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Community structure of arbuscular mycorrhizal fungi associated with *Robinia pseudoacacia* in uncontaminated and heavy metal contaminated soils

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ABSTRACT

The significance of arbuscular mycorrhizal fungi (AMF) in soil remediation has been widely recognized because of their ability to promote plant growth and increase phytoremediation efficiency in heavy metal (HM) polluted soils by improving plant nutrient absorption and by influencing the fate of the metals in the plant and soil. However, the symbiotic functions of AMF in remediation of polluted soils depend on plant–fungus–soil combinations and are greatly influenced by environmental conditions. To better understand the adaptation of plants and the related mycorrhizae to extreme environmental conditions, AMF colonization, spore density and community structure were analyzed in roots or rhizosphere soils of *Robinia pseudoacacia*. Mycorrhization was compared between uncontaminated soil and heavy metal contaminated soil from a lead–zinc mining region of northwest China. Samples were analyzed by restriction fragment length polymorphism (RFLP) screening with AMF-specific primers (NS31 and AM1), and sequencing of rRNA small subunit (SSU). The phylogenetic analysis revealed 28 AMF group types, including six AMF families: *Glomeraceae*, *Claroideoglomeraceae*, *Diversisporaceae*, *Acaulosporaceae*, *Pacisporaceae*, and *Gigasporaceae*. Of all AMF group types, six (21%) were detected based on spore samples alone, four (14%) based on root samples alone, and five (18%) based on samples from root, soil and spore. Glo9 (*Rhizophagus intraradices*), Glo17 (*Funneliformis mosseae*) and Acau3 (*Acaulospora* sp.) were the three most abundant AMF group types in the current study. Soil Pb and Zn concentrations, pH, organic matter content, and phosphorus levels all showed significant correlations with the AMF species compositions in root and soil samples. Overall, the uncontaminated sites had higher species diversity than sites with heavy metal contamination. The study highlights the effects of different soil chemical parameters on AMF colonization, spore density and community structure in contaminated and uncontaminated sites. The tolerant AMF species isolated and identified from this study have potential for application in phytoremediation of heavy metal contaminated areas.

1. Introduction

Soil contamination with toxic heavy metals is a serious and widespread issue resulting from both natural and anthropogenic activities. Mining, agriculture, smelting, electroplating, and other human activities are the major anthropogenic sources of heavy metals found in soils (Gómez-Sagasti et al., 2012). Heavy metals cannot be degraded easily like organic pollutants and can therefore constitute a persistent environmental hazard. Long-term impacts of heavy metal accumulation in soils include adverse effects on microbial biomass, activity, and diversity (Alguacil et al., 2011; Margesin et al., 2011). In most terrestrial ecosystems, soil microorganisms play a critical role in mineral cycling and organic material decomposition. Therefore, any adverse change in the

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environment impacting soil microbial communities can greatly affect ecosystem functions (Wang et al., 2010).

The arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota (Schüßler et al., 2001), form mutualistic symbioses with most terrestrial plants (Smith and Read, 2008). It is well established that mycorrhizal plants take up water and mineral nutrients, especially phosphorus (P), more efficiently than non-mycorrhizal plants (Smith and Read, 2008). In addition, AMF protect host plants against diverse biotic and abiotic stresses, such as heavy metals, because of their beneficial role in enhancing metal tolerance and nutrient acquisition (Doubková et al., 2012; Lehmann et al., 2014). However, the symbiotic functions of AMF are diverse on the level of AMF species, host plants species, soil properties and even AMF ecotypes (Hildebrandt et al., 2007; Hempel et al., 2009; Zarei et al., 2010). Distributions of AMF are also affected by many environmental parameters. Among them, mineral nutrients, especially phosphorus, seem to be more important than other environmental factors. Generally, higher content of phosphorus in soils is associated with lower mycorrhizal root colonization rates and lower AMF diversity (Cheng et al., 2013). Moebius-Clune et al. (2013) suggested that soil texture rather than phosphorus or pH may have an influence on AMF community structure. Although several studies on the effect of heavy metals on abundance and diversity of AMF have been conducted, we still have a poor understanding of the factors that determine AMF community structure, symbiotic functioning and adaptive evolution (Antunes et al., 2012). Therefore, a better understanding of AMF communities in heavy metal contaminated areas is important to fully evaluate the plant-AMF (König et al., 2010; Thoms and Gleixner, 2013), AMF–AMF (Jansa et al., 2008) and AMF-soil (Bedini et al., 2010) interactions. The effects of heavy metals on soil environments are complex because of the complex array of parameters (Wang et al., 2010), and therefore it is crucial to identify specific phylotypes or ecotypes of AMF and their relationship with specific soil environments (Zarei et al., 2010). Consequently, a comparative analysis of AMF community structure in polluted soils with different heavy metal concentrations is essential for identification of metal-tolerant AMF isolates and ecotypes to develop efficient phytoremediation techniques (Zarei et al., 2010; Hassan et al., 2011).

A variety of methods have been designed to analyze the diversity of AMF in field soils and plant roots (Sanders, 2004). Most current studies are based on spore counts and on morphological identification of AMF spores extracted directly from the field (Gai et al., 2006; Symanczik et al., 2014; Wetzel et al., 2014; de Oliveira Freitas et al., 2014). A limitation of this method is that spore density assessed in soils does not necessarily reflect the AMF population that is actually colonizing the plant roots (Clapp et al., 1995; Hempel et al., 2007). In fact, some AMF species may not be detected at all because they sporulate only infrequently or not at all in the field (Clapp et al., 1995; Sanders, 2004). Recently developed molecular tools for detection of AMF species directly in plant roots and soils result in a better understanding of the abundance and diversity in this group of fungi (Schüßler et al., 2001; Chen et al., 2014; Krishnamoorthy et al., 2014). Helgason et al. (1998) designed the primer AM1 to match all small subunit ribosomal RNA (SSU) sequences known at that time from the *Glomales*. In combination with the universal eukaryotic primer NS31 (Simon et al., 1992), the AM1 primer can amplify partial 18S rRNA sequences (approximately 550 bp) from the three traditional families of *Glomales*. Compared to other regions, AMF SSU sequences have relatively low variability, which makes phylogenetic analysis more convenient (Kohout et al., 2014). However, the primers for the SSU rRNA gene cannot cover the whole range of AMF, and usually co-amplify DNA from plants (Alguacil et al., 2011; Hazard et al., 2014) or non-target fungal groups (Borriello et al., 2012; Chen et al., 2014).

Nowadays, the use of molecular methods may provide a more detailed analysis of root or soil AMF communities compared with spore-based morphological approach. However, undoubtedly, sporulation is a key part of the AMF life-cycle, and therefore, the analysis of AMF diversity based on spore 18S rRNA can reflect an important life history strategy of AMF for surviving in abiotic environments. In order to obtain a more dynamic and more complete picture of the AMF abundance and diversity actually present in rhizosphere soils and roots, we have combined the sequence analysis based on the SSU region of rRNA genes of AMF in rhizosphere soil, root and spore samples extracted directly from study sites. In the present study, we compared AMF status and community structure in the root and rhizosphere of a metal tolerant tree, black locust (*Robinia pseudoacacia* L.), among study sites contaminated or uncontaminated with Pb, Zn, Cu or Cd. Black locust is a commonly planted legume tree in China with high drought tolerance (Yang et al., 2014) and has been extensively used for reforestation to control soil erosion in the Loess Plateau. Black locust can form symbiotic associations with both nitrogen-fixing rhizobia and phosphorus-acquiring AMF. As a pioneer species, *R. pseudoacacia* appeared to be a promising woody legume for phytoremediation in heavy metal polluted areas due to its fast growth, deep root system, heavy metal tolerance and ability to fix atmospheric nitrogen (Vlachodimos et al., 2013). In our previous investigation, we reported that *R. pseudoacacia* was frequently found in heavy metal polluted areas and commonly colonized by AMF (will be published separately). However, a detailed knowledge of AMF status in these bacteria-AMF-plant interactions under heavy metal polluted conditions is still lacking. The objectives of the present study was to (1) compare different experimental methods to analyze AMF community structure by molecular analysis of root, soil and spore samples collected directly from study sites; (2) evaluate the toxic effect of heavy metals (Pb, Zn, Cu and Cd) on AMF status, abundance and community structure; and (3) identify environmental factors controlling status, abundance and species compositions of AMF in forest soils. We hypothesize that (1) AMF status, richness and diversity would differ between heavy metal polluted and unpolluted areas due to different disturbance tolerance among AMF species; and (2) AMF community structures present in root, soil and spore samples in different study sites would differ because of different ecological preferences and life history strategies among AMF species.

2. Materials and methods

2.1. Study sites

The study sites are located in Qiandongshan lead and zinc region, Feng County, northwest part of China. Qiandongshan lead and zinc region is the largest and the most typical of five national nonferrous metal planning mines, and accounts for 25% of the total reserves of Feng County (Yao et al., 2004). The predominant pollution sources in this region are mine wastewater, beneficiation wastewater, and mine tailings (Hou et al., 2003). The region has a warm temperate semiarid climate with an annual average temperature of 11.4 °C. The annual average rainfall and frost-free period of Feng County are 613.2 mm and 188 days, respectively. The main soil type is cinnamon and brunisolic soil according to the traditional soil genesis classification in China, and the soil texture is from light to heavy (Xu et al., 2012). The mineral resources are very abundant and mining industry has become an important economic mainstay in Shaanxi Province. Specifically, mineral resources of lead and zinc are mostly distributed in this region and its annual output accounts

for 72% of the entire provincial output. Feng County, with an annual production capacity of 100,000 tons of zinc (Zn) concentrates, 30,000 tons of lead (Pb) concentrates, 10,000 tons of electrolytic lead, and 5000 tons of lead alloy, has become one of the four largest Pb–Zn bases in China. However, due to the traditional development model, which focuses more on economic growth using less advanced technologies and neglecting environmental protection, a large amount of waste from factories has caused serious environmental pollution.

The heavy metal polluted area was divided into sites of high (S9, 33°51.288′N, 106°39.429′E; S10, 33°51.290′N, 106°39.438′E), medium (S8, 33°51.034′N, 106°38.647′E), low (S6, 33°51.972′N, 106°39.312′E; S7, 33°49.810′N, 106°39.054′E), and non-polluted (S1–S5, 34°03′54″N–34°04′20″N, 106°46′30″E–106°48′38″E) levels. The separation between polluted and non-polluted soils was based on recommendation of environmental quality standard (Grade II) in soils of China (GB 15618-1995). The division of polluted soils into levels of high, medium, and low pollution was based on the variations of Pb and Zn concentrations in the study area.

