



# Heavy metal domestication enhances beneficial effects of arbuscular mycorrhizal fungi on lead (Pb) phytoremediation efficiency of *Bidens parviflora* through improving plant growth and root Pb accumulation

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## Abstract

Native arbuscular mycorrhizal fungi (AMF) generally provide more effective assistance for phytoremediation to remove heavy metal (HM) from polluted soils than non-native AMF. Nevertheless, it is a time-consuming work to isolate, identify, and propagate AMF inoculum for practical application. This study aims to explore an alternative method to improve the phytoremediation efficiency of *Bidens parviflora* using domesticated AMF under HM stress condition for a certain period of time. Our results showed that *Funneliformis mosseae* inoculation alleviated oxidative damage to plant membranes by enhancing activities of superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase. Furthermore, mycorrhizal plants had higher chlorophyll concentration, photosynthesis efficiency, and root Pb content to protect the aerial parts from damage. These protective mechanisms were found to be more efficient in domesticated AMF inoculation compared with non-domesticated AMF inoculation. Overall, this study suggests that *F. mosseae* domesticated for 12 months could greatly enhance plant root Pb accumulation and plant growth mainly through strengthening antioxidant defenses as well as the photosynthesis efficiency under Pb stress conditions. Plants inoculated with pre-domesticated AMF provided a promising new strategy to enhance phytoremediation of Pb-contaminated soils.

**Keywords** Arbuscular mycorrhizal fungi · *Bidens parviflora* · Heavy metal · Phytoremediation · Domestication · Antioxidant enzyme · Photosynthesis

## Introduction

Due to rapid industrialization and urbanization, heavy metal (HM) contamination is increasingly becoming a serious environmental problem around the world because of their toxic effects on living beings, food security, and the entire ecological function (Briffa et al. 2020). Although HMs are naturally occurring elements in soil, anthropogenic activities (mining, smelting, industrial emissions, agricultural

fertilization, etc.) are still responsible for excessive quantities of HM input to the environment. Among the HMs, Pb was found to be the most toxic heavy element in the environment due to its abundance, toxic, and non-biodegradable behavior (Ab Latif Wani and Usmani 2015). China has the second largest Pb reserves in the world and produced 1.9 million metric tons of Pb in 2020, accounting for 43% of the world's total mine production (Global No. 1 Business Data Platform 2021). Soil Pb contamination has become a serious problem in China due to its detrimental effects on human health and the potential threat to the construction of the ecological civilization proposed by Chinese government in 2017 (Hansen et al. 2018). Therefore, HM contamination of soils has become a worldwide problem which requires urgent remediation actions.

Various strategies are currently used for the remediation of HM-polluted soils, mainly including physical, chemical, and biological methods (Lajayer et al. 2019). Despite high efficiency, physical and chemical techniques suffer from serious limitations such as high

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cost, intensive labor, soil property destruction, and soil microorganism disturbance (Sharma et al. 2016). Phytoremediation is a promising technology that uses metal-accumulating plants to remove toxic metals or render them harmless from environment (Raskin et al. 1997). It is accepted as one of the safer, cost-effective, and environment-friendly techniques, which has attracted more and more attentions over the recent decades (Marques et al. 2009). However, there are several practical limitations associated to phytoremediation, mainly related to the slow growth rate, small biomass yields, and low HM accumulation of plants. The limitations of phytoremediation can be partly overcome using plants having large biomass, fast growth rate, high HM accumulation, and ability to adapt with wide range of environmental stress.

On the other hand, the application of plant-associated microbe can be considered as an alternatively promising strategy to enhance phytoremediation efficiency (Rajkumar et al. 2012). Arbuscular mycorrhizal fungi (AMF) are a group of soil microorganisms that form symbiotic association with most terrestrial plants (Smith and Read 2008). The benefits of AMF in phytoremediation of HM-contaminated soils has been widely accepted, as their ability to improve plant biomass, growth rate, stress resistance, and HM accumulation (Ezawa et al. 2002; Smith et al. 2011; Yang et al. 2015a; Salazar et al. 2018; Bhandana et al. 2021). The beneficial effects of AMF on phytoremediation efficiency are related to the source of AMF and their adaptability to HM-contaminated soils (Yang et al. 2016). Generally, the inoculation of native AMF species is more effective in promoting plant growth and HM accumulation compared with the non-native fungi (Klironomos 2003; Orłowska et al. 2005; Pellegrino et al. 2011). However, it is a time-consuming process to isolate native AMF species from HM-contaminated soils, select and identify the most effective AMF strain, and propagate it to obtain enough inoculum for practical application. Alternatively, a simple and feasible method known as stress-driven adaptive evolution experiments has been proposed to improve the adaptability and growth of microbial species to particular stress conditions (Sun et al. 2018). This is because microorganisms can adapt rapidly to changing environments through regulation of their gene expression in order to survive and reproduce (López-Maury et al. 2008). Recently, this strategy has been successfully used in a number of microbe (especially microalgal species) to obtain domesticated strain with high growth and stress resistance advantages in stress environments. However, whether the domestication treatment can enhance growth and stress tolerance of AMF and their beneficial effects on phytoremediation efficiency

is still unclear. We have a hypothesis here: AMF can gain the capacity to cope with HM stress and enhance positive impact on phytoremediation through consuming nutrients efficiently, regulating gene expression, and influencing HM accumulation.

*Bidens* is an annual herbaceous plant belonging to compositae family and distributes widely all over the world (Sun et al. 2009). It has been suggested that *Bidens parviflora* Willd. has the ability to accumulate large amount of HM and can be considered as a potential hyperaccumulator for restoration of Cd- and Pb-contaminated soil (Deng et al. 2019). In our field study, the roots of *B. parviflora* were found highly colonized by AMF in HM-polluted soil. The exact role of domesticated AMF with different domestication durations in phytoremediation efficiency remains unclear. Therefore, the objectives of this study were to (1) determine the ability of *B. parviflora* to accumulate and translocate Pb from soil to plant organs, (2) evaluate the impacts of AMF on growth and HM accumulation of *B. parviflora*, and (3) explore whether the domesticated AMF under HM stress had more beneficial effects on phytoremediation efficiency.

## Materials and methods

### Plant, AMF inoculum, and substrate

*B. parviflora* was selected as a host plant in this study because it is a potential used hyperaccumulator in north-east China. The seeds of *B. parviflora* were collected from Longwan National Natural Reserve, Jilin Province, China (42°20'56"N, 126°22'51"E) in October. The seeds were surface-sterilized with 0.5% sodium hypochlorite and 75% ethanol (v/v) for 10 min and 5 min, respectively. Subsequently, the seeds were washed carefully with sterile distilled water five times and then were sown on moistened filter paper arranged in 9 cm diameter Petri dishes with 7 mL distilled water. The Petri dishes were incubated in the dark at 27 °C for 2 days before the germinated seeds were transplanted into the sterilized pots.

The AM fungus *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler (BGC GZ01A, formerly *Glomus mosseae*) was purchased from Beijing Academy of Agriculture & Forestry Sciences (BAAFS). The fungus is a widely distributed AMF species all over the world and can form symbionts with most plants (e.g., *B. parviflora*) to enhance their resistance to stress conditions (Huang et al. 2019). The AMF isolate (*F. mosseae*) was chosen based on its widely distribution and beneficial effects on plant growth as well as its predominance in HM-contaminated soils (Yang et al. 2015b). The AMF strain was propagated in sterilized sand (diameter < 1 mm) pots using *Zea mays* L. and *Trifolium repens* L. as host plants for 6, 12, and 24 months.

The plants were uniformly irrigated with distilled water and Hoagland's nutrient solution every 2 days and 1 week, respectively. Pots were divided into two groups. In the first group, pots were irrigated with pure Hoagland's nutrient solution (no Pb addition), and the fungus was expressed as non-domesticated *F. mosseae* (ND<sub>6</sub>Fm, ND<sub>12</sub>Fm, and ND<sub>24</sub>Fm). In the second group, pots were irrigated with Hoagland's nutrient solution contained 1000 mg kg<sup>-1</sup> Pb, and the arbuscular mycorrhizal fungus was expressed as domesticated *F. mosseae* (D<sub>6</sub>Fm, D<sub>12</sub>Fm, and D<sub>24</sub>Fm). Finally, the spore density in sand substrate was determined using the wet-sieving and decanting methods under light dissecting microscope (Gerdemann and Nicolson 1963). The average spore densities in soils were shown in Table 1, and only dried substrate was mixed homogeneously to use as the AMF inoculum to ensure that all treatments could receive the same number of spores.

