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# Effects of metal lead on growth and mycorrhizae of an invasive plant species (*Solidago canadensis* L.)

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**Abstract**

It is less known whether and how soil metal lead (Pb) impacts the invasion of exotic plants. A greenhouse experiment was conducted to estimate the effects of lead on the growth and mycorrhizae of an invasive species (*Solidago canadensis* L.) in a microcosm system. Each microcosm unit was separated into HOST and TEST compartments by a replaceable mesh screen that allowed arbuscular mycorrhizal (AM) fungal hyphae rather than plant roots to grow into the TEST compartments. Three Pb levels (control, 300, and 600 mg/kg soil) were used in this study to simulate ambient soil and two pollution sites where *S. canadensis* grows. Mycorrhizal inoculum comprised five indigenous arbuscular mycorrhizal fungal species (*Glomus mosseae*, *Glomus versiform*, *Glomus diaphanum*, *Glomus geosporum*, and *Glomus etunicatum*). The $^{15}N$ isotope tracer was used to quantify the mycorrhizally mediated nitrogen acquisition of plants. The results showed that *S. canadensis* was highly dependent on mycorrhizae. The Pb additions significantly decreased biomass and arbuscular mycorrhizal colonization (root length colonized, RLC%) but did not affect spore numbers, N (including total N and $^{15}N$) and P uptake. The facilitating efficiency of mycorrhizae on nutrient acquisition was promoted by Pb treatments. The Pb was mostly sequestered in belowground of plant (root and rhizome). The results suggest that the high efficiency of mycorrhizae on nutrient uptake might give *S. canadensis* a great advantage over native species in Pb polluted soils.

**Key words**: *Solidago canadensis* L.; metal lead; mycorrhizae; N and P uptake; Pb accumulation

## Introduction

*Solidago canadensis* L. is one of the most destructive invasive weeds in southeastern China. It is commonly found in moist or dry fields, such as meadows, edges of forests, swamps, clearings, orchards, and roadsides, ponds-surrounding, stream-banks, fencerows and shorelines. Recently, it is considered as a weed in cultivated fields (Guo and Fang, 2003). Numerous evidences suggest that soil N, P and water availability, light, and temperature intensively influence the growth and spread of *S. canadensis* in China (Ruan *et al*., 2004; Guo, 2005; Huang and Guo, 2005; Lu *et al*., 2005). However, whether and how heavy metal polluted soil impacts the invasion of *S. canadensis* is poorly known.

Heavy metal contaminated soils are increasing in China in the last two decades due to anthropogenic activities such as mining, smelting, combustion of fossil fuels, and sewage irrigation, and so on (Jin *et al*., 2005; Li, 2006; Huang *et al*., 2007; Li *et al*., 2007; Zhu *et al*., 2007). Excessive heavy metals not only strongly suppress the growth and development of native species but also decrease the diversity and activity of soil microbial community (Hu *et al*., 2007; Lei *et al*., 2007; Wang *et al*., 2007). Arbuscular mycorrhizal fungi (AMF) are one of the most important soil microbes as they form mutualistic association with the majority of terrestrial plants (Smith and Read, 1997) and provide an interacting interface between plant root and soil. AMF promote plant growth, facilitate nutrient uptake, ameliorate tolerance to adverse conditions (Abdalla and Abdel-Fattah, 2000; Thingstrup *et al*., 2000; Jakobsen *et al*., 2001; Huang *et al*., 2002; Kowalchuk *et al*., 2002; Kaya *et al*., 2003), and alter heavy metal accumulation of host plants (Meharg and Cairney, 2000; Jamal *et al*., 2002). Furthermore, as a feedback to plant invasion local AMF communities facilitate or inhibit the establishment and flourish of exotic species (Wolfe and Klironomos, 2005). Philip *et al.* (2001) reported that AMF changed vegetative and reproductive traits of an exotic species (*Lythrum salicaria*) in the North America wetlands. Stampe and Daehler (2003) found that AMF significantly affected the invasion process of *Bidens pilosa*. More importantly, AMF showed a relatively high diversity and played an important role in the establishment of *S. gigantean* in a heavy metal polluted soil in Northern Italy (Vallino *et al*., 2006).

