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Methodological study on coal-based microbial modification of mineral black clay to overcome plant growth challenges on open-pit mine dumps in cold regions

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ABSTRACT

A critical challenge in ecological restoration of open-pit mine dumps in cold regions with limited topsoil resources is how to rapidly mitigate the plant growth-inhibitory effects of mineral black clay, thereby converting it into arable soil. Leveraging the high degradation capacity of coal seam-associated microorganisms on fossil carbon materials, combined with soil conditioning techniques, this study developed a microbial-based approach for modifying black clay. Seed germination experiments informed both laboratory and field trial designs. This approach focused on removing germination-inhibiting compounds, establishing a plant-compatible soil ecological environment, and employing composite strategies to reduce soil viscosity. Field experiments demonstrated that in-situ microbial modification of black clay effectively supports ecological restoration, enhances plant growth. To refine and implement this microbial-based bioremediation strategy in practical ecological restoration efforts, two key technical methods were employed:

- A comprehensive experimental protocol was established for black clay bioremediation, covering both laboratory-scale and field test procedures, ensuring the approach can be readily adapted to diverse environmental conditions.
- By incorporating the characteristics of local species, employing representative seed germination tests to assess plant compatibility can facilitate a rapid evaluation of the bioconversion of mineral substrates into arable soils.

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Specifications table

This table provides general information on the methodology you reviewed.

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Keywords:	*Bioremediation, Ecological Restoration, Mineral Black Clay, Open-pit Mine, Dump*
Resource availability:	*Data will be made available on request.*
Review question:	1. *Are the technical steps logical and described with sufficient detail?* • *Yes* • *No* *Please specify what should be improved including which revisions are essential.* 2. *Can others reproduce this method based on the protocol(s) provided?* • *Yes* • *No* *Please specify what should be improved including which revisions are essential.* 3. *Will this information be useful to others working in the field/with this method?* • *Yes* • *No* *Please specify what should be improved including which revisions are essential.* 4. *Please assess the manuscript's different sections:* • *Abstract (very good/acceptable/needs improvement)* • *Graphical Abstract (very good/acceptable/needs improvement/not included)* • *Methods (very good/acceptable/needs improvement)* • *References (very good/acceptable/needs improvement)* *Please specify what should be improved including which revisions are essential.* 5. *What is your recommendation?* • *Accept as it is* • *Accept with text revision* • *Reject* 6. *Please provide your comments (specify where the value of the paper lies).* *Please formulate review questions* and list here. (*All the mentioned questions should be answered in this review)*

Background

Open-pit mining has been crucial in meeting global demands for minerals and resources, driving economic growth. However, this activity has led to extensive environmental degradation, especially soil erosion and loss of vegetation cover. In regions like Inner Mongolia, the removal of surface soil and rock layers results in vast accumulations of spoil and slag, forming dumps that degrade the land [1].

Ecological restoration of these mine dumps in Inner Mongolia is particularly challenging due to the scarcity of fertile soil resources. Harsh climatic conditions—low temperatures, strong winds, and minimal rainfall—exacerbate soil degradation, erosion, and desertification, posing significant threats to the local ecosystem [2]. The lack of humus and organic matter further complicates efforts to reestablish vegetation and stabilize the soil.

To address the shortage of soil necessary for ecological restoration, mining companies like the Baorixile Mine have begun utilizing underground-excavated black clay as an alternative to natural topsoil. Extracted from depths over 50 m, this gray-black, argillaceous mineral has an average organic matter content of 28.42%. It becomes malleable when wet but cracks easily when dry (Fig. 1). Despite its high organic content, the black clay's complex structure and inherent toxicity to plant seedlings result in very low germination rates, making it unsuitable for supporting plant growth. Nevertheless, there is ongoing interest in leveraging this mineral to mitigate soil scarcity in ecological restoration projects.

While research on using mineral source clays like black clay for agricultural purposes is limited, significant studies have focused on modifying conventional clay soils to support plant growth. Various methods have been explored to improve the physicochemical properties of clayey soils, such as incorporating materials to enhance soil aeration and reduce viscosity [3–5]. Efforts have also been made to promote plant root growth by improving soil structure and nutrient availability [6,7].

Biological approaches have also been investigated to modify soil properties using microbiological methods. Beneficial microorganisms like rhizosphere arbuscular mycorrhizal fungi can form symbiotic relationships with plants, enhancing soil recovery and organic matter accumulation [8,9]. Certain bacterial volatile organic compounds (VOCs) exhibit antimicrobial and pesticidal activities, promoting plant growth and inducing stress tolerance [10].

However, significant differences exist between conventional clayey soils and mineral clay soils like black clay in terms of physicochemical structure and microbial community composition. These differences present challenges in directly applying existing soil improvement methods to black clay.

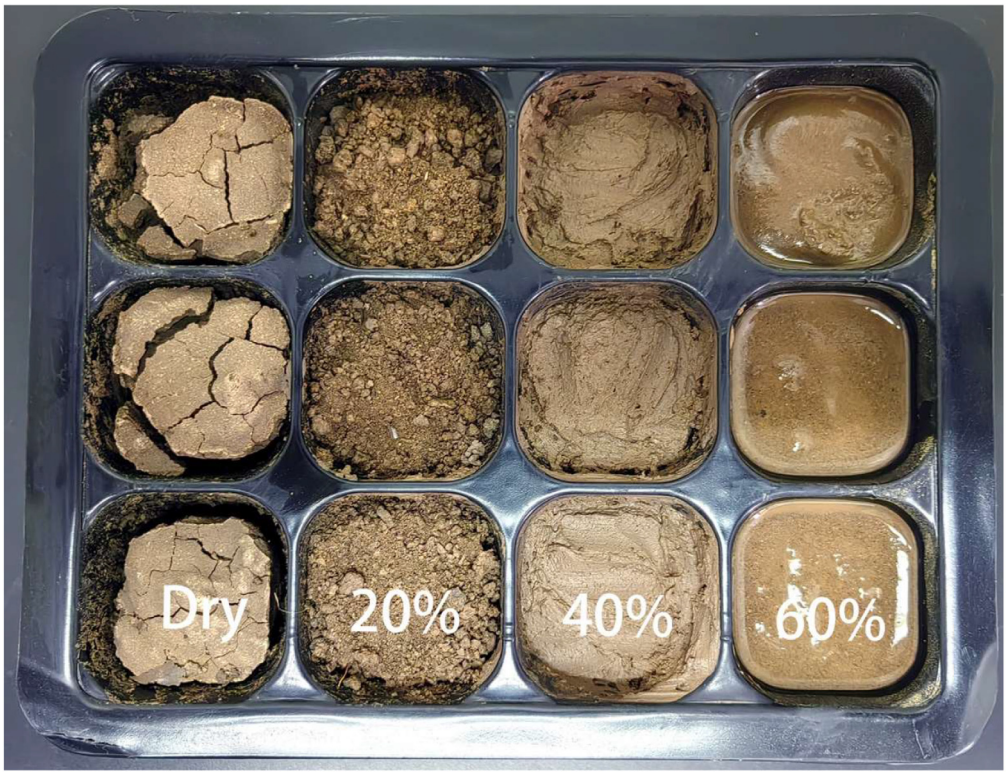

Fig. 1. Characteristics of black clay under different moisture content conditions.

In response to these challenges, this study aims to explore the feasibility of enhancing the soil micro-ecosystem of black clay by introducing exogenous microorganisms. By improving its physical and chemical properties through microbial inoculation, we seek to accelerate vegetation restoration on mine dumps and contribute to ecological rehabilitation efforts in regions facing soil scarcity.

Method details

From obtaining soil samples to establishing an optimized microbial treatment method for black clay and developing the best soil-blending strategy to achieve black clay bioremediation, ultimately enabling mineral-derived black clay to replace surface soil for ecological restoration, the following key steps are required:

(1) **Identify representative soil samples for experimental research**: Through seed germination experiments, analyze the impact of black clay storage duration on plant germination patterns and characteristic differences. Select soil samples that are both representative of engineering conditions and suitable for experimental research.
(2) **Optimize treatment timing**: Design three experimental methods—pre-treatment, concurrent treatment, and post-treatment. Evaluate the optimal timing for microbial treatment of black clay based on seed germination experiments.
(3) **Optimize blending methods**: Using orthogonal experimental design, analyze the best physical blending strategies to reduce the viscosity of black clay and optimize the combined treatment method with ERB.
(4) **Validate feasibility and mechanism**: Based on laboratory research results, conduct field experiments. Validate the treatment's effectiveness through in-situ planting tests, and explore the scalability and mechanism of black clay bioremediation using changes in soil physicochemical properties, microbial diversity analysis, and metabolomics studies.

