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Endophytic fungi and soil microbial community characteristics over different years of phytoremediation in a copper tailings dam of Shanxi, China

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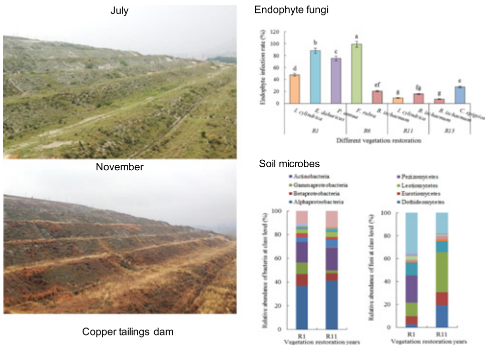
HIGHLIGHTS

- Endophyte infection frequency increased with years of phytoremediation.
- Endophyte infection rates of *Bothriochloa ischaemum* and *Festuca rubra* were positively related to levels of cadmium pollution levels.
- The structure and relative abundance of bacterial communities were varied little over years of phytoremediation, but there was a pronounced variation in soil fungi types.

GRAPHICAL ABSTRACT



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ABSTRACT

We conducted a survey of native grass species infected by endophytic fungi in a copper tailings dam over progressive years of phytoremediation. We investigated how endophytic fungi, soil microbial community structure and soil physiochemical properties and enzymatic activity varied in responses to heavy metal pollution over different stages of phytoremediation. endophyte infection frequency increased with years of phytoremediation. Rates of endophyte infection varied among different natural grass species in each sub-dam. Soil carbon content and soil enzymatic activity gradually increased through the years of phytoremediation. endophyte infection rates of *Bothriochloa ischaemum* and *Festuca rubra* were positively related to levels of cadmium (Cd) pollution levels, and fungal endophytes associated with *Imperata cylindrical* and *Elymus dahuricus* developed tolerance to lead (Pb). The structure and relative abundance of bacterial communities varied little over years of phytoremediation, but there was a pronounced variation in soil fungi types. Leotiomycetes were the dominant class of resident fungi during the initial phytoremediation period, but Pezizomycetes gradually became dominant as the phytoremediation period progressed. Fungal endophytes in native grasses as well as soil fungi and soil bacteria play different ecological roles during phytoremediation processes.

1. Introduction

Large amounts of heavy metals are disposed directly into soil via waste rocks, tailings, and other mineral dust in mining areas and their

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surrounding areas during metal mineral resource development. This has become a major source of environmental pollution in mining areas (Huang, 2015). The Zhongtiao Mountain copper mine, which is the largest underground copper mine in China, has been consistently increasing its mining of metal ore as well as gradually accelerating the speed of lift of its tailings dam. Such activities result in a great amount of heavy metals being introduced into soil, which not only leads to the degradation of soil ecosystems but also affects plant growth and development. With regards to research in ecological phytoremediation and practices in mining areas, researchers have been paying more attention to the mutual restoration of plants and microbes in ecosystems (Alfredo Narvaez-Ortiz et al., 2014; Mirzahosseini et al., 2014; Zamani et al., 2015). In recent years, plant-endophyte symbiosis, and in particular *Epichloë* and its host plants, as well as the physiological characteristics of symbionts have attracted widespread worldwide attention (Jia et al., 2014; Jia et al., 2015; Ren et al., 2014; Xia et al., 2015).

Endophytic fungi are ubiquitous, residing in healthy plant tissue without causing obvious disease (Arnold et al., 2000). Endophyte-infected host distribution is very broad, involving multiple groups of herbs, shrubs, conifers, and algae. It is particularly prevalent in many common grass species. During the lifecycle of a plant host, endophyte fungal hypha will asymptomatically colonize intercellular spaces of plant tissues and organs, such as leaf sheaths and blades, and hypha density in stem meristems will be higher than in leaf sheaths and blades (Schulthess and Faeth, 1998). However, frequencies of *Epichloë* infection in natural grass populations are variable, ranging from 0% to 100% of populations, even among populations of the same grass species (Schulthess and Faeth, 1998; Vinton et al., 2001). A few surveys related to the infection of endophytic fungi in natural grass species have been published (Li et al., 2004; Saikkonen et al., 2000; Cheplick and Faeth, 2009). Results have shown that there is a 100% endophyte infection rate in *Elymus canadensis* populations in prairie environments (Vinton et al., 2001). Infection frequencies of six different populations of *Elymus dahuricus* ranged from 4.4% to 100% (Zhang and Nan, 2007). Endophytic fungi are widely distributed in Inner Mongolia Steppe, and 63% of common natural grass species are infected by endophytic fungi (Wei et al., 2006). It has been reported that endophyte infection rates of *Betula platyphylla* depend on interactions between their environments and genotypes when environmental conditions change (Ahlholm et al., 2002).