2.2. Sampling and analysis

Sampling was conducted from August to September (rainy season), 2012. At each site, three healthy, similarly-sized *R. pseudoacacia* were randomly selected. The plants were young trees of 7–12 years old with 4–7 m height and 3.5–8.0 cm diameter at breast height (DBH). The soil samples were collected beneath each tree from at least three directions. In each direction, the top 3-cm was removed to eliminate part of the leaf litter and one large hole (diameter, 50 cm; depth, 30 cm) was excavated in order to collect enough root fragments. The large hole was within 100 cm of the trunk to ensure that the collected root samples belonged to the correct species. The roots were carefully excavated and traced from the originating tree to ensure identity. The soils loosely and tightly bound to the surface of roots were removed by clean tweezers and brush, and defined as rhizosphere soil samples for DNA extraction and spore density determination. The zone we studied here was sometimes more than 1 mm wide from the surface of the roots (rhizosphere soil) due to the moist soil during the rainy season, but this zone of soil was still influenced by root secretions and associated soil microorganisms to a certain extent. The remaining extracted soils from the hole at different directions were homogenized and then approximately 500–1000 g soil was placed in plastic bags for determination of soil physical–chemical properties. The soil samples were sieved (2 mm mesh size) to remove stones, coarse roots and other litter. In some sites heavily polluted by heavy metals, additional large-diameter excavations were necessary to collect adequate quantities of fine roots for DNA extraction and measurement of mycorrhizal colonization. For each tree, portions of each composite root sample were contained in 50 ml centrifuge tubes filled with formaldehyde-acetic acid alcohol (FAA) for determining mycorrhizal colonization; the remaining root samples and rhizosphere soil samples were kept in cooling boxes with ice cartridges during transport to the laboratory and were stored at 4 °C prior to DNA extraction.

2.3. Soil chemical properties

The soil samples were air dried at room temperature, ground to a fine powder in an agate mortar, and then sieved through a 10 mesh (<2 mm) and an 80 mesh (<180 μm). The finely ground powder (<180 μm) was used to determine the chemical properties and the coarse powder (<2 mm) was used to measure pH and electrical conductivity (EC). Soil chemical and physical analysis was conducted in the College of Natural Resources and Environment, Northwest A&F University, Yangling, P.R. China. The soil pH was determined using a combination glass electrode (Leici PHS-3D, Shanghai, China) according to the international analysis method of ISO 10390: 2005 (soil/water, 1:5). The air dried soil samples were shaken with deionized water (soil:solution ratio, 1:5) for 3 min and the EC of the supernatant was determined with a conductivity meter (DDSJ-308A, Zhejiang, China). Soil organic matter (OM) content was measured by dichromate oxidation and titration with ferrous sulfate (Nelson and Sommers, 1982). Total nitrogen (TN) content was determined according to the semi-micro Kjeldahl method (Bremner and Mulvaney, 1982). Soils were treated with HF-$HClO_4$, and then the total phosphorus (TP) content was measured colorimetrically after wet digestion with HF-$HClO_4$ (Jackson, 1958). Available nitrogen (AN) was determined with a micro-diffusion technique after alkaline hydrolysis (Page, 1982). Available phosphorus (AP) was determined by extraction with a buffered alkaline solution of 0.5 M sodium bicarbonate (pH = 8.5). The product was quantified colorimetrically in a spectrophotometer (Hitachi, UV2300) at 660 nm (Page, 1982). The total and DTPA-extractable heavy metal concentrations were determined by flame atomic absorption spectrometry (FAAS, Hitachi Z-2000, Tokyo, Japan), following the dissolution of a 0.5 g soil sample with aqua regia (HNO_3/HCl = 1:3) and $HClO_4$, DTPA solution (0.005 M diethylene triamine penta-acetic acid (DTPA), 0.01 M $CaCl_2$,0.1 M triethanolamine, pH = 7.3). The blank reagent and standard reference soils were analyzed for quality assurance and quality control. All of the results were calculated from triplicate analytical data.

2.4. Mycorrhizal colonization analysis

To evaluate mycorrhizal colonization, the root samples were washed with tap water, and then cut into about 1-cm length segments. The method modified from Koske and Gemma (1989) was used to clean and stain root samples. The segments were first softened in 2.5% KOH at 90 °C for 1 h, bleached in alkaline hydrogen peroxide at room temperature for 30 min, acidified in 1% HCl at room temperature for 1 h, and then stained with trypan blue (0.05%) at 90 °C for 20 min. The mycorrhizal colonization was estimated according to Trouvelot et al. (1986). More than two hundred root fragments per site were used to measure the mycorrhizal colonization (MC) under a light microscope (Olympus Bx51, Japan) at 200× magnification. The percentage of mycorrhizal structures in each 1 cm root fragment was assessed as 0, 10, 20 … 100%. The intensity of MC was measured as follows (Liu and Li, 2000):

$$MC\% = \frac{\sum(0\% \times N_0 + 10\% \times N_{10} + 20\% \times N_{20} + \cdots + 100\% \times N_{100})}{(N_0 + N_{10} + N_{20} + \cdots + N_{100})}$$

Where N is the number of root fragments.

2.5. Spore isolation

AMF spores were isolated from soil samples using wet sieving and sucrose density gradient centrifugation (Daniels and Skipper, 1982). Briefly, distilled water was added to 30 g of soil and the solution passed through a sequence of sieves (2000, 500 and 32 μm). The soil fraction in the last sieve was collected into plastic tubes. Spore suspensions (25 mL) were transferred to 50 mL centrifugation tubes, and another 25 mL of a 70% sucrose solution were added at the bottom of the tubes, which were then centrifuged for 2 min at 3000 rpm. Spores were collected from the water–sucrose interface, washed and transferred to Petri dishes with filter paper for sorting and quantification under a dissection microscope at up to ×400 magnification. Only spores that appeared

to be viable (based on color, shape, surface condition and examination of spore contents) were counted (Eom et al., 2001). AMF spore density was expressed as number of spores in one gram of dry soil. Spores from soil samples from each site were pooled and then grouped according to spore morphology and color using a dissecting microscope. Spore number of each morphotype in each pooled sample was counted, and the spore morphotypes were further identified using the molecular method as described below.

2.6. Molecular analysis

Fine roots and rhizosphere soil were homogenized in liquid nitrogen using ceramic mortar and pestle. Root DNA was extracted from approximately 100 mg lyophilized roots using the Plant DNA Extraction Kit (Tiangen Biotech, China) following the manufacturer's instructions. Soil DNA was extracted from approximately 500 mg lyophilized soils using the E.Z.N.A. Soil DNA Kit (Omega, USA) following the protocol of the manufacturer. A total of 60 samples, consisting of 30 soil samples and 30 root samples, were subjected for DNA extraction. The purity and the concentration of the DNA were measured by Smartspec™ Plus Spectrophotometer (Bio-Rad, USA), and the quality was checked by agarose gel electrophoresis (Invitrogen, USA) with ethidium bromide staining (Sigma, USA). The DNA samples were then diluted 1: 10 with ddH_2O to be used as PCR template. For spore samples, up to four clean and healthy-looking spores of each morphotype per pooled sample were transferred into microcentrifuge tubes and vortexed at maximum speed. Spores were further rinsed four times with sterilized distilled water. Single spores were transferred into tubes with 10 μL ddH_2O, and crushed with forceps under the dissecting microscope.

Partial AMF 18S ribosomal RNA (about 550 bp) was amplified from soil, root and spore DNA extracts with the universal eukaryotic primer NS31 (Simon et al., 1992) and primer AM1, designed to amplify AMF 18S ribosomal RNA sequences but not plant sequences (Helgason et al., 1998). The PCR reactions were carried out in a final volume of 25 μL, containing 12.5 μL 2 × Taq Master Mix (Beijing CoWin Biotech Co., Ltd), 0.5 μL each primer (10 μM, Invitrogen), 1 μL template and 10.5 μL RNase-Free Water with the following cycling conditions: 94 °C for 4 min, followed by 30 cycles of 94 °C for 50 s, 56 °C for 60 s, 72 °C for 70 s and extension at 72 °C for 10 min. All the PCR reactions were run in BioRad PCR cycler (USA). PCR products were examined on a 1.5% (w/v) agarose gel with ethidium bromide staining in the presence of DL2000 DNA ladder. PCR products were purified using Universal DNA Purification Kit (Tiangen Biotech CO., LTD, Beijing, China). Purified PCR products were cloned into the plasmid pGEM-T vector following the manufacturer's instructions of the pGEM-T Cloning Kit (Tiangen Biotech CO., LTD, Beijing, China), and then the ligation products were transformed into DH5α competent cells. Totally, 60 clone libraries were constructed (30 clone libraries for root samples and soil samples). Within each clone library, 40 positive clones were selected and restriction digested using *Hin*fI and *Hsp*92II enzymes according to the manufacturer's advice (Takara, Kyoto, Japan). Digested products were examined on a 2.0% (w/v) agarose gel. Representatives of each RFLP type detected were sequenced with primer T7 by GenScript Corporation (Nanjing, China).

2.7. Data analysis

All glomeromycotan sequences were aligned using DNAMAN version 6.0 (Lynnon Biosoft, USA), and clustered to species-level groups according to 97% sequence similarity. Each species-level group was regarded as an AMF group type. The AMF group types found in this study and the GenBank sequences most similar to clone sequences were included in the phylogenetic tree construction. Phylogenetic analysis was performed using MEGA version 5.0, and the neighbor-joining tree was constructed by performing 1000 replicates to produce bootstrap values. The 54 sequences obtained in this study had been deposited in the GenBank database with accession numbers KM233891–KM233916 and KM659233–KM659260.

The sequence group data were imported into SPSS v16.0 (Chicago, USA) to calculate the Shannon–Wiener index (H'), Margalef (d_{Ma}), Evenness (E_h) and Simpson index (D) as follows:

$$H' = -\sum_{i=1}^{S} P_i \ \ln P_i,\ P_i = \frac{n_i}{N}$$

$$d_{Ma} = \frac{S-1}{lnN}$$

$$E_n = \frac{H'}{H_{max}}$$

$$D = 1 - \sum_{i=1}^{S} P_i^2$$

where N is the total number of clones selected from each clone library, S is the total number of AMF sequence groups detected in each site, n_i is the number of the ith sequence group present in each clone library.

Redundancy analyses (RDA) were conducted to determine the multivariate relationships between AMF community compositions and environment variables using the software Canoco (version 4.5, Centre for Biometry, Wageningen, The Netherlands). Pearson's correlation coefficients were calculated to determine the relationships among environmental factors, mycorrhizal colonization, spore density and the biodiversity indices by using SPSS software version 16.0 ($P < 0.05$). Significant differences were detected by employing a one-way analysis of variance (ANOVA) ($P < 0.05$) and significant differences between means were determined by Duncan's test ($P < 0.05$). Principal component analysis (PCA) was performed to compare the AMF communities from root, soil and spore samples at family level. In this analysis, the presence or absence of each family was scored as “1” or “0”, respectively.

3. Results

3.1. Soil properties and AMF status

The main characteristics of the soil samples collected from ten study sites are presented in Table 1. The soil samples were all slightly alkaline with pH of the ten study sites ranging from 7.45 (S9) to 8.36 (S2). S10 had the highest EC (0.94 dS/m), TP (0.85 g/kg), TN (1.50 g/kg), AP (15.6 mg/kg) and AN (25.5 mg/kg), whereas the highest OM was found at S9 (12.2 g/kg). The values of pH, OM, EC, TP, TN, TP, AP and AN showed small but significant differences among study sites, but no obvious difference of AN could be found among the five study sites located in uncontaminated area ($P > 0.05$). The concentrations of heavy metals showed a large variability among the five study sites selected from contaminated area (Table 1). S10 had the most serious heavy metal pollution and the total concentrations of Pb and Zn at this site were 16.9 and 2.7 times higher than the environmental quality standard (Grade II) in soils of China (GB 15618-1995), whereas S6 was only slightly polluted by Pb (65.4 mg/kg), Zn (136 mg/kg), Cu (29.0 mg/kg) and Cd (0.32 mg/kg). The DTPA-extractable Pb, Zn, Cu and Cd presented

Table 1
Chemical properties of the rhizosphere soil samples and AMF colonization of *Robinia pseudoacacia* growing in uncontaminated area (S1–S5) and lead and zinc mining area (S6–S10).