In our preliminary experiment we found that *S. canadensis* was a strongly mycorrhizal host (Yang *et al*., 2007). Here we conducted a greenhouse experiment to quantify

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the effects of metal lead on the growth and mycorrhizae of *S. canadensis*. Specific objectives were to examine (1) whether soil Pb depresses growth of *S. canadensis* and formation of mycorrhizae, and (2) whether soil Pb affects the mycorrhizal facilitation on host plant (N and P uptake).

## 1 Materials and methods

### 1.1 Soil, mycorrhizal inoculum and plant for the experiment

The soil was collected from a citrus orchard situated at 28°54′N, 118°30′E in Zhejiang Province, southeastern China. It is a clayey red soil, which is equivalent to *Ultisols* in US soil taxonomy, with 70.50% clay, 10.63% silt, 18.79% sand, and pH 4.59 (determined in KCl). The soil contained 34.39 g/kg organic matter, 1.30 g/kg total N, and 0.95 g/kg total P, there were 48.08 mg/kg extractable N, 59.50 mg/kg extractable P, and 208.23 mg/kg extractable K in the soil. Pb concentration in the soil was 23.27 mg/kg.

Five indigenous arbuscular mycorrhizal fungal species (*Glomus mosseae* (BGC501, XJ-01), *Glomus versiforme* (BGC504, BJ08), *Glomus diaphanum* (BGC506, SC05), *Glomus geosporum* (BGC507, GZ01), and *Glomus etunicatum* (BGC505, TW01)) were used in the experiment. The spores were collected from the Glomales Germplasm Bank in China (Institute of Plant Nutrient and Resources, Beijing Municipal Academy of Agriculture and Forestry Science, China). The propagules of *S. canadensis* were collected in the field.

### 1.2 Experimental design and treatments

This experiment was a partially factorial design with four replicates. Three Pb concentrations (control, 300, 600 mg/kg) were setup in this study to simulate ambient soil and two Pb pollution sites where *S. canadensis* grows. Lead was applied as $Pb(AC)_2{\cdot}2H_2O$ in this experiment.

Special microcosms built with plexi-glass were designed to assess mycorrhizal contribution to N uptake of *S. canadensis* through isolating mycorrhizae from plant roots. Each microcosm (a modification from Hodge *et al.*, 2001; Tu *et al*., 2006) consists of two compartments (13 cm × 14 cm × 15 cm) that are separated by a replaceable mesh (20 μm in diameter, 11.5 cm × 8.0 cm, Tetko/Sefar mesh, Sefar America, New York, USA). The compartment with the plant and arbuscular mycorrhizal (AM) fungi was called the HOST compartment, and other compartment was the TEST. The mesh allowed AM hyphae but no plant roots to penetrate and obtain nutrients from another side.

Each compartment of the microcosm was filled with 3 kg sterilized quartz sand and soil mixture (2:1, *W/W*). For mycorrhizal microcosms, 50.0 g inoculum of constructed AMF community composed of the five *Glomus* species (equal spore numbers for each species) was incorporated into each HOST compartment. The remaining microcosm (non-mycorrhizal control, NMC) received 100 ml of washing filtrate from 50.0 g AM inoculum (without mycorrhizal spores) and the sterilized leftover of the inoculum to correct the possible difference in the microbial community between mycorrhizal and non-mycorrhizal treatments. Four propagules of *S. canadensis* were planted in each HOST compartment after surface disinfected using 3% NaClO twice for 5 min and then rinsed with distilled water.

The microcosms were covered with aluminum foil to minimize the growth of algae. Full strength of Hoagland's nutrient solution (Hoagland and Arnon, 1950) was added every two months to keep normal growth of host plant. The microcosms were arranged in the greenhouse in a completely randomized design and watered daily to keep a constant water capacity.

The $^{15}N$ tracer was introduced to quantify mycorrhizally-mediated N uptake two weeks before harvesting. The $^{15}N$ tracer was injected uniformly as $^{15}N$-enriched mineral N ($(NH_4)_2SO_4$, 99.7% atom $^{15}N$) in DI water at a rate of 3.0 mgN/kg soil into each TEST compartment.

### 1.3 Collection of plant and soil samples

Plants were harvested at maturity (7 months after seedling). Roots and rhizomes (below-ground sample) were cleaned with tap water and separated from shoots (above-ground sample).

