J Arid Land (2019) 11(1): 135–147
https://doi.org/10.1007/s40333-018-0019-9

Science Press Springer-Verlag

Arbuscular mycorrhizal fungi ameliorate the chemical properties and enzyme activities of rhizosphere soil in reclaimed mining subsidence in northwestern China

QIU Lang[1,2], BI Yinli[1,2*], JIANG Bin[1,2], WANG Zhigang[1,2], ZHANG Yanxu[1,2], Yryszhan ZHAKYPBEK[3]

[1] State Key Laboratory of Coal Resources and Safe Mining, China University of Mining and Technology (Beijing), Beijing 100083, China;
[2] College of Geoscience and Surveying Engineering, China University of Mining and Technology (Beijing), Beijing 100083, China;
[3] Satbayev University, Almaty 050013, Kazakhstan

Abstract: In semi-arid region of northwestern China, underground mining subsidence often results in decreased vegetation coverage, impoverishment of soil fertility and water stress. In addition, the physical-chemical and biological properties of soil also change, resulting in more susceptible to degradation. In particular, subsidence causes disturbance of the symbioses of plant and microbe that can play a beneficial role in the establishment of vegetation communities in degraded ecosystems. The objective of this study was to evaluate the effects of revegetation with exotic arbuscular mycorrhizal fungi (AMF) inoculum on the chemical and biological properties of soil over time in mining subsidence areas. Soils were sampled at a depth up to 30 cm in the adjacent rhizosphere of *Amorpha fruticose* Linn. from five reclaimed vegetation communities in northwestern China. In August 2015, a field trial was set up with five historical revegetation experiments established in 2008 (7-year), 2011 (4-year), 2012 (3-year), 2013 (2-year) and 2014 (1-year), respectively. Each reclamation experiment included two treatments, i.e., revegetation with exotic AMF inoculum (AMF) and non-AMF inoculum (the control). Root mycorrhizal colonization, glomalin-related soil protein (GRSP), soil organic carbon (SOC), soil nutrients, and enzyme activities were also assessed. The results showed that mycorrhizal colonization of inoculated plants increased by 33.3%–163.0% compared to that of non-inoculated plants (P<0.05). Revegetation with exotic AMF inoculum also significantly improved total GRSR (T-GRSP) and easily extracted GRSP (EE-GRSP) concentrations compared to control, besides the T-GRSP in 1-year experiment and the EE-GRSP in 2-year experiment. A significant increase in SOC content was only observed in 7-year AMF reclaimed soils compared to non-AMF reclaimed soils. Soil total N (TN), Olsen phosphorus (P) and available potassium (K) were significantly higher in inoculated soil after 1–7 years of reclamation (except for individual cases), and increased with reclamation time (besides soil Olsen P). The exotic AMF inoculum markedly increased the average soil invertase, catalase, urease and alkaline phosphatase by 23.8%, 21.3%, 18.8% and 8.6%, respectively (P<0.01), compared with the control. Root mycorrhizal colonization was positively correlated with soil parameters (SOC, TN and soil available K) and soil enzyme activities (soil invertase, catalase, urease and alkaline phosphatase) in both AMF and non-AMF reclaimed soils (P<0.05), excluding available

*Corresponding author: BI Yinli (E-mail: ylbi88@126.com)
The first and second authors contributed equally to this work.
Received 2017-08-01; revised 2018-05-11; accepted 2018-06-05

K in non-AMF reclaimed soils. T-GRSP (P<0.01) and EE-GRSP (P<0.05) were significantly correlated with the majority of edaphic factors, except for soil Olsen P. The positive correlation between root mycorrhizal colonization and available K was observed in AMF reclaimed soils, indicating that the AMF reclaimed soil with a high root mycorrhizal colonization could potentially accumulate available K in soils. Our findings concluded that revegetation with exotic AMF inoculum influenced soil nutrient availability and enzyme activities in the semi-arid ecosystem, suggesting that inoculating AMF can be an effective method to improve soil fertility and support restoration of vegetation communities under poor conditions like soil nutrient deficiency and drought.

Keywords: revegetation; mycorrhizal colonization; glomalin-related soil proteins; arbuscular mycorrhizal fungi; coal mining; *Amorpha fruticose*

Citation: QIU Lang, BI Yinli, JIANG Bin, WANG Zhigang, ZHANG Yanxu, Yryszhan ZHAKYPBEK. 2019. Arbuscular mycorrhizal fungi ameliorate the chemical properties and enzyme activity of rhizosphere soil in reclaimed mining subsidence in northwestern China. Journal of Arid Land, 11(1): 135–147. https://doi.org/10.1007/s40333-018-0019-9

1 Introduction

Although coal mining has contributed greatly to China's economy, it causes land subsidence that results in eco-environmental problems, such as disruption of hydrologic regimes, loss of topsoil, and degradation of landscape (Bell et al., 2000; Sidle et al., 2000; Yang et al., 2016). These serious impacts have threatened plants survival and the sustainability of natural vegetation communities, accompanying by the loss of physical-chemical and biological properties of soil (soil nutrient availability, organic matter content, and/or microbial community) (Shrestha and Lal, 2011; Li et al., 2014). In general, the physical-chemical and biological properties of soil determine soil quality and fertility, and soil degradation inhibits the potential for vegetation reestablishment.

The Shendong coalfield, located at the border of Shanxi, Shaanxi and Inner Mongolia, is currently the largest coal reserves in China (Lei et al., 2010). Subsidence in this coalfield has become a longstanding environmental issue since high-intensity exploitation began in the mid-1980s, and has led to a very fragile and degraded ecosystem, which is susceptible to both anthropogenic activities and climatic conditions in semi-arid region (Zhang et al., 2012). Moreover, there are problems of surface soil loose, groundwater level recession and soil nutrient loss, which exacerbate the soil degradation (Cheng et al., 2007; Lei et al., 2010). In particular, this process also inhibits the formation of mycorrhizal roots and its hyphal network in soils, resulting in a reduction of mycorrhizal propagules, which further attenuates the symbiotic relationship between plants and arbuscular mycorrhizal fungi (AMF).

