Arbuscular mycorrhizal fungi increase Pb uptake of colonized and non-colonized Medicago truncatula root and deliver extra Pb to colonized root

Haoqiang Zhang
Northwest A&F University
Wei Ren (renw@nwsuaf.edu.cn)
NWSUAF: Northwest Agriculture and Forestry University
Yaru Zheng
Northwest A&F University
Fei Zhao
Northwest A&F University
Ming Tang
Northwest A&F University

Manuscript

Keywords: Pb uptake, translocation, resource allocation, arbuscular mycorrhizal pathway for Pb uptake, transpiration pull

Posted Date: February 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-208596/v1

Abstract

Aims

Arbuscular mycorrhizal (AM) fungi form symbiosis with terrestrial plants and improve lead (Pb) tolerance of host plants. The AM plants accumulate more Pb in root than their non-mycorrhizal counterparts. However, the direct contribution of the mycorrhizal pathway to host plant Pb uptake was less reported.

Methods

In this study, the AM fungi colonized and non-colonized root of *Medicago truncatula* was separated by a split-root system, and their differences in responding to Pb application was compared.

Results

Inoculation of *Rhizophagus irregularis* increased shoot biomass accumulation and transpiration, and decreased both colonized and non-colonized root biomass accumulation. Application of Pb in the non-colonized root compartment increased the colonization rate of *R. irregularis* and up-regulated the relative expressions of *MtPT4* and *MtBCP1* in the colonized root compartment. Inoculation of *R. irregularis* increased the Pb uptake in both colonized and non-colonized plant root, while *R. irregularis* transferred Pb to the colonized root. The Pb transferred through the mycorrhizal pathway had low mobility move from root to shoot, and might be sequestrated and compartmented by *R. irregularis*.

Conclusions

The Pb uptake of plant root might follow water flow that facilitated by the aquaporin MtPIP2. The quantification of Pb transfer via mycorrhizal pathway and the involvement of MtPIP2 deserve further study.

Introduction

Heavy metal contamination in soil is a worldwide issue due to rapid urbanization, mining, sewage sludge, application of fertilizers, and other anthropogenic activities (Fan et al. 2020; Gonzalez-Alcaraz et al. 2018; Sidhu et al. 2017). Lead (Pb) is one of the most common heavy metal pollutants in China (Li et al. 2014b) and is a non-essential element that have an immense risk for human beings especially for children (Baloch et al. 2020; Wang et al. 2020). Phytoremediation is an efficient and noninvasive way to remediate soils (Chang et al. 2018; Ma et al. 2019). The application of microorganisms in phytoremediation of Pb has received extensive attention (Gonzalez-Chavez et al. 2009; Jan and Parray 2016; Jia et al. 2016).

Arbuscular mycorrhizal (AM) fungi can establish mutualistic symbioses with more than 80% of terrestrial plants in different ecosystems (Davison et al. 2015; Parniske 2008) including Pb polluted areas (Faggioli et al. 2019; Zhang et al. 2020a). With AM fungal colonization, plants usually have higher biomass (Chen et al. 2005; Huang et al. 2017), increased antioxidant enzymes activities and photosynthetic rates, and showed improved Pb tolerance (Huang et al. 2017; Zhang et al. 2019).

Establishment of AM symbiosis leads to enhancement of host plant photosynthetic rates, transpiration flow, and water uptake (Gavito et al. 2019; Huang et al. 2017; Kaschuk et al. 2009; Mortimer et al. 2008; Puschel et al. 2020). The water transport from soil to plant leaves require participation of aquaporins (AQPs), which are a class of membrane intrinsic proteins (MIPs) that mediate water transport across membranes following an osmotic gradient (Li et al. 2014a) and participate in hydraulic conductance regulation (Maurel et al. 2015; Watts-Williams et al. 2019). Plant AQPs include plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), NOD26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), and uncategorized intrinsic proteins (XIPs) (Maurel et al. 2015). In AM plants, the uptake of water and nutrients was suggested via two pathways, of which one pathway (plant root pathway) relies on plant root and the other pathway (mycorrhizal pathway) relies on the AM fungal hyphae (Ferrol et al. 2016; Smith and Smith 2011). It was confirmed that nutrients transportation via mycorrhizal pathway to plant needs participation of aquaporin (Kikuchi et al. 2016).

In previous studies, the AM plants accumulated more Pb in root and less Pb in shoot than their non-mycorrhizal counterparts (Huang et al. 2017; Sudova and Vosatka 2007; Yang et al. 2016). However, the direct contribution of mycorrhizal pathway to host plant Pb uptake has not been reported. In this study, we used a split-root system (Fig. 1a) to separate the AM fungi colonized and non-colonized root, and investigated the influence of AM fungi on the root Pb uptake and Pb transfer from root to shoot. We hypothesized that: (1) the AM fungi increase the Pb uptake in both colonized and non-colonized root part through improvement of plant transpiration; (2) the AM fungi deliver Pb to colonized root part through the mycorrhizal pathway; and (3) the plant Pb uptake involves participation of plant aquaporin. To our knowledge, this is the first study use split-root system to verify the contribution of AM fungi to plant Pb uptake.

Materials And Methods

Plant material, growth substrate, and AM fungal inoculum

Seeds of *M. truncatula* (Jemalong A17) were kindly provided by Prof. Philipp Franken (Plant Physiology Department, Humboldt University of Berlin). The seeds were soaked in concentrated sulfuric acid for 10 min and washed 5 times using sterile distilled water, then incubated in 3% (v/v) sodium hypochlorite solution for 3 min. After surface sterilization, seeds were washed 3 times with sterile distilled water. Sterilized seeds were germinated in Petri dishes with water agar (0.7%; w/v) at 4 °C for 4 days, and at room temperate in darkness for 2 days. Germinated seeds were transplanted into plastic pots (10 cm in diameter, 12 cm in height) with sterilized sands to grow roots. After 8 weeks, the roots were washed with

tap water and divided into two halves of a similar size. Then, the roots were planted in a split-root system consisting of two adjoining compartments with one root half in each (as Fig. 1a). Each root compartment was filled with 0.8 kg growth substrate. The split-root system was made of acrylic plate and was stuck by ABS plastic adhesive. Two compartments were separated by an acrylic plate to prevent Pb spill. The split-root system was raised 4 cm above the growth substrate on each side to prevent transfer of Pb and AM fungal inoculum between compartments.

The growth substrate was a mixture of sand and vermiculite (1: 1; v: v). The sand was sieved through a 2 mm sieve, thorough washed with tap water, and sterilized at 170 °C for 4 h. The vermiculite was autoclaved at 121 °C for 2 h for sterilization. The vermiculite was clay mineral with 2:1 crystalline structure which contain two silica tetrahedral sheets with a central alumina octahedral layer (dos Anjos et al. 2014).

The AM inoculum of *Rhizophagus irregularis* (Bank of Glomales in China, No. BGC BJ09), which consisted of a sandy substrate that contained spores (approximately 21 spores per gram), mycelium, and colonized root fragments, was provided by the Beijing Academy of Agriculture and Forestry Sciences (Beijing, China) and multiplied in pot cultures of *Zea mays* Linn.

Experimental design

The experiment consisted of 5 treatments (Fig. 1b) which include neither AM inoculum nor Pb application in root compartment (CK), only AM inoculum application in one root compartment (OA), only Pb application in one root compartment (OP), AM inoculum and Pb applications in separated root compartments (SE), and AM inoculum and Pb applications in the same root compartment together (TO). The seedling roots in different root compartments were also denominated according to its position, AM status, and Pb status (as Fig. 1b). Ten-gram inoculum was applied underneath the root of *M. truncatula* seedlings at transplanting into split-root system in mycorrhizal treatment, while sterilized inoculum (170 °C for 4 h) was applied in the non-mycorrhizal treatment. The Pb application was 4 weeks post the AM inoculum application to ensure AM fungal colonization, and was accomplished by applying 32 mL 20 g L^{-1} Pb $(NO_3)_2$ solution to the junction of root and growth substrate by syringe to reach 800 mg kg^{-1} Pb in growth substrate. Each treatment contained 3 replicates and each replicate included 4 seedlings.

Seedlings were grown in a greenhouse with 28 °C/24 °C day/night temperatures under 16 h daylight and 40-60% humidity. Twenty milliliters of modified Hoagland's nutrient solution (Hoagland and Arnon 1950) containing 10% phosphate (0.1 mM KH_2PO_4) was added twice a week to each root compartment before Pb application. After Pb application, only water (20 mL) was added to root compartment of all treatments once in 2 days to avoid direct precipitation of Pb.