Black clay sampling

Based on the duration of exposure, three types of black clay samples were collected: (1) newly exposed samples directly from the stratigraphy (designated as the J_0 group); (2) samples exposed for 6 months in surface stockpiles (designated as the J_6 group); and (3) samples exposed for 1 year in surface stockpiles (designated as the J_{12} group). To minimize surface contamination, the top 50 cm layer of each stockpile was removed prior to sampling. From each type of sample, five random points were selected, with approximately 10 kg collected from each point. These were combined directly at the landfill to minimize variability associated with single-point sampling. The total collected sample, exceeding 50 kg, was then placed into sterile bags. To ensure the representativeness of the sampling points, the site was selected only if there had been no rainfall in the previous five days, no standing water in the

Table 1
Experimental Design Program for Seedling Germination.

Family	Name	Seeds Size mm	Resources	Seed Usage per Cell	Germination Date	Germination Rate	Germination Impact Rate
Amaranthaceae	*Kochia scoparia* (L.) Schrad	1-2	Native Seedling	5			
	Salsola collina Pall.	1-2	Native Seedling	5			
Fabaceae	*Medicago sativa.*	1-2	Commercial Seedling	5			
	Melissitus ruthenicus (L.) Peschkoua,	2-3	Commercial Seedling	5			
Poaceae	*Bromus inermis* Leyss.	7-10	Commercial Seedling	3			
	Agropyron cristatum (L.) Gaertn.	5-7	Commercial Seedling	3			

vicinity, and no visible signs of water erosion or washout. And The sampling details are based on the sampling methods outlined in the “Collection, processing and storage of soil samples” (NY/T 1121–2006).

In the laboratory, plant fibers and stones were removed from the samples under a laminar flow hood to maintain sterility. The samples were then vacuum-dried at 45 ˚C and sealed for subsequent use.

Seedling germination experiment

The black clay samples were blended and passed through a 10-mesh sieve to achieve uniform particle size. For the seed germination experiment, seedling trays with dimensions of 3 × 4 cells were prepared, filling each cell with black clay to a depth of 5 cm. A control group was established using cleaned fine sand in place of black clay under the same conditions.

As outlined in Table 1, an adequate quantity of seeds was soaked in warm water at 30 ˚C for 4–8 h to promote uniform hydration (WB-500, Jingxue, China). Full and healthy seeds were selected and evenly distributed in both the experimental trays (containing black clay) and the control trays (containing fine sand), ensuring a seed spacing of at least twice the seed length to prevent competition. Five replicates were conducted for each treatment to ensure statistical validity.

The surfaces of the trays were moistened with distilled water, with the initial moisture content controlled at approximately 30%.

The initial moisture content of the trays were adjusted to approximately 30% using distilled water. They were then covered with perforated lids to minimize evaporation and placed in an incubator maintained at 25 ˚C (LHS-250, Jingxue, China). Observations were made every 12 h to monitor germination progress, and distilled water was added as necessary to maintain adequate moisture levels. The time to initial germination was recorded. After 3–5 days, when no additional seeds germinated, the final number of germinated seeds was documented.

The seed germination rate (a_{ij}) for each replicate was calculated using Eq. (1):

$$a_{ij} = \frac{M_{ij}}{M} \times 100\% \tag{1}$$

Where: a_{ij}: Seed germination rate (%) for the i th replicate of group j (j = j_0, j_6 or j_{12});

M_{ij}: Number of seeds germinated in the i th replicate of group j;

M: Total number of seeds sown in each seedling tray (constant across all trays).

To evaluate the impact of black clay on seed germination relative to the control, the germination impact rate (b_j) was calculated using Eq. (2):

$$b_j = \frac{\sum_{i=1}^{5} a_{ij}}{\sum_{i=1}^{5} a_{iC}} \times 100\% \tag{2}$$

Where: b_j: Germination impact rate (%) for group j (j = j_0, j_6 or j_{12});

a_{ij}: Seed germination rate (%) for the i th replicate of group j;

a_{ic}: Seed germination rate (%) for the i th replicate of the control group.

Statistical analyses were performed to determine significant differences in germination rates among the three types of black clay samples. This assessment aimed to evaluate the effects of 6-month and 12-month stockpiling on seed germination. The results provide foundational data for further research.

Black clay ecological restoration bacteria group modification experiment

Ecological restoration bacteria group preparation

The Ecological Restoration Bacteria Group (ERB) was prepared using microbial strains sourced from coal seams. Corn straw (moisture content <45%) and black clay (moisture content <30%) served as nutritional substrates under aerobic, medium-low temperature cultivation conditions (SS3316, Tengfang, China). A thermostatic aeration cultivator was employed, maintaining the temperature

Table 2
Experimental design for evaluating the bioremediation effect of soils with different stockpiling periods.

Group	7 days before seeding	At the time of seeding	At the time of first seed germination	Control
j_0 j_6 j_{12}	1th in experiment	7th in experiment	10th in experiment	–

at 40 °C, humidity at 50–55%, and pH between 5.5 and 6.5. The aeration rate was set to one cycle every 2 h. The substrate was thoroughly stirred every 3 days to ensure uniformity, and the cultivation period lasted for 60 days.

The bacteria present in the matured substrate constituted the ERB. The substrate was mixed with water at a 1:50 (v/v) ratio and fully dispersed using a high-speed disperser (ZHD-22, Zhongshi, China). The ERB solution was then obtained by filtering the mixture through a 120-mesh sieve.

Timing design for ERB application and effectiveness assessment

Based on the soil seedling tray preparation method described in Section 2 and following the experimental design in Table 2, three types of soil samples were prepared, with 120 samples for each type. According to the experimental setup in Table 3, ERB was applied at three different times: 7 days before seeding, at the time of seeding, and at the time of germination. Three application programs were designed on this basis (Table 3). One blank control experimental group was also set up for each type of soil. This approach allowed for both horizontal and vertical comparisons of the effects of different treatment timings on seedling germination in the black clay environment, considering both soil sample types and seedling species.

Differences in germination between species of the Amaranthaceae and Fabaceae families were used as the primary reference indices (Reference Indices Group I), while species from the Poaceae family served as secondary reference indices (Reference Indices Group II) to evaluate the optimal timing for ERB treatment in black clay soil.

The experiment revealed that applying ERB 7 days before seeding and at the time of seeding had similar effects on seedling germination. Considering the overall cost of ecological restoration at the disposal site, simultaneous application of ERB and seeds was ultimately selected as the preferred method.

Selection of soil samples for further experiments

Based on data from the blank Control group, the preliminary experiments revealed that the seedling germination rate in black clay soil improved with longer stockpiling periods (Table 4). This observation suggests that surface microorganisms may gradually enhance the quality of black clay soil during exposure. However, statistical analysis using an F-test indicated that these changes were not significant, particularly between soil samples stored for 6 months and those stored for 12 months. This lack of significant difference is attributed to the region's climatic conditions; located in China's Cold Zone II, the area experiences average temperatures below 0 °C for over half the year[11]. During these prolonged cold periods, microorganisms in the exposed black clay piles become dormant, resulting in minimal microbial activity and negligible changes in soil properties across the winter months compared to the warmer, 6-month stockpiling period.

Considering these findings, soil samples that had been stockpiled for 12 months were selected for subsequent experiments. This choice not only aligns with the largest stockpile available at the disposal site but also provides a more representative sample for further ecological restoration studies.

Meanwhile, The experimental results demonstrate that ERB treatment has a favourable effect on black clay soil at different periods on seedling germination surfaces. Both pretreatment in advance and simultaneous treatment at the time of sowing were found to enhance seedling germination. However, the lagging treatment was observed to be ineffective in this regard. In light of the feasibility of the project, it is recommended that microbiological treatment of black clay be prioritized at the same time of sowing (Table 4).

Investigation of black clay blending methods

Incorporating certain amounts of sand or humus into black clay can improve its physical properties under wet and dry conditions [3,5,12,13]. In this experiment, humus soil, yellow sand, and gravel from the test area were selected as blending materials to assess their effects on the modification of black clay and its water retention capacity at different blending ratios.