AMF are the most widespread mutualistic symbionts formed between plants roots and the surrounding soils. The fungal mycelium constructs a huge mycelium network through extending from the mycorrhizal roots (Rillig and Steinberg, 2002). The network system can enhance water and the mineral nutrition absorption (particularly phosphorus) under stressed conditions (nutrient deficiency, drought or degraded soils) (Cumming and Ning, 2003; Taheri and Bever, 2010; Lazcano et al., 2014). The mycelium also provides a bonding mechanism to the formation and stabilization of soil aggregates, thus contributing to soil quality (Zhang et al., 2014). There is considerable evidence showing that AMF have the potential to increase the tolerance of host plants against biotic and abiotic stresses. Arbuscular mycorrhizal (AM) symbiosis can alleviate the drought stress symptoms of host plants via altering the rates of water uptake and transport from AM (arbuscular mycorrhizal) hyphae to their host plants' roots (Marulanda et al., 2003), thereby regulating root hydraulic properties (e.g., root hydraulic conductivity) (Bárzana et al., 2014), accumulating more solutes involved in osmotic adjustment (Aroca et al., 2007; Bheemareddy and Lakshman, 2011), and enhancing the gas exchange of plant (Habibzadeh et al., 2013). AM symbiosis can plays a crucial role in plant development by improving nutrient uptake through N fixation (Sanâa et al., 2016), phosphorus acquisition (Liu et al., 2014), and contributing to the formation of water-stable soil aggregates (Rillig, 2004), especially when facing

nutrient-poor soils. In addition, AM symbiosis can increase carbon fixation of plants and the carbon inputs to soil through mycorrhizal fungi thereby enhancing ecosystem carbon storage (Orwin et al., 2011). Moreover, the activity and diversity of AMF can affect plant community composition, structure and dynamics to some extent (Barea et al., 2011). Therefore, AMF are considered critical for the stability of vegetation community in arid or semi-arid ecosystems (Zhang et al., 2012), including prairie, coal mine spoiled soils or subsided soils (White et al., 2008; Li et al., 2015; Zhao et al., 2015). Loss of mycorrhizal propagules in degraded soil can block the revegetation process in semi-arid environment, while the addition of exotic AMF agents may help plants establishment in these situations (Requena et al., 2001; Veresoglou et al., 2012; Bi et al., 2014). Thereby, revegetation with AMF agents may be beneficial for restoring vegetation communities and ameliorating soil properties in degraded ecosystem.

Once revegetation has been successfully established, it is necessary to investigate whether the changes of rhizosphere soil properties are associated with the inoculation of exotic AMF agents. The chemical properties and enzyme activities of soil are often considered as sensitive indicators for soil quality, which reflect the status of soil nutrient cycling (Caravaca et al., 2003; Pereira et al., 2008). However, there are few reports studying the changes of soil chemical and biological parameters during revegetation associated with both AMF inoculation and reclamation time in the mining subsidence areas. Hence, our study aims to evaluate the inoculation effects of exotic AMF agents on chemical properties and enzyme activities of rhizosphere soil in subsided land in northwestern China, in order to identify the significance of long-term effect of AMF inoculation on chemical and biological properties of the degraded soil.

2 Materials and methods

2.1 Study site and field experimental design

This study was carried out on reclaimed coal mining subsidence located in Shendong coal mining sites, Daliuda town, Shenmu County, Shaanxi Province of China (39°14′–39°17′N, 110°10′–110°16′E). The climate in the study area belongs to arid and semi-arid continental monsoon climate, with an approximate altitude of 1200 m a.s.l. The annual mean temperature is 8.9°C and annual mean sunshine time is 2876.0 h. According to local meteorological data, the mean annual precipitation is about 422.7 mm, which mainly occurs from June to September, whereas the mean annual evaporation is 2211.2 mm. Soil type is classified as aeolian sandy, and the soil characteristics are as follows: soil organic carbon (SOC) 3.65 g/kg, total nitrogen (TN) 0.39 g/kg, Olsen phosphorus (P) 2.81 mg/kg, available potassium (K) 45.80 mg/kg, maximum water-holding capacity 20.10% and pH value 8.51.

Land reclamation and ecological reconstruction were studied for ten years in this region, such as artificial revegetation, land reclamation and improvement of soil fertility (Bi et al., 2003; Yu et al., 2013; Li et al., 2015; Wang et al., 2016). Ten pre-existent trails in the years of 2008 (7-year), 2011 (4-year), 2012 (3-year), 2013 (2-year) and 2014 (1-year) at Daliuta Mine were revegetated with *Amorpha fruticose* Linn. *A. fruticose* is one of the drought-tolerant legumes and widely cultivated for sand-fixation on the semi-arid sand land in Northwest China (Qi et al., 2015). It has been shown to be highly dependent on arbuscular mycorrhizal microsymbionts. Two plots were set up in each reclamation year: AMF inoculation treatment (AMF) and control (CK). The inoculated plot received 50 g of inoculum per seedling at the roots area, while the control plot was added with equivalent sterilized AMF inoculum. The introduced AMF inoculum, *Funneliformis mosseae* BGCXJ01, was provided by Beijing Academy of Agriculture and Forestry Sciences, and cultivated with maize for three months in sterile sand, which consisted of spores (262 spores per 10 g soil), external mycelium, and infected root fragments (90% root mycorrhizal colonization). Each plot had an area of 60 m×100 m and was mainly covered by pure *A. fruticose*, except for a few native shrubs and grasses, such as *Artemisia ordosica*, *Hedysarum scoparium*, *Stipa grandis*, and *Salix psammophila*, etc. (Chen et al., 2002). *A. fruticose* seedlings with a height of 30–40 cm were obtained from local garden nursery base. Seedlings were annually transplanted at a density

of 2 m×2 m into the plots in May. Several times of irrigation were applied as required at initial growth stage, and no further management was provided.

2.2 Sampling

Soil samples were collected from ten plots in August, 2015. Six individual subplots (20 m×10 m) in each plot were selected for replicates. Rhizosphere soils and fine roots from ten *A. fruticose* plants at each subplot with an S-shaped pattern were collected as sub-samples and then they were mixed to form a pooled sample. We used a wide shovel to collect soils surrounding the roots in 0–40 cm soil profile. A total of sixty soil samples with each ca. 500 g were placed in sterilized plastic bags and then taken back to laboratory in ice cooled refrigerator. The plant residues or other visible impurities were removed from the soil samples, which were then divided into two parts and sieved with a 2-mm mesh. One part was air-dried and used for the measurement of soil chemical properties and mycorrhizal characteristics, and the other part was stored at 4°C for assessment of soil enzyme activities, i.e., soil invertase, catalase, urease, and alkaline phosphatase.

