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Diversity of arbuscular mycorrhiza fungi in rhizosphere soil and roots in *Vetiveria zizanioides* plantation chronosequence in coal gangue heaps

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Abstract
As key soil microorganisms of terrestrial ecosystems, arbuscular mycorrhiza fungi (AMF) play a key role in vegetation succession and mediation and stabilizing ecosystems. This study investigated the structures of AMF communities at the roots and rhizosphere soil of *Vetiveria zizanioides* of different ages (6-, 10-, 14-, and 17-year-old) in coal gangue heaps in Liupanshui City, Guizhou Province using high-throughput sequencing. The factors that affected the structures are also discussed. The results demonstrated that the roots and rhizosphere soil of *V. zizanioides* contained 109 and 173 AMF operational taxonomic units (OTUs). At the generic level, AMF communities in the roots were different from those in the rhizosphere soil. The roots of AMF communities mainly included *Glomus*, *Dominikia* and *Rhizophagus*, while the mainly included *Glomus*, *Dominikia*, *Rhizophagus*, *Septoglomus* and *Paraglomus*, *Glomus* and *Dominikia* were the dominant AMF communities in the roots and rhizosphere soil of *V. zizanioides*. The Shannon and Simpson diversity indexes of AMF communities in the roots and rhizosphere soil did not significantly change with *V. zizanioides* planting years. Redundancy analysis (RDA) revealed that the soil available phosphorus and pH were the main factors affecting AMF communities in the rhizosphere soil and roots. Our study provided references for the remedy of coal gangue heaps via mycorrhiza inoculation.

Keywords AMF communities · Coal gangue heap · Diversity · *Vetiveria zizanioides*

1 Introduction

Due to its abundant reserves, coal has been the primary energy source in China for an extended time (Chao et al., 2010; Qiu et al., 2018). However, while coal mining drives China's economic development, it also causes severe adverse effects on the environment and society (Kompała-Bąba et al., 2019). Coal gangues are solid by-products inevitably produced during coal mining and washing (Bell et al., 2000). The accumulation of coal gangues consumes considerable land resources and destroys the landscape. In addition, toxic and harmful substances in coal gangues are discharged into the air, soil, and waterbodies by weathering and leaching, thus posing a substantial threat to ecosystems and human health (Qiu et al., 2011; Zhang et al., 2015). The ecological issues caused by coal gangue heaps have been a major hidden danger threatening sustainable economic development and regional ecological security (Liu et al., 2017). Hence, efficient yet cost-effective remediation techniques for coal gangue heaps are urgently needed.

Vetiveria zizanioides is a perennial herb in the Gramineae family. It has fast growth and reproduction characteristics, a developed root system, wide adaptability, and strong resistance. It is widely used in soil and water conservation and soil reforestation (Nero et al., 2019; Kiamarsi et al., 2020; Mondal and Patel, 2020) and is one of the main species in vegetation reforestation of coal gangue hills (Meyer et al., 2016). *V. zizanioides* roots form mycorrhizal symbionts with arbuscular mycorrhizal fungi (AMF) (Wong 2003; Khan 2009). The symbiont explores the soil pores that cannot be touched by the root hair and establish material transport channels between the root and the soil, promoting the absorption of nutrients by the plants (Neumann and

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George 2004; Gloria et al., 2012; Smith and Smith 2012; Chitarra et al., 2016). Studies have shown that arbuscular mycorrhizae secrete H^+ and organic acids (such as citric acid and oxalic acid) to activate insoluble phosphate, thereby increasing the available phosphorus content in soil (Hong et al., 2002). The soil's acid phosphatase activity increased after plant inoculation with AMF, and P uptake by plants was positively correlated with acid phosphatase content (Ma et al., 2021). In plants that form arbuscular mycorrhizae, AMF can provide up to 100% P for plants (Smith et al., 2004), and can directly absorb NH_4^+ (Tanaka and Yano 2010), NO_3^- (Bago et al., 1996), and small molecular organic nitrogen (Barrett et al., 2011) from the soil to promote plant growth. In addition, AMF improves the stress resistance of host plants by maintaining the Na^+ and Cl^- contents balance, increasing the SOD, POD, and CAT activities and the soluble sugar and proline contents (Garg and Aggarwal 2012; He and Huang 2013; Grümberg et al., 2015; Zhu et al., 2015).

AMF belonging to the phylum Glomeromycota are an important part of natural and agricultural ecosystems widely found in soil and can establish a mutualistic symbiotic relationship with more than 80% of land plant roots (Smith and Read, 2008; Zu et al., 2019; Zhang et al., 2020). AMF diversity affects plant community structure, diversity, and productivity in different ecosystems and plays a vital role in vegetation succession and restoration (Heijden et al., 2010). AMF community composition and diversity are affected by those of the host plant and biological and abiotic factors, which often interact (Niall and Alison 2016). Johnson et al. (2004) found that grassland plant communities had a significant impact on AMF diversity, and different host plants have their unique AMF community (Hausmann and Hawkes 2010). Compared with saprophytic soil microorganisms, the AMF abundance in soil has a more pronounced effect on the composition and diversity of plant communities (Deyn et al., 2011), and the abundance of AMF in the roots of plants with higher community richness is relatively higher (Vanessa et al., 2016).

