Rhizophagus irregularis influences As and P uptake by alfafa and the neighboring non-host pepperweed growing in an As-contaminated soil

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ABSTRACT

It was documented that arbuscular mycorrhiza fungi (AMF) play an important role in protecting host plants against arsenic (As) contamination. However, most terrestrial ecosystems contain a considerable number of nonmycorrhizal plants. So far little information is available for the interaction of such non-host plants with AMF under As contaminations. By using a dual compartment cultivation system with a plastic board or a nylon mesh separating roots of non-host pepperweed from roots of the AM-host alfafa plants, avoiding direct root competition, the two plant species were grown separately or partially separated (with rhizosphere effects) in the presence or absence of the AMF Rhizophagus irregularis in As-contaminated soil. The results indicated that mycorrhiza caused phosphorus (P) concentration decrease in the non-host pepperweed, but promoted the P concentration of the AM host alfafa. Mycorrhiza is potentially helpful for non-host pepperweed to adapt to As contamination by decreasing root As concentration and showing no suppressing effect on biomass production. The study provides further evidence for the protective effects of AMF on non-host plants against As contamination, and improved our understanding of the potential role of AMF for non-host plant adaptation to As contaminated soils.

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Introduction

Arsenic (As) is ubiquitous in the environment, which is highly toxic even at low concentrations. In recent decades, public concern regarding this element has been increasing, because As is regarded as a well-known class 1, nonthreshold carcinogen (Smith et al., 2002). Arsenic mining industry (Zhu et al., 2008), irrigation with As-contaminated groundwater (Williams et al., 2006) and application of As-containing pesticides (Williams et al., 2007) have been found to be the main reasons for the elevated As concentration in soil. Plant uptake of As from contaminated soils may contribute a significant pathway of human exposure via the food chain. Understanding As uptake in plant is thus critical for formulating countermeasures to minimize the ecological risk of As contaminations (Meharg and Hartley-Whitaker, 2002).

It was documented that higher plants that are adapted to As-contaminated soils are generally symbiotic with arbuscular mycorrhizal fungi (AMF) (Sharples et al., 2000; Gonzalez-Chavez et al., 2002), while the mycorrhizal associations could influence

As uptake and accumulation by plants. However, up to date most of the investigations focused on As uptake, accumulation and distribution of a single mycorrhizal plant (Chen et al., 2007; Liu et al., 2009; Zhang et al., 2015), or the interactions between two mycorrhiza plants under As contamination (Dong et al., 2008). It has been well known that there are some plant families, an estimated 18% of all vascular species, that do not associate with AMF (Brundrett, 2009). These plants have been named as "non-host" or "non-mycorrhizal" (NM) plants and are widely distributed (Francis and Read, 1994). Brassicaceae is one of these families that have long been known as non-host for AMF except a few of plant species such as *Thlaspi praecox* and *Lepidium bonariense* (Giovannetti et al., 1994; Vogel-Mikus et al., 2005; Massenssini et al., 2014). Even though a functional mycorrhization does not occur in a non-host plant, the invasion of hyphae deriving from the host plant neighbors to the non-host plant roots is persistent (Tong et al., 2015). On the other hand, Brassicaceae family has been proven to have genetic and physiological adaptations that allow plants to accumulate, translocate, and resist high amounts of arsenic (Wang et al., 2009; Ramirez-Andreotta et al., 2013). Therefore, we specifically addressed the role that AMF play in non-host plant adaptation to As contamination. To our knowledge, the interaction of non-host plants with AMF under As contamination is so far poorly understood.

In the present study, a model mycorrhizal plant alfalfa (*Medicago sativa* L.) was selected as arbuscular mycorrhizal (AM) host plant, and pepperweed (*Lepidium apetalum* L.), a weed of the Brassicaceae family widely distributed in rubbly slopes, meadows, steppes, fallow lands, and along the roads (Prokopiev et al., 2013) was chosen as non-AM host plant, whose leaves can be eaten and the whole plant can be used as a medicine (Duke and Ayensu, 1984; Kunkel, 1984). By using a dual compartment cultivation system with a plastic board or a nylon mesh separating roots of pepperweed from roots of alfalfa plants and avoiding direct root competition, the two plant species were grown separately or partially separated (with rhizosphere effects) in the presence or absence of the AMF *Rhizophagus irregularis* in an As-contaminated soil. It was hypothesized that although non-host pepperweed could not form symbiosis with AMF, while the involvement of AMF might decrease As concentration of non-host pepperweed, thus potentially helpful for non-host pepperweed to adapt to As contamination. The aim of the present study is to shed light on the effects of AMF on growth, mineral nutrition, As uptake of non-AM host plant under As contaminations, which may contribute to our understanding of the potential role of AMF for non-host plant adaptation to As-contaminated soils.

1. Material and methods

1.1. Host plants

Seeds of alfalfa and pepperweed plants were respectively obtained from the Beijing Gold Garden Agriculture Technology Institution and Research Center of National Vege Engineering and Technology. The seeds were surface sterilized in 10% (V/V) H_2O_2 solution for 10 min, then immersed in deionized water for 10 hr. They were then pre-germinated on moist filter paper for about 48 hr at 27°C until emergence of radicles. The seeds were selected for uniformity before sowing.

