Postprint: Effects of AM Fungal Inoculation on Caragana Growth and Soil Remediation in Coal Mining Subsidence Land in the Western Loess Plateau

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Abstract

With Caragana as the host plant in coal mining subsidence areas, this study investigated the ameliorative effects of inoculating arbuscular mycorrhizal fungi (AM fungi) on Caragana growth and rhizosphere soil. The results showed that: in August, AM fungi inoculation significantly increased plant height, crown width, and ground diameter by 29.11%, 29.83%, and 14.81%, respectively, compared with non-inoculated Caragana; in September, root length, average diameter, root surface area, and root volume in the inoculated area increased by 151.0%, 34.2%, 116.0%, and 129.3%, respectively, compared with the control area. AM fungi inoculation enhanced the stress resistance of Caragana, with soluble sugar content and catalase activity in leaves of inoculated Caragana increasing by 13.4% and 111.1%, respectively, compared with the control area. In August, AM fungi inoculation improved the biological and physicochemical properties of soil, with organic matter, alkaline-hydrolyzable nitrogen, available phosphorus, and available potassium in the inoculated area increasing by 7.06 g/kg, 140.0 mg/kg, 1.82 mg/kg, and 16.72 mg/kg, respectively, compared with the control area; AM fungi inoculation significantly increased the quantities of fungi, actinomycetes, and bacteria, as well as acid phosphatase activity in rhizosphere soil. In summary, AM fungi inoculation promoted the growth of Caragana and soil amelioration in coal mining subsidence areas.

Full Text

Effects of Arbuscular Mycorrhizal Fungi on the Growth of *Caragana korshinskii* and Soil Improvement in Coal Mining Subsidence Areas of the Western Loess Region

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Abstract

This study investigated the effects of inoculating arbuscular mycorrhizal (AM) fungi on the growth of *Caragana korshinskii* and the improvement of rhizosphere soil in coal mining subsidence areas of the western Loess region, using *C. korshinskii* as the host plant. The results demonstrated that AM fungi significantly promoted plant growth. In August, the plant height, crown width, and ground diameter of inoculated *C. korshinskii* increased by 29.11%, 29.83%, and 14.81%, respectively, compared with non-inoculated plants. In September, the root length, average diameter, surface area, and volume of inoculated plants were greater by 151.0%, 34.2%, 116.0%, and 129.3%, respectively, than those of the control group. Inoculation with AM fungi enhanced plant stress resistance; the soluble sugar content and catalase activity in leaves of inoculated plants increased by 13.4% and 111%, respectively. Moreover, AM fungi improved the biological, physical, and chemical properties of soil. In August, organic matter, available nitrogen, phosphorus, and potassium in the inoculated zone increased by 7.06 g/kg, 140.0 mg/kg, 1.82 mg/kg, and 16.72 mg/kg, respectively, compared with the control. Inoculation significantly increased the number of fungi, actinomycetes, and bacteria in the rhizosphere soil, as well as acid phosphatase activity. Therefore, AM fungal inoculation promoted *C. korshinskii* growth and soil improvement in loess coal mining subsidence areas.

Keywords: arbuscular mycorrhizal fungi; *Caragana korshinskii*; rhizosphere soil

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1. Study Area Overview

The Ningtiaota mining area is located in the northwestern part of Shenmu County, Yulin City, Shaanxi Province, on the southeastern edge of the Mu Us Desert, in the transition zone between the Mu Us Desert and the loess hilly-gully region. It belongs to an arid and semi-arid continental monsoon climate zone (110°06 -110°20 E, 38°70 -39°10 N). The area experiences perennial drought with low rainfall, severe soil erosion, frequent strong winds, and sandstorms. Summers are hot and rainy with thunderstorms and gusty winds, while autumns are cool and humid with rapid temperature drops. The coal mining

subsidence area studied is located at the Ningtiaota mining face. Compared with the Daliuta mining area of equivalent scale, which is dominated by sandy soil with aeolian sand parent material, coarse texture, and extremely poor water and nutrient retention capacity, the study area is characterized by loess soil (loessal soil). Loess has well-developed vertical joints, good water permeability, and can accumulate substantial effective moisture after ripening, though its fertilizer retention capacity is relatively poor. Although total phosphorus content is relatively high, the enrichment of active calcium carbonate generally causes phosphorus deficiency during crop growth, and organic matter content is low.