Soil was collected from 0 to 20 cm soil depth at the Experimental Station of Jilin Agricultural University, Changchun, China (43°49'07"N, 125°23'56"E). The area is located in the temperate continental monsoon climate, with mean annual temperature of 6.7 °C and mean annual precipitation of 600–700 mm. The soil type was classified as black soil (Phaeozems) and has a silty loam texture. The collected soil samples were passed through a 2-mm mesh sieve to remove large coarse sand and gravel particles as well as to improve the homogeneity. The sieved soil was air-dried at room temperature for 20 days, added into a clean cloth bag, and then sterilized with two cycles of the autoclave at 121 °C, 0.11 Mpa for 2 h to eliminate all microorganisms. The basic properties of soil used for the substrate in the current study were listed in Table S1. The soil was mixed with equal volume of deionized water or Pb(NO<sub>3</sub>)<sub>2</sub> solution at final concentrations of 0, 500, and 2000 mg kg<sup>-1</sup> Pb. Subsequently, the soils were equilibrated for 2 weeks, undergoing five cycles of saturation with deionized water and air-drying.

## Experimental design

The pot experiment was carried out in a solar greenhouse of Northeast Normal University, Changchun city (Jilin province, China), and lasted 90 days. We carried out the study

in a randomized complete block design with five replications: AMF inoculation (three levels, without AMF inoculation, non-domesticated, and domesticated *F. mosseae* inoculation); domestication duration (three levels, 6, 12, and 24 months), and Pb addition (three levels, 0, 500, and 2000 mg kg<sup>-1</sup> soil). The plastic pots (15 cm diameter top, 12 cm diameter base, and 15 cm depth) filled with 2.5 kg sterilized soil with different concentrations of Pb. A total of 40 g of mycorrhizal inoculum (containing 1000 spores) was placed in each plastic pot at a depth of 2 cm below the seeds and covered with soil. Subsequently, eight germinated seeds of *B. parviflora* were planted into each plastic pot and then covered with a thin layer of the substrate. Plant positions were repositioned at random, and plants were watered every 2 days based on the pot weight to maintain the soil water field capacity (FC) at 75%.

## Plant and soil analysis

After 90 days of treatments, the net photosynthetic rate (Pn), intercellular CO<sub>2</sub> concentration (Ci), stomatal conductance (Gs), and transpiration rate (Tr) were assessed with a LI-COR LI-6400 portable gas exchange system on the fifth leaf of each plant from 8:30 to 11:00 am (Yang et al. 2014).

The fifth leaf was then harvested to measure the chlorophyll concentration (Chl a, Chl b, Chl a + b, and Chl a/b). Chlorophyll pigments were extracted by chilled acetone (80%, v/v) using a mortar and pestle at room temperature in the dark, and the optical density (OD) of the extract was read at 663 and 646 nm using a UV-5500PC spectrophotometer (Shanghai Metash Instruments Co., Ltd., China). The concentration of chlorophyll pigments was calculated according to the formulas of Lichtenthaler and Buschmann (2001).

The activities of antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR)) in leaves were determined according to the method used in our previous study (Yang et al. 2020). Briefly, fresh leaf samples were harvested separately, grinded in liquid nitrogen, and homogenized with 25 mM potassium phosphate buffer solution (pH 7.8) containing 0.5 mM EDTA-Na<sub>2</sub> and 1 mM ascorbate. The homogenate was then centrifuged at 10,000 rpm for 20 min, and the supernatant was stored in a refrigerator (4 °C) for the measurement of antioxidative enzyme activities. The CAT activity was assessed by monitoring the decrease in the absorbance at 240 nm as a consequence hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) decomposition (Aebi 1984). The SOD activity was assayed according to Aebi (1984) by measuring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. The APX activity was calculated by detecting the decrease of ascorbate

**Table 1** AMF spore density in the substrate after 6, 12, and 24 months' cultivation under different Pb stress levels

Pb stress level	Cultivation duration		
	6 months	12 months	24 months
0 mg kg <sup>-1</sup> Pb (ND)	477.4 ± 34.5	514.8 ± 50.7	538.4 ± 33.3
1000 mg kg <sup>-1</sup> Pb (D)	360.6 ± 39.6	419.4 ± 22.5	454.8 ± 19.9

ND, non-domesticated treatment; D, domesticated treatment. Data represent mean ± SD for biological replicates (n = 5)

oxidation at 290 nm (Nakano and Asada 1981). The GR activity was assayed according to Foyer and Halliwell (1976) based on the rate of glutathione at 420 nm.

The hydrogen peroxide ( $H_2O_2$ ) content in leaves was estimated as described by Patterson et al. (1984) by detecting the absorbance of the titanium–peroxide complex at 410 nm. The malondialdehyde (MDA) content in leaves was performed using the thiobarbituric acid method as described by Heath and Packer (1968).

Plant roots were washed with tap water, cleared in 10% KOH, bleached in  $H_2O_2$ , acidified with 1% lactic acid, and then stained with 0.05% trypan blue (Phillips and Hayman 1970). The mycorrhizal colonization was determined according to Biermann and Linderman (1981) by monitoring the proportion of root length colonized by AMF. AMF spores were extracted from 20 g soil using wet-sieving and decanting method (Gerdemann and Nicolson 1963), and the number was counted under a dissecting microscope. The extracted hyphae of AMF in soil were stained with a 0.05% (w/v) trypan blue solution, and the hyphal length density (HLD) was determined by grid-line intersection method (Jakobsen et al. 1992).

Plants were harvested and separated into roots, stems, and leaves. The samples were rinsed with distilled water three times, blotted with filter paper, and oven-dried at 80 °C until constant mass to recorded plant biomass. Plant organs (leaves, stems, and roots) were firstly digested in Teflon vessels with concentrated HF and  $HNO_3$  acid solutions, and then the Pb concentrations in different plant organs were determined using flame atomic absorption spectrometry (FAAS, AA-7003A, Beijing, China). The bioconcentration factor (BCF) and translocation factor (TF) were estimated to depict the ability of the plants to accumulate HM from soil, which is calculated as follows:

$$BCF = \frac{C_{root} \text{ or } C_{stem} \text{ or } C_{leaf}}{C_{soil}}$$

$$TF = \frac{C_{stem} \text{ or } C_{leaf}}{C_{root}}$$

where  $C_{soil}$ ,  $C_{root}$ ,  $C_{stem}$ , and  $C_{leaf}$  are the concentrations of Pb in the soil, plant root, stem, and leaf, respectively.

## Statistical analysis

Prior to data analysis, the Kolmogorov–Smirnov test and Levene test were used to evaluate the normality and homogeneity of data using SPSS 21 (SPSS Inc., Chicago, IL, USA) for Windows 10, respectively. The significant differences among the mean values of different treatments were compared using one-way ANOVA followed by the Duncan's multiple range tests using SPSS 21.  $p$

values less than 0.05 were considered statistically significant. The coefficient of variation of each parameter determined in this study could be seen in Table S2. The correlations among all observed variables were computed by applying the Pearson correlation method at a significance level of 0.05. Mantel test was performed to assess the correlations between AMF growth index and plant physiochemical parameters, and the results were visualized using the “ggplot2” package in R (version 4.0.4). A bubble plot was constructed using the R packages “ggplot2” and “reshape2.”