Soil physical and chemical properties are important factors affecting AMF diversity. Recent studies on karst ecosystems have shown that AMF abundance is sensitive to N addition, while diversity is sensitive to P addition (Xiao et al., 2019). Moreover, AMF abundance and diversity are negatively correlated with total phosphorous (TP) and available phosphorus (AP) in soil organic matter, and positively correlated with K (Lin et al., 2019; Wang et al., 2021). However, other studies showed that AMF richness was positively correlated with pH, TP, and AP and negatively correlated with N (Yang et al., 2015a; Xiao et al., 2019). In addition, AMF community composition changes with seasons (Wang et al., 2021), altitude (Zhao et al., 2020), root age (Kil et al., 2014), and spatio-temporal distribution (Kezia et al., 2020). Although soil properties (soil C, N, P, C: N, C: P, N: P) greatly influence AMF diversity in the rhizosphere (Zhao et al., 2020), there is no fixed model for the effect of soil properties on AMF community. At present, there are many studies on AMF community composition and diversity, but there are few reports on AMF community structure and diversity in vetiver roots and rhizosphere soil of different planting years in mining areas.

Since 2000, our research group has planted *V. zizanioides* in the coal gangue hill of the Dahe Coal Mine, Zhongshan District, Liupanshui City, Guizhou Province for ecological restoration. In the previous study, we discussed the effects of vetiver planting on the physicochemical properties of heavy metals (Cu, Zn, Cd, Pb, As) in the coal gangue matrix, showing that AMF might play an important role in vetiver's adaptation to coal gangue environment. Therefore, samples of *V. zizanioides* with different planting years (2002, 2005, 2009, and 2013) were collected, and the following aspects were investigated via spatiotemporal substitution: (1) the AMF communities in the roots and rhizosphere soil of *V. zizanioides*; (2) the effects of age on the diversity and community structure of AMF in the roots and rhizosphere soil of *V. zizanioides*; (3) the soil factors that affect the diversity and community structure of AMF in *V. zizanioides*.

2 Materials and methods

2.1 Sample collection

The sample sites were in the Dahe Mine, Liupanshui City, Guizhou Province, China (see Fig. 1). This area is characterized by a warm and humid northern subtropical monsoon climate. The annual average temperature is 14 °C, the annual precipitation is 1182.8 mm, and the frost-free period is 230-298 days. The abundant coal resources have become the local main economic pillar. Due to long-term over-exploitation, many coal gangues are exposed, resulting in a severe negative impact on the surrounding environment.

The Dahe Mine has been mined for a long time. In 2000, our research group initiated *V. zizanioides* mediated biological remediation of coal gangue heaps. *V. zizanioides* was planted on

Fig. 1 Locations of sampling sites

the gangue hills where coal was mined that same year. Seedlings were planted with topsoil soil and a small amount of water, and the failed seedlings were replanted after a month. After their survival, *V. zizanioides* and coal gangue mountain were no longer managed, and each *V. zizanioides* community grew in its natural state. To date, *V. zizanioides* communities of seven different ages are available. This study selected four vetiver communities from different planting years (2002, 2005, 2009, and 2013) as research objects. All samples were collected in March 2019. Three samples were collected from different planting years, and each sample consisted of three randomly selected *V. zizanioides* in good conditions. Large sand and other debris on the ground were removed, and fine root and soil samples were collected at 0-30 cm of the soil layer. The soil samples were divided into two groups: one group was stored at −80 °C for DNA extraction, and the other was naturally dried, screened by a 2-mm sieve for physicochemical properties determination. The roots were rinsed with clean water and stored at −80 °C for DNA extraction. After repeatedly rinsed with distilled water, the root samples were divided into two parts. One part was stored in a−80 °C for DNA extraction, and the other was placed in a glass bottle containing FAA fixative solution (5 mL formaldehyde, 5 mL acetic acid and 90 mL 70% ethanol) for AMF infection rate determination.

2.2 Detection of physicochemical properties of soils

The pH value, organic matter (OM), total nitrogen (TN), total phosphorous (TP), total potassium (TK), available potassium (AK), and alkaline phosphatase (AP) contents of the soil were respectively measured using the pH acidity method, high-temperature external heat potassium dichromate oxidation capacity method, Kjeldahl method, molybdenum antimony resistance colorimetric method, flame spectrophotometry, and the $NaHCO_3$ method (Saunders and Williams 1955; Dormaar 1964; Steward and Oades, 2006).

2.3 AMF spore density and mycorrhizal colonization rate

2.3.1 AMF spore extraction

AMF spores in the rhizospheric soil were separated by wet-sieving and decanting-sucrose centrifugation (Gerdemann and Nicolson 1963). The rhizospheric soil (10 g per sample) was suspended in distilled water and filtered through two sieves (upper 500 μm; middle 106 μm; lower 45 μm). The residue in the lower sieve was transferred to a 100 mL centrifuge tube and centrifuged (3000 rpm, 3 min). After discarding the supernatant, a sucrose solution was added to the centrifuge tube and mixed well. Then the mixture was centrifuged (1500 rpm, 1.5 min). The supernatant was sifted immediately through a clean sieve (45 μm). The residue on the sieve was washed by distilled water to ensure that no sucrose solution remained in the residue, then washed into a petri dish and examined with a dissecting microscope. The spore density was as:

Spore density = total number of AMF spores/total number of soil (samples)

2.3.2 AMF colonization rate

The AMF colonization rate was determined using the acid fuchsin stain (Phillips and Hayman 1970). *V. zizanioides* roots stored in FAA fixating solution were taken out, washed with distilled water and cut into 1-cm-long segments, which were put in 5% w/v KOH solution and heated at 90 °C for 30 min in a water bath until they were completely transparent. Hydrochloric acid at 2% v/v was then added to neutralize the KOH. The root segments were placed in the acid fuchsin solution and heated at 90 °C for 20 min in a water bath. After samples were washed in distilled water, they were placed in a lactic acid–glycerin solution to decolorize the tissue. The decolorized root segment was observed under a microscope on a slide, and the infection rate was determined by root segment method (Biermann and Linderman 1981). The mycorrhizal colonization rate was determined as:

Percentage of mycorrhizal colonization % = (number of mycorrhizal root segments observed) / (total number of root segments observed) × 100.

