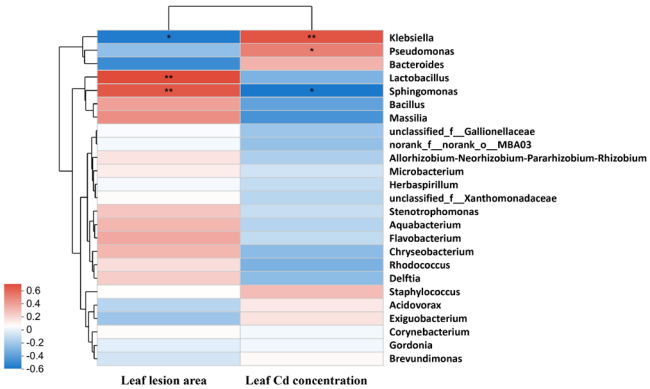


FIGURE 4 Redundancy analysis (RDA) and Spearman rank correlation heatmaps of the relative abundance of the top 25 abundant phyllosphere microbes at the genus level and leaf Cd concentration and leaf lesion area of *A. philoxeroides* and *A. sessilis* grown on control and Cd-containing soils. (a) RDA of epiphytic fungi; (b) heatmap of epiphytic fungal taxa; (c) RDA of endophytic fungi; (d) heatmap of endophytic fungal taxa; (e) RDA of epiphytic bacteria; (f) heatmap of epiphytic bacterial taxa; (g) RDA of endophytic bacteria; (h) heatmap of endophytic bacterial taxa. Spearman correlation two-tailed test; $N = 5$ plants for each treatment $^{*}0.01 < p \leq 0.05$; $^{**}0.001 < p \leq 0.01$; $^{***}p \leq 0.001$. *A. philoxeroides*−: *A. philoxeroides* from control soil, *A. sessilis*−: *A. sessilis* from control soil, *A. philoxeroides*+: *A. philoxeroides* from Cd-containing soil, *A. sessilis*+: *A. sessilis* from Cd-containing soil.

