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Response of arbuscular mycorrhizal fungi and phosphorus solubilizing bacteria to remediation abandoned solid waste of coal mine

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Abstract Coal is the vital resource of energy in China, but abandoned coal ash and gangue lead to the degradation of vegetation cover and reduce soil quality. Both arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing bacteria (PSB) play a key role in biogeochemical cycle such as soil organic matter decomposition, nutrition release, and energy flow. To improve and reclamation the soil quality and ecological efficiency of the coal mining waste, we investigated the effects of an AMF strain (*Glomus mosseae*) and a PSB strain (*Pantoesstewarti*) on phytate mineralization and subsequent transfer to the host plant (*Medicago sativa* L.) using a two-compartment microcosm with a central 30 mm nylon mesh barrier. The results showed that significantly higher available P (AP), above ground biomass (AGB) and underground biomass (UGB) were in combined inoculation of AMF-PSB than other treatments in root and hyphae compartment. The microbial inoculum of the AMF or PSB had a significant influence on soil acid phosphatase activities (ACP). AMF-PSB enhanced phytate mineralization, improved plant biomass. AP and ACP positively influenced the AGB and UGB. AMF-PSB could be used as bioinoculant to enhance sustainable production of the plant in abandoned solid waste of coal mine.

Keywords Arbuscular mycorrhizal fungi · Phosphorus solubilizing bacteria · Abandoned solid waste · Ecological reclamation · Coal mine

1 Introduction

Coal is the vital resource of energy in China (Lam 2005), which had great contributions to the economic and social development for many years (Liu and Diamond 2005; Miao and Marrs 2000). However, coal mine lead to the degradation of vegetation cover and reduced soil quality activities, especially abandoned coal mine with coal ash and gangue damage soil physicochemical property and disrupt local ecosystems (Mukhopadhyay et al. 2016; Bi et al. 2018). In addition, abandoned coal mine often has adverse effect on biodiversity of the affected area and soil moisture, leading to land degradation (Mukhopadhyay et al. 2014). Meanwhile, the influence of abandoned coal mine on social and economic facilities is a growing concern due to rapid urbanization and industrialization in China. Site recovery, which could restore soil fertility and increase biodiversity, play an important role in controlling the soil erosion and land degradation in the abandoned coal site (Singh and Singh 2006). People also studied and vegetation coverage change and stability in large open-pit coal mine dumps in China during 1990–2015 (Liu et al. 2016) and re-vegetation types improve soil enzyme activities and microbial biomass in coal mining subsidence areas of Northern China (Xiao et al. 2019). Nevertheless, how to sustainably and rapidly improve and reclamation the soil quality and ecological efficiency of the coal mining waste is unclear but vital for current society with rapid urbanization and industrialization.

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Soil microorganisms, an important component of soil ecosystem, play a key role in biogeochemical cycle such as soil organic matter decomposition, nutrition release, and energy flow (Bell et al. 2005; Zeng et al. 2017; Zhang et al. 2017). Arbuscular mycorrhizal fungi (AMF) are obligate symbionts of plants that are associated with the roots of more than 80% vascular land plants, which significantly enhances long-term success of mine site reclamation (Levy and Cumming 2014). Previous studies showed that AMF were useful for building a productive, healthy, and sustainable land ecosystem with vegetation cover. Previous studies found that rhizosphere microbial processes had diverse effects on performance of the planted tree and plant community succession promoted reclaimed soil fertility in degraded coal mining area (Sinha et al. 2009; Pedrol et al. 2010). Meanwhile, AMF are able to release protons to mobilize insoluble soil phosphates, extend their extensive hyphae from the phosphorus depletion zone to explore a greater soil volume for inorganic P sources (Smith and Smith 2011). Previous studied found that AMF alleviate root damage stress induced by simulated coal mining subsidence ground fissures (Bi et al. 2019b) and increase plant diversity in mining land remediation (Bi and Shen 2019). P is the second most important macronutrient in plants after nitrogen, as it plays an important role in energy transfer, cell division, photosynthesis, biological oxidation, metabolism, and reproduction (Sashidhar and Podile 2010). Phosphate solubilizing microorganisms mobilize insoluble phosphates in the soil and increase plant growth under conditions of poor P availability (Tripura et al. 2007). The relationship between phosphate solubilizing bacteria (PSB) and plants is synergistic in nature, as bacteria provide soluble phosphate and plants supply root borne carbon compounds (mainly sugars) that can be metabolized for bacterial growth (Pérez et al. 2007). PSB can increase soil available P through the release of organic acids and phosphatases, which enhances the mineralization of organic P sources (Rodríguez and Fraga 1999). Research found that PSB could achieve the effective use of coal fly ash in the mine reclamation (Bi et al. 2008). Meanwhile, through the development of vegetation and plants, mutualistic fungal and bacterial associations have provided key metabolites (e.g. phosphorus, nitrogen, microelements) to their hosts, and thereby play a major role in plant colonization in terrestrial ecosystems (Barker et al. 2017).

