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# Soil cadmium pollution facilitated the invasion of alligator weed through enhanced herbivore resistance and competitive ability over a congeneric species

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## Abstract

A number of invasive plant species, such as *Alternanthera philoxeroides*, have been documented to be able to accumulate trace metal elements in their tissues. Since metal accumulation in plants can serve as a defence against herbivores, we hypothesized that metal pollution will increase herbivore resistance of metal-accumulating invasive plant species and such a benefit will grant them a competitive advantage over local co-occurring plants. In this study, we compared the differences in plant growth and herbivore feeding preference between *A. philoxeroides* and its native congener *Alternanthera sessilis* in single and mixed cultures with and without soil cadmium (Cd) pollution. The results showed that *A. philoxeroides* plants were more tolerant to Cd stress and accumulated more Cd in the leaves than *A. sessilis*. Cd exposure increased the resistance of *A. philoxeroides* against a specialist and a generalist herbivore compared with *A. sessilis*. Competition experiments indicated that Cd stress largely increased the competitive advantage of *A. philoxeroides* over *A. sessilis* with or without herbivore pressures. The differences in herbivore resistance between the two plant species under soil Cd stress are most likely due to the deterring effect of Cd accumulation and Cd-enhanced mechanical defences rather than changes in leaf specialized metabolites.

KEYWORDS

chemical defence, elemental defence hypothesis, interspecific competition, leaf metabolome, mechanical defence

## 1 | INTRODUCTION

Due to globalization, biological invasions have intensified and caused tremendous impacts on the structure and functioning of ecosystems in the introduced ranges (Hoffmeister et al., 2005; Vilà et al., 2011). The invasion process of nonindigenous plants consists of three phases, including introduction, colonization and naturalization (Radosevich et al., 2003). Each of the phases is influenced by abiotic or biotic factors, which may determine the success or failure of the invasion (Zhang et al., 2020). In general, an introduced range with severe disruption and low biodiversity facilitates the colonization of invasive plants. Metal pollution poses significant threats to

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Tiantian Lin and Wanci He contributed equally to this study.

ecosystem balance, resulting in biodiversity loss and habitat degradation (Pujari & Kapoor, 2021). Several trace metal elements, such as cadmium (Cd), lead (Pb) or mercury (Hg), are toxic to plants since they can inhibit plant physiological and biochemical processes when present in concentrations that exceed the plant's tolerance level. This can result in a loss in biomass and fitness, and in severe cases can even lead to plant death (Morkunas et al., 2018). However, several invasive plant species have been observed to exhibit greater tolerance to metal pollution than indigenous plant species (Wang et al., 2018, 2020). Such metal-tolerant ability might provide a competitive advantage for invasive plants to outcompete indigenous species with lower tolerance. This further facilitates their colonization and subsequent establishment in the new habitat (Li et al., 2021). A number of invasive plant species were found to be more dominant and exhibited greater competitive ability than surrounding indigenous plant species in metal-contaminated conditions (Gulezian et al., 2012; Piola & Johnston, 2008; Zhang et al., 2008).

On the other hand, the elemental defence hypothesis postulates that plants can use metal accumulation as a defence mechanism to protect themselves against herbivores and pathogens since some metals such as Cd or Pb are toxic to herbivorous insects and pathogenic microbes (Boyd, 2007; Boyd & Martens, 1998). Although previous studies have documented the resistance-enhancing effect of metals accumulated in herbaceous and woody plant species (Behmer et al., 2005; Hanson et al., 2003; Kazemi-Dinan et al., 2014; Lin et al., 2020), the applicability of the elemental defence hypothesis in the framework of biological invasion has been rarely studied. As several invasive plants were documented to be capable of accumulating a certain amount of metals in their tissues (Chen et al., 2015, 2018; Yang et al., 2008), we hypothesize that invasive plant species that accumulate metals will exhibit greater resistance to herbivory compared with coexisting native plants in metal-contaminated habitats. We further propose that this difference in herbivore resistance will grant the invasive plants a competitive advantage in interspecific competitions with coexisting native plants.

In general, plants often encounter two different types of herbivores in nature. One type is a generalist herbivore that feeds on many different plant species, and the other type is a specialist herbivore that feeds only on a restricted set of closely related plant species (Lankau, 2007). Thus, plants have developed two types of defence, mechanical and chemical, to defend themselves against these two types of herbivores (Feeny, 1976; Rhoades & Cates, 1976). Mechanical defences are based on structural or anatomical traits, such as leaf toughness, thickness or thorns, that are generally present in high amounts in plant tissues. These traits can act as a defence against both types of herbivores (Lin et al., 2015a). Chemical defences, such as flavonoids and alkaloids, which are found in small quantities and function as toxins, have been suggested to serve as a deterrent against generalist herbivores and certain specialist herbivores that lack a coevolutionary history (Ali & Agrawal, 2012). However, it has been observed that the majority of specialist herbivores that coexist with these plants have developed mechanisms to tolerate or counteract these toxins (Macel & Vrieling, 2003). Since there is evidence suggesting that specialist and generalist herbivores may respond differently to the toxicity of metals (Konopka et al., 2013), it is necessary to test both types of herbivores and take two types of plant defences into account when exploring the underlying mechanism of metal-mediated plant defences against herbivores.

Here, we conducted a study on the aggressive invasive weed *Alternanthera philoxeroides* (alligator weed) and its coexisting native congeneric species *Alternanthera sessilis*. We examined the effects of soil Cd contamination on the changes in herbivore resistance and competitive ability. We specifically asked the following three questions: (1) Is the invasive *A. philoxeroides* more tolerant to Cd stress and can it accumulate more Cd in its leaves on Cd-amended soil? (2) Does higher Cd tolerance and accumulation of *A. philoxeroides* result in enhanced resistance against herbivory by a specialist or a generalist herbivore? (3) Does the invasive *A. philoxeroides* outcompete the native *A. sessilis* when grown on Cd-amended soil and exposed to herbivory by specialist or generalist herbivores? To further understand the mechanism underlying the changes in herbivore resistance between *A. philoxeroides* and *A. sessilis* further, we measured host plant traits related to mechanical defence and explored the leaf metabolome differences between the two plant species. This study aims to disentangle the ecological relationships between plant invasion and metal pollution within the framework of the elemental defence hypothesis. The results will provide insights into how metal contamination can potentially facilitate plant invasion.

## 2 | MATERIALS AND METHODS

### 2.1 | Study materials

### 2.1.1 | Plant materials

*A. philoxeroides* is a perennial, clonal invasive plant species that originated from South America and has been introduced to several countries such as the United States, China and Australia (Stohlgren et al., 2013). This species has invaded both terrestrial and aquatic habitats and is considered a serious weed in the introduced ranges due to its fast growth and colonization ability (Dugdale & Champion, 2012). *A. philoxeroides* has been documented as a superior competitor over various indigenous plant species (Liang et al., 2020; Shen et al., 2022; Wu et al., 2017). It has been shown that *A. philoxeroides* exhibits better tolerance to metal pollution due to its ability to accumulate a certain amount of metals in its tissues (Wang et al., 2021a; 2021b).

*A. sessilis* is a perennial, clonal herb that is naturally distributed in Asian countries (Liang et al., 2020). This species is the only native congener of *A. philoxeroides* in China, and these two species commonly occur together in wetland habitats (Chen et al., 2013). Recently, *A. sessilis* has been observed in areas contaminated with metals (Kananke et al., 2016). Previous studies have shown that the morphology and phenotypic plasticity of these two species are highly similar (Chen et al., 2013; Wang et al., 2021a). Twenty individual plants of *A. philoxeroides* and *A. sessilis* were collected from three populations, respectively, from a metal-polluted site near an abandoned mining field in Meigu county, Southwestern China (28°04′ N, 102°11′ E), with soil Cd concentrations ranging between 40 and 80 mg $Cd^{2+}$ $kg^{-1}$ dry soil. After collection, sampled plants of each species were grown individually in a 1-L pot. The potting soil was mixed with 30% potting soil and 70% sand. The plants were kept in a greenhouse until ramets were sampled from each plant for later soil Cd treatment.

### 2.1.2 | Herbivore species

The specialist herbivore *Agasicles hygrophila* (Coleoptera: Chrysomelidae) is a monophagous flea beetle. Its larvae and adults feed exclusively on the leaves of the *Alternanthera* genus (Zhao et al., 2015). *A. hygrophila* originates from South America and was introduced to the United States, Australia and China in the 1980s as a biological control agent for *A. philoxeroides* (Sun et al., 2010). We have observed a strong feeding preference of *A. hygrophila* for *A. philoxeroides* compared with *A. sessilis* in the field. Adults of *A. hygrophila* were collected from a natural population of *A. philoxeroides* in a forest park in Wenjiang, Southwestern China (30°41′ N, 103°51′ E). They were then reared in a climate chamber (25°C, 70% RH, 16: 8 h L/D) with *A. philoxeroides* leaves.

*Spodoptera litura* (Lepidoptera: Noctuidae) is a native generalist herbivore that is widely distributed in Asian countries. It is a highly polyphagous pest species that feeds on a wide variety of plants, resulting in severe agricultural losses worldwide (Xia et al., 2020). Eggs were obtained from a laboratory culture from the College of Agriculture, Sichuan Agriculture University (30°42′ N, 103°51′ E). The hatched larvae were then reared with an artificial diet in the same climate chamber.