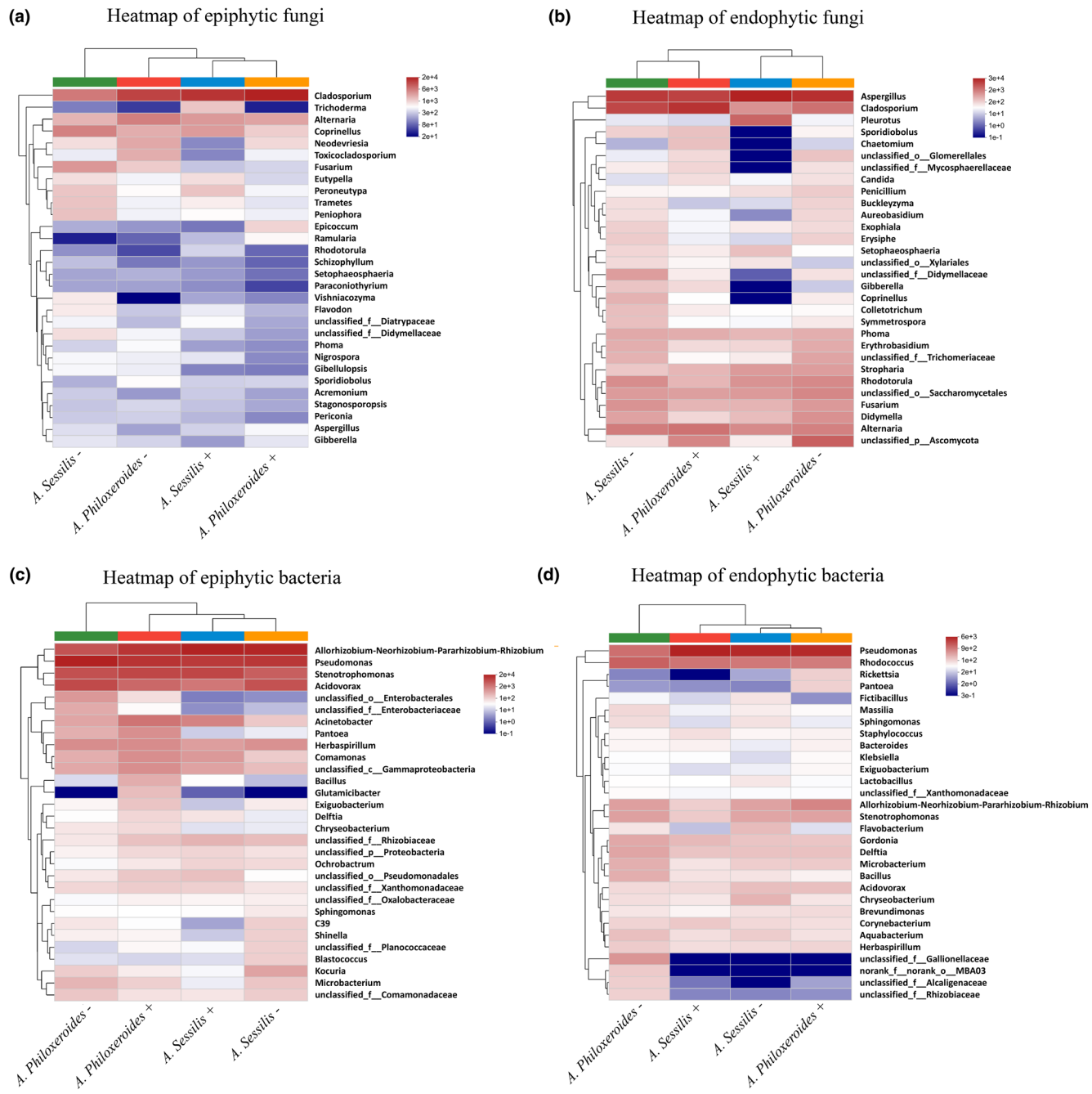


FIGURE 5 The heatmap clustering of the distribution of fungal and bacterial genera in the epiphytic or endophytic phyllosphere of *A. philoxeroides* and *A. sessilis* grown on control and Cd-containing soils. (a) epiphytic fungal genera; (b) endophytic fungal genera; (c) epiphytic bacterial genera; (d) endophytic bacterial genera. Cluster analysis was based on the Bray–Curtis method, and only the top 30 abundant genera were included for each heatmap. The clustering method for genera and samples was average. *A. philoxeroides*−: *A. philoxeroides* from control soil, *A. sessilis*−: *A. sessilis* from control soil, *A. philoxeroides*+: *A. philoxeroides* from Cd-containing soil, *A. sessilis*+: *A. sessilis* from Cd-containing soil.

