# Quantitative Simulation of Damage Roots on Inoculated Alfalfa by Arbuscular Mycorrhiza Fungi

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**Received: November 02, 2016** **Accepted: July 19, 2017**

**Published: December 31, 2017**

***Abstract:*** *Underground mining would cause ground subsidence damage and large amounts of cracks, which would result a loss of surface moisture and nutrient and intensifying drought. There are a few reports about damage to plant roots caused by coal mining. The irregular distribution of plant roots in soil and the different forces generated in process of surface subsidence are difficult to study comprehensively. The technologies to repair damaged plant roots have not been completely perfected yet. Based on quantitative simulation of alfalfa root cut-repair experiment, this paper discusses the influences of inoculated Arbuscular Mycorrhiza Fungi on alfalfa root and the mitigation effects of an inoculation on the growth of alfalfa. Root injured alfalfa were investigated by soil pot experiments. The result indicated that at the same cut degree, the growth situation of inoculated alfalfa is better than the contrast. Compared with the Olsen-P content, at cut level of 0 and 1/3, the sand of inoculated alfalfa has less Olsen-P than contrast, at cut degree of 1/2 and 2/3, the sand of inoculated alfalfa has more Olsen-P than contrast, at degree of 3/4, the sand of inoculated alfalfa has less Olsen-P than contrast, the change trend of Olsen-P content is concerned with the relative strength size of absorb Olsen-P by alfalfa root and dissolve Olsen-P by root exudates and hyphae interstate.*

***Keywords:*** *Quantitative, Damage roots, Arbuscular Mycorrhiza Fungi.*

## Introduction

Arbuscular Mycorrhiza Fungi (AMF) is a kind of beneficial symbiotic microorganism, commonly found in the rhizosphere of terrestrial plants [12], Mycorrhiza hyphae like the roots of plants, bring rhizosphere extended to the surrounding broader soil [9, 10], AMF form lots of hyphae in the soil and promote plant growth by accelerating nutrient transportation to the host plant [17], AMF can also relieving negative influence by heavy metal on host plants, improving soil physical and chemical properties [11], AMF still can make soil small particles into micro-aggregates, maintain soil large aggregates formation, and thus improving the stability of soil structure [18]. Therefore, it not only modifies soil ecological micro-environment, but also improves soil productivity. Bing the biggest coal output and the largest subsidence area in China, Shendong mining area is the largest coal field in China and one of the seven major coalfields in the world now. Large-scale underground mining could cause ground subsidence and large amounts of cracks, which would result a loss of surface moisture and nutrient and intensifying drought. There are a few reports about damage to plant roots caused by coal mining in China and abroad. The main reasons could be the plant roots in soil form a "black box" which is difficult to observe directly. Moreover, the irregular distribution of plant roots in soil and the different forces generated in process of surface subsidence are difficult to study comprehensively. The technologies to repair damaged plant roots have not been completely perfected yet. Although the physical and chemical methods would alleviate

the adverse effects of coal mining to some extent, they cannot fundamentally solve the environmental degradation caused by root damage in coal mining. In addition, these technologies are difficult to expand and apply in large mining area as their cost is too high. The bioremediation method is one of the most popular and advocated methods currently. As a good function of biological fertilizer AMF has greater value and potential in an ecological environment treatment. In the process of coal mining, surface subsidence leads to plant root injury. With the continuous development of the coal industry, underground mining would cause ground subsidence damage and large amounts of cracks, which would result a loss of surface moisture and nutrient and intensifying drought [13]. The soil structure of wasted lands is disturbed, then reduced soil microbial activity, severely damaged plant root and reducing the hold water ability of the soil, causing soil degradation and intensifying desertification. In recent years, there are a few reports about damage to plant roots caused by coal mining around the world, a few studies have been reported about AMF microbial reclamation which used to improve soil matrix and increase the land productivity [4, 14], as well using underground AMF hypha network to alleviate plant root damage has been rarely reported. This paper through quantitative simulation experiment to study effects of root damage by mining subsidence, primary discusses the influences of AMF rhizosphere for plant growth and rhizosphere environment.

