Effects of the mycorrhizal fungus *Glomus intraradices* on uranium uptake and accumulation by *Medicago truncatula* L. from uranium-contaminated soil

Baodong Chen[1], Iver Jakobsen[2], Per Roos[3], and Yong-Guan Zhu[1,4]

[1]*Department of Soil Environmental Science, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shang Qing Road, 100085, Beijing China.* [2]*Biosystems Department, Risø National Laboratory, DK-4000, Roskilde, Denmark.* [3]*Radiation Research Department, Risø National Laboratory, DK-4000, Roskilde, Denmark.* [4]*Corresponding author**

Abstract

Phytostabilization strategies may be suitable to reduce the dispersion of uranium (U) and the overall environmental risks of U-contaminated soils. The role of *Glomus intraradices*, an arbuscular mycorrhizal (AM) fungus, in such phytostabilization of U was investigated with a compartmented plant cultivation system facilitating the specific measurement of U uptake by roots, AM roots and extraradical hyphae of AM fungi and the measurement of U partitioning between root and shoot. A soil-filled plastic pot constituted the main root compartment (C_A) which contained a plastic vial filled with U-contaminated soil amended with 0, 50 or 200 mg KH_2PO_4–P kg^{-1}soil (C_B). The vial was sealed by coarse or fine nylon mesh, permitting the penetration of both roots and hyphae or of just hyphae. *Medicago truncatula* plants grown in C_A were inoculated with *G. intraradices* or remained uninoculated. Dry weight of shoots and roots in C_A was significantly increased by *G. intraradices*, but was unaffected by mesh size or by P application in C_B. The P amendments decreased root colonization in C_B, and increased P content and dry weight of those roots. *Glomus intraradices* increased root U concentration and content in C_A, but decreased shoot U concentrations. Root U concentrations and contents were significantly higher when only hyphae could access U inside C_B than when roots could also directly access this U pool. The proportion of plant U content partitioned to shoots was decreased by root exclusion from C_B and by mycorrhizas (M) in the order: no M, roots in C_B > no M, no roots in C_B > M, roots in C_B > M, no roots in C_B. Such mycorrhiza-induced retention of U in plant roots may contribute to the phytostabilization of U contaminated environments.

Introduction

Uranium (U) is one of the most toxic radionuclides and can disperse in environments due to the exploration and utilization of nuclear energy (Ebbs et al., 1998; Xu et al., 2002). U in environments endangers both human and animal health

* FAX No: 8610-62936940; E-mail: ygzhu@mail.rcees.ac.cn

(Fellows et al., 1998), and proper management of U-contaminated environments has become an urgent need. Traditional chemical and physical strategies turned out to be costly and have produced uncertain results and alternative pollution control techniques have been considered (Francis and Dodge, 1998; Zhu and Shaw, 2000). Phytoremediation has been widely accepted as an economic, environment-friendly and efficient

approach for management of soils contaminated by heavy metals or radionuclides (Dushenkov, 2003; Shahandeh et al., 2001; Zhu and Shaw, 2000). Phytoextraction of U was induced by amending soil with organic acids (Huang et al. 1998), but ultimate remediation or cleaning up of contaminants is in most cases impractical for economic and periodical reasons, especially on heavily contaminated sites or mine tailings. Phytostabilization is an alternative strategy, which could prevent U dispersion in environments. The feasibility of U phytostabilization is supported by the fact that only a few hyperaccumulating plant species have been identified (Whiting et al., 2004), whereas many plants can accumulate U in their roots despite large variation in U uptake capability (Sheppard and Thibault, 1984; Saric et al., 1995).