AMF and PSB are two key beneficial functional groups that are directly involved in P turnover and subsequent plant P acquisition (Zhang et al. 2014). Previous studies have shown positive effects of dual inoculation with AMF and PSB on increasing PSB numbers on root surfaces (Andrade et al. 1998), mycorrhizal colonization rates (Sabannavar and Lakshman 2009), and AMF hyphal promoting phosphatase activity (Mar Vázquez et al. 2000; Kohler et al. 2007). However, few studies focused on the effects of AMF and PSB on the abandoned solid waste of coal mine. The aim of the present study was to discuss whether AMF and PSB can improve the soil quality of abandoned coal mine waste. We hypothesized that AMF or PSB can ① improve soil quality, soil moisture of abandoned coal mine waste, ② the combination of AMF and PSB had more effective on facilitating soil nutrient and the utilization of organic P by plants as PSB will convert organic P to available P. The results could provide baseline data for applying the biological reclamation effects of AMF-PSB on abandoned solid of coal mining.

2 Method and material

2.1 Biological materials

The host plant used was *Medicago sativa* L., which had a relatively small biomass at the seedling stage and AMF could symbionts with the roots. The AMF strain *Funneliformis mosseae*, kindly provided by Beijing Academy of Agriculture and Forestry, is a widely studied strain that has often been used as a model AM fungus and propagated at China University of Mining and Technology (Beijing) (Bi et al. 2019a). The phosphorus solubilizing bacteria strain used was *Pantoesstewarti*, which was isolated from the coal ash of Ningxia Autonomous Region in the microbial reclamation laboratory of China University of Mining & Technology, Beijing. General primers (the primers were 27F and 1492R) of 16S rDNA were used for PCR amplification of the bacteria gene (Bi 2008).The Sequence of the PCR product was compared with BLASTN in the GenBank database, and the bacteria strain was determined as *Pantoesstewarti*.

2.2 Experiment design and management

Two-compartment microcosms were constructed to meet the experimental requirements (Fig. 1). Each microcosm was comprised of a 5 cm × 10 cm × 15 cm ($l \times h \times w$) root compartment (RC) and a 3 cm × 10 cm × 15 cm hyphae-only compartment (HC). The two compartments were separated from each other by a 30 μm nylon mesh. Thus, the soils in the two sections were referred to as root soil and hyphae soil (Fig. 1). Moderately acid (pH = 7.26) river sand soil was used as the growth medium for root compartment and a mixture of coal ash and gangue (from Ningxia Autonomous Region) at a ratio of 6:1 was used as the soil for hyphae-only compartment. The "soils" were passed through a 1-mm sieve, sterilized at high temperature and pressure (121 °C, 103 kP) for 2 h, and air-dried. The levels of fertilizers for root compartment