## Results

### AMF growth parameters

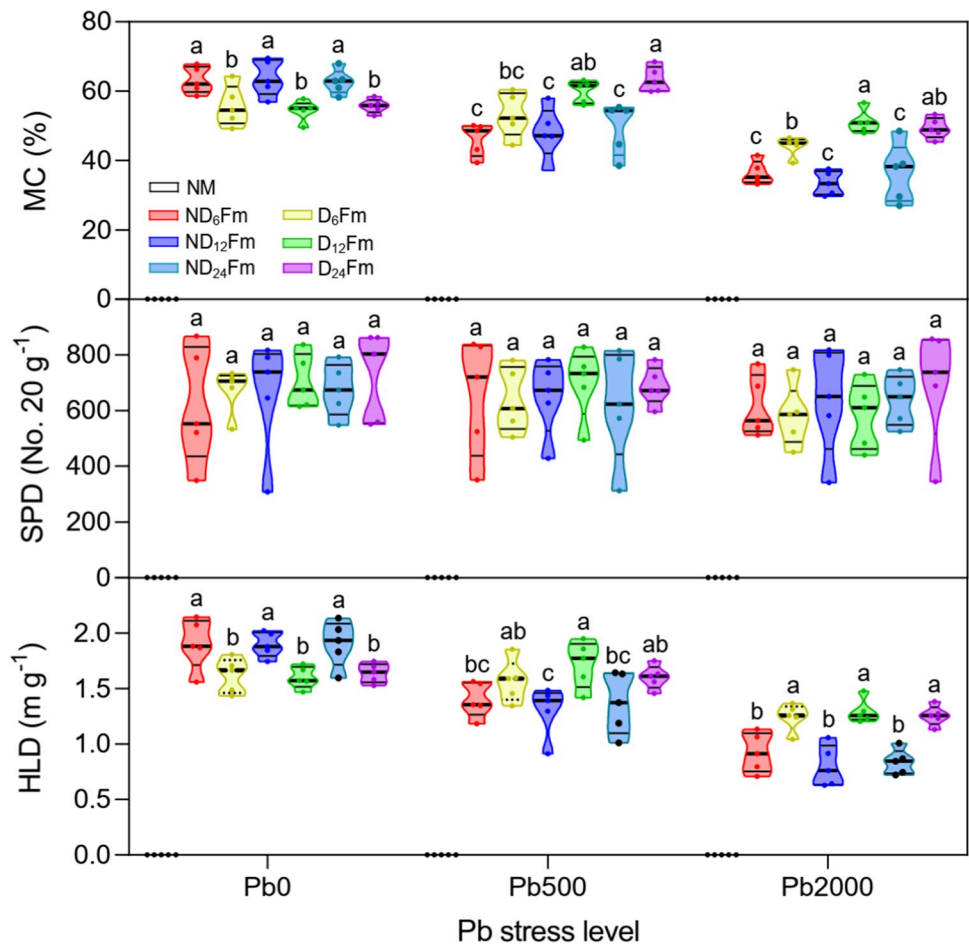
The good symbiosis between *F. mosseae* and *B. parviflora* could be detected using microscopic assessment, whereas no mycorrhizal structures were found in the roots of non-mycorrhizal plants. Pb2000 treatment (2000 mg  $kg^{-1}$ ) considerably decreased mycorrhizal colonization (MC) of plants colonized by non-domesticated *F. mosseae* (NDFm), while plants inoculated with domesticated *F. mosseae* (DFm) had significantly higher MC and HLD than NDFm plants (Fig. 1).  $D_{12}Fm$  and  $D_{24}Fm$  plants had higher MC compared with  $D_6Fm$  plants, while no significant difference was detected between  $D_{12}Fm$  and  $D_{24}Fm$  plants under Pb2000 treatment. By contrast, no significant difference in spore density (SPD) was found among all treatments ( $p > 0.05$ ).

### Plant growth parameters

Compared with non-mycorrhizal plants, AMF inoculation increased root, stem, and leaf dry weights under Pb stress conditions (Fig. 2). The magnitude of the growth response to domesticated AMF was more effective than non-domesticated AMF in terms of plant root, stem, and leaf dry weights under Pb500 and Pb2000 treatments. However, no significant difference in stem dry weight, leaf dry weight, and R/S ratio was detected between non- and mycorrhizal plants under Pb0 treatment. The R/S ratio of non-mycorrhizal plants was lower compared with the mycorrhizal plants under Pb2000 treatment, although no significant difference could be detected under Pb500 treatment ( $p > 0.05$ ). DFm plants had significantly higher leaf and stem dry weights than non-mycorrhizal and NDFm plants, while no difference was found between  $D_{12}Fm$  and  $D_{24}Fm$  plants under Pb2000 treatments ( $p > 0.05$ ).



**Fig. 1** Mycorrhizal colonization (MC, **a**), spore density (SPD, **b**), and hyphal length density (HLD, **c**) of *B. parviflora* inoculated with *F. mosseae* (Fm) under different Pb stress levels. Pb0, 0 mg kg<sup>-1</sup> Pb stress level; Pb500, 500 mg kg<sup>-1</sup> Pb stress level; Pb2000, 2000 mg kg<sup>-1</sup> Pb stress level; NM, non-mycorrhizal inoculation; NDFm, non-domesticated *F. mosseae* inoculation; DFm, domesticated *F. mosseae* inoculation. The subscript numbers present the duration of domestication (month). Data represent mean  $\pm$  SD for biological replicates ( $n=5$ ). The same letter indicates no significant difference at each Pb stress level (Duncan's test,  $p<0.05$ )



### Leaf gas exchange parameters

The leaf gas exchange parameters of *B. parviflora* among different treatments are showed in Fig. 3. The Pn, Gs, and Tr were negatively correlated with the increasing Pb concentration ( $R^2 = -0.582, -0.612$ , and  $-0.630$ , respectively; all  $p < 0.001$ ). Ci was positively correlated with Pb concentration ( $R^2 = 0.586$ ,  $p < 0.001$ ). The Pn, Gs, and Tr of non-mycorrhizal plants under Pb2000 versus Pb0 treatments decreased by 41.5, 46.4, and 42.8%, respectively. Plants inoculated with domesticated *F. mosseae* (D<sub>6</sub>Fm, D<sub>12</sub>Fm, and D<sub>24</sub>Fm) had higher Pn, Gs, and Tr but lower Ci compared with the plants colonized by non-domesticated *F. mosseae* (ND<sub>6</sub>Fm, ND<sub>12</sub>Fm, and ND<sub>24</sub>Fm) under Pb2000 treatment ( $p < 0.05$ ).

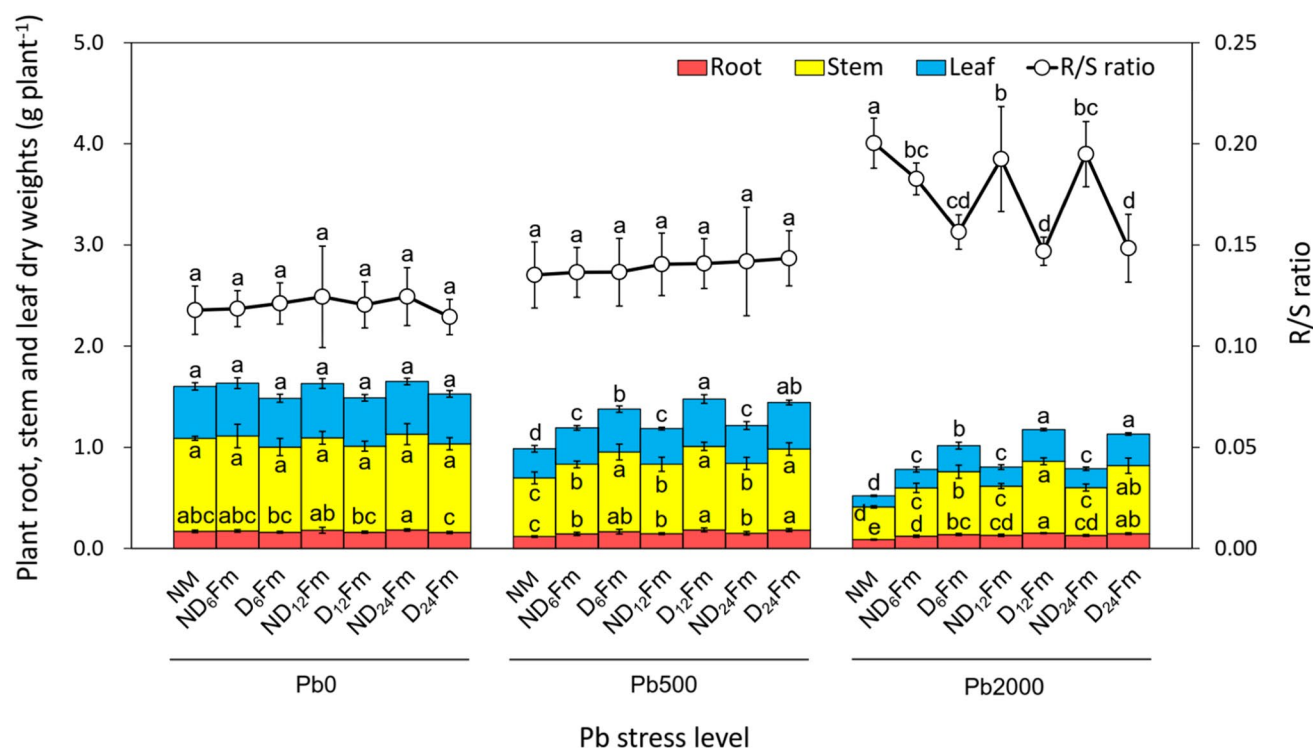
### Leaf chlorophyll concentration

With the increase of Pb stress level, the chlorophyll a, b, and a + b concentrations of DFm plants firstly

