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No significant transfer of the rare earth element samarium from spiked soil to alfalfa by *Funneliformis mosseae*

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Abstract

Rare earth elements including samarium have been widely used in modern technologies in recent decades. Following over-exploitation and soil contamination, they can accumulate in plants and be toxic at high concentrations. Arbuscular mycorrhizae benefit plants in metal-contaminated soils by improving their survival and growth and alleviating metal toxicity, but little information is available about soil contaminated by rare earth elements. We performed two experiments using samarium to study the role of arbuscular mycorrhizal fungi on plant growth and samarium transfer to alfalfa in a samarium-spiked soil. A pot experiment was conducted in a soil spiked with two concentrations of samarium and a non-spiked control, inoculated or not with a metal-tolerant *Funneliformis mosseae*. A compartmented pot experiment was then performed with a separated compartment containing samarium-spiked sand only accessible by *F.mosseae* fungal hyphae to further study the transport of samarium from the soil to alfalfa. The biomass of alfalfa grown on samarium-spiked soil was reduced, while it was significantly higher following arbuscular mycorrhiza inoculation in the pot experiment, both in the control and samarium-spiked soil. Although mycorrhizal plants had a higher phosphorus content than non-mycorrhizal ones, there was no significant difference in samarium concentrations between mycorrhizal and non-mycorrhizal plants. The compartment experiment confirmed that there was no significant samarium transfer to the plant by *F.mosseae*. Other fungi and plants should be tested, and field experiments performed, but our results suggest that arbuscular mycorrhizal plants might be considered in phytorestoration of rare-earth-contaminated soils.

Keywords

Rare earth element, *Funneliformis mosseae*, Compartment experiment, Metal tolerance

Introduction

Rare earth elements (REEs) are a group of elements that comprise 15 lanthanides and yttrium (Y). Despite their name, they are rather abundant in the earth crust, as abundant as some metallic trace elements (MTEs). Due to exceptional conductivity, magnetic and other properties, REEs have been increasingly used in advanced technologies and industries. The potential anthropogenic input of REEs to natural ecosystems and to the food chain will increase correspondingly in the near future (Jiang et al. 2012; Falandysz et al. 2017). In parallel, the over-exploitation of REE ores can cause a dramatic issue to local environment, with large amounts of REE deposits in soil (Li et al. 2010; Liang et al. 2014).

Samarium (Sm) is a lanthanide found half-way among the 15 lanthanides in the periodic table. It is the fifth most abundant REE with about 700 tons of Sm oxide production every year (Zhang et al. 2014). It has been used in lasers, magnets, screens, catalysts, and fertilizers (Zhang et al. 2014; Pagano 2016). This wide use has

made it one of the REEs with the highest commercial interest (Massari and Ruberti 2013). REEs can accumulate in crops and wild plants and be toxic at high concentrations (Carpenter et al. 2015; Pagano 2016). Sm is not only phytotoxic at high concentrations, but has also been reported to be toxic to mammals (Zhang et al. 2014). The toxicity of REEs depends on their bioavailability, which can be affected by their sources and forms and also by soil parameters like the pH, particle size and organic matter (Babula et al. 2008; MacMillan et al. 2019). Other elements such as calcium can affect the bioavailability of light REEs because the uptake of light REEs by plant roots involves Ca channels (Lansman 1990; Yuan et al. 2017).

Arbuscular mycorrhizal (AM) symbiosis represents the most common symbiotic association between fungi and plants and can be found in the roots of 90% of plants on earth. AM fungi bring remarkable benefits to plant growth, especially in stressful conditions like polluted soils. Many studies have been carried out on the effects of AM fungi on plants growing in MTE-polluted soils: AM fungi decrease MTE concentrations in plants and increase plant biomass (Haselwandter et al. 1994; Leyval et al. 1997; Hildebrandt et al. 2007). This beneficial effect of AM fungi on plant growth and a lower metal concentration in AM plants have been found for cadmium (Cd) in maize (Chen et al. 2004; Chang et al. 2018), alfalfa (Redon et al. 2008), and tobacco (Janoušková et al. 2005). They have also been shown for copper (Cu) in both Cu-mine-tailing soil (Da Silva et al. 2005; Chen et al. 2007) and spiked soil (Merlos et al. 2016); for zinc (Zn) in maize grown in Zn-spiked soil (Zhang et al. 2010), and in plants growing in Zn waste soil (Turnau et al. 2001); and finally for lead (Pb) (Hovsepyan and Greipsson, 2004). AM hyphae transported Cd from the soil to the roots in a compartmented pot and radioactive Cd experiment (Joner and Leyval 1997; Chen et al. 2018). However, no AM transport of caesium (Cs) or europium (Eu) to *Medicago truncatula* occurred using the same experimental approach (Joner et al., 2004). Despite the large number of studies carried out on the effects of AM fungi on plants in MTE-polluted soils, few data are available on the effects of AM fungi on plant growth and REE transfer to plants in REE-contaminated soils, and such studies concern only Eu and lanthanum (La) (Joner et al. 2004; Chang et al. 2018).

Many studies have compared different AM fungi for their tolerance to MTE and showed that AM fungi isolated from MTE-contaminated soils were more tolerant to Cd and Zn than isolates from non-contaminated soils (Weissenhorn et al. 1993; Joner et al. 2000; Joner and Leyval 2001; Rivera-Becerril 2002; Rivera-Becerril et al. 2005). Metal-tolerant AM fungi are more efficient to improve plant growth and reduce metal uptake by plants than less tolerant ones (Redon et al. 2009). To date, information on whether AM fungi can also be tolerant to other trace elements such as REEs is lacking.

The objective of our study was to investigate the effect of an AM fungus on plant growth and on REE transfer to plants in an REE-contaminated soil, using Sm as a model REE. We performed two experiments using the model legume alfalfa, inoculated or not with a metal-tolerant *Funneliformis mosseae*. Mycorrhizal and non-mycorrhizal alfalfa plants were first grown in pots with Sm-spiked soil. Then a compartment experiment was performed to determine whether hyphae could transfer Sm to plants. Alfalfa was grown in a top compartment without added Sm, separated from a bottom compartment spiked with Sm by two kinds of mesh to allow hyphal crossing and limit diffusion.

Materials and methods

Soil, plant and arbuscular mycorrhizal fungus

The soil used in both experiments was a Luvisol collected (5-25cm deep) in north-eastern France (Bouzule, 48°44'7.95"N, 6°19'9.64"E). The sampled soil was air-dried, ground, sieved to 2 mm and then homogenised and stored in a cool, dry and dark room prior to the experiments. Its physico-chemical characteristics are presented in Table 1. The soil was amended and homogenized with 2-mm, pre-sieved and washed sand (30% sand/70% soil) to prevent compaction. Both experiments were carried out using the model legume alfalfa (*Medicago sativa L. var. Europe*), which grows rapidly. The AM fungus used in the experiments was metal-tolerant *F. mosseae* strain BEG 69. It was isolated from a soil with a 60-year history of industrial metal pollution (Weissenhorn et al. 1993; Tonin et al. 2001), and is kept as a permanent culture on the contaminated soil using leek as host plant in our laboratory.