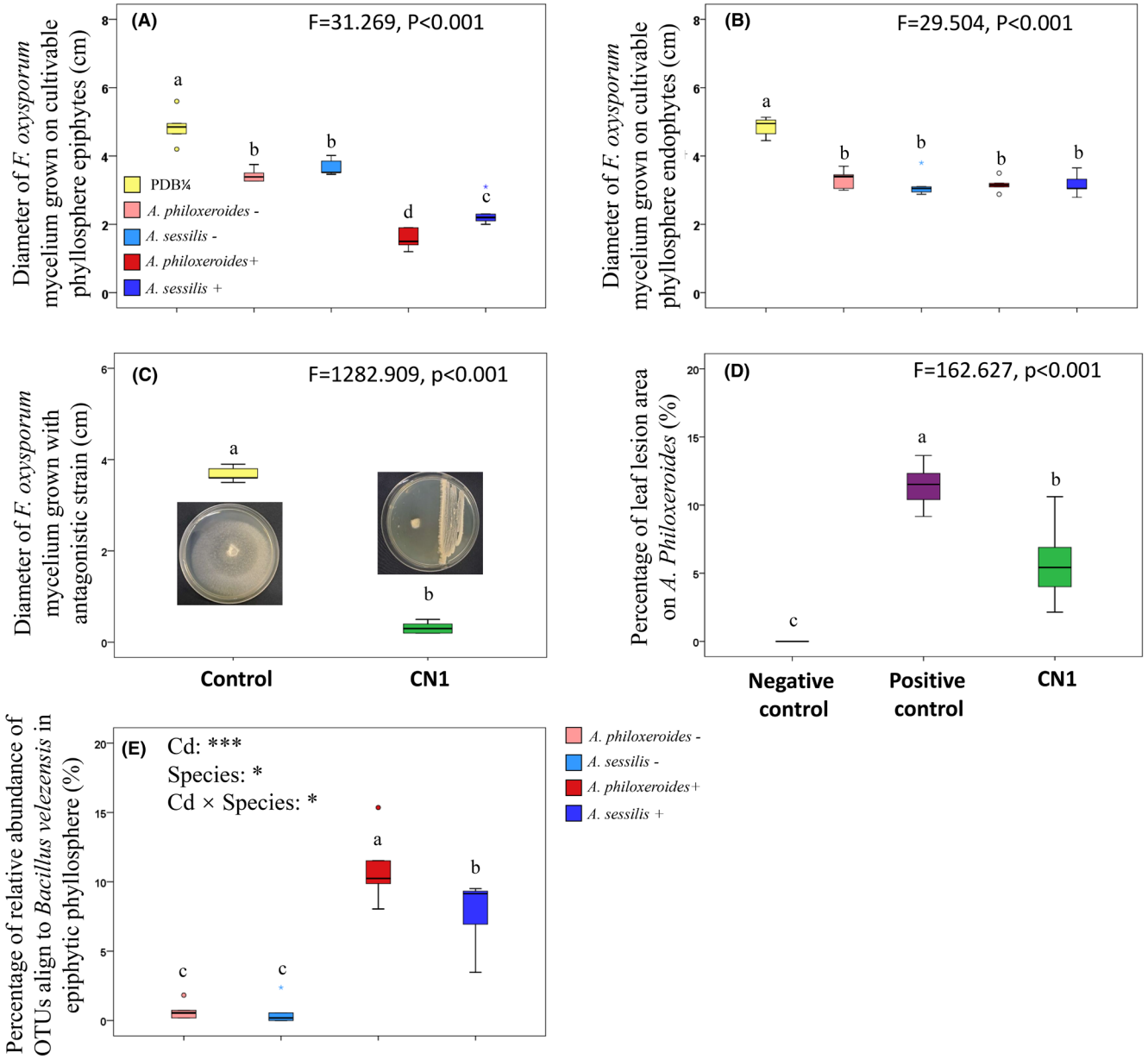


FIGURE 6 The inhibitory effect of cultivable phyllosphere microbes and the antagonistic strain on the growth and infection of the pathogenic fungus *Fusarium oxysporum*. (A) Inhibition effect of epiphytic microbes; (B) inhibition effect of endophytic microbes; (C) Inhibition effect of the antagonistic strain extracted from the phyllosphere on the growth of the pathogenic fungus in vitro. $N = 5$ replicated plates for each test. (D) Inhibition effect of the antagonistic strain on the infection of the pathogenic fungus on *A. philoxeroides* in vivo. $N = 15$ replicated plants for each treatment. The negative control was *A. philoxeroides* plants without *F. oxysporum* infection. The positive control was *A. philoxeroides* plants with *F. oxysporum* infection. CN1 was *A. philoxeroides* plants firstly inoculated with the antagonistic strain and then infected by *F. oxysporum*. Values are means ± SE. Different letters indicate significant differences among treatments at $p < 0.05$ according to one-way ANOVA. (E) Difference in percentage of relative abundance of OTUs aligned to the antagonistic strain *Bacillus velezensis* in epiphytic phyllosphere among treatments. Values are means ± SD. Different letters indicate significant differences among treatments at $p < 0.05$ according to GLMMs followed by Wald pairwise comparisons. n.s. = not significant; $^{*}p \leq 0.05$; $^{***}p \leq 0.001$. *A. philoxeroides*−: *A. philoxeroides* from control soil, *A. sessilis*−: *A. sessilis* from control soil, *A. philoxeroides*+: *A. philoxeroides* from Cd-containing soil, *A. sessilis*+: *A. sessilis* from Cd-containing soil.

from *A. philoxeroides* still demonstrated a 32.5% higher suppression of the growth of *F. oxysporum* than that from *A. sessilis*. In addition, the mycelium of *F. oxysporum* displayed a similar reduced diameter when grown with cultivable endophytic microorganisms from both plant species and treatments (Figure 6B).

3.9 | Screening and in vivo antagonistic test of the antagonistic strain

In total, two fungal strains and four bacterial strains were isolated from the epiphytic phyllosphere of *A. philoxeroides* plants and

cultured in PDA or NA medium for purification. However, only a particular bacterial strain CN1 demonstrated a notable inhibitory impact on the mycelium growth of the pathogenic fungus *F. oxysporum* in the in vitro antagonistic test (Figure 6C). The mycelium of *F. oxysporum* cultivated on the medium containing the antagonistic strain CN1 exhibited an 11.5-fold reduction in diameter compared with that grown on control plates. Furthermore, the antagonistic impact of CN1 on *F. oxysporum* infection was evaluated in vivo using individual *A. philoxeroides* plants. Additionally, *A. philoxeroides* plants inoculated with the antagonistic strain CN1 initially displayed a 49.4% decrease in the percentage of leaf lesion compared with the positive controls (Figure 6D).