Plant sampling, biomass, and AM fungal colonization

At harvest (8 weeks after Pb treatment), biomass of shoots and roots and fresh-to-dry mass ratio (Ma et al. 2014) were measured. After measuring fresh weights, part of leaves was dried in an oven at 105 °C

with forced air circulation for 15 min to inactivate enzymes and then turned to 65 °C until they reached a constant weight for Pb content measurement. The remaining part of leaves were immediately frozen in liquid nitrogen and stored at -80 °C. Roots were soaked with water for the root structure scanning (EPSON EXPRESSION 1680, Seiko Epson Corporation, Japan). After root structure scanning, part of roots was fixed in FAA solution (37% formaldehyde: glacial acetic acid: 95% ethanol, 9: 0.5: 0.5, v: v: v) for assessment of the AM colonization as Koske and Gemma (1989). The total colonization and arbuscule colonization were measured using magnified cross sections method as McGonigle et al. (1990). Part of roots were dried in an oven at 105 °C for 15 min and then at 65 °C with forced air circulation until they reached a constant weight for Pb concentration measurement. The remaining part of roots were immediately frozen in liquid nitrogen and stored at -80 °C.

Pb concentration and content

The dry sample was ground in a mortar and placed in the digestion tube (50 mL) with 5 mL mixture of HNO_3 + $HClO_4$ (4:1) to digest at a temperature that gradually increased to 220 °C. Pb concentration was measured using flame atomic absorption spectrometry (PinAAciie 900F, American). Pb content was calculated using Pb concentration, fresh-to-dry mass ratio, and plant biomass (Ma et al. 2014). The ratio of root Pb content to root surface area was calculated to indicate the root surface's contribution to Pb absorbing capacity.

Photosynthesis

The fifth leaf of each plant was used for the measurements. At the harvest day from 8:00 to 11:30 a.m., the net photosynthetic rate (Pn), intercellular CO_2 concentration (Ci), transpiration rate (Tr), and conduction to H_2O (Gs) were measured using Li-6400 portable open flow gas-exchange system (Li Corporation, American) and converted with measured leaf area. Leaf area was measured by ImageJ 1.38 (National Institutes of Health, American) after pictured by camera. The measurement conditions were as follows: photosynthetically active irradiation, 1000 μmol m^{-2} s^{-1}; temperature, 22 °C; relative humidity, 30%; and CO_2 concentration of sample cell, 419 μmol mol^{-1}.

Gene Relative Expression

Root samples that stored at -80 °C were ground and homogenized with mortar and pestle with liquid nitrogen. Total RNA was isolated from root samples by E.Z.N.A.^{TM} Plant RNA Kit (Omega Biotech, American) following the supplier’s instructions. After quantification of RNA yield by Nanodrop 2000 (Thermo Scientific, American), cDNA was synthesized from 1000 ng of RNA using FastKing RT Kit with gDNase (TIANGEN Biotech, China). The synthesized cDNA was diluted fivefold and used as the template for PCR reactions.

The primers used in the qRT-PCR were as Watts-Williams et al. (2019) and were listed in Supplementary Table S1. Gene relative expression was normalized to the *M. truncatula* housekeeping gene *MtEF1α*. The qRT-PCR reaction was conducted using the CFX96 real-time PCR detection system (Bio-Rad Laboratories,

American) and contained 5 μL ChamQ™ Universal SYBR® qPCR Master Mix (Vazyme Biotech, China), 0.5 μL (10 μM) of each primer, 1 μL a cDNA, and 3 μL ddH_2O. The PCR procedure consisted of a 3 min denaturation at 95 °C; 40 cycles of denaturation at 95 °C for 10 s, annealing at the annealing temperature (Supplementary Table S1) for 20 s, extension at 72 °C for 20 s; followed by heating from 60 to 95 °C to check the specificity of the PCR amplification. All samples were technically replicated twice. Negative controls without cDNA were run within each analysis. The relative quantity of transcripts was determined using the $2^{-\triangle CT}$ method (Livak and Schmittgen 2001).

Statistical analysis

Statistical analysis was performed using the SPSS 19.0 statistical programmed (SPSS, American). Data used for one-way ANOVA complied with the assumption of a normal distribution, and the variance equality was also tested by LSD test. Correlation analysis were analyzed by Spearman's (Supplementary Table S2). Figures were drawn with Origin 2018 (Origin Lab, American). Heatmap and cluster analysis of relative genes were using MetaboAnalyst 4.0 (Chong et al. 2018).

Results

Biomass and colonization

Eight weeks after Pb application in root compartments, the biomass of shoot and root in different compartments was recorded (Fig. 2a). In CK treatment, the biomass of root in two compartments showed no difference, which indicated that the spilt-root system divided roots into two part evenly. Inoculation of *R. irregularis* in one root compartment (comparing treatment OA with treatment CK) increased the shoot biomass (not significantly), but reduced root biomass both locally (OA-RAN) and systemically (OA-LNN). Application of Pb in one root compartment (comparing treatment OP with CK) significantly reduced shoot biomass and root biomass in the other root compartment (OP-LNN). When plants received both inoculation of *R. irregularis* and Pb (together and separated), the shoot biomass was not affected significant and the root biomass was reduced.

No AM fungal feature was observed in roots from non-mycorrhizal treatment and root compartments (Fig. 2b, c). Over 60% of root (OA-RAN) was colonized and the typical feature (arbuscules) was observed in treatment which received only AM fungal inoculum. Application of Pb showed little limitation on the total and arbuscular colonization of *R. irregularis* in the together treatment (TO), but promoted the colonization in the separated treatment (SE). The relative expression of *MtPT4* and *MtBCP1* that were used as the indicator of functional and numeric of arbuscules (Javot et al. 2007; Parádi et al. 2010) resembled the colonization results (Fig. 3a, b).

Root structure, Pb concentration and content

In CK treatment, root surface, length, and average diameter in two compartments showed no difference (Fig. 4a, b, c). Inoculation of *R. irregularis* or Pb application only in one root compartment (treatment OA

and treatment OP) did not show local and systemic influence on the root surface area, length, and average diameter, except for OA-RAN treatment in which the average root diameter was locally reduced. It indicated that inoculation of *R. irregularis* directly reduced average root diameter. When plant roots received both inoculation of *R. irregularis* and Pb application separately (treatment SE) and jointly (treatment TO), the root surface area, length, and average diameter were reduced (comparing with treatment CK).

Environmental Pb existed and was unable to eliminate as in previous study (Zhang et al. 2020b). The lowest concentration and content of Pb in root and shoot was shown in the CK treatment (Fig. 5a, b). Solely Pb application increased local root Pb concentration and shoot Pb concentration. The highest concentration and content of Pb in shoot was shown in separate treatment (SE). The highest concentration of Pb in root was shown in the SE-LNP root compartment, and the highest content of Pb in root was shown in the TO-RAP root compartment. Inoculation of *R. irregularis* in one root compartment increased Pb concentration in roots from the other compartment in which extra Pb solution was added (comparing SE-LNP with OP-RNP) or not (comparing OA-LNN and TO-LNN with CK-RNN). The Pb concentrations and contents in root from compartment that received Pb were increased compared with CK. Especially, the increment of Pb concentrations and contents in root was much higher when inoculation of *R. irregularis* involves (comparing SE-LNP and TO-RAP with OP-RNP). Besides, the Pb contents in root that have direct contact with *R. irregularis* (TO-RAP) was much higher than that in root that have indirect contact with *R. irregularis* (SE-LNP).

The ratio of root Pb content to root surface area was calculated to evaluate the contribution of plant root surface to Pb uptake (Fig. 5c). Compared with CK, the increased ratios were observed in the root compartment that received extra Pb (SE-LNP and TO-RAP). The highest ratio was shown in the root compartment (TO-RAP) which received both *R. irregularis* inoculum and Pb application.

Photosynthesis

Solely *R. irregularis* inoculation and Pb application increased Pn, Tr, and Gs respectively (Fig. 6a, c, d). Application of Pb increased Ci while *R. irregularis* inoculation decreased Ci (Fig. 6b). When Pb and *R. irregularis* inoculum were applied together in the same root compartment, the Gs was higher than they were applied separately in different root compartments.