2.4 Detection of AMF molecule diversity of roots and rhizosphere soil

2.4.1 DNA extraction, PCR, and sequencing

The total genomic DNA from the roots and rhizosphere soil samples was extracted using the cetyltrimethylammonium bromide (CTAB) method. DNA concentration and purity were monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/μL using sterile water.

Using diluted genomic DNA as template, the nuclear ribosomal internal transcribed spacer region (ITS rDNA gene) was amplified by polymerase chain reaction (PCR) using the fungal primer set for ITS1-1F-F (5'-CTTGGT CATTTAGAGGAAGTAA-3′) and ITS1-1F-R (5'-GCT GCGTTCTTCATCGATGC-3′) (Liang et al., 2020; Wang et al., 2020). All PCR reactions were carried out in a 30 μL total volume with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, elongation at 72 °C for 30 s, and final extension at 72 °C for 5 min. The same volume of 1X loading buffer (SYBR green) was mixed

with PCR products, and electrophoresis was performed on 2% agarose gel for detection. Then, the mixed PCR products were purified with a Gene JET Gel Extraction Kit (Thermo Scientific).

Sequencing libraries were generated using an Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific) following the manufacturer's recommendations. The library quality was assessed on a Qubit® 2.0 Fluorometer (Thermo Scientific). Finally, the library was sequenced on an Ion S5TM XL platform, and 400 bp/600 bp single-end reads were generated.

2.4.2 Processing of sequencing data

Single-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Quality filtering on the raw reads was performed under specific conditions to obtain high-quality clean reads according to the Cut adapt quality-controlled process. The reads were compared with the reference database using the UCHIME algorithm to detect chimera sequences, then removed. The clean reads ultimately obtained by the sequencing analysis were analyzed by UPARSE software. Sequences with ≥97% similarity were assigned to the same OTU. A representative sequence for each OTU was screened for further annotation. The UNITE Database was used to annotate taxonomic information for each representative sequence based on the Blast algorithm, calculated by QIIME software (version 1.9.1). Multiple sequence alignments were conducted using MUSCLE software (version 3.8.31) to study the phylogenetic relationships of different OTUs and the differences of the dominant species in different samples (groups). The OTU abundance information was normalized using a standard sequence number corresponding to the sample with the fewest sequences. All subsequent alpha and beta diversity analyses were performed based on this output normalized data. Alpha diversity was applied to analyze the species diversity complexity for a sample via 5 indices, namely Chao1, Shannon, Simpson, ACE, and Good's coverage. All indices were calculated with the QIIME software (version 1.7.0) and displayed with R software (version 2.15.3).

2.5 Statistical analysis

SPSS 18.0 was used for statistical data analysis. The single-factor analysis of variance and Duncan's test were used to determine the significance of differences in soils' physicochemical properties and diversity indices in different planting years. Mantel-test was used to test the significant difference of AMF spore density and infection rate with planting years and soil physical and chemical properties. The Alpha diversity index was calculated using QIIME (version 1.7.0) software. The bar chart of the community compositions, which was generated using SigmaPlot 12.5, reflected the abundances of AMF communities in different plots. Non-metric multidimensional scaling (NMDS) using R software reflected the differences in the structures of AMF communities in samples of different ages. The relationships between the structures and diversity of AMF communities and the physicochemical properties of soils were investigated using redundancy analysis (RDA).

3 Results

3.1 Changes in soil properties across different planting years

The main properties of the soil samples collected from all selected sites are presented in Table 1. The soil samples were all slightly alkaline, with the pH of all study sites ranging from 7.21 (6 years) to 8.48 (17 years). The soil's OM, TN, and TP were maximized in the 14- year-old samples and minimized 17-year-old samples. The TK and AK of the soil decreased and then increased as the sample age. The AP in the soil decreased with planting time was significantly lower inthe17-year-old soil sample than those in the 6- and 10-year-old soil samples ($P < 0.05$).

Table 1 Physicochemical properties of soil in different planting years

Sites (years)	PH	OM (g/kg)	TN/(g/k)	TP/(g/kg)	TK/(g/kg)	AP/(mg/kg)	AK /(mg/kg)
6a	7.21 ± 0.10c	82.63 ± 8.96b	1.08 ± 0.03c	0.97 ± 0.11ab	11.19 ± 1.09a	3.42 ± 0.73a	263.33 ± 35.12ab
10a	7.59 ± 0.43b	131.58 ± 12.47a	1.75 ± 0.25b	0.94 ± 0.17ab	10.89 ± 0.98a	3.37 ± 1.72a	230.00 ± 24.58ab
14a	7.43 ± 0.04bc	150.00 ± 18.92a	2.19 ± 0.19a	1.00 ± 0.06a	9.11 ± 1.00b	1.67 ± 0.28ab	204.33 ± 36.83b
17a	8.48 ± 0.08a	34.17 ± 2.37c	0.89 ± 0.31c	0.76 ± 0.08b	11.7 ± 0.23a	1.10 ± 0.14b	298.67 ± 59.55a