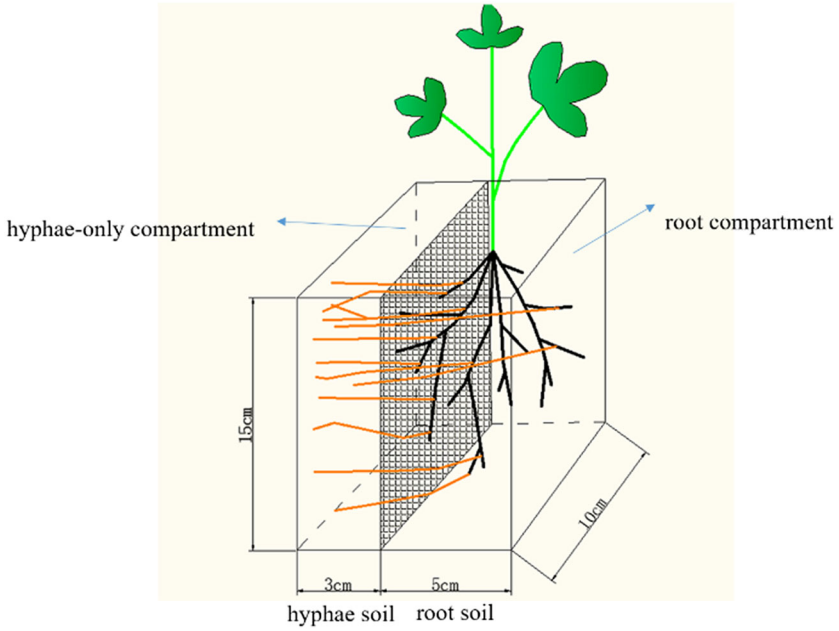

Fig. 1 Two-compartment microcosms

were N (NH_4NO_3) 10 mg/kg, P (KH_2PO_4) 2.5 mg/kg and K (KNO_3) 15 mg/kg.

In the root soil compartment, *Medicago sativa* L. seeds that were treated with 10% hydrogen peroxide for 10 min were planted and grew for 60 days after germination. The experiment contained three treatment groups: ① PSB were added to the hyphae-only compartment (−M/CA); ② AMF were added to the root compartment (+M/CK); ③ AMF were added to the root soil and PSB were added to the hyphae soil (+M/CA); ④ Plants grown in two-compartment microcosm without the addition of fungus and bacteria were used as the control (−M/CK). Each treatment had three replicates. Plants in these microcosms were grown in a campus greenhouse at China University of Mining & Technology, Beijing. The plants and soils were harvested after growth for 60 days. The roots and shoots of each plant were separated, and their dry weights were recorded after oven drying at 75 °C. Rhizosphere soil samples were removed roots, stones and animals for basic physical and chemical properties.

2.3 Measurement of parameters

Soil pH was measured using a pH meter after shaking the soil water (1:5 *w*/*v*) suspension for 30 min. Soil water content was determined by oven-drying to constant mass at 105°. Soil available phosphorus (AP) was extracted by 0.5 mol/L $NaHCO_3$ and measured by molybdenum-blue method (Olsen et al. 1982). Acid phosphatase activity (μg p-nitrophenyl phosphate min^{-1} g^{-1} DW soil) in the hyphae soil was determined according to Zhang et al. (2014). Mycorrhizal colonization of roots was measured using the reported method (Trouvelot et al. 1986). External mycorrhizal hyphae were extracted from two 5 g soil sub-samples from the hyphae soil compartment using the membrane filter technique (Staddon et al. 2010). Hyphal length was assessed using the gridline intercept method at 200 × magnification and then converted to hyphal length density (m/g DW soil). The amount of soil fungi and bacteria were determined by plate count method (Hattori 1985).

2.4 Data analysis

All results were reported as mean ± standard error. One-way ANOVA, Student's *t* test, and S-K-N multiple range comparison were used to compare the significant effects among crops using the IBM SPSS 18.0 software program (SPSS Inc., USA). The figures were adopted with origin 9.0. The non-metric multi-dimensional scaling (NMDS) was used with Primer 7. The spearman correlation analysis was performed to show the relationships between the soil microbial activity and soil physicochemical properties with R (3.4.1) for windows.

3 Results

3.1 Physicochemical properties and biomass

As shown in Table 1, all soil pH was neutral or weak acid, ranging from 6.82 to 7.68. Significantly higher AP, AGB

Table 1 Physicochemical properties and biomass

Item	Compartment	pH	MS	AP (mg/kg)	AGB (g)	UGB (g)
−M/CK	RC	7.46 ± 0.02b	0.03 ± 0.00b	0.91 ± 0.06b	0.28 ± 0.01c	0.10 ± 0.01c
	HC	6.82 ± 0.23a	0.35 ± 0.05a	5.05 ± 0.20c		
−M/CA	Root	7.59 ± 0.05b	0.04 ± 0.00a	1.15 ± 0.07b	0.34 ± 0.04b	0.13 ± 0.00b
	Hyphae	6.92 ± 0.07a	0.31 ± 0.05ab	6.33 ± 0.45b		
+M/CK	Root	7.67 ± 0.03a	0.02 ± 0.01b	0.90 ± 0.07b	0.33 ± 0.03b	0.12 ± 0.02b
	Hyphae	6.82 ± 0.06a	0.24 ± 0.01b	5.78 ± 0.21b		
+M/CA	Root	7.68 ± 0.03a	0.04 ± 0.02a	1.53 ± 0.33a	0.39 ± 0.01a	0.16 ± 0.02a
	Hyphae	6.89 ± 0.16a	0.29 ± 0.08ab	6.93 ± 0.23a		