Numerous studies have shown that the outcome of grass-endophyte interactions can be asymptomatic or antagonistic, depending on the grass and endophyte fungal species involved (Faeth and Sullivan, 2003; Jia et al., 2016). Different outcomes in host-endophyte interactions have been attributed to the different life histories of individual symbionts, patterns of endophyte fungal infection, genotypic variation, and ecological factors (Gonthier et al., 2008; Müller and Krauss, 2005; Photita et al., 2004; Saikkonen et al., 1998; Zhou et al., 2014). Given that endophyte-host interactions can also affect soil microorganisms, effects of endophyte infection are not only limited to the host plant (Rudgers and Orr, 2009). Soil microbes participate in promoting material circulation and energy flow, decomposition of organic matter, nutrient transformation, and other ecosystem-related biochemical processes (Fall et al., 2012; Lozupone et al., 2007; Logares et al., 2013). Previous work has shown soil microbial activity also can be inhibited by endophyte infection of aboveground plant material (Franzluebbers et al., 1999; Franzluebbers and Hill, 2005; Franzluebbers and Studemann, 2005; Buyer et al., 2011). Soil microbes are one of several sources within plants and soil nutrient repositories that can be used as important and effective biological indicators of soil fertility and nutrient resources (Fall et al., 2012; Zhang et al., 2016). This depends on variations in environmental factors as well as soil microbial community composition, structure, and diversity (Fall et al., 2012). Microbial community composition shifted somewhat in response to fungal endophyte infection: significantly higher fungal to bacterial ratios were observed in endophyte-free compared to endophyte infected stands (Iqbal et al., 2012).

Researchers have gradually come to understand the importance of the effects of endophytic fungi on soil microbial community structure and function (Buyer et al., 2011). In pot experiments, Zhou et al. (2014) reported that endophytic activity leads to an increase in bacteria, gram-negative bacteria, fungi, and total phospholipid fatty acid content in fungi. In field experiments, the phospholipid-derived fatty acid (PLFA) content of gram-positive bacteria and actinomycetes was significantly higher during endophyte infection treatments. Casas et al. (2011) found that there was an increase in fungal activity when *Lolium perenne* was infected by *Neotyphodium occultans*, which also affected the metabolic diversity of the soil microbial community.

Most previous studies on the ecological effects of plant-endophyte symbionts focused on pot experiments of agronomic grass or native grass species. There have only been limited studies on endophyte infection frequency response to heavy metal pollution and its impact on soil microbial communities in their native habitats. In this study, we conducted a survey of endophytic fungi infected natural grass species in four sub-dams of a copper tailings dam in the Zhongtiao Mountains each of which represent different years of phytoremediation. We also investigated physiochemical properties of soil and soil enzymatic activity in the sub-dams. Specifically, we investigated endophytic fungi and soil microbial community characteristics during different years of phytoremediation in an environment experiencing heavy metal pollution. We addressed the following questions: 1) How does the frequency of endophyte infection respond to environments experiencing heavy metal pollution? 2) How do physiochemical properties of soil and characteristics of enzymatic activity in soil vary between different years of phytoremediation in a copper tailings dam? 3) What are the characteristics of soil microbial communities during two different phytoremediation years? The aim of this study was to describe and evaluate the effects that different years of phytoremediation have on the frequency of endophyte infection rates and the development of soil properties and microbial characteristics.

2. Materials and methods

2.1. Site description and soil sampling

This study was conducted on Eighteen River tailings of Northern Copper Mine in Yuanqu County, Shanxi Province, China. The copper tailings dam was built in 1969. Its present height is 23.0 m and its crest elevation is 509.0 m. At this point in time, it is composed of 13 sub-dams. Each sub-dam has formed different vegetation domains after phytoremediation. In July 2015, we selected four sub-dams undergoing different phytoremediation stages (referred to as R1, R6, R11, and R13) for sampling (Table 1). We determined endophyte infection rates in common grass species in *Imperata cylindrical*, *Bothriochloa ischaemum*, *E. dahuricus*, *Calamagrostis epigeios*, *Poa annua*, and *Festuca rubra*, which were naturally by seed dispersal during phytoremediation processed. For each sub-dam, we randomly collected 30 grass samples chosen during transect walks (100 m × 10 m). A distance from 5 m to 10 m was left between sampled plants. For each sub-dam, we collected samples from the soil organic layer (at a 0 cm to 5 cm depth directly below the litter layer) at three random points using a sterile blade. They were then composited together into a single sample. Visible roots and residues were removed prior to homogenizing the soil fraction of each

Table 1
The wild natural grasses and different years of phytoremediation.

Plot number	Wild natural grasses	Start time (y)	Age of phytoremediation (a)
R1	*I. cylindrica*, *E. dahuricus*, *P. annua*	1969	45
R6	*F. rubra*, *B. ischaemum*	1989	26
R11	*I. cylindrica*, *B. ischaemum*	2009	7
R13	*B. ischaemum*, *C. epigeios*	2014	3

sample. Fresh soil samples were sieved through a 2 mm sieve and divided into two subsamples. One subsample was stored at 48 °C to determine physiochemical properties, while the other was stored at 20 °C prior to DNA extraction.

2.2. Detection of endophytic fungi

Five tillers were collected randomly from each plant and the outermost non-senescent leaf sheath of each tiller was used in this assay. A strip of epidermis was peeled from the inner surface of the leaf sheath close to the stem base. The strip was placed on a slide, mounted in aniline blue stain (Latch et al., 1987) and the slide was heated over a flame until the stain reached boiling point. It was then examined for hyphae under ×400 magnification.