Experimental design

Pot experiment

Alfalfa was grown in 1 kg pots, with (M) or without (NM) AM inoculation, filled with soil spiked with 2 concentrations of Sm (100 and 200 $mg \cdot kg^{-1}$, using $SmCl_3 \bullet 6H_2O$ solution) and a control without Sm addition. Four replicates were prepared for each treatment. The spiking concentrations were chosen according to the results of preliminary experiments (Hu et al. 2020). After spiking, the soil was dried at 60 °C and thoroughly homogenized. Spiked or non-spiked soil was used to fill the pots evenly, and deionized water was added to reach 70% of water holding capacity (WHC). The pots were stored at 4°C in a dark room (T0) for 21 days of ageing. Soil samples were collected at T0, dried at 80 °C and ground for Sm analysis. After the ageing period, 15 pre-germinated alfalfa (3-4-day-old) seedlings were transplanted into each pot. Prior to transplantation, mycorrhizal pots were inoculated with approximately 200 spores of *F. mosseae* BEG 69 extracted from a pot culture on leek and added to the soil at 3 cm depth. The non-inoculated pots received an equal amount of deionized water at the same depth. The planted pots were transferred to a growth chamber with a 16 h photoperiod, 22°C/18°C day/night, 250 µmol photons $m^{-2} \cdot s^{-1}$, and 80% relative humidity. Plants were watered twice a week to maintain 70% WHC.

Alfalfa shoots and roots were harvested 49 days after transplantation. The shoot samples were rinsed carefully 4 times with deionized water, then dried, weighed and finely ground for Sm, P, calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) analysis. The root samples were cleaned in the same way and were stained with the trypan blue method to determine mycorrhizal colonization (Koske and Gemma 1989). Thirty 1-cm pieces of stained root segments were sealed on slides, and arbuscular mycorrhizal colonization was observed and determined according to the protocol of Trouvelot et al. (1986).

Compartment experiment

Alfalfa plants inoculated or not with *F. mosseae* were grown in compartmented pots where only AM hyphae could access the bottom compartment containing 100 mg kg^{-1} spiked Sm. The compartmented pots consisted of a plant compartment made of a 150-mm tall polyvinyl chloride (PVC) tube (50 mm outer diameter, 45 mm inner diameter), and a Sm-spiking bottom compartment made of a 30-mm tall PVC tube with the same diameter separated by a nylon mesh (Supplementary Fig. 1). Two kinds of nylon mesh were used: a one-layer 37 µm mesh and a 3-layer (37µm, 1000 µm and 37 µm) mesh, called air-mesh below, forming a 0.5 mm air gap between the two compartments (Joner et al. 2004).

The bottom compartment was filled with 65 g of Fontainebleau sand (EINECS: 238-878-4) spiked with 100 $mg \cdot kg^{-1}$ Sm (365 $mg \cdot kg^{-1}$ $SmCl_3 \bullet 6H_2O$) and deionized water to reach 70% WHC. The main compartment contained 250 g of soil watered with deionized water to reach 70% WHC. Half of the pots were inoculated with approximately 200 spores of *F. mosseae* BEG 69 in the main compartment. Five pre-germinated alfalfa (3-4-day-old) seedlings were transplanted into each pot in the main compartment. Each of the four treatments was carried out in three replicates. All the pots were transferred to the growth chamber with the same conditions as the pot experiment. Plants were watered twice a week to maintain 70% WHC.

Alfalfa shoots and roots were sampled separately 42 days after transplantation. The shoot samples were rinsed 3 times with deionized water. The root samples were rinsed 3 times with deionized water, then transferred to 5-mM ice-cold $CaCl_2$ solution for 15 min to remove the soil particles and adsorbed mineral elements from the root surface, and washed with running deionized water (Han et al. 2004). The samples were dried at 60 °C and weighed before being ground for Sm, P and Ca analysis. Root samples were blotted dry with paper towels and weighed. One root sub-sample was preserved in 70% ethanol solution to measure arbuscular mycorrhizal colonization as described above. Another root sub-sample was weighed before drying at 60 °C, weighed and ground for Sm, P and Ca analysis. Total dry weight was calculated using the total fresh weight along with the fresh and dry weights of the sub-samples.

Sm and nutrient element analysis

The total Sm concentration in the soil samples from both experiments was assessed by *Aqua Regia* digestion. Two hundred mg of ground soil were digested with 10 mL of *Aqua Regia* (nitric acid: hydrochloric acid 1:3 v/v) in an acid-cleaned Teflon tube of the CEM Mars 5 Digestion Microwave System®, and then processed with the digestion program: 15 min digestion at 25°C and 10 min digestion at 170°C. Samples were then filtered (DigiFILTER, SCP Science® 010-500-070), and the volume was calibrated to 10 mL with ultra-pure deionized water 18 MΩ and preserved at 4 °C until analysis.

The soil available Sm concentration was determined using the DTPA extraction method (Carter and Gregorich 2007; Kim et al. 2015) based on preliminary experiments (Hu et al. 2020). One g of ground soil sample was added to 10 mL of DTPA reagent consisting of diethylenetriaminepentaacetic acid (DTPA) (0.005M), $CaCl_2$ (0.01M) and triethanolamine (0.1M). Samples were homogenized and centrifuged. The supernatants were filtered and calibrated to 10 mL with ultra-pure deionized water 18 MΩ and preserved at 4 °C until analysis.

The ground shoot and root samples (200 mg) were transferred into DigiTUBEs with 6 mL of 69% HNO_3 (CAS: 7697-37-2) and 3 mL of 30 % H_2O_2 (w/w H_2O) (CAS: 7722-84-1) at room temperature overnight. Then, the samples were processed in a DigiPREP MS 48 & TS (SCP Science® 010-500-205 & 010-500-275) hot plate system with a digestion program adapted to plant tissues with 5 min solubilization at 45 °C, 10 min pre-digestion at 65 °C and 60 min digestion at 95 °C. Then, the solutions were filtered and preserved at 4 °C until analysis.

For the pot experiment, shoot Sm, P, Na, Mg, K and Ca concentrations were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES), using certified soil NCS® Reference Material DDC 73030 Soil as a reference. The detection limits for Sm concentrations in the plant and soil samples by ICP-OES were 0.02 and 0.05 $mg \cdot kg^{-1}$, respectively. For the compartment experiment, Sm, P and Ca were analysed in the root and shoot samples using inductively coupled plasma mass spectrometry (ICP-MS), using the certified

reference material BCR®-667 estuarine sediment (rare elements, Th, U) as a reference. The detection limit for Sm concentration in the plant samples analyzed by ICP-MS was 0.0001 mg·kg^{-1}.

Data analysis

The data obtained in both experiments were analyzed using XLSTAT version 2020.3. Statistical analyses were performed using two-way analysis of variance (ANOVA) and Tukey's honestly-significant-difference (HSD) post-hoc test. For the non-normally distributed data parameters, we used rank-transformed data. Mycorrhizal colonization data were arcsine transformed before statistical analyses. The data presented in the figures are the means ± standard deviations (SDs). *P*-values are given in the figure legends.