U speciation is closely related to soil properties (especially pH) (Ebbs et al., 1998; Langmuir, 1978; Mortvedt, 1994;) and is one of the key factors influencing U uptake by plants. U is most mobile as U(VI) (Campbell and Biddle, 1977). U(VI) exists in solution predominantly as UO_2^{2+} and as soluble carbonate complexes (Ciavatta et al., 1981; Duff and Amrhein, 1996; Grenthe et al., 1992; Langmuir, 1978). Between pH 4.0 and 7.5, the pH range of most soils, U(VI) exists primarily in hydrolyzed forms (Meinrath et al., 1996). Plants take up U from the readily exchangeable and soluble fractions of the soil. However, according to previous work, negligible amount of U(VI) remain in the soluble and exchangeable forms over any significant time, thereby limiting the amount available for plant uptake and subsequent phytoremediation (Bunzl et al., 1995; Sheppard and Thibault, 1992). In addition, plant-associated microorganisms, including mycorrhizal fungi, may have significant effects on U uptake (Rufyikiri et al., 2002, 2003, 2004b). Arbuscular mycorrhizae (AM) are ubiquitous symbiotic associations between higher plants and soil fungi (Smith and Read, 1997) and their extraradical mycelium form bridges between plant roots and soil, and mediate the transfer of various elements into plants. It has been demonstrated that mycorrhizas could protect plants grown in metal-contaminated soils by enhancing metal retention in roots and reducing metal partitioning to shoots (Leyval et al., 2002). There is direct evidence for the strong binding capacity of fungal mycelium to heavy metals, such as Zn and Cd (Chen et al., 2001; Joner et al., 2000). Therefore, AM can possibly be used for the stabilization of metals in soil environments, revegetation and ecological restoration of metal-polluted sites.

As U behaviour in soil is similar to heavy metals such as Pb, it can be deduced that mycorrhizal fungi should enhance the immobilization of U by roots. It was recently shown that the extraradical AM fungal mycelium took up and translocated U towards root in *in vitro* culture system (Rufyikiri et al., 2002), and hyphae were more efficient in U translocation compared with roots (Rufyikiri et al., 2004a). Soluble uranyl cations or uranyl-sulphate species that are stable under acidic conditions were translocated to a higher extent to roots through fungal tissues, while phosphate and hydroxyl species dominating under acidic to near neutral conditions or carbonate species dominating under alkaline conditions were rather immobilized by hyphal structures (Rufyikiri et al., 2002). However, U transfer from roots to shoots could not be quantified by this model system and studies of U uptake and accumulation by intact plants are required in order to elucidate the potential role of AM in management of U contaminated environments. An *in vivo* study was therefore carried out to compare U uptake by non-mycorrhizal roots, mycorrhizal roots and extraradical hyphae and U redistribution in medic plants using a compartmented cultivation system. The study aimed to investigate mycorrhizal effects on U translocation in the plant-soil continuum, and to evaluate the potential role of AM fungi in phytoremediation of U contaminated soil. Different P application levels were included to investigate their interactions with plant accumulation of U.

Materials and methods

Host plant and AM fungi

Seeds of medic plants (*Medicago trunctula* L. cv Jemalong) were pre-germinated in aerated distilled water for 36 h. The AMF *Glomus intraradices* Schenck and Smith (BEG 87) was propagated on subterranean clover (*Trifolium subterraneum* L.) in

sand–soil mixture for 10 weeks and the inoculum was a mixture of growth medium, fungal spores, mycelium and colonized root fragments.

Compartmented cultivation system

A plastic pot constituted the main root compartment (C_A), which also contained a 50 mL plastic vial (C_B) filled with U-contaminated soil. The vial was sealed by 650 μm or 25 μm nylon net, which permitted the penetration of both roots and hyphae or of just hyphae, respectively, into the contaminated soil (Figure 1).

Cultivation media

The growth medium filled in C_A was a 1:1 (w/w) mixture of sand and soil, which was partially sterilized (10 kGy, 10 MeV electron beam). Basal nutrients without P (Pearson and Jakobsen, 1993) were added to the mixture. The soil–sand mixture (subsequently referred to as soil) had a pH of 6.7 (1: 2.5 soil to water) and a final extractable P content of 5.9 mg kg^{-1}, using the method of Olsen et al. (1954), detailed information of the soil properties was reported by Jakobsen and Nielsen, 1983). U contaminated soil (SCK U-A3) filled in C_B was collected from the storage of U tailings at Schlema U mine site, Germany and passed through a 2 mm sieve. The U-mining debris originates from carbonaceceous micaceous shist and has apparent density of 1.62 g cm^{-3}, pH

of 7.3 (1: 2.5 in water), extractable P of 1.3 mg kg^{-1}, total U of 53 mg kg^{-1}, organic matter content of 3.7% and cation exchange capacity of 5.7 cmol kg^{-1}.