Different letters in the same column indicate significant differences between different years ($P < 0.05$). 6a, 10a, 14a, and 17a refer to *V. ziza-nioides* planted in 2013, 2009, 2005 and 2002, respectively

3.2 The AMF spore density and mycorrhizal colonization

Many AMF spores were isolated from *V. zizanioides* rhizosphere soils, ranging from 104.33 (10 years) to 462.67 (17 years) spores per 10 g of soil. The spore density was significant in each planting year ($P < 0.05$) (Fig. 2), and significantly decreased and increased with increasing OM and pH, respectively ($P < 0.01$). However, no significant correlation could be found between spore density and soil TP, TK, AP levels ($P>0.05$) (Table 2). All the samples obtained were colonized by AMF and formed typical arbuscular structures, ranging from 48.66% (16 years) to 65.43% (10 years) (Fig. 2). Furthermore, no significant correlation could be found between mycorrhizal colonization and soil properties ($P>0.05$). Overall, AMF spore density first decreased and then increased with planting years, and mycorrhizal colonization was opposite to spore density. In addition, planting years were significantly correlated with spore density ($P < 0.05$) and no significantly correlated with mycorrhizal colonization ($P>0.05$) (Table 2).

Table 2 Mantel-test of AMF spore density and colonization rate in relation to planting years and physicochemical properties of soil

	Spore density		mycorrhizal colonization	
	r	p	r	p
Planting years	0.677	0.016	0.355	0.258
OM	−0.862	0.000	0.162	0.615
TN	−0.664	0.018	0.221	0.490
TP	−0.667	0.018	−0.307	0.332
TK	0.461	0.132	0.094	0.772
AP	−0.572	0.052	−0.135	0.676
AK	0.603	0.038	−0.011	0.974
pH	0.821	0.001	0.502	0.097

Significant correlation at the $P<0.05$ level; significant correlation at the $P<0.01$ level

3.3 Analysis of sequencing results and dilution curves

High-throughput sequencing of the roots and rhizosphere soil of *V. zizanioides* in the four plots generated 79,487 valid data points after quality control averaging. The sequences were clustered into OTUs with 97% similarity, and 178 AMF OTUs were obtained. The rhizosphere soil contained 173 OTUs in total, with 112, 92, 131, and 103 OTUs in the 6-, 10-, 14-, and 17-year-old samples, respectively. The roots contained 109 OTUs in total, with 86, 78, 61, and 100 OTUs in the 6-, 10-, 14-, and 17-year-old samples, respectively (Fig. 3). Data of a specific sequencing volume were randomly extracted from samples. In the dilution curves presented in Fig. 4, the x-axis refers to the number of sequences extracted, and the y-axis refers to the quantity of OTUs that can be established based on this sequence quantity. The curve was saturated as the sequence quantity increased, suggesting that the sequencing volume was sufficient. This sequencing reflected the diversity of AMF in the roots and rhizosphere soil of *V. zizanioides* of all ages, and the population abundance of the rhizosphere soil was higher than that of the roots.

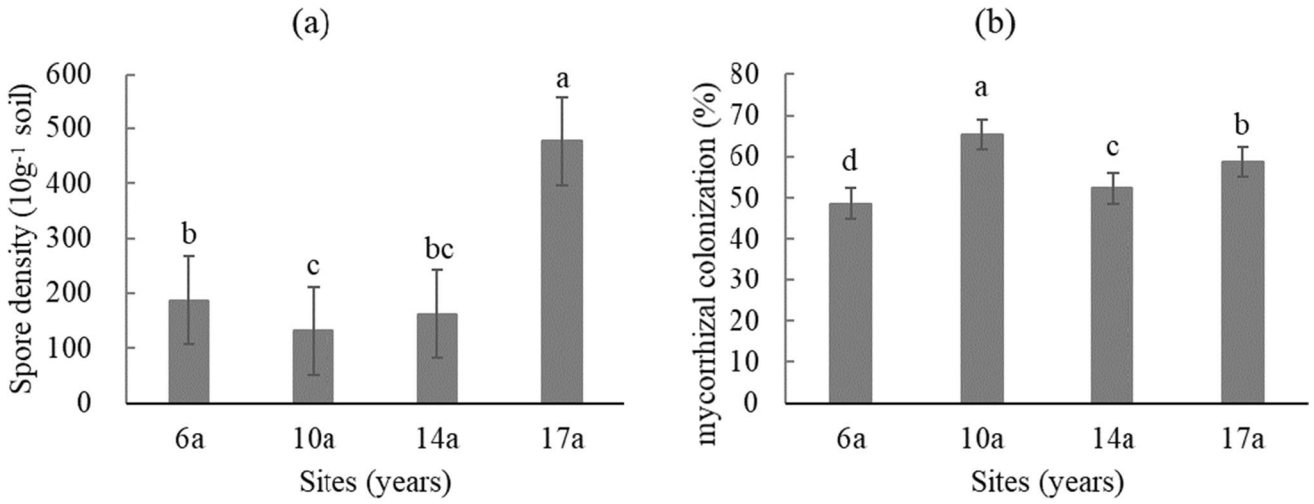

Fig. 2 Spore density (a) and mycorrhizal colonization (b) of AMF in the rhizosphere of *V. zizanioides* across different planting years. Values are the means (n = 3) for each study site Different lowercase letters are significantly different ($P < 0.05$)