## 2.2 | Experimental design

### 2.2.1 | First experiment: Single culture experiment

*Plant cultivation*

Similar-height ramets with five nodes of *A. sessilis* and *A. philoxeroides* were selected and planted individually into 1-L plastic pots (top diameter: 13.5 cm; bottom diameter: 10 cm; height: 11.5 cm). Each pot was filled with 30% potting soil and 70% sand, resulting in a pH of 7.11, an organic matter content of 4.34%, a total nitrogen of 0.64 g $kg^{-1}$, a soil total phosphorus of 0.78 g $kg^{-1}$ and a soil Cd concentration of 0.03 mg $kg^{-1}$. Based on the average Cd concentration detected at the plant collection site, a Cd concentration of 60 mg $Cd^{2+}$ $kg^{-1}$ dry soil was selected for the Cd treatment. The soil of pots used for the Cd treatment was mixed with $CdCl_2\,2.5H_2O$ to obtain a $Cd^{2+}$ concentration of 60 mg $Cd^{2+}$ $kg^{-1}$ dry soil. In total, there were four treatments: *A. philoxeroides* control, *A. philoxeroides* + Cd, *A. sessilis* control and *A. sessilis* + Cd. Each treatment contained 25 replicates, and all plants were grown in a climate chamber (25°C, 70% RH, 16: 8 h L/D) for 8 weeks.

*Herbivore feeding bioassays*

*Choice feeding experiment.* A choice feeding bioassay was conducted to investigate the feeding preferences of the specialist and generalist herbivores between the two plant species. *A. philoxeroides* and *A. sessilis* plants from the above four treatments were paired as follows: *A. philoxeroides* control versus *A. sessilis* control; *A. philoxeroides* control versus *A. philoxeroides* + Cd; *A. sessilis* control versus *A. sessilis* + Cd and *A. philoxeroides* versus Cd + *A. sessilis* + Cd. The 1st to 8th fully expanded leaves of *A. sessilis* and *A. philoxeroides* from each treatment were sampled from five individual plants and punched into discs ($d = 8$ mm). At maximum, three leaf discs were taken from one leaf. A leaf disc from one treatment was placed on moistened filter paper in a Petri dish (diameter = 90 mm) to be compared with a leaf disc from another treatment. One 3rd-instar larva of *S. litura* or one adult beetle of *A. hygrophila* was placed in the middle between the leaf discs in each Petri dish. For each herbivore species, 30 tests were carried out for each choice and totalling 240 tests (2 herbivores × 4 choices × 30 replicates). After 24 h, the remaining area of each leaf disc was scanned and the consumed leaf area was calculated.

*Nonchoice feeding experiment.* A nonchoice feeding bioassay was conducted to investigate the leaf consumption of the specialist and generalist herbivores on *A. philoxeroides* and *A. sessilis*. One 3rd-instar larva of *S. litura* or one adult beetle of *A. hygrophila* was placed into a nylon net (5 × 5 cm) and attached to the 1st or to the 2nd fully expanded leaf of a plant. Therefore, each plant received one generalist and one specialist herbivore, resulting in a total of 20 plants tested ($N = 20$ replicates per treatment per herbivore species). The nets were closed with rubber bands to prevent herbivores from escaping. After 24 h of feeding, all herbivores were retrieved and the remaining area of each leaf was scanned for calculating the consumed leaf area.

*Measurements of host plant traits*

All plants from the herbivore nonchoice feeding experiment were harvested to measure the dry mass of leaf, stem and root, number of ramets and total leaf area. Leaf chlorophyll content of each plant was measured from the 3rd to the 5th fully expanded leaves using a portable leaf chlorophyll meter (SPAD-502; Minolta). The average measurements of the three leaves were taken for statistical analysis. The same leaves were sampled for leaf Cd and nitrogen measurements. Leaf Cd concentration was measured using an inductively coupled plasma atomic emission spectrometer (ICP-AES; PerkinElmer) after acid digestion with 3 mL of $HNO_3$ (1:3, v/v) according to Lin et al. (2020). Leaf nitrogen concentration was determined using the Kjeldahl method (Čeh-Brežnik & Tajnšek, 2018).

Leaf mass–area ratio, leaf thickness, leaf tensile strength and leaf toughness were measured as mechanical defence-related traits of *A. philoxeroides* and *A. sessilis*. The leaf mass–area ratio was calculated as the ratio of total leaf dry mass and total leaf area of each plant. Leaf thickness was measured as described by Lin et al. (2015a). In short, sections were cut from the middle of a leaf using a hand microtome and images were acquired with a microscope (U-LHLEDC; Olympus). Leaf thickness was measured using ImageJ 1.42q, and five leaves were measured for each plant from the nonchoice feeding experiment. The average thickness of five leaves of a plant was used for statistical analysis. Leaf tensile strength and punch toughness were measured using a tensile strength testing machine (ZT969S; Xianglong), following the method described by Graça and Zimmer (2020). Leaf tensile strength refers to the amount of force required to tear a leaf sample apart. The testing machine consists of two pegs that secure a leaf disc. One peg remains stationary and the other one exerts an upward force until it tears the leaf apart. Leaf punch toughness was estimated by measuring the force required to penetrate a leaf sample using a steel needle. The needle was installed into the same testing machine used for measuring leaf tensile strength. When the leaf was fractured or pierced, a sharp increase in force was observed and the maximum force (N) was recorded. Three different leaves from each plant were measured for either leaf tensile strength or punch toughness. The average of the three measurements was taken for statistical analysis ($N = 20$ plants for each treatment).

#### *Metabolite extraction and profiling*

The remaining fully expanded leaves of *A. sessilis* and *A. philoxeroides* used for the herbivore choice feeding experiment were sampled and freeze-dried before being ground into a powder. The extraction and profiling of leaf metabolites were conducted according to Su et al. (2021). In short, 50 mg of fresh leaves was sampled from each plant and ground into a fine powder with liquid nitrogen. Before thawing, the leaf powder was extracted with 0.4 mL methanol/water (4:1, v/v) for 30 min using ultrasonication. The extracts were incubated at −20°C for 30 min coupled with an internal standard of 2-Chloro-ʟ-phenylalanine (0.02 mg $mL^{-1}$) before being centrifuged at 13,000 rpm at 4°C for 15 min. After that, 200 µL of the supernatant was filtered through a 0.2 µm filter and measured using UHPLC-Q Exactive HF-X Mass Spectrometer (ThermoFisher) equipped with an ACQUITY UPLC HSST3 (100 × 2.1 mm i.d., 1.8 µm; Waters) column. Mobile phase A consisted of 95% water + 5% acetonitrile (containing 0.1% formic acid) and mobile phase B consisted of 47.5% acetonitrile + 47.5% isopropyl alcohol + 5% water (containing 0.1% formic acid). The solvent gradient was changed as described by Hu et al. (2023). The sample injection volume was 2 µL and the flow rate was set to 0.4 mL $min^{-1}$. The column temperature was maintained at 40°C. The MS parameters for detection were as follows: the scan range was 70–1050 m/z; the sheath gas flow rate was 50 arb; the heater temperature was 425°C; the capillary temperature was 325°C; the ESI source voltages were +3.5 and −3.5 kV, respectively; and the normalized collision energy was set at 20, 40 and 60 eV, respectively. The optimal conditions were set the same as described by Hu et al. (2023).

### 2.2.2 | Second experiment: Competition experiment

The competition experiment consisted of 10 treatments with two plants in a pot according to Lin et al. (2015b), with a few modifications (Supporting Information: Figure S1). The 10 treatments were two pairs of monocultures and three pairs of mixed cultures with and without herbivores, which were subjected to control or Cd treatment, respectively. Similar-sized ramets with five nodes of *A. philoxeroides* and *A. sessilis* were paired and planted in the same plastic pots (1 L) as the single-culture experiment filled with the same proportion of potting soil and sand. Soil from pots used for Cd treatment was mixed with $CdCl_2$ $2.5H_2O$ to reach a $Cd^{2+}$ concentration of 60 mg $kg^{-1}$ dry soil. In total, there were 10 replicates per treatment resulting in 100 pots ([two monocultures and three mixed cultures] × two soil treatments). A priori, all individual plants in monoculture or mixed culture were labelled for later statistical analysis. The competition experiment was grown for 8 weeks in a climate chamber (25°C, 70% RH, 16: 8 h L/D).

After 8 weeks, all the pots of the 10 treatments from Supporting Information: Figure S1 were individually fit through a hole into a plastic cage (top diameter: 12.5 cm; bottom diameter: 14.5 cm; height: 10 cm) with a transparent nylon net to prevent herbivores from escaping. For the specialist and generalist herbivore treatments, five *A. hygrophila* adults and five 2nd-instar larvae of *S. litura* were released in each cage, respectively. We found no significant difference in the total fresh weight of either *A. hygrophila* or *S. litura* at the start of the herbivore treatment among treatments (one-way analysis of variance [ANOVA], *A. hygrophila*: $F_{1,8} = 1.696$, $p = 0.151$; *S. litura*: $F_{1,8} = 0.937$, $p = 0.464$). The *A. philoxeroides* and *A. sessilis* monoculture treatments and the no herbivore competition control without herbivores were all placed in the same type of cages as the herbivory treatments to maintain a consistent environment. The herbivore treatment lasted for 10 days in the climate chamber (25°C, 70% RH, 16: 8 h L/D). In the end, herbivores were removed from each cage and all the plants were harvested. The leaf, stem and root of each plant were weighed separately after being oven-dried at 60°C for 5 days.