Experiments

Nutrient-amended growth medium for each pot (C_A) was mixed with either 70 g of the *G. intraradices* inoculum or the equivalent amount of the soil–sand mixture was carefully mixed into the soil to obtain the inoculation treatments and the non-inoculation controls. KH_2PO_4–P was mixed into the SCK soil to obtain 0, 50 or 200 mg P kg^{-1}. The C_B vials received 50 g amended SCK soil, which was then covered by 20 g soil–sand mixture as a buffering soil layer. The combination of two inoculation treatments, three P levels in C_B and two nylon mesh sizes resulted in 12 treatments. Each treatment had four replicates and the 48 pots were set-up in a complete randomized block design. Medic seeds were selected for uniformity before sowing, and six pre-germinated seeds were sown in each pot. Three days after emergence seedlings were thinned to three per pot. The experiment was conducted in a controlled environment chamber with a 16h-21°C day and 8 h-16 °C night regime. Osram daylight lamps provided 550 μE m^{-2} s^{-1} PAR (400–700 nm). De-ionized water was added as required to maintain moisture content at 55% of water holding capacity by regular weighing. A solution of NH_4NO_3 was added to the pots 32, 37
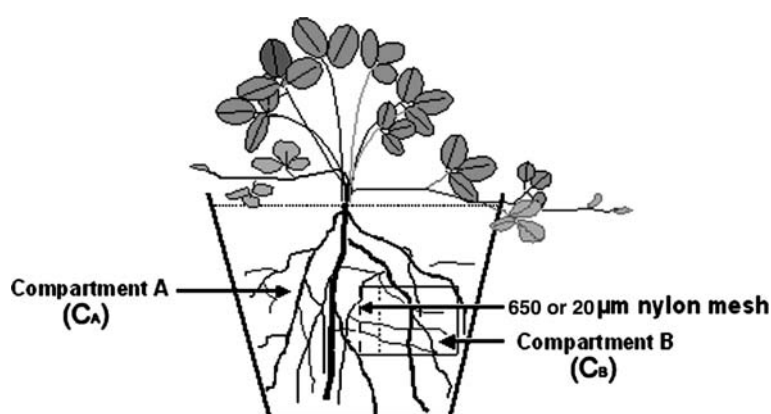

Figure 1. Diagram of the compartmented cultivation system. Coarse (25 μm mesh size) or fine (37 μm mesh size) nylon net was used to separate the cultivation system into two compartments: compartment A (plastic pot, the main compartment) for plant growth, and compartment B (a small container inside plastic pot) for hyphae, roots or mycorrhizal roots development depending on inoculation treatments and nylon net applied.

Table 1. Mycorrhizal colonization rates and hyphal length density of medic plants in association with *G. intraradices* as influenced by different compartmentation and P addition treatments

Inoculation treatment	Type of nylon mesh	P addition levels in Comp. B (mg kg^{-1})	Root colonization (%)		Hyphal length density (mg^{-1})	
			Compartment A	Compartment B	Compartment A	Compartment B
Non-inoculated	Coarse mesh	0	0	0	5.5	0.7
		50	0	0	5.6	0.8
		200	0	0	5.6	0.7
	Fine mesh	0	0	–	4.8	0.5
		50	0	–	4.8	0.9
		200	0	–	5.7	0.4
Inoculated	Coarse mesh	0	77	87	14.5	8.6
		50	78	74	13.6	8.7
		200	75	63	12.4	7.5
	Fine mesh	0	84	–	13.3	10.1
		50	84	–	11.4	10.4
		200	81	–	11.3	13.7
Significance[a] of						
Inoculation (I)			***	***	***	***
Mesh type (M)			***	–	*	**
P addition (P)			NS	**	NS	NS
I × M			***	–	NS	***
I × P			NS	**	NS	NS
M × P			NS	–	NS	NS
I × M × P			NS	–	NS	*

[a] By analysis of variance; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS, not significant.

and 42 days after sowing to provide a total of 120 mg N per pot.

Harvest and analysis

The experiment was harvested 7 weeks after seedling emergence. Shoots were carefully cut off from the stem base and soil samples (about 20 g fresh weight) were collected for determination of hyphal length density before washing out roots. Roots growing into C_B equipped with coarse nylon mesh were excised along the mesh and all C_B compartments were removed from pots and stored frozen for later quantification of roots and hyphae. Roots in C_A were washed with tap water and all plant samples were carefully washed with de-ionised water to remove adhering soil particles. 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 h. Oven-dried sub-samples were milled and digested in mixture of HNO_3 and $HClO_4$ (4:1 v/v) at 225°C. Along with the samples four procedure blanks were run.

