

EFFECTS OF COMBINED MICROBIAL AND PLANT REMEDIATION ON WATER TRANSPORT AND NUTRIENT UPTAKE IN HEAVY METALPOLLUTED MINING AREAS

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Abstract

This study investigated the changes in soil water transport and nutrient content under plant-microbe synergistic remediation in a mining area. To restore and improve heavy metal contaminated soils, three enriched plant species were used in combination with microbial agents. Results showed that herbaceous plants significantly improved soil texture in the short term. The highest soil moisture was observed in the 0-20 cm layer, especially in the AH treatment (EM fungi + Vetiver grass + Seabuckthorn). AH also reduced soil pH most effectively. The AX treatment (EM fungi + Vetiver grass) increased soil organic matter by 25.7% compared to the control. Rhizobia combined with shrub grass planting enhanced soil nitrogen. Both AX and AH significantly increased effective phosphorus (57.1 and 55.6 mg/kg) and available potassium (194 and 174 mg/kg), as well as total microbial counts (4.071×10^7 and 3.561×10^7 cells/g), showing increases of 94.8 and 70.4%, respectively, over the control. Overall, AH and AX treatments were most effective in improving soil moisture and quality in contaminated mining areas.

Introduction

The coal mining area in northern Shaanxi is a transitional area between the Loess Plateau and the Inner Mongolian Plateau. It is rich in energy and mineral resources and known as "Kuwait in China". Its unique geological conditions, geographical location, climate characteristics and the development of coal has led to multiple ecological and geological problems in the local area (Li *et al.* 2025, Zhang and Lv 2025).

The coal mining area is facing multiple challenges such as water and fertilizer scarcity, and ecological degradation and urgently needs breakthroughs in systematic restoration technologies. When heavy metals accumulate to a certain extent in soil, they can cause toxicity to the soil plant system. In addition, cadmium, arsenic, lead, copper, zinc and other substances can easily pose a threat to human health through crops and the food chain (Arora *et al.* 2008). The main purpose of heavy metal remediation in mining areas was to reduce pollution risks or decrease the total amount of heavy metals (Luo *et al.* 2025). At present, most studies have focused on exploring the changes in heavy metal content after heavy metal remediation in mining areas. Han *et al.* (2024) found that in soil contaminated with 400 mg/kg of Pb, joint remediation using plants and microbes reduced the heavy metal concentration by 19.61 and 19.48% after 30 and 60 days of treatment, respectively. Zhang (2021) found that microorganisms have a significant promoting effect on plant remediation of soil lead pollution. Pang *et al.* (2022) effectively enriched Cd, Cs, Pb, Zn and Cu by planting heavy metal accumulating plants, promoting the transport of water and nutrients in the soil. Li *et al.* (2021) found that microbial agents could accelerate plant uptake of heavy metals, reduce heavy metal residues in soil and improve soil fertility. Jin *et al.* (2018) studied the combined remediation of heavy metal contaminated soil by active bacteria and herbaceous plants,

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significantly improved the remediation rate and rapidly improved soil quality. Yin and Hu (2023) analyzed the combination of biochar and *Bacillus subtilis* effectively promoted the growth of *Artemisia annua* in Cd contaminated soil, improved soil quality and had a promoting effect on each other.

Planting tolerant or accumulating plants can promote the biological recovery of soil systems or reduce heavy metal content in soil, thereby improving soil quality. Microbial remediation can effectively improve the physical properties of soil, enhance volumetric mass, aeration and porosity. The combination of soil microorganisms and plant secretions can improve soil structure. While single biological restoration is common in mining areas, joint plant-microbe approaches remain underexplored. Therefore, this study investigated the combined effects of two plants, vetiver and seabuckthorn and three microbial agents, rhizobia, *Bacillus subtilis* and EM fungi, to improve soil fertility and soil moisture transport in the coal mining area of northern Shaanxi, aiming to clarify their role in ecological restoration.

Materials and Methods

The experimental site was located in the Fugu coal mining area of Shaanxi Province. Ten treatment groups were established (Table 1) and randomly arranged in 4m × 4m residential areas. The microbial agents Rhizobia, *Bacillus subtilis* and EM bacteria were obtained from Hebei New Century Zhoutian Biotechnology Co., Ltd. Vetiver and seabuckthorn were the test plants. The bacterial agent was applied at a rate of 50 kg/acre. The bacterial solution was diluted and the root systems of soil removed seedlings were immersed to ensure sufficient contact. After transplanting the roots were irrigated with residual solution. The row spacing of the planted plants was set at 40 cm × 50 cm.

