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

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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 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.

Full Text

Preamble

Arbuscular Mycorrhizal Fungi Ameliorate the Chemical Properties and Enzyme Activities of Rhizosphere Soil in Reclaimed Mining Subsidence in Northwestern China

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Abstract: In the 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, rendering the ecosystem more susceptible to degradation. In particular, subsidence disrupts the symbioses between plants and microbes that can play a beneficial role in establishing 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 depths up to 30 cm in the adjacent rhizosphere of *Amorpha fruticosa* Linn. from five reclaimed vegetation communities in northwestern

China. In August 2015, a field trial was established with five historical revegetation experiments initiated in 2008 (7-year), 2011 (4-year), 2012 (3-year), 2013 (2-year), and 2014 (1-year), respectively. Each reclamation experiment included two treatments: revegetation with exotic AMF inoculum (AMF) and non-AMF inoculum (control). Root mycorrhizal colonization, glomalin-related soil protein (GRSP), soil organic carbon (SOC), soil nutrients, and enzyme activities were assessed. The results showed that mycorrhizal colonization of inoculated plants increased by 33.3%–163.0% compared to non-inoculated plants ($P<0.05$). Revegetation with exotic AMF inoculum also significantly improved total GRSP (T-GRSP) and easily extracted GRSP (EE-GRSP) concentrations compared to the control, except for T-GRSP in the 1-year experiment and EE-GRSP in the 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 soils after 1–7 years of reclamation (except for individual cases) and increased with reclamation time (except for soil Olsen P). The exotic AMF inoculum markedly increased average soil invertase, catalase, urease, and alkaline phosphatase activities 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 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 AMF reclaimed soil with high root mycorrhizal colonization could potentially accumulate available K in soils.

Our findings demonstrate 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 such as soil nutrient deficiency and drought.

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

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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 landscape degradation (Bell et al., 2000; Sidle et al., 2000; Yang et al., 2016). These serious impacts threaten plant survival and the sustainability of natural vegetation communities, accompanied by loss of the 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, currently contains 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, leading to a very fragile and degraded ecosystem that is susceptible to both anthropogenic activities and climatic conditions in semi-arid regions (Zhang et al., 2012). Moreover, problems of surface soil looseness, groundwater level recession, and soil nutrient loss exacerbate soil degradation (Cheng et al., 2007; Lei et al., 2010). In particular, this process inhibits the formation of mycorrhizal roots and their 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 plant roots and surrounding soils. The fungal mycelium constructs a huge network by extending from mycorrhizal roots (Rillig and Steinberg, 2002). This network system can enhance water and mineral nutrition absorption (particularly phosphorus) under stressed conditions such as 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 for the formation and stabilization of soil aggregates, thus contributing to soil quality (Zhang et al., 2014). Considerable evidence shows that AMF have the potential to increase host plant tolerance against biotic and abiotic stresses. Arbuscular mycorrhizal (AM) symbiosis can alleviate drought stress symptoms in host plants by altering rates of water uptake and transport from AM hyphae to host plant 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 plant gas exchange (Habibzadeh et al., 2013). AM symbiosis 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 plant carbon fixation and carbon inputs to soil through mycorrhizal fungi, thereby enhancing ecosystem carbon storage (Orwin et al., 2011). Moreover, AMF activity and diversity 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 communities in arid or semi-

arid ecosystems (Zhang et al., 2012), including prairies, 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 environments, while the addition of exotic AMF agents may help plant establishment in these situations (Requena et al., 2001; Veresoglou et al., 2012; Bi et al., 2014). Thus, revegetation with AMF agents may be beneficial for restoring vegetation communities and ameliorating soil properties in degraded ecosystems.

Once revegetation has been successfully established, it is necessary to investigate whether changes in rhizosphere soil properties are associated with the inoculation of exotic AMF agents. Soil chemical properties and enzyme activities are often considered sensitive indicators of soil quality that reflect the status of soil nutrient cycling (Caravaca et al., 2003; Pereira et al., 2008). However, few reports have studied changes in soil chemical and biological parameters during revegetation associated with both AMF inoculation and reclamation time in 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 AMF inoculation effects on chemical and biological properties of degraded soil.