Relative expression of aquaporins

In order to test the hypothesis that inoculation of *R. irregularis* improves the capacity of Pb uptake in *M. truncatula* with the help of aquaporins, the relative expression of aquaporins were detected (Fig. 7). Inoculation of *R. irregularis* locally increased the relative expression of *MtAQP1* in root compartment SE-RAN, *MtPIP2* in root compartment TO-RAP, and *MtNIP1* in root compartment OA-RAN and TO-RAP (comparing with CK). Inoculation of *R. irregularis* also systemically increased the relative expression of *MtPIP1* in root compartment OA-LNN. Application of Pb locally increased the relative expression of *MtAQP1* in root compartment OP-RNP, *MtPIP2* in root compartment TO-RAP, and *MtNIP1* in root

compartment TO-RAP (comparing with CK). The relative expression of *MtNIP4* was higher in root compartment OA-RAN than that in root compartment TO-RAP.

Correlation analysis

From the Spearman correlation analysis (Supplementary Table S2), Pb concentration and content of shoot and root showed negative correlations with root biomass, root surface area, and root length, but positive correlations with the relative expression of *MtPIP2* in root. Moreover, root Pb concentration showed a positive correlation with the relative expression of *MtPT4*. Shoot Pb content showed positive correlations with the relative expressions of *MtPT4* and *MtBCP1* in root. The ratio of root Pb content to root surface area showed positive correlations with Pb concentration and content of shoot and root. In addition, the ratio of root Pb content to root surface area showed positive correlations with the relative expression of *MtPT4*, *MtBCP1*, and *MtPIP2* in root.

Discussion

AM fungi can survive in various environments including Pb polluted areas, improve growth and stress tolerance of host plants (Faggioli et al. 2019; Yang et al. 2015; Zhang et al. 2020b). Under Pb stress, AM fungi colonized plants were reported to have better growth (Chen et al. 2005; Dhawi et al. 2016; Huang et al. 2017) and accumulate more Pb in root than in shoot (Yang et al. 2016). To verify the influence of AM fungi on plant root Pb uptake, a spilt-root system was established to separate the colonized and non-colonized root and compare their differences in Pb uptake.

The evenly distributed root biomass in two root compartments of CK treatment demonstrated a success of the split-root system. The similar split-root system was used in other studies for the systemic influence of AM fungi in *M. truncatula* (Liu et al. 2007; Zhang and Franken 2014). When roots of *M. truncatula* were only colonized in one root compartment, the AM fungi showed improvement of plant shoot growth and systemic reduction of plant root growth (Fig. 2) as reported previously (Liu et al. 2007). It might be due to the diluted mycorrhizal effect on nutrient and water uptake (Weissenhorn and Leyval 1995). The systemic influence of AM fungi on root growth reduction was due to the carbon investment of plant in AM fungal hyphae, which require less carbon than root (Chen et al. 2016). Application of Pb had a negative effect on the biomass accumulation of non-AM plants confirming a sensitivity of *M. truncatula* to Pb toxicity in the treated concentration (Zhang et al. 2019). The beneficial effect and alleviation of Pb toxicity by AM fungi, which indicated by the higher shoot biomass and photosynthetic parameters of AM plants than those of the non-AM plants, was consistent with previous studies (Huang et al. 2017; Sudova and Vosatka 2007; Zhang et al. 2020b).

Inoculation of *R. irregularis* successfully established AM symbiosis in root compartments, and set the basis for this study. The colonization rate showed similar tendency with the relative expression of *MtPT4* and *MtBCP1* in root, and this support the view that the expression of these two genes was the indicator of AM symbiosis in root of *M. truncatula* (Javot et al. 2007; Parádi et al. 2010; Zhang and Franken 2014). When Pb and *R. irregularis* were applied in different root compartments, the colonization rate of *R.*

irregularis increased (Punamiya et al. 2010) and the relative expressions of *MtPT4* and *MtBCP1* up-regulated. This might be due to the increased reliance of plant on AM symbiosis, which maintains the balance of plant mineral elements uptake (Ferrol et al. 2016; Zheng et al. 2015), and Pb disturbs plant ion homeostasis through hindering permeability of root cell plasma membrane (Sharma and Dubey 2005; Yadav 2010). Nevertheless, when Pb and *R. irregularis* were applied in the same root compartment, the extension of extraradical hyphae was inhibited (Weissenhorn et al. 1993), the reliance of plant on AM symbiosis was limited, and the colonization rate was restored.

Although Pb is a non-essential element, the uptake of Pb by plant is inevitable (Baloch et al. 2020; Wang et al. 2020; Yabe et al. 2018). Compared with non-mycorrhizal plants, the mycorrhizal plants usually had higher shoot biomass and Pb content in shoot, which was explained by the bio-dilution effect (Chen et al. 2005; Gonzalez-Chavez et al. 2004; Joner et al. 2000; Weissenhorn and Leyval 1995; Zhang et al. 2020b). Similar result was observed in this study, the Pb content in shoots of treatment TO was higher than that of treatment OP and the relative expression of *MtPT4* and *MtBCP1* was positive correlated with Pb content in shoot, yet the Pb concentrations in shoot of these treatments were alike (Fig. 5a, b). When *R. irregularis* and Pb were applied in different root compartments, the Pb concentration and content in shoot were also increased, and this indicated an improvement of Pb transfer from non-colonized root to shoot by AM symbiosis (Chen et al. 2005; Xin et al. 2017).

In root, Pb application increased the Pb concentration and content (Fig. 5a, b) and this was consistent with previous study (Xin et al. 2017). The increment of Pb concentration and content in colonized and non-colonized *M. truncatula* roots by *R. irregularis* (comparing TO-RAP and SE-LNP with OP-RNP) (Fig. 5a, b) was in accordance with previous study that AM plants accumulated more Pb in root than non-mycorrhizal plants (Sudova and Vosatka 2007). The increased root Pb concentration and content by *R. irregularis* might be due to the increased water and nutrient uptake of root, which was proven by the higher shoot biomass and lower root biomass of treatment SE and TO than those of treatment OP (Fig. 2a) and the positive correlations of the relative expression of *MtPT4* and root Pb content. Moreover, the increment of root Pb content by *R. irregularis* in colonized root was higher than that in non-colonized root (comparing TO-RAP with SE-LNP) (Fig. 5a). This indicated an increased Pb accumulation capability of AM fungi colonized root, which have two nutrient and water uptake pathways (Ferrol et al. 2016; Smith and Smith 2011) and may have the mycorrhizal pathway delivers Pb to plant root (Sudova and Vosatka 2007).

The Pb accumulation capability of different roots was compared through the ratio of root Pb content to root surface area. The ratio in treatment TO-RAP was higher than that in treatment SE-LNP (Fig. 5c) and was positive correlated with the relative expression of *MtPT4* and *MtBCP1*. This result further confirmed that the AM fungi supply Pb to colonized root besides increase Pb accumulation capability of plant root. However, the Pb concentration and content in shoot of treatment TO was lower than those of treatment SE (Fig. 5a, b), and this indicated that the Pb supplied by mycorrhizal pathway to plant root have lower mobility than Pb absorbed from growth substrate by plant root itself (Fig. 8). The retention of Pb by AM

fungi might be the results of sequestration by their cell wall and proteins, and compartmentation of vacuoles (Ferrol et al. 2016; Salazar et al. 2018).

The nutrient delivery of mycorrhizal pathway was ascertained to follow the water flow (Cooper and Tinker 1981; Kikuchi et al. 2016), which involves participation of aquaporins (Maurel et al. 2015). The relative expression of gene encoding MtPIP2, which was suggested to have higher water permeability than PIP1 and form heterotetramer with PIP1 (Jozefkowicz et al. 2016), was up-regulated in root compartment TO-RAP (Fig. 7) and was positive correlated with the Pb content and concentration in root and shoot, and the ratio of root content to root surface area. This result fitted the hypothesis that the Pb uptake by plant root follows water flow (Fig. 8). The specific role of MtPIP2 in Pb uptake is under study.

To summarize, inoculation of *R. irregularis* had a beneficial effect on *M. truncatula* and could alleviate the Pb toxicity. The AM symbiosis increased the Pb uptake in both colonized and non-colonized plant root, while the AM fungi transferred extra Pb to the colonized root section. The Pb transferred from soil to plant root by the mycorrhizal pathway had low mobility move from root to shoot, and might be sequestrated and compartmented by AM fungi. The Pb uptake of plant root might follow water flow that facilitated by the aquaporin MtPIP2. Further researches will quantify the Pb that directly transfer of from *R.irregularis* to plant root, and decipher the role of MtPIP2 in root Pb uptake.

Declarations

Acknowledgements

This research was funded by the Key projects of Guangzhou science and technology plan (201904020022), and National Natural Science Foundation of China (31700530). The authors declare that they have no conflict of interest.