The dissolved samples were analysed for uranium (^{238}U) on a High Resolution – Inductively Coupled Plasma-Mass Spectrometry (HR-ICP-MS, PlasmaTrace 2, Micromass) without any radiochemical treatment. The uranium isotope ^{233}U was used as an internal standard. Preliminary study showed that the U signal in the plant material was well above background and typical procedure blank levels were two to three orders of magnitude lower than what was found in the plant materials. Phosphorus in plant samples was determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, Vista axial, Varian).

Sub-samples of fresh roots were cleared in 10% KOH and stained with Trypan blue by a modification of the procedure of Phillips and Hayman (1970), omitting phenol from solutions and HCl from the rinse. Percentage root colonization and root length were determined by the grid line intersect method (Giovannetti and Mosse, 1980). External hyphae were extracted from soil samples using a modified membrane filter technique (Jakobsen et al., 1992).

Statistical analysis

Root data from C_B were analysed by two-way ANOVA to compare mycorrhizal status and P application levels. All other experimental data were analysed by three-way ANOVA to compare mycorrhizal status, compartmentation pattern and P application levels using GenStat for PC/Windows 6.1 (GenStat Committee, 2002).

Results

Colonization of roots and soil by G. intraradices

Roots of inoculated plants were extensively colonized by *G. intraradices* while non-inoculated controls remained non-mycorrhizal (Table 1). For inoculated plants the colonization of roots in C_A was higher when C_B was sealed by the 25 μm nylon mesh than when C_B was sealed by the 650 μm nylon mesh ($P < 0.001$). Colonization of

C_B roots decreased with increasing P application ($P < 0.001$).

The size of mesh sealing C_B had different effects on hyphal length density in C_A and C_B: the coarse mesh resulted in the highest length densities in C_A ($P < 0.05$) while the fine mesh resulted in the highest length densities in C_B ($P < 0.01$) (Table 1). On the other hand, hyphal growth was unaffected by P levels in C_B. Some hyphae were also present in non-inoculated pots, but at lower densities in C_B than in C_A soil. These hyphae were probably dead or saprophytic and were assumed to be also present in the inoculated treatments.

Plant growth

Dry weights of both shoots and C_A roots were significantly increased by inoculation with *G. intraradices* ($P < 0.001$), but were unaffected either by compartmentation or by P application (Figure 2). However, dry weights of C_B roots
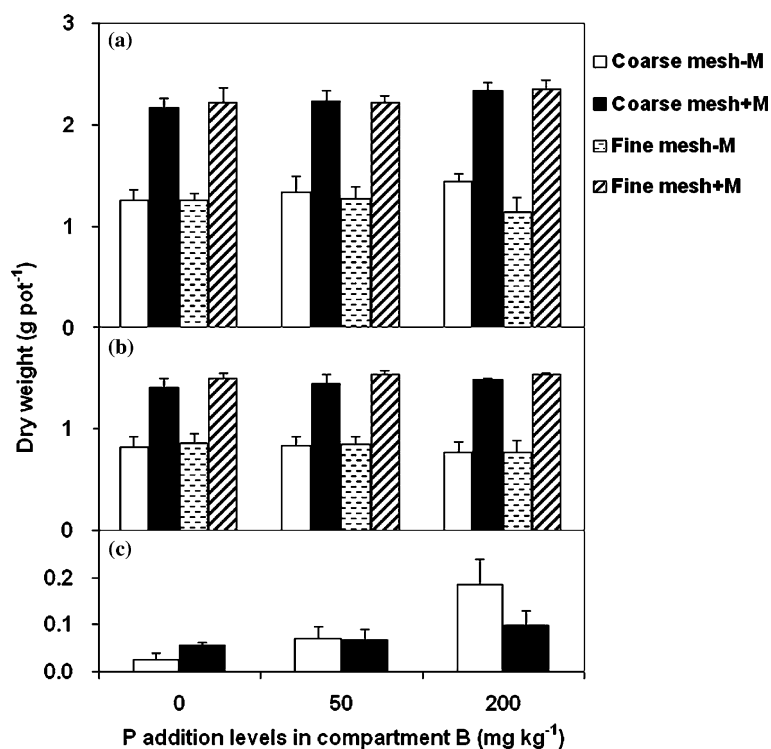