increased and then decreased, reaching a maximum under Pb500 treatment (Fig. 4). There were no significant differences in the chlorophyll a, b, and a + b concentrations or chlorophyll a/b ratio between non- and mycorrhizal plants under Pb0 treatment. Non-mycorrhizal plants had significantly lower chlorophyll a, b, and a + b concentrations compared with mycorrhizal plants, while the chlorophyll a/b ratio was considerably higher than mycorrhizal plants. The chlorophyll b concentration showed a similar change trend as the chlorophyll a concentration, and DFm plants had higher chlorophyll b concentration and chlorophyll a/b ratio than NDFm plants under Pb stress conditions ( $p < 0.05$ ).

### Antioxidant enzyme activities

The activities of SOD and CAT in leaves of D<sub>12</sub>Fm and D<sub>12</sub>Fm plants were higher than other plants under Pb500 treatment (Fig. 5). There was no significant difference in SOD, CAT, APX, and GR activities in leaves of non- or mycorrhizal plants under Pb0 treatment, but D<sub>12</sub>Fm and



**Fig. 2** Plant root dry weight, stem dry weight, leaf dry weight, and root:shoot (R/S) ratio of *B. parviflora* inoculated without or with *F. mosseae* (Fm) under different Pb stress levels. Pb0, 0 mg kg<sup>-1</sup> Pb stress level; Pb500, 500 mg kg<sup>-1</sup> Pb stress level; Pb2000, 2000 mg kg<sup>-1</sup> Pb stress level; NM, non-mycorrhizal inoculation;

NDFm, non-domesticated *F. mosseae* inoculation; DFm, domesticated *F. mosseae* inoculation. The subscript numbers present the duration of domestication (month). Data represent mean  $\pm$  SD for biological replicates ( $n=5$ ). The same letter indicates no significant difference at each Pb stress level (Duncan's test,  $p < 0.05$ )

D<sub>24</sub>Fm plants had considerably higher SOD, CAT, and APX activities compared with the control and other mycorrhizal plants under Pb2000 treatment ( $p < 0.05$ ). Furthermore, the SOD, CAT, and GR activities in leaves of mycorrhizal plants were significantly greater than non-mycorrhizal plants under Pb2000 treatment ( $p < 0.05$ ).

### Leaf hydrogen peroxide and malondialdehyde contents

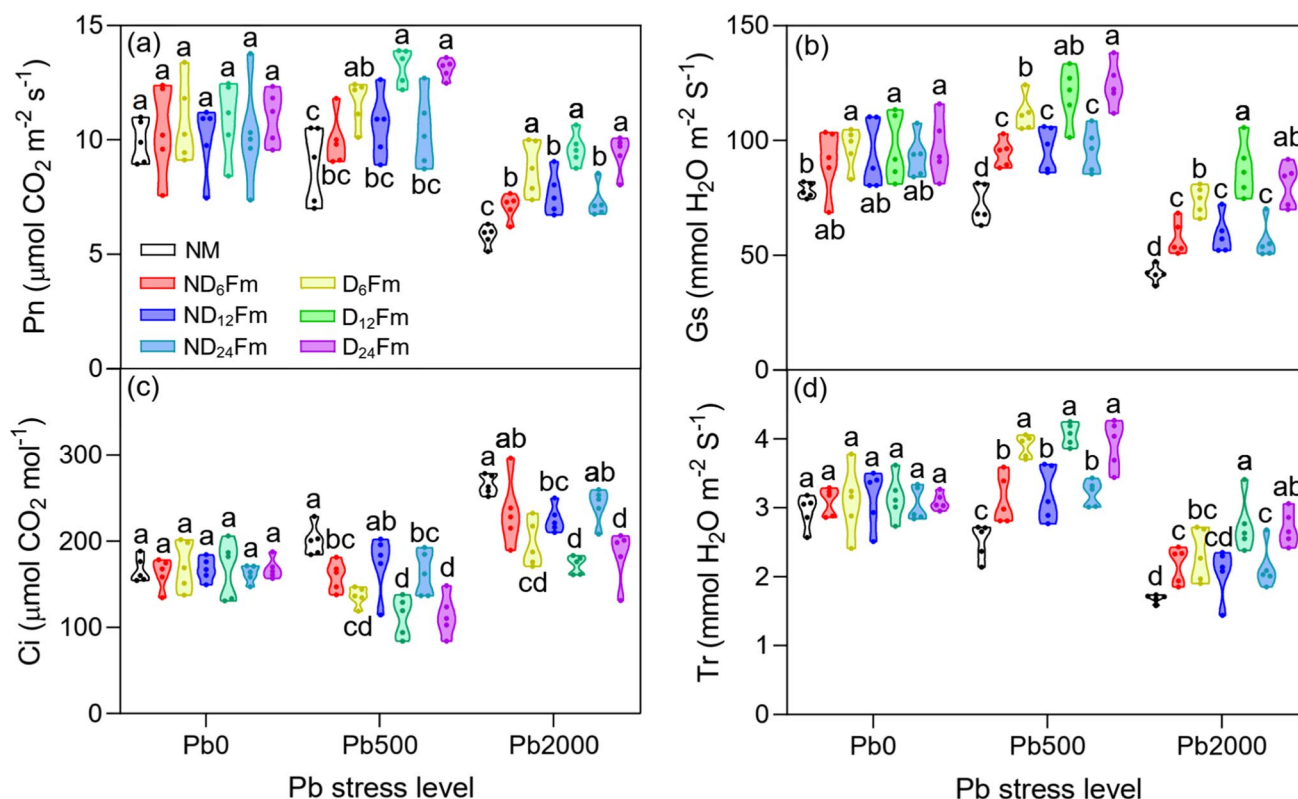
Pb stress significantly increased the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA) contents in plant leaves (Fig. 6), while there was no significant difference in the H<sub>2</sub>O<sub>2</sub> and MDA contents between non- and mycorrhizal plants under the control treatment (0 mg kg<sup>-1</sup> Pb). Non-mycorrhizal plants had higher H<sub>2</sub>O<sub>2</sub> content compared with mycorrhizal plants under Pb addition treatments. Interestingly, DFm plants had lower H<sub>2</sub>O<sub>2</sub> and MDA contents compared with NDFm plants, while no significant difference in H<sub>2</sub>O<sub>2</sub> and MDA contents among D<sub>6</sub>Fm, D<sub>12</sub>Fm, and D<sub>24</sub>Fm was found under Pb stress conditions.

### Pb accumulation in plant

The Pb concentrations in the roots, stems, and leaves of *B. parviflora* seedlings performed an increasing trend following an increase in Pb concentration in soil (Fig. 7). Plant roots had the highest Pb concentration followed by the leaves, while the stems showed the lowest amounts of Pb. Mycorrhizal plants accumulated significantly higher Pb in the roots compared with non-mycorrhizal plants at all Pb stress levels, but no difference in stem and leaf Pb concentrations was detected between non- and mycorrhizal plants under Pb0 treatment ( $p > 0.05$ ). Pb concentrations in the leaves and stems of D<sub>12</sub>Fm and D<sub>24</sub>Fm plants were lower compared with the NDFm plants under Pb stress conditions ( $p < 0.05$ ). However, there was no difference in leaf, stem, or root Pb concentrations between D<sub>12</sub>Fm and D<sub>24</sub>Fm plants under all treatments ( $p > 0.05$ ).