2.3 Mycorrhizal characteristic analyses

Fine roots were cut into 1.5-cm-long segments and then cleared in 10% KOH, stained with 0.05% w/v trypan blue for 24 h, and distained in acid glycerol (Phillips and Hayman, 1970). Root mycorrhizal colonization was measured by the glass slide method, in which two groups of 15 randomly selected root segments in each plot (n=180) were examined microscopically according to Giovannetti and Mosse (1980). The percentage of root mycorrhizal colonization was calculated by dividing the number of colonized roots by the total number of examined root samples. We measured glomalin-related soil protein (GRSP), including total GRSP (T-GRSP) and easily extracted GRSP (EE-GRSP), based on the method of Wright and Upadhyaya (1996). Briefly, EE-GRSP was extracted with citric alkaline (20 mM, pH 7.0) by autoclaving the samples for 30 min (121°C), followed by centrifuging at 10,000 *g* for 5 min and collecting the supernatant. For T-GRSP, the same soil sample was extracted with 50 mM citric alkaline at pH 8.0 and centrifuged at 10,000 *g* for 5 min, followed by four cycles of extraction and centrifugation until the supernatant was almost transparent. We described GRSP concentration as the protein content per gram of dried soil based on Bradford assay. Bovine serum albumin (BSA) was used as a standard.

2.4 Analyses of soil chemical properties and enzyme activities

SOC was analyzed by dichromate-sulphuric acid oxidation with heating. TN was measured using the semi-micro Kjeldahl method. Olsen P was determined by the sodium bicarbonate-extractable P colorimetric method. Soil available K was extracted with 1 mol/L ammonium acetate (pH 7.0) and determined using ICP-OES (Optima5300 DV, Perkin Elmer, Norwalk, CT, USA) (Bao, 1998).

The activity of soil invertase, catalase, urease, and alkaline phosphatase was determined according to Guan (1996). Soil invertase activity was measured by the method of 3, 5-dinitrosalicylic alkaline colorimetry. Soil catalase activity was determined by $KMnO_4$ titration method. Soil urease activity was measured using phenol sodium hypochlorite colorimetry. Soil alkaline phosphatase activity was detected by the method of p-nitrophenyl sodium dihydrogen phosphate colorimetry.

2.5 Data analysis

All data were statistically analyzed using SAS software (8.0). Two-way ANOVA was conducted to evaluate the effects of AMF inoculation and reclamation time by the Least Significant Difference (LSD) test at 5% significant level. Excel 2010 was used to process the data and generate figures.

3 Results

3.1 Mycorrhizal characteristics

Mycorrhizal colonization of *A. fruticose* roots in all plots was found to be infected due to the presence of indigenous AMF or introduced AMF agents, but the mycorrhizal plants showed significantly higher root mycorrhizal colonization than the control plants by 33.3% to 163.0% (P<0.05; Fig. 1a). Root mycorrhizal colonization in the inoculated group significantly increased with reclamation time, ranging from 32.2% to 63.3%, whereas the colonization in controls ranged

from 12.2% to 44.4%. T-GRSP and EE-GRSP concentrations were significantly higher in inoculated soils than in the control soils, besides the T-GRSP in 1-year reclamation experiment and the EE-GRSP in 2-year reclamation experiment (P<0.05; Figs. 1b and c). Among all inoculated treatments, there was a positive correlation between T-GRSP concentration and reclamation time, except for the 4-year reclamation. In contrast, EE-GRSP concentration reached peak in 3-year reclamation, but was not obviously different from that in 7-year reclamation. Similarly, the same trends of T-GRSP and EE-GRSP were observed in non-inoculated groups. The two-way ANOVA analysis showed that root mycorrhizal colonization, T-GRSP and EE-GRSP were significantly influenced by AMF inoculation and reclamation time (P<0.001; Table 1). In addition, T-GRSP and EE-GRSP concentrations were also significantly influenced by the interaction of AMF inoculation and reclamation time (P<0.05).

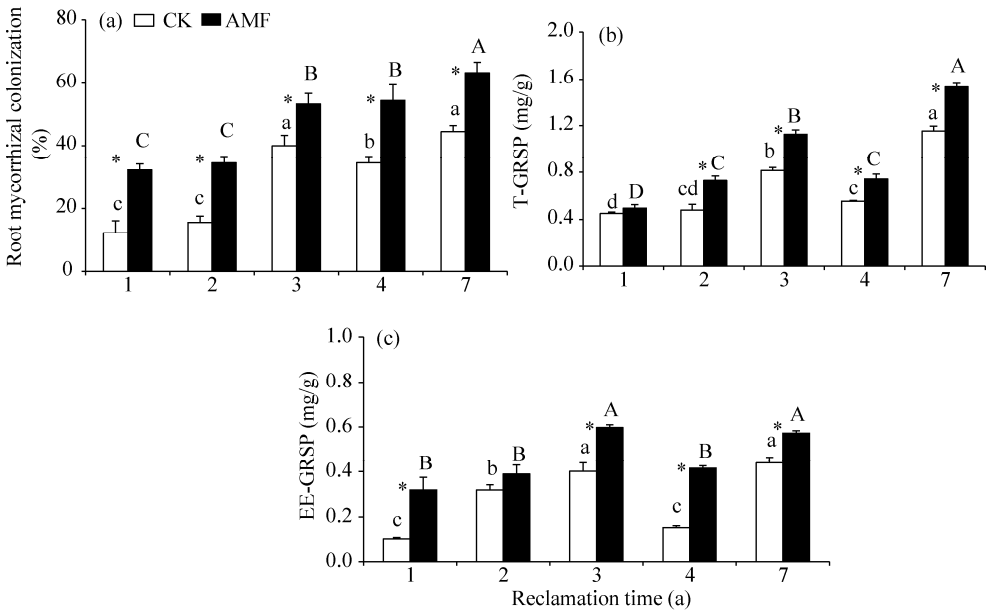

Fig. 1 Mycorrhizal characteristics of rhizosphere soil of *A. fruticose* under five reclamation times and two treatments. (a) root mycorrhizal colonization; (b) total glomalin-related soil protein (T-GRSP); (c) easily extractable glomalin-related soil protein (EE-GRSP). CK, control; AMF, arbuscular mycorrhizal fungi. Bars indicate standard errors (n=6). Different lowercase letters (among five CK treatments) or capital letters (among five AMF inoculation treatments) indicate significant differences (P<0.05) as determined by LSD test. * indicates significant differences between AMF inoculation and control at the same reclamation time.

3.2 Soil chemical properties

Compared to non-inoculated plants, SOC content was higher in the inoculated soils in the same reclamation year. However, no significant differences were observed between inoculated soils and controls, besides the 7-year reclamation experiment (P<0.05; Fig. 2a). A significant increase in SOC content was only observed in older AMF reclaimed soils at 7-year compared to non-AMF reclaimed soils, of which the increase of SOC content was more significant than those of newly AMF reclaimed soils. Contrary to SOC, TN content in soils was significantly higher in inoculated soils than in control soils, except for 4-year reclamation. Among all treatments, the highest contents of SOC and TN were found both in inoculated soils in 7-year reclamation with the maximum values of 8.45 and 0.50 g/kg, respectively (Figs. 2a and b).