The yellow sand, with particle sizes ranging from 2.0 to 2.7 mm, was sourced from a building materials market. The gravel was obtained from crushed sandstone exposed during open-pit mining and sieved through 2-mesh and 5-mesh screens to select particles sized 4–8 mm for the gravel samples. The black clay was dried, pulverized, and passed through a 10-mesh sieve to prepare the samples.

Three factors were chosen as indicators: 48 h saturated hygroscopicity, 24 h dehydration rate, and seed germination rate. In selecting species for the seed germination experiments, considerations included germination rate, complexity of germination environment requirements, and their importance to subsequent soil remediation. Among six plant species, alfalfa (Medicago sativa.) was chosen as the experimental subject. Mung beans (Vigna radiata (L.) Wilczek) were also included as a reference for further study.

Table 3
Experimental Design of Three Bioremediation Methods for Soil.

Group	Reference Indices Grouping	Plant	Sample	Day			Note
				1	7	10	
J_0	I	Kochia scoparia (L.) Schrad	J_0-1-1~5	√	O	O	Treading berfore seeding
			J_0-1-6~10	O	√	O	Treading when seeding
			J_0-1-11~15	O	O	√	Treading after germination
			J_0-1-16~20	O	O	O	Control group
		Salsola collina Pall.	J_0-2-1~5	√	O	O	Treading berfore seeding
			J_0-2-6~10	O	√	O	Treading when seeding
			J_0-2-11~15	O	O	√	Treading after germination
			J_0-2-16~20	O	O	O	Control group
		Medicago sativa.	J_0-3-1~5	√	O	O	Treading berfore seeding
			J_0-3-6~10	O	√	O	Treading when seeding
			J_0-3-11~15	O	O	√	Treading after germination
			J_0-3-16~20	O	O	O	Control group
		Melissitus ruthenicus (L.) Peschkoua	J_0-4-1~5	√	O	O	Treading berfore seeding
			J_0-4-6~10	O	√	O	Treading when seeding
			J_0-4-11~15	O	O	√	Treading after germination
			J_0-4-16~20	O	O	O	Control group
	II	Bromus inermis Leyss.	J_0-5-1~5	√	O	O	Treading berfore seeding
			J_0-5-6~10	O	√	O	Treading when seeding
			J_0-5-11~15	O	O	√	Treading after germination
			J_0-5-16~20	O	O	O	Control group
		Agropyron cristatum (L.) Gaertn.	J_0-6-1~5	√	O	O	Treading berfore seeding
			J_0-6-6~10	O	√	O	Treading when seeding
			J_0-6-11~15	O	O	√	Treading after germination
			J_0-6-16~20	O	O	O	Control group
J_6	I	Kochia scoparia (L.) Schrad	J_6-1-1~5	√	O	O	Treading berfore seeding
			J_6-1-6~10	O	√	O	Treading when seeding
			J_6-1-11~15	O	O	√	Treading after germination
			J_6-1-16~20	O	O	O	Control group
		Salsola collina Pall.	J_6-2-1~5	√	O	O	Treading berfore seeding
			J_6-2-6~10	O	√	O	Treading when seeding
			J_6-2-11~15	O	O	√	Treading after germination
			J_6-2-16~20	O	O	O	Control group
		Medicago sativa.	J_6-3-1~5	√	O	O	Treading berfore seeding
			J_6-3-6~10	O	√	O	Treading when seeding
			J_6-3-11~15	O	O	√	Treading after germination
			J_6-3-16~20	O	O	O	Control group
		Melissitus ruthenicus (L.) Peschkoua	J_6-4-1~5	√	O	O	Treading berfore seeding
			J_6-4-6~10	O	√	O	Treading when seeding
			J_6-4-11~15	O	O	√	Treading after germination
			J_6-4-16~20	O	O	O	Control group
	II	Bromus inermis Leyss.	J_6-5-1~5	√	O	O	Treading berfore seeding
			J_6-5-6~10	O	√	O	Treading when seeding
			J_6-5-11~15	O	O	√	Treading after germination
			J_6-5-16~20	O	O	O	Control group
		Agropyron cristatum (L.) Gaertn.	J_6-6-1~5	√	O	O	Treading berfore seeding
			J_6-6-6~10	O	√	O	Treading when seeding
			J_6-6-11~15	O	O	√	Treading after germination
			J_6-6-16~20	O	O	O	Control group
J_{12}	I	Kochia scoparia (L.) Schrad	J_{12}-1-1~5	√	O	O	Treading berfore seeding
			J_{12}-1-6~10	O	√	O	Treading when seeding
			J_{12}-1-11~15	O	O	√	Treading after germination
			J_{12}-1-16~20	O	O	O	Control group
		Salsola collina Pall.	J_{12}-2-1~5	√	O	O	Treading berfore seeding
			J_{12}-2-6~10	O	√	O	Treading when seeding
			J_{12}-2-11~15	O	O	√	Treading after germination
			J_{12}-2-16~20	O	O	O	Control group
		Medicago sativa.	J_{12}-3-1~5	√	O	O	Treading berfore seeding
			J_{12}-3-6~10	O	√	O	Treading when seeding
			J_{12}-3-11~15	O	O	√	Treading after germination
			J_{12}-3-16~20	O	O	O	Control group
		Melissitus ruthenicus (L.) Peschkoua	J_{12}-4-1~5	√	O	O	Treading berfore seeding
			J_{12}-4-6~10	O	√	O	Treading when seeding
			J_{12}-4-11~15	O	O	√	Treading after germination
			J_{12}-4-16~20	O	O	O	Control group
	II	Bromus inermis Leyss.	J_{12}-5-1~5	√	O	O	Treading berfore seeding
			J_{12}-5-6~10	O	√	O	Treading when seeding
			J_{12}-5-11~15	O	O	√	Treading after germination
			J_{12}-5-16~20	O	O	O	Control group
		Agropyron cristatum (L.) Gaertn.	J_{12}-6-1~5	√	O	O	Treading berfore seeding
			J_{12}-6-6~10	O	√	O	Treading when seeding
			J_{12}-6-11~15	O	O	√	Treading after germination
			J_{12}-6-16~20	O	O	O	Control group

Table 4
Example of germination results of seedlings in each experimental group.