2.3. Soil chemical properties and enzyme activities

Soil pH was measured after shaking a soil water (1:5 mass/volume) suspension for 30 min. Soil moisture was measured gravimetrically. Soil particle size was measured by using Mastersizer 3000 laser diffraction particle size analysis instrument (Malvern Co. Ltd., Malvern, UK). Before obtaining the particle size measurements, each sample was weighed at 3 g, and the sediments were immersed in 10% H_2O_2, and then in 12.7% HCl to remove any plant debris and to disperse the aggregates within the sediments. The sample residue was finally treated with 10 ml of 0.05 M $(NaPO_3)_6$ in an ultrasonic vibrator for 10 min to facilitate the dispersion prior to the particle size analysis. Only slight differences (0.5%) were found in the repeated particles size measurements on each sample. Total soil carbon (C), total nitrogen (N) and total sulfur (S) content were measured by using elemental analyzer (vario EL/MACRO cube, Elementar, Hanau, Germany). We used 3, 5-dinitrosalicylic acid colorimetry, potassium permanganate titration and indophenol-blue colorimetry to measure soil sucrose, catalase and urease activities, respectively (Guan, 1986). Heavy metal elements, which include As, Cd, Cr, Cu, Mn, Ni, Pb and Zn, were measured after shaking in the reagent (3.0 mL HNO_3, 1.0 mL HF and 2.0 mL H_2O_2) and left it for 30 min before microwave digestion. Samples were measured by Inductively Coupled Plasma-Atomic Emission Spectrometry (iCAP 6000, Thermo Fisher, UK). Three repetitions were performed in soil chemical properties and enzyme activities within each sub-dam.

2.4. Determination of the microbial community structure

Soil microbial total DNA was extracted using aDNA Isolation Kits for soil (Felix bio-tech, USA). Amplification of bacterial 16S rRNA is V3 fragment, and the primers are: 338F (5′-ACTC CTAC GGGA GGCA GCA-3′), 533R (5′-TTAC CGCG GCTG CTGG CAC-3′). Fungal amplification region is ITS1, and primer set as follows: ITS1F: (5′-TCCG TAGG TGAA CCTG CGG-3′), ITS2-Rev. (5′-GCTG CGTT CTTC ATCG ATGC-3′). In present study, soil samples were sent to Shanghai Personal Biotechnology Co., Ltd. for high-throughput sequencing.

2.5. Statistical analysis

The effects of phytoremediation processes on soil chemical properties, enzyme activities, endophyte infection rates and microbial characteristics were examined through one-way analysis of variance (ANOVA) with Duncan test. We used Person linear correlation to determine whether there was significant correlation among tested abiotic and biotic characteristics. These statistical analyses were performed using SPSS 13.0 for Windows. A matrix of environmental factors was applied into analyzing the relationships between the endophyte infected plants and environmental factors using redundancy analysis (RDA). RDA was performed using the CANOCO 4.5 (Ter Braak and Šamilauer 2002Li), and we used the Monte Carlo permutation test to test the significant level ($P < 0.05$) between environmental data and endophyte infection rates of different wild natural grasses.

3. Results

3.1. Soil chemical properties and soil enzyme activities

R1 showed the lowest particle size and soil pH, while highest N% and C% compared to other sites (Table 2). With the correlation analysis of physical and chemical properties of soil and soil enzyme activities, the results showed that the soil water content was significantly positively correlated with soil porosity and nitrogen content, and soil pH was significantly negatively correlated soil carbon content. These three soil enzyme activities had a highly significant negative correlation relationship with soil pH, and were significantly positively correlated with soil carbon content (Table 3). Urease and sucrase showed a significant negative correlation with soil particle size (Table 3). Thus, we speculated that the stronger soil alkaline, the greater inhibition of enzyme activity by the soil, however, the higher soil carbon content would promote soil enzyme activities. Soil carbon content increased with the increase of the recovery period, and the percentage of carbon content of R1 up to 1.75% (Table 3). Therefore, soil activities were gradually increased as increasing of phytoremediation period of copper tailings dam.

3.2. Endophyte fungi infection rate and its influence factors

In phytoremediation process, each sub-dam gradually consisted of different wild natural grasses. Endophyte infection rates of different natural grasses were significantly varied in each sub-dam. Endophyte infection rate of *Elymus dahuricus* was significantly higher than the infection rates of *Poa annua* and *Imperata cylindrica* in R1 plots, and the highest endophyte infection rate of *Festuca rubra* up to 100% in R6 plots, which was significantly higher than other natural grasses' endophytes infection rates in different phytoremediation processes of copper tailings dams. Endophyte rates of *Imperata cylindrical*, *Bothriochloa ischaemum* and *Calamagrostis epigeios* in R11 and R13 were significantly lower than each natural grass' endophyte infection rate in R1. *Bothriochloa ischaemum* had a significantly greater endophyte infection rate in R6 than in R13, but there was no significant difference between R6 and R11 (Fig. 1). The endophyte infection rates of grasses in copper tailings dam were associated by soil physical and chemical characteristics and soil enzyme activities. We evaluated these ecological factors on grasses infection rate with the Redundancy Analysis, and found the relationships among endophyte infection rates of wild natural grasses, soil physical and chemical properties and soil enzyme activities (Fig. 2). The results showed that 97.2% of the variations in endophyte infection rates could be explained by soil physical and chemical properties

Table 2
Soil porosity, particle size, soil pH, soil water content (SWC), total nitrogen (N), carbon (C), sulfur (S) and carbon nitrogen ratio (C/N) from different vegetation restoration stages in the copper tailing dam.