	Uncontaminated area					HM contaminated area				
Parameters	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
pH	8.16 ± 0.13bc	8.36 ± 0.13a	8.17 ± 0.15bc	8.21 ± 0.09b	8.18 ± 0.09bc	8.24 ± 0.15 ab	8.06 ± 0.11c	8.13 ± 0.20bc	7.45 ± 0.17e	7.88 ± 0.16d
OM (g/kg)	7.29 ± 0.28e	7.53 ± 0.38e	7.54 ± 0.33e	8.73 ± 0.43c	7.32 ± 0.43e	7.39 ± 0.53e	8.29 ± 0.29cd	8.15 ± 0.32d	12.2 ± 0.8a	10.3 ± 0.77b
EC (dS/m)	0.74 ± 0.05cd	0.74 ± 0.05cd	0.80 ± 0.04b	0.71 ± 0.03cde	0.75 ± 0.05c	0.73 ± 0.04cde	0.70 ± 0.04de	0.81 ± 0.04b	0.68 ± 0.04e	0.94 ± 0.07a
TP (g/kg)	0.64 ± 0.05cd	0.44 ± 0.05g	0.25 ± 0.05h	0.43 ± 0.07g	0.55 ± 0.10ef	0.72 ± 0.09b	0.59 ± 0.05de	0.67 ± 0.10bc	0.52 ± 0.05f	0.85 ± 0.07a
TN (g/kg)	1.08 ± 0.05cde	1.11 ± 0.07cde	1.16 ± 0.05bc	1.05 ± 0.05ef	1.02 ± 0.05f	1.12 ± 0.06cd	0.82 ± 0.10h	0.85 ± 0.06h	1.23 ± 0.20b	1.50 ± 0.07a
AP (mg/kg)	11.4 ± 0.9c	9.7 ± 1.0f	13.3 ± 1.4b	10.9 ± 0.6cde	11.1 ± 1.4cd	13.2 ± 2.2b	10.0 ± 0.9ef	14.1 ± 1.3b	11.5 ± 1.3c	15.6 ± 1.4a
AN (mg/kg)	16.7 ± 1.6cd	16.4 ± 2.8cd	15.6 ± 1.3d	17.1 ± 1.3cd	17.9 ± 1.9bc	19.4 ± 2.3b	12.1 ± 1.3e	10.2 ± 1.0f	15.6 ± 2.1d	25.5 ± 2.8a
TPb (mg/kg)	20.8 ± 3.7e	26.2 ± 3.9e	20.8 ± 3.6e	21.3 ± 5.4e	20.5 ± 4.1e	65.4 ± 9.3e	181 ± 32d	364 ± 38c	1982 ± 212b	5899 ± 250a
TZn (mg/kg)	62.0 ± 7.4g	79.0 ± 8.9 fg	58.2 ± 9.7g	65.9 ± 16.3g	105 ± 17e	136 ± 14e	94.1 ± 20.7ef	611 ± 41b	508 ± 34c	812 ± 21a
TCu (mg/kg)	29.4 ± 2.9c	20.0 ± 2.3e	18.1 ± 3.1e	24.5 ± 6.2d	24.2 ± 6.4d	29.0 ± 4.5c	36.6 ± 3.5b	26.0 ± 3.7cd	74.3 ± 5.3a	37.1 ± 4.5b
TCd (mg/kg)	0.27 ± 0.09de	0.18 ± 0.07e	0.23 ± 0.03de	0.20 ± 0.04e	0.22 ± 0.07de	0.32 ± 0.04d	0.77 ± 0.11c	1.51 ± 0.07a	1.31 ± 0.17b	0.80 ± 0.23c
DPb (mg/kg)	1.92 ± 0.22e	1.62 ± 0.33e	2.10 ± 0.25e	2.70 ± 0.62e	2.69 ± 0.24e	5.56 ± 0.46e	14.9 ± 1.5d	29.3 ± 2.8c	245 ± 13a	161 ± 11b
DZn (mg/kg)	1.47 ± 0.15f	1.87 ± 0.48ef	2.21 ± 0.34de	1.84 ± 0.38ef	1.69 ± 0.26ef	2.58 ± 0.41d	2.13 ± 0.33def	13.5 ± 1.2b	12.3 ± 1.0c	18.6 ± 0.7a
DCu (mg/kg)	0.81 ± 0.09e	1.04 ± 0.19d	0.93 ± 0.16de	0.89 ± 0.20de	0.88 ± 0.11de	1.64 ± 0.21c	0.96 ± 0.16de	2.08 ± 0.21b	2.99 ± 0.21a	1.71 ± 0.23c
DCd (mg/kg)	0.03 ± 0.01e	0.03 ± 0.00e	0.04 ± 0.01e	0.14 ± 0.01bc	0.03 ± 0.00e	0.15 ± 0.01b	0.12 ± 0.02c	0.09 ± 0.01d	0.25 ± 0.04a	0.15 ± 0.02b
MC (%)	38.6 ± 2.6a	34.8 ± 4.7 ab	39.3 ± 2.1a	40.0 ± 4.8a	38.8 ± 3.1a	29.3 ± 1.8bc	31.7 ± 3.6bc	27.6 ± 3.6c	21.7 ± 2.8d	12.6 ± 3.3e
SD (g^{-1} soil)	3.90 ± 0.38cd	4.93 ± 0.31b	4.13 ± 0.22c	2.95 ± 0.39ef	5.77 ± 0.46a	4.84 ± 0.33b	2.19 ± 0.33g	3.78 ± 0.23cd	3.32 ± 0.38de	2.48 ± 0.76 fg

Assay values are presented in means of three replicates ± standard deviations. Different letters indicate significant differences according to Duncan's test. Abbreviations: OM, organic matter; EC, electrical conductivity; TP, total phosphorus; TN, total nitrogen; AP, available phosphorus; AN, available nitrogen; TPb, total Pb; TZn, total Zn; TCu, total Cu; TCd, total Cd; DPb, DTPA-extractable Pb; DZn, DTPA-extractable Zn; DCu, DTPA-extractable Cu; DCd, DTPA-extractable Cd; MC, mycorrhizal colonization; SD, spore density.

the same patterns as total Pb, Zn, Cu and Cd (Table 1). In our study, the heavy metal concentrations in soils were found to decrease in the order Pb > Zn > Cu > Cd at all study sites. The increasing order of total/DTPA-extractable concentrations of Pb at five study sites located in polluted area was S6 < S7 < S8 < S9 < S10, whereas the increasing order of total/DTPA-extractable concentrations of Zn and Cu at these five study sites changed to S7 < S6 < S9 < S8 < S10 (Table 1). The concentrations of DTPA-extractable heavy metals in relation to total concentration were as follows: Pb: 9.1%, Zn: 2.4%, Cu: 4.5% and Cd: 23.5%, when all soil samples were taken into consideration.

Mycorrhizal colonization (MC) and AMF spores could be detected even at the most heavily contaminated site. The spore density (SD) varied among study sites, ranging from 2.19/g soil (S7) to 5.77/g soil (S5), but no significant correlations could be found between SD and any environmental parameters ($P > 0.05$) (Supplementary Table S1). The MC significantly decreased with increasing total/DTPA-extractable Pb, Zn concentrations ($P < 0.01$), however, no significant correlations could be found between MC and soil EC, TN, AP and AN levels ($P > 0.05$) (Supplementary Table S1). The overall trends were higher mycorrhizal colonization and AMF spore density in uncontaminated areas and lower in heavily contaminated areas, with intermediate values in moderately contaminated areas (Table 1).

3.2. AMF community structure based on molecular analysis

The AMF occurrence was analyzed in 30 root samples and 30 rhizosphere soil samples of *R. pseudoacacia*. Template DNA was successfully amplified with the PCR primers AM1-NS31, yielding PCR products of expected length (about 550 bp). For the 60 clone libraries, a total of 2400 clones were screened, and 2273 clones containing insert of correct size of SSU rRNA gene were investigated by *Hin*fI and *Hsp*92II RFLP typing. Between one and four representatives of each RFLP type were sequenced, yielding 54 AMF and nine non-AMF sequences. Representative sequences of each sequence phylotype were submitted to the NCBI database and were included in the phylogenetic tree shown in Fig. 1.

The neighbor-joining phylogenetic analyses of 54 representative AMF sequences from rhizosphere soil samples (Y1–Y19), root samples (Y23–Y45) and spore samples (Y19–Y22, Y45–54) revealed 28 AMF sequence groups. The sequence groups or phylotypes covered six families of *Glomeromycota*: the *Glomeraceae*, *Claroideoglomeraceae*, *Diversisporaceae*, *Acaulosporaceae*, *Pacisporaceae*, and *Gigasporaceae*. Of all the AMF sequence groups, 17 belonged to the *Glomus* group, three to *Acaulospora*, one to *Claroideoglomus*, two to *Diversispora*, two to *Pacispora*, one to *Entrophospora* and two to *Scutellospora*. All the AMF genera in the soil samples were also found in the root samples except for *Acaulospora*, *Pacipora* and *Scutellospora* (Fig. 1). The constructed phylogenetic tree included 38 sequences downloaded from GenBank and 54 different AMF sequences recorded in this study. All sequence groups had high similarity to AMF and belonged to the phylum *Glomeromycota*. Eleven AMF sequence groups were related to known AMF species because sequences of the same type were obtained from spores collected in our study. These were Glo10 and Glo11 for *Rhizophagus clarum*, Glo14 for *Glomus constritum*, Glo15 for *Funneliformis caledonium*, Glo17 for *Funneliformis mosseae*, Acau1 for *Acaulospora mellea*, Acau2 for *Acaulospora lacunose*, Dive1 for *Diversispora spurca*, Dive2 for *Diversispora celata*, Scut1 for *Scutellospora heterogama* and Scut2 for *Scutellospora dipurpurescens*. Two groups, Glo16 and Paci1, did not seem to be related to any sequences of AMF in the database since they did not cluster closely with any sequences of reference AMF isolates in international culture collections. Thus, they may be unique to this study.