## Materials and methods

### *Materials*

The experiment use alfalfa as host plant, *Glomus mosseae* (GM) is selected as inoculant. The culture medium is barren sand, the basic physical and chemical properties are: pH 7.56, Electro conductibility 37.7 μs/cm, Olsen-P 7.65 mg/kg, Rapidly-available potassium 26 mg/kg, and maximum water-holding capacity 17%.

### *Method-Alfalfa Cultivation and Root Treatment*

Two treatments are inoculated (GM) and contrast (CK). Put alfalfa seed soaked in 10% $H_2O_2$ for 10 min, cleaning with deionized water, then accelerating germination in constant temperature incubator and sowing in pot, final singling in 10 plants/pot, after 30 days, remove the root of alfalfa from sand, cut off the ground part and shook the adhesion sand of the root, cut from bottom of the root by length, at the degree of 0, 1/3, 1/2, 2/3, 3/4, and then replant the residual roots harvest after 45 days.

### *Experiment management*

Keeps soil moisture in the level of 60% to 80% of the maximum water-holding capacity. Fertilization level as: $NH_4NO_3$ 100 mg/kg, $KH_2PO_4$ 15 mg/kg, $KNO_3$ 150 mg/kg, respectively fertilized before sowing and after damage treatment.

### *Test items and methods*

Harvest the alfalfa respectively overground and belowground. Olsen-P and rapidly-available potassium content consult by Bao [2], pH and electro conductibility (EC) was adopted deionized water immersion method, root vitality adopts α-amine oxidation [20], acidic phosphatase activities adopts the nitro phenol colorimetric method [7], Mycorrhizal infection rate adopt the method of Phillips and Haymen [16], hyphae density used the grid intersection method [8].

## *Results − effects of alfalfa growth in different treatments*

Different treatment alfalfa after harvest of over ground and belowground fresh weight and dry weight is shown in Fig. 1. The inoculation increased the alfalfa over ground parts and root biomass, in root less degree of injury (1/3 and 1/2), inoculated alfalfa can alleviate the negative impact of the root injury of Mycorrhiza associations, but more than 1/2 root damage degree and the Mycorrhiza effect is reduced. At the same damage degree, growth situation of inoculated (GM) is better than the contrast (CK), with the cut degree of alfalfa root increased, over ground and belowground fresh weight of alfalfa is reduced, and the ratio of over ground and belowground fresh weight gradually increased. It is consistent with the gradually increase of quantitative cut root degree. The dry weight over ground between inoculated and contrast has no obvious difference, probably causes are cut over ground part and growth time after processing is short.

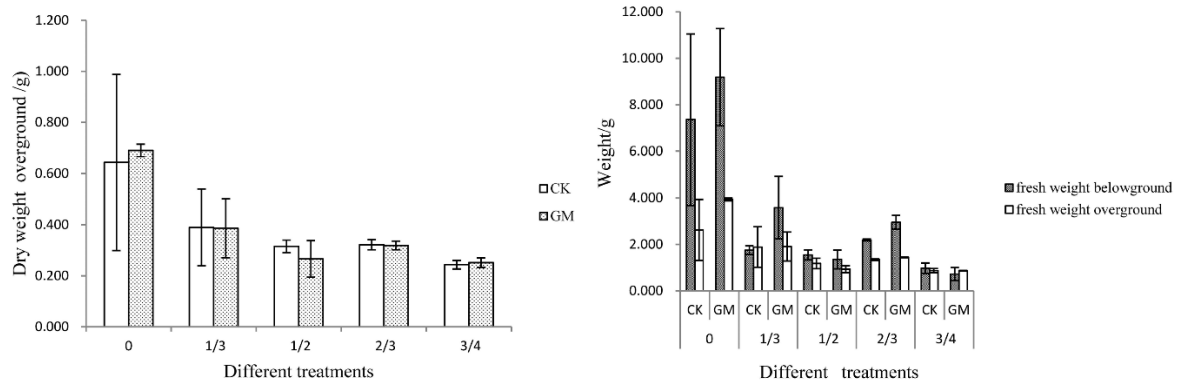


Fig. 1 Effect of AMF on dry weight over ground and fresh weight of alfalfa with damaged root