Table 1. Experimental scheme settings.

Main	Vetiver grass (X)	Seabuckthorn (S)	Vetiver grass + seabuckthorn (H)
	Rhizobium bacteria (GX)	Rhizobium bacteria (GS)	Rhizobium bacteria (GH)
Deputy	<i>Bacillus subtilis</i> (QX)	<i>Bacillus subtilis</i> (QS)	<i>Bacillus subtilis</i> (QG)
	EM fungi (AX)	EM fungi (AS)	EM fungi (AH)
Compare	Did not add any materials (CK)		

Soil samples were collected and background values were measured before conducting the experiment. After planting, the growth of the experimental plants was monitored monthly. Plant and soil samples were collected to investigate the changes in plant community characteristics at the end of the experiment. Ten plant samples were randomly collected to determine biomass, length of the main root system, number and weight of fibrous roots. Undisturbed soil was sampled using the ring knife method to measure saturated moisture content. Soil samples were collected using a snake shaped pattern from four points per plot, every 20 cm to a depth of 0-40 cm. Two kg of mixed soil samples from each layer were divided into two equal parts. One part of soil sample was sealed in plastic bags and stored cold for enzyme activity, soil microbial species, and total microbial count. Samples were sealed, dried indoors in thin layers, regularly turned, cleaned of debris, ground, sieved and stored as required for testing.

According to the method proposed by the Editorial Committee of the State Environmental Protection Administration (2002), moisture content, organic matter, pH texture, total nitrogen, available phosphorus, and available potassium were tested. Microorganisms were measured by Shanghai Meiji Biomedical Technology Co., Ltd.

Results and Discussion

The entire experimental site had sandy loam soil, but remediation treatments increased clay content compared to the control (CK), with GX showing the highest clay and lowest sand content, followed by AX (Table 2). At the same time, planting seabuckthorn also increased the clay content in the soil to a certain extent. However, overall, herbaceous plants improved soil texture more rapidly, mainly due to their fast growth, active roots enhancing soil structure and root exudates promoting beneficial biochemical and physicochemical soil reactions. Wang *et al.* (2024) showed that vetiver had a large root system, which not only held the soil but also allowed dead branches, fallen leaves and broken fibrous roots, which remained in the soil, increased soil organic matter and improved soil physical and chemical properties.

Table 2. Soil texture under different treatments.

Treatment	Clay (<2 μm) (%)	Silt (2-50 μm) (%)	Sand (50-2000 μm) (%)	Texture
CK	0.46	31.16	68.38	Sandy loam soil
AX	0.51	33.71	65.78	Sandy loam soil
AS	0.49	33.03	66.48	Sandy loam soil
AH	0.46	32.29	67.25	Sandy loam soil
QX	0.50	33.54	65.96	Sandy loam soil
QS	0.45	32.91	66.64	Sandy loam soil
QH	0.44	32.99	66.57	Sandy loam soil
GX	0.52	35.03	64.45	Sandy loam soil
GS	0.48	33.55	65.97	Sandy loam soil
GH	0.50	34.72	64.78	Sandy loam soil

The soil moisture of different microorganisms and vetiver plants showed a decreasing trend (Fig. 1). This indicated that after planting shrub seabuckthorn, root water absorption occurred in deeper soil layers. The root system of herbaceous plants mainly grew at 30-50 cm, resulting in lower water content than other soil layers. Overall analysis showed that the AH treatment had relatively higher soil layers compared to other treatments. This was beneficial for improving soil quality in mining areas, promoting plant growth, and ultimately enhancing the efficiency of mining area restoration.

After applying microbial agents, the soil pH in the mining area was restored (Fig. 2). Based on the control group (CK) and the soil pH, the soil in the coal mining area was strongly alkaline with a pH above 8.5. Compared to control (CK), the pH value of different microbial agent plant combined remediation treatments decreased significantly by 1.01-1.51, which was basically neutral, indicating that microbial agent plant combined remediation could improve the acidity and alkalinity of soil in coal mining areas. Among the treatments, AH had the most significant effect on regulating soil pH value, with the lowest pH value of only 7.14 followed by AX and QX, with pH values of 7.32 and 7.35, respectively. Overall, soil remediation agents combined herbaceous plants significantly improved soil pH.