Compliance with ethical standards

References

Baloch S, Kazi TG, Baig JA, Afridi HI, Arain MB (2020) Occupational exposure of lead and cadmium on adolescent and adult workers of battery recycling and welding workshops: Adverse impact on health. Sci Total Environ 720:137549. https://doi.org/10.1016/j.scitotenv.2020.137549

Chang Q, Diao FW, Wang QF, Pan L, Dang ZH, Guo W (2018) Effects of arbuscular mycorrhizal symbiosis on growth, nutrient and metal uptake by maize seedlings (*Zea mays* L.) grown in soils spiked with Lanthanum and Cadmium. Environ Pollut 241:607-615. https://doi.org/10.1016/j.envpol.2018.06.003

Chen WL, Koide RT, Adams TS, DeForest JL, Cheng L, Eissenstat DM (2016) Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. P Natl Acad Sci USA 113:8741-8746. https://doi.org/10.1073/pnas.1601006113

Chen X, Wu CH, Tang JJ, Hu SJ (2005) Arbuscular mycorrhizae enhance metal lead uptake and growth of host plants under a sand culture experiment. Chemosphere 60:665-671. https://doi.org/10.1016/j.chemosphere.2005.01.029

Chong J, Soufan O, Li C, Caraus I, Li SZ, Bourque G, Wishart DS, Xia JG (2018) MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res 46:486-494. https://doi.org/10.1093/nar/gky310

Cooper KM, Tinker PB (1981) Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas: IV. Effect of environmental variables on movement of phosphorus. New Phytol 88:327-339. https://doi.org/10.1111/j.1469-8137.1981.tb01728. x

Davison J, Moora M, Opik M, Adholeya A, Ainsaar L, Ba A, Burla S, Diedhiou AG, Hiiesalu I, Jairus T, Johnson NC, Kane A, Koorem K, Kochar M, Ndiaye C, Partel M, Reier U, Saks U, Singh R, Vasar M, Zobel M (2015) Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science 349:970-973. https://doi.org/10.1126/science.aab1161

Dhawi F, Datta R, Ramakrishna W (2016) Mycorrhiza and heavy metal resistant bacteria enhance growth, nutrient uptake and alter metabolic profile of sorghum grown in marginal soil. Chemosphere 157:33-41. https://doi.org/10.1016/j.chemosphere.2016.04.112

dos Anjos VE, Rohwedder JR, Cadore S, Abate G, Grassi MT (2014) Montmorillonite and vermiculite as solid phases for the preconcentration of trace elements in natural waters: Adsorption and desorption studies of As, Ba, Cu, Cd, Co, Cr, Mn, Ni, Pb, Sr, V, and Zn. Appl Clay Sci 99:289-296. https://doi.org/10.1016/j.clay.2014.07.013

Faggioli V, Menoyo E, Geml J, Kemppainen M, Pardo A, Salazar MJ, Becerra AG (2019) Soil lead pollution modifies the structure of arbuscular mycorrhizal fungal communities. Mycorrhiza 29:363-373. https://doi.org/10.1007/s00572-019-00895-1

Fan YZ, Xu ZH, Huang YK, Wang TB, Zheng SK, DePasquale A, Brueckner C, Lei Y, Li BK (2020) Long-term continuous and real-time in situ monitoring of Pb(II) toxic contaminants in wastewater using solid-state ion selective membrane (S-ISM) Pb and pH auto-correction assembly. J Hazard Mater 400:123299. https://doi.org/10.1016/j.jhazmat.2020.123299

Ferrol N, Tamayo E, Vargas P (2016) The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. J Exp Bot 67:6253-6265. https://doi.org/10.1093/jxb/erw403

Gavito ME, Jakobsen I, Mikkelsen TN, Mora F (2019) Direct evidence for modulation of photosynthesis by an arbuscular mycorrhiza-induced carbon sink strength. New Phytol 223:896-907. https://doi.org/10.1111/nph.15806

Gonzalez-Alcaraz MN, Loureiro S, van Gestel CAM (2018) Toxicokinetics of Zn and Cd in the earthworm *Eisenia andrei* exposed to metal-contaminated soils under different combinations of air temperature and soil moisture content. Chemosphere 197:26-32. https://doi.org/10.1016/j.chemosphere.2018.01.019

Gonzalez-Chavez MC, Carrillo-Gonzalez R, Gutierrez-Castorena MC (2009) Natural attenuation in a slag heap contaminated with cadmium: The role of plants and arbuscular mycorrhizal fungi. J Hazard Mater 161:1288-1298. https://doi.org/10.1016/j.jhazmat.2008.04.110

Gonzalez-Chavez MC, Carrillo-Gonzalez R, Wright SF, Nichols KA (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut 130:317-323. https://doi.org/10.1016/j.envpol.2004.01.004

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil, 2nd ed. Circular. vol 347. California Agricultural Experiment Station. https://doi.org/10.1016/S0140-6736(00)73482-9.

Huang L, Zhang HQ, Song YY, Yang YR, Chen H, Tang M (2017) Subcellular compartmentalization and chemical forms of lead participate in lead tolerance of *Robinia pseudoacacia* L. with *Funneliformis mosseae*. Front Plant Sci 8:517. https://doi.org/10.3389/fpls.2017.00517

Jan S, Parray JA (2016) Use of mycorrhiza as metal tolerance strategy in plants. In: Approaches to heavy metal tolerance in plants. Springer Singapore, Singapore, pp 57-68. https://doi.org/10.1007/978-981-10-1693-6_4

Javot H, Penmetsa RV, Terzaghi N, Cook DR, Harrison MJ (2007) A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. P Natl Acad Sci USA 104:1720-1725. https://doi.org/10.1073/pnas.0608136104

Jia X, Zhao YH, Liu T, Huang SP, Chang YF (2016) Elevated CO_2 increases glomalin-related soil protein (GRSP) in the rhizosphere of *Robinia pseudoacacia* L. seedlings in Pb- and Cd-contaminated soils. Environ Pollut 218:349-357. https://doi.org/10.1016/j.envpol.2016.07.010

Joner EJ, Briones R, Leyval C (2000) Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant Soil 226:227-234. https://doi.org/Doi 10.1023/A:1026565701391

Jozefkowicz C, Sigaut L, Scochera F, Soto G, Ayub N, Pietrasanta LI, Amodeo G, Flecha FLG, Alleva K (2016) PIP water transport and its pH dependence are regulated by tetramer stoichiometry. Biophys J 110:1312-1321. https://doi.org/10.1016/j.bpj.2016.01.026

Kaschuk G, Kuyper TW, Leffelaar PA, Hungria M, Giller KE (2009) Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? Soil Biol Biochem 41:1233-1244. https://doi.org/10.1016/j.soilbio.2009.03.005

Kikuchi Y, Hijikata N, Ohtomo R, Handa Y, Kawaguchi M, Saito K, Masuta C, Ezawa T (2016) Aquaporin-mediated long-distance polyphosphate translocation directed towards the host in arbuscular mycorrhizal symbiosis: application of virus-induced gene silencing. New Phytol 211:1202-1208. https://doi.org/10.1111/nph.14016

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res 92:486-488. https://doi.org/10.1016/S0953-7562(89)80195-9

Li G, Santoni V, Maurel C (2014a) Plant aquaporins: roles in plant physiology. BBA-Gen Subjects 1840:1574-1582. https://doi.org/10.1016/j.bbagen.2013.11.004

Li ZY, Ma ZW, van der Kuijp TJ, Yuan ZW, Huang L (2014b) A review of soil heavy metal pollution from mines in China: Pollution and health risk assessment. Sci Total Environ 468:843-853. https://doi.org/10.1016/j.scitotenv.2013.08.090

Liu JY, Maldonado-Mendoza I, Lopez-Meyer M, Cheung F, Town CD, Harrison MJ (2007) Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. Plant J 50:529-544. https://doi.org/10.1111/j.1365-313X.2007.03069.x

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402-408. https://doi.org/10.1006/meth.2001.1262

Ma Y, Rajkumar M, Oliveira RS, Zhang C, Freitas H (2019) Potential of plant beneficial bacteria and arbuscular mycorrhizal fungi in phytoremediation of metal-contaminated saline soils. J Hazard Mater 379:120813. https://doi.org/10.1016/j.jhazmat.2019.120813

Ma YL, He JL, Ma CF, Luo J, Li H, Liu TX, Polle A, Peng CH, Luo ZB (2014) Ectomycorrhizas with *Paxillus involutus* enhance cadmium uptake and tolerance in *Populus* x *canescens*. Plant Cell Environ 37:627-642. https://doi.org/10.1111/pce.12183

Maurel C, Boursiac Y, Luu DT, Santoni V, Shahzad Z, Verdoucq L (2015) Aquaporins in plants. Physiol Rev 95:1321-1358. https://doi.org/10.1152/physrev.00008.2015