2.1 Study Site and Field Experimental Design

This study was carried out on reclaimed coal mining subsidence located in Shendong coal mining sites, Daliuta town, Shenmu County, Shaanxi Province of China (39°14 -39°17 N, 110°10 -110°16 E). The climate in the study area is classified as arid and semi-arid continental monsoon, with an approximate altitude of 1200 m a.s.l. The annual mean temperature is 8.9°C and annual mean sunshine duration is 2876.0 h. According to local meteorological data, mean annual precipitation is about 422.7 mm, which occurs mainly from June to September, whereas mean annual evaporation is 2211.2 mm. Soil type is classified as aeolian sandy, with the following characteristics: 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 have been studied for ten years in this region, including 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-existing trails established in 2008 (7-year), 2011 (4-year), 2012 (3-year), 2013 (2-year), and 2014 (1-year) at Daliuta Mine were revegetated with *Amorpha fruticosa* Linn. *A. fruticosa* is a drought-tolerant legume widely cultivated for sand-fixation in semi-arid sandy lands of Northwest China (Qi et al., 2015) and has been shown to be highly dependent on arbuscular mycorrhizal microsymbionts. Two plots were established in each reclamation year: AMF inoculation treatment (AMF) and control (CK). The inoculated plot re-

ceived 50 g of inoculum per seedling at the root zone, while the control plot received 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, consisting 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. fruticosa*, except for a few native shrubs and grasses such as *Artemisia ordosica*, *Hedysarum scoparium*, *Stipa grandis*, and *Salix psammophila* (Chen et al., 2002). *A. fruticosa* seedlings 30–40 cm in height were obtained from a local nursery and annually transplanted at a density of 2 m×2 m into the plots in May. Several irrigation events were applied as required during the initial growth stage, with no further management 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 as replicates. Rhizosphere soils and fine roots from ten *A. fruticosa* plants at each subplot were collected in an S-shaped pattern as sub-samples and then mixed to form a pooled sample. A wide shovel was used to collect soils surrounding the roots in the 0–40 cm soil profile. A total of sixty soil samples of approximately 500 g each were placed in sterilized plastic bags and transported to the laboratory in an ice-cooled refrigerator. Plant residues and 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 measurement of soil chemical properties and mycorrhizal characteristics, while the other part was stored at 4°C for assessment of soil enzyme activities (soil invertase, catalase, urease, and alkaline phosphatase).

2.3 Mycorrhizal Characteristic Analyses

Fine roots were cut into 1.5-cm-long segments, cleared in 10% KOH, stained with 0.05% w/v trypan blue for 24 h, and destained 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. Glomalin-related soil protein (GRSP), including total GRSP (T-GRSP) and easily extracted GRSP (EE-GRSP), was measured 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. GRSP concentration was expressed as protein content per gram of dried soil based on Bradford assay, with bovine serum albumin (BSA) used as a standard.

2.4 Analyses of Soil Chemical Properties and Enzyme Activities

SOC was analyzed by dichromate-sulfuric 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).

Soil invertase, catalase, urease, and alkaline phosphatase activities were determined according to Guan (1996). Soil invertase activity was measured by 3,5-dinitrosalicylic alkaline colorimetry. Soil catalase activity was determined by KMnO titration method. Soil urease activity was measured using phenol sodium hypochlorite colorimetry. Soil alkaline phosphatase activity was detected by 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% significance level. Excel 2010 was used to process the data and generate figures.

3.1 Mycorrhizal Characteristics

Mycorrhizal colonization of *A. fruticosa* roots in all plots was observed due to the presence of indigenous or introduced AMF agents, but mycorrhizal plants showed significantly higher root colonization than control plants by 33.3% to 163.0% ($P<0.05$; Fig. 1a [Figure 1: see original paper]). Root mycorrhizal colonization in the inoculated group significantly increased with reclamation time, ranging from 32.2% to 63.3%, whereas colonization in controls ranged from 12.2% to 44.4%. T-GRSP and EE-GRSP concentrations were significantly higher in inoculated soils than in control soils, except for T-GRSP in the 1-year reclamation experiment and EE-GRSP in the 2-year reclamation experiment ($P<0.05$; Figs. 1b and c). Among all inoculated treatments, a positive correlation existed between T-GRSP concentration and reclamation time, except for the 4-year reclamation. In contrast, EE-GRSP concentration peaked in the 3-year reclamation but was not significantly different from that in the 7-year reclamation. Similar trends for T-GRSP and EE-GRSP were observed in non-inoculated groups. 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 significantly influenced by the interaction of AMF inoculation and reclamation time ($P<0.05$).