1.2. AMF inoculums

The AMF *Rhizophagus irregularis* Schenck and Smith (BJ09) was provided by Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry. The fungus was propagated in pot culture with maize plants grown in a sandy soil for 10 weeks. Inoculum from pot culture was a mixture of spores, mycelium, sandy soil and root fragments containing approximately 6000 spores per 100 g soil.
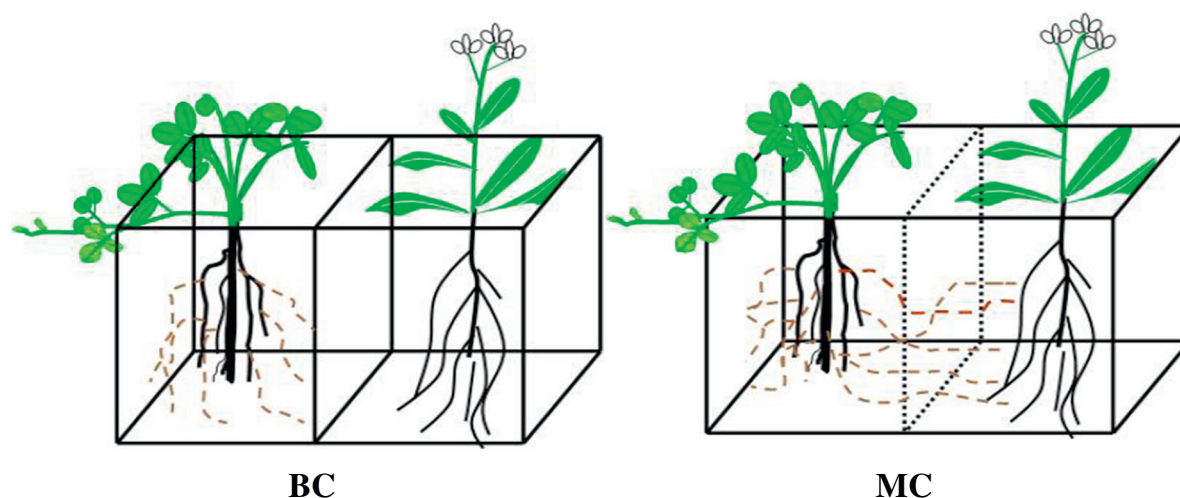
1.3. Cultivation system

Two alfafa and three pepperweed seedlings were grown in each individual compartmented PVC boxes (12 cm × 8 cm × 10 cm). Roots of both plant species were separated by a PVC board (board compartment mode, BC), to avoid that both roots and hyphae get intermingled with the other plant species, or by a nylon net with 37 μm mesh size (mesh compartment mode, MC), which only allows the penetration by AM fungal hyphae (Fig. 1). The hyphae penetrating freely through the nylon mesh can directly interact with the root of pepperweed, which is convenient for investigating the interactions of AMF with non-hosts in the present study. In contrast, in the BC modes, neither the roots nor the hyphae of alfafa could reach the rhizosphere of pepperweed, which was regarded as a control in the present study.

1.4. Cultivation media

The experimental soil was collected from the experimental field of Chinese Agriculture University, Beijing, China. The soil had a pH value of 7.47 (1:2.5 soil to water (m/V)), extractable phosphorus (P) content of 10.50 mg/kg (extracted by 0.5 mol/L $NaHCO_3$ following the methods described by Olsen et al., 1954) and extractable As of 0.21 mg/kg (extracted by 0.5 mol/L $NaHCO_3$). The soil contained 5.6 mg/kg As, 27.39 mg/kg Cu, 125.51 mg/kg Mg, 527.92 mg/kg Mn and 84.31 mg/kg Zn. Total As concentration were analyzed by inductively coupled plasma-mass spectroscopy (ICP-MS, Agilent7500, Agilent Technology, USA) and other total metal concentrations were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 2000 DV, Perkin Elmer, USA) following HNO_3-HF digestion. Before the experiment, the soil was passed through a 2-mm sieve and received basal nutrients without P as recommended by (Pearson and Jakobsen, 1993). A 2:1 (W/W) mixture of the soil and river sand, also passed through 2-mm sieve and sterilized by irradiation (20 kGy, 10 MeV electron beam), was used as growth medium, and this mixture is referred to as "soil" hereinafter.

1.5. Experimental procedure

The 5 mg/kg As in the form of $Na_3AsO_4 \cdot 12H_2O$ (As(V)) were added to the soil and then carefully mixed to ensure uniformity. The soil was incubated for one month to allow metal equilibrium. A mixture of 900 g soil and 30 g fungal inoculum was divided equally and put into the two compartments of each box for AM

Fig. 1 – Schematic illustration of the dual-compartment cultivation system. BC: board compartment mode; MC: mesh compartment mode, nylon net with 37 μm mesh was used to separate the two compartments, which only allowed penetration by AM fungal hyphae (dashed lines). AM: arbuscular mycorrhizal.

inoculation, or 900 g soil and 30 g sterilized inoculum for non-inoculated controls. Inoculation treatments and compartment modes gave a total of 4 treatments. There were 4 replicates for each treatment, resulting in a total of 16 pots. Each pot was sown with 4 pre-germinated pepperweed seeds in one compartment and 6 pre-germinated alfafa seeds in the other compartment. Seven days after emergence pepperweed seedlings and alfafa seedlings were thinned to 2 and 3, respectively. The experiment was conducted in a greenhouse with 16 hr/25°C day, 8 hr/18°C night, and a light intensity of 700 μmol/(m²·sec) provided by supplementary illumination. During the experimental period, de-ionized water was added as required to maintain soil moisture content of *ca.* 60% water holding capacity by regular weighing.

1.6. Harvest and chemical analysis

Plant shoots and roots were harvested separately. Root samples were first carefully washed with tap water to remove adhering soil particles and rinsed in ice-cold phosphate solution containing 1.0 mmol/L K_2HPO_4, 5.0 mmol/L MES and 0.5 mmol/L $Ca(NO_3)_2$ for 10 min to remove As in the apoplast of the roots (Abedin *et al.*, 2002). Roots and shoots were then carefully washed with de-ionized water, blotted dry and weighed. Sub-samples of fresh roots were collected for the determination of AM colonization. Dry weights of shoots and roots were determined after oven-drying at 70°C for 48 hr.

Sub-samples of fresh roots were cleared in 10% KOH and stained with Trypan Blue following a modification procedure of (Phillips and Hayman, 1970) by omitting phenol from solutions and HCl from the rinse. Percentage root colonization and root length were determined by the magnified grid-intersect method (McGonigle *et al.*, 1990) and the presence of fungal structures (intraradical mycelia, vesicles, and arbuscules) were identified at 200× magnification under a light microscope.

Approximately 0.2 g dried samples were weighed and digested with 10 mL HNO_3 by using a microwave accelerated reduction system (Mars 5, CEM Co. Ltd., USA). The dissolved samples were analyzed by ICP-OES for P and by ICP-MS for As. Soil pH was measured with a potentiometer (Thermo Orion Model 868) at a 1:2.5 (*m/V*) soil:water ratio. Soil-available P was extracted for 30 min at a 1:10 (*m/V*) soil: water ratio and available P was determined as described above.