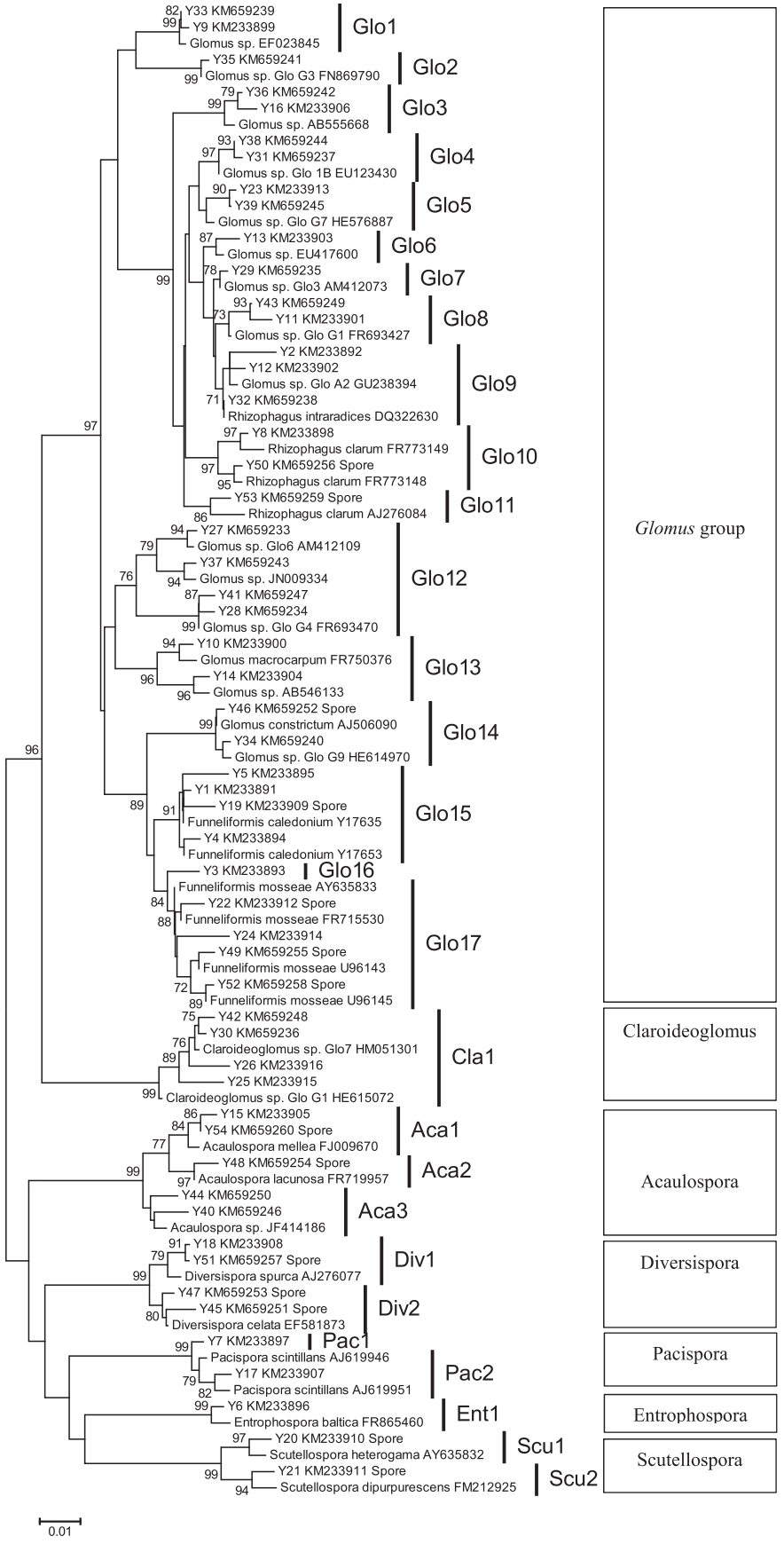

Fig. 1. Phylogenetic tree showing AMF sequences isolated from roots, spores and rhizosphere soils of *Robinia pseudoacacia* based on partial small subunit sequences (SSU). The tree was obtained by neighbor joining method. Numbers above branches denote bootstrap values from 1000 replications. Bootstrap values >70% were shown. Sequences obtained in the present study were labeled with the database accession number and the internal identification number.

The remaining groups clustered with sequences of uncultured AMF species (Fig. 1).

3.2.1. AMF communities in roots of R. pseudoacacia

The AMF communities in roots of *R. pseudoacacia* in all 30 samples were determined based on the percentages of clones of the 28 AMF sequence types (Fig. 1). Among the AMF group types detected by the RFLP analysis of root samples, 15 were found in roots of plants growing on uncontaminated sites, with an average of 1.0 group types per sample, while 14 group types were detected in roots of plants growing on contaminated sites, with an average 0.9 group types per sample. In root samples collected from uncontaminated sites, the most frequent group type was Glo9 (33.2%), followed by Glo17 (27.2%). In addition, five group types of Glo2 (0.67%), Glo4 (0.67%), Glo12 (0.67%), and Glo14 (1.33%) were only recorded in roots from uncontaminated sites. In contrast, the abundant AMF group types in root samples collected from HM contaminated sites were Glo9 (35.8%) and Glo17 (37.7%). The third most common group was Acau3, which could not be assigned to any morphologically described species. Acau3 was present in 16.5% of all clones in uncontaminated sites, but was significantly less common in HM contaminated sites (13.8%). Three group types of Glo6 (0.50%), Glo8 (0.17%) and Dive1 (0.67%) were only detected from roots in HM contaminated sites. Eleven AMF group types of Glo3, Glo5, Glo7, Glo9, Glo10, Glo13, Glo15, Glo17, Clar1, Acau3 and Entr1 were found in roots of plants growing on both HM contaminated and uncontaminated sites (Fig. 2).

3.2.2. AMF communities in rhizosphere soils of R. pseudoacacia

Rhizosphere soils of *R. pseudoacacia* had a characteristic AMF community that was clearly different from these of the roots. Significant differences were found in the relative frequencies of two sequence groups. Sequence group Glo17 is of particular interest since it was found more frequently in soil compared with root samples, accounting for 41.0% of all clones in uncontaminated sites. However, Glo9 was seldom found in soil samples, and only 4.2% of all clones had this sequence group in uncontaminated sites. Soil samples contained more sequence groups (22 types) than root samples (18 types). In soil, 22 AMF group types were detected in samples from uncontaminated sites, with up to 4.4 group types per site. Nineteen AMF group types were detected in HM contaminated soil samples, with an average of 3.8 group types per site. Glo17 and Acau3 were the two most dominant AMF group types in soils contaminated or uncontaminated by heavy metal (Fig. 2).

3.2.3. AMF community structure based on molecular identification of spores

In our study, only 14 out of 36 single spores were successfully amplified and then subjected to RFLP analysis. One representative of each spore-derived RFLP type was sequenced and a total of 11 sequence groups were detected. A BLAST search showed that five sequence groups belonged to *Glomeromycota*, two groups belonged to *Acaulosporaceae*, two groups belonged to *Diversisporaceae*, and two groups were related to *Gigasporaceae*, partly representing AMF community in spore level (Fig. 1). The two most common AMF sequence groups were Glo17 and Acau1 both in contaminated (44.8% for Glo17 and 16.0% for Acau1) and uncontaminated sites (41.0% for Glo17 and 12.7% for Acau1) when all the spores identified by both molecular and morphological methods were taken into consideration (Fig. 2).

3.3. Ecological measures of diversity

The Species richness (S), Shannon–Wiener index (H'), Margalef (d_{Max}), and Evenness (E_h), Simpson index (D) were calculated for each study site (Supplementary Table S2). Species richness (S) were 8.5 for root samples, 11.4 for soil samples, and 5.2 for spore samples (molecular identification) collected from contaminated and uncontaminated sites. The diversity indices of S, H' and d_{Max} showed significant differences between uncontaminated and contaminated sites for soil samples, whereas no differences could be found for root and spore samples ($P > 0.05$). Significant differences were showed in diversity indices of E_h and D for root samples between uncontaminated and contaminated sites; however, they did not show differences for soil and spore samples ($P > 0.05$, Fig. 3).

Linear correlation analysis was used to determine the relationship between diversity indices of AMF communities and soil properties (Table 2). For root and soil samples, the results showed that Species Richness index (S) and Margalef index (d_{Max}) had significantly negative correlations with soil available P content, total Pb, Zn, Cd and DTPA-extractable Zn concentrations ($P < 0.05$), while the Shannon–Wiener index (H') index had obviously negative correlation with soil OM content, total and DTPA extractable Pb and Zn concentrations ($P < 0.05$). For root samples, Evenness index (E_h) and Simpson index (D) had significantly positive correlations with soil pH, but negative correlations with soil OM content, total Pb, Zn, Cd and DTPA-extractable Pb, Zn and Cu concentrations ($P < 0.05$). On the contrary, Evenness index (E_h) and Simpson index (D) of soil samples only had significantly negative correlations with soil EC, total Pb, Zn and DTPA-extractable Zn concentrations

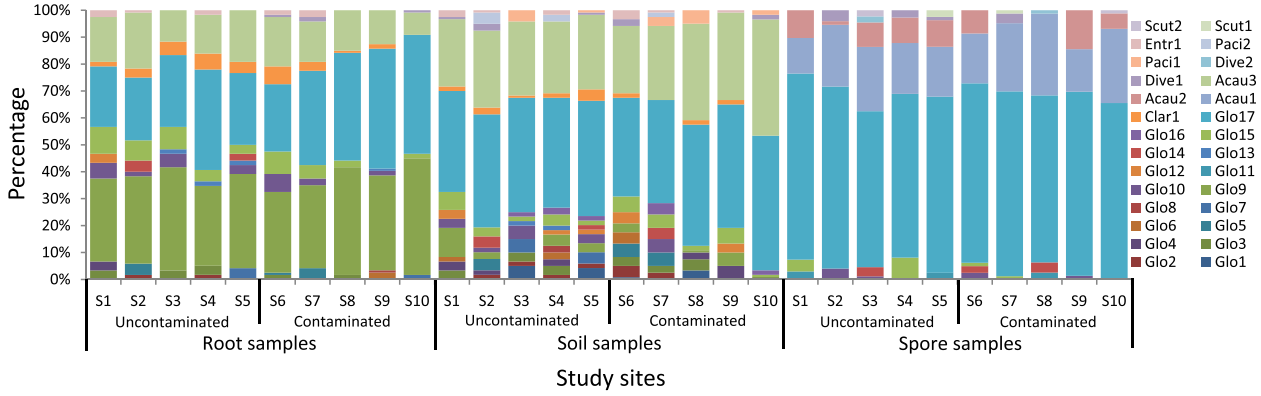

Fig. 2. AMF communities in root, soil and spore samples collected from different study sites. AMF communities are represented by the percentages of total clones belonging to each AMF sequence group. Each color corresponds to a sequence group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

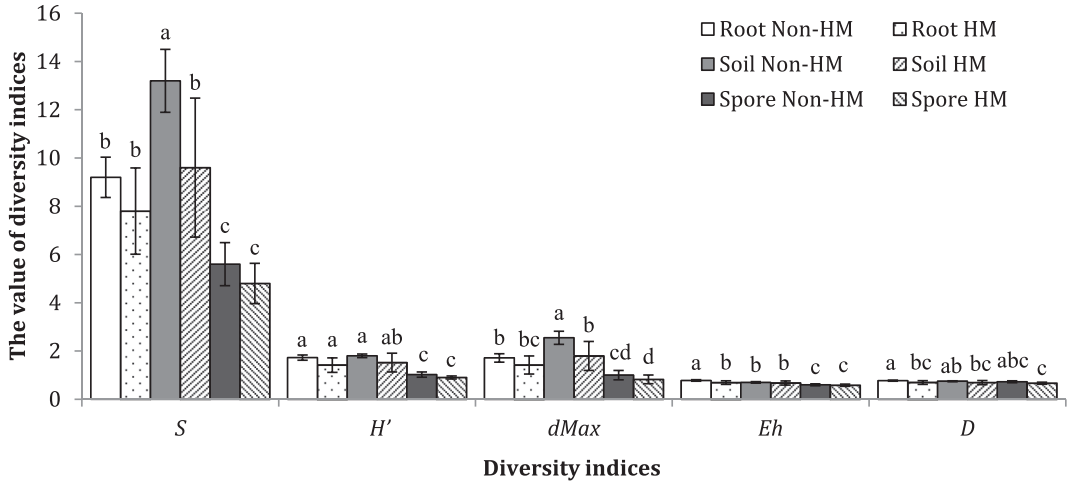

Fig. 3. Species richness (S), Shannon–Wiener index (H'), Margalef (d_{Max}), and Evenness (E_h), Simpson index (D) of AMF community in ten sampling sites. Different letters indicate significant differences according to Duncan's test ($P < 0.05$).