Soil Olsen P and available K contents were also notably affected by the addition of AMF inoculation after 2 to 7 years of reclamation (Figs. 2c and d). Soil Olsen P under AMF inoculation ranged from 1.41 to 2.89 mg/kg, and was significantly higher than that of control soils besides the 1-year reclamation. Interestingly, the highest Olsen P appeared in inoculated soil at 2-year reclamation time, and did not increase further with longer reclamation time. Soil available K was significantly higher in inoculated soil for 7-year reclamation than any new reclamation time in either AMF inoculated or non-AMF inoculated soils (P<0.05; Fig. 2d). In addition, the averaged

SOC, TN, soil Olsen P and available K in inoculated soils were significantly increased by 46.2%, 41.2%, 43.3%, and 31.6% compared to CK, respectively. Moreover, these soil factors showed a positive correlation with reclamation time since their maximum values were found in 7-year reclaimed soil, regardless of whether the plants were AMF inoculated or not, except for soil Olsen P. In addition, AMF inoculation, reclamation time and their interaction had significant effects on the contents of SOC, TN, Olsen P, and available K (Table 1).

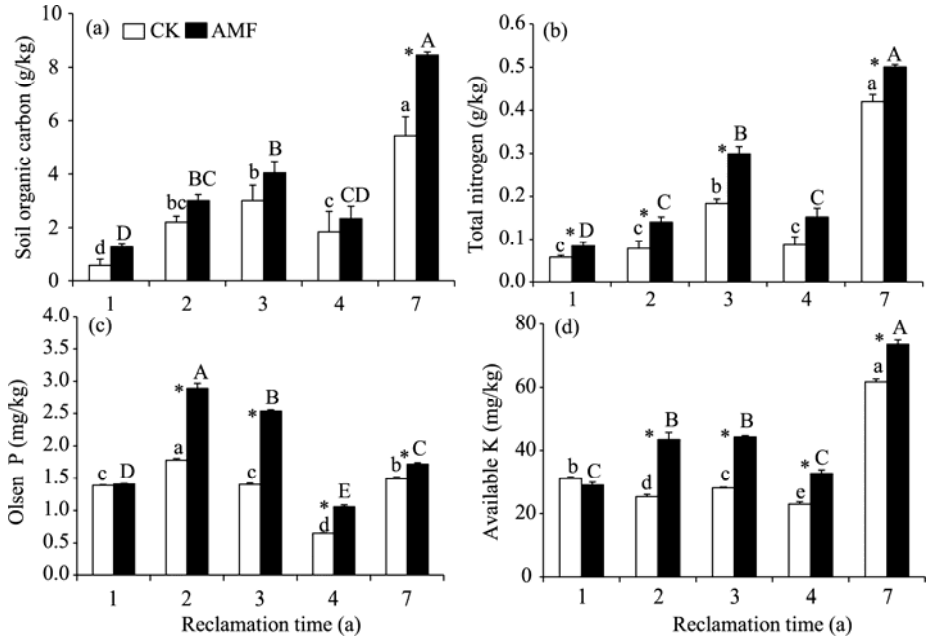

Fig. 2 Chemical properties of rhizosphere soil of *A. fruticose* under five reclamation times and two treatments. (a) soil organic carbon; (b) total nitrogen; (c) Olsen P; (d) available K. CK, control; AMF, arbuscular mycorrhizal fungi. Bars indicate standard errors (n=6). Different lowercase letters (among five CK treatments) or capital letters (among five AMF inoculation treatments) indicate significant differences (P<0.05) as determined by LSD test. * indicates significant differences between AMF inoculation and the control at the same reclamation time.

Table 1 *F*-values of two-way ANOVA analysis on the impacts of AMF inoculation and reclamation time on mycorrhizal characteristics, soil chemical properties and enzyme activities under the canopy of *A. fruticose* in Shendong coalfield, China

Dependent variable	Reclamation ti(me T)	AMF inoculation (I)	T×I
Mycorrhizal colonization	126.46***	266.83***	1.25NS
T-GRSP	203.33***	114.43***	6.73*
EE-GRSP	45.63***	101.10***	3.72*
SOC	73.11***	25.58***	3.68*
TN	274.73***	64.30***	2.96*
Olsen P	618.24***	817.94***	129.80***
Available K	448.92***	244.68***	26.47***
Invertase	1252.31***	154.90***	65.17***
Catalase	324.87***	63.01***	14.17***
Urease	2435.88***	50.95***	13.78***
Alkaline phosphatase	201.51***	19.04**	1.70NS

Note: *, **, and *** indicate significances at P<0.05, P<0.01, and P<0.001 levels, respectively. NS represents no significance. T-GRSP and EE-GRSP represent total and easily extracted glomalin-related soil proteins, respectively. SOC, soil organic carbon; TN, total nitrogen; Olsen P, available phosphorus; K, potassium.

3.3 Soil enzyme activities

The addition of AMF inoculation increased soil invertase activities compared to controls, except for 1-year reclamation (Fig. 3a). However, no significant difference was observed for 7-year

reclamation between inoculated and non-inoculated soils. Catalase activities were significantly higher in inoculated soils for 1-, 4-, and 7-year of reclamation than those controls (P<0.05; Fig. 3b). Similarly, the inoculated soils reclaimed for 2-, 4-, and 7-year had significantly higher urease activities than controls (P<0.05; Fig. 3c), whereas inoculated soils with 3-year reclamation showed insignificantly lower catalase and urease activities compared to controls (Figs. 3b and c).

The inoculated soils with 7-year reclamation showed the highest alkaline phosphatase activities among all treatments, but not significantly compared to controls. There were significant differences in soil alkaline phosphatase activities between inoculation treatments and controls with 2- and 3-year reclamations (Fig. 3d). The averaged soil invertase, catalase, urease, and alkaline phosphatase activities across five reclamation years were 23.8%, 21.3%, 18.8%, and 8.6% in inoculated soils, respectively, which are significantly higher than controls. Overall, soil enzyme activities increased with reclamation time in both AMF reclaimed and non-AMF reclaimed soils, and reached the maximum values in inoculated soils at 7-year reclamation. AMF inoculation, reclamation time and their interaction had significant effects on soil invertase, catalase, urease, and alkaline phosphatase activities of *A. fruticose* (Table 1), except for the effect of their interaction on alkaline phosphatase.