1.7. Data analysis

Data were subjected to two-way analysis of variance (ANOVA) to examine significance of mycorrhizal inoculation and compartment modes by using windows-based SPSS 16.0 statistical package (SPSS Inc., USA). The differences between means were examined by Duncan's multiple-range Test at 0.05 probability level.

2. Results

2.1. Mycorrhizal colonization

No root colonization (intraradical mycelia, vesicles, and arbuscules) was detected in uninoculated alfalfa plants but the roots of mycorrhizal alfalfa plants were extensively colonized, with the mean colonization rate of 47% ± 0.9% (*n* = 4) and 50% ± 1.3% (*n* = 4) for BC and MC, respectively. There was no significant difference between BC and MC for alfafa plants. Pepperweed plants were not colonized irrespective of inoculation treatments.

2.2. Plant biomass

The MC mode increased both shoot (*p* < 0.01, Fig. 2a), root (*p* < 0.01, Fig. 2b) and total biomass (*p* < 0.001, Table 1) of pepperweed, but inoculation had no effect on the pepperweed biomass. In contrast, both shoot (*p* < 0.01, Fig. 2a), root (*p* < 0.05, Fig. 2b) and total dry weight (*p* < 0.01, Table 1) of alfafa plants were significantly increased by mycorrhizal colonization, but unaffected by the compartment mode. The total dry weight of
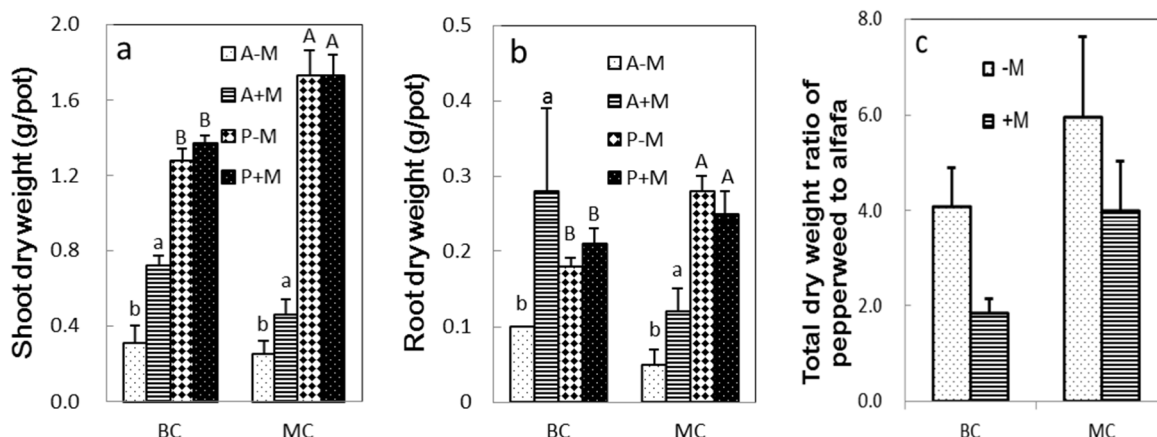
Fig. 2 – Shoot (a), root (b) dry weights of alfafa and pepperweed plants, and the total dry weight ratio of pepperweed to alfafa (c) as affected by different inoculation treatments and compartment modes. +M and –M represent inoculated and uninoculated treatments. "A" stands for alfafa plant and "P" stands for pepperweed plant, respectively. Data are means ± SE ($n = 4$). The statistical results were shown in Table 4. Different lower case letters "a, b" or upper case letters "A, B" above the error bars indicate significant differences ($p < 0.05$) between treatments for alfafa or pepperweed by Duncan's multiple-range test, respectively. There are no significant differences between treatments in Fig. 2c.

pepperweed is almost 1.8–5.9 folds higher than that of corresponding alfafa plant (Fig. 2c).

2.3. Plant P uptake

Generally, inoculation had no influence on the either shoot or root P content of pepperweed, but the MC modes increased both shoot ($p < 0.01$, Table 1) and root ($p < 0.01$, Table 1) P contents of pepperweed. Differently, mycorrhizal colonization significantly increased both shoot ($p < 0.01$, Table 1) and root ($p < 0.05$, Table 1) P contents of alfalfa plants, while compartment modes showed no effect on the alfafa P contents. As for P concentration, mycorrhiza decreased pepperweed's shoot ($p < 0.01$, Fig. 3a) and root ($p < 0.001$, Fig. 3b) P concentration, but increased alfafa's shoot ($p < 0.05$, Fig. 3a) and root P ($p < 0.001$, Fig. 3b) concentration. The MC mode significantly increased shoot P concentration of alfafa ($p < 0.05$, Fig. 3a), but not for

pepperweed. There were significant interactions between inoculation and compartment modes on the root P concentration of both alfafa plant ($p < 0.001$, Fig. 3b) and pepperweed ($p < 0.05$, Fig. 3b). The positive effect of AMF on P concentration in alfafa roots is stronger in MC treatment than that in BC treatment, while the negative effect of AMF on P concentration in pepperweed roots is stronger in MC than in BC treatment. The highest root P concentrations were recorded for inoculated alfafa plant under MC modes, and in contrast, pepperweed in presence of AMF under MC modes contained the lowest root P concentration.

2.4. Plant As uptake

Mycorrhiza and BC modes decreased root As concentration of pepperweed ($p < 0.001$, Fig. 4a2), but showed no significant effect on shoot As concentration. In addition, mycorrhizal

Table 1 – Total dry weight, shoot and root P content of alfafa and pepperweed as affected by different inoculation treatments and compartmentation modes (Data presented are means of 4 replicates).