3.10 | Identification of the antagonistic strain

The morphological traits of the antagonistic strain were illustrated in Figure S4. The CN1 strain displayed irregular, opaque, milky, and wrinkled features with a central depression in its colony. The cells were rod-shaped, ranging from 1.2 to 4.1 μm in length and 0.4 to 0.6 μm in width, existing either individually or in pairs, and producing nearly mesophytic spores. Additionally, a multi-gene phylogenetic analysis indicated a 100% similarity between strain CN1 and *Bacillus velezensis* (Figure S5). Consequently, based on its morphological characteristics and sequence alignment of gyrA, gyrB and rpoB genes, strain CN1 was tentatively identified as *Bacillus velezensis*.

In addition, we determined the relative abundance of OTUs aligned to the antagonistic strain *B. velezensis* in the epiphytic phyllosphere of either *A. philoxeroides* or *A. sessilis* from the microbiome analysis. The epiphytic phyllosphere of *A. philoxeroides* and *A. sessilis* harboured a similar abundance of *B. velezensis* OTUs in the control (Figure 6E). Soil Cd treatment largely promoted the relative abundance of *B. velezensis* OTUs in the epiphytes of both plant species, while *A. philoxeroides* leaves still harboured 1.4 times higher abundance of *B. velezensis* OTUs than *A. sessilis* leaves.

4 | DISCUSSION

In the present study, we demonstrated that the invasive plant species *A. philoxeroides* accumulated higher levels of Cd in its leaves and displayed reduced leaf lesion when artificially infected with a pathogenic fungus, in comparison to the native congener species *A. sessilis*, under conditions of soil Cd treatment. Subsequent investigation of the phyllosphere microbiome and toxicity assessments indicated that the increased pathogen resistance of *A. philoxeroides* following exposure to Cd could be attributed to both the direct Cd toxicity and indirect recruitment of antagonistic microbes in the epiphytic phyllosphere. To the best of our knowledge, our study represents one of the first attempts to elucidate the potential mechanisms underlying enhanced plant resistance to pathogens in the framework of EDH. The results offer significant insights for the fields of plant invasion biology and microbial ecology by establishing connections between phytopathogens, phyllosphere microbial assemblies, and plant performance in the condition of heavy metal contamination.

Although the soil Cd treatment significantly decreased the growth performance of both plant species, *A. philoxeroides* still performed better compared with *A. sessilis*. The combination of a greater Cd accumulation in the leaves of *A. philoxeroides* and relatively better growth in the soil Cd treatment suggested that *A. philoxeroides* had a better metal-tolerant ability than *A. sessilis*. Similarly, several invasive plant species have demonstrated a superior tolerant and accumulation ability to heavy metals when cultivated on heavy metal contaminated soil (Chen et al., 2017; Li et al., 2021; Wang, Chen, et al., 2021). Moreover, since heavy metals have been documented to exhibit toxic effects on insects and microorganisms, the EDH postulates that the accumulation of heavy metals within plant tissues may serve as a defensive strategy against herbivores and pathogens (Boyd, 2007; Boyd & Martens, 1998). Evidently, several invasive plant species demonstrated enhanced resistance to herbivores when cultivated in environments polluted with heavy metals (Lin, He, et al., 2023; Wang et al., 2022; Zhou et al., 2023). However, it remains uncertain how these advantages may further influence the interactions between invasive plants and their associated pathogens within the framework of the EDH. In this study, we showed that upon artificial infection with the leaf pathogenic fungus *F. oxysporum*, both plant species exhibited increased pathogen resistance under Cd treatment, with *A. philoxeroides* displaying greater resistance than *A. sessilis*. This finding is in line with the predictions of the EDH. While a number of studies have demonstrated such heavy metal-induced resistance to pathogens in different plant species (Hanson et al., 2003; Hörger et al., 2013), only a few focused on invasive plants. For instance, Dai et al. (2020) demonstrated that Cd hyperaccumulation in the invasive *Ageratina adenophora* reduced its susceptibility to leaf pathogen infections. However, the precise mechanism underlying the enhanced pathogen resistance of invasive plants in response to Cd exposure warrants further exploration.

