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Effects of Arbuscular Mycorrhizal Fungi on Ecological Restoration in Coal Mining Areas[1]

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Abstract—In the coal mining subsidence areas in arid and barren locations in western China where plants are difficult to grow, the root of *A. fruticosa* was inoculated with arbuscular mycorrhizal fungi (AMF). The effects of inoculation with AMF on the growth of *A. fruticosa* and improvement of the degraded soil were determined. Results showed that the selected AMF and *A. fruticosa* had a strong symbiotic relation. Sixteen months after inoculation, the root colonization rate in *A. fruticosa* reached 88%. Inoculation with AMF was shown to promote the growth and root development of *A. fruticosa* and improve rhizospheric soil and fertilization of *A. fruticosa*. After the inoculation, the contents of organic matters and glomalin-associated proteins in the rhizospheric soil of *A. fruticosa* increased significantly. Sixteen months after the inoculation, the acidic phosphatase activity in the rhizosphere of *A. fruticosa* in the inoculated plots increased by 44% compared with that of the control plot, and the content of available phosphorus was 2.5 times that of the control. Hence, inoculation with AMF improves the rhizosphere of *A. fruticosa*, promotes a stable ecosystem in the mining area.

Keywords: arbuscular mycorrhizae; *A. fruticosa*; restoration, Amelioration; coal mining area

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1. INTRODUCTION

China is the world's largest coal producing and consuming country. Coal, which dominates China's energy structure, is an important pillar and driving force for the country's rapid socio-economic development (Tang et al., 1999). Although coal brings huge economic benefits to China, coal mining causes numerous environmental problems, such as drastic reduction in the number of biological communities in the reconstructed soil as a result of disturbances in coal mining, plant root damage caused by mining site subsidence, reduced topsoil fertility, water-fertilizer disharmony in soil, and pollution from filling materials. Currently, China's coal resources are mainly concentrated in arid and semi-arid regions in western China, among which Shendong mining area has the largest proven reserves of coal. Shendong mining area is among the world's top seven coalfields and has the country's highest coal production and largest subsidence area (Singh et al., 2006). The mining area in Shendong is drought, and the contemporary coal mining area has intensified desertification and soil impoverishment. The vegetation coverage is gradually decreasing and thus severely restricts the sustainable eco-environment development of western China.

Revegetation is a systematic engineering approach. A sound ecological environment requires the perfect co-existence of plants, soil, microorganisms, and animals. The mining subsidence area in Shendong is characterized by low biodiversity. Hence, achieving ecological restoration merely by physical or chemical means is difficult, and the environmental issues of the mining area cannot be solved just by providing funding. Fortunately, biological treatments have been well developed and applied. Therefore, it is important to improve the soil micro-environment in the subsidence area and achieve self-healing of ecological environment in the mining area by implementing appropriate biological measures. Arbuscular mycorrhizal fungi (AMF) is a ubiquitous soil microorganism in nature. AMF, being a good biological "bacterial manure", has a great value and potential in ecological environment improvement. In recent years, AMF is increasingly used for environment improvement and has been shown to improve the structure, evolution, and stability of phytocoenoses (van der Heijden et al., 1998; Hajboland et al., 2010; Streitwolf et al., 2001). When mycelium is formed between AMF and its host plant, the soil contact area of host plant roots is expanded, resulting in improved nutrient and water absorption of plant, and increased stress tolerance (Fokoma et al., 2012; Spohn et al., 2010; Auge, 2001). For Shendong mining area, use of AMF is a new breakthrough in the environmental and ecological restoration. In this study, we investigated the effect of AMF inoculation on the growth of *A. fruticosa* and improvement of its

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rhizospheric soil. The results would provide theoretical and technical basis for better use of AMF in the reclamation of mining areas.

2. MATERIAL AND METHODS

2.1. Experimental Sites

Typical coal mining areas of Western China were selected as the experimental sites. The live chicken and live rabbit mining subsidence areas of Shendong Coal, a branch company of the Shenhua Corporation (40°01.500′ N, 116 16.500′ E) are located in the arid and semi-arid areas. The area is located in the borderland Maowusu, and is sandy with an annual average temperature of 7.3°C and annual precipitation of 365 mL. The total area of coal mining subsidence areas in the experimental region was 4059 m^2. The vegetation coverage is low, evaporation was strong, and the average annual evaporation capacity is 4.55–6.72 times that of the average annual precipitation.