Compartmentation mode	Inoculation treatment	Total dry weight (g/pot)		Shoot P content (mg/pot)		Root P content (mg/pot)	
		Alfafa	Pepperweed	Alfafa	Pepperweed	Alfafa	Pepperweed
BC	Non-inoculated	0.41 ± 0.09	1.45 ± 0.07	0.74 ± 0.23	6.18 ± 0.26	0.31 ± 0.01	0.71 ± 0.05
	Inoculated	0.93 ± 0.14	1.58 ± 0.04	1.81 ± 0.16	5.98 ± 0.37	0.70 ± 0.37	0.69 ± 0.06
MC	Non-inoculated	0.30 ± 0.09	2.01 ± 0.15	0.64 ± 0.18	8.14 ± 0.63	0.16 ± 0.07	1.11 ± 0.09
	Inoculated	0.58 ± 0.11	1.98 ± 0.14	1.36 ± 0.23	7.51 ± 0.60	0.45 ± 0.13	0.82 ± 0.09
Significance[a] of							
Compartmentation (C)		NS	**	NS	**	NS	**
Inoculation (I)		**	NS	**	NS	*	NS
C × I		NS	NS	NS	NS	NS	NS

NS: not significant ($p > 0.05$); P: phosphorus.
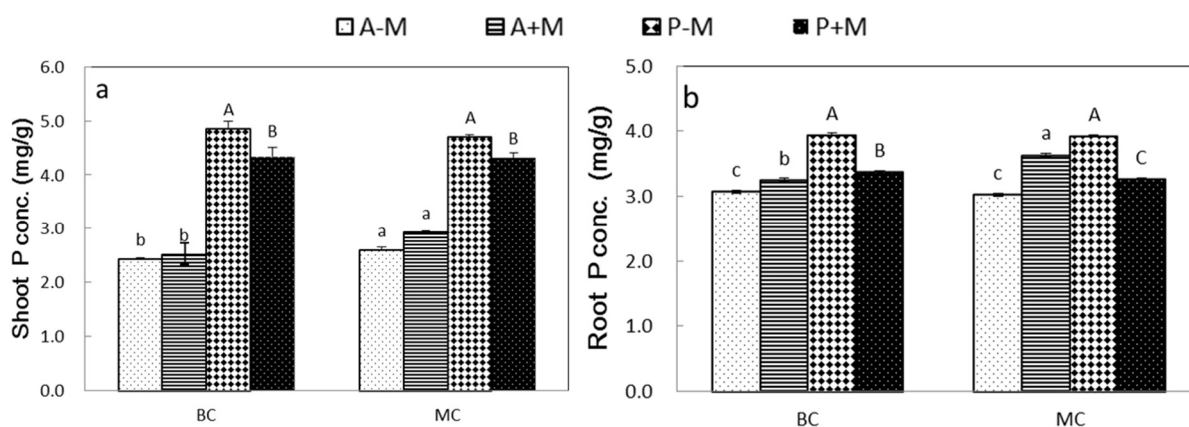* $p < 0.05$;
** $p < 0.01$.
[a] By analysis of variance.

Fig. 3 – Shoot (a) and root (b) P concentration of alfafa and pepperweed plants as affected by different inoculation treatments and compartmentation modes. +M and –M represent inoculated and uninoculated treatments. "A" stands for alfafa plant and "P" stands for pepperweed plant, respectively. Data are means ± SE (n = 4). The statistical results were shown in Table 4. Different lower case letters "a, b, c" or upper case letters "A, B, C" above the error bars indicate significant differences ($p < 0.05$) between treatments for alfafa or pepperweed by Duncan's multiple-range test, respectively.
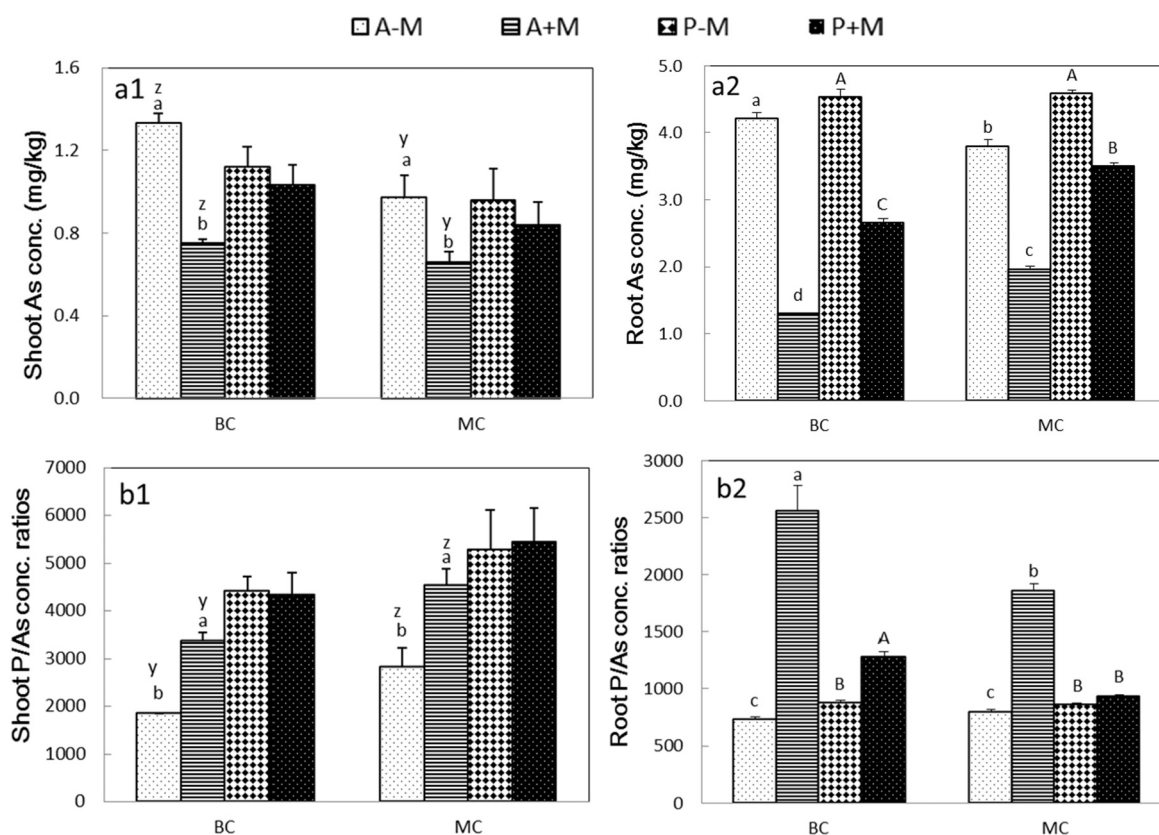