Group	Plant	Sample	Day			Number of germinated seeds						Germination rate
			1	7	10	1	2	3	4	5	均值	
J_0	Kochia scoparia (L.) Schrad	J_0-1-1~5	√	O	O	56	40	47	50	48	48.20	80.33%
		J_0-1-6~10	O	√	O	55	45	49	50	53	50.40	84.00%
		J_0-1-11~15	O	O	√	5	4	3	3	6	4.20	7.00%
		J_0-1-16~20	O	O	O	3	5	2	3	4	3.40	5.67%
	Salsola collina Pall.	J_0-2-1~5	√	O	O	44	43	44	42	39	42.40	70.67%
		J_0-2-6~10	O	√	O	55	41	45	52	48	48.20	80.33%
		J_0-2-11~15	O	O	√	2	2	1	2	2	1.80	3.00%
		J_0-2-16~20	O	O	O	1	0	1	1	2	1.00	1.67%
	Medicago sativa.	J_0-3-1~5	√	O	O	49	43	48	48	50	47.60	79.33%
		J_0-3-6~10	O	√	O	57	52	54	55	58	55.20	92.00%
		J_0-3-11~15	O	O	√	2	1	1	2	2	1.60	2.67%
		J_0-3-16~20	O	O	O	0	0	1	1	2	0.80	1.33%
	Melissitus ruthenicus (L.) Peschkoua	J_0-4-1~5	√	O	O	29	25	23	27	28	26.40	44.00%
		J_0-4-6~10	O	√	O	30	32	41	40	43	37.20	62.00%
		J_0-4-11~15	O	O	√	1	2	1	2	2	1.60	2.67%
		J_0-4-16~20	O	O	O	1	1	1	1	0	0.80	1.33%
	Bromus inermis Leyss.	J_0-5-1~5	√	O	O	25	21	24	20	31	24.20	67.22%
		J_0-5-6~10	O	√	O	30	35	33	36	39	34.60	96.11%
		J_0-5-11~15	O	O	√	2	1	1	2	1	1.40	3.89%
		J_0-5-16~20	O	O	O	0	0	1	1	1	0.60	1.67%
	Agropyron cristatum (L.) Gaertn.	J_0-6-1~5	√	O	O	52	42	40	52	53	47.80	132.78%
		J_0-6-6~10	O	√	O	58	46	56	54	55	53.80	149.44%
		J_0-6-11~15	O	O	√	2	1	2	1	1	1.40	3.89%
		J_0-6-16~20	O	O	O	0	1	0	0	1	0.40	1.11%
J_6	Kochia scoparia (L.) Schrad	J_6-1-1~5	√	O	O	56	43	48	50	52	49.80	83.00%
		J_6-1-6~10	O	√	O	56	46	51	55	53	52.20	87.00%
		J_6-1-11~15	O	O	√	11	8	10	9	13	10.20	17.00%
		J_6-1-16~20	O	O	O	9	11	7	6	9	8.40	14.00%
	Salsola collina Pall.	J_6-2-1~5	√	O	O	46	44	50	40	39	43.80	73.00%
		J_6-2-6~10	O	√	O	55	42	46	52	51	49.20	82.00%
		J_6-2-11~15	O	O	√	2	3	2	1	3	2.20	3.67%
		J_6-2-16~20	O	O	O	0	1	1	2	2	1.20	2.00%
	Medicago sativa.	J_6-3-1~5	√	O	O	50	41	51	49	53	48.80	81.33%
		J_6-3-6~10	O	√	O	56	53	59	61	58	57.40	95.67%
		J_6-3-11~15	O	O	√	1	2	2	3	2	2.00	3.33%
		J_6-3-16~20	O	O	O	1	0	1	1	2	1.00	1.67%
	Melissitus ruthenicus (L.) Peschkoua	J_6-4-1~5	√	O	O	36	30	29	31	35	32.20	53.67%
		J_6-4-6~10	O	√	O	37	41	42	41	43	40.80	68.00%
		J_6-4-11~15	O	O	√	2	3	2	3	0	2.00	3.33%
		J_6-4-16~20	O	O	O	0	0	1	2	2	1.00	1.67%
	Bromus inermis Leyss.	J_6-5-1~5	√	O	O	33	32	33	28	35	32.20	89.44%
		J_6-5-6~10	O	√	O	34	38	39	37	40	37.60	104.44%
		J_6-5-11~15	O	O	√	1	2	1	3	1	1.60	4.44%
		J_6-5-16~20	O	O	O	1	0	1	1	1	0.80	2.22%
	Agropyron cristatum (L.) Gaertn.	J_6-6-1~5	√	O	O	54	44	42	54	55	49.80	138.33%
		J_6-6-6~10	O	√	O	59	49	57	56	56	55.40	153.89%
		J_6-6-11~15	O	O	√	2	1	2	1	3	1.80	5.00%
		J_6-6-16~20	O	O	O	0	1	0	2	0	0.60	1.67%
J_{12}	Kochia scoparia (L.) Schrad	J_{12}-1-1~5	√	O	O	54	46	53	52	53	51.60	86.00%
		J_{12}-1-6~10	O	√	O	54	51	55	55	54	53.80	89.67%
		J_{12}-1-11~15	O	O	√	13	12	11	12	14	12.40	20.67%
		J_{12}-1-16~20	O	O	O	11	10	9	11	12	10.60	17.67%
	Salsola collina Pall.	J_{12}-2-1~5	√	O	O	47	45	50	44	42	45.60	76.00%
		J_{12}-2-6~10	O	√	O	52	48	50	54	53	51.40	85.67%
		J_{12}-2-11~15	O	O	√	3	2	3	2	4	2.80	4.67%
		J_{12}-2-16~20	O	O	O	3	2	1	1	0	1.40	2.33%
	Medicago sativa.	J_{12}-3-1~5	√	O	O	51	48	53	51	53	51.20	85.33%
		J_{12}-3-6~10	O	√	O	60	54	60	61	62	59.40	99.00%
		J_{12}-3-11~15	O	O	√	2	3	3	2	3	2.60	4.33%
		J_{12}-3-16~20	O	O	O	0	1	2	2	1	1.20	2.00%
	Melissitus ruthenicus (L.) Peschkoua	J_{12}-4-1~5	√	O	O	38	30	33	32	35	33.60	56.00%
		J_{12}-4-6~10	O	√	O	43	44	43	41	43	42.80	71.33%
		J_{12}-4-11~15	O	O	√	1	3	2	3	4	2.60	4.33%
		J_{12}-4-16~20	O	O	O	1	2	0	0	3	1.20	2.00%
	Bromus inermis Leyss.	J_{12}-5-1~5	√	O	O	34	32	33	31	37	33.40	92.78%
		J_{12}-5-6~10	O	√	O	39	36	39	38	42	38.80	107.78%
		J_{12}-5-11~15	O	O	√	3	2	3	1	3	2.40	6.67%
		J_{12}-5-16~20	O	O	O	0	1	2	1	2	1.20	3.33%
	Agropyron cristatum (L.) Gaertn.	J_{12}-6-1~5	√	O	O	56	47	40	56	57	51.20	142.22%
		J_{12}-6-6~10	O	√	O	61	48	57	58	59	56.60	157.22%
		J_{12}-6-11~15	O	O	√	2	2	3	3	2	2.40	6.67%
		J_{12}-6-16~20	O	O	O	1	0	1	1	3	1.20	3.33%

Table 5
Factor design for orthogonal tests of black clay blending.

Level	Factor			
	A Humus	B Sand	C Gravel	D ERB (g/m^2)
1	0	0	0	0
2	10%	10%	10%	0.2
3	25%	25%	25%	0.4

Table 6
Orthogonal experimental design for black clay blending.

Experiment number	Column number			
	A	B	C	D
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

Based on the geological structure and surface hydrological characteristics of the dump site, methods were designed to measure soil 48 h saturated hygroscopicity and 24 h dehydration rate. Specifically, following the orthogonal experimental design methods outlined in Tables 5 and 6, each experimental group completed blending according to the design. After applying the exogenous microbial agent (ERB), the samples were stored at room temperature for 7 days. Subsequently, each sample was thoroughly mixed again and quartered to obtain subsamples for determining maximum saturated hygroscopicity and 24 h dehydration rate.

Measurement of 48 h saturated hygroscopicity

(1) Place the samples in a vacuum drying oven at 60 ˚C for 24 h.
(2) Weigh a sample of V_0=10.00±0.2 g using an analytical balance (LE2002E, METTLER TOLEDO, Switzerland), place it in an evaporating dish, and record the total weight as V_1.
(3) Place the sample in a desiccator with distilled water at the bottom, maintain at 30 ˚C (JK-HI-9272, Jingxue, China, and let it stand for 48 h. Afterward, weigh each sample and record the weight as V_2.
(4) Calculate the 48 h saturated hygroscopicity h using Eq. (3):

$$h = \frac{V_2 - V_1}{V_0} \times 100\% \tag{3}$$

Measurement of 24 h dehydration rate

(1) After completing the 48 h saturated hygroscopicity measurement, place the sample in a desiccator lined with superabsorbent resin equal to twice the total weight of the sample.
(2) Maintain at 20 ˚C and let it stand for 24 h, then weigh each sample and record the weight as V_3.
(3) Calculate the 24 h dehydration rate p using Eq. (4):

$$p = \frac{V_2 - V_3}{V_2 - V_1} \times 100\% \tag{4}$$

Here, the 48 h saturated hygroscopicity represents the maximum moisture absorption capacity of the soil samples under saturated humidity conditions at 30 ˚C. A higher h value indicates better water absorption, which is advantageous for acquiring moisture from the air under the arid and water-deficient conditions of the dump site. The 24 h dehydration rate reflects the soil's water retention capacity at 20 ˚C. A higher p value indicates poorer water retention, which is unfavorable for retaining moisture to support plant growth.

Seed germination rate measurement

(1) After ERB treatment, place the sample in a petri dish with a layer thickness of 0.8–1.0 cm.

Table 7
Weighted design of germination indicators.

Ranking of indicators		Weight 1	Weight 2	Weight 3	Weight 4
First indicator	Medicago sativa.	a_{iM}>50%	a_{iM}>50%	a_{iM}<50%	a_{iM}<50%
Second indicator	Vigna radiata (L.) Wilczek	a_{iV}>50%	a_{iV}<50%	a_{iV}>50%	a_{iV}<50%
Weight for Vigna radiata (L.) Wilczek		1	3/4	1/2	1/4

Table 8
ERB application and sampling schedule in the H3 experimental area.