Note RC root compartment, *HC* hyphae compartment, *MS* moisture, *AP* available phosphorus, *AGB* above-ground biomass, *UGB* under-ground biomass, different lowercase letters in each column refer to significant differences among treatments in the same compartment ($P < 0.05$), the same below

and UGB were found in +M/CA. The levels of AP, AGB, and UGB in the four groups were +M/CA > + M/CK ≈ −M/CA > −M/CK, with similarity regulation between root compartment (root) and hyphae compartment (hyphae). The content of AP in +M/CA was 6.93 mg/kg and 1.53 mg/kg in root and hyphae, respectively, which were 1.37 and 1.68 times of those in −M/CK.

3.2 Soil biological properties

The details of the hyphae density, the rate of mycorrhizal colonization and acid phosphatase were presented in Fig. 2. We found that PSB significantly increased the hyphae density of AMF (Fig. 2a). The value of hyphae density was 0.81 and 0.65 m g^{-1} in root compartment and hyphae compartment in +M/CA, which was about twice as much as those in the +M/CK. The rate of mycorrhizal colonization in the +M/CK and +M/CA were 0.48 and 0.31, with significant variations between different isolates ($P < 0.05$) (Fig. 2b).The microbial inoculum of the AMF and PSB had a significant influence on soil acid phosphatase activities (Fig. 2c). In the hyphae compartment, soil acid phosphatase activities were found to be significantly higher in groups containing AMF and/or PSB (+M/CA > −M/CA > + M/CK > −M/CK), ranging from 1.46 to 1.61 μg^{-1} g^{-1} h^{-1}. Soil acid phosphatase activities in the root compartment soil showed a similar trend as that in the hyphae compartment, but had the lowest value in –M/CK. One-way ANOVA analysis results indicated that the amount of soil fungi and bacteria were principally affected by AMF and PSB (Table 2). The amount of soil fungi and bacteria were significantly higher in +M/CA and lower in –M/CK both in root and hyphae compartments, with the rank of +M/CA > −M/CA > + M/CK > −M/CK.

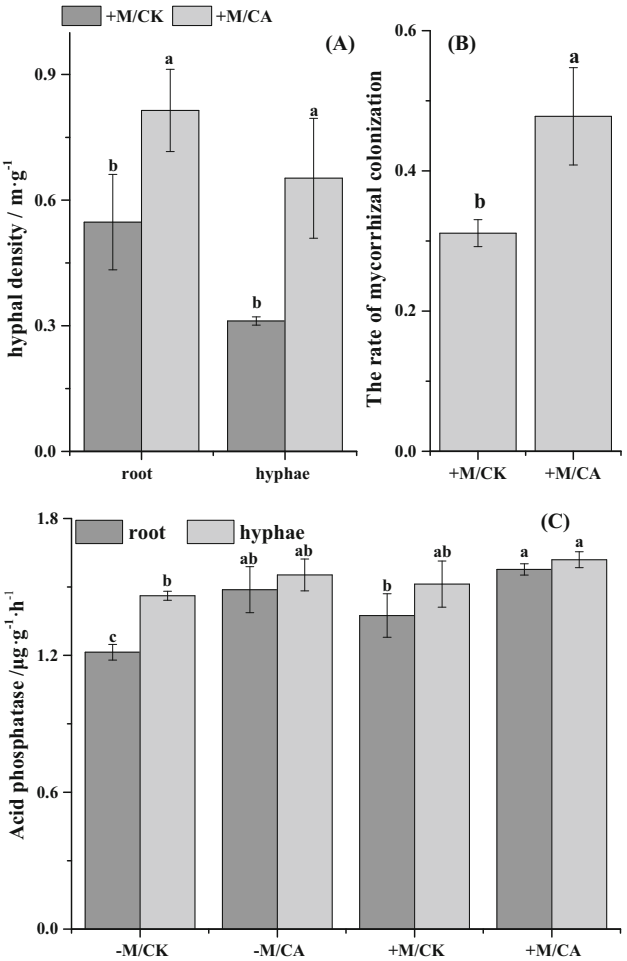

Fig. 2 Hyphae density (**a**), the rate of mycorrhizal colonization (**b**) and soil acid phosphatase (**c**) under different treatments. *Note*: different lowercase letters refer to significant difference among treatments in the same compartment ($p < 0.05$)