Fig. 4 – Shoot (a1) and root (a2) As concentration and P/As concentration ratios in shoot (b1) and root (b2) of alfafa and pepperweed plants as affected by different inoculation treatments and compartmentation modes. +M and –M represent inoculated and uninoculated treatments. "A" stands for alfafa plant and "P" stands for pepperweed plant, respectively. Data are means ± SE (n = 4). The statistical results were shown in Table 4. Different letters above error bars indicate significant differences ($p < 0.05$) between treatments by Duncan's multiple-range test. For shoot As (in Fig. 4a1) and P/As (in Fig. 4b1) concentration, letters written as "a, b" indicate significant differences ($p < 0.05$) between inoculated and non-inoculated alfafa; letters written as "z, y" indicate significant differences ($p < 0.05$) between BC and MC modes for alfafa. For root As (in Fig. 4a2) and P/As (in Fig. 4b2) concentration, lower case letters "a, b" or upper case letters "A, B" indicate significant differences ($p < 0.05$) between treatments for alfafa or pepperweed, respectively.

Table 2 – As contents and shoot to root ratios of As contents of alfafa and pepperweed as affected by different inoculation treatments and compartmentation modes (Data presented are means of 4 replicates).

Compartmentation mode	Inoculation treatment	Shoot As content (mg/pot)		Root As content (mg/pot)		S/R As content (mg/pot)	
		Alfafa	Pepperweed	Alfafa	Pepperweed	Alfafa	Pepperweed
BC	Non-inoculated	0.41 ± 0.13	1.42 ± 0.10	0.43 ± 0.02	0.81 ± 0.08	1.02 ± 0.40	1.79 ± 0.21
	Inoculated	0.54 ± 0.03	1.41 ± 0.12	0.29 ± 0.16	0.54 ± 0.04	2.52 ± 1.05	2.67 ± 0.34
MC	Non-inoculated	0.26 ± 0.09	1.62 ± 0.20	0.20 ± 0.09	1.29 ± 0.10	1.49 ± 0.26	1.28 ± 0.20
	Inoculated	0.30 ± 0.06	1.45 ± 0.20	0.24 ± 0.06	0.88 ± 0.10	1.39 ± 0.18	1.67 ± 0.23
Significance[b] of							
Compartmentation(C)		*	NS	NS	[a]	NS	*
Inoculation (I)		NS	NS	NS	**	NS	*
C × I		NS	NS	NS	NS	NS	NS

NS: not significant ($p > 0.05$). As: arsenic; S/R: shoot/root.
  * $p < 0.05$;
  ** $p < 0.01$.
  [a] $p < 0.001$.
  [b] By analysis of variance.

colonization significantly decreased both shoot ($p < 0.001$, Fig. 4a1) and root ($p < 0.001$, Fig. 4a2) As concentration of alfafa plants, and MC modes also decreased alfafa's shoot As concentration ($p < 0.01$, Fig. 4a1). There were also significant interactions between inoculation and compartment modes on the root As concentration of both pepperweed and alfafa plants ($p < 0.001$, Fig. 4a2). The positive effects of AMF on As concentration in both pepperweed and alfafa roots are stronger in BC treatment than that in MC treatment, as indicated by the fact that inoculated pepperweed or alfafa under BC modes has the lowest root As concentration, followed by those inoculated treatments under MC modes.

Mycorrhizal colonization significantly decreased root As content of pepperweed ($p < 0.01$, Table 2), but increased shoot to root As content ratios of pepperweed ($p < 0.05$, Table 2). Moreover, MC modes decreased shoot to root As content ratios of pepperweed ($p < 0.05$, Table 2). In addition, mycorrhiza increased root P/As concentration ratios of pepperweed plants ($p < 0.001$, Fig. 4b2) and P/As concentration ratios in alfalfa plants ($p < 0.001$, Fig. 4b1, b2). There were also significant interactions between inoculation and compartment modes on root P/As concentration ratios of both pepperweed ($p < 0.001$, Fig. 4b2) and alfafa plants ($p < 0.01$, Fig. 4b2). The positive effects of AMF on

P/As concentration ratios in both pepperweed and alfafa roots are stronger in BC treatment than that in MC treatment.

2.5. Changes in soil properties

MC mode significantly increased soil pH values ($p < 0.01$, Table 3) and decreased water-extractable P concentration of alfafa plant ($p < 0.01$, Table 3). There is a tendency that the soil pH values of inoculated treatments were higher than those in corresponding non-inoculated controls for both pepperweed and alfafa plant (Table 3), although it was statistically insignificant. Similarly, there is also a trend that mycorrhiza decreased water-extractable P concentration for both plant species (Table 3).

3. Discussion

The present study investigated the interactions of AMF with non-AM host plant pepperweed and the results indicated that the involvement of AMF laid some effect on plant growth, P nutrition, As uptake of non-host pepperweed. Although pepperweed could not form symbiosis with AMF, mycorrhiza is potentially helpful for non-host pepperweed to adapt to As

Table 3 – Soil pH and water extractable soil P concentration as affected by different inoculation treatments and compartmentation modes (Data presented are means of 4 replicates).

Compartmentation mode	Inoculation treatment	pH		Water extractable P (mg/kg)	
		Alfafa	Pepperweed	Alfafa	Pepperweed
BC	Non-inoculated	7.49 ± 0.04	7.55 ± 0.04	4.43 ± 0.01	3.04 ± 0.07
	Inoculated	7.59 ± 0.05	7.56 ± 0.05	3.99 ± 0.01	2.73 ± 0.05
MC	Non-inoculated	7.70 ± 0.02	7.49 ± 0.04	3.23 ± 0.04	3.12 ± 0.04
	Inoculated	7.72 ± 0.05	7.60 ± 0.03	2.97 ± 0.03	2.91 ± 0.02
Significance* of					
Compartmentation (C)		**	NS	**	NS
Inoculation (I)		NS	NS	NS	NS
C × I		NS	NS	NS	NS

NS: not significant ($p > 0.05$);
  * By analysis of variance.
  ** $p < 0.01$.