### Discussion

Lead (Pb) contamination in soils has been reported in many countries throughout the world, with the most severe problems found in Asia. Using plants that can hyperaccumulate



**Fig. 3** Leaf net photosynthetic rate (Pn, **a**), stomatal conductance (Gs, **b**), intercellular CO<sub>2</sub> concentration (Ci, **c**), and transpiration rate (Tr, **d**) of *B. parviflora* inoculated without or with *F. mosseae* (Fm) under different Pb stress levels. Pb0, 0 mg kg<sup>-1</sup> Pb stress level; Pb500, 500 mg kg<sup>-1</sup> Pb stress level; Pb2000, 2000 mg kg<sup>-1</sup> Pb stress level; NM, non-mycorrhizal inoculation; NDFm, non-domesticated

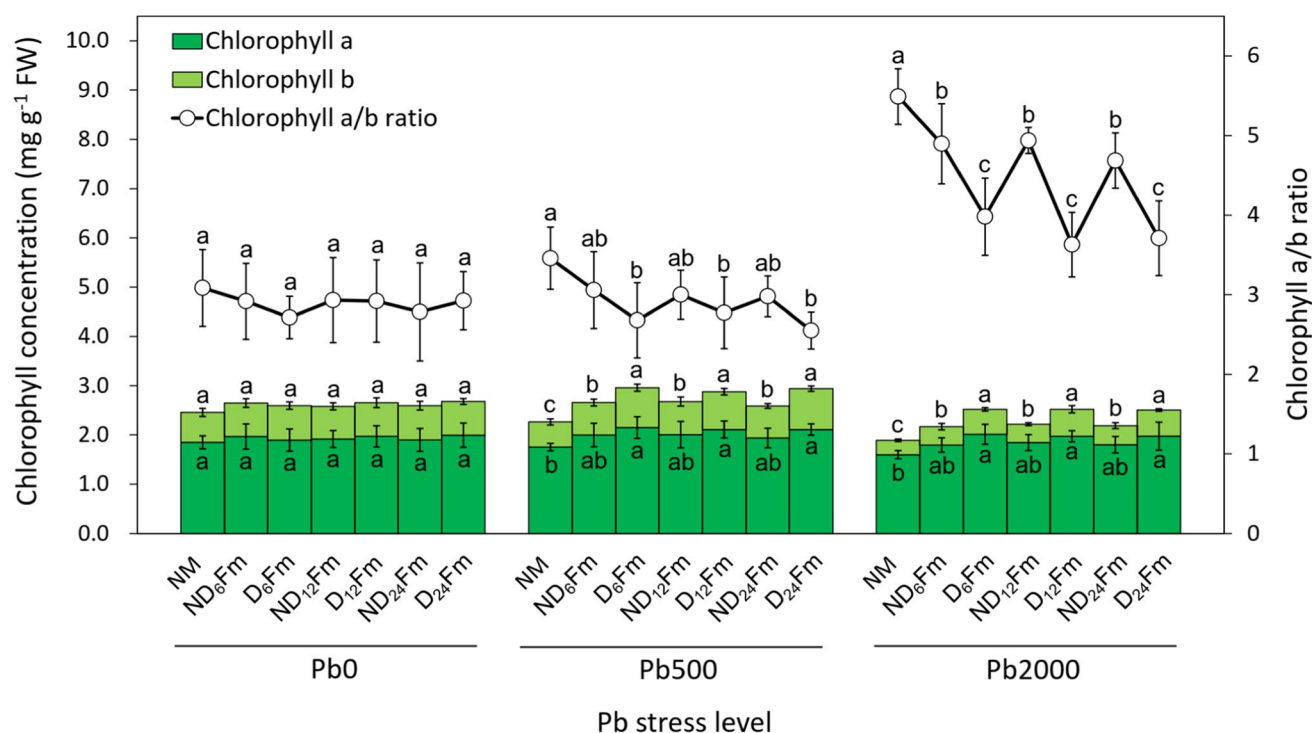
*F. mosseae* inoculation; DFm, domesticated *F. mosseae* inoculation. The subscript numbers present the duration of domestication (month). Data represent mean  $\pm$  SD for biological replicates ( $n=5$ ). The same letter indicates no significant difference at each Pb stress level (Duncan's test,  $p < 0.05$ )

specific metals has been considered as a promising, environmentally friendly, and low-cost technology for remediation of Pb-contaminated soils (Salt et al. 1995). In northeast China with cold temperature, *B. parviflora* is a more suitable phytoremediator for Pb than the other hyperaccumulator because of its high tolerance to cold stress. The native AMF isolates have beneficial effects on phytoremediation efficiency, but it is a time-consuming and laborious process to isolate and identify the most effective AMF strain from HM-contaminated soils (Vivas et al. 2003). Our study provided an alternative strategy to enhance positive impact of AMF strain on phytoremediation efficiency by domesticating it under HM-contaminated soil for 12 months.

The Pb hyperaccumulator is defined as being able to accumulate more than 1,000 mg kg<sup>-1</sup> Pb in plant aerial organs on a dry-weight basis (Baker and Brooks 1989). Furthermore, it is suggested that only species with both BCF and TF greater than one had the potential to be used for phytoextraction (Baker 1981), while the BCF is greater than one, and TF less than one in the plants had the potential for phytostabilization (Yoon et al. 2006). The current study showed that

*B. parviflora* grown in the Pb-contaminated soils contained higher concentration of Pb than that of the uncontaminated soils (Fig. 7). Although plants had the ability to accumulate Pb, the Pb concentrations in plant roots and leaves among all treatments were below but similar to the above-mentioned criterion. Based on the average BCFs of different plant organs (Table S2), the Pb was most efficiently accumulated in plant leaves (BCF=0.67) and followed by plant roots (BCF=0.65) under Pb500 treatment. Based on the average TFs (Table S2), the Pb was most efficiently translocated from roots to leaves under Pb2000 treatment (TF=1.10) and followed by Pb500 treatment (TF=1.08). The results indicated that *B. parviflora* could be identified as a potential Pb hyperaccumulator (Fig. S2).

Several studies have indicated that AMF inoculation can promote plant growth, nutrient uptake, and tolerance to HM stress (Yang et al. 2015a; Adeyemi et al. 2021). Zhan et al. (2018) reported that the root and shoot biomass of maize inoculated with *F. mosseae* were significantly higher than that of non-mycorrhizal plants in HM-contaminated soils. Our findings were consistent with these



**Fig. 4** Chlorophyll concentration and chlorophyll a/b ratio in leaf of *B. parviflora* inoculated without or with *F. mosseae* (Fm) under different Pb stress levels. Pb0, 0 mg kg<sup>-1</sup> Pb stress level; Pb500, 500 mg kg<sup>-1</sup> Pb stress level; Pb2000, 2000 mg kg<sup>-1</sup> Pb stress level; NM, non-mycorrhizal inoculation; NDFm, non-domesticated *F. mos-*

*seae* inoculation; DFm, domesticated *F. mosseae* inoculation. The subscript numbers present the duration of domestication (month). Data represent mean  $\pm$  SD for biological replicates ( $n=5$ ). The same letter indicates no significant difference at each Pb stress level (Duncan's test,  $p < 0.05$ )