Figure 2. Dry weight of shoots (a), roots in compartment A (b) and compartment B (c) of medic plants under different compartmentation and P application treatments. –M and +M represent non-inoculation treatment and inoculation with mycorrhizal fungus *G. intraradices*. By analysis of variance, inoculation was highly significant ($P < 0.001$) for both shoot and root in compartment A. Interaction between inoculation and mesh size was significant ($P < 0.05$) for shoot. Phosphorus ($P < 0.001$) and interaction of P application with inoculation ($P < 0.01$) was highly significant for root in compartment B.

increased with increasing P levels ($P < 0.001$), and this response was larger in non-inoculated controls than in inoculated treatments.

Plant phosphorus nutrition

P concentrations in plant shoots and in C_A roots were affected by inoculation only ($P < 0.001$) (Table 2), while P concentrations in C_B roots were increased not only by inoculation with *G. intraradices* ($P < 0.01$), but also by P applications ($P < 0.01$).

Mycorrhizal colonization increased plant P content in shoots and in C_A roots ($P < 0.001$) (Figure 3). Shoot P content also increased with increasing P levels in C_B ($P < 0.05$) while P content in C_A roots was higher with the fine than with the coarse mesh sealing C_B ($P < 0.001$), and the P content in C_B roots increased with increasing P application ($P < 0.001$) but was unaffected by mycorrhizal inoculation.

Uranium uptake and partitioning

Shoot U concentrations were generally lower in inoculated plants than in non-inoculated controls ($P < 0.001$), and also lower when C_B was sealed by a fine instead of a coarse mesh ($P < 0.001$) (Table 2). In contrast, root U concentrations were higher in inoculated plants than in controls ($P < 0.001$), and higher with a coarse than with a fine mesh ($P < 0.01$). Root U concentrations in C_B were 10–50-fold the concentrations in C_A roots, but were unaffected by inoculation or P applications (Table 2).

Shoot U content was increased by root penetration into the U contaminated C_B soil ($P < 0.001$) (Figure 4a) and treatment interactions were complex among P application, inoculation and compartmentation ($P < 0.05$): With the 650 μm mesh sealing C_B, shoot U contents was increased by both P application in C_B and mycorrhiza, while when the 25 μm mesh was used, mycorrhiza decreased shoot U contents.

Table 2. P and ^{238}U concentrations in medic plants in association with *G. intraradices* as influenced by different compartmentation and P addition treatments

Inoculation treatment	Type of nylon mesh	P addition levels in Comp. B (mg kg^{-1})	Shoot P concentration (mg g^{-1})	Root P concentration (mg g^{-1})		Shoot U concentration (ng kg^{-1})	Root U concentration (ng kg^{-1})	
				Comp. A	Comp. B		Comp. A	Comp. B
Non-inoculated	Coarse mesh	0	0.83	1.03	0.53	10.63	123	5972
		50	0.78	0.91	0.87	7.62	122	5948
		200	0.90	1.07	1.28	12.87	152	1798
	Fine mesh	0	0.81	0.97	–	8.42	142	–
		50	0.78	1.08	–	11.64	164	–
		200	0.79	0.98	–	5.18	130	–
Inoculated	Coarse mesh	0	0.89	1.46	0.98	7.09	182	5218
		50	0.92	1.46	1.80	8.25	141	4049
		200	0.97	1.46	1.59	7.58	188	6023
	Fine mesh	0	0.90	1.44	–	3.82	203	–
		50	0.98	1.60	–	4.29	202	–
		200	0.98	1.63	–	3.33	183	–
Significance[a] of								
Inoculation (I)			***	***	**	***	***	NS
Mesh type (M)			NS	NS	–	***	**	–
P addition (P)			NS	NS	**	NS	NS	NS
I × M			NS	NS	–	NS	NS	–
I × P			NS	NS	NS	NS	NS	***
M × P			NS	NS	–	**	***	–
I × M × P			NS	NS	–	**	NS	–