2.2. Materials

The tested strains used in the present study were endophytes *Glomus mosseae* (G.m), which was used for the proliferation and culture, and provided by the Microbiology Laboratory, Plant Nutrition and Natural Resources Institute, Beijing Academy of Agriculture and Forestry Science. The tested plant was the pioneer plant *A. fruticosa*, which is used for local greening and ecological governance. In May, 2011, *A. fruticosa* was selected for the study. The experimental soil was sandy soil, and the basic characteristics were as follows: pH 7.48; electrical conductivity 35.4 μs cm^{-1}; maximum water holding capacity 22.86%; organic matter 6.07 g kg^{-1}; total nitrogen 0.34 g kg^{-1}; total phosphorus 0.41 g kg^{-1}; available phosphorus 7.2 mg kg^{-1}; and available potassium of 50 mg kg^{-1}.

2.3. Experimental Design and Management

A comparative study on the effect of mycorrhizal inoculation on the selected *A. fruticosa* was conducted. Two treatments were performed: treatment with inoculation of mycorrhizal fungi treatment (+M), and that without inoculation of mycorrhizal fungi treatment (CK). 372 *A. fruticosa* were inoculated. 50 g of mixed mycorrhizal fungi inoculants were inoculated in each tree. The CK region contained 385 *A. fruticosa*. *A. fruticosa* with equal size were selected according to the "S" method, in both the +M area and CK area. At the same time rhizospheric soil of *A. fruticosa* was also collected. The repeated number of *A. fruticosa* was 10. Plant root monitoring pipes were buried within the roots of the targeted plants. The horizontal sextant angle between the root canal and the ground was 45°. Six months after the burying of the root canal, the microenvironment around the root tubes tended to be stable, at which time the CI-600 roots in situ monitoring system was used to monitor the local root system of the targeted plants.

2.4. Test Items and Methods

2.4.1. Root system monitoring method. The working principle of the CI-600 roots in situ monitoring system is as follows: The CI-600 root system growth monitoring system is designed for the scanning of the growth dynamic of soil living roots. The scanning head can scan the root system distribution at different depths or in soil profile images. The scanning head is mainly composed of a vertical rotation type linear scanning head, notebook computer, and transparent plastic tubes. The tubes must be buried or inserted into the soil, then the CCD linear scanning head is placed into the tube. Scanning is conducted automatically by the scanning head along the wall tube, and controlled by computer. The plant growth state images around the root tubes can then be obtained. Various systematic growth parameters can be obtained by the analysis software of the root system, which thus reflects the response of the plant roots to the external environment change.

CI-600 scanning images were analyzed by the root system analysis program WinRHIZO Tron MF to obtain the root length, surface area, projection area, volume, mean diameter, number and other indicators.

2.4.2. Extraction and measurement method. Glomalin is isolated from a soil sample, and includes easily extractable glomalin (EEG) and total glomalin (TG), according to the method of Wright and Janos with slight modification (Rilling et al., 2007; Janos et al., 2008). The mycorrhizal infection rate was determined by the trypan blue staining method (Phillips and Hayman, 1970). Organic matter was detected by the high temperature and external heating of oxidation of ferrous titration method (Qian et al., 2011). The soil available phosphorus content was measured by the molybdenum antimony colorimetric method (Bao, 1999). The determination of soil phosphatase activity was conducted by the improved Tabatabai and Brimner method (Zhao and Jiang, 1986).

2.5. Data Analysis

This study used SAS statistical software to analyze the experimental data (t-test), and the significant level was set to 5%.