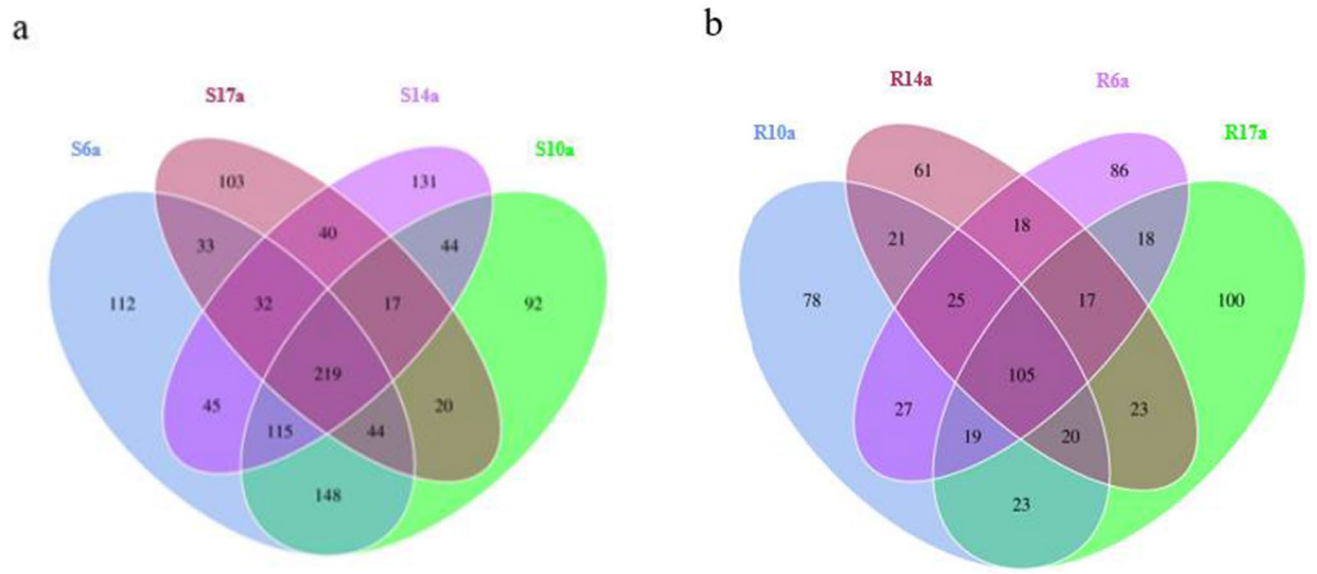

Fig. 3 Venn diagrams of AMF OTUs in the (a) roots and (b) rhizosphere soil. S6a, S10a, S14a, and S17a refer to the rhizosphere soils of the 6-, 10-, 14-, and 17-year-old samples, respectively. R6a, R10a, R14a, and R17a refer to the roots of the 6-, 10-, 14-, and 17-year-old samples, respectively

3.4 The structures of AMF communities

3.4.1 Compositions of AMF communities in roots and rhizosphere soil of *V. zizanioides*

Figure 5 illustrates the compositions of AMF communities in the roots and rhizosphere soil of *V. zizanioides* of different planting years at the genus level. In the four plots, the roots' AMF communities mainly consisted of *Glomus*, *Dominikia,* and *Rhizophagus*. Those in rhizosphere soil mainly consisted of *Glomus*, *Dominikia*, *Rhizophagus*, *Septoglomus*, and *Paraglomus*. *Dominikia* was the dominant genus in the roots (96.38%) and rhizosphere soil (95.77%) in the 17-year-old *V. zizanioides* sample. *Glomus* was the dominant genus in the roots and rhizosphere soil of the other samples. *Septoglomus* was only observed at the root of the 17-year-old sample (0.18%); *Diversispora* was only observed at the rhizosphere soil of the 10-year-old sample (0.38%).

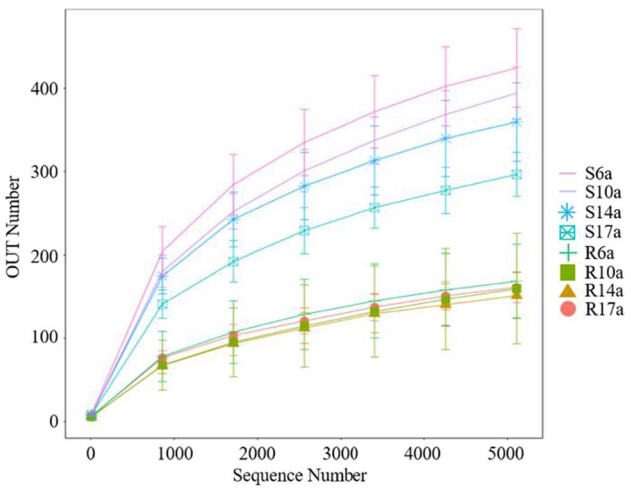

Fig. 4 Dilution curves of AMF OTUs in the roots and rhizosphere soil

3.4.2 Diversity of AMF communities in roots and rhizosphere soil of *V. zizanioides*

Calculations of the AMF communities' diversity in the roots and rhizosphere soil of *V. zizanioides* in the four plots revealed that the Shannon and Simpson indices of the rhizosphere soil were minimized in the 17- and 10-year-old samples, respectively, and maximized in the 6-year-old sample. Additionally, samples of different ages exhibited negligible differences ($P > 0.05$). The Chao1 and ACE indices values decreased with sample age, while those of the 17-year-old sample were significantly lower than those of the 6- and 10-year-old samples ($P < 0.05$). The Shannon, Simpson, Chao1, and ACE indices of the root samples of different ages exhibited negligible differences. Additionally, the coverage indices of roots and rhizosphere soil of all samples were above 97% (Table 3).