($P < 0.05$). For spore samples, the Species Richness index (S) and Margalef index (d_{Max}) showed significantly negative correlations with soil OM content, total Zn, Cu and DTPA-extractable Pb and Zn concentrations ($P < 0.05$), while the Shannon–Wiener index (H') index only had negative correlations with soil total P content ($P < 0.01$). However, no correlation could be found between Evenness index (E_h), Simpson index (D) and soil properties ($P > 0.05$).

3.4. Redundancy analysis (RDA)

To further resolve the effects of environmental variables on the AMF communities, fifteen environmental factors were analyzed by using Redundancy analysis (RDA). RDA analysis was conducted on each type of samples separately, due to the differences in AMF community structure between root and soil samples (Fig. 2). Spore samples were removed from the analysis because most of the single spores failed to amplify in this study. Results from RDA are shown in Fig. 4. The length of the arrows indicate the relative importance of each environmental factor in explaining variation of community structures, while the angle between the arrows or axis indicate the degree to which they are correlated (Fig. 4). In root samples, more than 84.2% of the variance in AMF communities could be explained by the two canonical axes. The first canonical axis explained 71.1% of the detected AMF diversity and was negatively correlated with soil pH. The second axis represented 13.1% of variance and was positively correlated with soil OM content, total/DTPA-extractable Cu and Cd concentrations. Soil properties related to pH, EC, OM content, total/DTPA-extractable Cd and Zn concentrations had a strong influence on the AMF composition structure in root samples as indicated by the length of their arrows in the RDA plots, but still a large proportion of the variance remained unexplained. Soil total Cu and DTPA-extractable Cd concentrations were positively associated with Glo6, Glo8 and Dive1, but showed negative correlation with Glo14 in root samples (Fig. 4a). Glo9 was significantly associated with soil EC, total N and available P contents, while Glo17 was abundant within roots of *R. pseudoacacia* growing in soils polluted by Pb, Cu and Cd. Glo4, Glo10, Glo12 and Glo15 were positively related to soil pH, but negatively correlated with soil OM content, DTPA-extractable Pb and Cu concentrations. AMF groups Glo2, Glo3, Glo5, Acau3 and Clar1 found in the upper left part of the graph were negatively correlated with soil total Pb, Zn and DTPA-extractable Zn concentrations, and Entr1 was only negatively correlated with EC and available P (Fig. 4a). Eight of the fifteen environmental variables fitted as vectors onto the RDA plot were significantly correlated with the AMF community structure in root

Table 2
Correlation coefficients among soil properties and biodiversity indices.

Biodiversity index		pH	OM	EC	TP	TN	AP	AN	TPb	TZn	TCu	TCd	DPb	DZn	DCu	DCd
Root samples	*S*	NS	NS	(−)*	NS	NS	(−)*	NS	(−)*	(−)**	NS	(−)*	NS	(−)**	NS	NS
	H′	(+)*	(−)*	NS	NS	NS	NS	NS	(−)*	(−)**	NS	(−)**	(−)*	(−)**	(−)*	NS
	dMax	NS	NS	(−)*	NS	NS	(−)*	NS	(−)*	(−)**	NS	(−)*	NS	(−)**	NS	NS
	Eh	(+)*	(−)**	NS	NS	NS	NS	NS	(−)**	(−)**	(−)*	(−)**	(−)**	(−)**	(−)**	(−)*
	D	(+)*	(−)*	NS	NS	NS	NS	NS	(−)**	(−)**	NS	(−)**	(−)*	(−)**	(−)*	NS
Soil samples	*S*	(+)*	(−)*	NS	NS	NS	(−)*	NS	(−)**	(−)**	NS	(−)*	(−)**	(−)**	(−)*	NS
	H′	NS	(−)*	(−)*	NS	NS	NS	NS	(−)**	(−)**	NS	(−)*	(−)*	(−)**	NS	NS
	dMax	(+)*	(−)*	NS	NS	NS	(−)*	NS	(−)**	(−)**	NS	(−)*	(−)**	(−)**	(−)*	NS
	Eh	NS	NS	(−)**	NS	NS	NS	NS	(−)**	(−)**	NS	NS	NS	(−)**	NS	NS
	D	NS	(−)*	(−)*	NS	NS	(−)*	NS	(−)**	(−)**	NS	NS	(−)*	(−)**	NS	NS
Spore samples	*S*	NS	(−)*	NS	NS	NS	NS	NS	NS	(−)*	(−)*	NS	(−)*	(−)*	NS	NS
	H′	NS	NS	NS	(−)*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	dMax	NS	(−)*	NS	NS	NS	NS	NS	NS	(−)*	(−)*	NS	(−)*	(−)*	NS	NS
	Eh	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Abbreviations: S, Species richness; H', Shannon–Wiener index; d_{Max}, Margalef; E_h, Evenness; D, Simpson index; OM, organic matter; EC, electrical conductivity; TP, total phosphorus; TN, total nitrogen; AP, available phosphorus; AN, available nitrogen; TPb, total Pb; TZn, total Zn; TCu, total Cu; TCd, total Cd; DPb, DTPA-extractable Pb; DZn, DTPA-extractable Zn; DCu, DTPA-extractable Cu; DCd, DTPA-extractable Cd; (−), negative correlation; (+), positive correlation; *$P < 0.05$, **$P < 0.01$, NS, no significance.

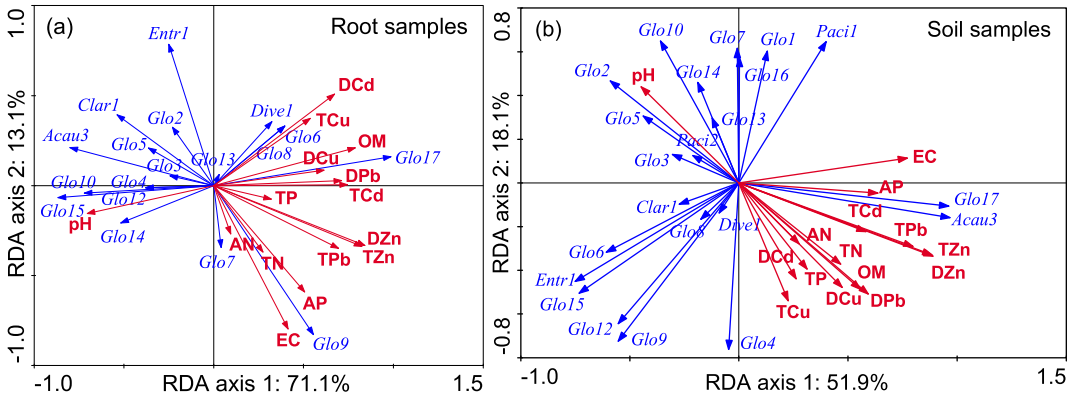

Fig. 4. Redundancy analysis (RDA) of AMF group types, their distribution among the root samples (a) and soil samples (b) collected from ten study sites, and correlation with soil chemical parameters. Blue arrows indicate the AMF group types and red arrows represent soil chemical parameters. Abbreviations: OM, organic matter; EC, electrical conductivity; TP, total phosphorus; TN, total nitrogen; AP, available phosphorus; AN, available nitrogen; TPb, total Pb; TZn, total Zn; TCu, total Cu; TCd, total Cd; DPb, DTPA-extractable Pb; DZn, DTPA-extractable Zn; DCu, DTPA-extractable Cu; DCd, DTPA-extractable Cd. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

samples; of these, soil pH, OM, total/DTPA-extractable Zn concentrations were most strongly related to the AMF community composition (Supplementary Table S3).

In the soil samples, a total of 70.0% of the cumulative variance in the AMF community data set was explained by the first two canonical RDA axes (Fig. 4b). The first canonical axis explained 51.9% of AMF composition in soil samples and was negatively correlated with soil pH, while, the second axis explained 18.1% of variance and was positively correlated with soil pH and EC. Soil available P, DTPA-extractable Zn and total Pb, Zn and Cd concentrations showed positive correlations with Glo7 and Acau3, but were negatively related to Glo2, Glo3, Glo5 and Paci2. Glo4 was associated with soil total Cu concentration, however, AMF group Glo6, Glo8, Glo9, Glo12, Glo15, Clar1, Dive1 and Entr1 were represented in the bottom left part of the graph without clear associations with environmental parameters (Fig. 4b). Glo10, Glo13 and Glo14 were negatively associated with Cu and Cd contaminations, and AMF groups Glo1, Glo7 and Glo16 found in the upper right part of the graph were influenced by both soil pH and EC. Five of the fifteen environmental variables fitted as vectors onto the RDA plot were significantly correlated with the AMF community structure in soil samples; of these, soil total Pb, Zn and DTPA-extractable Zn concentrations were most strongly related to the AMF community composition (Supplementary Table S4).