3. RESULTS AND DISCUSSION

3.1 Effects of AMF Inoculation on the Growth of A. fruticosa

Inoculation with AMF promoted the growth of *A. fruticosa*. As shown in Table 1, the ecological effects of AMF began to appear four months after mycorrhizal inoculation, exhibiting the fact that the growth of *A. fruticosa* rate in the +M treatment was clearly

Table 1. Effect of AMF on the growth of *A. fruticosa* and its symbiotic relationship

Time	Treatment	Plant height, cm	Crown diameter, cm	Infection rate, %	Hyphae length, m g^{-1}
May-11	ck	28.46e	18.75e	36.80d	2.67d
	+M	31.34de	19.64e	37.60d	2.68d
Sep-11	ck	35.06d	26.20e	36.67d	2.67d
	+M	44.99c	26.43e	74.00b	3.83b
May-12	ck	44.19c	33.29d	45.33c	2.93c
	+M	54.52b	41.56c	80.00b	4.02ab
Sep-12	ck	52.90b	51.68b	41.33cd	2.92c
	+M	72.33a	69.65a	88.00a	4.22a

Different letter in a treatment means significant difference at 5% level. The labeling method in table was the same as follow.

Table 2. Effect of mycorrhizal inoculation on the growth of roots of *A. fruticosa*

Time	Treatment	Total length, cm	Total projected area, cm^2	Total average diameter, mm	Total volume, cm^3	Total number of tips	Total surface area, cm^2
May-2011	ck	157.03e	11.90d	2.49b	0.77c	41.00c	34.52e
	+M	262.60bc	22.39b	3.39a	1.54b	67.00a	70.35c
Sep-2011	ck	199.95d	12.90cd	2.54b	0.81c	48.00bc	36.80e
	+M	280.48b	23.80b	3.59a	2.35a	67.00a	80.40b
May-2012	ck	207.15d	13.50cd	2.55b	0.82c	50.00b	40.50de
	+M	286.32b	24.30b	3.62a	2.43a	70.00a	84.60ab
Sep-2012	ck	245.65c	15.44c	2.56b	0.88c	66.00a	48.52d
	+M	318.16a	29.51a	3.83a	2.68a	75.00a	92.71a

higher than that of the CK treatment. There were increasing trends of the growth in the +M treatment and CK treatment with increase of time, and the growth rate of *A. fruticosa* in the +M treatment was better than that of the CK treatment at all times. 16 months later, the plant height and crown width of *A. fruticosa* reached their maximums. There were significant differences in the plant height and crown width of *A. fruticosa* in the +M treatment and CK treatment. The inoculation of AMF promoted the growth of *A. fruticosa*.

The growth of *A. fruticosa* was quick and formed a symbiotic relationship with AMF, which enlarged the contact area between the *A. fruticosa* root and soil and was helpful in the absorption of water and nutrients in the soil by the *A. fruticosa* root system. As illustrated in Table 1, the mycorhiza successfully infected the root system of *A. fruticosa* in September, 2011. The colonization rate of the *A. fruticosa* roots in the +M treatment was higher than that of the CK treatment. The inoculation significantly increased the root colonization rate. The inoculation of AMF dramatically increased the hypha density of rhizospheric soil. The hypha density of rhizospheric soil of the inoculated region reached its maximum in September, 2012. At the beginning of inoculation, root colonization rate and hyphal density increased gradually with the increase of time. One year after inoculation with mycorhiza, the increasing rate of the *A. fruticosa* root colonization and hyphal density both decelerated. There was also infection of *A. fruticosa* root in the CK treatment region, which is mainly due to the existence of indigenous AMF in nature. The high infection rate of *A. fruticosa* root after mycorrhizal fungal inoculation also indicated that mycorhiza and *A. fruticosa* root formed a mutually beneficial symbiont. In the present study, the adaptation options of the selected AMF and *A. fruticosa* were good, thus the popularization and application of the selected AMF and *A. fruticosa* in mining areas were in favor of vegetation restoration and ecological reconstruction of the Shendong mining subsidence areas.

3.2. Effect of AMF Inoculation on the Growth of Roots of A. fruticosa

Plant roots not only play the role of repairing plants, but are also the only channel for absorbing water inorganic nutrients and a small amount of organic nutrition which crops need for growth and development. Inoculation of AMF was in favor of the growth of *A. fruticosa* roots, as revealed by the *A. fruticosa* root in situ scanning through the CI-600 root monitoring system (Table 2). In May 2012, the *A. fruticosa* root length, projection area, surface area, root

Table 3. Effects of AMF on chemical properties of rhizospheric soil of *A. fruticosa*