	Porosity %	Particle size (μm)	N%	C%	C/N	S%	pH	SWC%
R1	0.474 ± 0.010a	83.700 ± 4.701	0.031 ± 0.015	1.570 ± 0.263a	81.224 ± 35.887	0.044 ± 0.003	7.966 ± 0.082c	0.168 ± 0.023ab
R6	0.502 ± 0.016a	145.467 ± 27.533	0.026 ± 0.003	0.923 ± 0.118b	38.004 ± 6.521	0.040 ± 0.003	8.362 ± 0.087b	0.181 ± 0.014a
R11	0.423 ± 0.016b	150.267 ± 31.019	0.010 ± 0.002	0.642 ± 0.069b	67.099 ± 9.331	0.044 ± 0.004	8.768 ± 0.036a	0.106 ± 0.011c
R13	0.357 ± 0.016c	119.900 ± 12.192	0.021 ± 0.006	0.718 ± 0.041b	41.008 ± 10.801	0.037 ± 0.001	8.211 ± 0.045b	0.122 ± 0.008bc

Data are means ± standard errors. The different case letters indicate that the means are significantly different among vegetation restoration stages ($P < 0.05$) with Duncan test.

Table 3
The Pearson correlations between soil chemical properties and enzyme activities in reclaimed tailing dam.

	Porosity	Particle size	N	C	C/N	S	pH	SWC	Catalase	Urease
Particle size	0.122									
N	0.344	−0.301								
C	0.474	−0.449	0.159							
C/N	0.107	−0.098	**−0.587***	**0.643***						
S	0.117	−0.268	−0.405	0.422	0.543					
pH	−0.119	0.491	−0.364	**−0.772****	−0.233	−0.074				
SWC	**0.746****	0.048	**0.593***	0.524	−0.058	−0.194	−0.514			
Catalase	0.176	−0.549	0.473	**0.662***	0.168	0.238	**−0.717****	0.251		
Urease	−0.233	**−0.661***	0.259	**0.593***	0.213	0.051	**−0.855****	0.072	**0.757****	
Sucrase	0.178	**−0.693***	0.434	**0.795****	0.363	0.182	**−0.746****	0.307	**0.884****	**0.762****

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

and soil enzyme activities. Axis 1 of the RDA plot explained nearly 67.8% of the variation; Axis 2 explained a further 29.4%. Endophyte infection rates of *Elymus dahuricus* and *Poa annua* were positively correlated with soil carbon content, catalase, urease and sucrase, but negatively correlated with particle size and soil pH (Fig. 2 and Table 4). Similarly, endophyte infection rates of *Imperata cylindrica* was positively correlated with soil carbon content, catalase and sucrase. In contrast to other wild natural grasses, endophyte infection rate of *Bothriochloa ischaemum* was positively correlated with soil pH and negatively correlated with soil enzyme activities. Endophyte infection rates of *Festuca rubra* and *Calamagrostis epigeios* were only correlated with soil porosity (Fig. 2 and Table 4). Ecological factors and correlations of endophyte infection rate with soil physical and chemical properties varied among different natural grasses in copper tailings.

We measured endophyte infection rates of six wild natural grasses and nine soil heavy metals. The results showed that 99.72% of the variations in endophyte infection rates could be explained by heavy metal factors. Axis 1 of the RDA plot explained nearly 66.9% of the variation, and Axis 2 explained a further 27.8%. Cd content was positively correlated with endophyte infection rates of *Bothriochloa ischaemum* and *Festuca rubra*, and Pb content was negatively correlated with endophyte infection rates of *Imperata cylindrical* and *Elymus dahuricus* (Fig. 3 and Table 5). These suggested that endophyte infection rates of *Bothriochloa ischaemum* and *Festuca rubra* could be as indicators of Cd pollution levels, and the endophyte fungi of *Imperata cylindrical* and *Elymus dahuricus* had a certain tolerance to Pb.

3.3. Soil microbial community structure and characteristics

The dominant soil bacteria were Alphaproteobacteria in different vegetation phytoremediation stages of copper tailings dam, and their relative abundance accounted for 36.74% of overall bacterial microbes in R1 and 41.15% in R11. The sub-dominant bacteria were Actinobacteria, and their relative abundances were 17.11% in R1 and 18.92% in R11 separately. The relative abundance of Betaproteobacteria (9.88%) and Gammaproteobacteria (9.96%) had little difference in R1, but the soil bacteria relative abundance of Thermoleophilia (6.42%) in R11 was higher than in R1 plot (3.38%) (Fig. 4a). The structure and relative abundance of bacterial communities and varied little over years of phytoremediation, but there was a pronounced variation in soil fungi types.