Results

Pot experiment

The Sm concentration was analyzed in the soil just after spiking (T0). It was 83.07 ± 14.88 mg·kg^{-1} and 158.48 ± 24.65 mg·kg^{-1} in the 100 and 200 mg·kg^{-1} Sm spiked treatments, respectively. In the non-spiked soil, it was 4.47 ± 0.49 mg·kg^{-1}. DTPA-extractable Sm in the spiked soil was 27.31 ± 0.83 mg·kg^{-1} and 66.12 ± 5.61 mg·kg^{-1} in the 100 and 200 mg·kg^{-1} spiked soils, respectively. In the non-spiked soil, it was 0.53 ± 0.29 mg·kg^{-1}.

Alfalfa shoot biomass was significantly higher (~ 80%) for the mycorrhizal plants grown on the non-spiked and 100 mg·kg^{-1} spiked soils than for the non-mycorrhizal ones (P<0.0001) (Fig. 1a). However, it was significantly lower (~ 50%, P<0.0002) for the mycorrhizal plants grown on the 200 mg·kg^{-1} spiked soil (Fig. 1a). No mycorrhizal colonization was observed in the non-inoculated plants. Nodules were observed on alfalfa roots in most of the pots, without any clear differences among treatments.

There was a high frequency of mycorrhiza-colonized roots and arbuscules in the inoculated plants, indicating that *F.mosseae* colonized the roots of alfalfa and was active, despite variations among replicates (Table 2). Significantly higher arbuscular frequency and intensity were found in the plants grown on 100 mg·kg^{-1} Sm spiked soil than in those grown on non-spiked soil. Mycorrhizal intensity, arbuscule frequency and intensity in roots were significantly lower in the 200 mg·kg^{-1} than in the 100 mg·kg^{-1} Sm spiked soil, but not significantly different from the non-spiked treatment.

The shoot Sm concentration increased with Sm addition and with the spiking concentration. Sm was detected in alfalfa shoots (0.16 ± 0.02 mg·kg^{-1}) in the non-spiked soil, but it increased to 1.08 ± 0.38 mg·kg^{-1} in the 100 mg·kg^{-1} spiked soil and up to 2.70 ± 0.51 mg·kg^{-1} in the 200 mg·kg^{-1} spiked soil. There was no significant difference between mycorrhizal and non-mycorrhizal plants (Fig. 2a and b).

The alfalfa shoot P concentration was not significantly affected by Sm spiking or AMF inoculation, and no significant difference was found among the treatments (Supplementary fig. 2). However, the P content was significantly affected by both Sm spiking and AMF inoculation (Fig. 1b, P<0.001). The shoot P content was significantly higher in mycorrhizal than in non-mycorrhizal plants in the non-spiked and 100 mg·kg^{-1} spiked soil and not significantly higher in the 200 mg.kg^{-1} spiked soil. The shoot P content was significantly lower in the plants grown on 200 mg·kg^{-1} spiked soil than in those grown on non-spiked soil.

Shoot Na, Mg, K and Ca concentrations were not affected by Sm spiking, while AMF inoculation significantly decreased shoot Ca concentrations in all Sm treatments, shoot Na concentration in the 100 mg.kg^{-1} spiked soil, and K and Mg concentrations in the non-spiked soil (Supplementary fig. 2). The shoot contents in these elements decreased with increased Sm spiking. However, significant differences in Mg and Na contents

were only observed between mycorrhizal plants grown on non-spiked soil and non-mycorrhizal plants grown on 200 $mg \cdot kg^{-1}$ Sm spiked soil. Shoot K and Ca contents were significantly lower in the plants grown on 200 $mg \cdot kg^{-1}$ spiked soil than in those grown on non-spiked soil. The shoot contents of these elements tended to increase with AMF inoculation, but differences among treatments were not significant.

Compartment experiment

Shoot biomass did not significantly differ among the four treatments in the compartment experiment (Fig. 3a). Root biomass was significantly affected by mesh size ($P<0.05$), but it was only significantly different between mycorrhizal plants grown in 37-µm mesh pots and non-mycorrhizal ones grown in air-mesh pots ($P<0.05$, Fig. 3a). As in the first experiment, a good level of mycorrhizal root colonization by *F.mosseae* BEG 69 was observed, with the presence of arbuscules in pots with the two kinds of mesh (both above 25% mycorrhizal frequency) (Table 2).

The alfalfa root Sm concentrations and the Sm contents in both alfalfa shoots and roots were not significantly different among the treatments (Fig. 4), although higher values were observed in the plants grown in the 37-µm mesh pots than in those grown in the air-mesh pots. However, the Sm concentration in alfalfa shoots was significantly higher in the plants grown in the 37-µm mesh pots than in those grown in the air-mesh pots ($F=28.174$, $P=0.001$), especially for non-mycorrhizal plants. But no significant difference in Sm shoot and root concentration or content was observed between mycorrhizal and non-mycorrhizal plants.

The P concentration and content in alfalfa shoots and roots were both affected by mycorrhizal inoculation (two-way ANOVA, $P<0.05$ for shoot concentration and $P\leq0.006$ for shoot content). However, significant differences were observed only for the shoot content with both kinds of mesh and for the root content in air-mesh pots, with higher values in mycorrhizal plants (Fig. 3b). The shoot and root P contents were also significantly affected by the mesh type, and significantly higher with the 37-µm mesh than the air mesh for non-mycorrhizal plants (Fig. 3b). When it comes to the root Ca concentration and content, no significant difference was observed among treatments because of high variance among replicates (Supplementary Fig. 3). However, the shoot Ca concentration was significantly lower in mycorrhizal than non-mycorrhizal plants in air-mesh pots (Supplementary Fig. 3).

Discussion

In the pot experiment, the alfalfa shoot dry weight was significantly higher due to AM inoculation, indicating a beneficial effect of *F.mosseae* on alfalfa growth with or without Sm contamination. When the plants were grown on the Sm-spiked soil, although their biomass was reduced at the highest Sm concentration, there was an 80-100% increase of the shoot biomass of *F. mosseae*-inoculated plants as compared to the non-inoculated ones. There was no significant effect of *F.mosseae* on alfalfa biomass in the compartment experiment, but the rootable soil volume was more limited than in the pot experiment and the plants did not grow on a Sm-contaminated soil (only a limited amount of Sm may have been transferred to the main compartments by diffusion). AM fungi had a beneficial effect on plant growth in REE-contaminated soil in a pot experiment with maize and sorghum grown on mine tailing soil with multiple metal contamination including REEs (Guo et al. 2013), where *Glomus versiforme* substantially promoted plant biomass (over 200%). To our knowledge, no other publication has reported such an effect of AM fungi on plant growth in REE-contaminated soils. Large numbers

of studies have reported this beneficial effect on plants grown on soils historically polluted by metals. However, results differed depending on plants and AM fungi. Redon et al. (2009) tested the effect of AM fungi on barrel medics (*Medicago trunculata*) grown on heavy metal (Pb, Zn, Cd) polluted agricultural soil; 4 out of the 5 fungi used in the experiment significantly increased shoot weight. Janoušková et al. (2005) studied the effects of two isolates of *G.intraradices* on a variety of tobacco in Cd-spiked soils and found a beneficial effect of AM fungi on tobacco plant growth, with differences among isolates. *Rhizophagus irregularis* helped maize to survive in soil spiked with a high concentration of Cu (500 $mg \cdot kg^{-1}$) by increasing shoot and root fresh weight by 15-90% (Merlos et al., 2016), but results differed depending on the Cu concentration and maize cultivar. For REE-contaminated soils, this beneficial effect of AM fungi on plant growth also may be fungus and host-plant dependent, because another AM fungus – *G.mosseae* – did not increase maize and sorghum growth (Guo et al. 2013) .