3.2 Soil Chemical Properties

Compared to non-inoculated plants, SOC content was higher in inoculated soils within the same reclamation year. However, no significant differences were observed between inoculated and control soils, except in the 7-year reclamation experiment ($P<0.05$; Fig. 2a [Figure 2: see original paper]). A significant increase in SOC content was only observed in older AMF reclaimed soils at 7 years compared to non-AMF reclaimed soils, with the increase being more pronounced than in newly AMF reclaimed soils. Contrary to SOC, TN content in soils was significantly higher in inoculated soils than in control soils, except for the 4-year reclamation. Among all treatments, the highest SOC and TN contents were both found in inoculated soils in the 7-year reclamation, with 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 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 except in the 1-year reclamation. Interestingly, the highest Olsen P appeared in inoculated soil at 2-year reclamation and did not increase further with longer reclamation time. Soil available K was significantly higher in inoculated soil for 7-year reclamation than at any newer reclamation time in either AMF-inoculated or non-AMF-inoculated soils ($P<0.05$; Fig. 2d). In addition, 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 controls, respectively. Moreover, these soil factors showed positive correlation with reclamation time since their maximum values were found in 7-year reclaimed soil, regardless of AMF inoculation, except for soil Olsen P. AMF inoculation, reclamation time, and their interaction had significant effects on SOC, TN, Olsen P, and available K contents (Table 1).

3.3 Soil Enzyme Activities

AMF inoculation increased soil invertase activities compared to controls, except in the 1-year reclamation (Fig. 3a [Figure 3: see original paper]). 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 reclamations than in controls ($P<0.05$; Fig. 3b). Similarly, inoculated soils reclaimed for 2-, 4-, and 7 years 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).

Inoculated soils with 7-year reclamation showed the highest alkaline phosphatase activities among all treatments, but not significantly different from controls. Significant differences in soil alkaline phosphatase activities existed between inoculation treatments and controls with 2- and 3-year reclamations (Fig. 3d). Averaged soil invertase, catalase, urease, and alkaline phosphatase activities across five reclamation years were 23.8%, 21.3%, 18.8%, and 8.6% higher in inoculated soils, respectively, which were significantly greater than controls. Overall, soil enzyme activities increased with reclamation time in both AMF and non-AMF reclaimed soils, reaching 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. fruticosa* (Table 1), except for the effect of their interaction on alkaline phosphatase.

3.4 Relationships Among Soil Properties in AMF and Non-AMF Reclaimed Mine Soils

Correlation coefficient analysis showed that root mycorrhizal colonization had positive correlation with most soil properties in both AMF and non-AMF reclaimed soils, except soil Olsen P (Table 2). Similarly, both T-GRSP and EE-GRSP were positively correlated with SOC, TN, available K, and invertase, catalase, urease, and alkaline phosphatase activities. A close correlation was observed between T-GRSP and EE-GRSP in both AMF and 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 significantly positive association with other soil properties except for soil Olsen P. Interestingly, soil Olsen P was either not correlated or only weakly correlated with other soil parameters in both AMF and non-AMF reclaimed soils.

4 Discussion

The 7-year trial results supported the hypothesis that revegetation associated with AMF inoculation could improve soil chemical and biological properties 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. fruticosa* was significantly higher in AMF-inoculated treatments than in non-AMF-inoculated treatments each year, suggesting that exotic AMF ecotypes can adapt well to local sandy soil. Taheri and Bever (2011) found that AMF species or ecotypes originating from mined areas showed greater colonization ability in mine soil than in native clay soil, suggesting that specifically adapted fungi can better promote plants under original environmental conditions. The non-indigenous AMF inoculum from laboratory collection was functionally com-

patible with the sandy soil at the study site, making it highly competitive for colonizing plant roots. Furthermore, high mycorrhizal colonization during the revegetation period can contribute to greater nutrient and water uptake from distant soil through AM hyphae and support *A. fruticosa* growth in lean and drought-prone environments (He et al., 2010).

GRSP, quantified as glomalin-related soil protein, is mainly produced by AM fungal hyphae and spores and accumulates in soils with turnover times of 7–42 years (Rillig, 2004). This compound plays an important role in translocating carbon from plant roots to soil. Previous studies have found positive correlation between GRSP and SOC in soils from agricultural ecosystems (Zhang et al., 2014), pastures (Franzluebbers et al., 2000), and rangelands (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. T-GRSP and EE-GRSP contents in this study ranged from 0.10 to 1.15 mg/g, consistent with the study by Bai et al. (2009) on the rhizosphere soil of *Astragalus adsurgens* Pall. in Mu Us Sandy Land near our target area, suggesting that soils in this subsided area are disturbed and heavily degraded.