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol 115:495-501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x

Mortimer PE, Pérez-Fernández MA, Valentine AJ (2008) The role of arbuscular mycorrhizal colonization in the carbon and nutrient economy of the tripartite symbiosis with nodulated *Phaseolus vulgaris*. Soil Biol Biochem 40:1019-1027 https://doi.org/10.1016/j.soilbio.2007.11.014

Parádi I, Tuinen Dv, Morandi D, Ochatt S, Robert F, Jacas L, Dumas-Gaudot E (2010) Transcription of two blue copper-binding protein isogenes is highly correlated with arbuscular mycorrhizal development in *Medicago truncatula*. Mol Plant Microbe In 23:1175-1183 https://doi.org/10.1094/MPMI-23-9-1175

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763-775. https://doi.org/10.1038/nrmicro1987

Punamiya P, Datta R, Sarkar D, Barber S, Patel M, Das P (2010) Symbiotic role of *Glomus mosseae* in phytoextraction of lead in vetiver grass [*Chrysopogon zizanioides* (L.)]. J Hazard Mater 177:465-474. https://doi.org/10.1016/j.jhazmat.2009.12.056

Puschel D, Bitterlich M, Rydlova J, Jansa J (2020) Facilitation of plant water uptake by an arbuscular mycorrhizal fungus: a Gordian knot of roots and hyphae. Mycorrhiza 30:299-313. https://doi.org/10.1007/s00572-020-00949-9

Salazar MJ, Menoyo E, Faggioli V, Geml J, Cabello M, Rodriguez JH, Marro N, Pardo A, Pignata ML, Becerra AG (2018) Pb accumulation in spores of arbuscular mycorrhizal fungi. Sci Total Environ 643:238-246. https://doi.org/10.1016/j.scitotenv.2018.06.199

Sharma P, Dubey RS (2005) Lead toxicity in plants. Brazilian Journal of Plant Physiology 17:35-52. https://doi.org/10.1590/S1677-04202005000100004

Sidhu GPS, Singh HP, Batish DR, Kohli RK (2017) Tolerance and hyperaccumulation of cadmium by a wild, unpalatable herb *Coronopus didymus* (L.) Sm. (Brassicaceae). Ecotox Environ Safe 135:209-215. https://doi.org/10.1016/j.ecoenv.2016.10.001

Smith SE, Smith FA (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annu Rev Plant Biol 62:227-250. https://doi.org/10.1146/annurev-arplant-042110-103846

Sudova R, Vosatka M (2007) Differences in the effects of three arbuscular mycorrhizal fungal strains on P and Pb accumulation by maize plants. Plant Soil 296:77-83. https://doi.org/10.1007/s11104-007-9291-8

Wang CR, Rong H, Zhang XB, Shi WJ, Hong X, Liu WC, Cao T, Yu XX, Yu QF (2020) Effects and mechanisms of foliar application of silicon and selenium composite sols on diminishing cadmium and lead translocation and affiliated physiological and biochemical responses in hybrid rice (*Oryza sativa* L.) exposed to cadmium and lead. Chemosphere 251:126347. https://doi.org/10.1016/j.chemosphere.2020.126347

Watts-Williams SJ, Cavagnaro TR, Tyerman SD (2019) Variable effects of arbuscular mycorrhizal fungal inoculation on physiological and molecular measures of root and stomatal conductance of diverse *Medicago truncatula* accessions. Plant Cell Environ 42:285-294. https://doi.org/10.1111/pce.13369

Weissenhorn I, Leyval C (1995) Root colonization of maize by a Cd-sensitive and a Cd-tolerant *Glomus mosseae* and Cadmium uptake in sand culture. Plant Soil 175:233-238. https://doi.org/10.1007/Bf00011359

Weissenhorn I, Leyval C, Berthelin J (1993) Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. Plant Soil 157:247-256. https://doi.org/10.1007/Bf00011053

Xin JL, Dai HW, Huang BF (2017) Assessing the roles of roots and shoots in the accumulation of cadmium in two sweet potato cultivars using split-root and reciprocal grafting systems. Plant Soil 412:413-424. https://doi.org/10.1007/s11104-016-3079-7

Yabe J, Nakayama SMM, Ikenaka Y, Yohannes YB, Bortey-Sam N, Kabalo AN, Ntapisha J, Mizukawa H, Umemura T, Ishizuka M (2018) Lead and cadmium excretion in feces and urine of children from polluted townships near a lead-zinc mine in Kabwe, Zambia. Chemosphere 202:48-55. https://doi.org/10.1016/j.chemosphere.2018.03.079

Yadav SK (2010) Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S Afr J Bot 76:167-179. https://doi.org/10.1016/j.sajb.2009.10.007

Yang YR, Liang Y, Ghosh A, Song YY, Chen H, Tang M (2015) Assessment of arbuscular mycorrhizal fungi status and heavy metal accumulation characteristics of tree species in a lead-zinc mine area: potential applications for phytoremediation. Environ Sci Pollut R 22:13179-13193. https://doi.org/10.1007/s11356-015-4521-8

Yang YR, Liang Y, Han XZ, Chiu TY, Ghosh A, Chen H, Tang M (2016) The roles of arbuscular mycorrhizal fungi (AMF) in phytoremediation and tree-herb interactions in Pb contaminated soil. Sci Rep 6:20469. https://doi.org/10.1038/srep20469

Zhang HQ, Franken P (2014) Comparison of systemic and local interactions between the arbuscular mycorrhizal fungus *Funneliformis mosseae* and the root pathogen *Aphanomyces euteiches* in *Medicago truncatula*. Mycorrhiza 24:419-430. https://doi.org/10.1007/s00572-013-0553-4

Zhang XY, Lou X, Zhang HQ, Ren W, Tang M (2020a) Effects of sodium sulfide application on the growth of *Robinia pseudoacacia*, heavy metal immobilization, and soil microbial activity in Pb-Zn polluted soil. Ecotox Environ Safe 197:110563. https://doi.org/10.1016/j.ecoenv.2020.110563

Zhang XY, Zhang HJ, Zhang YX, Liu YQ, Zhang HQ, Tang M (2020b) Arbuscular mycorrhizal fungi alter carbohydrate distribution and amino acid accumulation in *Medicago truncatula* under lead stress. Environ Exp Bot 171:103950. https://doi.org/10.1016/j.envexpbot.2019.103950

Zhang XY, Zhang HQ, Lou X, Tang M (2019) Mycorrhizal and non-mycorrhizal *Medicago truncatula* roots exhibit differentially regulated NADPH oxidase and antioxidant response under Pb stress. Environ Exp Bot

164:10-19. https://doi.org/10.1016/j.envexpbot.2019.04.015

Zheng S, Wang C, Shen Z, Quan Y, Liu X (2015) Role of extrinsic arbuscular mycorrhizal fungi in heavy metal-contaminated wetlands with various soil moisture levels. Int J Phytoremediat 17:208-214. https://doi.org/10.1080/15226514.2013.876968