Soil fungi dominance and composition differed in the copper tailings dam with years of phytoremediation, and their relative abundances were also varied. The dominant fungi was Pezizomycetes at class level in R1 plots, and its relative abundance was 23.85%, and the relative abundances of Leotiomycetes, Sordariomycetes and Eurotiomycetes were 11.73%, 11.27% and 7.31% respectively. In contrast, the dominant fungi was Leotiomycetes at class level in R11 plots, which relative abundance accounted for 34.90% total fungal microbes, and then Dothideomycetes, Eurotiomycetes and Sordariomycetes relative abundances were 19.42%, 11.18% and 9.65% respectively (Fig. 4b). The dominant fungal was Leotiomycetes during initial phytoremediation period, but as the recovery period increased, Pezizomycetes was gradually preponderance.

4. Discussion

4.1. Relationships between soil factors and enzymes

Previous studies clearly indicated that soil organic carbon (SOC) varied according to plant type used in phytoremedial recovery and the time that elapsed (Li et al., 2015). This was in accordance with our results that the soil C content increased as the period of phytoremediation increased (Table 2). Furthermore, soil enzymatic sucrase, which is widely

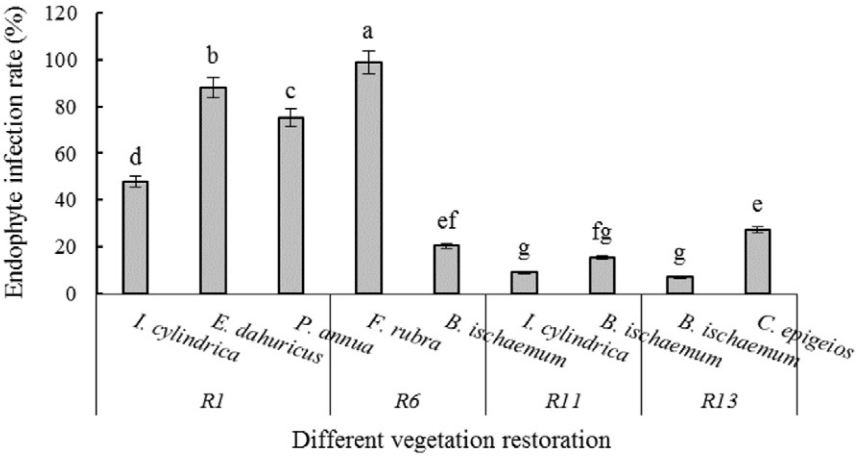

Fig. 1. Endophyte infection rates of natural grasses in different vegetation restoration years.

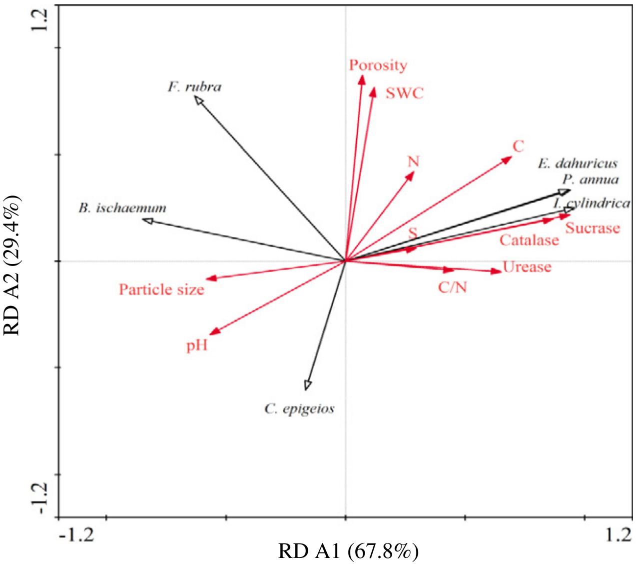

Fig. 2. Redundancy analysis (RDA) bi-plot of endophyte infection rate and explanatory variables. Explanatory variables include endophyte infection rate of six different grasses. Soil physical and chemical properties and enzyme activity include total nitrogen (N), total carbon (C), total sulfur (S), ration of carbon and nitrogen (C/N), and soil water content (SWC).

present in soils, is involved in ecosystem C cycling and plays an important role in increasing soil soluble substances. Moreover, its byproducts are a nutrient source for plants and microorganisms. In this study, we found that enzymatic urease and sucrase also had a significant negative correlation to soil particle size, which may be attributable to the different soils sampled during different years of phytoremediation. Moreover, soil water, gas, heat as well as other related conditions gradually restricted soil microbial activity along with changes in soil porosity. Thus, this affected the capacity of soil microbial metabolic enzyme production and decreased overall soil enzymatic activity (Zhang et al., 2010). Sucrase played an important role in increasing the number of small-molecule soil nutrients, and typically, the better the soil fertility was, the more robust sucrase activity would be. In our study, enzymatic sucrase activity was significantly correlated to soil C content, which is in agreement with a previous study that reported that enzymatic activity was positively correlated to C concentrations (Ciarkowska et al., 2014). The effects of years of phytoremediation on enzymatic activity could be potentially determined by two major drivers. One driver could be due to substrate induction resulting from the fact that litter inputs of aboveground vegetation seem to influence soil enzymatic activity (Tischer et al., 2014); another driver could be due to the fact that plant roots produce a variation of stimuli for enzymatic activity. This is the result of the different effects on microbial activity and production of exudates rich in substrates (Salam et al., 1998).