## 2.3 | Statistical analysis

To compare the differences in host plant traits related to growth and mechanical defence, leaf water and chlorophyll content, leaf nitrogen and Cd concentration as well as herbivore feeding in the nonchoice feeding bioassay between *A. philoxeroides* and *A. sessilis* plants in single culture subject to Cd or control treatments, generalized linear mixed models (GLMMs) were performed with species and Cd treatment as fixed factors and population as a random factor, followed by a Wald pair-wise comparison. Further, two-tailed Spearman rank correlation tests were used for testing the correlations between the consumed leaf area of each herbivore species and the leaf parameters related to growth and mechanical defence of the same plant used in the herbivore nonchoice feeding bioassays. In addition, a Wilcoxon test was used to compare the herbivore preferences between paired plants in the choice bioassay. Spearman correlation two-tailed test was employed to examine the correlations between consumed leaf area by each herbivore in the nonchoice bioassay and host plant traits related to leaf chemicals and leaf structural defence.

As for plant growth in the competition experiment, we first compared the mixed-culture with monoculture controls under Cd-free and Cd treatment (Supporting Information: Figure S1a–f). GLMMs were used with plant population and pot as random factors to test the effects of culture type, plant species and Cd treatment on leaf, stem, root and total dry mass of individual plants without herbivore feeding. Second, we compared the mixed cultures subjected to control or Cd treatment under the conditions of no herbivore feeding, *A. hygrophila* feeding and *S. litura* feeding (Supporting Information: Figure S1e–j). GLMMs were used with plant population and pot as random factors to test the effect of species, Cd treatment and herbivore treatment on leaf, stem, root and total dry mass of individual plants. Lastly, the difference in total dry mass between *A. philoxeroides* and *A. sessilis* plants from the same treatment was analysed by a Wilcoxon test. We further calculated the replacement rate of *A. philoxeroides* over *A. sessilis* as total dry mass of *A. philoxeroides* divided by the total dry mass of *A. sessilis* in the mixed culture. The difference in the replacement rate of *A. philoxeroides* among different herbivore pressures in the mixed culture was tested using GLMMs with Cd treatment and herbivore type as fixed factors and population as a random factor, followed by a Wald pair-wise comparison. All the above analyses were carried out using SPSS 21.0 (IBM).

The metabolome data matrix from the UPLC-MS was analysed using the free online platform of Majorbio (www.majorbio.com). After the mass spectrometry detection was completed, the raw data of LC/MS was preprocessed as described by Li et al. (2023). Variables with relative standard deviation (RSD) > 30% of QC samples were removed, and log10 logarithmization was performed to obtain the final data matrix for further analysis. After normalization by sum and log transformation, a principal component analysis (PCA) with unit variance scaling was performed to classify all samples based on the metabolomic data. Furthermore, a partial least squares discriminant analysis (PLS-DA) with pareto (Par) scaling was performed for the metabolomic data to classify the observations into groups from giving the largest predicted indicator variable. The relative abundance of the top 30 variable importance in the projection (VIP) based on the PLS-DA was listed in the heatmaps.

# 3 | RESULTS

## 3.1 | Host plant traits of *A. philoxeroides* and *A. sessilis* grown on control and Cd soils

### 3.1.1 | Growth-related parameters

Both plant species and Cd treatment but not their interaction had significant effects on the dry mass of leaf, stem and root and the total dry mass of *A. philoxeroides* and *A. sessilis* plants (Figure 1a–d). Although *A. philoxeroides* showed a relatively higher leaf, stem, root and total dry mass than *A. sessilis* without Cd stress, such differences were not significant. Cd treatment significantly reduced the growth performance of both species, while *A. philoxeroides* still showed 67.2% ($p = 0.001$), 115.9% ($p = 0.001$), 82.0% ($p = 0.026$) and 88.0% ($p < 0.001$) higher leaf, stem, root and total dry mass than *A. sessilis*, respectively. Cd stress significantly reduced the number of ramets and nodes of *A. sessilis* by 54.7% ($p = 0.001$) and 38.8% ($p < 0.001$), respectively, compared with control plants, whereas those of *A. philoxeroides* were not strongly affected by Cd stress (Figure 1e,f). Interestingly, Cd exposure largely decreased the number of leaves and total leaf area of *A. philoxeroides*, which resulted in 19.2% ($p = 0.042$) and 38.5% ($p = 0.003$) higher number of leaves and total leaf area, respectively, in *A. sessilis* compared with *A. philoxeroides* (Figure 1g,h).

Leaf Cd concentration was significantly affected by plant species and soil Cd treatment as well as their interaction (Figure 2a). More specifically, leaves of *A. philoxeroides* and *A. sessilis* from the control treatment only contained trace amounts of Cd, whereas Cd exposure drastically increased the leaf Cd concentration in both plant species, resulting in 1.8 times more Cd accumulation in *A. philoxeroides* leaves than in *A. sessilis* leaves ($p < 0.001$). Soil Cd treatment significantly increased the leaf water content of *A. sessilis* ($p < 0.001$), while it had no significant effect on that of *A. philoxeroides* ($p = 0.916$) (Figure 2b). In addition, plant species and soil Cd treatment alone but not their interaction had significant effects on leaf chlorophyll and nitrogen content (Figure 2c,d). *A. philoxeroides* contained significantly higher levels of chlorophyll ($p < 0.001$) and nitrogen ($p < 0.001$) in the leaves than *A. sessilis* from control treatment, whereas Cd stress largely decreased both of them in a similar way in both plant species.

### 3.1.2 | Mechanical defence

With regard to host plant traits related to mechanical defence, plant species alone and species × Cd significantly affected leaf thickness, leaf tensile strength and leaf punch toughness, whereas the leaf mass–area ratio was strongly influenced by all three factors (Figure 3a–d). It was observed that the leaf mass–area ratio, leaf thickness, leaf tensile strength and leaf punch toughness of *A. philoxeroides* plants were similar to those of *A. sessilis* plants from the control treatment. However, Cd treatment significantly increased the mechanical defensive levels of *A. philoxeroides* but reduced that of *A. sessilis*, resulting in an average 2.3, 1.7, 2.4 and 3.0 times higher leaf mass–area ratio ($p < 0.001$), leaf thickness ($p < 0.001$), leaf tensile strength ($p < 0.001$) and leaf punch toughness ($p < 0.001$) for *A. philoxeroides*, respectively, than *A. sessilis* after Cd exposure.

### 3.1.3 | Leaf metabolome

The nontargeted UPLC-QTOF/MSA in total annotated 1442 compounds. Hierarchical cluster analysis (HCA) indicated two main clusters separated by the plant species (Supporting Information:

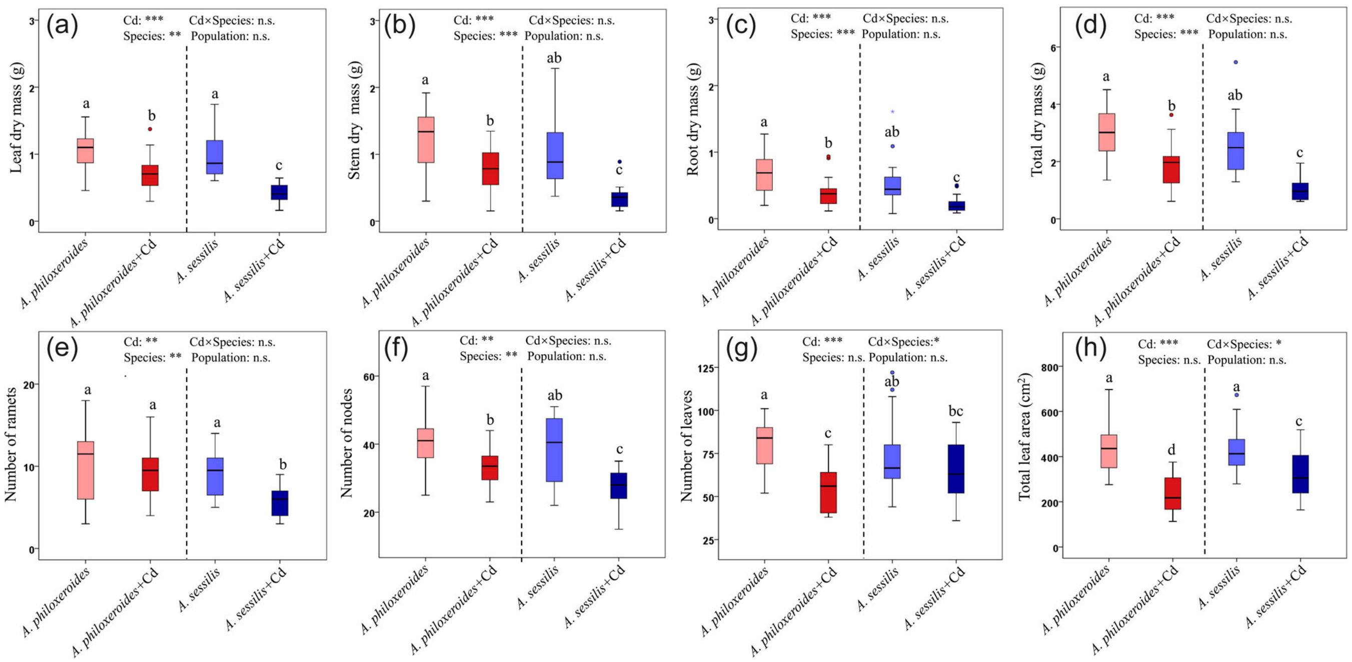

**FIGURE 1** Host plant traits of *Alternanthera philoxeroides* and *Alternanthera sessilis* grown on control and Cd-containing soils. (a) Leaf dry mass, (b) stem dry mass, (c) root dry mass, (d) total dry mass, (e) number of ramets, (f) number of nodes, (g) number of leaves and, (h) total leaf area. $N = 20$ for each treatment. Values are means ± SD. Different letters indicate significant differences among treatments at $p < 0.05$ according to generalized linear mixed models followed by Wald pair-wise comparisons. The significance values of the generalized linear models are shown as follows: n.s., not significant; $^{*}p \le 0.05$; $^{**}p \le 0.01$; $^{***}p \le 0.001$.

Figure S2a). The heatmap further showed that the metabolite composition of the leaves differed between *A. philoxeroides* and *A. sessilis*. The PCA plot only showed a clear separation in the leaf metabolites between the two plant species, whereas that of the same plant species from the control and Cd treatments were overlapping (Supporting Information: Figure S2b). Further PLS-DA plot showed that the metabolome of the two plant species was different but also that the metabolome of plants grown in control soil was distinct from the metabolome of plants grown in Cd-containing soils. The first two components explained up to 72.5% of the variance, while the first and the second components represented 65.1% and 7.4%, respectively, of the total variance (Supporting Information: Figure S2c).

We used the PLS-DA to compare the metabolome differences between paired plants from the herbivore choice feeding experiment. The metabolites in the leaves of *A. philoxeroides* and *A. sessilis* without Cd treatment were clearly separated by the first component, which explained 64.4% of the variance, whereas the second component explained 11.3% of the variance (Figure 4a). Of the metabolites with the 30 highest VIP values, three metabolites belonged to the class of flavonoids potentially acting as defensive metabolites, including apigenin 6-C-glucosyl-O-arabinoside, hyacinthin and multiflorin A (Figure 4b). Interestingly, the relative amounts of all these three compounds were drastically higher in the leaves of *A. philoxeroides* than those of *A. sessilis*. In addition, the PLS-DA plot showed that the metabolites in the leaves of *A. sessilis* that were subjected to control and Cd treatment were clearly separated by the first component that explained 26.2% of the variance, whereas the second component explained 19.8% of the variance (Figure 4c). The VIP plot further indicated six metabolites that might play a defensive role, including apigenin 6-C-glucosyl-O-arabinoside, kaempferol 3-arabinofuranoside 7-rhamnofuranoside, violanthin, diospyrin and patuletin 3-gentiobioside that all belong to the class of flavonoids and biochanin A 7-(6-malonylglucoside) that belongs to the class of isoflavonoids. The relative abundance of the six compounds was significantly decreased by Cd treatment in *A. sessilis* leaves (Figure 4d). Similarly, metabolites in the leaves of *A. philoxeroides* from Cd treatment were clearly separated from control plants by the first component that explained 39.8% of the variance, whereas the second component explained 16.6% of the variance (Figure 4e). However, Cd exposure largely induced a higher amount of 2-cinnamoyl-1,6-digalloyl-β-D-glucopyranose, a defensive compound belonging to the class of tannins, in *A. philoxeroides* leaves compared with control treatment (Figure 4f). Lastly, the metabolites in *A. philoxeroides* and *A. sessilis* leaves subjected to Cd treatment were clearly separated by the first two components, while the first and the second components explained 64.4% and 11.0%, respectively, of the total variance (Figure 4g). More specifically, the levels of six defensive metabolites that belong to flavonoids class in *A. philoxeroides* leaves were significantly higher than *A. sessilis* leaves, namely epiafzelechin-(4alpha->8)-pelargonidin 3′-glucoside, 6”‘-feruloylspinosin, hyacinthin, apigenin 6-C-glucosyl-O-arabinoside, kaempferol 3-arabinofuranoside 7-rhamnofuranoside and multiflorin A (Figure 4h).

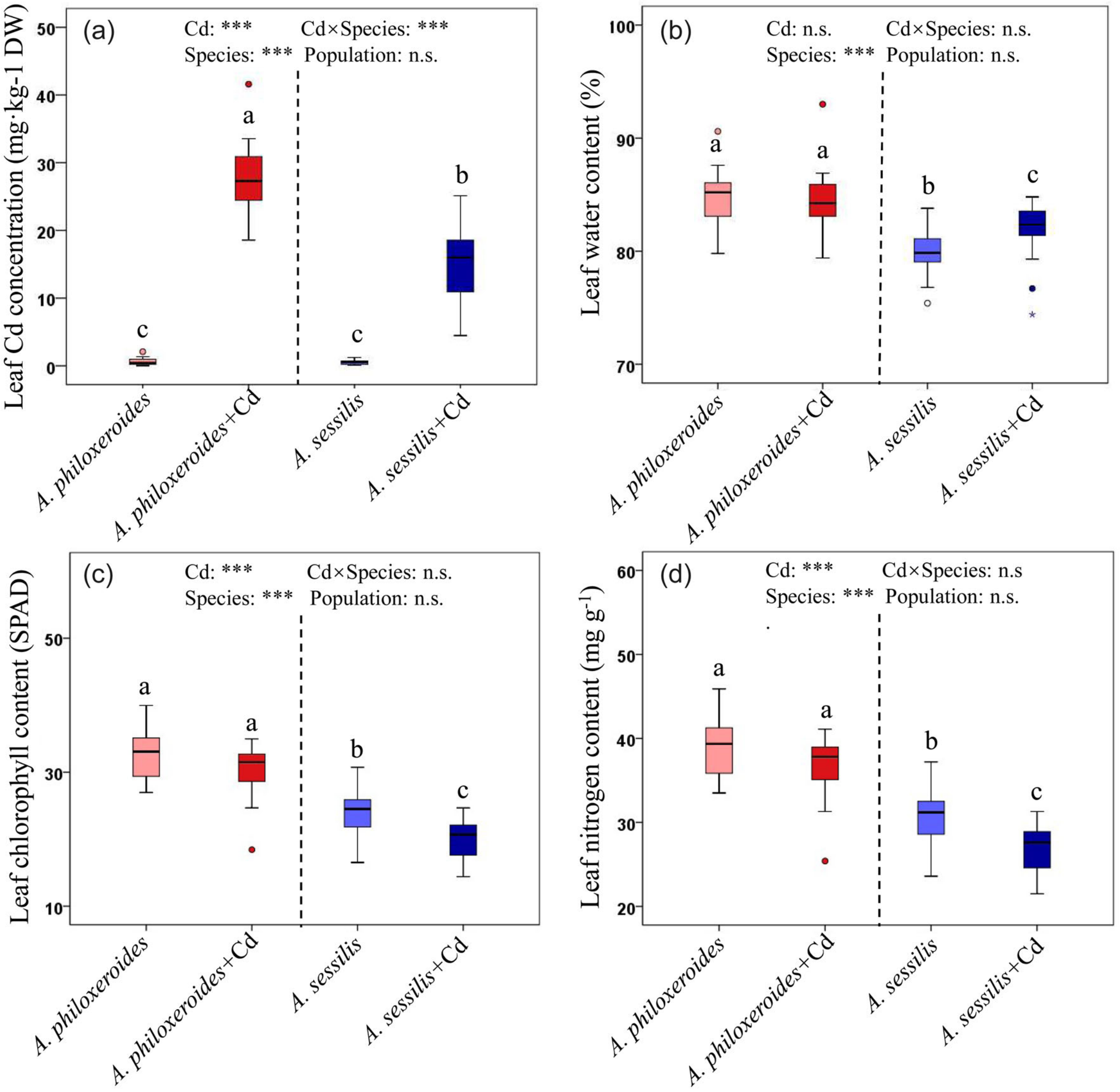

**FIGURE 2** Leaf chemical traits of *Alternanthera philoxeroides* and *Alternanthera sessilis* grown on control and Cd-containing soils. (a) Leaf Cd concentration, (b) leaf water content, (c) leaf chlorophyll content and (d) leaf nitrogen content ($N = 20$ for each treatment). Values are means ± SD. Different letters indicate significant differences among treatments at $p < 0.05$ according to generalized linear mixed models followed by Wald pair-wise comparisons. The significance values of the generalized linear models are shown as follows: n.s., not significant; $^*p \leq 0.05$; $^{***}p \leq 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