4. Discussion

Under natural conditions, 80%–90% of plants are colonized by AMF leading to mutualistic associations that have been found in most vegetative systems and climates, including some aquatic ecosystems (Gamalero et al., 2009). The extensive extraradical hyphal network produced by AMF allows the plants to access a greater volume of the soil, resulting in the enhancement of plant nutrient absorption and translocation (Fellbaum et al., 2014). The first step of our current study was to analyze the mycorrhizal structures such as hyphae, arbuscules (*Arum*-type and *Paris*-type) and vesicles in the inner root parenchyma cells where most of these fungal structures reside (Hildebrandt et al., 2007). Intracellular hyphae, arbuscules and vesicles were commonly found in all study sites and *Arum*-type was the dominant structure (Supplementary Fig. S1a, b, d and e). A few roots forming mycorrhizal structures of *Paris*-type could be also observed in our investigation (Supplementary Fig. S1c). Higher percentage of arbuscules was observed at the sites heavily polluted by Pb, Zn, Cu and Cd (Supplementary Fig. S1e), which may be attributed to alleviation of heavy metal toxicity in *R. pseudoacacia*. The previous studies conducted by our lab in the Pb and Zn mining area showed a negative effect of Pb concentration on mycorrhizal colonization (Xu et al., 2012). With the present analysis, we extended the focus of our work by taking additional environmental factors into account. The correlation analysis showed that MC had a significantly positive relationship with soil pH ($P = 0.038$), but was negatively associated with soil OM content ($P = 0.019$), total P content ($P = 0.033$) and strongly inhibited by Zn and Pb contaminations (Supplementary Table S1). These results are in agreement with Vogel-Mikuš et al. (2005) who reported that mycorrhizal colonization levels were significantly lower or absent on the most Pb, Zn and Cd polluted plots. Wu et al. (2010) indicated that elevated concentrations of As, Pb, Zn, Cd and Cu exerted harmful effects on spore numbers of AMF in abandoned As/Pb/Zn mines. However, our results did not support that and no significant difference could be found between AMF spore density and Pb, Zn, Cu, Cd concentrations or with any of the other studied environmental factors (Supplementary Table S1). The inhibition of mycorrhizal colonization in the contaminated soil could be partly due to the high availability of metals caused by low pH (Table 1), inhibition of fungal spread in the soil (Pawlowska and Charvat, 2004), disturbance of vegetation and erosion of the locations (Liu et al., 2011). Heavy metals have been reported to reduce, delay, or even eliminate AMF colonization and spore density (Lingua et al., 2008; Wei et al., 2014). However, in our study, AMF propagules never disappeared completely even in soils contaminated by high concentrations of heavy metals (Supplementary Fig. S1) and other studies have likewise found positive, negative or neutral effects on mycorrhizal colonization and spore density in Pb–Zn mining area (Zarei et al., 2008a; Khade and Adholeya, 2009; Wu et al., 2010). These conflicting observations indicated that the effects of heavy metals on the extent of AMF colonization may depend on other environmental factors, such as AMF species, host plants, season of the year, stage of plant development, plant nutritional status and environmental conditions (Bever et al., 2001; Oehl et al., 2003; Pande and Tarafdar, 2004).

The phylogenetic analysis revealed that 17 out of 28 AMF groups belonged to *Glomus*, suggesting that it was the most dominant genus in the AMF community associated with *R. pseudoacacia* at all study sites. The dominance of *Glomus* species has been commonly found in surveys on AMF diversity in various habitats, such as Mediterranean soils (Alguacil et al., 2014), tropical forest soils (Husband et al., 2002), agricultural soils (Dai et al., 2014), grassland

soils (Birgander et al., 2014), and heavy metal contaminated soils (Zarei et al., 2008a, 2008b). Yang et al. (2010) found that all the sequences detected in the roots of *Elsholtzia splendens* growing in Cu-contaminated soil were related to *Glomus* species. Similar to our results, Hassan et al. (2011) indicated that *Glomus* was one of the most common AMF genera detected in trace metal polluted soils. The *Acaulospora* group was the second most dominant genus in our study (Fig. 2), although only 3 out of 28 AMF groups belonging to this genus were found (Fig. 1). Prevalence of *Acaulospora* species in some Chinese natural ecosystems was widely reported (Li et al., 2004). Our results are also in accordance with a study that detected *Glomus* and *Acaulospora* abundantly distributed in heavy metal polluted soils (Ortega-Larrocea et al., 2001). Three possible reasons could explain why *Glomus* and *Acaulospora* groups became the most dominant genera in our study. Firstly, *Glomus* and *Acaulospora* species have the ability to propagate by mycelial fragments and mycorrhizal root fragments, unlike other AMF species that require spore germination (Klironomos and Hart, 2002; Hassan et al., 2011). Secondly, as predominance genera (Daniell et al., 2001), *Glomus* and *Acaulospora* species already adapted to the local environment, and it became much easier for them to develop their own ability to survive in heavily disturbed environments compared with other AMF species. Thirdly, it is quite common for *Glomus* to form anastomoses between mycelia, which may therefore have the ability to re-establish an interconnected network in disrupted environments (de la Providencia et al., 2005).

The large differences in the diversity of AMF among root, soil and spore samples collected from ten study sites were observed in the present investigation (Fig. 2). Of particular note was the distribution of Glo9, Glo17 and Acau3. Glo9, showing a high similarity to *Rhizophagus intraradices*, is known as a generalist fungal species since it has been found in diverse environments (Öpik et al., 2003) and host species (Helgason et al., 2007). In the current study, Glo9 was found primarily in root samples (>30%), but only 4% abundance of this group type was observed in soil samples and it was almost not detected in spore samples. Glo17, which showed a high homology with *F. mosseae*, can also be considered a generalist fungal species because it is very frequent in diverse ecosystems (Jansa et al., 2014). However, this group species presented an almost opposite pattern in distribution among root, soil and spore samples compared with Glo9. Glo17 was found to be 32% of all clones in root samples, but this value increased to 42% of all clones in soil samples (Fig. 2). AMF group Acau3, showing a high homology with *Acaulospora* sp. has been commonly found in China (Zhang et al., 2004). Similarly, the percentage of all clones for this group increased from 15% in root samples to 30% in soil samples (Fig. 2). Our results were supported by previous studies conducted by Chen et al. (2014) and Hempel et al. (2007) who reported large differences in the AMF community among root, soil and even spore samples at AMF species level. Wilde et al. (2009) observed that *R. intraradices* occurred abundantly in roots of host plants but could hardly be retrieved in batches of spores and not at all in single spores of soil samples by molecular analyses. Hempel et al. (2007) suggested that this type of group may generally extend short distances in soils relative to its abundance in roots. Another important aspect that needs to be considered is the properties of the host plant (*R. pseudoacacia*). Black locust can form symbiotic associations with both nitrogen-fixing rhizobia and phosphorus-acquiring AMF. The fixed nitrogen provides legumes with an additional nitrogen source, but at the same time, they require large amounts of energy and phosphorus to balance the nutrients. Black locust may preferentially associate with specific AMF species that are particularly efficient in supplying phosphorus (Scheublin et al., 2004). It is a reasonable assumption that *R. intraradices*, *F. mosseae* and *Acaulospora* sp. might be good candidates for this role, although research on the differences in P acquisition efficiency among AMF species is lacking.

The principal component analysis (PCA) revealed a distinct clustering of the AMF communities in root, soil and spore samples at family level (Fig. 5). The AMF diversity in soil samples showed a much wider distribution in the PCA plot, suggesting the heterogeneity of the soils in our study sites (Table 1). This result was consistent with Martínez-García et al. (2011) who found much higher AMF genetic diversity in soils compared with that in roots. A strong overlap among the community structure of AMF in root, soil and spore samples was noticed in the current study (Fig. 5), suggesting that the plant, AMF and soil formed a unified whole, with each component influencing one another.

Although distinct patterns of heavy metal tolerance among AMF groups were found, redundancy analysis (RDA) showed that heavy metal contamination were not the only soil parameters influencing AMF sequence group type distribution (Fig. 4). In root samples, the multivariate analysis showed that, apart from the total Pb, Zn, Cd and DTPA-extractable Pb and Zn concentrations, soil pH, organic matter content (OM) and electrical conductivity (EC) greatly influenced the AMF community structure (Fig. 4a, Supplementary Table S3). Detailed information showed that, in soil samples, the AMF composition was highly associated with soil EC, AP, total Pb, Zn and DTPA-extractable Zn concentrations (Fig. 4b, Supplementary Table S4). Although factors influencing AMF community structure are varied (Zarei et al., 2010), some studies have found heavy metal contaminations (Pb, Zn, Cu and Cd) to be the dominant parameters influencing AMF community structure (Del Val et al., 1999; Zarei et al., 2008b). In addition to understanding how AMF communities are influenced by soil factors, we need to determine the relationships among soil parameters in the local heavy metal polluted area with unique and special geological and climate conditions. Generally, the effects of heavy metals on plants and soil microorganisms are dependent both on chemical speciation and mobilization of the metal available for uptake (Krishnamurti et al., 2013). It has been widely noted that the availability and toxicity of heavy metals to plants and AMF were associated with soil P content, soil

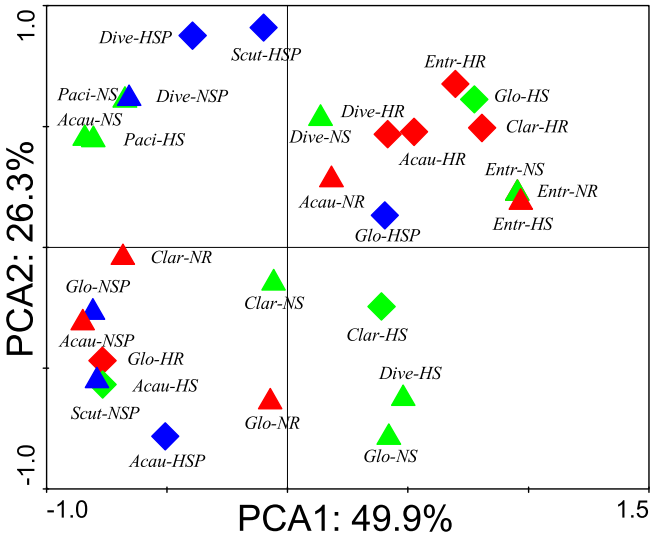

Fig. 5. Principal component analysis (PCA) of AMF communities in root (R, red symbols), soil (S, green symbols) and spore (SP, blue symbols) samples in response to different environmental factors. The percentages in the axes show the percentage of variation explained by the analysis. Triangle represents uncontaminated soils (N); diamond represents heavy metal contaminated soils (H). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pH and organic matter content (OM) (Cao et al., 2003; Meharg, 2003; Antoniadis et al., 2008). Among these factors, soil pH plays the most important role in determining metal speciation, solubility from mineral surfaces, movement, and eventual bioavailability of metals, due to its strong effects on solubility and speciation of metals both in the soil as a whole and particularly in the soil solution (Zeng et al., 2011). Our data also provide evidence that soil pH was negatively correlated with DTPA-extractable Pb ($P < 0.01$), Zn ($P < 0.05$), Cu ($P < 0.01$), Cd ($P < 0.01$) concentrations, but no correlation was found between soil pH and total Pb or Zn concentrations ($P > 0.05$) (Supplementary Table S5). Apart from soil pH, organic matter content (OM) is also one of the most important soil properties affecting heavy metal availability. The current study found that organic matter content (OM) was positively correlated with soil DTPA-extractable Pb ($P < 0.01$), Zn ($P < 0.05$), Cu ($P < 0.01$) and Cd ($P < 0.01$) (Supplementary Table S5). Similar results were demonstrated by Dai et al. (2004) who estimated DTPA-extractable Pb, Zn and Cd concentrations in heavy metal contaminated soils and found that the concentrations of these metals were positively correlated with organic matter content (OM) in soils. Bending et al. (2002) reported that soil organic matter content could influence the function of the soil microbial community by impacting soil structural properties and types of nutritional substrates available. Soil EC provides an evaluation of soil stress level and is typically correlated with pH. In agreement with our findings, Bainard et al. (2014) reported that soil pH or pH-driven changes in soil chemistry and EC were highly correlated with the composition of the AMF community in both soils and crop roots. In natural and agricultural ecosystems, the variability in soil pH has been shown to be one of the most important factors influencing the structure and composition of AMF communities (An et al., 2008; Dumbrell et al., 2011). A high P level is another factor that is known to reduce AMF diversity (Gosling et al., 2013). The explanation for this is that the relationship between AMF and host plant was greatly regulated by soil P level, which could directly or indirectly affect AMF status in plant roots and soils (Sheng et al., 2013). However, more studies are still required to get a better understanding of the detailed relationship among soil factors and their specific impacts on *R. pseudoacacia* growth and AMF community structure in heavy metal contaminated areas.