The improved growth of AM plants is often attributed to nutritional effects, especially P nutrition. Together with the promotion of plant growth, *F.mosseae* increased the P content of alfalfa shoots in our pot experiment. The shoot P content almost doubled in AM-inoculated plants compared to the non-inoculated ones. This increased P content was found even in the highly Sm-contaminated mycorrhizal pots. The same increase in P content was also found in the compartment experiment with the two kinds of mesh, and in alfalfa roots in the air-mesh pots, although plant growth was not increased. In an experiment with REE mine tailings (Guo et al. 2013), *G. versiforme* increased P content by more than 2 fold in maize and 6 fold in sorghum shoots and roots, whereas the other AM species investigated, *G.mosseae*, had no effect.

The beneficial effect of AM fungi on plant nutrition is mainly documented for P, which is a diffusion-limited element in the soil, while the effect on other nutrients is not usually as great. The promotion of nutrient uptake due to AM fungal colonization has been found in mining soil contaminated by Zn, Cd and Pb (Vogel-Mikuš et al. 2005): shoot and root P and S concentrations almost doubled, and the concentrations of mineral nutrients such as K, Ca, Ni and Cu also were significantly higher in AM-inoculated plants. The Ca concentration was analyzed in our experiments because it may interact with REEs. It was not significantly affected in AM-inoculated alfalfa in either experiment. However, the same results were obtained for the concentrations of other mineral nutrients such as K, Mg and Na, suggesting that there was no specific interaction between Ca and Sm uptake. The contents of these elements decreased with increased Sm concentration, probably due to the decreased biomass. In the study of Vogel-Mikuš et al. (2005), the soil was contaminated by several metals, while in our experiment only Sm was present. Chang et al. (2018) showed that maize plants grown in pots in La- and Cd-spiked soil had higher N, P and K concentrations than when grown on single metal-spiked soil and than the controls grown on non-spiked soil. However, there were no increased N, P, K concentrations in plants due to AM symbiosis and even a decrease in some of the treatments. Therefore, further experiments on multi-REE contamination could be considered to clarify the effect of AM fungi on plant nutrient uptake in REE-contaminated soils.

The influence of REE contamination on nutrient element uptake from the soil rarely has been studied, but a few reports indicate that application of REEs decreases nutrient concentrations in plants. La application to maize seedlings decreased root K concentration (Chang et al. 2018), while at a high concentration (>1g REEs/plant, including La, Ce, Pr and Nd) P and Zn uptake by coconut palm was significantly reduced (Wahid et al. 2000). The concentrations of a few plant nutrients (K, Ca, Mg, Zn, Cu, Mn) decreased in wheat seedlings

grown on liquid media following exposure to La and Ce (Hu et al. 2002). In our study, Sm addition had no significant effect on the P concentration in alfalfa shoots, or on the shoot K, Ca, Na, and Mg concentrations. However, compared to experiments with plants grown on nutrient solution (Hu et al. 2002), our study was performed with Sm-spiked soil without any nutrient added. The shoot P, K and Ca contents were significantly lower in the alfalfa plants grown on Sm-spiked soil at the highest Sm concentration, but this could be due to the lower shoot biomass. Wang and Liang (2014) studied the effect of a REE mixture on P adsorption and desorption in 5 different soils and showed that it depended on their respective concentrations and on competitive adsorption between REE and phosphate ions on soil colloids. Grosjean et al. (2019) studied the effects of REEs on the growth and micro- and macro-nutrients of 5 hyperaccumulator *Phytolacca* species; they showed that the leaf P concentration was positively correlated with Sm concentrations, although it was more strongly correlated with heavy REEs. There was no evidence of interactions between Sm and nutrient uptake in plants in our study, but other REEs and soil types should be considered.

Besides promoting plant survival and growth in contaminated soils, AM fungi alleviate metal toxicity by decreasing plant uptake, although conclusions may differ (Pagano 2016). As far as REEs are concerned, no contribution of *G. intraradices* hyphae to Eu uptake by plants has been found (Joner et al., 2004). *Claroideoglomus etunicatum* had no significant effect on root and shoot La concentrations in a pot experiment with spiked soil (Chang et al., 2018). Results differed in a pot experiment with mine tailing soil contaminated with a mixture of REEs and heavy metals, depending on the plant and the fungal species (Guo et al. 2013). *G. versiforme* decreased La, cerium, praseodymium and neodymium in maize roots and shoots, but increased them in the roots of sorghum. However, no data were available for Sm. We observed no significant effect of *F. mosseae* on Sm uptake in either alfalfa shoots from the pot experiment or alfalfa shoots and roots from the compartment experiment. A higher Sm concentration was observed in the plants grown in the 37-µm mesh pots than in those grown in the air-mesh pots, regardless of the presence of AM fungi. However, this higher concentration was likely induced by Sm diffusion through the single-layer mesh, which was prevented in the air mesh pots by a 0.5-mm air gap. A lot of studies have been carried out on the effect of metal contaminant uptake by AM fungi, but produced different results. Vogel-Mikuš et al. (2006) studied AM fungal colonization of the metal hyperaccumulator *Thlaspi praecox* grown on soil long contaminated by Cd, Zn and Pb. They compared AM-inoculated and non-inoculated plants in a pot experiment: AM colonization significantly decreased Cd, Zn and Pb shoot and root concentrations. The question of the direct uptake of metal pollutants by AM fungi has been a challenging issue because these fungi cannot to be cultured in the absence of host plants and it is difficult to distinguish fungal uptake from root uptake (Leyval et al. 1997). Therefore, some researchers used compartmented pots to only allow fungal hyphae access to the contaminant, and radiolabeled elements to have a strong signal and a low detection limit. A radioelement labeling experiment was carried out with a lateral compartment using ^{109}Cd to study Cd transfer through AM hyphae to plants (Joner and Leyval 1997). The shoot Cd concentration was not significantly influenced by ^{109}Cd spiking, but the root Cd concentration was significantly higher in AM-inoculated roots than in non-inoculated ones. This experiment showed that AM hyphae can transport Cd from the soil to the roots, but Cd translocation from the roots to the shoots was not increased by AM colonization. The hypothesis was that AM fungi can decrease MTE uptake by plants by immobilizing metals in the soil through sorption and by binding metals to intraradical fungal structures (Joner and Leyval 1997; Joner et al. 2000; Chen et al. 2018) or to extracellular polymeric substances possibly including