Date	F-ERB (Full)	S-ERB (Single)	N-ERB (Control)	Temperature (˚C)	Weather
10-May-19	ERB Applied	ERB Applied	No ERB	4–19	Cloudy
24-May-19	Sampling	Sampling	–	8–29	Sunny
30-Jun-19	ERB Applied	–	–	12–26	Sunny
13-Jul-19	Sampling	Sampling	–	16–28	Sunny
22-Aug-19	ERB Applied	–	–	11–18	Cloudy
5-Sep-19	Sampling & Plant Survey	Sampling & Plant Survey	Sampling & Plant Survey	13–26	Cloudy

(2) Soak Medicago sativa. and Vigna radiata (L.) Wilczek seeds at 30 ˚C for 4–8 h, then select plump seeds and place 30 seeds on the surface of each petri dish.
(3) After spraying water on the petri dishes, place them in a desiccator with distilled water at the bottom and incubate at 25 ˚C. Observe seed germination every 12 h.
(4) Starting from the emergence of any germinated seed, continue the experiment for 5 days. Count the number of normally germinated seeds and calculate the seed germination rate using Eq. (1).

The seed germination rate reflects the differences in how various blending methods affect the germination of the two leguminous plants. A higher germination rate (a_{ij}) indicates that the corresponding black clay blending method is more conducive to ecological restoration. Alfalfa germination rate serves as the primary evaluation index, while mung bean germination rate is the secondary index. By integrating both indicators and using the primary index as the baseline, weighted calculations were performed (Table 7).

Methods for ecological restoration of soil in the field at discharge sites

Based on laboratory results, a field experimental method was designed for ecological restoration. Medicago sativa. was selected for artificial sowing at a rate of 15 kg/ha. Kochia scoparia and Salsola collina were allowed to establish through natural seeding.

Considering the full lifecycle of plant growth—from germination to flowering and fruiting—the application of ERB was scheduled in three stages: at germination, pre-flowering, and pre-fruiting. Control groups included a single ERB application at the germination stage and a no-treatment group. The specific methods are detailed below.

Preparation and application of ERB solution

The ERB solution was prepared by diluting the stock solution tenfold with distilled water. Aeration was performed using a 20 L bucket, with air pumped through a 0.22 μm microporous membrane at a flow rate of 5.0 L/min for 20 min. The diluted ERB solution was applied at a rate of 1000 L/ha (equivalent to 0.2 g/m^2 of ERB) to minimize disturbances to the soil's primary elements.

Field experiment setup

Ecological restoration research was conducted in an area designated as H3. Based on laboratory findings, a synthetic soil was prepared by mixing black clay and surface soil in a 4:1 ratio, applied to a thickness of approximately 25 cm.

To assess the restorative effects, the H3 experimental area was divided into three zones (Fig. 1):

- **F-ERB:** Full-process ERB treatment zone (three applications)
- **S-ERB:** Single ERB treatment zone (application at germination)
- **N-ERB:** Control zone with no ERB application

ERB application schedule and sampling

ERB applications were conducted from May to August 2019, aligning with optimal treatment protocols established in the laboratory. The first application occurred after the frost-free date, the second was scheduled 1–2 weeks before the expected flowering period based on historical data, and the third was applied 1–2 weeks after flowering (Table 8). Application dates were chosen based on weather forecasts to avoid significant rainfall within two days post-application.

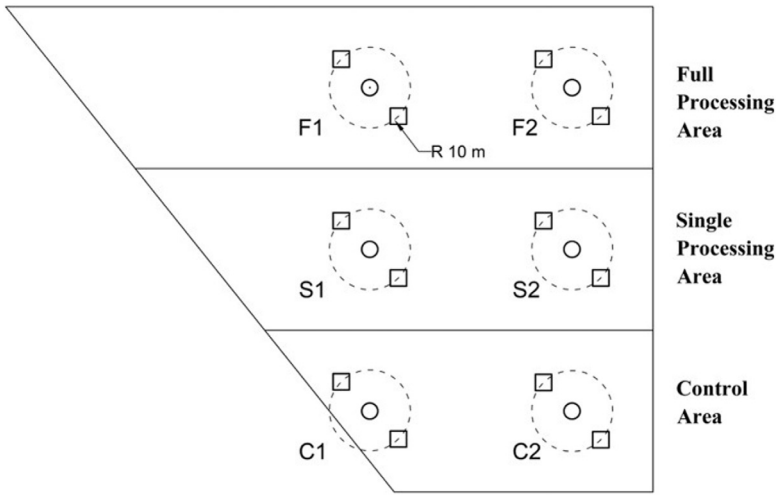

Fig. 2. Zoning of the H3 experimental area for black clay biomodification.
Note: The boxes represent plant diversity sampling locations within each zone, with two sampling sites per zone.

Table 9
Species diversity survey record sheet.

Species	F-ERB	S-ERB	N-ERB
Kochia scoparia (L.) Schrad	F1–1	S1–1	C1–1
	F1–2	S1–2	C1–2
	F2–1	S2–1	C2–1
	F2–2	S2–2	C2–2
Salsola collina Pall.	F1–1	S1–1	C1–1
	F1–2	S1–2	C1–2
	F2–1	S2–1	C2–1
	F2–2	S2–2	C2–2
Medicago sativa.	F1–1	S1–1	C1–1
	F1–2	S1–2	C1–2
	F2–1	S2–1	C2–1
	F2–2	S2–2	C2–2

Note: F-ERB refers to the full-process ERB treatment zone; S-ERB refers to the single ERB treatment zone; N-ERB refers to the control zone without ERB application. The numbers represent specific sampling points within each zone.

This experimental methodology provides a detailed framework for evaluating the effectiveness of ERB treatments and soil blending methods on the ecological restoration of black clay soils in mining disposal sites. The integration of laboratory and field experiments aims to develop practical solutions for improving soil quality and promoting sustainable vegetation growth in degraded environments.

Evaluation of restoration effect

Plant diversity and soil samples were collected according to the schedule in Table 8. Sampling locations within each zone were predetermined, with two sites per zone for comprehensive analysis.

Plant diversity analysis

To conduct a plant diversity survey (Fig. 2), four sampling points were established in each of the three experimental areas. Each sampling quadrat measured 1.00 × 1.00 m. Data on plant species distribution from these sampling points were collected (Table 9) and used to compute various diversity indices, including the Patrick richness index (D_p), Shannon-Wiener's diversity index (H), Pielou's evenness index (E_H), Margalef's richness index (F), and Simpson's dominance index (D_S).

Soil sample collection

The experimental area, covering approximately 1 hectare, featured flat terrain and was shaped like a right-angled trapezoid. Ten sampling points were established in each experimental zone. A Luoyang shovel was used for soil sampling (Fig. 3); it was cleaned and disinfected with 75% ethanol before each use to prevent cross-contamination. In the F-ERB and S-ERB zones, each sampling point

Fig. 3. Luoyang shovels used for vertical soil sampling.

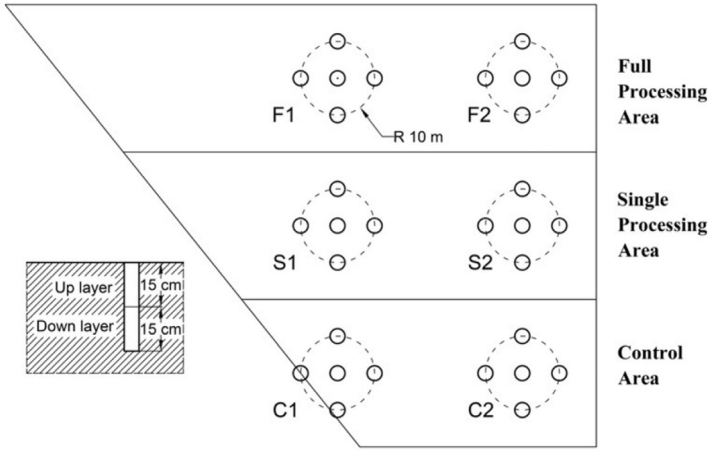

Fig. 4. Soil sampling methodology design.

Table 10
Methods for analysis of soil physico-chemical indicators, microbial diversity, and metabolomics.

Parameter	Indicator	Analytical Method	Reference
Nitrogen (N)	Total Nitrogen (T_N)	Kjeldahl method	[14]
	Ammonium Nitrogen (A_N)	Nessler's reagent colorimetry	[15]
Phosphorus (P)	Total Phosphorus (T_P)	Spectrophotometry (Molybdenum blue method)	[16]
	Soluble Phosphorus (S_P)	Spectrophotometry	[17]
Potassium (K)	Potassium (K)	Flame photometry	[18]
Soil Enzymes	Urease Activity	Phenol-sodium hypochlorite colorimetry	[19]
Microbial Diversity Analysis		High-throughput sequencing	[20]
Metabolomics Analysis		LC-MS (Liquid Chromatography-Mass Spectrometry), HPLC (High-Performance Liquid Chromatography)	[21]

was divided into upper and lower soil layers, each with a thickness of 15 cm (Fig. 4). In contrast, only surface black clay samples (0–15 cm) were collected in the N-ERB zone due to the absence of ERB treatment.