Previous studies have shown that revegetation can increase productivity of degraded or disturbed soil by developing extensive root systems via soil stabilization or stimulating soil microbial activity, thus providing 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 soil chemical and biological properties was more evident in this semi-arid region. Our results showed marked increases in SOC and available nutrient contents in AMF reclaimed soils. This finding accords with other studies that have quantitatively addressed 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 in SOC content in 7-year AMF reclaimed soils was mainly due to decomposed litter from fallen leaves and branches (Qi et al., 2015), but was also related to the extent of exotic AMF colonization of roots, because the majority of glomalin secreted by hyphae and spores is also a component of organic matter (Haddad and Sarkar, 2003). Meanwhile, vegetation inoculated with AMF tends to reduce decomposition rates by increasing nutrient limitation of saprotrophs, thereby promoting C accumulation in soil (Read et al., 2004). The increase in TN in the rhizosphere of shrub legume can be ascribed to improved nodulation and nitrogen fixation capacity caused by AMF inoculation (Requena et al., 2001; Sanâa et al., 2016).

Symbiotic plants can produce more enzymes from excess carbon through direct photosynthesis of carbohydrates (Orwin et al., 2011). Soil enzyme activities directly affect available nutrient content in rhizosphere soil (He et al., 2010), which in turn provides soil microbes with more energy and nutrients to increase enzyme activities (Vázquez et al., 2000). This result indicates that AMF may

indirectly influence soil nutrient levels. Generally, invertase is involved in soil organic matter metabolism and catalyzes production of glucose and fructose from sucrose. Catalase decomposes hydrogen peroxide into water and oxygen to inhibit toxicity to plant organisms. Urease catalyzes NH -N release from urea and soil organic nitrogen, and alkaline phosphatase hydrolyzes both organic phosphorous esters and anhydrides of phosphoric acid into inorganic phosphorous. 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 previous studies showing AMF have higher affinity for phosphate ions and facilitate phosphorous uptake by host plants (Smith et al., 2000; Liu et al., 2014). This may be because phosphorus content was notably low at the reclaimed site and *A. fruticosa* was in a rapid growth period with greater nutrient demand after 7 years of reclamation, but this requires further testing. Moreover, 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 potential to accumulate available potassium in rhizosphere soil.

Furthermore, significant improvements in soil chemical properties and enzyme activities of *A. fruticosa* rhizosphere soil occurred in all inoculation treatments over time, except for soil Olsen P. Generally, 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 in nutrient and enzymatic activities of AMF-inoculated soil. Mycorrhizal colonization can differentially induce additional qualitative or quantitative changes in root exudates in rhizosphere soil, thus exerting indirect effects on rhizosphere microbial populations (Vázquez et al., 2000). It is also possible that increased soil glomalin may have important consequences for individual plant species in improving soil aggregation, thus contributing to 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 Requena et al. (2001), who found that exotic AMF could contribute to plant survival and soil TN and organic matter content in a 5-year trial in a desertified Mediterranean ecosystem. Additionally, an interesting finding was that 7-year reclaimed soil had the highest alkaline phosphatase activity while corresponding Olsen P was low, which may support the hypothesis that when P availability in soil is low and becomes a limiting factor, it causes an overall increase in phosphatase activity secreted by roots (Azcón and Barea, 1998).

5 Conclusions

This study demonstrated that revegetation associated with exotic AMF inoculum could improve soil nutrient availability and enzyme activities in mining subsidence regions under nutrient deficiency and water shortage conditions. AMF-inoculated plants showed higher root mycorrhizal colonization, T-GRSP and EE-GRSP concentrations, as well as increased SOC, TN, Olsen P, available K, and enzyme activities compared to controls. A significant increase in SOC content was found in 7-year AMF reclaimed soils compared to non-AMF reclaimed soils. 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 potential to accumulate soil available K. Among vegetation inoculated with exotic AMF, older reclaimed soils displayed significantly higher levels of soil nutrients (except Olsen P) and enzyme activities compared to newly reclaimed soils. These results tracking changes in soil chemical properties and enzyme activities over time led to better understanding of long-term effects of exotic AMF on mitigating soil erosion in restored ecosystems. 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 compatible with *A. fruticosa* or other indigenous plants may help acclimatize plants to the semi-arid and infertile environment in subsided soils.

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Note: Figure translations are in progress. See original paper for figures.