Time	Treatment	TG, g kg^{-1}	EEG, g kg^{-1}	Organic matter, g kg^{-1}	Acid phosphatase activity, mol h^{-1} g^{-1}	Available P, g kg^{-1}
May-2011	ck	2.03f	0.77de	5.59c	2.29cd	1.21e
	+M	2.00f	0.76e	5.64c	2.08d	1.20e
Sep-2011	ck	2.15ef	0.75e	6.04bc	2.45cd	1.49d
	+M	3.38c	1.11c	7.37a	3.73a	2.74c
May-2012	ck	2.29de	0.78de	6.11bc	2.19d	1.56d
	+M	3.69b	1.47b	7.57a	3.05b	3.35b
Sep-2012	ck	2.44d	0.94d	6.49b	2.62c	1.60d
	+M	4.26a	1.89a	7.93a	3.78a	4.03a

volume and root tip number in the +M group were all shown to be higher than those of the CK group, and there were statistical differences. In September 2012, the indexes of *A. fruticosa* roots in the two treatments both showed increasing trends, exhibiting the fact that the increase rate of *A. fruticosa* root of the +M treatment group was higher than that of the CK group. With the exceptions of the root volume and root tip number, there were significant differences in the other indicators of the root system. Drought, water shortage and root damage are the main environment problems in the coal mining subsidence lands of the Shendong mining area. The inoculation of AMF may reduce the harm of other organisms on the host plant. In addition, the AMF hyphae may extend to the areas which the plant roots cannot reach, thus expanding the contact area of *A. fruticosa* root and the surrounding soil, which is conducive to the absorption of water and nutrients by *A. fruticosa*, improves the drought resistance of the plant, and increases the plant length (Newsham et al.,1995; Eissenstat et al., 2000). AMF can also improve the tolerance of the host under drought conditions, which is conducive to the absorption of water by the host plants (Kohler et al., 2009; Miller et al., 2002).

3.3. Effects of AMF Inoculation on A. fruticosa Rhizospheric Glomalin and Organic Matter

With the deepening of research, AMF is becoming more and more widely used. AMF can promote plant growth on degraded soil, and its secreted glomalin related protein can improve the quality of soil (Rillig and Mummey, 2006). glomalin is a specific glycoprotein containing metal ions, and is produced by AMF. glomalin is insoluble in water and is difficult to decompose. Under natural conditions, glomain is very stable. Recently, glomalin was renamed as "glomalin related soil protein". The main effects of glomalin are increase of soil organic carbon pools and improvement of soil aggregates (Karanika et al., 2008). glomalin is produced in the AMF and settles in the internal root hyphae in the roots of the host plants and surface of extraradical mycelium in the rhizospheric soil. Furthermore, the content of glomalin in the soil ecosystem is quite high, thus it provides the AMF with new ecological functions. In the present study, the levels of glomalin and easily extractable glomalin in the *A. fruticosa* root soil in the +M treatment both increased, while there was no significant change in the CK treatment group of September 2011. With the increase of inoculation time, the glomalin and easily extractable glomalin in the *A. fruticosa* root rhizospheric soil both exhibited increasing trends. In September 2012, the contents of glomalin and easily extractable glomalin in the *A. fruticosa* root rhizosphere soil of the +M treatment region both reached their respective maximum values. Four months after mycorrhizal inoculation, the increase of the organic matter content in the rhizospheric soil of *A. fruticosa* was the most significant in the +M treatment group. Although the organic matter content in the rhizospheric soil of *A. fruticosa* in the +M treatment group showed an increasing trend, the changes of organic matter of the rhizospheric soil of *A. fruticosa* were not significant during the different observation periods (Table 3). There were also glomalin and easily extractable glomalin in the *A. fruticosa* root rhizosphere soil of the CK group, which was mainly due to the existence of indigenous arbuscular mycorrhiza in the mining subsidence. In the mycorrhizal symbiont, the extraradical mycelium provided the essential nutrients and water for plant growth, and the plants provided the carbohydrates needed for fungal growth and metabolism. This study also verified the fact that the inoculation of AMF could significantly improve the content of *A. fruticosa* rhizospheric glomalin and organic matter. glomalin may greatly improve the stability of soil particles (Wright and Upadhyaya, 1998). At present, a large number of studies have confirmed that the glomalin content in soil was positively correlated with the condensate soil stability and soil culvert water. Mycorrhizal inoculation is conducive to maintaining the soil moisture, and can alleviate the effects of drought on crop growth (Wright and Anderson, 2000). At the same time, glomalin is a positive response mechanism of the adjustment and adaptation of AMF to its host plant growth environment and microorganism metabolism (Rillig and Steinberg, 2002). Miller and Jastrow (2000) also found that AMF external hyphae with adhered glomalin could survive very well in the soil water interface. These hyphae can reduce the destruction of large soil aggregates during the wetting and drying cycles, thereby improving the soil structural stability and anti-erosion ability, and improving the soil properties. The mining subsidence areas of western China are dry and suffer from water shortage, and coal mining has destroyed the soil structure. Soil desertification is very serious in this region, and its anti-erosion ability is poor. Therefore, there is important significance for the application of mycorrhizal in mining subsidence areas for soil improvement and ecological environmental treatments.