Notably, our correlation analysis showed a negative relationship between leaf lesion area and leaf Cd concentration in both plant species, while leaf lesion area had no significant correlations with the three primary leaf defensive chemicals (Table S4). This suggests that the difference in pathogen resistance between *A. philoxeroides* and *A. sessilis* under Cd treatment may be due to direct Cd toxicity rather than shifts in the levels of leaf defensive compounds. To validate this hypothesis, we conducted the in vitro toxicity test and found a strong inhibitory effect of Cd on the growth of *F. oxysporum* in the PDA medium. Similarly, many studies also noted a strong biological toxicity of heavy metals such as nickel or zinc on phytopathogens under laboratory conditions (Hörger et al., 2013). Interestingly, the Cd treatment resulted in a substantial decrease of 70.4% and 47.67% in leaf lesion area in *A. philoxeroides* and *A. sessilis* plants, respectively, surpassing the inhibitory effect observed in the in vitro Cd toxicity test. This suggests that the Cd-enhanced resistance to pathogens in both plant species might be influenced by additional defence mechanisms beyond direct Cd toxicity such as microorganisms inhabiting the phyllosphere.

Microbiomes associated with plants, found in both the phyllosphere and rhizosphere, have been demonstrated to confer protection to the host plant against pathogen infection (Trivedi et al., 2020). While microbes associated with the rhizosphere can influence the interactions between plants and air-borne pathogens indirectly through the activation of plant immune responses, those in the phyllosphere can enhance plant resistance to leaf pathogens through direct pathogen inhibition and resource competition (Du et al., 2024; Vorholt, 2012). In this study, we employed a leaf pathogenic fungus to artificially infect the plants, thereby highlighting that the defensive compounds produced by the leaves, along with the microbial assemblies harboured in the phyllosphere, serve as the primary line of defence against leaf pathogen infection. Therefore, we only examined the changes in composition of the phyllosphere microbes, and the results indicated that microbial assemblies harboured in the epiphytes, rather than endophytes, were more impacted by soil Cd treatment. In addition, several fungal genera such as *Ramularia* and *Cladosporium*, as well as bacterial taxa like *Bacillus*, *Delftia* and *Glutamicibacter* in the epiphytic phyllosphere of *A. philoxeroides* plants, were found to have a positive correlation with leaf Cd concentration but a negative correlation with leaf lesion area. Several species within these microbial genera have been identified for their antagonistic or biological control properties. For example, numerous bacterial species within the *Bacillus* genus have been reported to exhibit strong antagonistic effects against plant pathogenic fungi, bacteria and viruses (Fira et al., 2018; Maral-Gül & Eltem, 2024), whereas few species from the *Glutamicibacter* genus are able to enhance plant growth and exert a strong antagonistic effect on plant pathogens (Fu et al., 2021). Hence, it is probable that the accumulation of Cd in plant leaves may promote the proliferation of these advantageous beneficial genera in the epiphytic phyllosphere, potentially enhancing plant resistance to pathogen infection. Although several studies also documented the heavy metal stress-induced shifts in the composition of plant-associated microbes (Coats & Rumpho, 2014; Jia et al., 2018; Lin, Lu, et al., 2023; Liu et al., 2021), the ecological consequences behind such changes have been rarely explored.

The subsequent in vitro toxicity test further confirmed this possibility and revealed that the cultivable microbes collected from the epiphytic phyllosphere of both plant species following Cd exposure exhibited an inhibitory effect on the growth of the leaf pathogen compared with those derived from the endophytic phyllosphere. Furthermore, the cultivable microbes extracted from the leaf surface of *A. philoxeroides* demonstrated greater resistance against *F. oxysporum* than those isolated from *A. sessilis* leaves. Altogether, it suggested that the epiphytic microbes existing in the phyllosphere of *A. philoxeroides* may significantly contribute to the enhancement of plant resistance against pathogen infection. Similarly, other studies have also demonstrated that phyllosphere-associated microorganisms can improve plant resistance to various diseases (Harish & Bhargavi, 2021; Ritpitakphong et al., 2016).

We further isolated and identified one bacterial strain, *B. velezensis*, from the epiphytic phyllosphere of *A. philoxeroides* after Cd exposure, exhibiting a robust antagonistic effect on the growth of the leaf pathogen *F. oxysporum* under in vitro and in vivo conditions. Earlier research has also underscored the potent antagonistic properties of this bacterial strain against *F. oxysporum* in either laboratory or greenhouse conditions (Abdelkhalek et al., 2020; Cao et al., 2018). Intriguingly, we showed that the relative abundance of OTUs aligned to *B. velezensis* in the sequenced microbiome of the epiphytic phyllosphere of both plant species was higher in soil Cd-exposed plants, with a notably higher prevalence observed in the phyllosphere of *A. philoxeroides*. Therefore, we postulated that *A. philoxeroides* plants might enhance their resistance to pathogen infection by recruiting antagonistic microbes in the epiphytic phyllosphere under soil Cd pollution.