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2. Experimental Materials

The host plant was *Caragana korshinskii* Kom., a leguminous shrub and pioneer species in the mining area, known for its drought tolerance, sand burial resistance, and good branching and fruiting characteristics. The AM fungal inoculant was *Glomus mosseae* (G.m), provided by the Microbiology Laboratory of the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, and proliferated in the Microbial Reclamation Laboratory of China University of Mining and Technology. The inoculant had a hyphal density of 5.97 m/g and a spore density of [value missing]/10g.

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3. Experimental Design

The experiment consisted of an inoculated zone (G.m) and a control zone (CK) with identical topography and soil conditions. *C. korshinskii* was planted in May 2014. The total area of both zones was 11,512 m^2, with row and plant spacing of [value missing]. Seeds were buried in planting holes. In May 2015, 50 g of inoculant was reapplied along with *C. korshinski* seedlings, followed by weekly watering to reach soil maximum water-holding capacity. After three months, watering was stopped to allow natural conditions. In August and September 2015, randomly selected *C. korshinskii* plants were measured for height, ground diameter, and leaf color values (SPAD), with marked plants monitored in both inoculated and control zones.

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4. Sample Collection

In August and September, five sampling points with similarly sized plants were randomly selected and marked in both inoculated and control zones. Fresh rhizosphere soil samples were collected, numbered, and sealed in ziplock bags for laboratory analysis. After air-drying, rhizosphere soil was sieved to remove debris. Fresh soil and leaves were also collected: leaves were sealed in ziplock bags and stored at 4°C for analysis of stress resistance indicators (soluble sugar

content and catalase activity), while fresh soil samples were used to determine microbial numbers and acid phosphatase activity.

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5. Measurement Indicators and Methods

Plant Growth and Root Indicators: Plant height and crown width were measured with a steel ruler; ground diameter was measured with vernier calipers. Root morphological characteristics were obtained using a CI-600 root monitoring system, which scans images of root distribution at different depths or soil profiles. Root analysis software RootSnap Version 1.2.9.30 was used to obtain various root growth indicators.

Mycorrhizal Infection Rate and Hyphal Density: Mycorrhizal infection rate was determined using the Phillips and Hayman method: roots were stained with trypan blue, and the number of infected root segments was examined under a microscope. Infection rate = (number of infected root segments / total root segments examined) × 100%. Hyphal density was measured using a vacuum pump micropore membrane filtration method and grid intersection method.

Rhizosphere Soil Biological and Physical-Chemical Indicators: Soil pH was measured with a glass electrode at a soil:water ratio of 2.5:1. Soil electrical conductivity (EC) was determined by electrical conductivity meter. Organic matter content was measured using the potassium dichromate external heating method (K Cr O -H SO). Available nitrogen was determined by the alkaline hydrolysis diffusion method. Available phosphorus was measured by the molybdenum-antimony anti-colorimetric method with 0.5 mol/L NaHCO . Available potassium was determined by flame photometry with 1.0 mol/L NH OAc. Soil microbial numbers were measured using conventional dilution plate methods: bacteria on beef extract peptone medium, actinomycetes on modified Gause' s No. 1 medium, and fungi on Bengal red medium. Acid phosphatase activity was measured using the improved Tabatabai & Brimner method, expressed as phenol production per gram of soil per hour (mg/g · h). Leaf color values were measured with a SPAD-502 chlorophyll meter.

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6. Other Indicator Calculations

Leaf Color Value: The mean value of leaf color measurements from different canopy layers was used as the whole plant leaf color value.

Photosynthetic Rate: Measured using an LI-6400XT photosynthesis system.

Soluble Sugar Content: Determined by anthrone colorimetry.

Catalase Activity: Measured by spectrophotometry using phosphate buffer extraction of crude enzyme solution.

Mycorrhizal Contribution Rate (%) = [(Inoculated plant indicator - Non-inoculated plant indicator) / Inoculated plant indicator] × 100%.

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7. Data Processing Methods

Data were processed using Excel 2007 and SPSS 17.0. Single-factor ANOVA (LSD) was performed on processed data with significance level at $p < 0.05$.

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1. Effects of AM Fungi Inoculation on *C. korshinskii* Growth

AM fungi significantly promoted *C. korshinskii* growth. In August, plant height, crown width, and ground diameter in the inoculated zone (G.m) were significantly greater than in the control zone (CK), increasing by 29.11%, 29.83%, and 14.81%, respectively ($p < 0.05$). In September, these parameters also showed inoculated zone > control zone, but differences were not significant. The mycorrhizal effect gradually weakened as the plant grew into later stages. shows the detailed effects of AM fungi on *C. korshinskii* growth.