3.4. Effect of AMF Inoculation on the Available Phosphorus and Acid Phosphatase in Rhizospheric Soil

It was shown that, under nutrient deficient conditions, the AMF could significantly improve the uptake of mineral nutrients by plants, especially phosphorus (Chen et al., 2005). The study results also showed that the mycorrhizal ecological effect appeared 4 months after mycorrhizal inoculation. The acid phosphatase activity in the mycorrhizal inoculation group was higher than that in the CK group, and the difference was significant. As demonstrated in Table 3, the fluctuation of acid phosphatase activity in the rhizospheric soil of *A. fruticosa* in the +M region was great throughout the entire observation period. The acid phosphatase activity obtained from the rhizospheric soil of *A. fruticosa* in September was higher than that of May each year. This is mainly due to the fact that the temperature of the experimental region in May was low, whereas that in September, the rainy season, was high. Regardless of the changes in temperature, the activity of acid phosphatase in the rhizosphere soil of *A. fruticosa* inoculated with AMF was higher than that of the CK group. Rapidly available phosphorus is the main phosphorus which *A. fruticosa* may obtain from soil. The inoculation of AMF significantly increased the content of available phosphorus in the rhizospheric soil of *A. fruticosa*. The content of available phosphorus in the rhizospheric soil of *A. fruticosa* in the +M group was dramatically higher than that of the CK group. With the increase of time, the content of available phosphorus in the rhizospheric soil of *A. fruticosa* in the +M group increased gradually. In September 2012, the content of available phosphorus in the rhizospheric soil of *A. fruticosa* reached its maximum value, while that in the rhizospheric soil in the CK group showed no significant change. The increase of the available phosphorus in rhizospheric soil may be a result of the significantly improved content of organic matter in rhizospheric soil due to mycorrhizal inoculation. Soil organic matter is an important material foundation of soil fertility, and to a great extent the existing number and morphology determined the soil fertility condition. In addition, soil enzymes play an important biological catalyst role in the cycles of soil carbon, nitrogen, phosphorus and other elements, which are important factors for soil metabolism, and are closely related to the formation and transformation of soil fertility. Acid phosphatase in soil may promote the transformation of organic phosphorus to inorganic phosphorus, and its activity can characterize the soil fertility status, especially the phosphorus status. Soil organic matter can be decomposed, and releases nitrogen, phosphorus, sulfur and other nutritional elements which are required for the growth of plants, under the participation of soil enzymes.

4. CONCLUSION

With continuous improvement of industrialization degree, the application of AMF in such fields as heavy metal in soil, organic pollution and soil salinization is concerned widely so as to achieve better ecological efficiency. It is discovered from a large amount of study that the inoculation of AMF may notably improve the plant growth and the biodiversity of wasteland in the soil polluted by different heavy metals. AMF can produce the holding function for metal via Hypha as well as reinforce the barrier action for heavy metal by root system via PH variation of rhizospheric bacteria, reduce the running that the heavy metal is towards the upper part of plant ground and effectively improve the mineral nutrition status of plant simultaneously so as to strengthen the adaptability of plant on threatening for pollution of heavy metal (Joner et a l.,2002; Xiao et al., 2006). In the soil by different organic substances, that the AMF may improve the restoring capability of plant for soil polluted by organic substances mainly benefits from the organic substance degradation promoted by Hypha of AMF, which may originate from immobilization of organic substances and promoting for variation of rhizospheric enzyme system (Gao et al., 2010, Schnabel et al., 2001). The AMF can accelerate the growth of plant in the salinized soil, increase the leaf area and the chlorophyll content, strengthen the resistance on salt threatening by host plant, improve the biomass of host plant and perfect the environmental quality of salinized soil (Kaya et al., 2003).