Table 2 The amount of soil fungi and bacteria

Item	Fungi amount 10^3 CFU g^{-1}		Bacteria amount 10^6 CFU g^{-1}	
	Root	Hyphae	Root	Hyphae
−M/CK	1.55 ± 0.24c	4.14 ± 0.80b	0.79 ± 0.26a	9.81 ± 1.12c
−M/CA	3.07 ± 1.09ab	9.16 ± 4.93a	1.58 ± 0.98a	16.78 ± 4.82ab
+M/CK	1.94 ± 0.83bc	8.77 ± 1.43a	1.18 ± 0.69a	13.18 ± 4.37bc
+M/CA	3.15 ± 1.15a	12.50 ± 4.64a	1.78 ± 1.30a	21.06 ± 3.09a

3.3 Relationship among the treatments and soil parameters

The non-metric multi-dimensional scaling (NMDS) of soil parameters and biological properties among different treatments was presented in Fig. 3. Different treatments took the formation of four distinguishing clusters, indicating that soil parameters had a significant composition among the four treatments. The spearman correlation analysis was used to demonstrate the relationship between physicochemical and biological properties (Fig. 4). Correlation matrix analysis of soil properties revealed that R-AP, H-AP, R-ACP and H-ACP positively influenced the AGB and UGB. As a result, soil acid phosphatase activities (H-ACP and R-ACP) were significantly and positively correlated with H-AP and R-AP. Meanwhile, R-pH had a significant positive correlation with AGB and UGB. However, H-pH had a negative correlation with H-ACP.

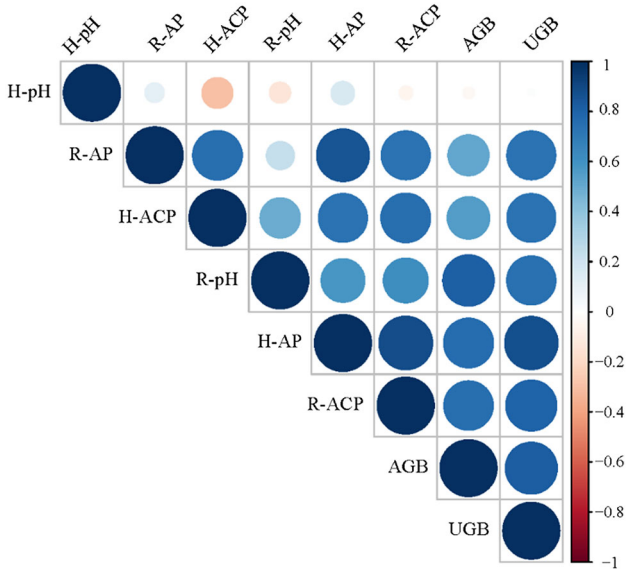

Fig. 4 The spearman correlation analysis between physicochemical and biological properties. *Note*: the prefix capital letter of H: hyphae compartment; the prefix capital letter of R: root compartment; *AP* available phosphorus, *ACP* acid phosphatase, *AGB* aboveground biomass, *UGB* underground biomass