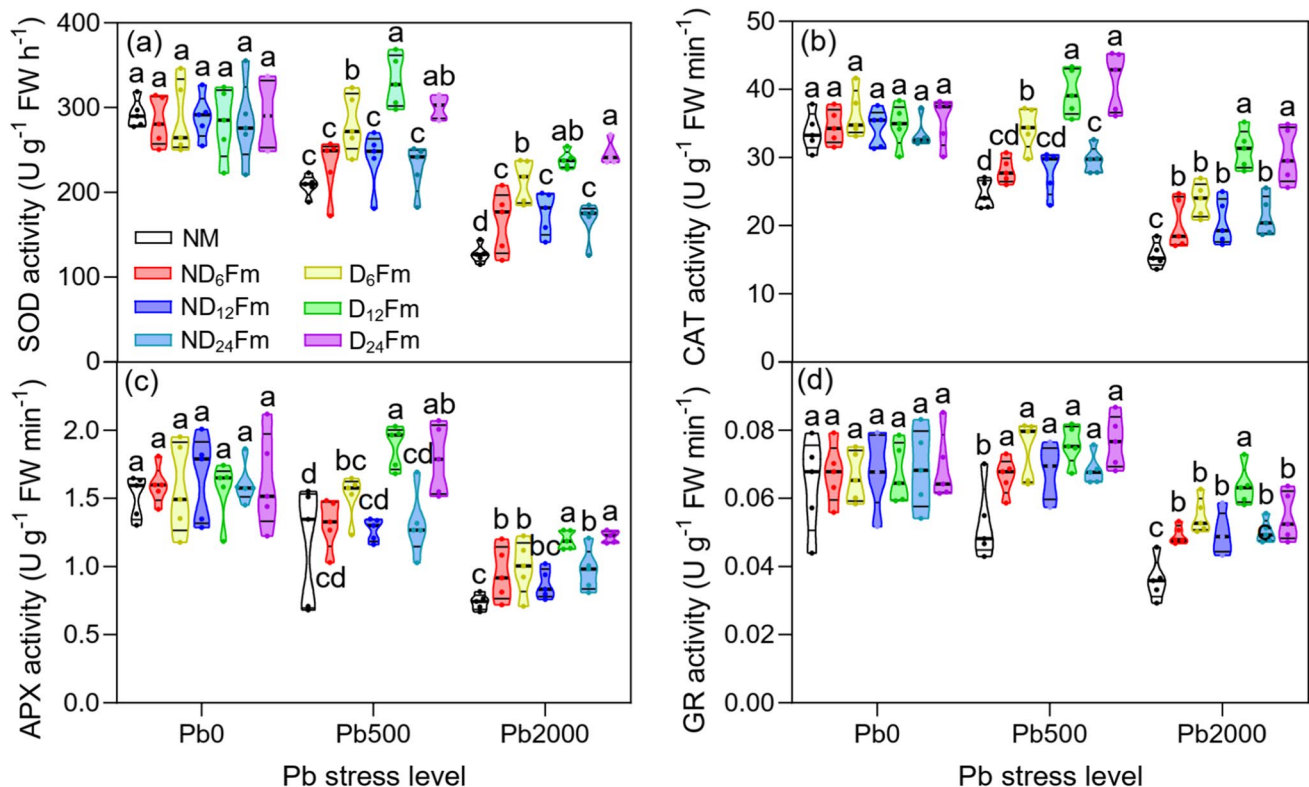
previous studies and indicated that the beneficial effects of AMF on plant growth could be attributed to MC and HLD based on the mantel test (Fig. 8). Additionally, the current study further revealed that the domesticated AMF was more effective than the non-domesticated fungi in promoting plant growth under Pb stress treatments (Figs. 2, S1). This indicated that an **ecophysiological adaptation of *F. mosseae* to the Pb-contaminated soil may have occurred, and the non-domesticated AMF may not function well even when colonizing plants to high levels in sterile soils.** However, our study found that the domesticated *F. mosseae* showed negative effect on plant biomass under the control treatment (0 mg kg<sup>-1</sup> Pb) (Fig. 2). It is quite possible that the domesticated *F. mosseae* had adapted well to the Pb-contaminated soil and could not form a functional symbiosis with *B. parviflora* in uncontaminated soils. This speculation could be supported by the lower MC and HLD in the roots of DFm plants compared with the NDFm plants under the control treatment (Fig. 1).

The more beneficial effect of domesticated *F. mosseae* on plant growth and stress tolerance compared with non-domesticated fungi was mainly attributed to the maintenance of the photosynthesis and improvement of antioxidant enzyme

activities under Pb stress conditions. In the present study, the chlorophyll concentration, Pn, Gs, and Tr of plants decreased significantly under Pb2000 treatment, and the decrease of chlorophyll concentration, Pn, Gs, and Tr of NDFm plants was higher than that of DFm plants (Figs. 3, 4). This indicated that NDFm plants were more sensitive to Pb stress and showed a greater reduction in photosynthetic rate, and DFm plants were able to maintain photosynthetic functionality for longer in response to Pb-contaminated soil. The inoculation of domesticated *F. mosseae* probably resulted in upregulated chloroplast gene expression, thereby contributing to higher PSII efficiency and enhancing photosynthetic capacity under Pb stress conditions (Chandrasekaran et al. 2019). Furthermore, chlorophyll is able to absorb energy from sunlight and then transform water and carbon dioxide to carbohydrates and oxygen. Therefore, chlorophyll is essential for plant photosynthesis, and high concentration of chlorophyll in DFm plants can lead to high photosynthetic activity (Yang et al. 2014; Mahama et al. 2016).

**Exposure of plants to stress environment is known to induce formation of reactive oxygen species (ROS), which are involved in damage mechanisms and then results in the inhibition of plant growth and development (Smimoff 1995;**





**Fig. 5** The activities of SOD (a), CAT (b), APX (c), and GR (d) in leaf of *B. parviflora* inoculated without or with *F. mosseae* (Fm) under different Pb stress levels. Pb0, 0  $\text{mg kg}^{-1}$  Pb stress level; Pb500, 500  $\text{mg kg}^{-1}$  Pb stress level; Pb2000, 2000  $\text{mg kg}^{-1}$  Pb stress level; NM, non-mycorrhizal inoculation; NDFm, non-domesticated

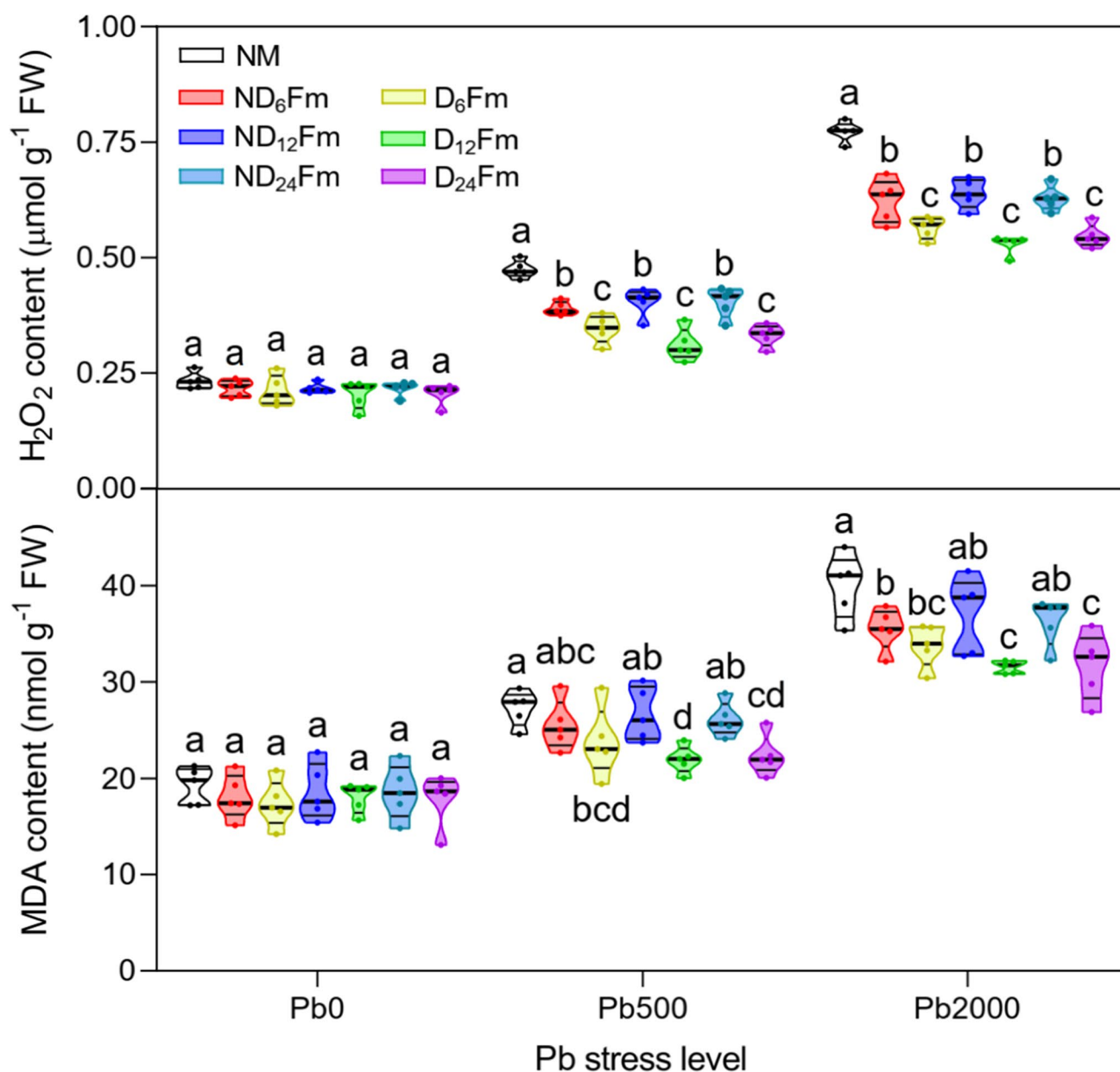
*F. mosseae* inoculation; DFm, domesticated *F. mosseae* inoculation. The subscript numbers present the duration of domestication (month). Data represent mean  $\pm$  SD for biological replicates ( $n=5$ ). The same letter indicates no significant difference at each Pb stress level (Duncan's test,  $p < 0.05$ )