Figures

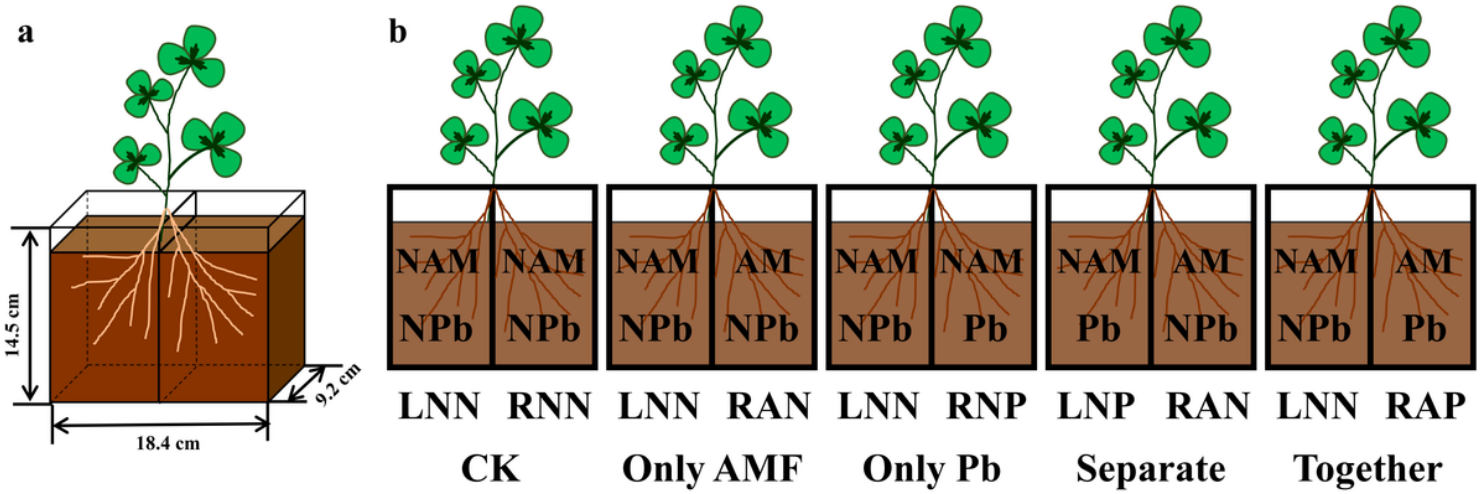

Figure 1

a Split root system used for the study of influence of R. irregularis on Pb uptake by M. truncatula. b The two compartments of the root systems were inoculated with/ without R. irregularis and after 4 weeks of mycorrhizal colonization two compartments applied with/ without Pb. NM= non-mycorrhizal treatment; AMF= arbuscular mycorrhizal fungi inoculation; Pb= Pb treatment; NPb= non-Pb treatment. CK= control treatment; Only Pb= Pb was added to only one compartment; Only AMF= AM fungi was added to only one compartment; Separate= Pb and AM fungi were added into split-root system separately in 2 compartments; Together= Pb and AM fungi were added into split-root system together in same compartment.

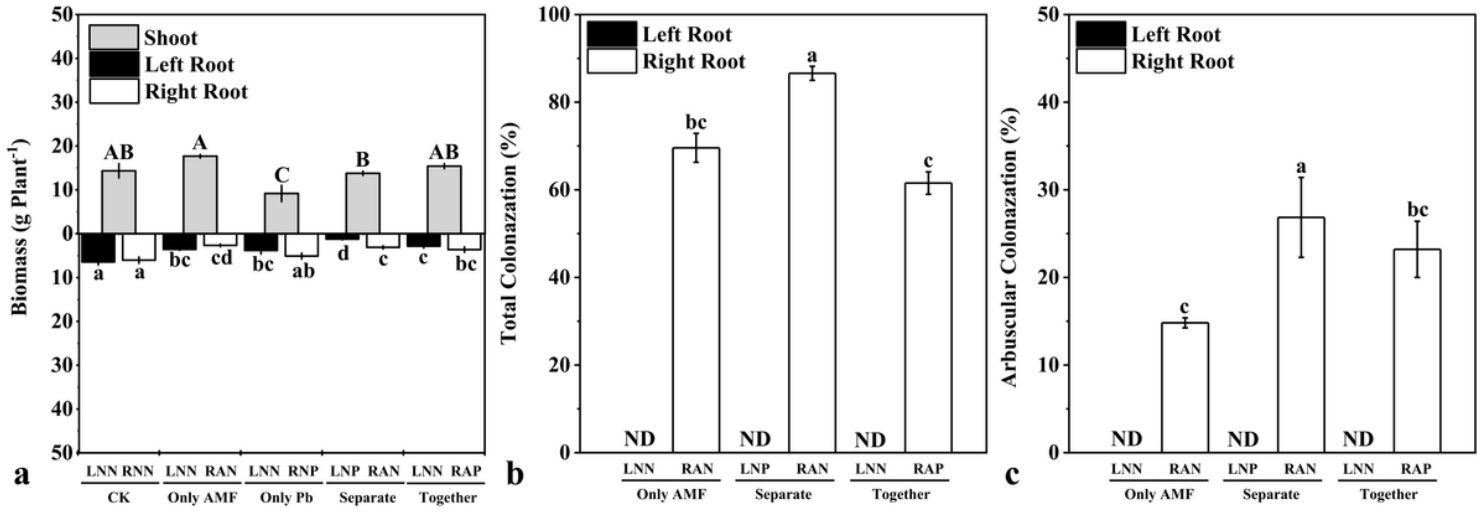

Figure 2

a The fresh weight of shoots and roots of M. truncatula in different treatments. The total colonization (b) and the arbuscule colonization (c) of R. irregularis in different treatments. The data were shown as means ± standard error (n = 3). Different uppercase and lowercase letters above the columns indicated significant difference among the means by LSD test (P < 0.05), respectively. ND=not detected; CK = control treatment; Only Pb= Pb was added to only one compartment; Only AMF= AM fungi was added to only one compartment; Separate = Pb and AM fungi were added into split-root system separately in 2 compartments; Together = Pb and AM fungi were added into split-root system together in same compartment; LNN = no AM fungi or Pb was added in the left compartment; RNN = no AM fungi or Pb was added in the right compartment; RAN= AM fungi was added in the right compartment without Pb; RNP= Pb was added in the right compartment without AM fungi; LNP= Pb was added in the left compartment without AM fungi; RAP= AM fungi and Pb were added in the right compartment.

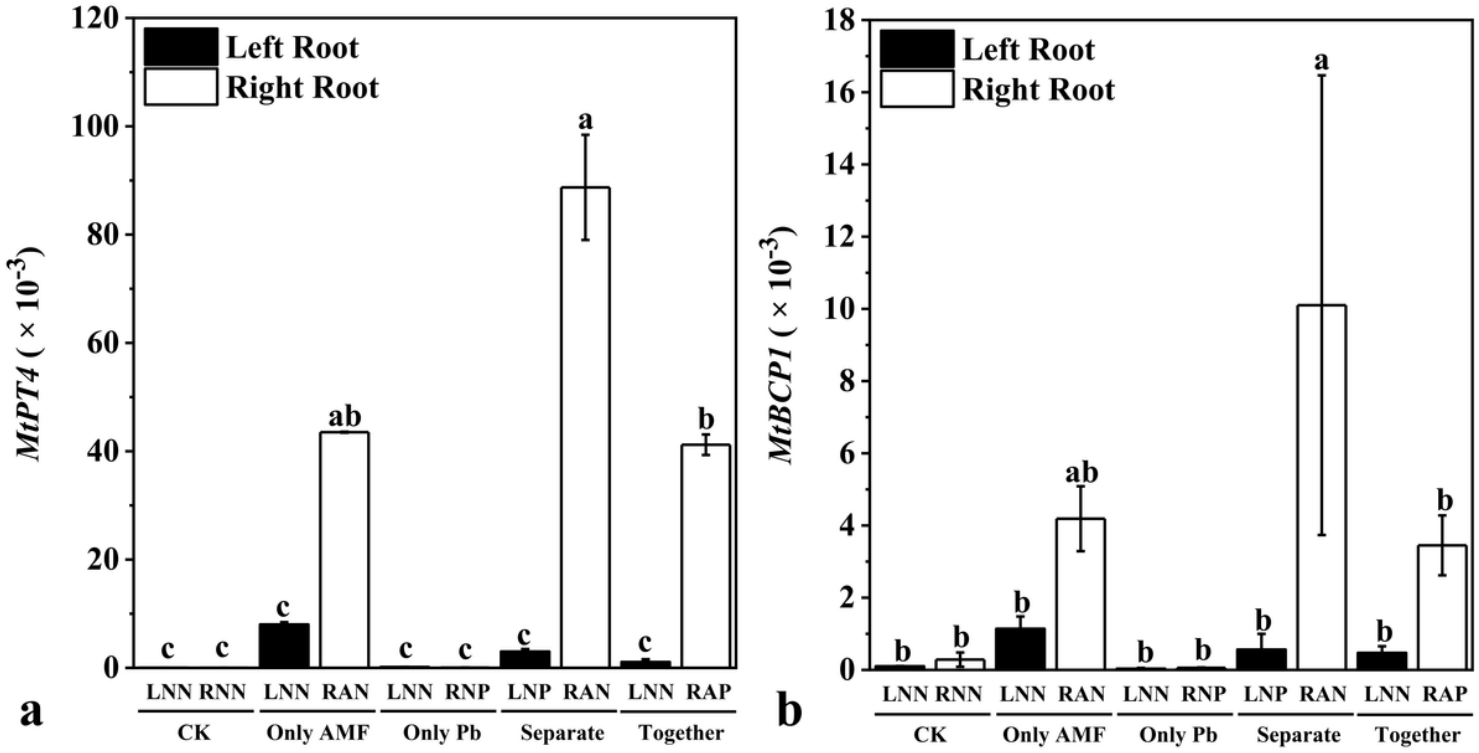

Figure 3

Effects of Pb and R. irregularis inoculation on expression of MtPT4 and MtBCP1 in root of M. truncatula. Expression of the MtEFα in root of M. truncatula was used as an internal control for normalization. The data are the means ± standard error (n = 3). Different letters within each gene indicate significant differences by LSD test (P < 0.05), respectively. The abbreviation is consistent with Fig.2.