## 3.2 | Herbivore preference of plants grown on control and Cd-containing soils

In the herbivore choice feeding experiment, the specialist and generalist herbivores exhibited opposite feeding preferences for *A. philoxeroides* and *A. sessilis* in the control treatment (Figure 5a,b). More specifically, the specialist herbivore, *A. hygrophila*, consumed on average 22.0% more area of the leaf discs of *A. philoxeroides* than *A. sessilis* (Wilcoxon test: $t = 2.696$, $p = 0.007$). In contrast, leaf discs of *A. sessilis* received on average 67.5% more damage than the leaf discs from *A. philoxeroides* after being fed by the generalist herbivore, *S. litura* (Wilcoxon test: $t = 1.976$, $p = 0.048$). Moreover, the leaf discs of both plant species under Cd treatment received less herbivory by both the specialist herbivore (Wilcoxon test: *A. philoxeroides*: $t = 3.035$, $p = 0.002$; *A. sessilis*: $t = 2.892$, $p = 0.004$) and the generalist herbivore (Wilcoxon test: *A. philoxeroides*: $t = 2.819$, $p = 0.005$; *A. sessilis*: $t = 3.552$, $p < 0.001$) than the control discs. When given a choice between leaves of *A. philoxeroides* and *A. sessilis* exposed to Cd treatment, both herbivore species strongly preferred *A. sessilis*. More specifically, the consumed leaf areas of *A. sessilis* were 1.9 and 2.6 times larger than that of *A. philoxeroides* after being fed by *A. hygrophila* (Wilcoxon test: $t = 3.868$, $p < 0.001$) and *S. litura* (Wilcoxon test: $t = 3.892$, $p < 0.001$), respectively.

In the nonchoice feeding bioassay, a significant interaction effect of Cd × species was present for the specialist herbivore *A. hygrophila* (Figure 5c). More specifically, *A. philoxeroides* plants grown on control soil were 44.5% more eaten by *A. hygrophila* than *A. sessilis* plants ($p < 0.001$), whereas *A. philoxeroides* plants

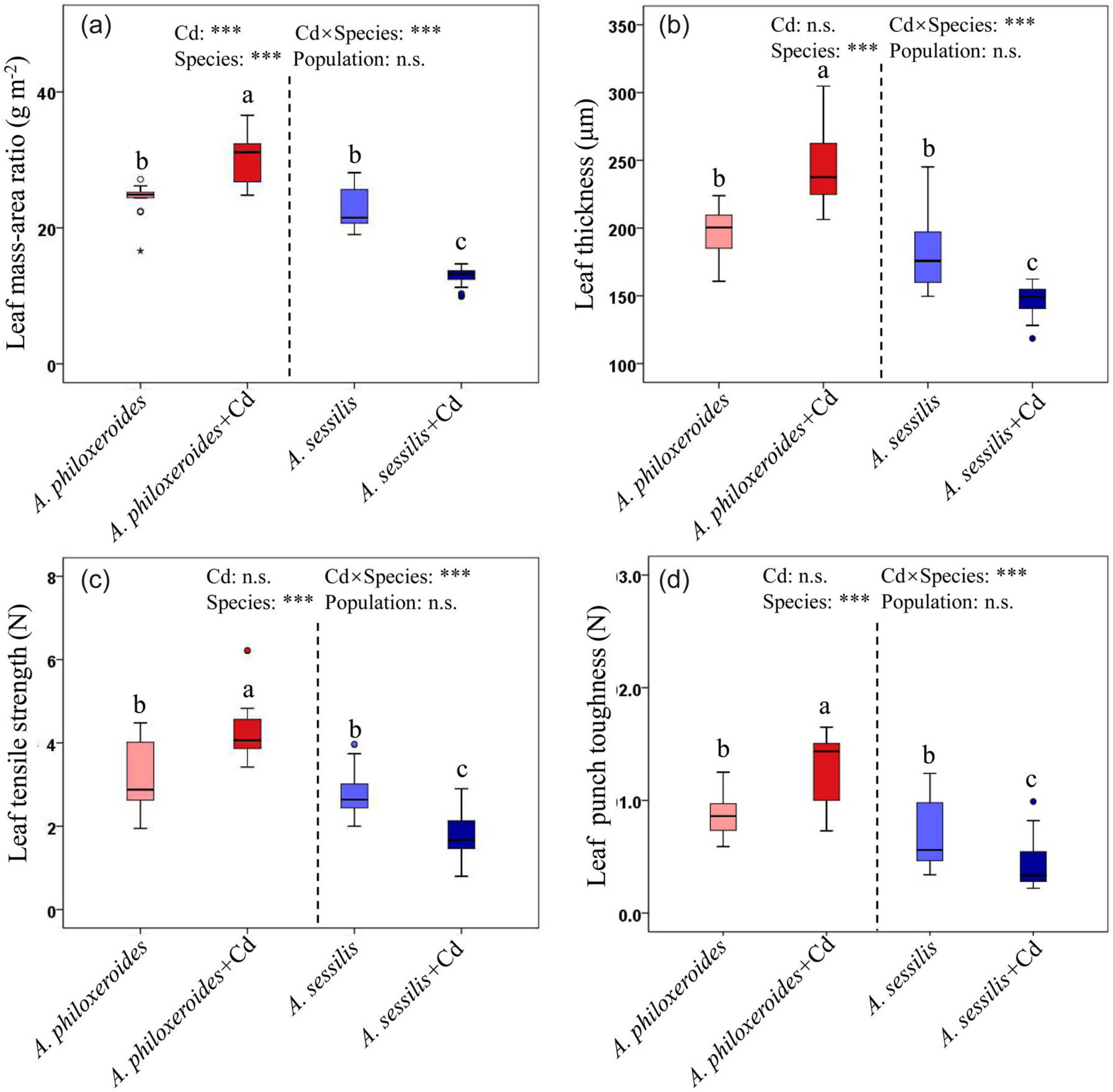

**FIGURE 3** Host plant traits related to structural defence of *Alternanthera philoxeroides* and *Alternanthera sessilis* grown on control and Cd-containing soils. (a) Leaf mass–area ratio, (b) leaf thickness, (c) leaf tensile strength and (d) leaf punch toughness ($N = 20$ plants for each treatment). Values are means ± SD. Different letters indicate significant differences among treatments at $p < 0.05$ according to generalized linear mixed models followed by Wald pair-wise comparisons. The significance values of the generalized linear models are shown as follows: n.s., not significant; ***$p \le 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

raised on Cd soil were less preferred by *A. hygrophila* than *A. sessilis* plants from Cd soil ($p = 0.018$). The feeding of the generalist herbivore *S. litura* was affected by both plant species and Cd in the soil but a significant interaction effect was not present (Figure 5d). On average, *S. litura* fed 24.3% more leaf area of *A. sessilis* than *A. philoxeroides* in the control treatment ($p < 0.001$), while *S. litura* consumed 202% more leaf area of *A. sessilis* than *A. philoxeroides* after Cd exposure ($p < 0.001$). Interestingly, the consumed leaf areas of *A. philoxeroides* and *A. sessilis* by either *A. hygrophila* or *S. litura* feeding decreased with increasing leaf Cd concentration, leaf mass–area ratio, leaf thickness, leaf tensile strength and leaf toughness (Supporting Information: Tables S1 and S2).

### 3.3 | Competitive ability of *A. philoxeroides* and *A. sessilis* grown on control and Cd-containing soils with and without herbivores

To explore whether the difference in herbivore resistance between *A. philoxeroides* and *A. sessilis* will affect their competitive ability, we conducted a competition experiment. First, we compared the mixed-culture with monoculture controls under Cd-free or Cd treatment to interpret the effects of culture type, plant species and Cd treatment on the growth of *A. philoxeroides* and *A. sessilis* without herbivore pressure (Supporting Information: Figure S1a–f). Leaf, stem, root and total dry mass of individual plants were significantly affected by plant species, soil type and whether they were grown in monoculture or