Das and Roychoudhury 2014). In our study, domesticated *F. mosseae* inoculation increased the SOD, CAT, APX, and GR activities significantly in the leaves of *B. parviflora* compared with the non-domesticated *F. mosseae* inoculation in Pb-contaminated soils, resulting in low  $\text{H}_2\text{O}_2$  and MDA contents (Figs. 5, 6). SOD dismutates superoxide radicals to  $\text{H}_2\text{O}_2$ , which is then converted into  $\text{H}_2\text{O}$  and  $\text{O}_2$  by CAT and APX, while GR sustains the reduced status of GSH via ascorbate–glutathione pathway and plays an essential role in maintenance of sulfhydryl (–SH) group (Yousuf et al. 2012). Therefore, we can infer that the domesticated *F. mosseae* inoculation was more effective than the commercial *F. mosseae* inoculation in promoting plant growth and resistance to oxidative damage caused by reducing excessive accumulation of ROS in Pb stress conditions. This might be attributed to the strong ability of domesticated AMF in activating the expression of genes encoding antioxidant enzymes to maintain the cellular oxidative equilibrium and reduce the free radical damage (Riaz et al. 2020).

The beneficial effects of the domesticated *F. mosseae* on phytoremediation efficiency were attributed to not only large

plant biomass but also high Pb accumulation in plant organs (Fig. S2). In the present study, the higher Pb concentration in the roots of DFm plants was observed compared with that of NDFm or non-mycorrhizal plants under Pb stress treatments (Fig. 7). Several studies indicated that mycorrhizal plants could confer greater Pb concentration in the roots through mechanisms such as immobilization and chelation of metals in hyphae and compartmentalization within fungal cells (Zhang et al. 2010). Additionally, AMF-produced glomalin, tightly bound in AMF hyphae and spore walls, is a kind of glycoprotein, which has the ability to efficiently sequester Pb in soils (Malekzadeh et al. 2016).

In this study, *B. parviflora* presented to be a good candidate for the Pb phytoaccumulator (Fig. 9), due to the high metal accumulation in plant roots and leaves (Deng et al. 2004). Stoltz and Greger (2002) reported that mycorrhizal inoculation increased exclusion strategy of plants on the basis of translocation factor. It is supported by our previous study where it was found that AMF inoculation significantly increased Pb concentration in the roots but decreased Pb concentration in the stems and leaves of *Robinia pseudoacacia*

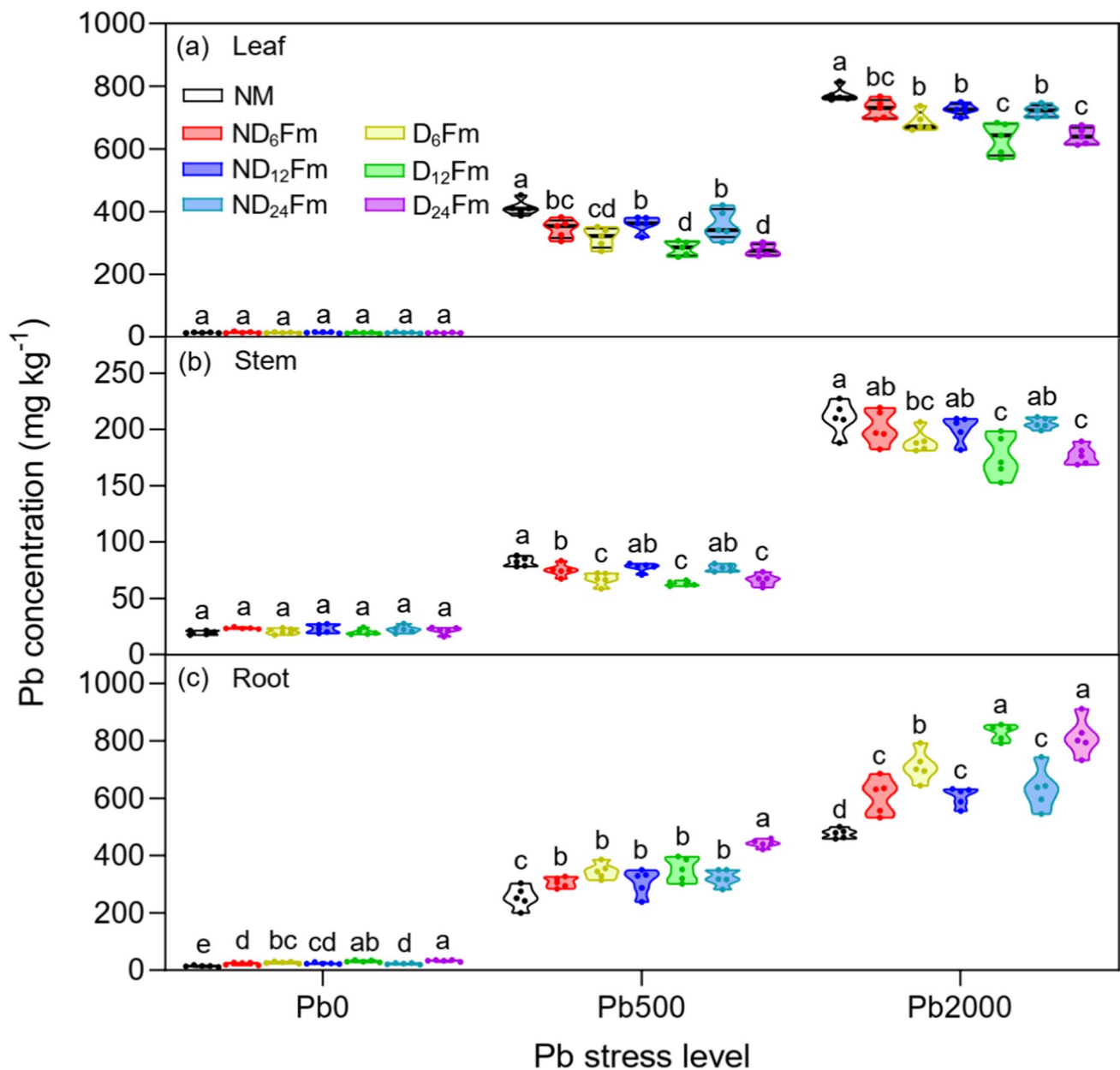


**Fig. 6** The hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, **a**) and malondialdehyde (MDA, **b**) contents in leaf of *B. parviflora* inoculated without or with *F. mosseae* (Fm) under different Pb stress levels. Pb0, 0 mg kg<sup>-1</sup> Pb stress level; Pb500, 500 mg kg<sup>-1</sup> Pb stress level; Pb2000, 2000 mg kg<sup>-1</sup> Pb stress level; NM, non-mycorrhizal inoculation; NDFm, non-domest-

cated *F. mosseae* inoculation; DFm, domesticated *F. mosseae* inoculation. The subscript numbers present the duration of domestication (month). Data represent mean ± SD for biological replicates (*n* = 5). The same letter indicates no significant difference at each Pb stress level (Duncan's test, *p* < 0.05)

seedlings (Yang et al. 2015a). Our study further confirmed that the inoculation of domesticated *F. mosseae* could greatly enhance Pb accumulation in plant roots compared with the non-domesticated fungus (Fig. 9). This phenomenon might be partly explained by the high MC and HLD of domesticated *F. mosseae* under Pb stress treatments (Fig. 1). However, DFm plants had a significantly lower stem and leaf Pb concentrations than NDFm plants grown in Pb-contaminated

soils (Fig. 7). The result was consistent with Vogel-Mikuš et al. (2006) who suggested that a large amount of HMs could be concentrated in mycorrhizal structures, such as fungal mycelia and vesicle, thereby minimizing metal translocation from the root to the aerial parts of the plant. The domesticated *F. mosseae* was more effective in providing a barrier against Pb transfer to plant leaf and protecting the photosystems from Pb-induced damage.