Half of each root sample was fixed in FAA (37% formaldehyde/glacial acetic acid/50% ethanol, 9:0.5:0.5, *V/V/V*) for quantification of AM fungal colonization (root length colonized, RLC%). The remaining sample and rhizomes were oven-dried (80°C for 48 h) and weighed. Shoots were oven-dried at 65°C for 48 h and weighed.

Soil samples was collected from both HOST and TEST compartments, transported to the laboratory and stored in the refrigerator at 4°C until further analysis.

### 1.4 Measurements

Root samples were stained with acid fuchsin in lactoglycerol (modified from Koske and Gemma, 1989) and mycorrhizal colonization was quantified from 200 root fragments using the gridline intersect method (Giovannetti and Mosse, 1980) under a stereomicroscope at 10× magnification. Spores were separated from the soil using wet sieving method (Gerdemann and Nicolson, 1963) and spore numbers of each species was counted according to spore morphology (Guo and Bi, 1989).

A subsample (10.0 g) of each dried shoot sample was ground using a stainless steel micronizing miller. Shoot N concentration and N isotope ratios ($^{14}N$ and $^{15}N$) were determined using a continuous flow isotope ratio mass spectrometer (CF-IRMS, Thermo Finnigan DELTA Plus, USA). Sample $^{15}N$ (%) was converted to excess N isotope (mg) based on the atom ratio of atmospheric N. Sample $^{15}N$ content was then calculated from fractional abundance ($^{15}N/(^{14}N+^{15}N)$) and total N content (Hu *et al*., 2001). Total $^{15}N$ in shoot biomass was defined as the $^{15}N$ excess, and the amount of plant $^{15}N$ uptake mediated by mycorrhizae was calculated by $^{15}N$ in mycorrhizal samples minus $^{15}N$ in non-mycorrhizal samples.

The oven-dried root and rhizome samples were milled with the same method as the above. The fine-ground samples were dried to ash at 600°C for 2 h, then dissolved

in 1:1 nitric acid (Lu *et al*., 2000). Pb concentration in the solutions extracted from plant materials (recovery rate 99.5%) was analyzed by flame atomic absorption spectroscopy (FAAS) (Model AA-6650, Shimadzu, Japan).

The above- and below-ground P concentrations were measured spectrophotometrically (UV-1600 spectrophotometer, Beijing, China) according to the method by Murphy and Riley (1962).

The available P in TEST soil was extracted with dilute hydrochloric acid (0.05 mol/L HCl) and sulfuric acid (0.025 mol/L $H_2SO_4$) (Olson and Sommers, 1982) and determined using the molybdate blue ascorbic acid method.

### 1.5 Data analysis

Mycorrhizal colonization was arcsine-square root transformed whereafter normalty and homoscedasticity test were performed prior to any treatment. Comparison between control and NMC was conducted using *t*-test. The rest data were submitted to one-way ANOVA with SPSS 10.0 for Windows software (SPSS Inc., Standard Version). LSD was performed for comparisons of means derived from control and Pb treatments at a significant level of 0.05.

## 2 Results

### 2.1 Mycorrhizal colonization and spore number

Almost no colonization was found in NMC (< 0.1%). Pb significantly decreased mycorrhizal colonization, but no difference was found between the two Pb treatments (Fig.1). Arbuscular mycorrhizal fungi (AMF) responded differently in spore numbers to Pb additions however no significant changes were detected between control and Pb treatments (Fig.2).

### 2.2 Biomass

Compared to non-mycorrhizal control (NMC), mycorrhizae increased above- and below-ground biomass of *S. canadensis* by 30.61% and 35.90%, respectively ($P < 0.05$). The treatment of 300 mg/kg Pb decreased above-ground biomass by 16.56% ($P < 0.05$) and treatment of 600 mg/kg Pb decreased above- and below-ground biomass by 31.22% and 28.26%, respectively ($P < 0.05$, Fig.3).