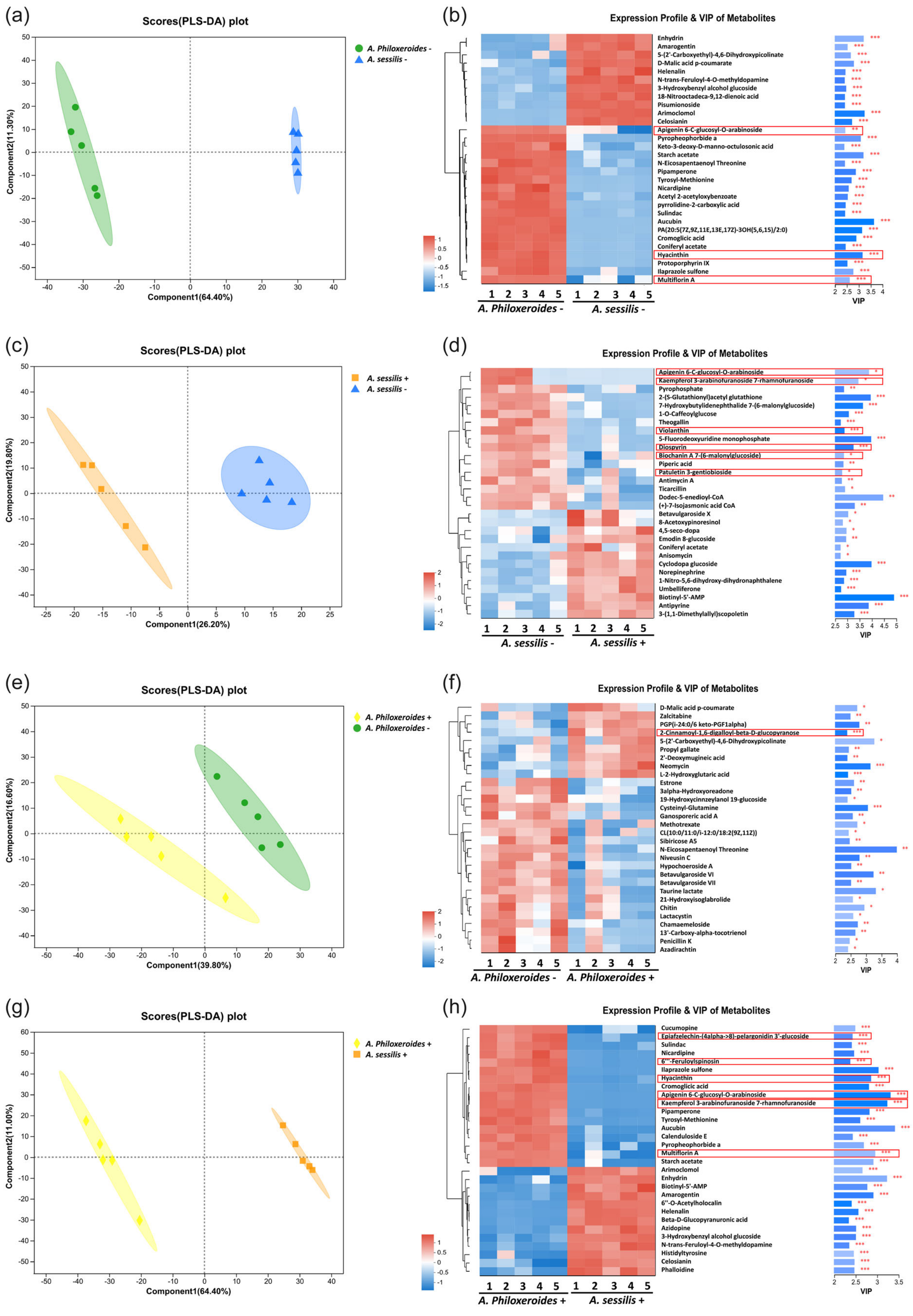

**FIGURE 4** (See caption on next page).

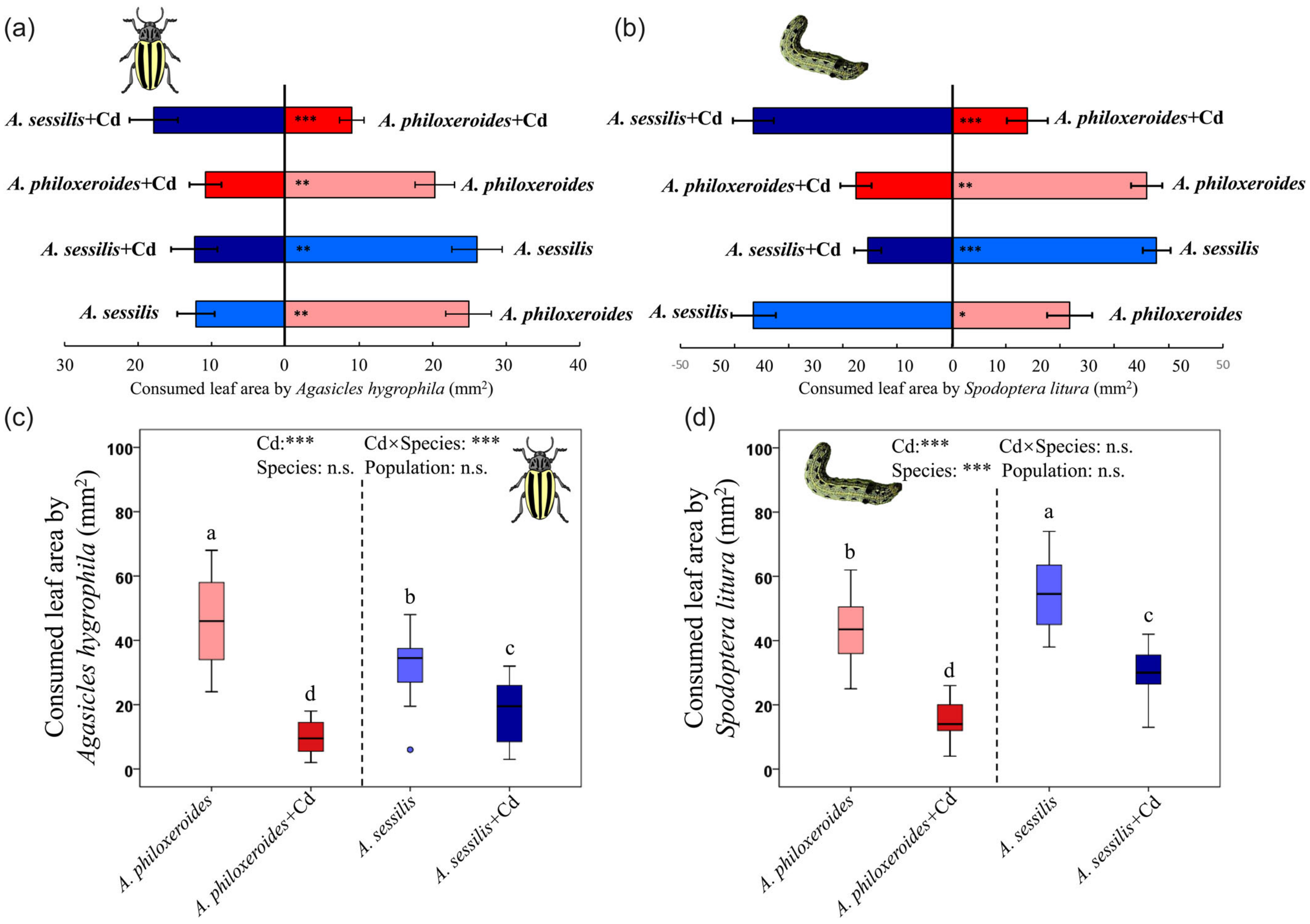

**FIGURE 5** Herbivore feeding preference in paired-choice feeding bioassays on leaf discs and nonchoice feeding bioassays on whole plants of *Alternanthera philoxeroides* and *Alternanthera sessilis* grown on control and Cd-containing soils. Consumed leaf area of *Agasicles hygrophila* (a) and *Spodoptera litura* (b) larvae in paired-choice bioassays. *N* = 30 replicates for each pair of treatments per herbivore species. Values are means ± SE. Asterisks above the bars indicate significant differences between the two choices according to a Wilcoxon test: $^{*}0.01 < p \le 0.05$; $^{**}0.001 < p \le 0.01$; $^{***}p \le 0.001$. *A. philoxeroides*-, *A. philoxeroides* grown on control soil; *A. philoxeroides*+, *A. philoxeroides* grown on Cd-containing soil; *A. sessilis*-, *A. sessilis* grown on control soil; *A. sessilis*+, *A. sessilis* grown on Cd-containing soil. Consumed leaf area of *A. hygrophila* (c) and *S. litura* larvae (d) in the nonchoice feeding bioassay. *N* = 20 replicates for each treatment for both herbivore species. Values are means ± SD. Different letters indicate significant differences among treatments at $p < 0.05$ according to generalized linear mixed models followed by Wald pair-wise comparisons. The significance values of the generalized linear models are shown as follows: n.s., not significant; $^{*}0.01 < p \le 0.05$; $^{***}p \le 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

mixed culture. Also, the interaction between culture × plant species was significant for leaf, root and total dry mass (Table 1, Figure 6a). Second, we compared the dry mass of plants from mixed cultures that were grown on control or Cd-containing soils, both with and without the herbivores *A. hygrophila* and *S. litura*, to test the effects of plant species, Cd treatment and herbivore species on the growth of *A. philoxeroides* and *A. sessilis* (Supporting Information: Figure S1e–j). The results indicated that the leaf, stem, root and total dry mass were

**FIGURE 4** Leaf metabolome of *Alternanthera philoxeroides* and *Alternanthera sessilis* grown on control and Cd-containing soils. (a, c, e, g) Partial least squares discriminant analysis (PLS-DA) plot of all detected metabolites in plants from different treatments subjected to herbivore bioassays (*N* = 5 plants for each treatment). (b, d, f, h) Heatmaps of top 30 variable importance in the projection (VIP) based on the PLS-DA. Metabolites in the red boxes are identified as defensive compounds involved in plant resistance to herbivores. Colour coding represents the intensity of induction (red) or suppression (blue) of metabolites in the leaves of each tested plant. Asterisk represented significant difference between plant pairs according to one-way ANOVA. $^{*}0.01 < p \le 0.05$; $^{**}0.001 < p \le 0.01$; $^{***}p \le 0.001$. *A. philoxeroides*-, *A. philoxeroides* with control treatment; *A. philoxeroides*+, *A. philoxeroides* with Cd treatment; *A. sessilis*-, *A. sessilis* with control treatment; *A. sessilis*+, *A. sessilis* with Cd treatment. [Color figure can be viewed at wileyonlinelibrary.com]