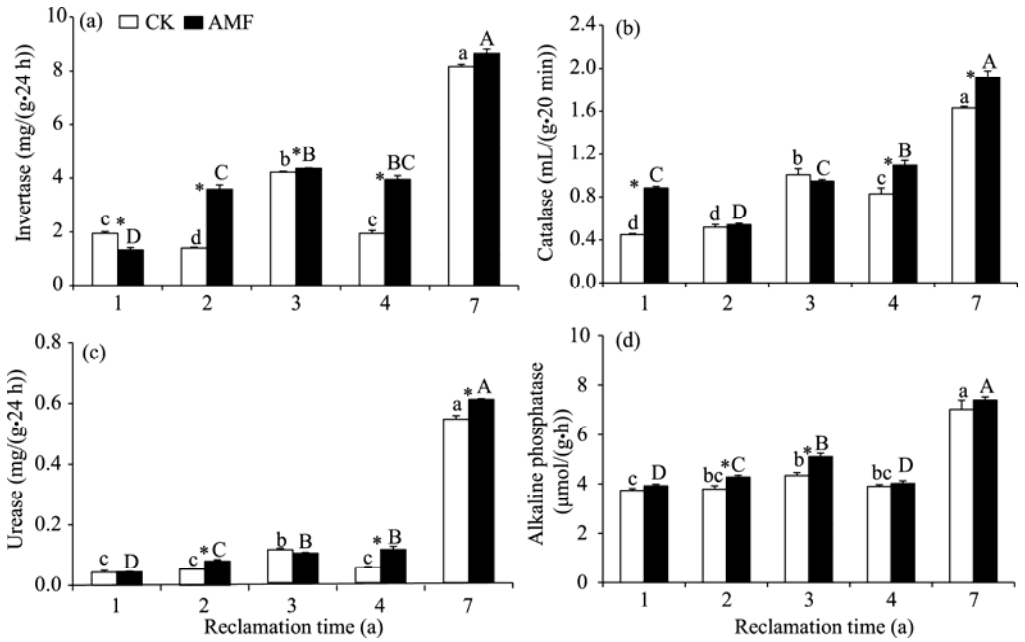

Fig. 3 Enzyme activities of rhizosphere soil of *A. fruticose* under five reclamation times and two treatments. (a) invertase; (b) catalase; (d) urease; (d) alkaline phosphatase. CK, control; AMF, arbuscular mycorrhizal fungi. Bars indicate standard errors (n=6). Different lowercase letters (among five CK treatments) or capital letters (among five AMF inoculation treatments) indicate significant differences (P<0.05) as determined by LSD test. * indicates significant differences between AMF inoculation and control at the same reclamation time.

3.4 Relationships among soil properties in AMF and non-AMF reclaimed mine soils

Correlation coefficient analysis showed that root mycorrhizal colonization had a positive correlation with most of the soil properties in both AMF and non-AMF reclaimed soils, besides soil Olsen P (Table 2). Similarly, both T-GRSP and EE-GRSP were positively correlated with SOC, TN, available K, invertase, catalase, urease, and alkaline phosphatase activities. In addition, a close correlation was observed between T-GRSP and EE-GRSP in either AMF or non-AMF reclaimed soils. However, root mycorrhizal colonization was positively correlated with available K in AMF reclaimed soils (R^2=0.641, P<0.01) but not in non-AMF reclaimed soils (R^2=0.500, P>0.05). SOC, TN, available K, and soil enzyme activities displayed a significantly positive

Table 2 Pearson's correlation coefficients for various parameters of AMF reclaimed and non-AMF reclaimed soils under the canopy of *Amorpha fruticose* in Shendong coal mining sites, China

	Mycorrhizal colonization	T-GRSP	EE-GRSP	SOC	TN	Olsen P	K_{avi}	Invertase	Catalase	Urease
AMF reclaimed soils										
T-GRSP	0.797**									
EE-GRSP	0.733**	0.827**								
SOC	0.725**	0.928**	0.682**							
TN	0.804**	0.971**	0.792**	0.944**						
Olsen P	–0.240	0.149	0.277	0.105	0.076					
K_{avi}	0.641**	0.923**	0.672**	0.946**	0.924**	0.212				
Invertase	0.827**	0.931**	0.690**	0.957**	0.940**	0.030	0.935**			
Catalase	0.798**	0.782**	0.530*	0.825**	0.839**	–0.433	0.764**	0.840**		
Urease	0.707**	0.867**	0.545*	0.927**	0.905**	–0.147	0.929**	0.934**	0.924**	
Alkaline phosphatase	0.710**	0.948**	0.705**	0.968**	0.970**	0.062	0.965**	0.934**	0.860**	0.952**
Non-AMF reclaimed soils										
T-GRSP	0.809**									
EE-GRSP	0.597*	0.770**								
SOC	0.753**	0.894**	0.786**							
TN	0.739**	0.969**	0.737**	0.915**						
Olsen P	–0.303	0.137	0.494	0.212	0.207					
K_{avi}	0.500	0.844**	0.546*	0.786**	0.919**	0.282				
Invertase	0.760**	0.973**	0.703**	0.875**	0.979**	0.177	0.921**			
Catalase	0.883**	0.951**	0.684**	0.914**	0.951**	–0.029	0.824**	0.949**		
Urease	0.649**	0.920**	0.638*	0.881**	0.971**	0.204	0.969**	0.961**	0.918**	
Alkaline phosphatase	0.655**	0.908**	0.647**	0.862**	0.969**	0.192	0.946**	0.949**	0.913**	0.975**

Note: * and ** indicate significances at $P<0.05$ and $P<0.01$ levels, respectively. T-GRSP and EE-GRSP represent total and easily extracted glomalin-related soil protein, respectively. K_{avi}, available phosphorus.

association with the other soil properties except for soil Olsen P. Interestingly, soil Olsen P was either not correlated or only weakly correlated with the other soil parameters in both AMF and non-AMF reclaimed soils.

4 Discussion

The results of 7-year trial supported the hypothesis that revegetation associated with AMF inoculation could improve the chemical and biological properties of soil during restoration. Mycorrhizal colonization rate reflects the ability of AMF to colonize host plant roots and is affected by various factors, such as AMF species, soil environment and human activities (Bai et al., 2009; Liu et al., 2014; Bonanomi et al., 2017). In this research, root mycorrhizal colonization of *A. fruticose* was significantly higher in AMF-inoculated treatments than in non-AMF inoculated treatments in each year, suggesting the exotic AMF ecotypes can well adapt to local sandy soil. Taheri and Bever (2011) found that AMF species or ecotypes originating from the mined area showed greater colonization ability in mine soil than in native clay soil, suggesting that specific adapted fungi can better promote plants under original environmental conditions. The nonindigenous AMF inoculum from laboratory collection was functionally compatible to the sandy soil in the study site, which makes it highly competitive for plant roots colonization. Furthermore, high mycorrhizal colonization during the revegetation period can contribute to more nutrient and water uptake from distant soil through AM hyphae and support the growth of *A. fruticose* in the lean and droughty environments (He et al., 2010).