For chemical indicator analysis and microbial biodiversity assessment, five samples from each upper or lower layer in each group were thoroughly mixed separately after removing plant roots and stones. For metabolomics analysis, two random topsoil samples from the circular sampling points were mixed after removing plant roots and debris. All prepared samples were stored at −80 °C until analysis.

Soil samples were analyzed for physico-chemical properties (nitrogen, phosphorus, potassium), microbial diversity, and metabolomics. The specific analyses are listed in Table 10.

Sample preparation for physico-chemical analysis

(1) Preparation of digest solution
The digest solution for nitrogen, phosphorus, and potassium (N-P-K) determination was prepared following the Chinese National Standards GB/T 17,767–2008 and NY/T 1121–2006. A 50.00 g composite soil sample was vacuum-dried at 45 °C and passed through a 0.25 mm sieve. A 2.00 g subsample was obtained using the quartering method and weighed with an accuracy of

Table 11
Results of orthogonal experiments on 48 h saturated hygroscopicity and 24 h dehydration rate.

Experiment number	A Humus	B Sand	C Gravel	D ERB	48 h saturated hygroscopicity	24 h dehydration rate
1	0%	0%	0%	0	27	30
2	0%	10%	10%	0.2	29	28
3	0%	25%	25%	0.4	30	26
4	10%	0%	10%	0.4	33	22
5	10%	10%	25%	0	31	26
6	10%	25%	0%	0.2	32	24
7	25%	0%	25%	0.2	35	21
8	25%	10%	0%	0.4	37	19
9	25%	25%	10%	0	33	23

±0.0001 g. The sample was then mixed with 5.00 mL H_2SO_4 (ρ1.84), 1.50 mL 30 % H_2O_2, and 0.50 g $CuSO_4{\cdot}5H_2O$ in a Kjeldahl flask. The mixture was covered with a small funnel and allowed to stand at room temperature for 15 hours. Subsequently, the samples were transferred to a preheated Kjeldahl digestion block at 370 °C and digested for 75 minutes. After cooling, the digest solution was diluted to a final volume of 100.00 mL with deionized water, preparing it for the determination of total nitrogen (T_N), total phosphorus (T_P), and potassium (K).

(2) Preparation of leaching solution
For the preparation of the leaching solution, 10.00 ± 0.10 g of the dried and sieved (0.25 mm) soil sample was collected using the quartering method and mixed with 25.00 mL of deionized water. The mixture was dispersed using a high-speed disperser at 1200 rpm for 5 minutes. After standing for 1 hour to reach equilibrium, the mixture was centrifuged at 8500 × g for 10 minutes. The supernatant was collected as the leaching solution, which was used to determine ammonium nitrogen (A_N) and soluble phosphorus (S_P).

Effectiveness evaluation

(1) Based on seedling germination experiments with black clay in its natural state at different stockpiling periods, it was formalized that stockpiling for more than 6 months slightly reduced the inhibition of seed germination (Table 4). To summarize the production characteristics of the mine, the black clay soil with 12 heaps of storage was selected as the experimental soil sample in the experiment.

(2) As evidenced by the experimental results presented in Table 4, the application of ERB via the pretreatment or simultaneous treatment method is an effective approach for achieving optimal soil-plant suitability modification. In consideration of the comprehensive construction cost, the simultaneous treatment of black clay soil by microbial method during the same period of seeding represents a viable solution for the project.

(3) Orthogonal design was used in this research to optimize the conditions for soil blending and microbial modification of black clay. Four factors, which included: humus (A), sand (B), gravel (C), and ERB (D), were each set at three levels, and nine experiments were conducted to examine how various factor combinations influenced the 48 h saturated hygroscopicity and 24 h dehydration rate of the soil (Table 11).
The results (table omitted) indicate that different factor combinations produce significant variations in the soil's moisture retention properties. Overall, increasing the humus content (A) notably enhanced both water absorption and retention capacities. For example, when A increased from 0% to 25%, the 48 h saturated hygroscopicity rose from about 27% to 37%, while the 24 h dehydration rate decreased from around 30% to between 19% and 23%. This suggests that the organic matter and aggregated structure provided by humus effectively increase the soil's effective porosity, improving both absorption and retention properties. The addition of sand (B) and gravel (C) expanded the macropores in the soil, moderately increasing initial water absorption. However, the larger pore channels also facilitated water loss over time, meaning that the 24 h dehydration rate remained relatively high. In the absence of humus (A = 0%), when B and C were both at higher levels (25%), the 48 h saturated hygroscopicity rose to about 30%, slightly above the baseline condition. Nonetheless, the 24 h dehydration rate remained at around 26%–28%.
The application of ERB (D) showed some incremental improvement in water retention, particularly when combined with a high proportion of humus. For instance, under conditions of high humus content and 0.4 g/m^2 ERB (Experiment 8), the 48 h saturated hygroscopicity reached the highest value (approximately 37%), while the 24 h dehydration rate dropped to 19%, representing one of the most favorable combinations in this series of experiments. This indicates that ERB can further refine soil microstructure and porosity, enhancing water retention when an abundant organic and aggregate structure is already present.
In summary, the orthogonal test results suggest that increasing humus content is the primary factor in improving soil water absorption and retention. The addition of ERB confers an additional benefit, especially under conditions of high humus content, while sand and gravel mainly affect initial water absorption through changes in macropore structure, providing limited improvement to overall water retention. These findings offer valuable data and insights for optimizing black clay bio-modification formulations and guiding their practical application in ecological restoration.

Table 12
Results of orthogonal experiments on seedling germination experiments.

Experiment number	A Humus	B Sand	C Gravel	D ERB	M(%)	V(%)	Weight	Rerslut
1	0%	0%	0%	0	2	9	0.25	4.25
2	0%	10%	10%	0.2	18	25	0.25	24.25
3	0%	25%	25%	0.4	75	80	1	155
4	10%	0%	10%	0.4	90	88	1	178
5	10%	10%	25%	0	30	40	0.25	40
6	10%	25%	0%	0.2	51	55	1	106
7	25%	0%	25%	0.2	68	72	1	140
8	25%	10%	0%	0.4	98	94	1	192
9	25%	25%	10%	0	70	80	1	150

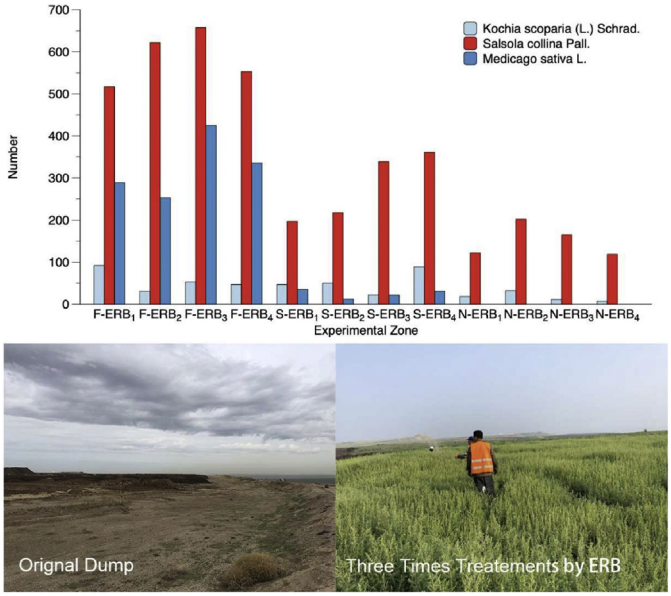

Fig. 5. Differences in the amount of plans in different ERB treatment zones in the open-pit mining dump.

By analyzing the influencing factors and their optimal levels, it is evident that for M(%) the factor order is D(53.67) > C(52.66) > A(47) > B(16.66), while for V(%) the order is C(47.33) > D(44.33) > A(44) > B(18.67). Thus, both indicators indicate that factors C and D play dominant roles, followed by A, with B having the least influence.