## *Effects of different treatments on nutrient absorption*

The phosphorus nutrition of plants is affected by the content of available phosphorus in soil and by the interaction of various elements of soil nutritious system [9]. Different damage treatments of rhizosphere soil nutrient absorption are shown in Fig. 2.

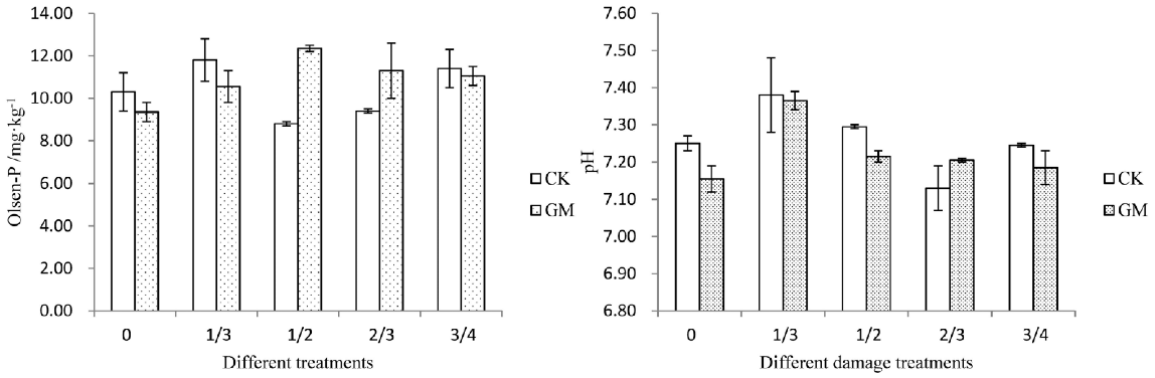


Fig. 2 Olsen-P content and pH in different treatments

At 0 and 1/3 damage treatments, Olsen-P content of inoculated was lower than contrast, since the damage degree of alfalfa root is low and the absorption intensity of alfalfa on Olsen-P is high. At 1/2 and 2/3 damage treatments, Olsen-P content of inoculated was higher than contrast, this situation reflect that root exudates increased after treatment and soil acidification by hyphae rhizosphere decreased soil pH; undissolved phosphorus increased in soil; and the damaged root absorption capacity reduced. In 3/4 damage treatment, Olsen-P content of inoculated was lower than contrast, it is because that excessive root damage degree affects the

ability of carbohydrate supply by root to soil hyphae network, reduced the hyphae acidification capability, decreased the undissolved phosphorus content. Along with the increase of damage degree, the relatively strength size between Olsen-P adsorption by alfalfa root and undissolved phosphorus dissolved by rhizosphere presents the corresponding change, AMF is the main factor which influence the change.

## *Effects of different treatments on Mycorrhiza infection*

Mycorrhiza infection rate is an indicator which reflects the extent to plant roots were infected by Mycorrhiza Fungi, it also reflects the Arbuscular Mycorrhiza plants of affinity degree index. Mycorrhiza Fungi can form good infection effect of alfalfa root, under the same root damage process, the inoculation alfalfa Mycorrhiza infection rate and the density of hyphae were greater than controls. In different root damage treatments, Mycorrhiza infection rate and hyphae density increase and presents the tendency of decrease with the cut degree, Mycorrhiza infection rate from 0 wounded root processing 3/4 to 60% of the 20% of root treatment; hyphae density root damage dealing with 2.47 m/g from 0 to 3/4 root damage dealing with 1.61 m/g. The contrast has no Mycorrhiza infection and hyphae density (Table 1), in the inoculant alfalfa both infection rate and hyphae density decreased along with cut root degree increased, while the root damage affected the combination of Arbuscular Mycorrhiza and roots, the damage affects alfalfa root growth, and then affects carbohydrate supply which hypha growth needed, reduced the soil hyphae network activity.