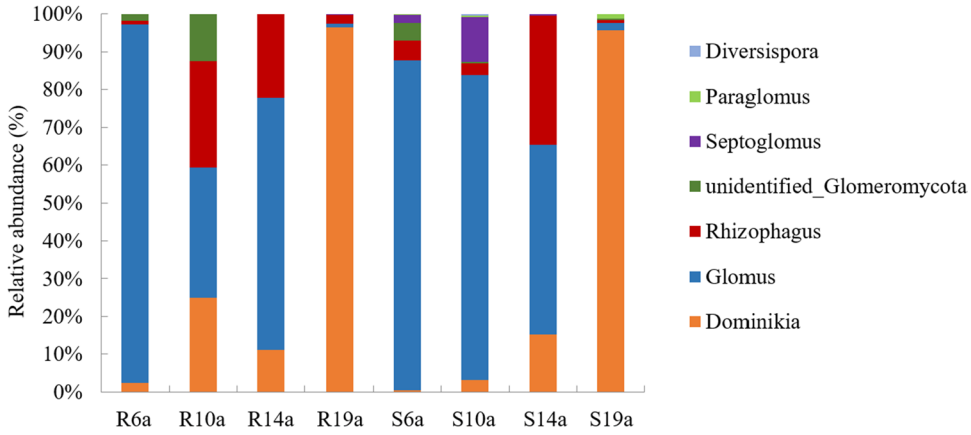

Fig. 5 Genera distribution of AMF communities in the roots and rhizosphere soil of *V. zizanioides* by planting age

Table 3 Diversity index of root and rhizosphere soil AMF in different planting years

Sites (years)		Shanon index	Simpson index	Chaol index	ACE index	Coverage index
Soils	6	6.51 ± 0.74a	0.96 ± 0.03a	564.38 ± 51.20a	561.25 ± 63.78a	0.98 ± 0.00a
	10	5.91 ± 0.83a	0.91 ± 0.08a	539.32 ± 43.90a	540.28 ± 22.27ab	0.97 ± 0.01a
	14	6.16 ± 0.31a	0.95 ± 0.02a	481.5 ± 67.17ab	470.14 ± 54.90bc	0.98 ± 0.02a
	17	5.53 ± 0.50a	0.93 ± 0.03a	425.09 ± 54.24b	416.58 ± 27.65c	0.98 ± 0.02a
Roots	6	4.03 ± 1.37a	0.85 ± 0.12a	223.78 ± 25.57a	237.60 ± 16.82a	0.99 ± 0.03a
	10	3.23 ± 1.79a	0.66 ± 0.30a	234.49 ± 138.95a	262.24 ± 141.54a	0.99 ± 0.02a
	14	3.72 ± 0.33a	0.83 ± 0.36a	221.18 ± 47.66a	237.86 ± 64.70a	0.99 ± 0.01a
	17	4.23 ± 0.18a	0.88 ± 0.35a	254.95 ± 18.76a	263.25 ± 16.07a	0.99 ± 0.00a

Different lowercase letters are significantly different ($P < 0.05$)

3.5 NMDS analysis of AMF communities in roots and rhizosphere soil of *V. zizanioides* of different planting years

PERMANOVA analysis revealed that the AMF communities in the roots and rhizosphere soil of *V. zizanioides* were significantly different ($P < 0.01$), but that there were no significant differences among planting years ($P > 0.05$). NMDS analysis demonstrated that the AMF communities in the roots and rhizosphere soil varied along the first axis from the 6- to the 17-year-old sample. For samples of different planting years, compositions of the AMF communities in the roots and rhizosphere soil were different, and these communities were distributed in different locations (Fig. 6).

3.6 Diversity of AMF communities and physicochemical properties of soils

Spearman's correlation analysis (Table 4) indicated that the Simpson index of the rhizosphere soil of *V. zizanioides* was significantly positively related to the TP content ($P < 0.05$). The Chao1 and ACE indices were significantly positively related to the AP content ($P < 0.05$). The Shannon and Simpson indices were significantly negatively related to the pH value of soil ($P < 0.05$). The four diversity indices of the roots were negatively related to the OM and TN contents and positively related to TP and TK contents.

Due to the different structures of the AMF communities in the rhizosphere soil and roots of *V. zizanioides* (Fig. 5),

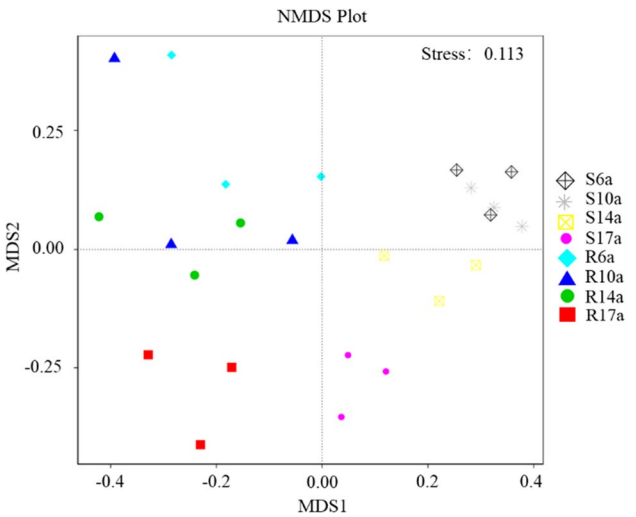

Fig. 6 NMDS analysis of AMF communities in the roots and rhizosphere soil of *V. zizanioides* of different planting years