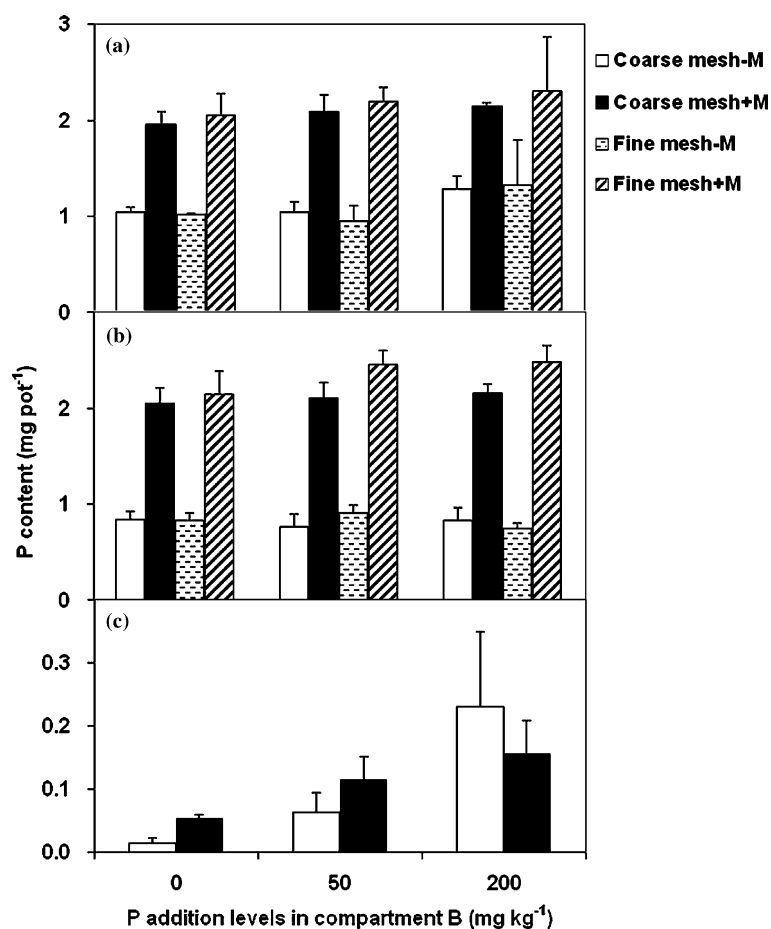[a] By analysis of variance; ***$P < 0.001$; **$P < 0.01$; NS, not significant.

Figure 3. P content of shoots (a), roots in compartment A (b) and compartment B (c) of medic plants under different compartmentation and P application treatments. –M and +M represent non-inoculation treatment and inoculation with mycorrhizal fungus *G. intraradices*. By analysis of variance, inoculation was highly significant for both shoot and root in compartment A ($P < 0.001$). Shoot P content was also significantly influenced by P addition in compartment B ($P < 0.05$), and P content of root in compartment A also significantly influenced by mesh size of the nylon net ($P < 0.001$), and the interaction of inoculation with mesh size ($P < 0.01$) and inoculation with P addition ($P < 0.05$). P content of root in compartment B was only influenced by P addition ($P < 0.001$).

In contrast, *G. intraradices* significantly increased U content in C_A roots ($P < 0.001$), furthermore the root U contents were significantly lower with the coarse than with the fine mesh ($P < 0.001$) (Figure 4b). On the other hand, U Content in C_B roots increased with increasing P levels ($P < 0.05$) (Figure 4c) in accordance with P effects on root growth. Interactions were detected between inoculation and P application treatments ($P < 0.05$).

Medic plants partitioned less than 15% of the total U uptake from roots to shoots (Figure 5) and non-mycorrhizal controls had much higher shoot to root ratios than the corresponding inoc-

ulated treatments ($P < 0.001$). Compared with fine mesh compartmentation, the U partitioning to shoots was higher in the coarse mesh treatments with root accessing the C_B soil than in the corresponding fine mesh treatment ($P < 0.001$). This was most consistent in inoculated plants.