Table 4 – The ANOVA results for data displayed in Figs. 2 to 4.

Factors	Dry weight				P concentration			
	Shoot		Root		Shoot		Root	
	Alfafa	Pepperweed	Alfafa	Pepperweed	Alfafa	Pepperweed	Alfafa	Pepperweed
Significance [a] *of*								
Compartmentation (C)	NS	**	NS	**	*	NS	b	**
Inoculation (I)	**	NS	*	NS	*	**	b	b
C × I	NS	NS	NS	NS	NS	NS	b	*

Factors	As concentration				P/As concentration			
	Shoot		Root		Shoot		Root	
	Alfafa	Pepperweed	Alfafa	Pepperweed	Alfafa	Pepperweed	Alfafa	Pepperweed
Significance [a] *of*								
Compartmentation (C)	**	NS	NS	b	**	NS	*	b
Inoculation (I)	b	NS	b	b	b	NS	b	b
C × I	NS	NS	b	b	NS	NS	**	b

NS: not significant ($p > 0.05$); ANOVA: analysis of variance;
  * $p < 0.05$;
 ** $p < 0.01$.
 [a] By analysis of variance.
 [b] $p < 0.001$.

contamination as indicated by decreasing root As concentration of pepperweed without plant yield reduction. The possible reason was that in the presence of AM host alfafa, the unavoidable invasion of AM hyphae deriving from alfafa to the non-host pepperweed is persistent. Continuously invading AM hyphae may induce a continuous defense response in the pepperweed (Matsumura et al., 2007). Moreover, the higher yields of pepperweed and different P acquisition strategy in comparison with alfafa plant might be another reason.

As demonstrated by previous studies, a negative effect of mycorrhiza on non-host growth and survival was observed (Sanders and Koide, 1994; Veiga et al., 2012). The enforced invasion of AM hyphae around non-host plant roots leading to plant growth inhibition could be due to a competitive disadvantage compared with mycorrhizal plants (Veiga et al., 2013) or release of allelopathic compounds by the AM mycelium which inhibit the growth of non-host plants (Francis and Read, 1994; Veiga et al., 2012). However, different results were obtained in the present study that the whole plant yield were not affected by the mycorrhizal inoculation, and shoot or root biomass of non-host pepperweed also showed no biomass reduction. This was possibly due to that in a dual compartment cultivation system, pepperweed was the dominating species with up to 4 times higher total dry matter than the corresponding mycorrhizal alfafa plant (Fig. 2c). Nutrient uptake was probably limited for the alfafa plants, while pepperweed plants can allocate sufficient resources for the production of defense-related compounds without a loss of plant fitness under As contaminations as demonstrated by Tong et al. (2015). Similar results were also demonstrated in Brassicaceae family species such as cabbage (Santos et al., 2002) and broccoli (Tong et al., 2015) when intercropped with pea or sesame, respectively. Furthermore, the results in our study showed that use of nylon mesh barrier between the two plant species generally increased pepperweed's total biomass compared with that in BC modes.

Nylon mesh barrier (MC modes) was used in the present study to obstruct possible physical overlap of alfafa roots with pepperweed roots, but allowed water and nutrients to exchange freely through the nylon mesh. This possible explanation might be that water, nutrient and some other acid exuding by the roots could be transported by mass flow and diffusion in the whole soil volume (Teste et al., 2015). Moreover, pepperweed is more competitive in nutrient uptake compared with alfafa plants, and thus resulted in the elevated amount of nutrients to be available for pepperweed.

Due to the similarity between phosphate and arsenate, understanding the role of AMF interaction with non-host plant in terms of As uptake is essential to realize the effects of AMF in plant uptake of P. AM-host and non-host plant have different strategies for P acquisition. For AM-host plant, AMF is considered to be of great importance in promoting P uptake (Pasqualini et al., 2007; Smith and Read, 2008). The mycelium can extend to the area outside the rhizosphere, connect roots with the surrounding soil microhabitats and enlarge the area that roots can absorb nutrients (Smith et al., 2003; Meng et al., 2015). Differently, non-host pepperweed appear to acidify their rhizosphere by exuding malic and citric acid (Hoffland et al., 1992). Organic acid exudation is regarded as a highly effective strategy to increase phosphate uptake from rock phosphate for non-host plant (Hoffland et al., 1992). Such strategy make pepperweed a P-efficient plant and benefit more when intercropped with P-inefficient plant alfafa, as indicated by the fact that total P content of pepperweed is almost 2.6–4.6 fold higher than the corresponding mycorrhizal alfafa plant (Table 1).

Owing to the different P acquisition strategy, mycorrhiza decreased P concentration of non-host pepperweed in the present study (Fig. 2a, b). The possible reason was that AM fungus may have contributed to the alkalization by releasing OH^- as a consequence of active uptake of NO_3^--N involving the NO_3^-/H^+ symport or NO_3^-/OH^- antiport mechanisms (Bago et al.,

1996), as shown in the present study that there is a trend that mycorrhiza increased soil pH values (Table 3). Therefore, the alkalization of the rhizosphere by AMF seemed to inhibit the P acquisition of non-host pepperweed. Furthermore, MC modes used in the present study allowed hyphae to penetrate freely through the nylon mesh and directly interact with the root of pepperweed. We speculated the other reason might be that in the MC modes, the external hyphae from alfafa plants could reach the rhizosphere of pepperweed and take away P in the whole soil volume as indicated by that soil P concentration of inoculated treatments were lower than in corresponding non-inoculated controls for pepperweed plants (Table 3), thus the P available for pepperweed might be decreased. Dong et al. (2008) also found that clover could benefit from hyphal uptake of nutrients from the whole soil volume when competed with ryegrass. However, in the present study that the P uptake by hyphae from the rhizosphere of pepperweed was just a hypothesis, further research is necessary to provide direct evidences for the involvements of hyphae in P uptake from the rhizosphere of pepperweed. Taken together, this might be the reason that under MC modes pepperweed contained lowest P concentration in the presence of mycorrhizal alfafa.