**TABLE 1** $\chi^2$ values of generalized models with plant species (*Alternanthera philoxeroides*/*Alternanthera sessilis*), mono- or mixed culture, soil treatment (control or Cd-containing soil) as fixed factors and leaf, stem, root and total dry mass as variables without herbivores.

| Mode | *Df* | Leaf dry mass | Stem dry mass | Root dry mass | Total dry mass |
|---|---|---|---|---|---|
| Without herbivore feeding | | | | | |
| Culture | 1, 112 | 0.665 | 1.335 | 1.764 | 0.034 |
| Species | 1, 112 | 28.699*** | 14.244*** | 23.356*** | 38.316*** |
| Cd | 1, 112 | 11.619*** | 3.852* | 19.219*** | 10.551** |
| Culture × Species | 1, 112 | 6.317* | 1.769 | 5.478* | 13.398*** |
| Culture × Cd | 1, 112 | 0.061 | 0.858 | 0.557 | 0.645 |
| Species × Cd | 1, 112 | 0.145 | 0.340 | 0.348 | 0.749 |
| Culture type × Species × Cd | 1, 112 | 0.007 | 0.166 | 0.525 | 0.184 |
| Population | | 0.424 | 0.608 | 0.412 | 0.216 |
| Pot | | 0.534 | 0.307 | 0.382 | 0.178 |
| With herbivore feeding and this is only for the mixed cultures | | | | | |
| Species | 1, 108 | 48.397*** | 33.279*** | 57.091*** | 94.831*** |
| Cd | 1, 108 | 11.142*** | 11.136*** | 10.570** | 50.682*** |
| Herbivore | 2, 108 | 19.396*** | 2.489* | 4.470* | 16.663*** |
| Species × Cd | 1, 108 | 10.530** | 8.365** | 22.367*** | 26.853*** |
| Species × herbivore | 2, 108 | 37.845*** | 13.215*** | 10.960*** | 40.291*** |
| Cd × herbivore | 2, 108 | 1.029 | 0.526 | 0.859 | 1.031 |
| Species × Cd × herbivore | 2, 108 | 19.561*** | 7.832*** | 8.866*** | 24.265*** |
| Population | | 0.278 | 0.332 | 0.091 | 0.389 |
| Pot | | 0.508 | 0.771 | 0.977 | 0.132 |

*Note*: The second part of the table shows $\chi^2$ with plant species (*A. philoxeroides*/*A. sessilis*), soil treatment (control or Cd-containing soil) and herbivory (specialist, generalist, no herbivores) as fixed factors and leaf, stem, root and total dry mass for mixed cultures only. Plant population and pot were used as random factors.

Abbreviations: Cd, control or Cd treatment; Culture, monoculture or mixed culture; Herbivore, no herbivore feeding, *Agasicles hygrophila* feeding or *Spodoptera litura* feeding; Species, *A. philoxeroides* or *A. sessilis*.

Values are $\chi^2$ from the generalized linear mixed models. The significances are shown as follows:
*$0.01 < p \leq 0.05$; **$0.001 < p \leq 0.01$; ***$p \leq 0.001$.

significantly affected by plant species, soil treatment and herbivores. Also, the two-way interaction species × herbivore as well as the three-way interaction species × Cd × herbivore were all significant.

Lastly, we compared the differences in total dry mass between individual plants and their neighbours grown in monoculture or mixed-culture under different herbivore pressures. *A. philoxeroides* or *A. sessilis* individuals grown in monocultures were not significantly differed from their neighbouring plants in either the control (*A. philoxeroides*: $p = 0.646$; *A. sessilis*: $p = 0.575$) or the Cd treatment (*A. philoxeroides*: $p = 0.508$; *A. sessilis*: $p = 0.959$). However, the Cd treatment reduced the average total dry weight of *A. philoxeroides* and *A. sessilis* by 57.4% ($p < 0.001$) and 76.7% ($p < 0.001$), respectively, compared with their corresponding controls (Figure 6a). On the other hand, *A. philoxeroides* individuals grew on average 58.4% more total dry mass than *A. sessilis* when they grew in mixed culture (Wilcoxon test: $t = 6.113$, $p = 0.005$), whereas Cd exposure further increased this difference to 144.5% (Wilcoxon test: $t = 7.940$, $p = 0.005$). The feeding of the two herbivores reduced the growth of both plant species. More specifically, the total dry mass of *A. philoxeroides* plants was only 38.1% compared with that of their competitor, *A. sessilis* (Wilcoxon test: $t = 7.613$, $p < 0.001$), after being fed by the specialist herbivore *A. hygrophila*. Cd exposure significantly reversed such difference, resulting in a 148% higher total dry mass of *A. philoxeroides* than that of *A. sessilis* (Wilcoxon test: $t = 10.671$, $p < 0.001$). In contrast, *A. philoxeroides* had 1.35 times more total dry mass than *A. sessilis* after the feeding of the generalist herbivore *S. litura* (Wilcoxon test: $t = 6.909$, $p < 0.001$), while Cd treatment enlarged this difference to 2.1 times (Wilcoxon test: $t = 10.359$, $p < 0.001$). We also examined the effect of the two herbivores and Cd treatment on the competitive ability of *A. philoxeroides* by calculating

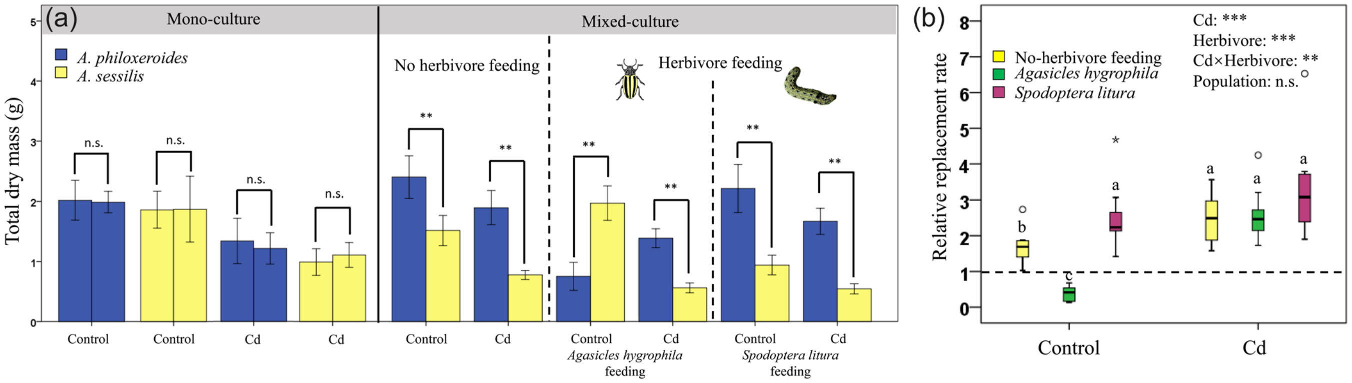

**FIGURE 6** The effect of herbivore pressure on competitive ability of *Alternanthera philoxeroides* and *Alternanthera sessilis* grown on control and Cd-containing soils. (a) Total dry mass of *A. philoxeroides* and *A. sessilis* in monoculture or mixed-culture grown on either control soil or Cd-containing soil with and without herbivores. $N = 10$ replicates for each plant species in the same treatment. Values are means ± SE. Asterisks above the bars indicate significant differences between *A. philoxeroides* and *A. sessilis* from the same treatment according to a Wilcoxon test: $^{**}p \le 0.01$. (b) Relative replacement rate of the *A. philoxeroides* (= total dry mass of *A. philoxeroides*/total dry mass of *A. sessilis* per pot). Dashed line indicates a relative replacement rate = 1. A relative replacement rate >1 indicates that the *A. philoxeroides* outcompetes *A. sessilis*. A relative replacement rate <1 indicated that *A. sessilis* outcompetes *A. philoxeroides*. Values are means ± SD. Different letters indicate significant differences among treatments at $p < 0.05$ according to generalized linear mixed models followed by Wald pair-wise comparisons. The significance values of the generalized linear models are shown as follows: $^{**}p \le 0.01$, $^{***}p \le 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

the relative replacement rate of *A. philoxeroides* over *A. sessilis* using total dry mass. Cd treatment, herbivore type and their interaction significantly affected the relative replacement rate of *A. philoxeroides* (Figure 6b). Without Cd treatment, the average value of the relative replacement rate of *A. philoxeroides* was significantly reduced by 76.7% after consumption by the specialist *A. hygrophila* ($p < 0.001$), while it increased by 49.4% after consumption by the generalist *S. litura* ($p = 0.014$) compared with mixed cultures without herbivores. In contrast, Cd treatment increased the relative replacement rate of *A. philoxeroides* by 3.6% ($p = 0.786$) and 28.7% ($p = 0.013$) under the feeding of *A. hygrophila* and *S. litura*, respectively, compared with the no-herbivore feeding controls.