GRSP, quantified as glomalin-related soil protein, is mainly produced by the hyphae and spores of AM fungi, and accumulates in soils with up to 7–42 years of turnover time (Rillig, 2004). This compound plays an important role in translocating carbon from plant roots to the soil. Previous studies have found a positive correlation between GRSP and SOC in soils from agricultural ecosystem (Zhang et al., 2014), pastures (Franzluebbers et al., 2000), or rangeland (Bird et al., 2002). Our study also demonstrated that SOC was highly correlated with T-GRSP and EE-GRSP across all treatments in degraded soils (R^2=0.962, P<0.01; R^2=0.764, P<0.05, respectively), indicating that GRSP was also important for C cycling and sequestration, especially in semi-arid ecosystems. In addition, T-GRSP and EE-GRSP contents in this study ranged from 0.10 to 1.15 mg/g, which was consistent with the study from Bai et al. (2009) on the rhizosphere soil of *Astragalus adsurgens* Pall. in Mu Us Sandy Land near our target area, suggesting that the soils in this subsided area are disturbed and heavily degraded.

Previous studies have shown that revegetation can increase the productivity of degraded or disturbed soil through developing the extensive root systems via soil stabilization, or stimulation of soil microbial activity, thus providing the nutrients and SOC accumulation to the soil (Conesa et al., 2007; Sheoran et al., 2010; Levy and Cumming, 2014). Moreover, the effect of revegetation with exotic AMF addition on improving chemical and biological properties of soil was more evident in this semi-arid region. Our results showed that there was a marked increase in SOC and available nutrient contents in AMF reclaimed soils. This finding was in accordance with other studies which have quantitatively addressed the AMF-mediated effects on degraded soil properties (Marschner and Baumann, 2003; Rillig and Mummey, 2006; Singh et al., 2008; Li et al., 2015). The increase of SOC content in 7-year AMF reclaimed soils was mainly due to the decomposed litter from fallen leaves and branches (Qi et al., 2015), but it had also been related to the extent of exotic AMF colonization to the root, because the majority of glomalin secreted by hyphae and spores was also a component of organic matter (Haddad and Sarkar, 2003). Meanwhile, vegetation inoculated with AMF tends to reduce its decomposition rate through increasing the nutrient limitation of saprotrophs, thereby promoting C accumulation in soil (Read et al., 2004). The increase of TN in the rhizosphere of shrub legume can be ascribed to an improvement in nodulation and nitrogen fixation capacity caused by AMF inoculation (Requena et al., 2001; Sanâa et al., 2016).

In addition, the symbiotic plants can produce more enzymes from the excess carbon by directly photosynthesizing carbohydrate (Orwin et al., 2011). The soil enzyme activities directly affected

the available nutrient content in the rhizosphere soil (He et al., 2010), which in turn provided soil microbes with more energy and nutrients to increase enzyme activities (Vázquez et al., 2000). This result indicates that AMF may indirectly influence the level of soil nutrients. Generally, invertase is involved in the metabolism of soil organic matter and catalyzes the production of glucose and fructose from sucrose. Catalase decomposes hydrogen peroxides into water and oxygen so as to inhibit the toxicity on plant organisms. Urease catalyzes NH_4^+-N released from urea and soil organic nitrogen, and alkaline phosphatase hydrolyzes both organic phosphorous esters and anhydrides of phosphoric acid into inorganic phosphorous. The positive correlations were also found between soil carbon, nitrogen, potassium, and soil invertase, catalase, urease, and alkaline phosphatase activities in the current study. However, soil Olsen P was not related to other soil parameters in this study, which is inconsistent with the previous studies that have shown AMF have a higher affinity for phosphate ions and facilitate of phosphorous uptake of host plants (Smith et al., 2000; Liu et al., 2014). This is possibly that the level of phosphorus content was notably low at reclaimed site and *A. fruticose* was in a rapid growth period with greater nutrients demand after 7 years of reclamation. But this needs further testing. Moreover, a positive correlation between root mycorrhizal colonization and available potassium was observed in AMF reclaimed soils ($P<0.01$), but not in non-AMF reclaimed soils, indicating that inoculated plants with high root mycorrhizal colonization have the potential to accumulate available potassium in the rhizosphere soil.

Furthermore, there were also significant improvements in soil chemical properties and enzyme activities of *A. fruticose* rhizosphere soil in all inoculation treatments over time, except for soil Olsen P. In general, improvement in physical-chemical and biological properties of soil in degraded land can relate to revegetation period (Xu et al., 2009; Zhao et al., 2013), vegetation species (Marcin and Maria, 2010) or soil type (Qi et al., 2015). In this study, an evident increase in AMF colonization during vegetation establishment was observed, which could have critical effects on plant development and improvements of nutrients and enzymatic activities of AMF-inoculated soil. Mycorrhizal colonization can differentially induce additional qualitatively or quantitatively changes in root exudates in rhizosphere soil, thus exerting an indirect effect on rhizosphere microbial populations (Vázquez et al., 2000). It is also possible that an increase in soil glomalin may have important consequences for individual plant species in the improvement of soil aggregation, thus contributing to the maintenance of good water infiltration rates and adequate aeration for plant development, which in turn improves soil quality (Requena et al., 2001). This is consistent with the study of Requena et al. (2001) who found that exotic AMF could contribute to plant survival and soil TN and organic matter content from a 5-year trial in desertified ecosystem of Mediterranean region. Additionally, there was an interesting finding that 7-year reclaimed soil had the highest alkaline phosphatase activity, while the corresponding Olsen P was low, which may be supported by the hypothesis that when P availability in soil is low and becomes a limiting factor, it will cause an overall increase in the activity of phosphatase secreted by roots (Azcón and Barea, 1998).