In the present study, we also observed that root As concentration (Fig. 4a2) and also root As content (Table 2) of pepperweed was dramatically decreased in comparison with the corresponding non-inoculated control. The similar results were also found for the mycorrhizal plant growing under As-contaminated soil (Chen et al., 2007; Dong et al., 2008; Zhang et al., 2015). As been well documented, one of the mechanisms was that after AMF and plant set good association with each other, AMF could effectively improve plant P nutrition and growth, resulting in a "dilution effect" on As in plant tissues (Chen et al., 2007; Dong et al., 2008; Zhang et al., 2015). However, for non-host pepperweed in the present study, neither the plant biomass nor the P nutrition was enhanced when AMF interacted with pepperweed (Figs. 2b and 3b). Therefore, the reduction of pepperweed's root As concentration was due to the different strategy to cope with As-contaminated environment compared with mycorrhizal plant. The main reason could be that mycorrhiza increased soil pH values (Table 3), and thus the alkalization of the rhizosphere by AMF seemed to inhibit the As acquisition of non-host pepperweed. The restricted As uptake by pepperweed seems to be a strategy for them to cope with high As toxicity. The similar results were also found that intercropping of upland kangkong and Alfred stonecrop inoculated with AM fungi significantly decreased Cd phytoavailability and kangkong Cd concentration via elevating soil pH (Hu et al., 2013a, 2013b, 2014). Moreover, mycorrhiza also increased pepperweed's root P/As concentration ratios (Fig. 4b2). Arsenate is a phosphate analogue and is transported across the plasma membrane of root cells via high affinity phosphate transporters (Meharg and Macnair, 1990, 1992), it indicated that non-host pepperweed had selective uptake and transfer of P over As. This may be another mechanism underlying the adaptation of the non-host plant to As contamination in the presence of AMF. More As partitioning to shoots of pepperweed in the presence of AMF were also observed in the present study (Table 2). It seemed to be a strategy for pepperweed to adjust the stress of high root As concentration by transporting more As from root to shoot.

4. Conclusions

The present study investigated the interactions of AMF with non-AM host plants under As contamination and provided further evidence for the role of AMF for non-host plant adaptation to As-contaminated soil. Clearly, mycorrhiza was helpful for non-host pepperweed to cope with As toxicity by decreasing root As concentration and without any suppressing effect on biomass production. Our study highlighted that AMF had some effect for pepperweed to adapt to As contamination, which provides further evidence for the protective effects of AMF on non-host plants against As contamination. Moreover, owing that pepperweed can be eaten or used as a medicine, the decreased root As concentration of pepperweed would finally guarantee the food or medicine security of humans. Given the fact that there is little information available on the mechanisms of AMF role in non-host plant resistance to As, further studies to provide direct evidences for the interaction of AMF to non-host plants in As metabolism are warranted.

Acknowledgments

REFERENCES

Abedin, M.J., Feldmann, J., Meharg, A.A., 2002. Uptake kinetics of arsenic species in rice plants. Plant Physiol. 128, 1120–1128.

Bago, B., Vierheilig, H., Piche, Y., AzconAguilar, C., 1996. Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus Glomus intraradices grown in monoxenic culture. New Phytol. 133, 273–280.

Brundrett, M.C., 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320, 37–77.

Chen, B., Xiao, X., Zhu, Y.G., Smith, F.A., Xie, Z.M., Smith, S.E., 2007. The arbuscular mycorrhizal fungus Glomus mosseae gives contradictory effects on phosphorus and arsenic acquisition by Medicago sativa Linn. Sci. Total Environ. 379, 226–234.

Dong, Y., Zhu, Y.G., Smith, F.A., Wang, Y., Chen, B., 2008. Arbuscular mycorrhiza enhanced arsenic resistance of both white clover (Trifolium repens Linn.) and ryegrass (Lolium perenne L.) plants in an arsenic-contaminated soil. Environ. Pollut. 155, 174–181.

Duke, J.A., Ayensu, E.S., 1984. Medicinal Plants of China. Michigan, Reference Publications, Inc., Algonac (ISBN 0-917256-20-4).

Francis, R., Read, D.J., 1994. The contributions of mycorrhizal fungi to the determination of plant community structure. Plant Soil 159, 11–25.

Giovannetti, M., Sbrana, C., Logi, C., 1994. Early processes involved in host recognition by arbuscular mycorrhizal fungi. New Phytol. 127, 703–709.

Gonzalez-Chavez, C., Harris, P.J., Dodd, J., Meharg, A.A., 2002. Arbuscular mycorrhizal fungi confer enhanced arsenate resistance on *Holcus lanatus*. New Phytol. 155, 163–171.

Hoffland, E., Vandenboogaard, R., Nelemans, J., Findenegg, G., 1992. Biosynthesis and root exudation of citric and malic-acids in phosphate pape plants. New Phytol. 122, 675–680.

Hu, J., Chan, P.T., Wu, F., Wu, S., Zhang, J., Lin, X., et al., 2013a. Arbuscular mycorrhizal fungi induce differential Cd and P acquisition by Alfred stonecrop (*Sedum alfredii* Hance) and upland kangkong (*Ipomoea aquatica* Forsk.) in an intercropping system. Appl. Soil Ecol. 63, 29–35.

Hu, J., Li, J., Wu, F., Wu, S., Ye, Z., Lin, X., et al., 2013b. Arbuscular mycorrhizal fungi induced differential Cd and P phytoavailability via intercropping of upland kangkong (*Ipomoea aquatica* Forsk.) with Alfred stonecrop (*Sedum alfredii* Hance): post-harvest study. Environ. Sci. Pollut. Res. 20, 8457–8463.

Hu, J., Wu, F., Wu, S., Lam, C., Lin, X., Wong, M.H., 2014. Biochar and glomus caledonium influence Cd accumulation of upland kangkong (*Ipomoea aquatica* Forsk.) intercropped with Alfred stonecrop (*Sedum alfredii* Hance). Sci Rep 4, 4671.

Kunkel, G., 1984. Plants for Human Consumption. Koeltz Scientific Books, Koeni-Gatein, West Germany 3874292169.

Liu, Y., Christie, P., Zhang, J.L., Li, X.L., 2009. Growth and arsenic uptake by Chinese brake fern inoculated with an arbuscular mycorrhizal fungus. Environ. Exp. Bot. 66, 435–441.