TABLE:1 Effect of AM fungi on growth of *Caragana*

Monitoring Index	August (G.m)	August (CK)	September (G.m)	September (CK)
Plant Height	29.36±1.37a	22.74±1.10b	32.76±1.26ab	30.12±0.62a
Crown Width	24.46±1.16a	18.84±0.71b	26.64±1.27ab	23.22±1.26a
Ground Diameter	0.31±0.009a	0.27±0.009b	0.33±0.010ab	0.32±0.007a

Values are means of replicates ± SE; different letters indicate significant differences at p* < 0.05.*

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2. Effects of AM Fungi Inoculation on Root Infection Rate and Hyphal Density

Root infection rate reflects the intimate association between AM fungi and plants. This study found that infection rates in the inoculated zone were significantly higher than in the control zone: 56.0% in August and 61.3% in September, with hyphal densities of 4.09 m/g and 4.36 m/g, respectively. AM fungi formed a good mutualistic symbiosis with *C. korshinskii*, establishing a dense hyphal network in soil that enhances nutrient absorption. [Figure 1: see original paper] and [Figure 2: see original paper] illustrate these effects.

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3. Effects of AM Fungi Inoculation on *C. korshinskii* Root Growth

Roots are vital organs for water and nutrient absorption, and their development affects plant growth and survival. AM fungi stimulated *C. korshinskii* root growth. Root length, average diameter, surface area, and volume in the inoculated zone were significantly greater than in the control zone, with increases of 151.0%, 34.2%, 116.0%, and 129.3%, respectively. Although root tip numbers were higher in the inoculated zone, differences were not significant. The mycorrhizal contribution rates for root length, average diameter, surface area, volume, and tip number were 60.2%, 25.5%, 53.7%, 56.4%, and 6.9%, respectively. details the effects on root growth.

TABLE:2 Effect of AM fungi on growth of *Caragana* root

Parameter	Inoculated	Control
Root Length (cm)	194.30±20.51a	77.41±9.45b
Mean Diameter (mm)	2.00±0.19a	1.49±0.03b
Root Surface Area (cm^2)	158.34±11.76a	73.32±21.76b
Root Volume (cm^3)	6.58±1.18a	2.87±0.92b
Root Tips Number	100±25.48a	87±27.27a

Values are means ± SE; different letters indicate significant differences at $p^* < 0.05$.*

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4. Effects of AM Fungi Inoculation on *C. korshinskii* Stress Resistance

Leaf color value reflects chlorophyll content and is an important factor determining photosynthetic rate. Inoculated *C. korshinskii* showed significantly higher leaf color values and photosynthetic rates than the control group. Soluble sugar content and catalase activity in inoculated leaves increased by 13.4% and 111.1%, respectively, compared with the control. Soluble sugar is an important osmotic regulator; under drought stress, its accumulation reflects adaptation to adverse environments. Catalase activity indicates the capacity to scavenge harmful oxygen free radicals. The mycorrhizal contribution rates for soluble sugar and catalase activity were 6.9% and 68.4%, respectively, while leaf color value contribution was smaller (11.8%), likely because plants were in the leaf-fall period when mycorrhizal effects diminished. presents these results.

TABLE:3 Effect of AM fungi on stress resistance of *Caragana*

Parameter	Inoculated	Control	Mycorrhizal Contribution Rate (%)
Leaf Color Value (SPAD)	40.34±0.527a	37.54±1.112b	11.8
Soluble Sugar Content (g/kg)	0.38±0.022a	0.12±0.018b	6.9

Parameter	Inoculated	Control	Mycorrhizal Contribution Rate (%)
Catalase Activity (U/kg · min)	1.27±0.035a	1.12±0.049b	68.4
Photosynthetic Rate (mol/m² · s)	0.19±0.018a	0.09±0.018b	-

Values are means ± SE; different letters indicate significant differences at p* < 0.05.*

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5. Effects of AM Fungi Inoculation on Soil Properties in Mining Areas

Soil microorganisms play crucial roles in element transformation and nutrient cycling in the rhizosphere microenvironment. Inoculation significantly increased soil microbial numbers: fungi, actinomycetes, and bacteria in the inoculated zone were 2.23, 1.53, and 2.98 times those in the control zone, respectively. Acid phosphatase activity, which is key to releasing plant-available phosphorus, increased by [value missing] in the inoculated zone, explaining the higher available phosphorus content.