The organic matter content treated with AX was the highest, increasing by 25.7% compared to CK (Fig. 6). The soil organic matter content under the treatment of vetiver grass was significantly higher than that under the planting of sea buckthorn and mixed planting treatments, indicating that microorganisms + vetiver grass have a significant promoting effect on the increase of soil organic matter content.

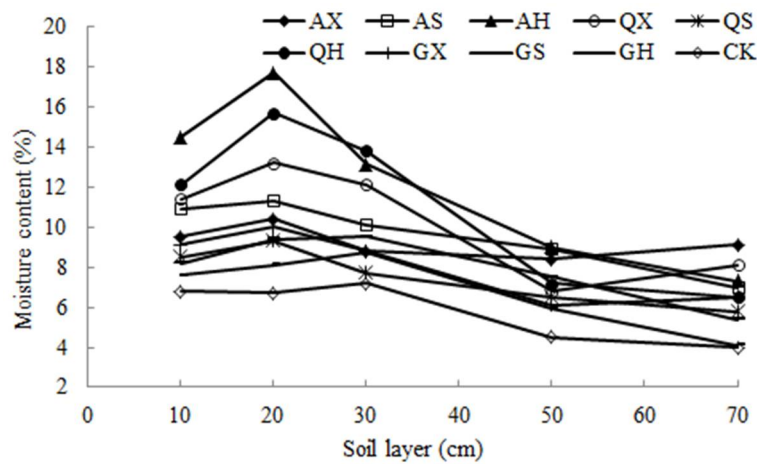


Fig. 1. Impact of different remediation methods on the moisture content.

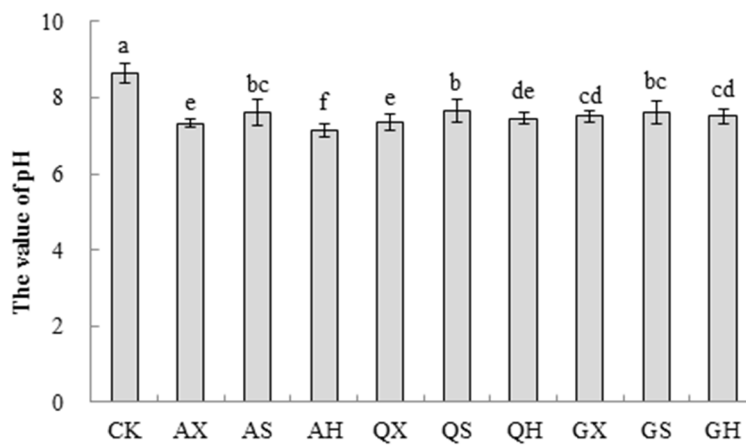


Fig. 2. Effects of different microbial-plant combined remediation on soil pH.

The content of total nitrogen, available phosphorus, and potassium in soil is a key indicator for measuring soil fertility. The total nitrogen content of different treatments was significantly higher than that of the control CK, but there were differences in the enhancement effect of different microbial agents (Fig. 3). The synergistic effect of rhizobia was most prominent, followed by EM bacteria, but the effect of *B. subtilis* was relatively weak. In terms of plant types, the soil nitrogen accumulation in seabuckthorn treatment was significantly higher than that in vetiver treatment, with GH and GS treatments reaching 0.54 and 0.51 g/kg, respectively. Comprehensive data showed that the rhizobia combined with irrigation grass mixed planting had the best nitrogen fixation effect. The nitrogen promoting effect of seabuckthorn under the same bacterial agent treatment was always better than that of vetiver grass. This might be due to the efficient symbiotic system between rhizobia and seabuckthorn roots, while their interaction with vetiver was weaker. In addition, the effective phosphorus and available potassium contents of each treatment were significantly increased compared to CK, with AX showing the largest increase. Its effective phosphorus was increased by 139.9% (57.1 mg/kg) and available potassium by 139.9%

(194 mg/kg), compared to CK. AH ranked second, with effective phosphorus and available potassium contents of 55.6 and 178 mg/kg, respectively (Fig. 3).