Table 4 Spearman's correlation analysis of the AMF diversity indices and physicochemical factors of soil

Diversity	Index	OM	TN	TP	TK	AP	AK	PH
Soil	Shannon	0.217	0.021	0.538	−0.483	0.249	−0.231	−0.706*
	Simpson	0.119	0.088	0.602*	−0.560	0.128	−0.259	−0.609*
	Chao1	0.336	0.091	0.441	−0.098	0.628*	−0.105	−0.790**
	ACE	0.301	0.084	0.406	−0.056	0.653*	0.000	−0.811**
Root	Shannon	−0.406	−0.350	0.049	0.189	−0.126	−0.245	0.203
	Simpson	−0.413	−0.343	0.112	0.224	0.007	−0.224	0.035
	Chao1	−0.294	−0.252	0.210	0.224	0.189	−0.287	−0.028
	ACE	−0.364	−0.308	0.105	0.308	0.140	−0.182	0.098

*=significant correlation at the $P<0.05$ level; **=significant correlation at the $P<0.01$ level

the correlations between the AMF and the physicochemical properties of the soil were investigated by RDA (Fig. 7). As presented in Fig. 7a, the cumulative interpretation rate for both axes was 61.08%. The first axis (RDA1) had an interpretation rate of 34.95% and was significantly positively related to the TP, AP, OM, and TN. The second axis (RDA2) had an interpretation rate of 26.13% and was significantly negatively related to the OM, TN, and TP content. The soil's pH value, AP, and OM were found to have the most significant effects on the diversity of AMF communities in the rhizosphere soil. As shown in Fig. 7b, the accumulated interpretation rate of the two axes was 78.72%. The first axis (RDA1) had an interpretation rate of 45.54% and was significantly positively related to the pH value of the soil. The second axis (RDA2) had an interpretation rate of 33.18% and was significantly negatively related to the TP content. The pH value of the soil, TP and AP contents were found to have the most significant effects on the diversity of AMF communities in the roots.

4 Discussion

In this study, the structures of AMF communities in the roots and rhizosphere soil of *V. zizanioides* of different planting years (6-, 10-, 14-, and 17-year-old) in coal gangue heaps were investigated using high-throughput sequencing. A total of 178 AMF OTUs of *Dominikia*, *Diversispora*, *Glomus, Paraglomus*, *Rhizophagus*, *Septoglomus*, *Rhizophagus*, and *unidentified_Glomeromycota* were obtained. In the 6-, 10-, and 14-year-old roots and rhizosphere soil samples of *V. zizanioides*, *Glomus* was dominant with contents between 34.37% and 94.95%. Yang et al. (2015) showed that *Glomus* was the dominant genus in the *Robinia pseudoacacia* AMF communities in the roots and rhizosphere soil of in a lead-zinc mine. Mehrotra (1998) investigated AMF communities in an Indian coal mine via morphological methods and claimed that the *Glomus* genus was dominant. The present study further demonstrated that the *Glomus* genus was widely observed in coal mines in the Karst areas

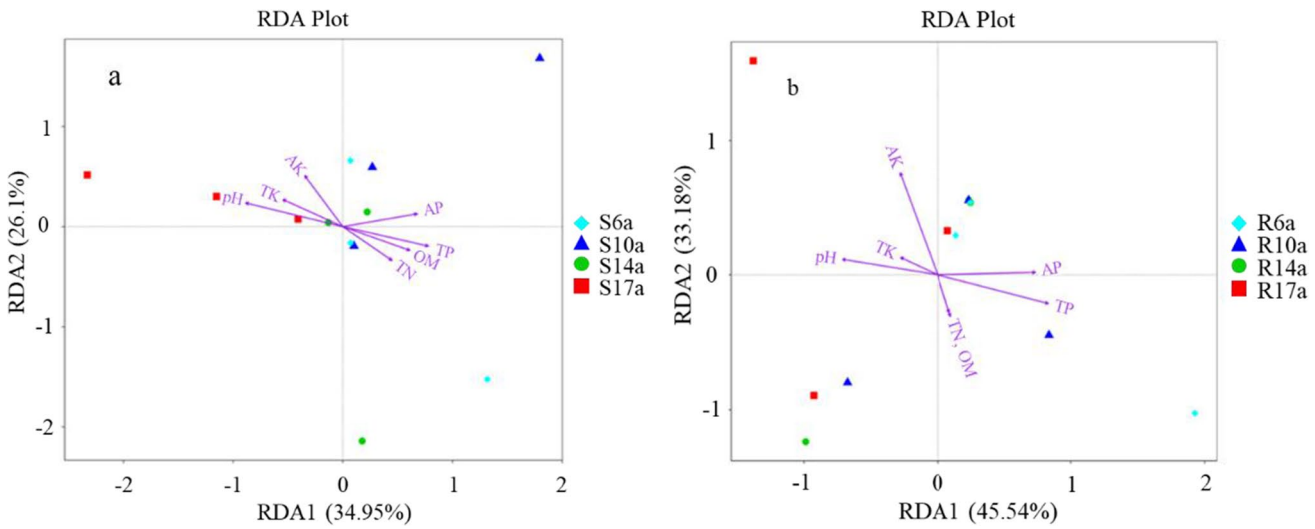

Fig. 7 Correlations between the AMF communities in the (a) rhizosphere soil and (b) roots of *V. zizanioides* and the physicochemical properties of soil as determined by RDA