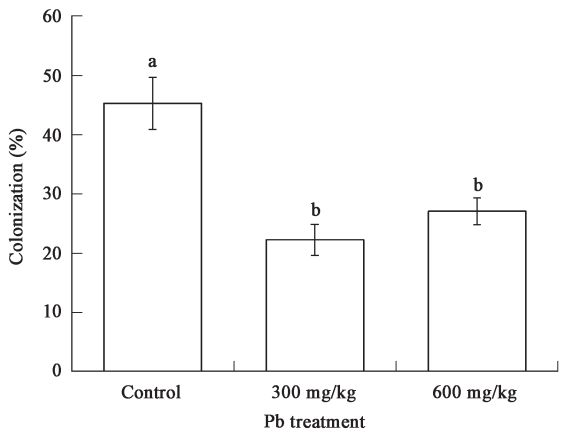


**Fig. 1** Effects of excessive Pb on arbuscular mycorrhizal fungi (AMF) colonization. Values are means ± S.E. The means marked by the same letters are not significantly different according to LSD multiple range test at the level $P \leqslant 0.05$.

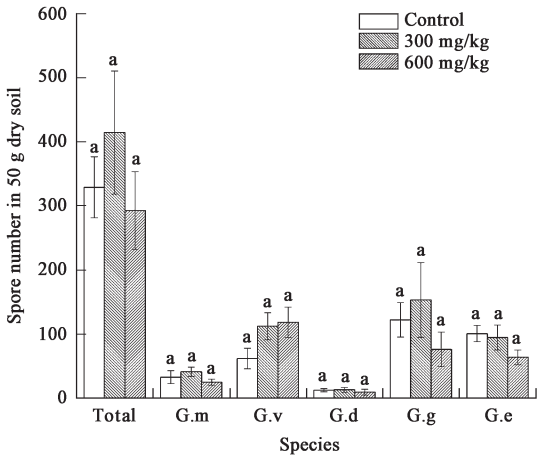


**Fig. 2** Effects of excessive Pb on total spore numbers and individual species spore numbers. The means marked by the same letters are not significantly different according to LSD multiple range test at the level $P \leqslant 0.05$. G.m: *Glomus mosseae*; G.v: *Glomus versiform*; G.d: *Glomus diaphanum*; G.g: *Glomus geosporum*; G.e: *Glomus etunicatum*.

### 2.3 Shoot biomass $^{15}$N and biomass N

No significant difference was detected between control and Pb treatments for shoot total N and mycorrhizally mediated $^{15}$N uptakes of *S. canadensis* (Fig.4).

### 2.4 Biomass P and soil soluble P in TEST compartment

Compared to NMC, mycorrhizae increased above- and below-ground P uptake by 44.18% ($P < 0.05$) and 27.25% ($P > 0.05$), respectively. However, Pb treatments did not significantly affect P uptake compare to control (Fig.5). Compared to NMC, soil soluble P in TEST compartment of control decreased by 5.18% ($P > 0.05$). Pb treatments decreased soluble P in TEST soil by 11.58% ($P > 0.05$) and 17.95% ($P < 0.05$), respectively, compared to control (Fig.6).

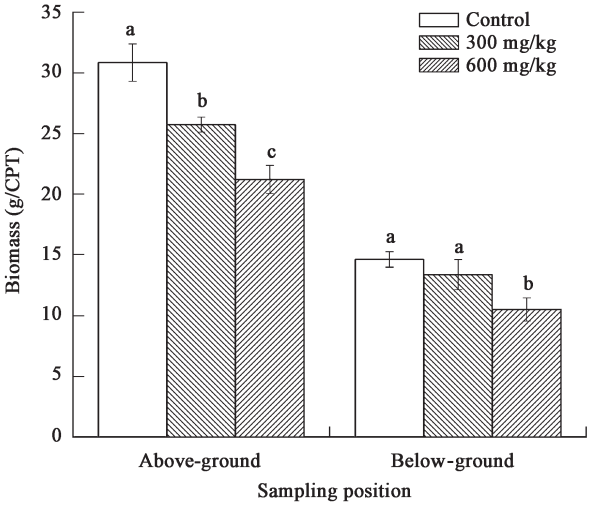


**Fig. 3** Biomass of *S. canadensis* under Pb treatments. Values are means ± S.E. The means marked by the same letters are not significantly different according to LSD multiple range test at the level $P \leqslant 0.05$. CPT: abbreviation of compartment.