5 Conclusions

In this study, we showed that revegetation associated with exotic AMF inoculum could improve soil nutrient availability and enzyme activities in mining subsidence region under nutrient deficiency and water shortage conditions. The AMF inoculated plant showed higher root mycorrhizal colonization, T-GRSP and EE-GRSP concentrations as well as an increase in SOC, TN, Olsen P, available K and enzyme activities compared to control. A significant increase in SOC content was found in 7-year AMF reclaimed soils compared to non-AMF reclaimed soils. A positive correlation between root mycorrhizal colonization and available K was observed in AMF reclaimed soils, indicating that AMF reclaimed soil with high root mycorrhizal colonization had the potential to accumulate soil available K. Among the vegetation inoculated with exotic AMF, the older reclaimed soil displayed significantly higher levels of soil nutrients (except for Olsen P) and enzyme activities compared to the newly reclaimed soil. These results which tracked the

changes of soil chemical properties and enzyme activities with time led to a better understanding of the long-term effects of exotic AMF on mitigating soil erosion in restored ecosystem. Given the effort and expense of producing AMF, our results suggest that introducing AMF inoculation may be a recommended strategy to improve soil quality and support restoration of vegetation communities under soil nutrient deficiency and drought conditions in mining subsidence regions. Furthermore, selecting adapted strains of indigenous AMF that are compatible with *A. fruticose* or other indigenous plants may be able to acclimatize plants to the semi-arid and infertile environment in subsided soils.

Acknowledgements

The study was funded by the National Natural Science Foundation of China (51574253), the National High Technology Research and Development Program of China (2013AA102904), and the Open Research Project of the State Key Laboratory of Coal Resources and Safe Mining, China University of Mining and Technology (Beijing) (SKLCRSM16KFA01).

References

Aroca R, Porcel R, Ruiz-Lozano J M. 2007. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? New Phytologist, 173(4): 808–816.

Azcón R, Barea J. 1998. Mycorrhizal dependency of a representative plant species in Mediterranean shrublands (*Lavandula spica* L.) as a key factor to its use for revegetation strategies in desertification-threatened areas. Applied Soil Ecology, 7(1): 83–92.

Bai C M, He X L, Tang H L, et al. 2009. Spatial distribution of arbuscular mycorrhizal fungi, glomalin and soil enzymes under the canopy of *Astragalus adsurgens* Pall. in the Mu Us sandland, China. Soil Biology & Biochemistry, 41(5): 941–947.

Bao S D. 1998. Soil Agricultural and Chemical Analysis. Beijing: China Agriculture Press, 56–83. (in Chinese)

Barea J M, Palenzuela J, Cornejo P, et al. 2012. Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain. Journal of Arid Environments, 75(12): 1292–1301.

Bárzana G, Aroca R, Bienert G P, et al. 2014. New insights into the regulation of aquaporins by the arbuscular mycorrhizal symbiosis in maize plants under drought stress and possible implications for plant performance. Molecular Plant-microbe Interactions, 27(4): 349–363.

Bell F G, Stacey T R, Genske D D. 2000. Mining subsidence and its effect on the environment some differing examples. Environmental Geology, 40(1–2): 135–152.

Bheemareddy V S, Lakshman H C. 2011. Effect of AM fungus *Glomus fasciculatum* on metabolite accumulation in four varieties of *Triticum aestivum* L. under short-term water stress. Vegetos, 24(1): 41–49.

Bi Y L, Li X L, Christie P, et al. 2003. Growth and nutrient uptake of arbuscular mycorrhizal maize in different depths of soil overlying coal fly ash. Chemosphere, 50(6): 863–869.

Bi Y L, Wang J, Feng Y B, et al. 2014. Effect of arbuscular mycorrhiza on root self-repairing action of *Amorpha fruticose* L. in coal mining subsidence land in arid areas. Journal of China Coal Society, 39(8): 1758–1764. (in Chinese)

Bird S B, Herrick J E, Wander M M, et al. 2002. Spatial heterogeneity of aggregate stability and soil carbon in semi-arid rangeland. Environmental Pollution, 116(3): 445–455.

Bonanomi G, Chirico G B, Palladino M, et al. 2017. Combined application of photo-selective mulching films and beneficial microbes affects crop yield and irrigation water productivity in intensive farming systems. Agricultural Water Management, 184: 104–113.

Caravaca F, Alguacil M M, Figueroa D, et al. 2003. Re-establishment of *Retama sphaerocarpa* as a target species for reclamation of soil physical and biological properties in a semi-arid Mediterranean area. Forest Ecology and Management, 182: 49–58.

Chen Y F, Yu F H, Dong M. 2002. Scale-dependent spatial heterogeneity of vegetation in Mu Us sandy land, a semi-arid area of China. Plant Ecology, 162(1): 135–142.

Cheng, X, An S, Chen J, et al. 2007. Spatial relationships among species, above-ground biomass, N, and P in degraded grasslands in Ordos Plateau, northwestern China. Journal of Arid Environments, 68: 652–667.

Conesa H M, García G, Faz Á, et al. 2007. Dynamics of metal tolerant plant communities' development in mine tailings from the Cartagena-La Unión Mining District (SE Spain) and their interest for further revegetation purposes. Chemosphere, 68(6):

1180–1185.

Cumming J R, Ning J C. 2003. Arbuscular mycorrhizal fungi enhance aluminium resistance of broomsedge (*Andropogon virginicus* L.). Journal of Experimental Botany, 54(386): 1447–1459.

Franzluebbers A J, Wright S F, Stuedemann J A. 2000. Soil aggregation and glomalin under pastures in the Southern Piedmont USA. Soil Science Society of America Journal, 64(3): 1018–1026.

Giovannetti M, Mosse B. 1980. An evaluation of technique for measuring vesicular-arbuscular mycorrhizae infection in roots. New Phytologist, 84(3): 489–500.

Guan S Y. 1996. Soil Enzymes and Research Methods. Beijing: China Agriculture Press, 274–321. (in Chinese)

Habibzadeh Y, Pirzad A, Zardashti M R, et al. 2013. Effects of arbuscular mycorrhizal fungi on seed and protein yield under water-deficit stress in mung bean. Agronomy Journal, 105(1): 79–84.

Haddad M J, Sarkar D. 2003. Glomalin, a newly discovered component of soil organic matter: Part II Relationship with soil properties. Environmental Geosciences, 10(3): 99–106.

He X L, Li Y P, Zhao L L. 2010. Dynamics of arbuscular mycorrhizal fungi and glomalin in the rhizosphere of *Artemisia ordosica* Krasch. in Mu Us sandland, China. Soil Biology & Biochemistry, 42(8): 1313–1319.

Lazcano C, Barrios-Masias F H, Jackson L E. 2014. Arbuscular mycorrhizal effects on plant water relations and soil greenhouse gas emissions under changing moisture regimes. Soil Biology & Biochemistry, 74: 184–192.

Lei S G, Bian Z F, Daniels J L, et al. 2010. Spatio-temporal variation of vegetation in an arid and vulnerable coal mining region. Mining Science and Technology, 20(3): 485–490.