Soil nutrient content also improved significantly. In August, organic matter, available phosphorus, and available potassium in the inoculated zone increased by 7.06 g/kg, 140.0 mg/kg, and 16.72 mg/kg, respectively, compared with the control. Soil pH was significantly lower in the inoculated zone, reducing alkalinity and activating base cations. Electrical conductivity was also significantly higher in the inoculated zone in August, though differences diminished by September, possibly because mycorrhizal effects weakened during the plant' s leaf-fall period when spores gradually formed. and summarize soil property changes.

TABLE:4 Effects of AM fungi on physical and chemical properties of soil in mining area

Parameter	August (G.m)	August (CK)	September (G.m)	September (CK)
pH	8.66±0.099a	9.04±0.060b	8.69±0.075a	8.79±0.052a
EC (mS/cm)	0.15±0.006a	0.12±0.002b	0.16±0.005a	0.15±0.004a
Organic Matter (g/kg)	12.12±1.41a	5.06±0.949b	16.15±1.69a	12.13±1.87b
Available N (mg/kg)	260.0±21a	120.0±11b	210.0±15a	130.0±8b

Parameter	August (G.m)	August (CK)	September (G.m)	September (CK)
Available P (mg/kg)	3.49±0.165a	1.67±0.202b	3.55±0.237a	2.33±0.148b
Available K (mg/kg)	52.92±6.46a	36.20±2.89b	54.46±1.99a	44.26±3.35b

TABLE:5 Effects of AM fungi on soil micro-environment in mining area

Parameter	Inoculated	Control
Fungi (×10 cfu/g)	2.90±0.690a	1.30±0.125b
Actinomycetes (×10 cfu/g)	5.67±0.369a	3.71±0.275b
Bacteria (×10 cfu/g)	4.08±0.717a	1.37±0.254b
Acid Phosphatase (g/g · h)	3.87±0.225a	3.09±0.107b

Values are means ± SE; different letters indicate significant differences at p* < 0.05.*

Discussion

Coal mining at Ningtiaota causes surface subsidence and fissures that damage *C. korshinskii* roots, severely impacting vegetation in this already fragile ecosystem. This study demonstrates that AM fungi can form an intimate symbiosis with *C. korshinskii*, establishing extensive hyphal networks that increase the absorption area for nutrients and water, thereby promoting both root and shoot growth. Similar results have been reported for *Artemisia sphaerocephala* and *Amorpha fruticosa* inoculated with AM fungi.

Drought is a primary environmental stress factor in mining areas. Under drought stress, plants accumulate soluble sugars to regulate cellular osmotic pressure and protect enzyme systems. Catalase, a protective enzyme, scavenges H O to reduce damage to leaf cell membranes. This study found that AM fungi significantly increased soluble sugar content and catalase activity in *C. korshinskii*, greatly enhancing drought resistance. These findings align with previous studies on wheat, *Onobrychis viciifolia*, maize, and peony.

The Ningtiaota mining area, located in a loess gully region, suffers severe soil nutrient and water loss due to coal mining. Soil organic matter, nitrogen, phosphorus, and potassium are essential nutrients for plant growth and indicators of soil fertility. Soil microorganisms are the most active components, participating in organic matter decomposition, humus formation, and nutrient transformation.

Soil enzyme activity reflects biological activity and biochemical reaction intensity, indicating metabolic vigor. This study found that AM fungi significantly increased available nutrients, microbial numbers, and acid phosphatase activity, consistent with previous research. AM fungi thus provide an effective approach for improving degraded soils in ecologically fragile mining areas.

Conclusions

AM fungi exhibited excellent remediation effects on degraded loess soil and *C. korshinskii* growth in the Ningtiaota coal mining subsidence area. The main conclusions are:

1. AM fungi formed a strong mutualistic symbiosis with *C. korshinskii*, significantly increasing plant height, crown width, and ground diameter compared with the control.
2. AM fungi stimulated root growth, significantly increasing root length, average diameter, volume, and tip numbers in the inoculated zone.
3. AM fungi enhanced plant stress resistance, significantly increasing soluble sugar content and catalase activity, thereby improving drought tolerance.
4. AM fungi improved soil biological and physicochemical properties, increasing organic matter, available nitrogen, phosphorus, and potassium, as well as significantly enhancing fungal, actinomycete, and bacterial populations and acid phosphatase activity.

This study focused on the remediation effects of AM fungi on *C. korshinskii* in loess mining areas. Further research should investigate effects on other vegetation types and different soils to fully understand the ecological restoration potential of AM fungi in coal mining subsidence areas.

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