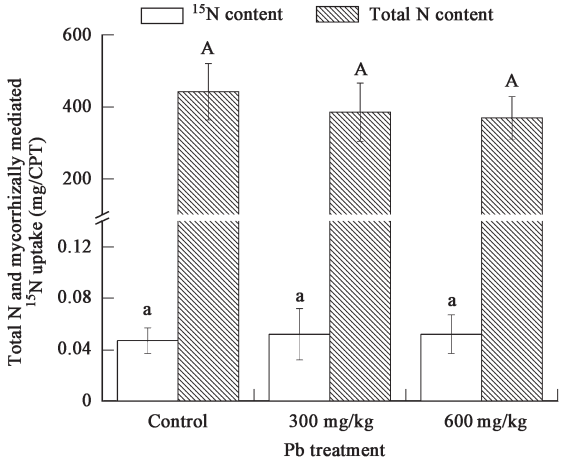


**Fig. 4** Total N and $^{15}$N contents (minus values in non-mycorrhizal control, NMC) in the shoots under Pb treatments. Values are means ± S.E. The means marked by the same letters are not significantly different according to LSD multiple range test at the level $P \leqslant 0.05$.

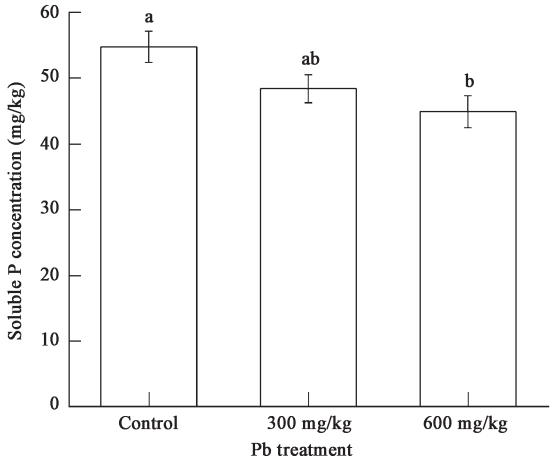


**Fig. 6** Soil soluble P in TEST compartment under Pb treatments. Values are means ± S.E. The means marked by the same letters are not significantly different according to LSD multiple range test at the level $P \leqslant 0.05$.

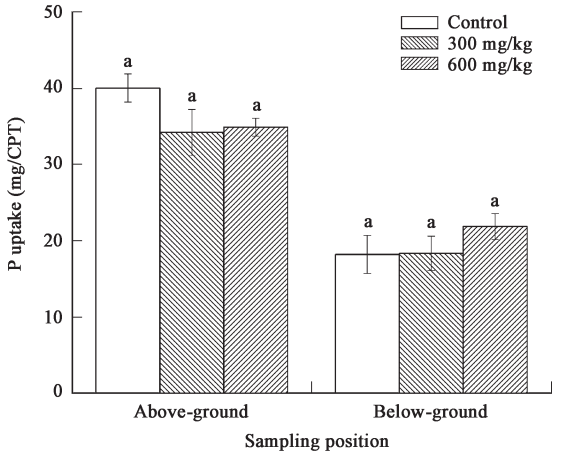


**Fig. 5** Biomass P of above-ground and below-ground under Pb treatments. Values are means ± S.E. The means marked by the same letters are not significantly different according to LSD multiple range test at the level $P \leqslant 0.05$.

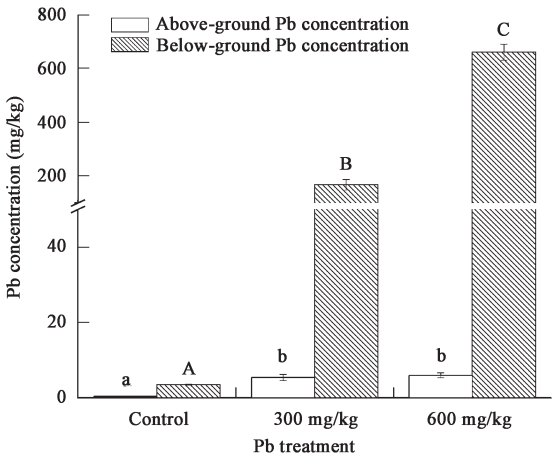


**Fig. 7** Pb accumulation in above-ground and below-ground of *S. canadensis* under Pb treatments. Values are means ± SE. The means marked by the same letters are not significantly different according to LSD multiple range test at the level $P \leqslant 0.05$.

### 2.5 Pb accumulation

Pb accumulations of *S. canadensis* in NMC and control were very low. Pb treatments significantly increased above- and below-ground Pb concentrations compared to control (Fig.7). Pb was mostly sequestered in below-ground and the Pb concentration ratio of below-ground to above-ground was 31.07 for 300 mg/kg Pb treatment and 110.70 for 600 mg/kg Pb treatment, respectively.