Massenssini, A.M., Araujo Bonduki, V.H., Totola, M.R., Ferreira, F.A., Costa, M.D., 2014. Arbuscular mycorrhizal associations and occurrence of dark septate endophytes in the roots of Brazilian weed plants. Mycorrhiza 24, 153–159.

Matsumura, A., Horii, S., Ishii, T., 2007. Effects of arbuscular mycorrhizal fungi and intercropping with bahiagrass on growth and anti-oxidative enzyme activity of radish. J. Jpn. Soc. Hortic. Sci 76, 224–229.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective-measure of colonizaiton of roots by vesicualr mycorrhizal fungi. New Phytol. 115, 495–501.

Meharg, A.A., Hartley-Whitaker, J., 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytol. 154, 29–43.

Meharg, A.A., Macnair, M.R., 1990. An altered phosphate-uptake system in arsenate-tolerant *Holcus-Lanatus* L. New Phytol. 116, 29–35.

Meharg, A.A., Macnair, M.R., 1992. Suppression of the high-affinity phosphate-uptake system-a mechanism of arsenate tolerance in *Holcus-Lanatus* L. J. Exp. Bot. 43, 519–524.

Meng, L., Zhang, A., Wang, F., Han, X., Wang, D., Li, S., 2015. Arbuscular mycorrhizal fungi and rhizobium facilitate nitrogen uptake and transfer in soybean/maize intercropping system. Front. Plant Sci. 6, 339.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular: No. 939, 1–19.

Pasqualini, D., Uhlmann, A., Sturmer, S.L., 2007. Arbuscular mycorrhizal fungal communities influence growth and phosphorus concentration of woody plants species from the Atlantic rain forest in South Brazil. Forest Ecol. Manag. 245, 148–155.

Pearson, J.N., Jakobsen, I., 1993. The relative contribution of hyphae and roots to phosphorous uptake by arbuscular mycorrhizal plants, measured by dual labeling with P-32 and P-33. New Phytol. 124, 489–494.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parastitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–161.

Prokopiev, I.A., Zhuravskaya, A.N., Filippova, G.V., 2013. Influence of technogenic dust pollution on changes in the physiological and biochemical adaptation and radioresistance of pepperweed (*Lepidium apetalum* Wild.) seedlings. Contemp. Probl. Ecol. 6, 199–202.

Ramirez-Andreotta, M.D., Brusseau, M.L., Artiola, J.F., Maier, R.M., 2013. A greenhouse and field-based study to determine the accumulation of arsenic in common homegrown vegetables grown in mining-affected soils. Sci. Total Environ. 443, 299–306.

Sanders, I.R., Koide, R.T., 1994. Nutrient acquisition and community structure co-occurring mycotrophic and non-mycotrophic old-field annuals. Funct. Ecol. 8, 77–84.

Santos, R.H.S., Gliessman, S.R., Cecon, P.R., 2002. Crop interactions in broccoli intercropping. Biol. Agric. Hortic. 20, 51–75.

Sharples, J.M., Meharg, A.A., Chambers, S.M., Cairney, J.W.G., 2000. Evolution - symbiotic solution to arsenic contamination. Nature 404, 951–952.

Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis. Academic Press, London, UK.

Smith, A.H., Lopipero, P.A., Bates, M.N., Steinmaus, C.M., 2002. Public health - arsenic epidemiology and drinking water standards. Science 296, 2145–2146.

Smith, S.E., Smith, F.A., Jakobsen, I., 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol. 133, 16–20.

Teste, F.P., Veneklaas, E.J., Dixon, K.W., Lambers, H., 2015. Is nitrogen transfer among plants enhanced by contrasting nutrient-acquisition strategies? Plant Cell Environ. 38, 50–60.

Tong, Y., Gabriel-Neumann, E., Krumbein, A., Ngwene, B., George, E., Schreiner, M., 2015. Interactive effects of arbuscular mycorrhizal fungi and intercropping with sesame (*Sesamum indicum*) on the glucosinolate profile in broccoli (*Brassica oleracea var. Italica*). Environ. Exp. Bot. 109, 288–295.

Veiga, R.S.L., Howard, K., van der Heijden, M.G.A., 2012. No evidence for allelopathic effects of arbuscular mycorrhizal fungi on the non-host plant *Stellaria media*. Plant Soil 360, 319–331.

Veiga, R.S.L., Faccio, A., Genre, A., Pieterse, C.M.J., Bonfante, P., van der Heijden, M.G.A., 2013. Arbuscular mycorrhizal fungi reduce growth and infect roots of the non-host plant *Arabidopsis thaliana*. Plant Cell Environ. 36, 1926–1937.

Vogel-Mikus, K., Drobne, D., Regvar, M., 2005. Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. Environ. Pollut. 133, 233–242.

Wang, B., Liu, L., Gao, Y., Chen, J., 2009. Improved phytoremediation of oilseed rape (*Brassica napus*) by *Trichoderma* mutant constructed by restriction enzyme-mediated integration (REMI) in cadmium polluted soil. Chemosphere 74, 1400–1403.

Williams, P.N., Islam, M.R., Adomako, E.E., Raab, A., Hossain, S.A., Zhu, Y.G., et al., 2006. Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. Environ. Sci. Technol. 40, 4903–4908.

Williams, P.N., Raab, A., Feldmann, J., Meharg, A.A., 2007. Market basket survey shows elevated levels of as in South Central US processed rice compared to California: consequences for human dietary exposure. Environ. Sci. Technol. 41, 2178–2183.

Zhang, X., Ren, B.H., Wu, S.L., Sun, Y.Q., Lin, G., Chen, B.D., 2015. Arbuscular mycorrhizal symbiosis influences arsenic accumulation and speciation in *Medicago truncatula* L. in arsenic-contaminated soil. Chemosphere 119, 224–230.

Zhu, Y.G., Sun, G.X., Lei, M., Teng, M., Liu, Y.X., Chen, N.C., et al., 2008. High percentage inorganic arsenic content of mining impacted and nonimpacted Chinese rice. Environ. Sci. Technol. 42, 5008–5013.