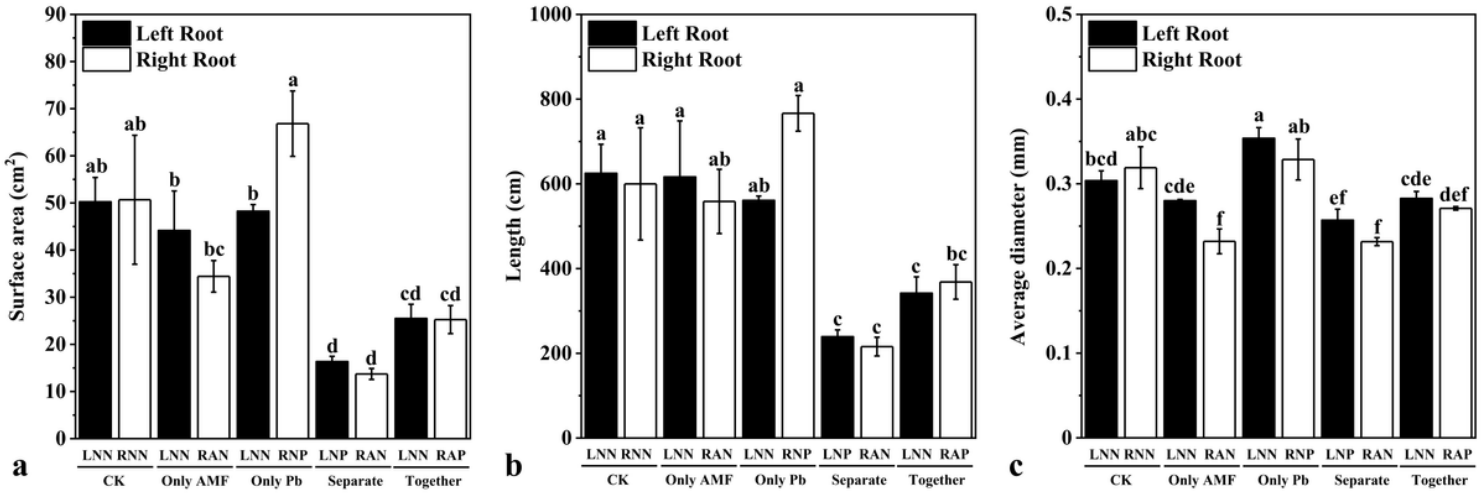

Figure 4

The root surface area (a), length (b) and average diameter (c) of M. truncatula in different treatments. The data are the means ± standard error (n = 3). Different letter above the columns indicates significant difference among the means by LSD test (P < 0.05), respectively. The abbreviation is consistent with Fig.2.

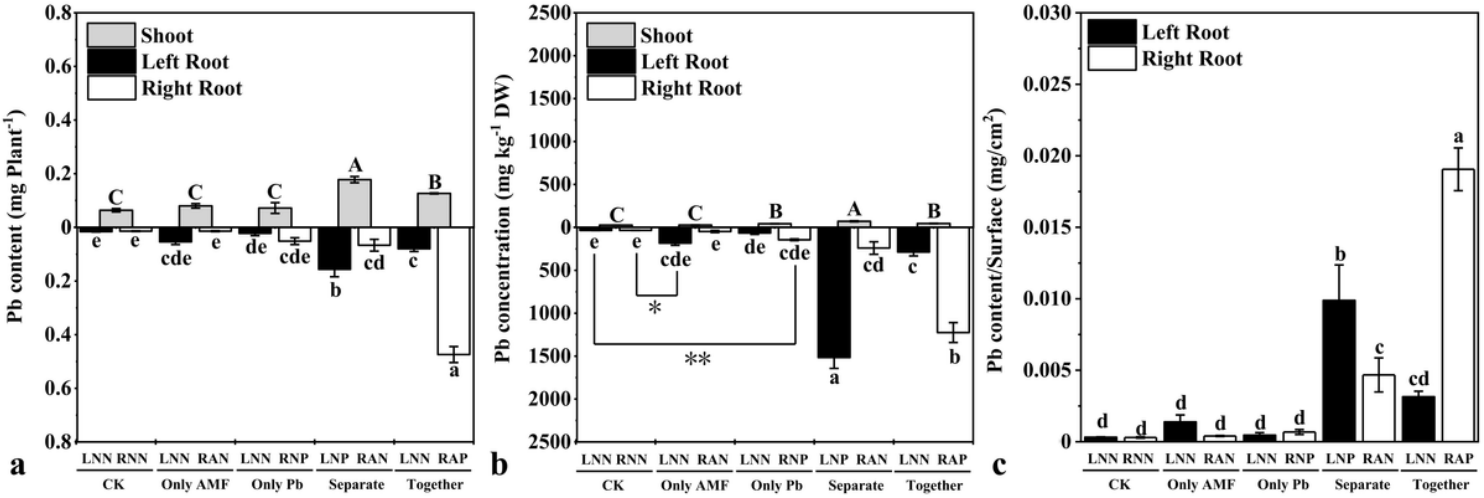

Figure 5

The Pb content (a), total Pb concentration (b) and ratio of Pb content to surface in M. truncatula plants in different treatments. The data are the means ± standard error (n = 3). Different uppercase and lowercase above the columns indicate significant difference among the means by LSD test (P < 0.05), respectively. The abbreviation is consistent with Fig.2.

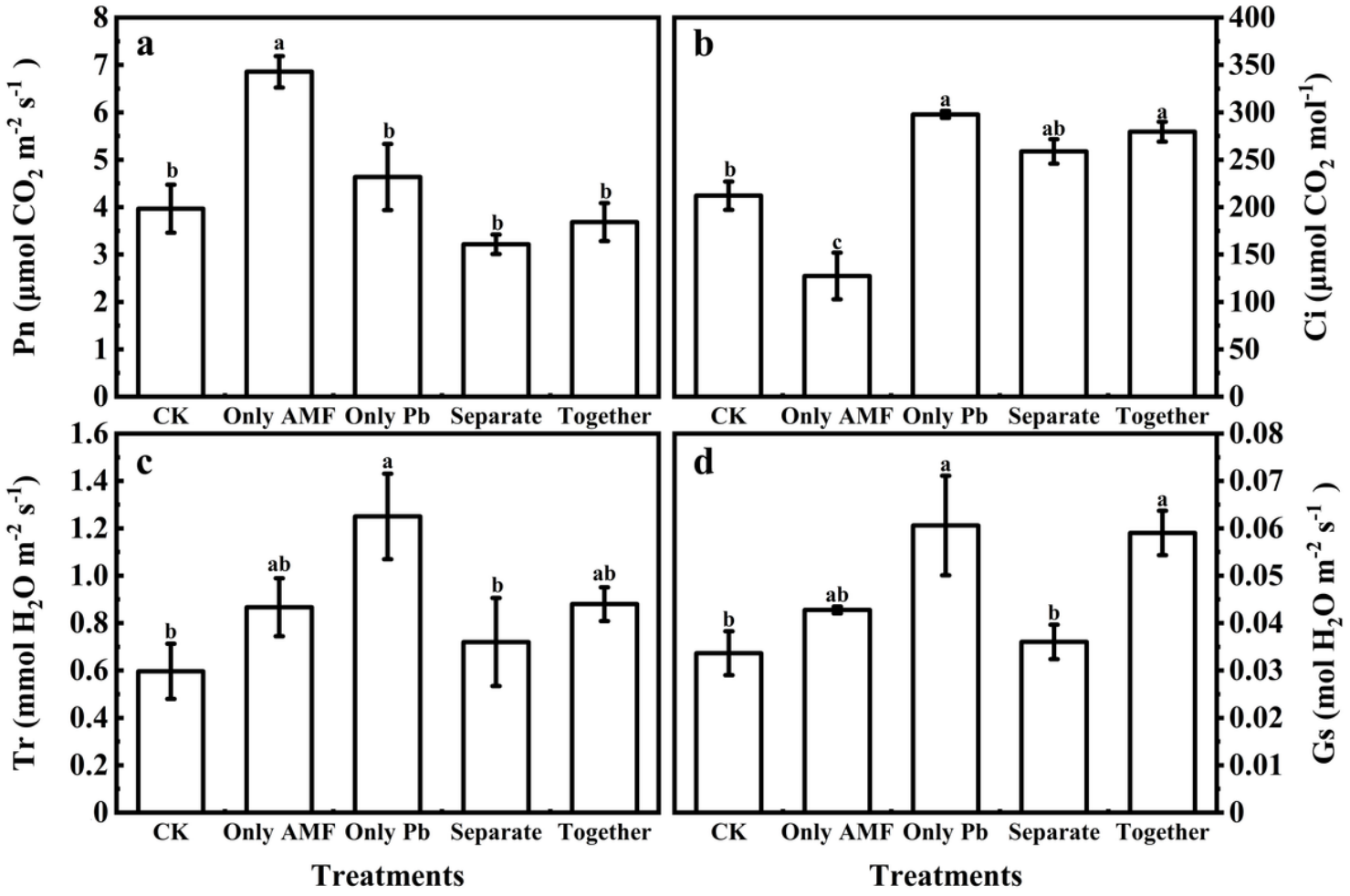

Figure 6

Effects of Pb and R. irregularis inoculation on net photosynthetic rate (Pn) (a), intercellular CO2 concentration (Ci) (b), transpiration rate (Tr) (c) and stomatal conductance (Gs) (d) in leaves of M. truncatula. The data are the means ± standard error (n = 3). Different letter above the columns indicates significant difference among the means by LSD test ($P < 0.05$), respectively. The abbreviation is consistent with Fig.2.