## 3 Discussion

### 3.1 Effects of AMF inoculation on *S. canadensis*

Many experiments have well illuminated the correlation between the occurrence of exotic plant invasion and soil abiotic characteristics (Brooks, 2003; Hawkes *et al*., 2005; Lindsay and French, 2005; Kalkhan *et al*., 2007; Siemann and Rogers, 2007). However, whether soil microbes contribute to the prevalence of *S. canadensis* in China is poorly understood. We estimated the effect of the most important soil microbial functional group, AMF, on the growth and nutrient uptake of *S. canadensis* through microcosm experiment. The result revealed that high AMF colonization (45.28%) conferred great benefit on *S. canadensis*. The biomass enhancement was probably due to the facilitation of AMF on mineral nutrient acquisition such as N and P. Although shoot total N uptake was the same between control and NMC, mycorrhizally mediated $^{15}$N and P uptake were increased significantly by AMF inoculation. Since the importance of AMF in exotic invasion has been explicitly illustrated (Callaway *et al*., 2001, 2004; Klironomos, 2002) the benefit from AMF might be a potential mechanism for *S. canadensis* to be a successful invader in China.

### 3.2 Effects of Pb on growth and mycorrhizae of *S. canadensis*

Anthropogenic disturbances decrease the stability of ecosystems and in turn increase the possibility of invasion (Hobbs, 1992; Rodgers and Parker, 2003; Chown *et*

*al*., 2005; Liu *et al*., 2005). Since excessive Pb strongly suppressed the majority of native plants, whether Pb contaminated soils were more vulnerable than ambient environment to invasion would depend on how *S. canadensis* responded to Pb additions. The results showed that growth and mycorrhizal colonization of *S. canadensis* were significantly inhibited by Pb contamination. However, the growth inhibition was more likely to be resulted from high Pb accumulation (Fig.7) rather than decreased mycorrhizal colonization. It is well known that the greatest benefit from AMF is the facilitation on nutrient uptake. Whereas N (including shoot total N and mycorrhizally mediated $^{15}N$) and P uptakes were not changed (Figs.4 and 5) in Pb treatments compared to control. If take biomass into account, N and P concentrations were both significantly increased by Pb treatments (data not shown). Andrade *et al*. (2004) also found that P concentration of soybean was increased by Pb addition. It is assumed that P plays an important role in the process of heavy metal phytotoxicity detoxification and provision of metabolic energy (Patra *et al*., 2004). Mycorrhizally mediated $^{15}N$ provided direct evidence that facilitation of AMF on N uptake was not susceptible to Pb treatments. Furthermore, the depletion of soluble P in TEST compartment, which reflected the magnitude of P uptaken by mycorrhizal hyphae, increased under Pb treatments (Fig.6). This means that Pb treatments motivate the facilitating function of AMF on P uptake.

Pb was mostly sequestered in below-ground (root and rhizome), which could have something to do with mycorrhizae. Glomalin, a kind of glycoprotein produced by AMF hyphae, was found to effectively sequester heavy metals (González-Chávez *et al*., 2004). However, the high Pb accumulated in below-ground and rhizospheric soil could in turn subsequently affect colonizing process (such as spore germination) and formation of mycorrhizae. Unlike mycorrhizal colonization, individual spore numbers showed various responses to Pb treatments although no pronounced difference was found (Fig.2). Considering the low colonization, Pb treatments did not impact or even stimulate sporulation of AMF compared to control.

The invasibility of a recipient ecosystem depends on the stability of itself and the superiority of exotic species to native species based on their responses to the given environment. Funk and Vitousek (2007) found that high resource-use efficiency posed a great advantage for invaders to outperform native species in resource-limiting environments. Actually, it is also the case for a stress environment such as Pb polluted soil. Although growth and mycorrhizal colonization were decreased, nutrient acquisition was not changed and the facilitating efficiency of mycorrhizae even increased in Pb treatments. This undoubtedly increased the invasive ability of *S. canadensis*.

## 4 Conclusions

Our results suggest that *S. canadensis* is highly dependent on mycorrhizae, and the facilitating efficiency on nutrient uptake is promoted by Pb treatments, which might have important implications for *S. canadensis* to invade such polluted environments.

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