## 4 | DISCUSSION

In this study, we found that although soils amended with Cd decreased plant growth of *A. philoxeroides* and *A. sessilis*, *A. philoxeroides* still exhibited a higher leaf, stem, root and total dry mass, as well as a greater number of ramets than *A. sessilis* grown on Cd-containing soils. Piola and Johnston (2007) found that the diversity of native plants was decreased by between 33% and 50% in response to increased metal stress, whereas the diversity of the invasive plant populations showed no evident change. Therefore, it was suggested that a higher growth performance of an invasive plant species in metal-polluted habitats may further facilitate its invasion success (Li et al., 2021). Despite the better growth performance in response to soil Cd stress, *A. philoxeroides* was also able to accumulate 1.8 times higher Cd concentration in the leaves than its native congener, *A. sessilis*. Similarly, several invasive plants have been found to accumulate larger amounts of metals in their tissues than their local congeners (Qian et al., 2008; Wang et al., 2021b). Since the presence of metals in plant tissues can serve as a protective mechanism against herbivore attack (Martens & Boyd, 1994), we proposed that the differences in leaf Cd accumulation between *A. philoxeroides* and *A. sessilis* may contribute to the changes in herbivore resistance between these two plant species.

The herbivore bioassay indicated that the specialist *A. hygrophila* and generalist *S. litura* exhibited opposite feeding preferences between *A. philoxeroides* and *A. sessilis* leaves without Cd pollution. We expected that such feeding preferences of different herbivore species could be attributed to the differences in leaf defensive compounds as these two species exhibited similar levels of mechanical defence. However, further leaf metabolomic analysis indicated that *A. philoxeroides* leaves contained significantly higher amounts of three specialized metabolites that all belong to the class of flavonoids compared with *A. sessilis* under Cd-free conditions. Flavonoids have been suggested as a common defensive chemical employed by plants to defend nonadapted generalist herbivores, while some specialized herbivores are adapted to them or even can sequester plant flavonoids in their body cuticle as a defence against predators (Treutter, 2005). The differences in the levels of the three specialized metabolites between the two plant species could explain the feeding preferences and performance of both herbivores between *A. sessilis* and *A. philoxeroides*.

Plants growing on Cd-containing soils changed the feeding preference of the specialist herbivore and resulted in a preference of both herbivores for the leaves of *A. sessilis* over *A. philoxeroides*. Combined with the fact that the leaf Cd concentration was negatively correlated with the consumed leaf areas of both herbivore species, we suggest that Cd accumulation in plant leaves has a direct deterrent effect on herbivores. Convergent with our findings, several studies testing the elemental defence hypothesis showed the protective role of metals against both herbivory by specialist and generalist herbivores (Kazemi-Dinan et al., 2014; Lin et al., 2020). We also found a significantly higher total leaf area in *A. sessilis* plants than in *A. philoxeroides* plants grown on Cd-containing soils. The fact that the leaf dry mass of *A. philoxeroides* was much higher than that of *A. sessilis* plants grown on Cd-containing soils suggests that *A. philoxeroides* might develop thicker leaves compared with *A. sessilis*. Indeed, we found that the leaf mass–area ratio and leaf thickness of *A. philoxeroides* were all higher than those of *A. sessilis* plants grown on Cd-contaminated soil. Leaf mass–area ratio is considered a leaf structural trait that confers toughness (Hanley et al., 2007), while leaf thickness was found to be negatively correlated with the densities of leaf-chewing insects (Peeters, 2002). Therefore, leaf mass–area ratio and leaf thickness could be considered as leaf mechanical defence-related traits that are involved in plant resistance to herbivorous insect assemblages. In addition, we also observed a significantly higher leaf tensile strength and leaf punch toughness of *A. philoxeroides* leaves than *A. sessilis*. In general, tougher leaves can act as a physical barrier to deter the feeding or oviposition of herbivorous insects (Kogan, 1994; Salgado-Luarte et al., 2023). As the mechanical defence can serve as digestibility reducers against both types of herbivores (Hanley et al., 2007), we found these four traits were all significantly negatively correlated with the herbivore-consumed leaf area of *A. hygrophila* and *S. litura* in the nonchoice-feeding bioassay. These results suggested that *A. philoxeroides* plants grown on Cd-containing soil can increase the levels of mechanical defence to be better defended against herbivores. Besides that, we found that growth on Cd-containing soils increased the amounts of specialized metabolites in *A. philoxeroides* leaves while decreasing the amounts of specialized metabolites in *A. sessilis* leaves. Such differences eventually resulted in greater levels of six specialized metabolites that belong to the class of flavonoids in the leaves of *A. philoxeroides* in comparison to *A. sessilis*. As both of the two herbivore species preferred the *A. sessilis* leaves regardless of the reduced levels of defensive metabolites, we suggest that such differences in the observed defensive metabolites between *A. philoxeroides* and *A. sessilis* might be largely conquered by the direct deterrent effect of Cd accumulated in plant leaves and Cd stress enhanced mechanical defence. Since there are trade-offs between plant resistance and tolerance to herbivory, Salgado-Luarte et al. (2023) suggested that mechanical defences could outweigh chemical defences under certain stressful circumstances. Besides that, it is important to note that the changes of other specialized metabolites or the composition of nutritious primary metabolites may have also contributed to the observed difference in herbivore resistance between these two plant species.

Moreover, we showed that *A. philoxeroides* exhibited a competitive advantage over its local congener *A. sessilis* when grown in the mixed-culture condition. In general, invasive plant species were observed to have a stronger competitive ability over local competitors when grown in a mixed-culture condition (Sheppard, 2019; Vila & Weiner, 2004; Zou et al., 2008). As suggested by the enemy release hypothesis, invasive plant species are freed from their native coexisting specialist herbivores while still encountering local generalist herbivores and occasional specialist herbivores of congeneric plant species after invasion (Keane & Crawley, 2002). Such a shift in herbivore pressure is expected to result in evolutionary changes in invasive plants with decreased investment in costly antiherbivore strategies such as mechanical defence but increased competitive ability, as proposed in the evolution of increased competitive ability hypothesis (Blossey & Nötzold, 1995; Joshi & Vrieling, 2005). However, as the feeding preferences of the generalist and specialist herbivores showed the opposite pattern, the competitive advantage of *A. philoxeroides* was increased by *S. litura* feeding while it was decreased by *A. hygrophila* feeding. Several studies have documented the contrasting effects of herbivory by generalist and specialist herbivores on the competitive ability of native and invasive plants, while the effects of specialists were mostly stronger on invasive plants (Huang et al., 2012; Lin et al., 2015b). Therefore, we suggest that the outcome of the competition between native and invasive plants depends on the herbivore types (Lin et al., 2015b).

Although Cd exposure decreased the growth of both plant species in the mixed-culture treatment, it increased the competitive advantage of *A. philoxeroides* over *A. sessilis* from 58.4% to 114.5% when there was no herbivore. We argue that such an increased competitive advantage of *A. philoxeroides* under Cd exposure might be due to its better tolerance to Cd stress, as *A. philoxeroides* showed higher growth than *A. sessilis* in the monoculture treatment. These findings are consistent with a similar study which found that soil Pb concentrations significantly enhanced the competitive ability of invasive *Solidago canadensis* over native *Kummerowia striata* grown in mixed cultures (Zhang et al., 2008). Interestingly, the replacement rate of *A. philoxeroides* was higher under herbivore pressure from both herbivore species, especially under the pressure of the specialist *A. hygrophila*. As we observed a higher deterrent effect of Cd-treated *A. philoxeroides* than Cd-treated *A. sessilis* on either the specialist or generalist herbivore in the single culture experiment, it strongly suggests that such changes in the outcome of the competition between the two plant species can be attributed to the Cd-induced difference in herbivore resistance between *A. philoxeroides* and *A. sessilis*.

## 5 | CONCLUSIONS

In this study, the invasive *A. philoxeroides* grew higher in response to Cd stress while accumulating more Cd in the leaves than its native congener *A. sessilis* when grown in a single culture condition. The herbivore bioassay further indicated that although Cd exposure largely reduced the consumed leaf area of the generalist and specialist herbivores, *A. philoxeroides* still exhibited better resistance to both herbivore species over *A. sessilis*. Measurements of a suite of host plant traits related to leaf mechanical and chemical defences suggest that such differences in herbivore resistance between the two plant species under Cd contamination were most likely due to the direct deterring effect of Cd accumulation and Cd-enhanced levels of mechanical defences in *A. philoxeroides*. Furthermore, the competition tests showed that Cd stress significantly enhanced the competitive advantage of *A. philoxeroides* over *A. sessilis* with or without herbivory. The results expand the application of the elemental defence hypothesis on invasive plant species and provide a fundamental basis for evaluating the invasiveness of nonindigenous plants that are introduced into metal-contaminated habitats. However, it is worthwhile to point out that further studies with more plant species and associated herbivores are needed to be investigated before drawing a certain conclusion.

## ACKNOWLEDGEMENTS

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

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## SUPPORTING INFORMATION

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

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