5. Conclusion

The current study described the local AMF community composition and structure in heavy metal contaminated and uncontaminated regions. The results showed that *R. intraradices*, *F. mosseae* and *Acaulospora* sp. were the three most dominant AMF group types associated with *R. pseudoacacia* in the study sites. Soil Pb and Zn concentrations were found to be the most important soil factors, which severely affected AMF abundance and community structure. However, other soil chemical parameters, such as soil pH, OM, EC and P level, which showed variation among the sampling sites in the study area, also greatly contributed to soil feedbacks on AMF diversity, as detected by redundancy analysis (RDA). It can be concluded from the current study that several AMF group types presented in the lead–zinc mining region were able to survive and overcome heavy metal polluted conditions and possessed some degree of adaptation to heavy metal stress. Further studies are required to explore the phytoremediation potential of the dominant AMF isolates (*F. mosseae*, *R. intraradices* and *Acaulospora* sp.) associated with *R. pseudoacacia* in both pot culture and field studies.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.03.018.

References

Alguacil, M.M., Torrecillas, E., Caravaca, F., Fernández, D.A., Azcón, R., Roldán, A., 2011. The application of an organic amendment modifies the arbuscular mycorrhizal fungal communities colonizing native seedlings grown in a heavy-metal-polluted soil. Soil Biology and Biochemistry 43 (7), 1498–1508.
Alguacil, M.M., Torrecillas, E., García-Orenes, F., Roldán, A., 2014. Changes in the composition and diversity of AMF communities mediated by management practices in a Mediterranean soil are related with increases in soil biological activity. Soil Biology and Biochemistry 76, 34–44.
An, G.H., Miyakawa, S., Kawahara, A., Osaki, M., Ezawa, T., 2008. Community structure of arbuscular mycorrhizal fungi associated with pioneer grass species *Miscanthus sinensis* in acid sulfate soils: habitat segregation along pH gradients. Soil Science and Plant Nutrition 54 (4), 517–528.
Antoniadis, V., Robinson, J.S., Alloway, B.J., 2008. Effects of short-term pH fluctuations on cadmium, nickel, lead, and zinc availability to ryegrass in a sewage sludge-amended field. Chemosphere 71 (4), 759–764.
Antunes, P.M., Lehmann, A., Hart, M.M., Baumecker, M., Rillig, M.C., 2012. Long-term effects of soil nutrient deficiency on arbuscular mycorrhizal communities. Functional Ecology 26 (2), 532–540.
Bainard, L.D., Bainard, J.D., Hamel, C., Gan, Y., 2014. Spatial and temporal structuring of arbuscular mycorrhizal communities is differentially influenced by abiotic factors and host crop in a semi-arid prairie agroecosystem. FEMS Microbiology Ecology 88 (2), 333–344.
Bedini, S., Turrini, A., Rigo, C., Argese, E., Giovannetti, M., 2010. Molecular characterization and glomalin production of arbuscular mycorrhizal fungi colonizing a heavy metal polluted ash disposal island, downtown Venice. Soil Biology and Biochemistry 42 (5), 758–765.
Bending, G.D., Turner, M.K., Jones, J.E., 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. Soil Biology and Biochemistry 34 (8), 1073–1082.
Bever, J.D., Schultz, P.A., Pringle, A., Morton, J.B., 2001. Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why the high diversity of ecologically distinct species of arbuscular mycorrhizal fungi within a single community has broad implications for plant ecology. Bioscience 51 (11), 923–931.
Birgander, J., Rousk, J., Olsson, P.A., 2014. Comparison of fertility and seasonal effects on grassland microbial communities. Soil Biology and Biochemistry 76, 80–89.
Borriello, R., Lumini, E., Girlanda, M., Bonfante, P., Bianciotto, V., 2012. Effects of different management practices on arbuscular mycorrhizal fungal diversity in maize fields by a molecular approach. Biology and Fertility of Soils 48 (8), 911–922.
Bremner, J.M., Mulvaney, C.S., 1982. Total nitrogen. In: Page, A.L. (Ed.), Methods of Soil Analysis: Part 2, Agronomy Monograph 9, second ed. American Society of Agronomy and Soil Science Society of America, Madison, pp. 595–624.
Cao, R.X., Ma, L.Q., Chen, M., Singh, S.P., Harris, W.G., 2003. Phosphate-induced metal immobilization in a contaminated site. Environmental Pollution 122, 19–28.
Chen, Y., Zhang, X., Ye, J., Han, H., Wan, S., Chen, B., 2014. Six-year fertilization modifies the biodiversity of arbuscular mycorrhizal fungi in a temperate steppe in Inner Mongolia. Soil Biology and Biochemistry 69, 371–381.
Cheng, Y., Ishimoto, K., Kuriyama, Y., Osaki, M., Ezawa, T., 2013. Ninety-year-, but not single, application of phosphorus fertilizer has a major impact on arbuscular mycorrhizal fungal communities. Plant and Soil 365 (1–2), 397–407.
Clapp, J.P., Young, J.P.W., Merryweather, J.W., Fitter, A.H., 1995. Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. New Phytologist 130, 259–265.
Dai, J., Becquer, T., Rouiller, J.H., Reversat, G., Bernhard-Reversat, F., Lavelle, P., 2004. Influence of heavy metals on C and N mineralisation and microbial biomass in Zn-, Pb-, Cu-, and Cd-contaminated soils. Applied Soil Ecology 25 (2), 99–109.
Dai, M., Hamel, C., Bainard, L.D., Arnaud, M.S., Grant, C.A., Lupwayi, N.Z., Malhi, S.S., Lemke, R., 2014. Negative and positive contributions of arbuscular mycorrhizal fungal taxa to wheat production and nutrient uptake efficiency in organic and