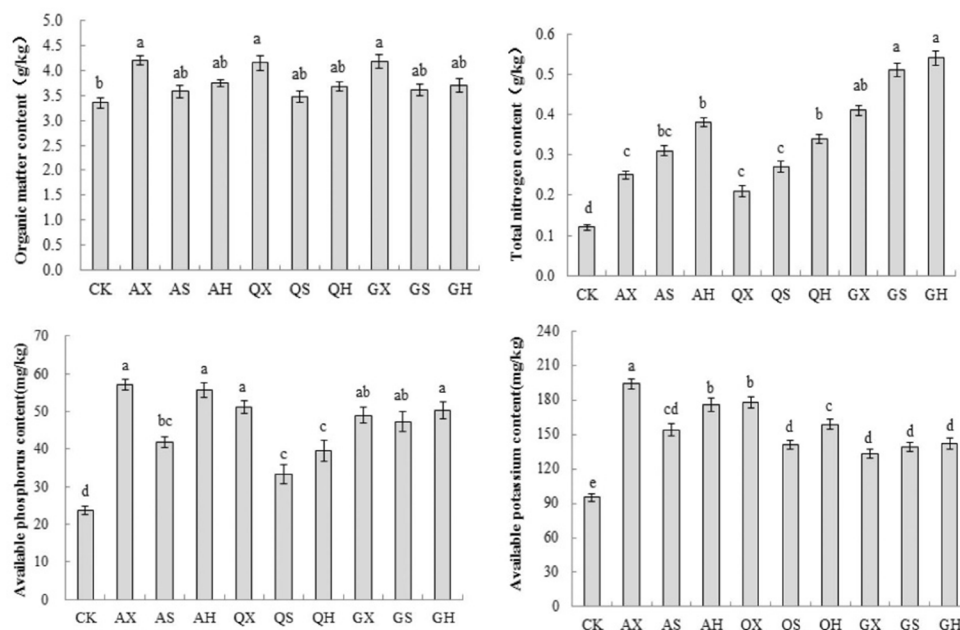


Fig. 3. Impact of different remediation methods on soil nutrients in mining areas.

Through comprehensive analysis, it was found that the combined remediation of different microorganisms and plants significantly improved the soil nutrients in coal mining areas. Among them, herbaceous plants showed better effects than shrubs, possibly due to their shorter planting years and rapid growth, which might facilitate nutrient exchange with the soil and achieved balanced symbiosis. Rhizobia could significantly increase nitrogen content among different microbial agents, but the comprehensive effect of EM bacteria was the best. Considering the overall nutrient level of the soil, AX and AH treatments had the best effect on soil nutrients.

The combined planting and restoration of microorganisms and plants had a significant impact on the number of bacteria and fungi in the soil of the mining area (Fig. 4). Under different planting methods, the treatment of a single vetiver significantly increased the number of bacteria in the soil of the mining area compared to other planting methods. AX, GX and QX increased by 35.0, 51.4 and 24.5%, respectively. Comparative analysis showed that under the combined action of microorganisms and plants, AX and AH treatments had the most significant impact on the total amount of soil bacteria and achieved significant differences compared to other treatments ($P < 0.05$). The variation pattern of fungal abundance in different soils was like that of bacteria, manifested as $AX > AH > GX > GH > AS > QS > QX > GS > CK > QH$. From the analysis of planting methods, vetiver > mixed species > seabuckthorn. From the analysis of microbial species, EM bacteria > *Bacillus subtilis* > Rhizobia. Through comprehensive analysis, it was found that AX and AH had the highest total microbial count, at 4.071×10^7 and 3.561×10^7 cells/g, respectively, which increased by 94.8 and 70.4%.

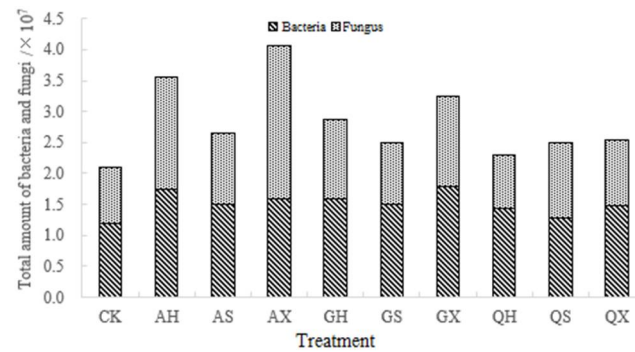


Fig. 4. Impact of different remediation methods on soil microbial biomass.

The soil microbial diversity index could be used to represent changes in environmental quality and analyze the stability of communities. Different experimental treatments increased the microbial diversity of the soil in the mining area, compared to control (Fig. 5). The mixed planting treatment had a significant impact on the soil microbial diversity index, with GH and AH treatments having the highest Shannon indices of 6.31 and 4.62, respectively, and reaching significant differences compared to other treatments ($P < 0.05$). The dominance index of CK treatment changed the most, with significant differences compared to other treatments ($P < 0.05$), while the diversity index of other treatments showed no significant differences (Fig. 6).