In this study it is shown that mycorrhizal inoculation significantly promotes the growth of both the aboveground parts and roots of *A. fruticosa*. Strengthened mycorrhizal inoculation is conducive to the establishment of the symbiotic relationship between AMF and plants. The infection rate of *A. fruticosa* mycorhiza in the +M region can reach 88%. The mycorhiza screened in the study is suitable for the popularization and application in typical Mining areas of western China. After mycorrhizal inoculation, the total glomalin, easily extractable glomalin and organic matter in the rhizospheric soil of *A. fruticosa* were all shown to increase significantly, and the acid phosphatase activity and available phosphorus content in the rhizospheric soil were much higher than those of the control area. In addition, mycorrhizal inoculation is conducive to the activation of rhizosphere soil nutrients, modification of the degraded soil, and improvement of fertilizers in the coal mining subsidence area, which is helpful for plant growth. Mycorrhizal inoculation also significantly increases the content of microorganisms in the rhizospheric soil, and improves the rhizospheric micro environment of the host plants, which has great practical significance for the restoration and improvement of soil productivity and ecological reconstruction.

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REFERENCES

Auge, R.M., Water relations, drought and vesicular arbuscular mycorrhizal symbiosis, *Mycorrhiza*, 2001, vol. 11, pp. 3–42.

Bao, S.D., *Analysis of Soil Agriculture Chemistry*, Beijing: China Agriculture Press, 1999, pp. 81–87.

Chen, X., Tang, J.J., Zhi, G.Y., and Hu, S.J., Arbuscular mycorrhizal colonization and phosphorus acquisition of plants: Effects of coexisting plant species, *Appl. Soil Ecol.*, 2005, vol. 28, pp. 359–369.

Eissenstat, D.M., Wells, C.E., and Yanai, R.D., Building roots in a changing environment: Implications for root longevity, *New Phytol.*, 2000, vol. 147, pp. 3–42.

Fokoma, R., Adamou, S., Teugwa, M.C., Begoude Boyogueno, A.D., Nana, W.L., Ngonkeu, M.E.L, Tchameni, N.S, Nwaga, D., Tsala Ndzomo, G., and Amvam Zollo, P.H., Glomalin-related soil protein, carbon, nitrogen and soil aggregate stability as affected by land use variation in the humid forest zone of south Cameroon, *Soil Tillage Res.,* 2012, vol. 120, pp. 69–75.

Gao, Y.Z., Cheng, Z.X., Ling, W.T., and Huang, J., Arbuscular mycorrhizal fungal hyphae contribute to the uptake of polycyclic aromatic hydrocarbons by plant roots, *Bioresource Technol.*, 2010, vol. 101, pp. 6895–6901.

Hajboland, R., Aliasgharzadeh, N., Laiegh, S.F., and Charlotte, P., Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants, *Plant Soil*, 2010, vol. 331, pp. 313–327.

Janos, D.P., Garamszegi, S., and Beltran, B., Glomalin extraction and measurement, *Soil Biol. Biochem.*, 2008, vol. 40, pp. 728–739.

Joner, E.J., Briones, R., and Leyval, C., Metal-binding capacity of arbuscular mycorrhizal mycelium, *Plant Soil*, 2002, vol. 226, pp. 227–234.

Karanika, E.D., Mamolos, A.P., Alifragis, D.A., Kalburtji, K.L., and Veresoglou, D.S., Arbuscular mycorrhizas contribution to nutrition, productivity, structure and diversity of plant community in mountainous herbaceous grassland of northern Greece, *Plant Ecol.,* vol. 199, pp. 225–234.

Kaya, C., Higgs, D., Kima, K.H., and Tas, I.,Mycorrhizal colonization improves fruit yield and water use efficiency in water melon (*Citrullus lanatus* Thunb.) grown under well-watered and water-stressed conditions, *Plant Soil*, 2003, vol. 253, no. 2, pp. 287–292.