in China, suggesting that it has strong adaptability in coal gangue heaps. Indeed, this genus can reproduce directly through hypha and mycorrhiza and has resistance to various adverse environments (Hassan et al., 2011). Compared with other AMF species, the reproduction strategy of *Glomus* is more suitable for survival in coal gangue heaps. Interesting finding in our analyses was that the number of *Dominikia* genus increases with planting age and became dominant genus in the roots and rhizosphere soil of *V. zizanioides* of the age of 17-year-old. Colombo et al. (2020) studied arbuscular mycorrhizal fungi in soils heavily contaminated with heavy metals in the Riachuelo River basin and found that *Dominikia* genus is one of the most representative arbuscular mycorrhizal fungi with a relative abundance of 26.5%. *Dominikia* genus may be an AMF with strong tolerance to heavy metals. Therefore, the reproduction pattern of the *Dominikia* genus in coal gangues and its effect on *V. zizanioides* requires further investigation. The changes of relative abundance between roots and rhizosphere soil in different planting years demonstrated that the sample age had a significant effect on the dominant genus of AMF communities the in roots and rhizosphere soil of *V. zizanioides*. However, Herrmann et al. (2016) claimed that the sample age has negligible effects on the dominant genus of AMF in the soil of a rubber tree plantation in Thailand. These different conclusions may be attributed to multiple factors such as the type of host plant, the physicochemical properties of the soils, and the climate.

In general, AMF communities were generally not characteristic of individual plant species, but those associated with ecological groups of plant species – habitat generalists and forest specialists – were nonrandom subsets of the available pool of fungal taxa (Davison et al., 2011). In this study, the OTU quantity of the rhizosphere soil was higher than the roots, and the AMF communities' compositions in the roots and rhizosphere soil were significantly different, demonstrating that AMF colonizing the roots of *V. zizanioides* may be a subgroup of AMF communities in rhizosphere soil. This is consistent with previous studies (Sheng et al., 2017), although other researchers have claimed that the diversity of AMF communities in rhizosphere soil is no greater than that of AMF communities in roots (Verbruggen et al., 2012; Saks et al., 2014; Deepika and Kothamasi, 2015). This may be attributed to three reasons. First, the identification of AMF communities in the roots of *V. zizanioides* has excluded dormant spore propagules, extra rhizomes, and dead mycorrhiza segments (Liu et al., 2015). Secondly, host plants prefer some AMF genera (De Souza and Santos, 2018). The *V. zizanioides* in this study may have preferred AMF genera in the rhizosphere soil, resulting in a low diversity of AMF communities in the roots. Finally, the relative abundances of the internal or external structures of AMF groups are different, and spore generation is seasonal (Bever et al., 2001; Jansa et al., 2002).

The time scale and physicochemical properties of soils are the dominant factors that affect the structures of AMF communities (Helgason et al., 2014; Alguacil et al., 2015). Cui et al. (2016) reported that the vegetation succession period affects the diversity and abundancy indices of AMF. In this study, the AMF diversity index in the *V. zizanioides* rhizosphere soil decreased, while in the roots increased as with planting years, demonstrating that the AMF diversity is affected by the planting years. The Simpson index of the rhizosphere soil was significantly positively related to the TP content and significantly negatively related to the pH value of soil. Chao1 and ACE indices were significantly positively related to the AP content, suggesting that the pH value of soil, TP and AP contents have significant effects on the structures of AMF communities in the rhizosphere soil of *V. zizanioides*. Previous studies have revealed that a high P content and low pH value of soil would inhibit spore germination and mycelial growth, thus hindering AMF growth and development. Hence, these factors directly or indirectly affect the construction of AMF communities (Bever et al., 2001; Hart et al., 2001). Additionally, heavy metal content is also a key factor that affects the structures of AMF communities (Faggioli et al., 2019). Our previous study revealed that Cu and Zn were the main heavy metals in the coal gangue heap. Yang et al. (2015) believed that Zn was the dominant factor that affects the structure of AMF communities in the roots and rhizosphere soil of *R. pseudoacacia*. Therefore, to fully understand the relationship between the local environment of coal gangue heaps and AMF communities, the relationship between the heavy metal content and diversity of AMF communities in coal gangues will be investigated in future research. RDA also demonstrated that the available phosphorus in the soil and the pH value of soil have the most significant effects on the diversity of AMF communities in the rhizosphere soil and roots. Be Enhouwer et al. (2015) studied AMF communities in soils under Arabica coffee trees in Ethiopia and demonstrated that the soil's AP in the soil and the pH value significantly affect AMF communities' diversity.

In summary, this study discussed the structures and diversity of AMF communities in the roots and rhizosphere soil of *V. zizanioides* in coal gangue heaps in Liupanshui, China. The results demonstrated that the diversity of AMF communities in rhizosphere soil was superior to that in the roots. *Glomus* and *Dominikia* were found to be the dominant genera of AMF communities in the rhizosphere soil and roots of *V. zizanioides* in coal gangue heaps, and the available phosphorus and pH value of the soil were found to be the dominant factors that affect AMF communities in the rhizosphere soil and roots. Additionally, the heavy metal content and activity

of microorganisms and enzymes in coal gangues affect AMF communities. Future studies will investigate these factors, as will the reproduction pattern of identified *Dominikia* in coal gangue heaps.

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