glomalin-like proteins (Wu et al. 2016) reducing their root-to-shoot translocation. Joner et al. (2004) used radiocesium and bottom compartmented pots to study cesium (Cs) transfer by AM fungi to plants. They found no significant difference in Cs radioactivity between mycorrhizal and non-mycorrhizal roots and concluded that there was no significant transfer of Cs from fungal hyphae to plants. On the contrary, radiocesium was present in the roots and shoots of mycorrhizal *Medicago truncatula* plants in *in vitro* culture pots with mycorrhizal plants (Dupré De Boulois et al., 2006). The results of these *in vitro* and *in vivo* studies imply that the sorption capacity of soil clay, K bioavailability in the soil solution and the plant K nutritional status could explain the different results (Dupré de Boulois et al. 2008). We did not find evidence of any Sm transfer *via* AM hyphae to alfalfa, and the mechanisms involved are to be further investigated, but interactions with soil characteristics may have to be considered.

Among the studies on AM fungi in metal-polluted soils, Leyval et al. (1997) and Hildebrandt et al. (2007) reviewed the tolerance of AM fungi to metals and illustrated that certain AM fungi can adapt to high metal concentrations. *F. mosseae* BEG 69 was isolated from a site polluted with MTEs, and has been cultured since then on that metal-contaminated soil. This *F.mosseae* isolate has been found tolerant to Cu (Weissenhorn et al. 1995), Cd (Weissenhorn and Leyval 1995; Joner and Leyval 1997), and multiple heavy metals (Cd, Zn, Pb) (Redon et al. 2009). The metal-binding capacity of AM hyphae has been shown, especially for metal-tolerant isolates (Joner et al. 2000). This could contribute to explain the beneficial effect of these fungi on plant growth in metal-contaminated soils, as they retain toxic metals in mycorrhizal root structures and reduce metal uptake by the plants. However, the beneficial effect of *G. intraradices* on plant growth in a metal-contaminated soil was instead due to the elevated frequency of N-fixing nodules (Redon et al., 2009). In the work of Janoušková et al. (2005), one isolate was more efficient to promote plant growth in the absence of Cd, while another decreased the negative effect of Cd on plant growth. Therefore, different mechanisms may explain the improved growth of mycorrhizal plants in metal-contaminated soils. In our study, *F. mosseae* BEG 69 was tolerant to elevated soil Sm concentrations. The high colonization rate did not significantly decrease at 200 $mg.kg^{-1}$. *F. mosseae* BEG 69 even showed higher arbuscule frequency and intensity in 100 $mg{\cdot}kg^{-1}$ Sm spiked soil than in the control without Sm added. No information is available on the ability of AM fungal structures to bind REEs, but the compartment experiment showed that there was no significant transfer of Sm through *F. mosseae* BEG69 hyphae.

Conclusion

The AM fungus *F. mosseae* BEG 69 colonized alfalfa roots even at the highest spiked concentration of Sm. and significantly promoted alfalfa growth in Sm-spiked and non-spiked soil. This fungus, formerly isolated from MTE-contaminated soil, can tolerate high concentrations of Sm. Other AM fungi from different soils could be tested for their tolerance to REEs, including light and heavy REEs, and their ability to promote plant growth in REE-contaminated soils. This AM fungus increased the P content in both our pot and compartment experiments. The compartment experiment showed that Sm was not transferred from the soil to alfalfa roots by *F. mosseae* extraradical hyphae, in accordance with results previously reported on Eu. In order to have a better understanding of REE transfer in soil-AM fungi-plant systems, further studies should consider a combination of REEs and the interactions between REEs and soil constituents. Other fungi and plants should be tested, and field experiments performed, but our results suggest that the beneficial effect of AM fungi on plants in REE-contaminated soils could be considered for applications in phytoremediation or restoration strategies.

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Table 1 Physico-chemical characteristics of the soil used in the experiment

Soil parameter	Value	Methods
pH	6.68	ISO 10390
sand: silt: clay (%)	167:513:320	NF X 31-107
Organic carbon ($g \cdot kg^{-1}$)	15.8	ISO 14235
Total nitrogen ($g \cdot kg^{-1}$)	1.65	ISO 14235
Organic matter ($g \cdot kg^{-1}$)	27.4	ISO 14235
C/N	9.6	ISO 14235
Olsen P ($mg \cdot kg^{-1}$)	91	ISO 11263
Ca ($cmol+ \cdot kg^{-1}$)	13.1	ISO23470
Mg ($cmol+ \cdot kg^{-1}$)	1.79	ISO23470
Na ($cmol+ \cdot kg^{-}1$)	0.11	ISO23470
K ($cmol+ \cdot kg^{-}1$)	1.14	ISO23470
Fe ($cmol+ \cdot kg^{-}1$)	0.01	ISO23470
Mn ($cmol+ \cdot kg^{-}1$)	0.3	ISO23470
Al ($cmol+ \cdot kg^{-}1$)	0.050	ISO23470

Table 2 Mycorrhizal colonization of roots in the two experiments (Trouvelot et al.1986). Means followed by the same letter do not differ significantly using using one-way ANOVA on arcsine transformed data ($p<0.05$). M: *Funneliformis mosseae* inoculated alfalfa plants, NM: non inoculated alfalfa plant

	treatment	**Mycorrhizal frequency (F%,% of colonized roots)**	**Mycorrhizal intensity (M%, % of root length colonized)**	**Arbuscule frequency (a%)**	**Arbuscule intensity (A%)**
Pot experiment	0 M	42.5±29.7 a	16.1±10.2 ab	10.1±7.1 a	2.1±1.8 a
	0 NM	0	0	0	0
	100 M	60.0±20.0 a	30.4±10.9 a	28.7±13.2 b	8.0±2.9 b
	100NM	0	0	0	0
	200 M	15.0±16.9 a	5.7±7.0 b	2.4±3.2 a	0.1±0.2 a
	200 NM	0	0	0	0
Compartment device experiment	37-M	28.9±8.4 a	17.3±2.7 a	32.7±14.8 a	5.4±1.9 a
	37-NM	0	0	0	0
	Air-M	25.6±3.8 a	16.4±5.4 a	22.8±16.1 a	3.7±2.4 a
	Air-NM	0	0	0	0