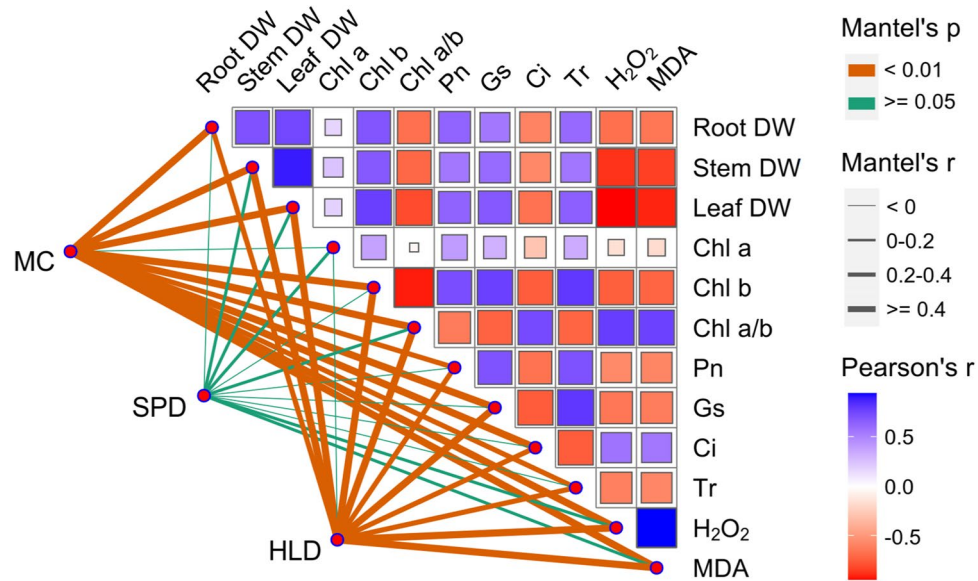
**Fig. 7** Pb concentration in leaf (a), stem (b), and root (c) of *B. parviflora* inoculated without or with *F. mosseae* (Fm) under different Pb stress levels. Pb0, 0 mg kg<sup>-1</sup> Pb stress level; Pb500, 500 mg kg<sup>-1</sup> Pb stress level; Pb2000, 2000 mg kg<sup>-1</sup> Pb stress level; NM, non-mycorrhizal inoculation; NDFm, non-domesticated *F. mosseae* inocu-

lation; DFm, domesticated *F. mosseae* inoculation. The subscript numbers present the duration of domestication (month). Data represent mean  $\pm$  SD for biological replicates ( $n=5$ ). The same letter indicates no significant difference at each Pb stress level (Duncan's test,  $p<0.05$ )

## Conclusions

Phytoremediation is receiving more and more attention in recent years. The potential importance of AMF in phytoremediation of Pb-contaminated soils has been demonstrated due to their beneficial effects on plant growth and Pb accumulation. The present study demonstrated that the domesticated AMF were more potentially useful

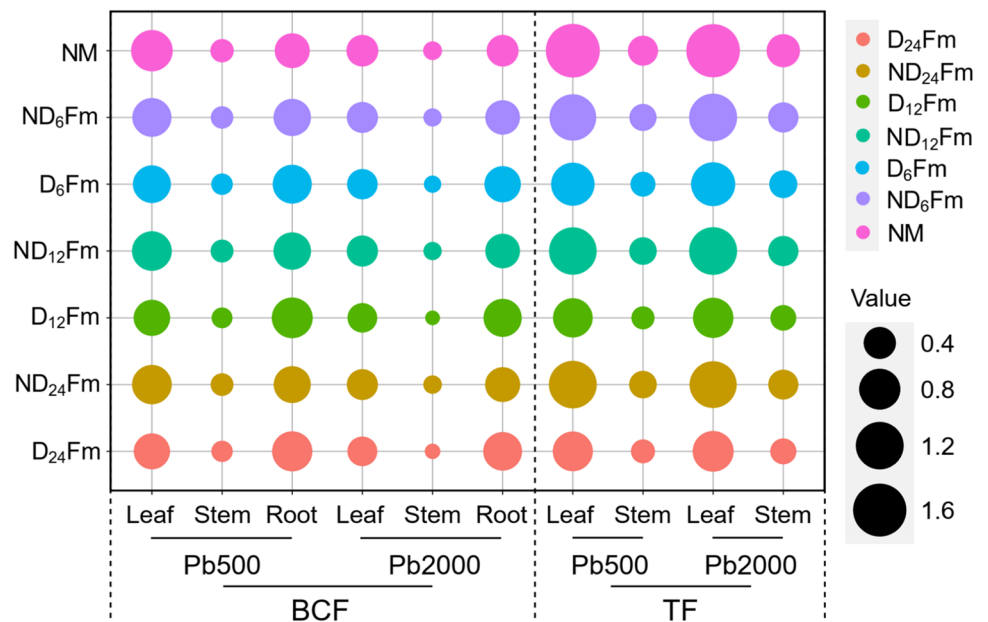
for the phytostabilization of Pb-contaminated soils than non-domesticated AMF, for possible control of Pb translocation in food chains, and subsequently for reducing environmental risks. For phytostabilization purposes, further studies were required to identify domesticated AMF species having more positive responsiveness to host plant. There is also a need to conduct systematic studies to screen for host-AMF compatibility and to understand



**Fig. 8** Pairwise comparisons of correlations between AMF growth index (MC, SPD, and HLD) and plant physiochemical parameters (dry weight, chlorophyll concentration, leaf gas exchange parameters,  $\text{H}_2\text{O}_2$ , and MDA concentrations). Pb0, 0  $\text{mg kg}^{-1}$  Pb stress level; Pb500, 500  $\text{mg kg}^{-1}$  Pb stress level; Pb2000, 2000  $\text{mg kg}^{-1}$  Pb stress level; MC, mycorrhizal colonization; SPD, spore density; HLD, hyphal length density; DW, dry weight; Pn, net photosynthetic rate; Ci, intercellular  $\text{CO}_2$  concentration; Gs, stomatal conductance; Tr,

transpiration rate; Chla, chlorophyll a concentration; Chlb, chlorophyll b concentration; Chla/b, chlorophyll a/b ratio;  $\text{H}_2\text{O}_2$ , hydrogen peroxide content; MDA, malondialdehyde. Pearson's correlation is shown in a color gradient. AMF growth index based on Bray–Curtis distance were correlated to plant physiochemical parameters by Mantel test, with edge representing Mantel's  $r$  for correlations, and the color corresponding to the significance

**Fig. 9** The bioconcentration factor (BCF) and translocation factor (TF) of Pb in different organs of *B. parviflora* inoculated without or with *F. mosseae* (Fm) under different Pb stress levels. Pb0, 0  $\text{mg kg}^{-1}$  Pb stress level; Pb500, 500  $\text{mg kg}^{-1}$  Pb stress level; Pb2000, 2000  $\text{mg kg}^{-1}$  Pb stress level; NM, non-mycorrhizal inoculation; NDFm, non-domesticated *F. mosseae* inoculation; DFm, domesticated *F. mosseae* inoculation. The subscript numbers present the duration of domestication (month). The same letter indicates no significant difference at each Pb stress level (Duncan's test,  $p < 0.05$ )





the mechanisms of how domesticated AMF contribute to the enhancement of phytostabilization.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11356-022-18588-2>.

**Author contribution** YY and XW designed and conceived the experiment. YY, BH, and JX carried out the experiments and collected the empirical data. ZT and ZL performed the data analysis. YY and XW wrote the paper with contributions from ZT and ZL.

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**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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