Table 1. AMF infection situation in different treatments

| Treatments | Damage | Infection, [%] | Hyphae density, [m/g] |
|---|---|---|---|
| CK | 0 | 0c | 0c |
| | 1/3 | 0c | 0c |
| | 1/2 | 0c | 0c |
| | 2/3 | 0c | 0c |
| | 3/4 | 0c | 0c |
| GM | 0 | 60a | 2.47a |
| | 1/3 | 30b | 2.12ab |
| | 1/2 | 30b | 2.02ab |
| | 2/3 | 30b | 1.64bc |
| | 3/4 | 20b | 1.61bc |

## *Effects of different treatments on acid phosphatase activity and root activity*

Soil enzymes is the one of the most important factor for the formation of soil fertility, soil acid phosphatase (APases) can contribute to the transformation of organic phosphorus to the inorganic phosphorus, how much did it content can reflect the soil fertility status of soil phosphorus in particular phosphate fertilizer [15]. Through Fig. 3, in the no-cutting treatment, it is showed that inoculated has higher root activity and acid phosphatase activity than contrast, but not reached significant difference, for the reasons that shook the rhizosphere soil affects root microenvironment; while in cutting treatment, inoculated slightly improved, but the effect was not significant, probably due to the short growth time and lower growth temperature growth. Plant root organs are active absorption and synthesis, root growth and energy level directly affects the growth of aboveground and nutrition and yield levels. Root activity is one of the main indicators to measure the root function, under the condition of alfalfa root damaged, root activity of inoculated alfalfa is significantly higher than the control group, and their difference is remarkable. The main reason is the Arbuscular Mycorrhiza changed root damage alfalfa rhizosphere microenvironment; at the same time improve the

activity of acid phosphatase in the rhizosphere which contributed to the soil, improved soil phosphorus activation.

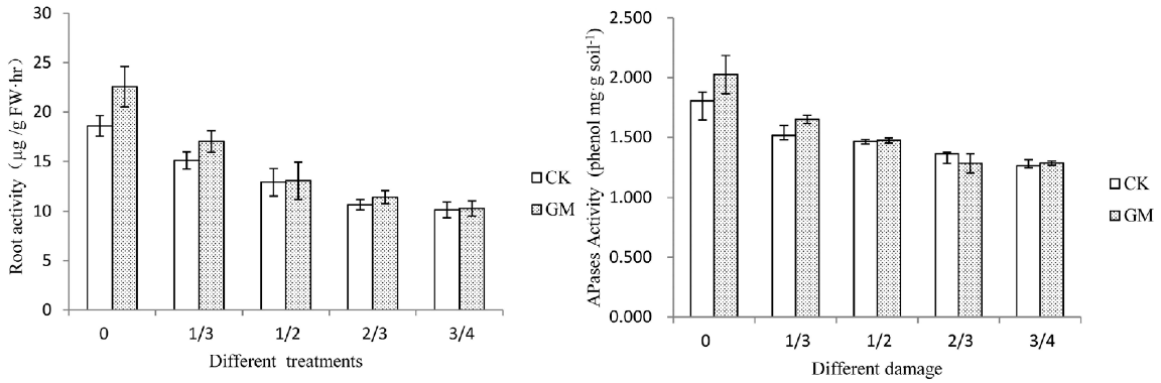


Fig. 3 APases activity and root activity in different treatments

## Discussion

The experiment through quantitative simulation of plant roots damage caused by mining subsidence to study the ecological restoration effect of AMF, due to plant roots in soil form a black box which is difficult to observe. In addition, the irregular distribution of plant roots in soil and the different forces generated in process of surface subsidence are difficult to study comprehensively [1]. The coal mining damage the strata structure, cause distortions, stretching, forming surface crack, it is difficult to quantitative analysis the influences of plant roots damage caused by subsidence, so this experiment used the root length to quantify cut root to simulate the real root damage [14]. Although adopted by weight of root to cut alfalfa root, but in practice, how many roots should be cut is relatively, otherwise it is difficult to get the exact conclusion, in the present research it is difficult to adopt a standard of root damage to simulate practical circumstance, so this experiment has certain practical significance.