Mao et al. (2010) found that soil enzymatic activity was significantly positively correlated to total nitrogen in salt marsh vegetation and crops in a coastal wetland. This indicates that the effects of stages of phytoremediation and types of plant species on soil activity are closely related to soil characteristics and plant community structure. It also indicates that higher soil alkalinity could inhibit soil enzymatic activity. Additionally, higher soil C content would promote soil enzymatic activity. Furthermore, soil pH is an important regulator of soil microbial communities and enzymatic activity on continental or global scales (Lauber et al., 2009; Sinsabaugh et al., 2008). In our study, soil pH had a significant negative correlation to soil enzymatic activity. Moreover, pH and enzymatic activity were significantly different for all four phytoremediation stages investigated, which was consistent with previous studies (Li et al., 2015). Lastly, it should be noted that soil pH is one of the main factors for functional soil microbial diversity, which affects C use efficiency of soil microbes (White et al., 2005).

4.2. Endophyte infection rates of natural grasses

In Mediterranean savannahs, endophyte infection rates of *F. rubra* were from 44% to 92% of the population (Zabalgogeazcoa et al., 1999). Similarly, the present study found endophyte infection rates up to 99% of the population for *F. rubra* in sub-dam R6, which was significantly higher than endophyte infection rates found in the other natural grass species in different years of phytoremediation. Wei et al. (2006) found that the endophyte infection rate of *Achnatherum sibiricum* was different between different populations. Moreover, the distribution between endophyte infected plant species may be influenced by the seasons, atmospheric moisture levels, plant height, and other residing plant species populations (Petrini, 1991). In addition, our study found that endophyte infection rates of natural grass species were relatively higher as the years of phytoremediation progressed. Thus, we speculated that the longer phytoremediation processed of copper tailings dam, the higher endophyte infection rates of natural grasses, which might be a co-evolution result between the endophytic fungi and its host. The variation in endophyte infected grass populations may be the result of different ecological functions, which play an important role in ecosystem stability, and this could also be the result of the different stages of ecosystem evolution. Lewis et al. (1997) found that the endophyte infection rate of L. *perenne* in natural populations was significantly correlated to five specific environmental factors. In our study, endophyte infection rates of *E. dahuricus* and *P. annua* were significantly positively correlated to soil C content, and they also showed a significant positive correlation to catalase, urease, and sucrase. *Bothriochloa ischaemum*, however, showed an opposite effect. One possible explanation for this was that endophyte fungi in *E. dahuricus* and *P. annua* indirectly improved soil C content and promoted the accumulation of soil C (Iqbal et al., 2012). Another possible explanation was that different host plants input different types of litter to soil and thus redistribute different nutrient sources for microbial growth. Consequently, microbial species and composition varied, which resulted in differences in the quality and quantity of soil enzymatic activity (Xiao, 1996). In our study, endophyte infected plant species indirectly effected soil enzymatic activity by altering soil properties and characteristics of soil microbes (Ushio et al., 2010).

Under cadmium (Cd) stress, one study found that tall fescues infected by endophytes could enhance the host's absorption rate of Cd (Ren et al., 2011). However, this differed from our findings. We found that the

Table 4
Correlation of endophyte infection frequency and soil properties.

	Porosity	Particle size	N	C	C/N	S	pH	SWC	Catalase	Urease	Sucrase
Elymus dahuricus	0.340	**−0.586***	0.378	**0.830****	0.425	0.310	**−0.681***	0.365	**0.901****	**0.634***	**0.962****
Imperata cylindrica	0.325	−0.542	0.288	**0.786****	0.490	0.393	−0.550	0.260	**0.861****	0.538	**0.923****
Poa annua	0.354	**−0.588***	0.491	**0.799****	0.347	0.227	**−0.657***	0.436	**0.867****	**0.593***	**0.959****
Bothriochloa ischaemum	0.225	0.502	−0.190	−0.540	−0.360	−0.090	**0.681***	−0.067	**−0.767****	**−0.769****	**−0.814****
Festuca rubra	**0.617***	0.294	0.155	−0.050	−0.330	−0.160	0.066	0.562	−0.387	−0.426	−0.416
Calamagrostis epigeios	**−0.807****	−0.068	−0.040	−0.330	−0.280	−0.460	−0.220	−0.338	−0.095	0.445	−0.118

* Means significant difference at 0.05 level.
** Means strong significant difference at 0.01 level.