(4) In orthogonal experiment, two indicators, which like Medicago sativa (M%) and Vigna radiata (L.) Wilczek (V%), were evaluated to determine factor significance. The results indicated that, for M(%), the order of factor importance was D(ERB) > C(Gravel) > A(Humus) > B(Sand). For V(%), the order was C(Gravel) > D(ERB) > A(Humus) > B(Sand). Both indicators consistently identified C (Gravel) and D (ERB) as the dominant factors, followed by A (Humus), with B (Sand) being the least influential (Table 12).
Data confirmed that appropriate increases in gravel and ERB additions significantly improved germination rates, while humus also produced beneficial effects, though to a slightly lesser extent than factors C (gravel) and D (ERB). Although sand contributed to initial pore structure adjustments, its overall influence on germination remained relatively limited. In other words, incorporating large rock particles into the black clay at the dump site positively influenced its suitability for plant growth.

(5) Based on the experimental results, an initial mixture of black clay and sandy soil was prepared and laid to a thickness of approximately 25 cm at the dump site. A soil-turning machine was then used to incorporate a certain amount of gravel into the mixture. Following this preparation, ERB applications were administered in stages according to the growth characteristics of the plants, successfully promoting vegetation growth at the site (Fig. 5).
The results revealed a significant increase in plant diversity. With a single application of ERB, the distribution of Kochia scoparia (L.) Schrad. increased by 3.06 times, and Salsola collina Pall. increased by 16.40 times. After three ERB applications, these increases reached 3.28 and 34.56 times, respectively. The data indicate that ERB's growth-promoting effect on Salsola collina Pall. is notably stronger than on Kochia scoparia (L.) Schrad.
To enhance soil nitrogen cycling, Medicago sativa L. was introduced. Under the original conditions, this species could not survive. However, after a single ERB treatment, its distribution in the S-ERB area reached 25.25 plants/m^2. Multiple ERB treatments further improved its survival rate, with the distribution reaching 325.75 plants/m^2 after three treatments (Fig. 6).

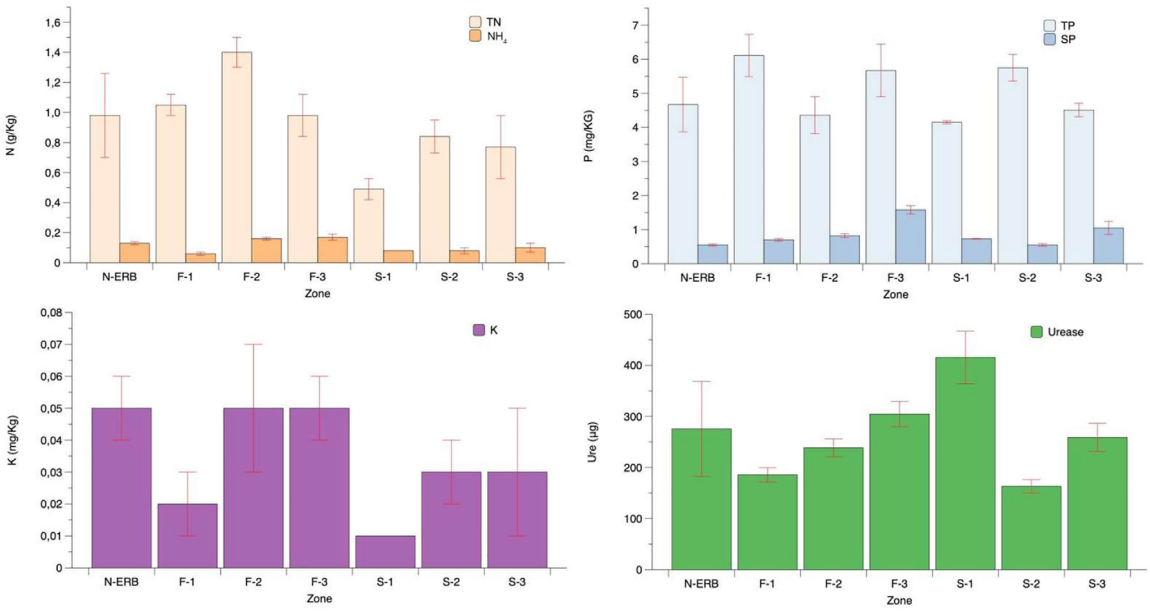

Fig. 6. Changes in N-P-K and urease indices in topsoil in different treatment groups.

(6) Changes of N-P-K and Urease indexes before and after different ERB treatments

In this research F (F-1, F-2, F-3) and S (S-1, S-2, S-3) groups represent multiple ERB application strategies sampled at three key growth stages: seedling emergence (F-1/S-1), pre-flowering (F-2/S-2), and fruiting (F-3/S-3). The N-ERB group serves as a baseline with no ERB treatment, maintaining relatively low and stable soil nutrient and enzyme activity levels throughout the plant growth cycle.

Compared to N-ERB, both F and S groups demonstrate that multiple ERB applications can influence soil conditions over time. The F group exhibits a relatively balanced and sustained improvement in nutrient availability (TN, TP, SP) and enzyme activity (e.g., urease) across all three stages. Notably, enhancements at the pre-flowering (F-2) and fruiting (F-3) stages suggest that the chosen multiple-application strategy supports stable nutrient cycling and microbial processes throughout the plant's developmental milestones. This stability likely fosters a more favorable environment for plant diversity and community structure from early growth through reproduction.

In contrast, the S group, although also applying ERB multiple times, presents greater variability. While early-stage (S-1) measurements indicate a pronounced boost in microbial activity and certain nutrient parameters, subsequent stages (S-2, S-3) show fluctuating nutrient levels and less consistent improvements. This suggests that the particular multiple-application pattern for the S group may provide strong initial stimulation but falls short of maintaining a consistently improved soil environment into the reproductive phases.

Integrating the plant diversity results, both F and S groups surpass the N-ERB baseline in promoting vegetation growth and diversity. However, the F group's more stable, sustained improvements likely support richer and more persistent plant communities over the entire growing season, whereas the S group's variability may limit long-term gains in diversity and overall plant performance.

(7) Analysis of Microbial Diversity and Metabolomics Changes with Different ERB Treatments

The biodiversity analysis revealed the composition and abundance changes in soil microbial communities at the genus level under different treatment conditions. The data indicated that the microbial communities in the original black clay soil (N-ERB) were relatively stable but structurally simple. After multiple and single ERB treatments, the abundance of certain key genera in black clay soil changed significantly (Fig. 7). For example, genera such as Arthrobacter and Streptomyces, belonging to Actinobacteriota, which play important roles in organic matter decomposition and nutrient cycling, increased significantly in the F group (multiple ERB treatments). This contributes to improving soil organic matter decomposition and nutrient release efficiency. Additionally, genera such as Sphingomonas also exhibited higher abundance in the F group, which are often associated with promoting plant growth and enhancing plant stress tolerance. In contrast, while the S group (single ERB treatment) showed an initial increase in certain functional genera, the overall improvement magnitude and sustainability were less pronounced than in the F group.

The co-evolutionary relationships of microbial communities under different treatments corresponded to the observed changes in community structure. Samples from the F group formed tighter microbial interaction clusters in the network analysis, indicating the establishment of potential mutualistic relationships among microorganisms, which help stabilize the soil micro-ecosystem. In contrast, the microbial interaction network of the S group was more dispersed, suggesting that single ERB applications, while capable of short-term stimulation of certain functional microorganisms, were insufficient to form a stable and synergistic microbial community structure over the long term (Fig. 8). This result aligns with the differences in microbial abundance observed among treatment groups in the biodiversity analysis.