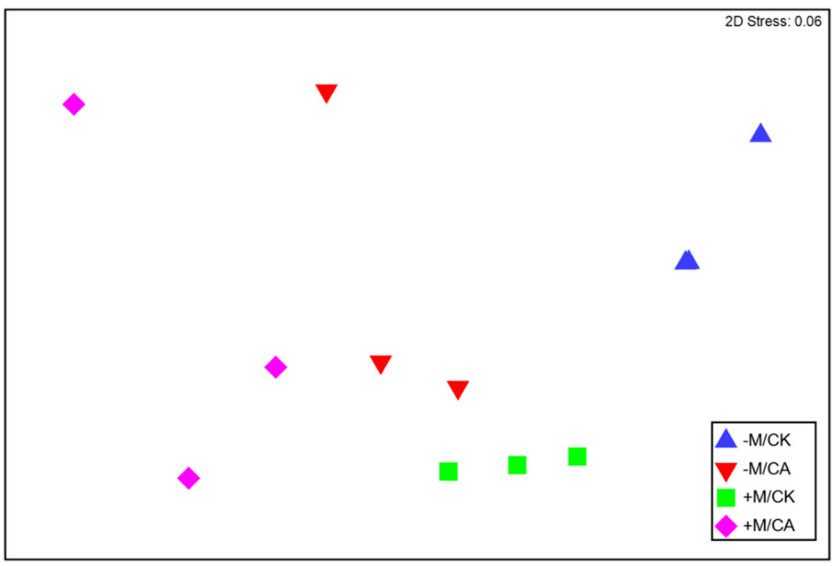

Fig. 3 The non-metric multi-dimensional scaling (NMDS) for soil parameters among treatments

4 Discussion

Low available P is often observed in highly acidic soils, which may be attributed to phosphate adsorption by minerals and phosphate precipitation as Fe and Al phosphates (Barroso and Nahas 2005). Among the treatments, the lowest content of available P was found in the –M/CK, which also showed the lowest pH (Table 1). The content of AP in +M/CK were higher than –M/CK. Similarly, it was higher in–M/CA than in –M/CK. These results indicated that AMF and PSB are able to mobilize insoluble soil phosphates, extend their extensive hyphae from the phosphorus depletion zone to explore a greater soil volume for inorganic P sources (Smith and Smith 2011; Rodríguez and Fraga 1999). Castagno et al. (2011) reported the ability of PSB to solubilize phosphate and to promote plant growth. In our study, AGB and UGB were the lowest in –M/CK, consist with that AMF symbiosis contributes significantly to global phosphate and carbon cycling and influences primary productivity in terrestrial ecosystems (Fitter 2005). Soil enzymes are important components of soil microbial activity, participate in many vital soil biochemical reaction, and have important effects on soil fertility (Thomson et al. 2015; Yousuf et al. 2012; Zhang et al. 2016a; Zhu et al. 2012). The beneficial effect of PSB inoculation may be direct due to an increased supply of available P, or indirect through changes in the growth rate and metabolic activities of the crop (Kaur and Reddy 2015). The microbial inoculum of the AMF and PSB had a significant influence on soil acid phosphatase activities (Fig. 2-C). Soil acid phosphatase activities were significantly higher in +M/CA in both root and hyphae compartments. Our present results also show that the mineralization of phytate and the amount of bacteria and fungus were significantly enhanced when both AMF and PSB were present in the soil. Previous study found that The AMF trigger PSB growth and activity, Inreturn, the PSB enhanced mineralization of organic P and the availability for AMF. When additional P was added to increase soil available P, the PSB enhanced AMF hyphal growth, and PSB activity was also stimulated by the fungus (Zhang et al. 2016b). These results imply that the PSB and AMF play an important role in the mineralization of phytate and plant growth.

Studies found that soil physicochemical properties, as key factors for soil microbes, affect soil microbial community composition and diversity though changing the composition and chemical properties of soil matrix and the efficiency of nutrient use (Yuan et al. 2014; Yao et al. 2014). Soil pH has been recently proved as the major factor to determine the soil microbial activity and composition (Chu et al. 2010; Shen et al. 2013). The relationship between the physicochemical properties and microbial composition of soil and soil biology provides a guide for soil management (Dick et al. 1996). In the present study, the ability of PSB to solubilize insoluble phosphate was related to the soil available P content. This result agreed with what was reported by Mander et al. (2012). PSB and AMFcan improve plant growth, yield and phosphorus content of several crops, and may be used as bioinoculant to enhance sustainable production (Viruel et al. 2014).

5 Conclusions

Here, we examined the remediation effects of AMF and PSB on abandoned solid waste of coal mine. AMF-PSB Combined inoculation were significantly promoted AP, AGB and UGB in root and hyphae compartments. The microbial inoculum of the AMF and PSB had a significant influence on soil acid phosphatase activities. R-AP, H-AP, R-ACP and H-ACP positively influenced the AGB and UGB. AMF and PSB improved plant growth and phosphorus content. PSB enhanced mineralization of organic P and the availability for AMF. Thus, AMF-PSB could be used as bioinoculant to enhance sustainable production of the plant in abandoned solid waste of coal mine.

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