A number of studies have documented the greater competitive advantage of several invasive plant species over native plant species when exposed to soil heavy metal contamination (Lin, He, et al., 2023; Wang, Chen, et al., 2021; Wang, Xiong, et al., 2021; Zhang et al., 2008). It has been proposed that these invasive plant species may modify their adaptive strategies regarding dry matter accumulation and resource allocation in response to heavy metal contamination (Cui et al., 2022). Furthermore, local consumers, including herbivores and pathogens, are recognised as significant biotic factors that can impede the spread and establishment of non-indigenous plant species (Allen et al., 2021; Harvey et al., 2010). Given that heavy metal pollution has been shown to improve the resistance of invasive plants to both herbivores and pathogens, it is posited that this phenomenon may diminish the impact of local consumers on invasive species, thereby facilitating their successful invasion. Consequently, it is imperative to re-evaluate the invasiveness of alien plant species that have established themselves in habitats contaminated by heavy metal pollution.

5 | CONCLUSIONS

In summary, our study reveals that exposure to soil Cd treatment strongly enhanced pathogen resistance of both plant species, while *A. philoxeroides* plants displayed less leaf lesion area compared with *A. sessilis* plants. We suggested that the Cd pollution increased the pathogen resistance of *A. philoxeroides* may be attributed to direct Cd toxicity and indirect recruitment of antagonistic microbes within the epiphytic phyllosphere. These findings hold significance for predicting the changes in disease incidence and the relationships with local plants of alien plant species that have been introduced to habitats polluted with heavy metals. Nevertheless, it is crucial to point out that this study only examined one pair of invasive plants and their native congener. Further research incorporating a larger species set representing different phylogenetic histories, along with field studies in habitats polluted with heavy metals, is necessary before drawing a comprehensive conclusion.

AUTHOR CONTRIBUTIONS

Tiantian Lin, Klaas Vrieling and Bo Li conceived the ideas and designed the methodology; Abdul Manan and Shuya Yang collected the data; Guoqing Zhu, Yan Wang and Zhicong Dai contributed to

material collection and method development; Abdul Manan and Tiantian Lin analysed the data; Tiantian Lin, Klaas Vrieling and Bo Li led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

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 article.

DATA AVAILABILITY STATEMENT

Data are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.h44j0zpv1 (Lin et al., 2024).

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SUPPORTING INFORMATION

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

Table S1. Soil characteristics for plant cultivation in the pot.

Table S2. PCR amplification primer sequences of the fungal ITS1 region and the bacterial 16S rRNA region.

Table S3. PCR amplification primer sequences of the antagonistic strain.

Table S4. PCR reaction system and reaction procedure.

Table S5. Accession numbers of the nucleotide sequences of the three antagonistic microbial strains.

Table S6. Spearman rank correlation coefficients and corresponding *p* values of leaf lesion area and leaf chemical traits of *A. philoxeroides* and *A. sessilis* grown on control and Cd containing soils.

Table S7. Taxonomically classification of OTUs of epiphytic and endophytic fungi and bacteria.

Table S8. Results of a one-way analysis of similarity (ANOSIM) based on Bray–Curtis distances for the compositions of phyllosphere microbial taxa at OTU level between soils with and without Cd addition for two plant species.

Table S9. Effects of leaf lesion area and leaf Cd concentration on the clustering of the phyllosphere microbial taxa based on the RDA analysis in Figure 4.

Figure S1. Diagram of experiment procedures.

Figure S2. Plant growth-related traits of the two species grown on control and Cd containing soils.

Figure S3. Venn diagrams showing the number of specific and shared OTUs on the phyllosphere.

Figure S4. Morphological characteristics of the antagonistic bacterial strain CN1.

Figure S5. Multigene phylogenetic trees of the antagonistic strain CN1 based on rpoB, gyrA and gyrB sequences of reference strains.

Appendix S1. DNA extraction and Illumina MiSeq sequencing of phyllosphere microorganisms.

Appendix S2. In vitro toxicity test of cultivable phyllosphere microorganisms on the growth of leaf pathogenic fungus.

Appendix S3. DNA extraction and identification of the antagonistic microbial strain.

Appendix S4. In vivo antagonistic effect of the antagonistic strain on the infection of *F. oxysporum*.

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