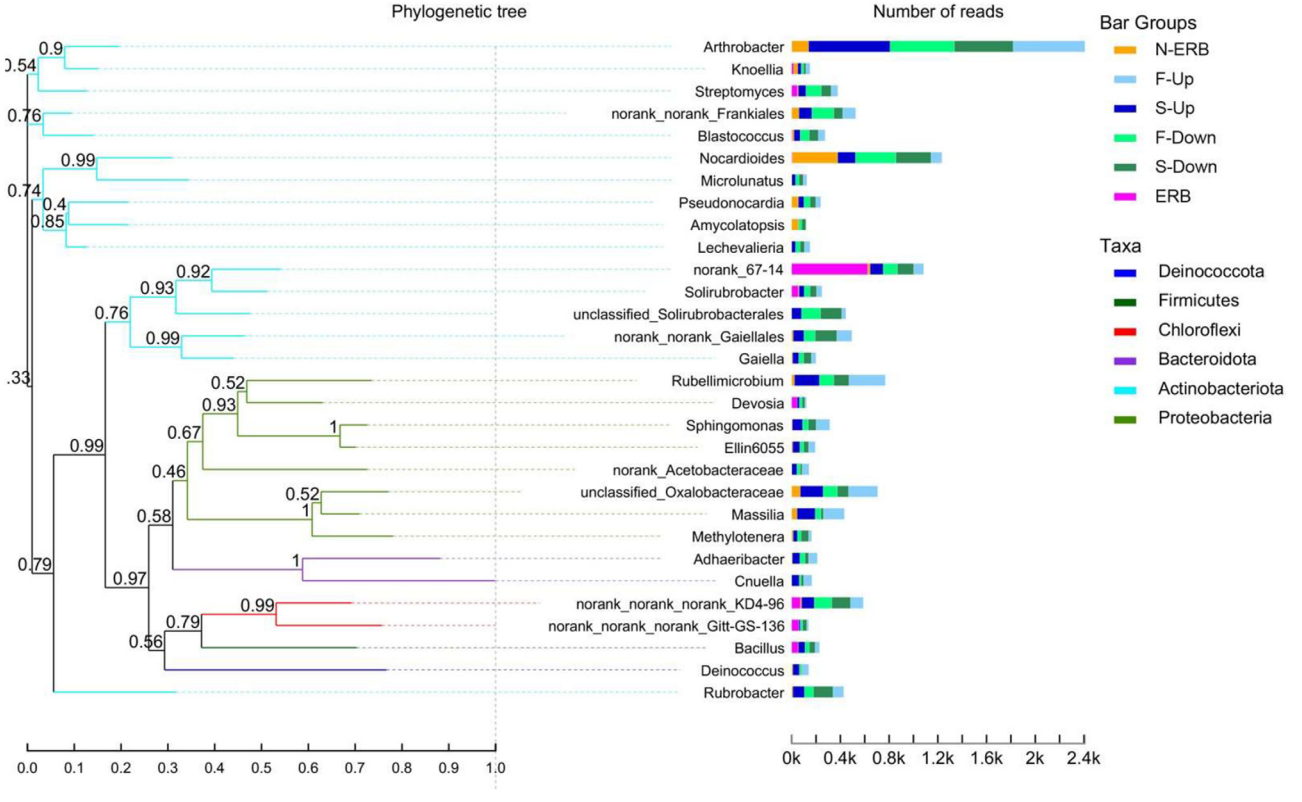

Fig. 7. Evolutionary tree of microflora in the upper and down soil layers of the blocks with different treatments at the end of the experiment.

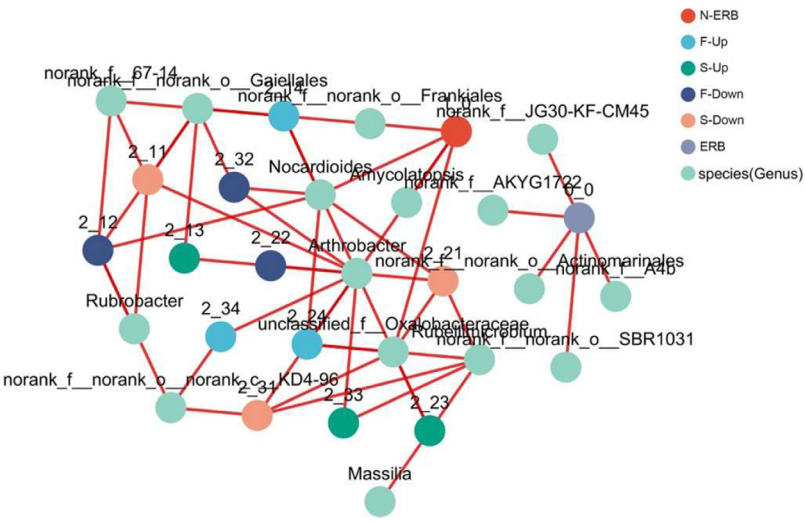

Fig. 8. Covariant network diagram.

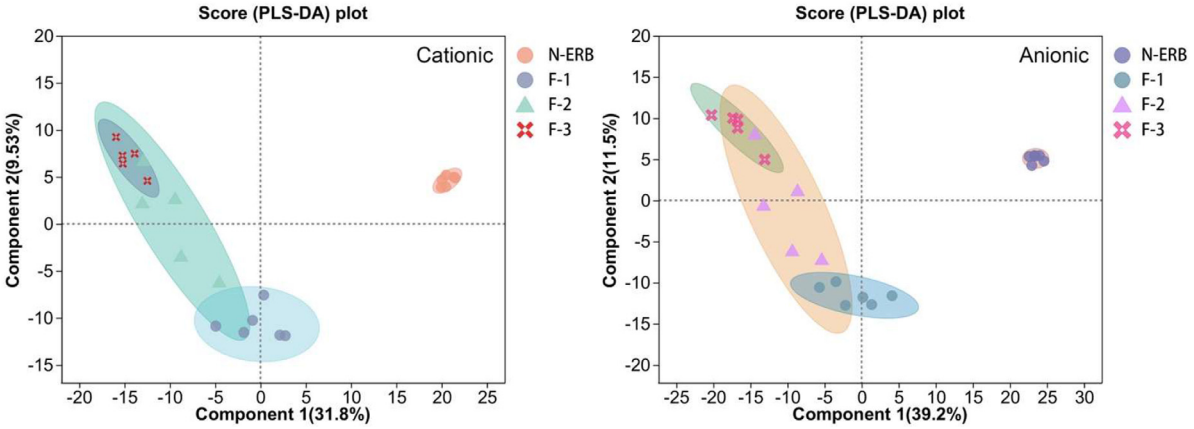

Fig. 9. PLS-DA anion and cation metabolite profiles of soil samples from different treatment batches of F-ERB.

The PLS-DA analysis further confirmed the sustained improvement in soil metabolic characteristics brought about by multiple ERB treatments. In the F group, from F-1 to F-3 sequential sampling, the soil metabolic pattern significantly diverged from the original state (N-ERB) and tended to stabilize. This indicates that nutrient transformation and metabolic pathways are progressively evolving in a direction more conducive to plant growth (Fig. 9). When combined with Figs. 7 and 8, the dynamic changes in metabolomics are closely related to the optimization of microbial community structure and the enhancement of cooperative relationships. The microbial network established through F group treatments facilitates sustained improvement in soil nutrient availability and biological activity, thereby providing a more favorable soil environment for vegetation restoration and increased biodiversity.

Conclusion

The ecological restoration of mining dump sites is vital for mitigating the environmental damage caused by open-pit mining operations. In regions where topsoil is scarce, restoration efforts face significant challenges due to the lack of suitable substrates for vegetation and soil recovery. To overcome this limitation, we explored the feasibility of using mine-derived black clay as a substitute for topsoil in ecological restoration projects. However, initial experiments revealed that untreated black clay significantly inhibits plant germination and growth, largely due to its unfavorable physical and chemical properties.

In response to these findings, we developed a comprehensive bioremediation method that involves amending the black clay with sand, humus, and gravel, as well as introducing an exogenous microbial agent (ERB) to enhance soil properties and establish a beneficial microbial community. This approach was systematically validated through a series of laboratory experiments and field trials, which demonstrated significant improvements in soil hygroscopicity, water retention capacity, and seed germination rates.

By providing a complete technical solution—from laboratory validation to practical field application—this study addresses a critical gap in ecological restoration techniques for mining areas lacking topsoil. The methodologies and insights gained offer valuable references for similar ecological restoration challenges elsewhere, contributing to sustainable environmental management and rehabilitation practices in mining-impacted regions.

Ethics statements

This study complied with all relevant environmental and biosafety guidelines. We obtained necessary permissions from local authorities and the mining company to collect soil samples and conduct field experiments on designated sites. No endangered or protected species were involved in the research.

All microbial strains used were safe and managed according to standard biosafety protocols to prevent any environmental impact. Efforts were made to minimize disturbance to the local ecosystem during both laboratory and field activities. Data collected were used solely for scientific purposes, and there are no conflicts of interest to declare.

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Supplementary material *and/or* additional information [OPTIONAL]

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

María Laura Gigena: Methodology, Writing – original draft, Data curation, Funding acquisition. **Dong Xiao:** Conceptualization, Writing – original draft, Funding acquisition. **Fangzhou Li:** Writing – review & editing. **Chengyu Wu:** Software, Data curation. **Yuhong Zhang:** Data curation.

Data availability

Data will be made available on request.

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