Levy M A, Cumming J R. 2014. Development of soils and communities of plants and arbuscular mycorrhizal fungi on West Virginia surface mines. Environmental Management, 54(5): 1153–1162.

Li S P, Bi Y L, Kong W P, et al. 2015. Effects of arbuscular mycorrhizal fungi on ecological restoration in coal mining areas. Russian Journal of Ecology, 46(5): 431–437.

Li Y Y, Chen LQ, Wen H Y, et al. . 454 pyrosequencing analysis of bacterial diversity revealed by a comparative study of soils from mining subsidence and reclamation areas. Journal of Microbiology and Biotechnology, 24(3): 313–323.

Liu H L, Tan Y, Nell M, et al. 2014. Arbuscular mycorrhizal fungal colonization of *Glycyrrhiza glabra* roots enhances plant biomass, phosphorus uptake and concentration of root secondary metabolites. Journal of Arid Land, 6(2): 186–194.

Marcin C, Maria N. 2010. The effect of different tree species on the chemical and microbial properties of reclaimed mine soils. Biology and Fertility of Soils, 46(6): 555–566.

Marschner P, Baumann K. 2003. Changes in bacterial community structure induced by mycorrhizal colonisation in split-root maize. Plant and Soil, 251(2): 279–289.

Marulanda A, Azcón R, Ruiz-Lozano J M. 2003. Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. Physiologia Plantarum, 119(4): 526–533.

Orwin K H, Kirschbaum M U, St John M G, et al. 2011. Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. Ecology Letters, 14(5): 493–502.

Pereira R, Marques S M, Antunes S C, et al. 2008. Comparison of portuguese soils from different geographical regions using physicochemical, biological and biochemical parameters. Journal of Soils and Sediments, 8(2): 106–115.

Phillips J M, Hayman D S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society, 55(1): 158–161.

Qi Y B, Yang F Q, Shukla M K, et al. 2015. Desert soil properties after thirty years of vegetation restoration in Northern Shaanxi Province of China. Arid Land Research and Management, 29(4): 454–472.

Read D J, Leake J R, Perez-Moreno J. 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Canadian Journal of Botany, 82(8): 1243–1263.

Requena N, Perez-Solis E, Azcón-Aguilar C, et al. 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Applied and Environmental Microbiology, 67(2): 495–498.

Rillig M C. 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. Canadian Journal of Soil Science, 84(4): 355–363.

Rillig M C, Mummey D L. 2006. Mycorrhizas and soil structure. New Phytologist, 171(1): 41–53.

Rillig M C, Steinberg P D. 2002. Glomalin production by an arbuscular mycorrhizal fungus: a mechanism of habitat modification? Soil Biology & Biochemistry, 34(9): 1371–1374.

Sanâa W, Tasnime M, Mohamed H, et al. 2016. Enhanced transfer of biologically fixed N from faba bean to intercropped wheat through mycorrhizal symbiosis. Applied Soil Ecology, 107: 91–98.

Sheoran V, Sheoran A S, Poonia P. 2010. Soil reclamation of abandoned mine land by revegetation: a review. International Journal of Soil, Sediment and Water, 3(2): 1–20.

Shrestha R K, Lal R. 2011. Changes in physical and chemical properties of soil after surface mining and reclamation. Geoderma,

161(3–4): 168–176.

Sidle R C, Kamil I, Sharma A, et al. 2000. Stream response to subsidence from underground coal mining in central Utah. Environmental Geology, 39(3–4): 279–291.

Singh B K, Nunan N, Ridgway K P, et al. 2008. Relationship between assemblages of mycorrhizal fungi and bacteria on grass roots. Environmental Microbiology, 10(2): 534–541.

Smith F A, Jakobsen I, Smith S E. 2000. Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. New Phytologist, 147(2): 357–366.

Taheri W I, Bever J D. 2010. Adaptation of plants and arbuscular mycorrhizal fungi to coal tailings in Indiana. Applied Soil Ecology, 45(3): 138–143.

Taheri W I, Bever J D. 2011. Adaptation of *Liquidambar styraciflua* to coal tailings is mediated by arbuscular mycorrhizal fungi. Applied Soil Ecology, 48(2): 251–255.

Vázquez M M, César S, Azcón R, et al. 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Applied Soil Ecology, 15(3): 261–272.

Veresoglou S D, Chen B D, Rillig M C. 2012. Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biology & Biochemistry, 46: 53–62.

Wang Z G, Bi Y L, Jiang B, et al. 2016. Arbuscular mycorrhizal fungi enhance soil carbon sequestration in the coalfields, northwest China. Scientific Reports, 6: 34336.

White J A, Tallaksen J, Charvat I. 2008. The effects of arbuscular mycorrhizal fungal inoculation at a roadside prairie restoration site. Mycologia, 2008: 100(1): 6–11.

Wright S F, Upadhyaya A. 1996. Extraction of an abundant and usual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science, 161(9): 575–586.

Xu Q F, Jiang P K, Wang H L. 2009. Improvement of biochemical and biological properties of eroded red soil by artificial revegetation. Journal of Soils and Sediments, 10(2): 255–262.

Yang D J, Bian Z F, Lei S G. 2016. Impact on soil physical qualities by the subsidence of coal mining: a case study in Western China. Environmental Earth Sciences, 75: 652.

Yu M, Bi Y L, Zhang C Q. 2013. Lasting improvement effects of arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* on rhizosphere soil environment in mining subsidence. Transactions of the Chinese Society of Agricultural Engineering, 29(8): 242–248. (in Chinese)

Zhang F W, Song Y X, Zhao H M, et al. 2012. Changes of precipitation infiltration recharge in the circumstances of coal mining subsidence in the Shen-Dong Coal Field, China. Acta Geologica Sinica (English Edition), 86(4): 993–1003.

Zhang T, Tian C Y, Sun Y, et al. 2012. Dynamics of arbuscular mycorrhizal fungi associated with desert ephemeral plants in Gurbantunggut Desert. Journal of Arid Land, 4(1): 43–51.

Zhang X K, Wu X, Zhang S X, et al. 2014. Organic amendment effects on aggregate-associated organic C, microbial biomass C and glomalin in agricultural soils. Catena, 123: 188–194.

Zhao R X, Guo W, Bi N, et al. 2015. Arbuscular mycorrhizal fungi affect the growth, nutrient uptake and water status of maize (*Zea mays* L.) grown in two types of coal mine spoils under drought stress. Applied Soil Ecology, 88: 41–49.

Zhao Z Q, Shahrour I, Bai Z K, et al. 2013. Soils development in opencast coal mine spoils reclaimed for 1–13 years in the West-Northern Loess Plateau of China. European Journal of Soil Biology, 55: 40–46.