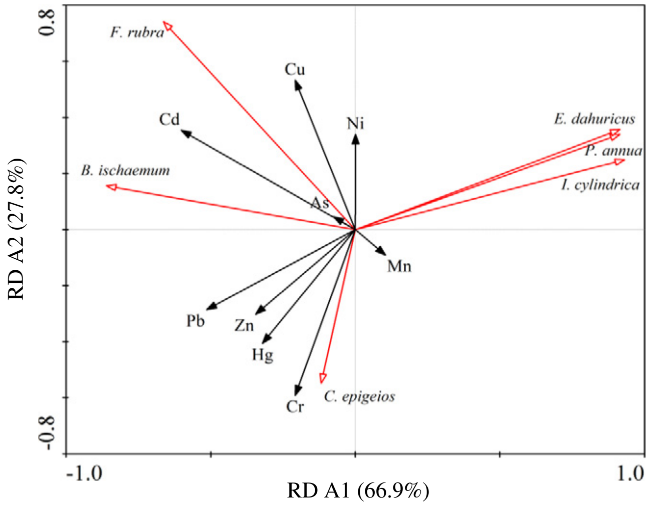

Fig. 3. Redundancy analysis (RDA) bi-plot of endophyte infection rate and explanatory variables. Explanatory variables include endophyte infection rate of six different grasses and soil heavy metals.

distribution of endophyte infected natural grass species was influenced by heavy metals content during different years of phytoremediation, and Cd content significantly positively correlated to endophyte infection rates of *B. ischaemum* and *F. rubra*. We assumed that this difference may have be associated with the contribution of different endophyte fungal species in natural grasses, and incidences of endophytic infection in these two particular natural grass species could also predict the degree of contamination of the copper tailings dam. It has reported that endophyte of tall fescue improved host plants drought and heat tolerance (Marks and Clay, 1996; West, 1994), enhanced photosynthesis (Marks and Clay, 1996; Newman et al., 2003; Richardson et al., 1993), and increased nutrient-deficiency tolerance (Malinowski and Belesky, 2000). Endophyte fungi and soil microbial population may have important implications for the functioning of soils, such as carbon storage, in copper tailings dam. Ban (2013) reported that plant mycorrhiza infected by *Gaeumannomyces cylindrosporus* had a strong tolerance to lead (Pb), and *G. cylindrosporus* promoted the absorption of greater amounts of Pb in roots while preventing its transfer to shoots, which reduced Pb content in the aboveground components of the plant species, thereby alleviating the toxic effects of Pb. Even though we did not focus on whether *B. ischaemum* was infected by mycorrhizal fungi in our study, we found that Pb content was significantly negatively correlated to endophyte infection rates of *E. dahuricus* and *I. cylindrical*, indicating that endophytic fungi that infects these two grass species may possess a tolerance to Pb. Furthermore, endophytic fungi mainly exist in the sheath of plants, but endophytic behavior within plants remains unclear. Three mechanisms are possible: 1) Endophytic fungi could promote the growth of host plants and could accumulate heavy metals inside mycelia. Zapotoczny et al. (2007) found that for *Acremonium pinkertoniae*, metal ions were mostly incorporated into the chitin-glucan complex present in fungal cell walls by the formation of coordinate bonds with N and oxygen (O) atoms of amide and hydroxyl groups of polysaccharides. Therefore, endophyte infected plants have greater biomass, the ability to absorb greater amounts of heavy metals, and possess greater root activity, which play an important role in the phytoremediation of heavy metal contaminated soil. 2) Endophyte infection could greatly improve host tolerance to abiotic stresses, such as drought and mineral scarcity, which often occurs in heavy metal-polluted areas (Ren et al., 2007); 3) Endophyte fungi might be secretion of metal-chelating molecules such as organic acids or phenolic compounds into the rhizosphere. Furthermore, it has been proposed that heavy metal mobilization was due to the behavior of siderophores in endophytic bacteria (Abou-Shanab et al., 2003). Taking these points into consideration, it is predicted that endophyte infected grass species will be used to phytoremediate heavy metal pollution in tailings dams in the future.

4.3. Characteristics of soil microbial community structure

Shi found that the abundance of soil bacteria and fungi were affected by vegetation type, humidity, temperature, soil structure, nutrients, pH, and other environmental factors (Shi and Yu, 2012). Rudgers and Clay (2007) reported that the impact of endophytic fungi on a community level is greater than on a population level. Studies have shown that tall fescues infected by *Neotyphodium coenophialum* alter soil microbial communities (Rudgers and Orr, 2009). In addition, one study has shown that bacterial communities are extremely sensitive to variations in soil water content, and, between them, they exhibit a negative correlation (Ahn and Peralta, 2009). However, this study did not arrive at the same conclusion. Even though soil water content was significantly different between R1 and R11, both sub-dams had the same dominant bacteria community. The probable reason for this was that soil water content mainly effected soil fungi community structure (Schimel et al., 1999). Moreover, it has reported that the presence of a fungal endophyte may enhance rhizodeposition by tall fescue and could consequently influence microbial mineralization processes in the soil (Hecke et al., 2005), and Rojas et al. (2016) found that tripartite interactions exist between the shoot endophyte *E. coenophiala*, tall fescue, and soil fungi that may have important implications for the functioning of soils, such as carbon storage, in fescue-dominated grasslands (Rojas et al., 2016). In our study, there was little change in soil bacteria between the different phytoremediation stages of the copper tailings dam, but soil fungal communities differed from each other. One explanation for this may have been that the effect of endophytic fungi on soil fungi community structure was greater than the effect on soil bacteria. Another possible explanation could be that endophytic fungi may alter the chemical composition of senescent leaves, which have different decomposition rates than most plant litter (Lemons et al., 2005; Siegrist et al., 2010). Thus, the effect of endophytic fungi on the structure and activity of soil microbial communities will be considerably different. Moreover, soil microbial communities are closely related to soluble C and soluble N (Drenovsky et al., 2004; Tabuchi et al., 2008; Zak et al., 2003). In present study, soil C content was significantly different between all phytoremediation years, and this was the result of the microbial communities present. As the phytoremediation period progressed, the preponderance of the dominant fungal Pezizomycetes gradually increased.