Discussion

This *in vivo* study supports the findings from previous studies (Chen et al., 2005; Rufyikiri et al. 2003, 2004b;) and indicates the potential role of AM fungi in phytostabilization of U contaminated
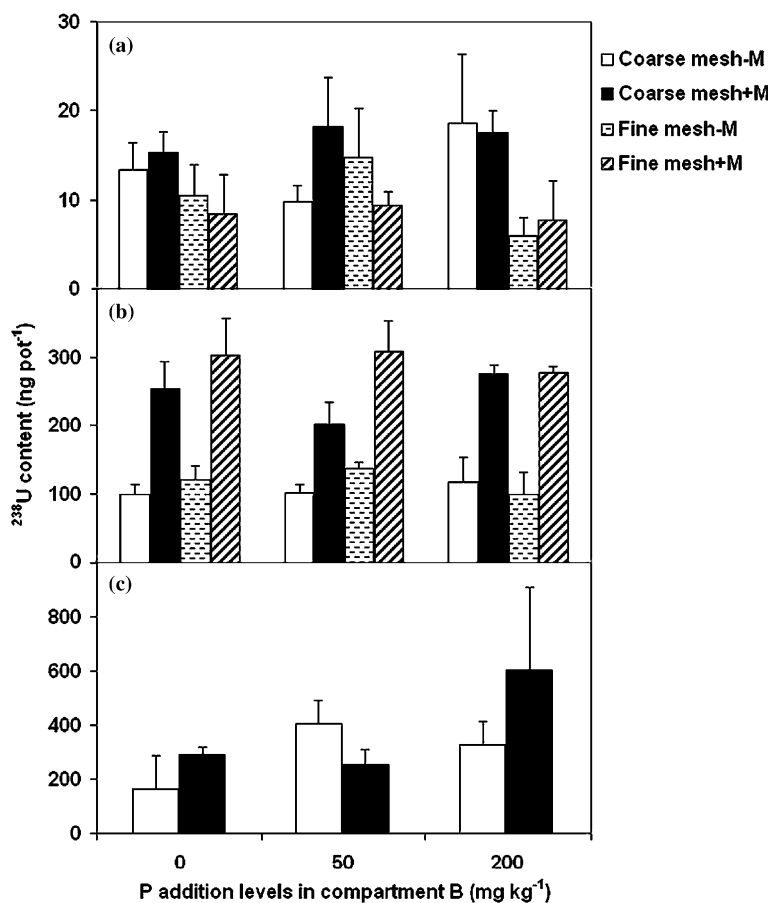
Figure 4. ^{238}U content of shoots (a), roots in compartment A (b) and compartment B (c) of medic plants under different compartmentation and P application treatments. –M and +M represent non-inoculation treatment and inoculation with mycorrhizal fungus *G. intraradices*. By analysis of variance, mesh size was highly significant for both shoot and root in compartment A ($P < 0.001$); Inoculation was only highly significant ($P < 0.001$) for root in compartment A; Interaction of inoculation with mesh size was significant ($P < 0.05$) and that of mesh size with P addition was highly significant ($P < 0.01$) for both shoot and root in compartment A. The interactions among inoculation, mesh size and P addition were also significant ($P < 0.05$) for shoot. U content of root in compartment B was influenced by P addition ($P < 0.05$), and the interaction of inoculation with P addition ($P < 0.05$).

environments. However, the compartmented cultivation system used in the present study has obvious advantages (Smith et al., 2003): C_B occupied only 5% volume of C_A and the system allows investigations on elemental uptake and translocation by mycorrhiza or hyphae under nearly natural conditions without serious interference from spatial restriction of root growth.