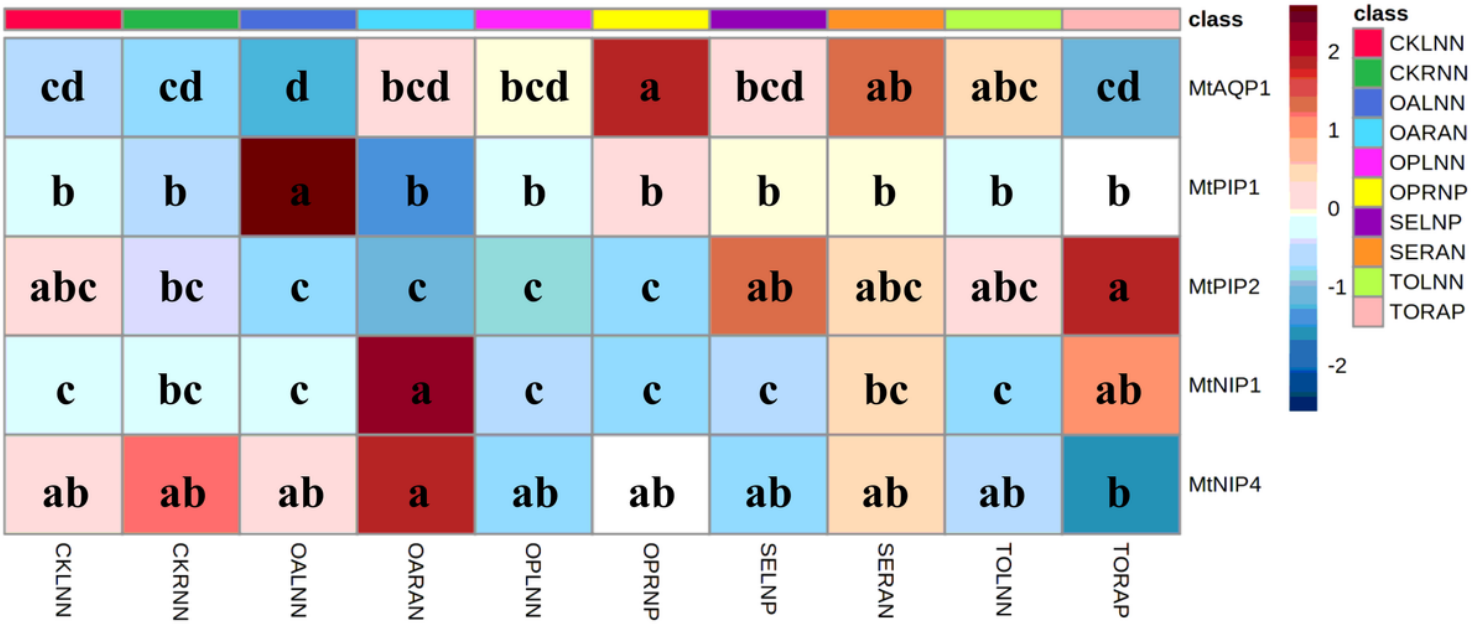

Figure 7

Effects of Pb and R. irregularis inoculation on expression of MtAQP1, MtPIP1, MtPIP2, MtNIP1 and MtNIP4 in root of M. truncatula. Expression of the MtEFα in root of M. truncatula was used for normalization. Different letters within each gene indicated significant differences by LSD test ($P < 0.05$), respectively. The abbreviation was consistent with Fig. 2.

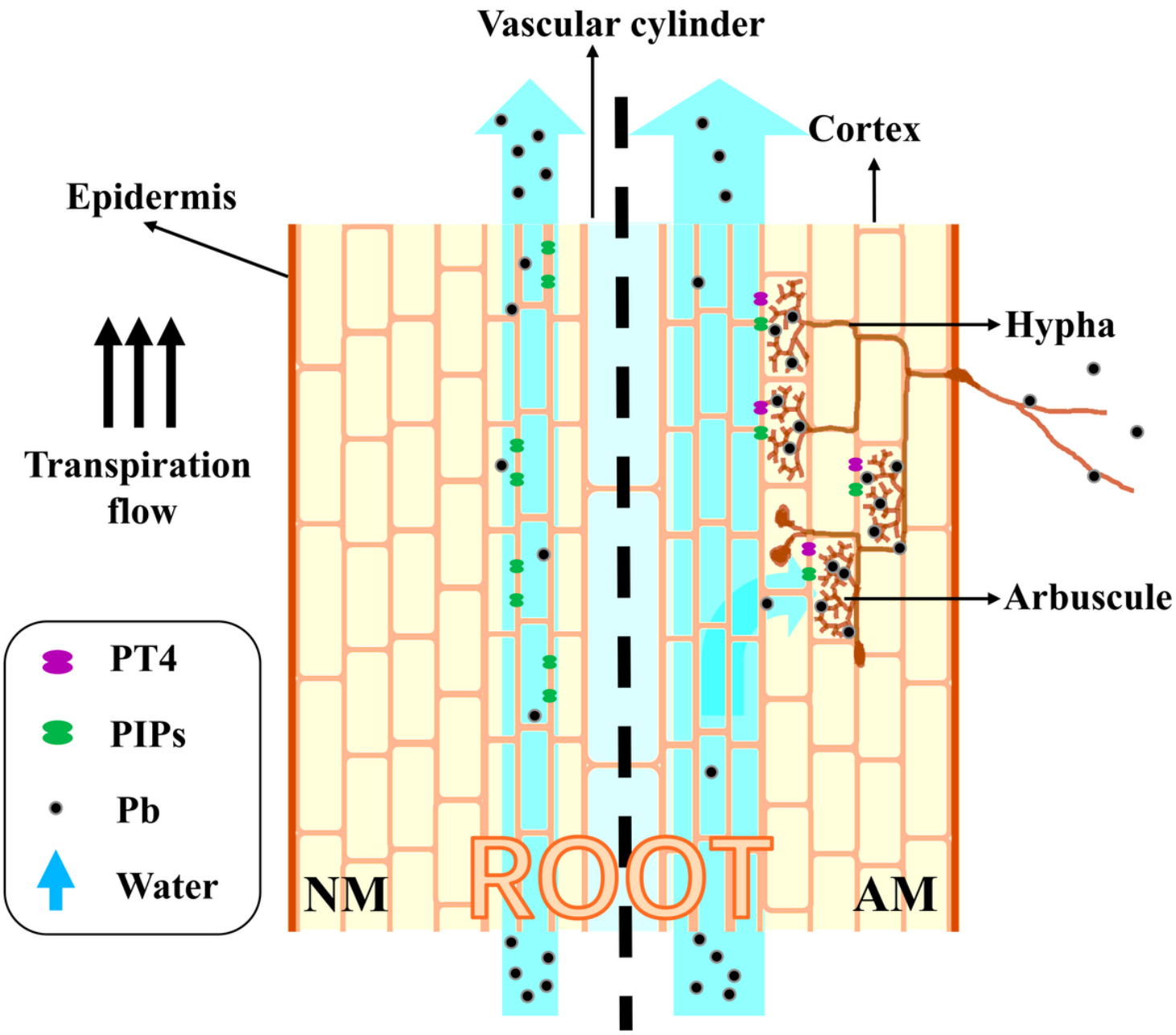

Figure 8

AM fungi can immobilize Pb that R. irregularis in inoculated compartment as a sifter reduced content of Pb in plant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CreditAuthorStatement.docx
- Supplementary.xlsx