Table 5
Correlation of endophyte infection frequency and heavy metal factors.

	As	Cd	Cr	Cu	Ni	Pb	Zn	Hg	Mn
Elymus dahuricus	−0.014	−0.431	−0.39	−0.021	0.139	**−0.584**[*]	−0.402	−0.416	0.110
Imperata cylindrica	−0.135	−0.392	−0.348	−0.016	0.097	**−0.610**[*]	−0.436	−0.476	0.021
Poa annua	−0.062	−0.47	−0.396	−0.016	0.09	−0.524	−0.412	−0.417	0.050
Bothriochloa ischaemum	−0.397	**0.651**[*]	0.120	0.256	−0.003	0.300	0.289	−0.076	−0.171
Festuca rubra	0.112	**0.648**[*]	−0.301	0.523	0.255	0.136	0.013	−0.069	−0.118
Calamagrostis epigeios	0.518	−0.507	0.381	−0.548	−0.143	0.417	0.478	0.690	0.510

[*] Means significant difference at 0.05 level.

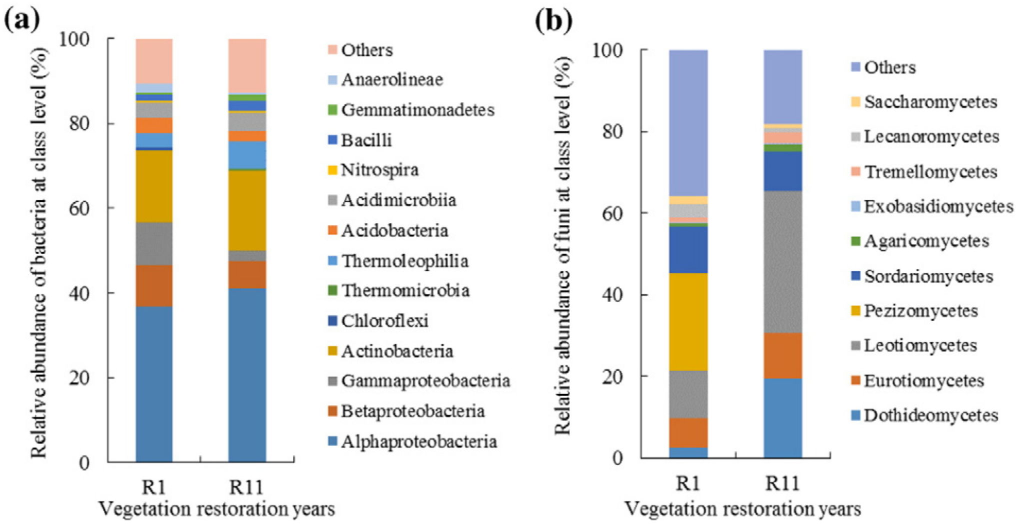

Fig. 4. Relative abundance of bacteria (a) and fungi (b) at class level in different vegetation restoration years.

What remains unclear is how endophytic fungi affect soil microbial community structure. It may be that endophytic fungi alter soil microbial community structure by altering host grass root exudates as well as physiochemical properties of soil (Van Hecke et al., 2005). Moreover, plant species infected by endophytes may also control litter accumulation and thus directly influence organic C and N inputs into soils (Bai et al., 2009), which would cause differences in soil characteristics, microbial biomass, and soil micro-environments, and indirectly lead to differences in the microbial growth conditions, structure, and diversity in soil microorganisms (Fu et al., 2009).

5. Conclusions

This study has shown that, endophyte infection frequency increased with years of phytoremediation, as well as rates of endophyte infection varied among different natural grass species in each sub-dam. Additionally, endophyte infection rates of *Bothriochloa ischaemum* and *Festuca rubra* were positively related to levels of cadmium pollution levels, and fungal endophytes associated with *Imperata cylindrical* and *Elymus dahuricus* developed tolerance to lead. The structure and relative abundance of bacterial communities and varied little over years of phytoremediation, but there was a pronounced variation in soil fungi types. Leotiomycetes were the dominant class of resident fungi during the initial phytoremediation period, but Pezizomycetes gradually became dominant as the phytoremediation period progressed. Over all, endophyte fungi and soil microbial population can play an important role in plant survival and growth, and using infected plants might help to reclaim and stabilize tailings more effectively.

Conflict of interest

The authors declare they have no competing financial interests.

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