conventional systems in the *Canadian prairie*. Soil Biology and Biochemistry 74, 156–166.
Daniell, T.J., Husband, R., Fitter, A.H., Young, J.P.W., 2001. Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. FEMS Microbiology Ecology 36 (2–3), 203–209.
Daniels, B.A., Skipper, H.D., 1982. Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck, N.C. (Ed.), Methods and Principles of Mycorrhizal Research. American Phytopathological Society, St. Paul, Minn., pp. 29–35.
de la Providencia, I.E., De Souza, F.A., Fernández, F., Delmas, N.S., Declerck, S., 2005. Arbuscular mycorrhizal fungi reveal distinct patterns of anastomosis formation and hyphal healing mechanisms between different phylogenic groups. New Phytologist 165 (1), 261–271.
de Oliveira Freitas, R., Buscardo, E., Nagy, L., dos Santos Maciel, A.B., Carrenho, R., Luizão, R.C., 2014. Arbuscular mycorrhizal fungal communities along a pedo-hydrological gradient in a Central Amazonian terra firme forest. Mycorrhiza 24 (1), 21–32.
Del Val, C., Barea, J.M., Azcon-Aguilar, C., 1999. Diversity of arbuscular mycorrhizal fungus populations in heavy-metal-contaminated soils. Applied and Environmental Microbiology 65 (2), 718–723.
Doubková, P., Suda, J., Sudová, R., 2012. The symbiosis with arbuscular mycorrhizal fungi contributes to plant tolerance to serpentine edaphic stress. Soil Biology and Biochemistry 44 (1), 56–64.
Dumbrell, A.J., Ashton, P.D., Aziz, N., Feng, G., Nelson, M., Dytham, C., Fitter, A.H., Helgason, T., 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytologist 190 (3), 794–804.
Eom, A.H., Wilson, G.W., Hartnett, D.C., 2001. Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. Mycologia 233–242.
Fellbaum, C.R., Mensah, J.A., Cloos, A.J., Strahan, G.E., Pfeffer, P.E., Kiers, E.T., Bücking, H., 2014. Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. New Phytologist 203 (2), 646–656.
Gai, J.P., Christie, P., Feng, G., Li, X.L., 2006. Twenty years of research on biodiversity and distribution of arbuscular mycorrhizal fungi in China: a review. Mycorrhiza 16, 229–239.
Gamalero, E., Lingua, G., Berta, G., Glick, B.R., 2009. Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Canadian Journal of Microbiology 55 (5), 501–514.
Gómez-Sagasti, M.T., Alkorta, I., Becerril, J.M., Epelde, L., Anza, M., Garbisu, C., 2012. Microbial monitoring of the recovery of soil quality during heavy metal phytoremediation. Water, Air, & Soil Pollution 223 (6), 3249–3262.
Gosling, P., Mead, A., Proctor, M., Hammond, J.P., Bending, G.D., 2013. Contrasting arbuscular mycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. New Phytologist 198 (2), 546–556.
Hassan, S.E.D., Boon, E., St-Arnaud, M.A.R.C., Hijri, M., 2011. Molecular biodiversity of arbuscular mycorrhizal fungi in trace metal-polluted soils. Molecular Ecology 20 (16), 3469–3483.
Hazard, C., Boots, B., Keith, A.M., Mitchell, D.T., Schmidt, O., Doohan, F.M., Bending, G.D., 2014. Temporal variation outweighs effects of biosolids applications in shaping arbuscular mycorrhizal fungi communities on plants grown in pasture and arable soils. Applied Soil Ecology 82, 52–60.
Helgason, T., Daniell, T.J., Husband, R., Fitter, A.H., Young, J.P.W., 1998. Ploughing up the wood-wide web? Nature 394, 431.
Helgason, T., Merryweather, J.W., Young, J.P.W., Fitter, A.H., 2007. Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. Journal of Ecology 95 (4), 623–630.
Hempel, S., Renker, C., Buscot, F., 2007. Differences in the species composition of arbuscular mycorrhizal fungi in spores, root and soil communities in grassland ecosystem. Environmental Microbiology 9, 1930–1938.
Hempel, S., Stein, C., Unsicker, S.B., Renker, C., Auge, H., Weisser, W.W., Buscot, F., 2009. Specific bottom-up effects of arbuscular mycorrhizal fungi across a plant-herbivore-parasitoid system. Oecologia 160, 267–277.
Hildebrandt, U., Regvar, M., Bothe, H., 2007. Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68, 139–146.
Hou, E.K., Xue, X.C., Liu, G.M., Ma, Z.K., Zhao, K., 2003. The mine environmental geology and conservation strategy of Fengxian County. Northwest Geology 36, 26–30. http://www.cqvip.com/qk/93232x/2003c00/9048339.html.
Husband, R., Herre, E.A., Turner, S.L., Gallery, R., Young, J.P.W., 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Molecular Ecology 11 (12), 2669–2678.
Jackson, M.L., 1958. Soil Chemical Analysis. Prentice-hall, Inc., Englewood cliffs, NJ, pp. 111–133.
Jansa, J., Erb, A., Oberholzer, H.R., Šmilauer, P., Egli, S., 2014. Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. Molecular Ecology 23 (8), 2118–2135.
Jansa, J., Smith, F.A., Smith, S.E., 2008. Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? New Phytologist 177 (3), 779–789.
Khade, S.W., Adholeya, A., 2009. Arbuscular mycorrhizal association in plants growing on metal-contaminated and noncontaminated soils adjoining Kanpur tanneries, Uttar Pradesh, India. Water, Air, and Soil Pollution 202 (1–4), 45–56.
Klironomos, J.N., Hart, M.M., 2002. Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. Mycorrhiza 12 (4), 181–184.
Kohout, P., Sudová, R., Janoušková, M., Čtvrtlíková, M., Hejda, M., Pánková, H., Slavíková, R., Štajerová, K., Vosátka, M., Sýkorová, Z., 2014. Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: is there a universal solution? Soil Biology and Biochemistry 68, 482–493.
König, S., Wubet, T., Dormann, C.F., Hempel, S., Renker, C., Buscot, F., 2010. TaqMan real-time PCR assays to assess arbuscular mycorrhizal responses to field manipulation of grassland biodiversity: effects of soil characteristics, plant species richness, and functional traits. Applied and Environmental Microbiology 76 (12), 3765–3775.
Koske, R.E., Gemma, J.N., 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycological Research 92, 486–488.
Krishnamoorthy, R., Kim, K., Kim, C., Sa, T., 2014. Changes of arbuscular mycorrhizal traits and community structure with respect to soil salinity in a coastal reclamation land. Soil Biology and Biochemistry 72, 1–10.
Krishnamurti, G.S., Subashchandrabose, S.R., Megharaj, M., Naidu, R., 2013. Assessment of bioavailability of heavy metal pollutants using soil isolates of *Chlorella* sp. Environmental Science and Pollution Research 1–7.
Lehmann, A., Veresoglou, S.D., Leifheit, E.F., Rillig, M.C., 2014. Arbuscular mycorrhizal influence on zinc nutrition in crop plants-A meta-analysis. Soil Biology and Biochemistry 69, 123–131.
Li, T., Li, J., Zhao, Z., 2004. Arbuscular mycorrhizas in a valley-type savanna in southwest China. Mycorrhiza 14 (5), 323–327.
Lingua, G., Franchin, C., Todeschini, V., Castiglione, S., Biondi, S., Burlando, B., Parravicini, V., Torrigiani, P., Berta, G., 2008. Arbuscular mycorrhizal fungi differentially affect the response to high zinc concentrations of two registered poplar clones. Environmental Pollution 153 (1), 137–147.
Liu, R.J., Li, X.L., 2000. Arbuscular Mycorrhizae and Application. Science Press, Beijing.
Liu, Y., He, J., Shi, G., An, L., Öpik, M., Feng, H., 2011. Diverse communities of arbuscular mycorrhizal fungi inhabit sites with very high altitude in Tibet Plateau. FEMS Microbiology Ecology 78 (2), 355–365.
Margesin, R., Płaza, G.A., Kasenbacher, S., 2011. Characterization of bacterial communities at heavy-metal-contaminated sites. Chemosphere 82 (11), 1583–1588.
Martínez-García, L.B., Armas, C., Miranda, J.D.D., Padilla, F.M., Pugnaire, F.I., 2011. Shrubs influence arbuscular mycorrhizal fungi communities in a semi-arid environment. Soil Biology and Biochemistry 43 (3), 682–689.
Meharg, A.A., 2003. The mechanistic basis of interactions between mycorrhizal associations and toxic metal cations. Mycological Research 107 (11), 1253–1265.
Moebius-Clune, D.J., Moebius-Clune, B.N., van Es, H.M., Pawlowska, T.E., 2013. Arbuscular mycorrhizal fungi associated with a single agronomic plant host across the landscape: community differentiation along a soil textural gradient. Soil Biology and Biochemistry 64, 191–199.
Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon and organic matter. In: Page, A.L. (Ed.), Methods of Soil Analysis: Part 2, Agronomy Monograph 9, second ed. American Society of Agronomy and Soil Science Society of America, Madison, pp. 539–579.
Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T., Wiemken, A., 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Applied and Environmental Microbiology 69 (5), 2816–2824.
Öpik, M., Moora, M., Liira, J., Kõljalg, U., Zobel, M., Sen, R., 2003. Divergent arbuscular mycorrhizal fungal communities colonize roots of *Pulsatilla* spp. in boreal Scots pine forest and grassland soils. New Phytologist 160 (3), 581–593.
Ortega-Larrocea, M.P., Siebe, C., Becard, G., Mendez, I., Webster, R., 2001. Impact of a century of wastewater irrigation on the abundance of arbuscular mycorrhizal spores in the soil of the Mezquital Valley of Mexico. Applied Soil Ecology 16 (2), 149–157.
Page, A.L., 1982. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Soil Science Society of America.
Pande, M., Tarafdar, J.C., 2004. Abuscular mycorrhizal fungal diversity in neem-based agroforestry systems in Rajasthan. Applied Soil Ecology 26, 233–241.
Pawlowska, T.E., Charvat, I., 2004. Heavy-metal stress and developmental patterns of arbuscular mycorrhizal fungi. Applied and Environmental Microbiology 70 (11), 6643–6649.
Sanders, I.R., 2004. Plant and arbuscular mycorrhizal fungal diversity-are we looking at the relevant levels of diversity and are we using the right techniques? New Phytologist 164 (3), 415–418.
Scheublin, T.R., Ridgway, K.P., Young, J.P.W., Van Der Heijden, M.G., 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Applied and Environmental Microbiology 70 (10), 6240–6246.
Schüßler, A., Schwarzott, D., Walker, C., 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycological Research 105 (12), 1413–1421.
Sheng, M., Lalande, R., Hamel, C., Ziadi, N., 2013. Effect of long-term tillage and mineral phosphorus fertilization on arbuscular mycorrhizal fungi in a humid continental zone of Eastern Canada. Plant and Soil 369 (1–2), 599–613.
Simon, L., Lalonde, M., Bruns, T.D., 1992. Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. Applied and Environmental Microbiology 58 (1), 291–295.
Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis, third ed. Academic Press, New York, USA, pp. 1–145.

Symanczik, S., Błaszkowski, J., Koegel, S., Boller, T., Wiemken, A., Al-Yahya'Ei, M.N., 2014. Isolation and identification of desert habituated arbuscular mycorrhizal fungi newly reported from the Arabian Peninsula. Journal of Arid Land 6 (4), 488–497.

Thoms, C., Gleixner, G., 2013. Seasonal differences in tree species' influence on soil microbial communities. Soil Biology and Biochemistry 66, 239–248.

Trouvelot, A., Kough, J.L., Gianinazzi-Pearson, V., 1986. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson, V., Gianinazzi, S. (Eds.), Physiological and Genetical Aspects of Mycorrhizae. INRA Press, Paris, pp. 217–221.

Vlachodimos, K., Papatheodorou, E.M., Diamantopoulos, J., Monokrousos, N., 2013. Assessment of *Robinia pseudoacacia* cultivations as a restoration strategy for reclaimed mine spoil heaps. Environmental Monitoring and Assessment 185 (8), 6921–6932.

Vogel-Mikuš, K., Drobne, D., Regvar, M., Zn, 2005. 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. Environmental Pollution 133 (2), 233–242.

Wang, F., Yao, J., Si, Y., Chena, H.L., Russel, M., Chena, K., Qian, Y.G., Zaray, G., Bramanti, E., 2010. Short-time effect of heavy metals upon microbial community activity. Journal of Hazardous Materials 173, 510–516.

Wei, Y., Hou, H., Li, J., ShangGuan, Y., Xu, Y., Zhang, J., Zhao, L., Wang, W., 2014. Molecular diversity of arbuscular mycorrhizal fungi associated with an Mn hyperaccumulator-*Phytolacca americana*, in Mn mining area. Applied Soil Ecology 82, 11–17.

Wetzel, K., Silva, G., Matczinski, U., Oehl, F., Fester, T., 2014. Superior differentiation of arbuscular mycorrhizal fungal communities from till and no-till plots by morphological spore identification when compared to T-RFLP. Soil Biology and Biochemistry 72, 88–96.

Wilde, P., Manal, A., Stodden, M., Sieverding, E., Hildebrandt, U., Bothe, H., 2009. Biodiversity of arbuscular mycorrhizal fungi in roots and soils of two salt marshes. Environmental Microbiology 11 (6), 1548–1561.

Wu, F.Y., Bi, Y.L., Leung, H.M., Ye, Z.H., Lin, X.G., Wong, M.H., 2010. Accumulation of As, Pb, Zn, Cd and Cu and arbuscular mycorrhizal status in populations of *Cynodon dactylon* grown on metal-contaminated soils. Applied Soil Ecology 44 (3), 213–218.

Xu, Z.Y., Tang, M., Chen, H., Ban, Y.H., Zhang, H.H., 2012. Microbial community structure in the rhizosphere of *Sophora viciifolia* grown at a lead and zinc mine of northwest China. Science of the Total Environment 435, 453–464.

Yang, R., Zan, S., Tang, J., Chen, X., Zhang, Q., 2010. Variation in community structure of arbuscular mycorrhizal fungi associated with a Cu tolerant plant-*Elsholtzia splendens*. Applied Soil Ecology 44 (3), 191–197.

Yang, Y., Tang, M., Sulpice, R., Chen, H., Tian, S., Ban, Y., 2014. Arbuscular mycorrhizal fungi alter fractal dimension characteristics of *Robinia pseudoacacia* L. seedlings through regulating plant growth, leaf water status, photosynthesis, and nutrient concentration under drought stress. Journal of Plant Growth Regulation 1–14.

Yao, Y.J., Tian, M.M., Wu, S.L., 2004. Mineral resources exploitation and sustainable development of Fengxian County in Shanxi province. Mineral Resources and Geology 18, 470–475.

Zarei, M., Hempel, S., Wubet, T., Schäfer, T., Savaghebi, G., Jouzani, G.S., Buscot, F., 2010. Molecular diversity of arbuscular mycorrhizal fungi in relation to soil chemical properties and heavy metal contamination. Environmental Pollution 158 (8), 2757–2765.

Zarei, M., König, S., Hempel, S., Nekouei, M.K., Savaghebi, G., Buscot, F., 2008a. Community structure of arbuscular mycorrhizal fungi associated to *Veronica rechingeri* at the Anguran zinc and lead mining region. Environmental Pollution 156 (3), 1277–1283.

Zarei, M., Saleh-Rastin, N., Jouzani, G.S., Savaghebi, G., Buscot, F., 2008b. Arbuscular mycorrhizal abundance in contaminated soils around a zinc and lead deposit. European Journal of Soil Biology 44 (4), 381–391.

Zeng, F., Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F., Zhang, G., 2011. The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environmental Pollution 159 (1), 84–91.

Zhang, Y., Guo, L.D., Liu, R.J., 2004. Survey of arbuscular mycorrhizal fungi in deforested and natural forest land in the subtropical region of Dujiangyan, southwest China. Plant and Soil 261 (1–2), 257–263.