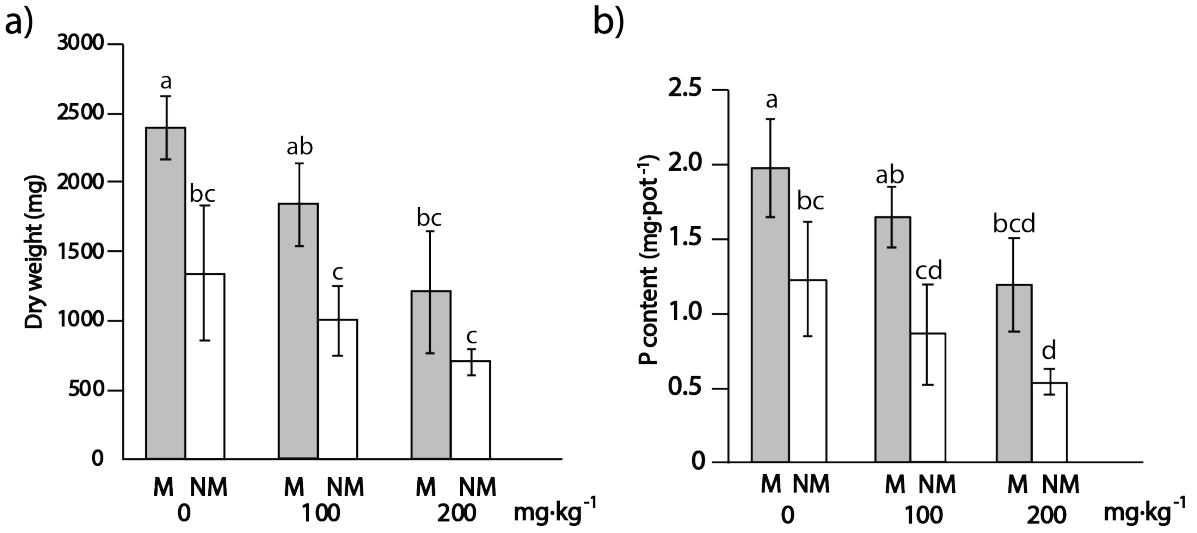


Fig. 1 a) Alfalfa shoot dry weight (total per pot) and **b)** P content of alfalfa shoots as a function of spiked Sm concentration (0, 100 and 200 mg kg^{-1}) and with (M) and without (NM) arbuscular mycorrhizal inoculation in pot experiment. Means followed by the same letter do not differ significantly using two-way ANOVA and Tukey (HSD) post-hoc test (P<0.05)

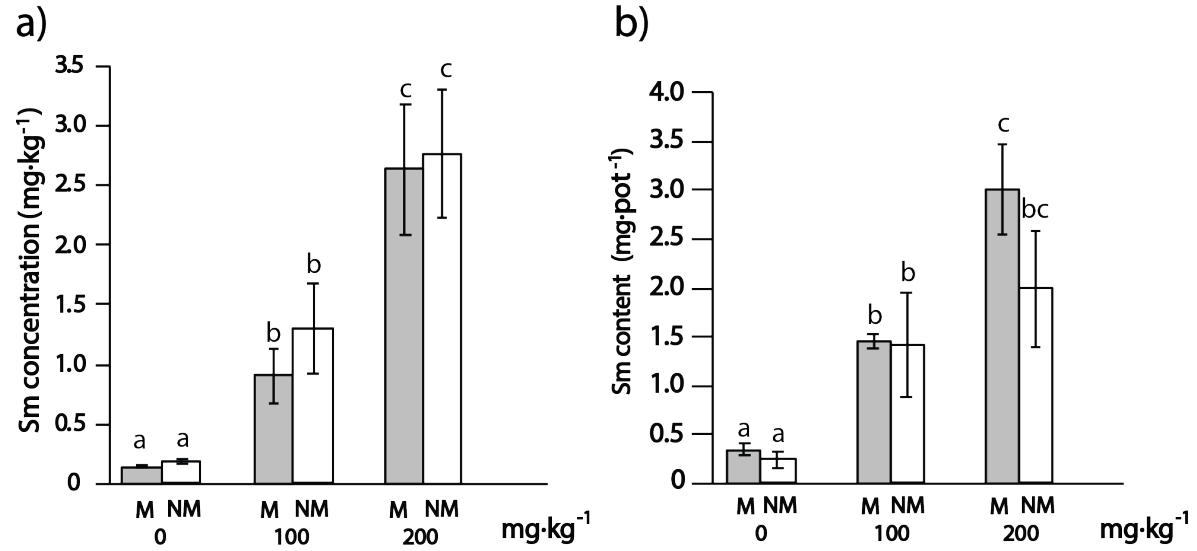


Fig. 2 Sm **a)** concentration and **b)** content of alfalfa shoots as a function of Sm spiked concentration (0, 100 and 200 mg kg^{-1}) with (M) and without (NM) arbuscular mycorrhizal inoculation in pot experiment. Means followed by the same letter do not differ significantly using two-way ANOVA on rank-transformed data and Tukey (HSD) post-hoc test (P<0.05)