If plant shoots are not taken into account, the compartmented system used here is in principle similar to the model system used by Rufyikiri et al. (2003). The influence of mycorrhizas on U uptake and accumulation by roots was rather similar in the two systems: U concentrations and contents of roots from C_A were significantly higher in *G. intraradices*-inoculated plants than in the corresponding non-inoculated controls. Rufyikiri et al. (2003) demonstrated that U concentration in external hyphae was 5.5 and 9.6-fold higher than those in mycorrhizal and non-mycorrhizal roots respectively. External hyphae had higher U flux rates than host roots, but internal hyphae may contribute largely to U immobilization in the mycorrhiza. In the present work, there were also data to show that when only hyphae could access U in C_B, U concentration and contents of roots in C_A were higher than when roots could access C_B.
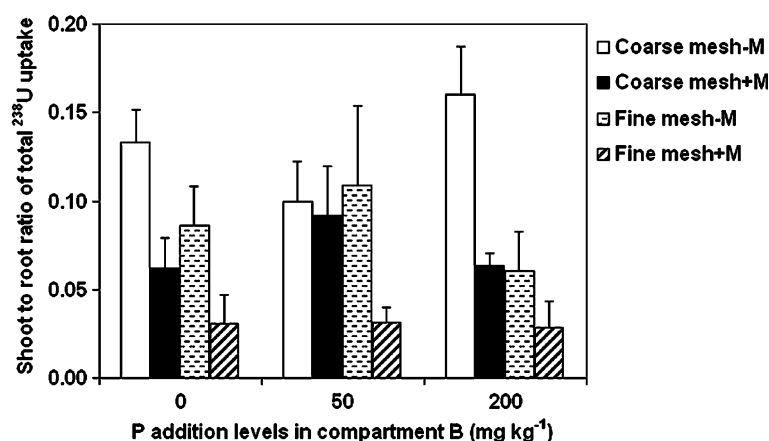
Figure 5. Shoot to root (from compartment A) ratio of total uranium uptake by medic plants as influenced by different compartmentation and P addition treatments. −M and +M represent non-inoculation treatment and inoculation with mycorrhizal fungus *G. intraradices*. By analysis of variance, inoculation and mesh size ($P < 0.001$), and the interactions among inoculation, mesh size and P addition ($P < 0.01$) were highly significant.

No large amount of U was translocated to the plant shoots, and U immobilization in roots was greatly enhanced by mycorrhizal colonization. This was shown by lower shoot U concentrations in inoculated plants and by shoot to root ratios of total U uptake. It was furthermore noticeable that more U had been partitioned to plant shoots when 650 μm net was used than when 25 μm net was used. In other words, U uptake through the root pathway could be more easily translocated to plant shoots than U taken up though the fungal pathway. The mycorrhiza-mediated U uptake was most likely immobilized in root-internal fungal structures as suggested by the observed accumulation of U in fungal vesicles of mycorrhizal *Cynodon dactylon* roots (Weiersbye et al., 1999).

Separation of the two compartments by different mesh sizes influenced root colonization and fungal growth. Where roots could not grow into C_B to take up P, root colonization in C_A was significantly higher than where roots could grow into C_B, and at the same time hyphal length density in C_B was higher. It seems that plants were regulating mycorrhizal development according to accessibility/availability of P sources. With low P supply, host plants would rely more on mycorrhizal fungi for P acquisition. When only hyphae could grow into C_B, P contents in roots in C_A were significantly higher than when there was root access to P in C_B. This indicates that hyphae could not only efficiently take up P (Li et al., 1991; Rufyikiri et al., 2004a), but also efficiently transfer P to host plants (Viereck et al., 2004).

It was noticeable that due to the low soil P supply, the shoot P concentrations of medic plants were generally quite low (<1 mg g^{-1}) even for inoculated plants (by obvious dilution effects). Despite that no typical symptoms of P deficiency were found among inoculated pots, the potential effects of plant P nutrition status on U accumulation could not be neglected. It is documented that plant uptake of U is affected by various factors, such as soil properties (Shahandeh and Hossner, 2002) and uranium–phosphorus interactions (Ebbs et al., 1998; Jerden Jr. et al., 2003). When P was present at high concentrations, complexes between U and P could most likely be formed in the soil. However, U and P may also interact during transportation processes within the plants. Though the experimental data from present study indicated that P addition in C_B did not influence fungal development or U accumulation by plants, plant P nutrition status could still indirectly influence U accumulation by influencing plant growth (root physiological activities) and the development of mycorrhizal symbiosis. Thus there is a need to conduct further investigations on U–P interactions both in soil and plants as mediated by mycorrhizal associations.

To understand the underlying mechanisms is no doubt important for proper manipulation of the combination of plants with associated microbes or fertilization strategies for maximizing the efficiency of phytoremediation.

In conclusion, this study further demonstrated that AM may play an important role in plant adaptation to U contaminated soils by not only improving plant P acquisition ability, but also enhancing U immobilization by roots, thus reducing U partitioning into plant shoots and environmental risks. There is no doubt that AM can play a role in future phytoremediation of U contaminated environments.

Acknowledgements

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