Phosphorus is one of the important nutrients in growth and development of plants. In global land reclamation, deficiency of soil available phosphorus is a major factor restricting plant production. The conclusion are Olsen-P content affected by two factors (1) the ability of Olsen-P absorbed, (2) how much Olsen-P dissolve by root secretion and hypha interstate, but the two factors was difficult to define, and root control mechanism is unclear [3, 5]. The pot experiment is discussed along with the increase of root cut degree, the change trend of alfalfa rhizosphere Olsen-P content between contrast and inoculated was discussed, the absorptive action role of hyphae in increasing Olsen-P content and how much it works are still unknown (Fig. 4) in the next step research we need to more on the mechanistic study of it.

Coal mining subsidence area soil ecological management is a complicated system engineering, with the continuous development of science and technology, in view of the mining subsidence area and poor low soil fertility and structure characteristics of various treatment methods are also appeared, including physical and chemical method is one of the main means of soil improvement, the method by changing the permeability of the soil, increase the effective element content of soil, increase the maturity of the soil. But the physical and chemical methods do not have sustainability, can't essentially changing soil maturity in coal mining area [6, 19]. Therefore, it is necessary to look for a biological soil improvement method, realizing mining area soil restoration, change of rhizosphere microenvironment. Due to plant roots in the soil are harder to observation, in the subsidence area in the soil and root distribution regularity, not so in the study of plant roots to repair damaged coal mining subsidence area is difficult to adopt a standard way of root damage

simulation of the actual root damage situation. In this article, through quantitative simulation of plant root system damage of Mycorrhiza Fungi in the soil of mining subsidence area management to provide the reference.

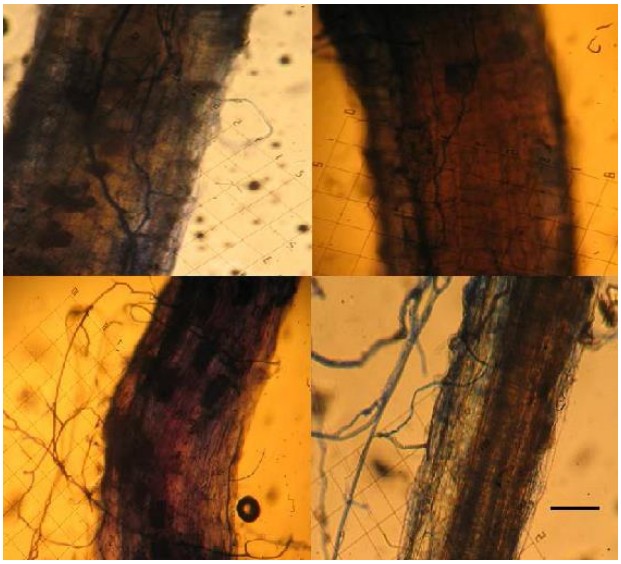

Fig. 4 GM infection on alfalfa root bars = 50μm

## Conclusion

In this study, we taking alfalfa as an object of study and degenerated soil in coal-mining subsidence as culture substrate, analyzed the impact of AMF on mitigating damage to the roots through quantitative simulation that simulates the damage of such subsidence to the root system by man-made root cutting. AMF inoculation improved the micro-environment of rhizosphere and made a contribution to the amelioration and fertilization of degraded soil in the mining area. It will provide technical support for land reclamation and land vegetation reconstruction by studying the ecological effects of AMF on the growth of damaged plants.

## Acknowledgements

*This paper was funded by Natural Science Foundation of China (41401496), Student's Platform for Innovation and Entrepreneurship Training Program (201610704123).*

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