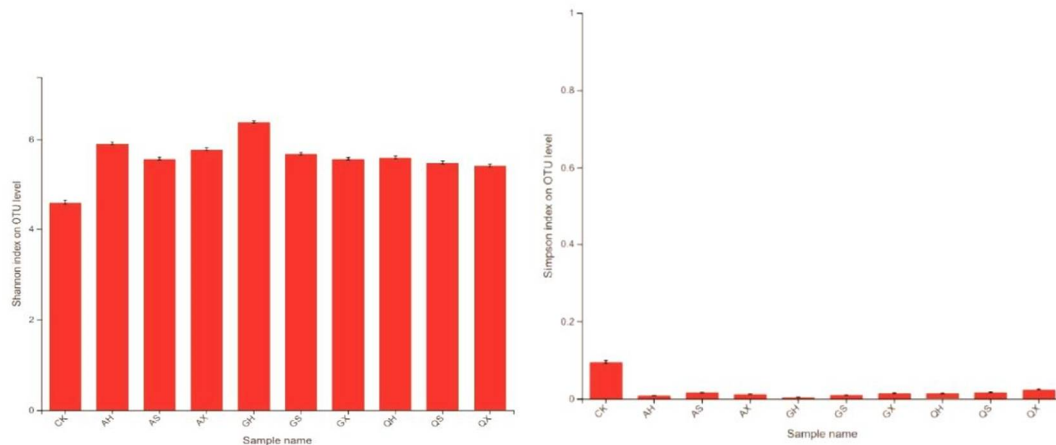


Fig. 5. Impact of different treatments on microbial diversity and dominance.

The formation of soil is one of the most important factors for the restoration of ecological functions in mining areas. However, the low fertility of reconstructed soil after mining in mining areas leads to difficulties in plant growth and serious disruption of ecological balance (Huang *et al.* 2019, Bi *et al.* 2020). According to research, implementing microbial remediation or long-term plant remediation in mining areas can effectively improve soil fertility and increase plant diversity (Yan *et al.* 2007). This is consistent with the results of this study. Research has shown that microbial plant joint remediation can significantly improve soil nutrients in coal mining areas and increase plant diversity in the sample area. Among them, the coverage of plants in the sample area

planted with a mixture of microbial agents and grass irrigation is the highest. The application of microbial fertilizers can increase the organic matter content in soil, improve the living environment of microorganisms, continuously provide nutrients for microorganisms, and promote their growth (Quan *et al.* 2016, Mao *et al.* 2019). The root exudates and metabolic processes of plants can cause an increase in organic matter content. The CO₂ released by plant root respiration and soil microbial activity dissolved in water to form carbonic acid can promote the dissolution of phosphorus, potassium salts, etc., which is beneficial for comprehensively improving soil fertility and creating a virtuous cycle (Wu *et al.* 2014). Soil enzymes participate in biochemical processes in soil and are essential in the decomposition of organic matter and nutrient cycling in ecosystems (Duan *et al.* 2012). Previous studies have shown that the application of microbial agents is beneficial for improving soil physical and chemical properties and microbial communities, increasing the number of microorganisms (Ezekoli *et al.* 2019, Zhang *et al.* 2021). This is consistent with the results of this study. As plants grow, their root exudates and humus formed by root decomposition can enhance soil microbial activity and increase the number of soil microorganisms. And the overall effect of microbial agents combined with herbaceous plants is better than that of shrubs. This may be because in the early stages of the experiment, herbaceous plants grow rapidly and have significantly higher metabolic activity than shrubs, so root exudates can efficiently increase soil microbial activity. And herbaceous plants can grow rapidly and complete the material capacity cycle with the soil. Therefore, this study shows that vetiver has a better effect on soil nutrient improvement. Sea buckthorn can significantly increase the nitrogen content in soil through nitrogen fixation (Fu 2017).

Field experiments on microbial plant interaction showed that microbial treatment increased soil moisture content (AH optimal), joint remediation reduced pH value and improved alkaline environment, significant enhancement of nutrient/enzyme activity (herbs > shrubs) and rhizobium had a significant nitrogen promoting effect and EM bacteria had outstanding comprehensive advantages.

Through comprehensive analysis, the combination of microbial agents and mixed planting mode could effectively increase the soil moisture content and nutrients in mining areas, while improving the soil environment. Among them, AH and AX had the best effects.

Acknowledgments

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