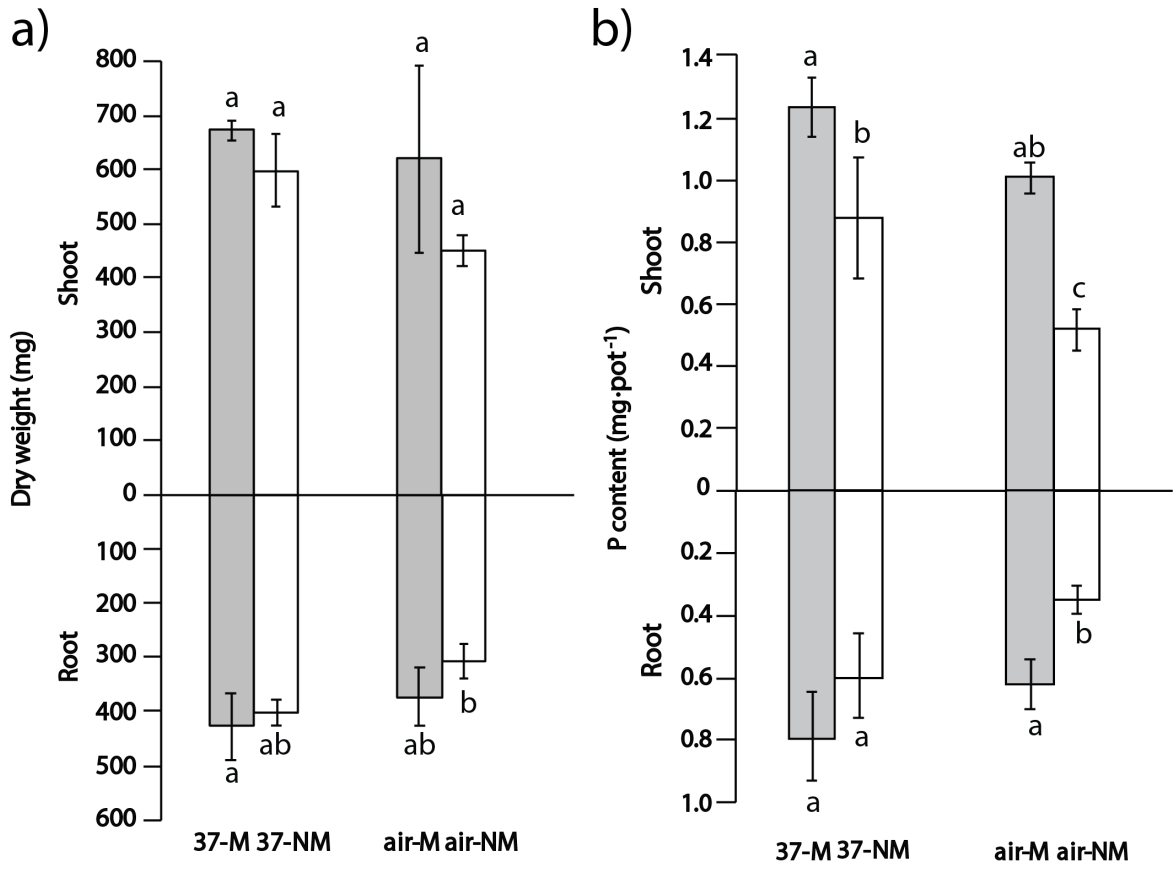


Fig. 3 a) Alfalfa shoot dry weight (total per pot) and **b)** P content of alfalfa shoots in compartment devices with 37 µm (37) or 37-1000-37 µm (air) mesh with (M) and without (NM) arbuscular mycorrhizal inoculation. Means followed by the same letter do not differ significantly using two-way ANOVA and Tukey (HSD) post-hoc tests (P<0.05)

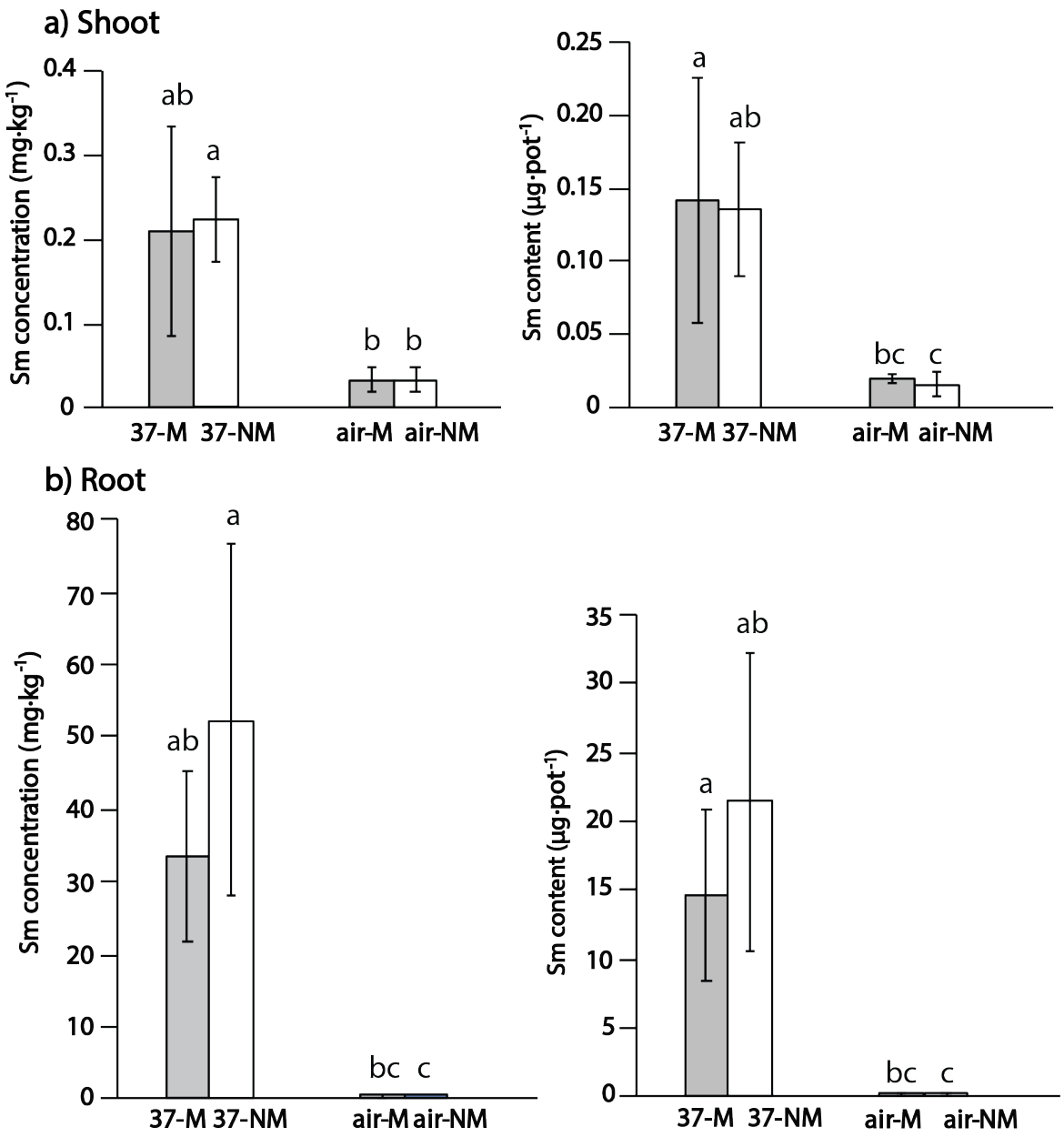
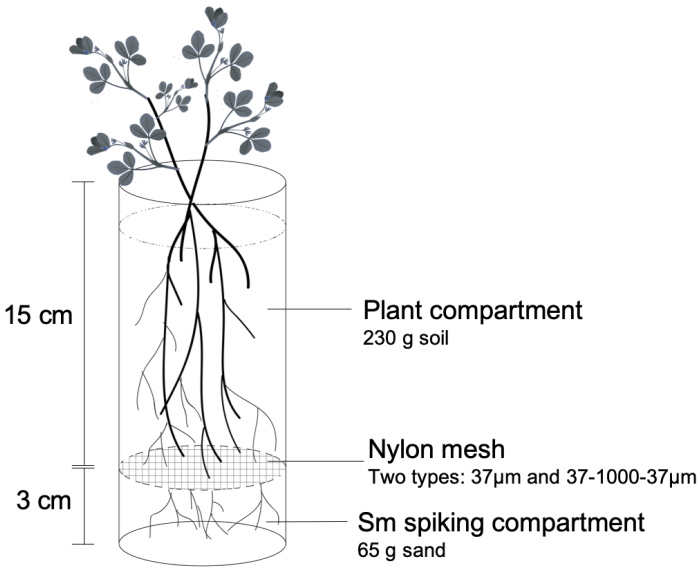
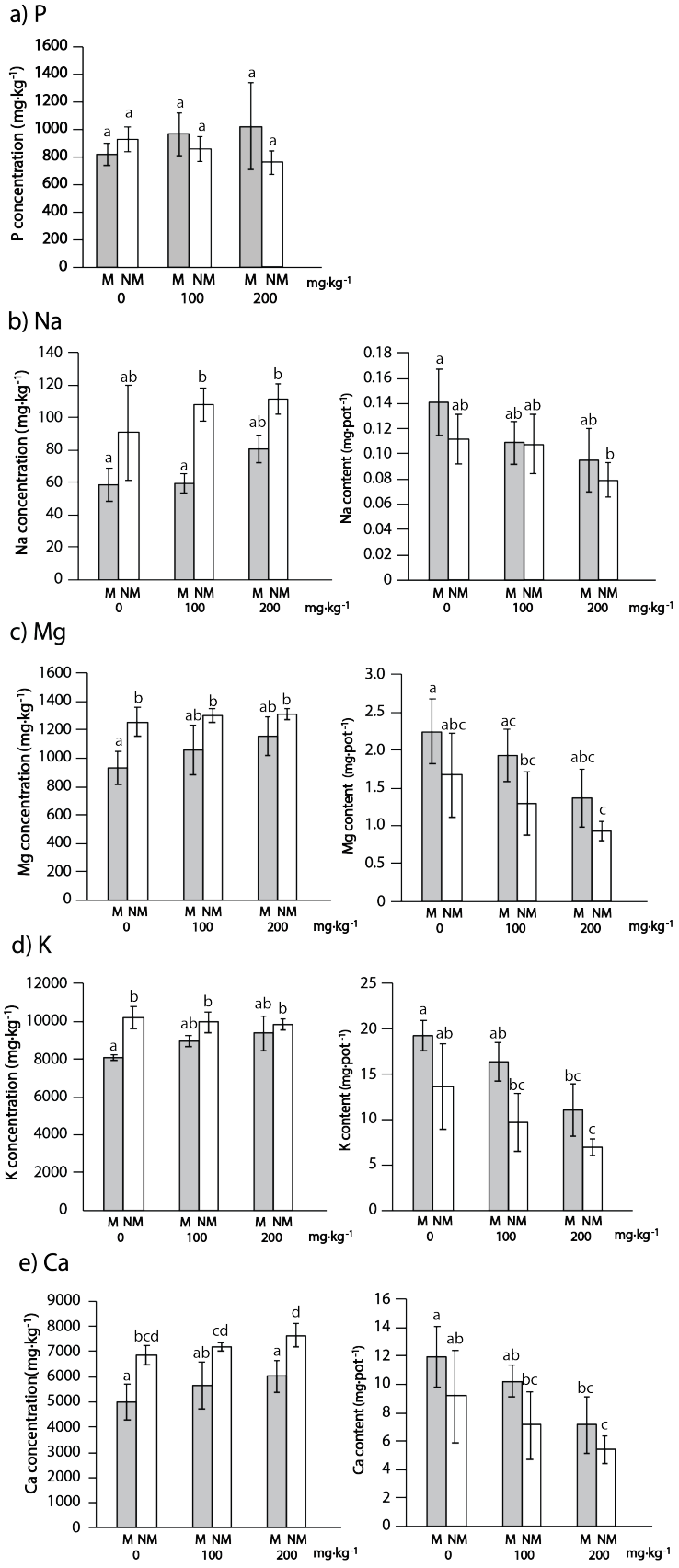