Kohler, J., Caravaca, F., and Roldan, A., Effect of drought on the stability of rhizosphere soil aggregates of *Lactuca sativa* grown in a degraded soil inoculated with PGPR and AM fungi, *Appl. Soil Ecol.*, 2009, vol. 42, pp. 160–165.

Miller, R.M. and Jastrow, J.D., Mycorrhizal fungi influence soil structure, in *Arbuscular Mycorrhizas: Physiology and Function*, Kapulnik, Y., Ed., Dordrecht: Kluwer, 2000, pp. 4–18.

Miller, R.M., Miller, S.P., Astrow, C.B., and Rivetta J.D., Mycorrhizal mediated feedbacks influence net carbon gain and nutrient uptake in *Andropogon gerardii, New Phytol.*, vol. 155, pp. 149–162.

Newsham, K., Fitter, A.H., and Watkinson, A.R., Multifunctionality and biodiversity in arbuscular mycorrhizas, *Trends Ecol. Evol.*, 1995, vol. 110, pp. 407–411.

Phillips, J.M. and Hayman, D.S., Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection, *Trans. Br. Mycol. Soc.*, 1970, vol. 55, pp. 158–161.

Qian, B., Liu, L., and Xiao, X., Comparative tests on different methods for content of soil organic matter, *J. Hehai Univ., Nat. Sci.*, 2011, vol. 39, no. 1, pp. 34–38.

Rillig, M.C. and Steinberg, P.D., Glomalin production by an arbuscular mycorrhizal fungus: A mechanism of habitat modification, *Soil Biol.Biochem.*, 2002, vol. 34, pp. 1371–1374.

Rillig, M.C. and Mummey, D.L., Mycorrhizas and soil structure, *New Phytol.*, 2006, vol. 171, pp. 41–53.

Rilling, M.C., Wosten, H.A.B., and Philip, S., Role of proteins in soil carbon and nitrogen storage: Controls on persistence, *Biogeochemistry*, 2007, vol. 85, pp. 25–44.

Schnabel, W.E. and White, D.M., The effect of mycorrhizal fungi on the fate of aldrin: Phytoremediation potential, *Int. J. Phytoremed.*, 2001, vol. 3, pp. 221–241.

Singh, A.N. and Singh, J.S., Experiments on ecological restoration of coal mine spoil using native trees in a dry tropical environment, India: A synthesis, *New Forests*, 2006, vol. 31, pp. 25–39.

Spohn, M. and Giani, L., Water-stable aggregates, glomalin-related soil protein, and carbohydrates in a chronosequence of sandy hydromorphic soils, *Soil Biol. Biochem.,* 2010, vol. 42, pp. 1505–1511.

Streitwolf-Engel, R., Heijden, M.G.A., Wiemken, A., and Sanders, I.A., The ecological significance of arbuscular mycorrhizal fungal effects on clonal reproduction in plants, *Ecology*, 2001, vol. 8, pp. 2846–2859.

Tang, Y.G., Dai, S.F., and Tang, Z.A., *Study on the Properties and Dynamic Evaluation of Coal in Wugan Coal Ming Area*, Xuzhou: China Univ. of Ming and Technology Press, 1999, pp. 1-8.

Van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolfengel, R., Boller T., Wiemken, A., and Sanders, I.R., Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity, *Nature*, 1998a, vol. 396 (5), pp. 69–72.

Van der Heijden, M.G.A., Bollerr, T., and Wiemken, A., Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure, *Ecology*, 1998b, vol. 79, pp. 2082–2091.

Wright, S.F. and Anderson, R.L., Aggregate stability and glomalin in alternative crop rotations for the central Great Plains, *Biol. Fertil. Soils*, 2000, vol. 31, pp. 249–253.

Wright, S.F. and Upadhyaya, A., A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi, *Plant Soil*, 1998, vol. 198, pp. 97–107.

Xiao, X.Y., Chen, B.D., and Zhu, Y.G., The influences of arbuscular mycorrhizal fungi on growth and mineral nutrition of plants grown in copper mine tailing, *Acta Sci. Circumstant.*, 2006, vol. 26, no. 2, pp. 312–317.

Zhao, L.P. and Jiang, Y., Study of soil phosphatase activity determination method, *Chin. J. Soil Sci.*, 1986, vol. 17, no. 3, pp. 138–142.