Fig. 4 Sm concentration and content of alfalfa **a)** shoots and **b)** roots in compartment devices with 37 µm (37) or 37-1000-37 µm (air) mesh with (M) and without (NM) arbuscular mycorrhizal inoculation. Rank transformed data were used for shoot Sm content, root Sm concentration and root Sm content. Means followed by the same letter do not differ significantly using two-way ANOVA and Tukey (HSD) post-hoc test (P<0.05)

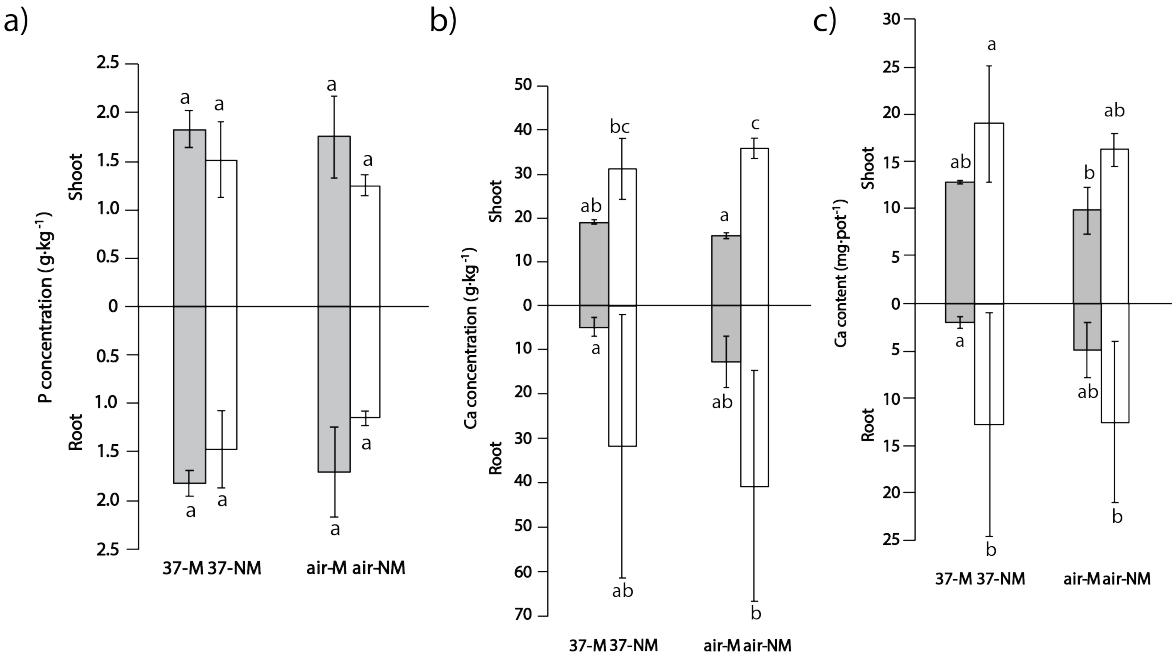


Supplementary Fig. 1 Compartmented pots used in the second experiment



Supplementary Fig. 2 Concentrations of **a)** P **b)** Na **c)** Mg **d)** K **e)** Ca and content of **b)** Na **c)** Mg **d)** K **e)** Ca of alfalfa shoots as a function of Sm spiked concentration (0, 100 and 200 mg kg^{-1}) with (M) and without (NM) arbuscular mycorrhizal inoculation in pot experiment. Means followed by the same letter do not differ

significantly using two-way ANOVA and Tukey (HSD) post-hoc tests (P<0.05). Rank transformed data were used for K, Mg and P concentrations.



Supplementary Fig. 3 a) P concentration **b)** Ca concentration and **c)** Ca content of alfalfa shoots and roots in compartmented pots with 37 µm (37) or 37-1000-37 µm (air) mesh with (M) and without (NM) arbuscular mycorrhizal inoculation. Means followed by the same letter do not differ significantly using two-way ANOVA and Tukey (HSD) post-hoc tests (P<0.05). Rank transformed data were used for shoot Ca concentration, root Ca concentration and root Ca content.