Research Article

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Effect of microbial combination with organic fertilizer on *Elymus dahuricus*

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Abstract: The objective of this study was to compare the growth using an organic fertilizer culture comprising wheat straw, mushroom residue or sawdust and dry dung, or plant growth-promoting microbes (PGPM) on the growth conditions and nutritional status of *Elymus dahuricus* to provide a set of feasible plans for the treatment and restoration of abandoned land exhibiting lower organic matter, calcification, and alkaline soil of the Qilianshan coal mine. Pot experiments were conducted on four groups to study the effect of the characteristics of nutrient absorption of *E. dahuricus*: (1) original soil with or without the addition of soil bacteria and compound bacteria (nitrobacteria and *Pleurotus*), (2) different ratios of original soil mixed with different proportions of organic fertilizer, (3) different proportions of original soil mixed with different proportions of organic fertilizer and soil bacteria, and (4) different proportions of original soil mixed with different proportions of organic fertilizer and compound bacteria. Results showed that original soil supplemented with different PGPM, organic fertilizer treatment, and the organic fertilizer combined with different PGPMs was an obvious increase in the growth of *E. dahuricus*. In particular, 40% of organic fertilizers mixed with the compound bacteria (nitrobacteria and lateral bacteria) exhibited the best growth trend, significantly improving the soil nutrients, the growth of *E. dahuricus*, and the nutritional status, and providing a reliable scientific foundation for the treatment and restoration of the abandoned land of the Qilianshan coal mine.

Keywords: *Elymus dahuricus*, coal mine, vegetation, nutrient elements, microbial

1 Introduction

The vegetation in Qilian County in Qinghai Province has a simple plant community structure, short growth cycle, slow exchange of material energy within the ecosystem, poor ability to resist external interference by natural recovery, and high sensitivity and instability to external interference. Under the influence of undesirable man-caused factors, the vegetation and land have shown a progressing trend of destruction and degradation. Considering the local geographical and climatic conditions, appropriate herbs and woody plants must be selected to achieve a primarily natural restoration supplemented by artificial greening. Such tactics should facilitate the development of a suitable community structure to achieve integration and reduction with the surrounding vegetation, prevention of soil erosion, and improvement in the ecological environment. Therefore, it is necessary to select vegetation with developed root systems and drought tolerance for restoration. Although coal mining yields remarkable economic benefits, it also causes serious social and environmental issues in the surrounding areas. The exploration and development of mineral resources have caused a series of geological and environmental issues in the Qinghai–Tibet Plateau [1,2]. The difficulty in restoration study area is much greater than that in the eastern area owing to the higher costs, depending on the high altitude, cold climate, and short plant-growth cycle [3–5]. If the vegetation cannot quickly recover during and after coal mining, the natural environment and ecological conditions of the surrounding areas deteriorate, resulting in a series of problems, such as decline in soil fertility, nutrient imbalance, and environmental pollution [6,7]. Therefore, it is necessary to adjust and improve the soil structure and restore the vegetation coverage. To this end, the improvement in species richness,

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the structure of soil microbial communities, and the vitality of crop roots are essential.

According to an investigation of the surrounding vegetation of the treatment area before mining, the main herbaceous vegetation is *Miscanthus* spp., *E. dahuricus* (*Elymus dahuricus*), and cold grass. Of these, *E. dahuricus*, an important member of the *Poaceae* (*Gramineae*) wheat family, is a meso-dry meridian perennial fine forage grain found in grasslands and meadows. It is an important component of an extremely high-value feeding material. Its collection of resistance genes confers *E. dahuricus* disease resistance, insect resistance, drought resistance, and salt tolerance, which wheat crops lack, and it is an important germplasm resource for modern wheat breeding. For these reasons, *E. dahuricus* was selected for vegetation restoration in the treatment zone [8]. In 2018, Zhang et al. [1] demonstrated that the grass species in the abandoned mining land of the Qinghai–Tibet Plateau should use pioneer plants with well-developed root systems, barren tolerance, drought tolerance, and a short growth cycle. These plants mainly include *E. dahuricus*, lambgrass, *Leymus chinensis*, *Poa crymophila*, and hulless barley. In 2010, Zhou [2] reported that artificial restoration shows that using native plants, including *Roegneria thoroldiana* and *E. dahuricus*, supplemented by appropriate artificial plant vegetation, enabled rapid restoration of vegetation in the project site of the Qinghai–Tibet high-year frozen soil area. Chen et al. [9] also confirmed that *E. dahuricus* has good cold resistance, drought resistance, and salt-alkali tolerance. The natural emergence rate in borrow soil can reach 60%, and the survival rate of overwintering and the average second-year plant community coverage degree is more than 50%. *E. dahuricus* also showed good adaptability in the borrow ground of the Qinghai–Tibet railway. Thus, it is effective and feasible for quick restoration of the vegetation of the secondary bare land on the borrow site of the Qinghai–Tibet railway.

In addition, the application of organic fertilizer is an important means to adjust the balance of soil nutrients, realize the combination of land use and nutrition, and improve soil fertility [10]. Meanwhile, plant growth-promoting microbes (PGPM) and organic fertilizer comprise a bio-organic type of fertilizer that has both organic fertilizer and microbial efficacy. It contains a variety of nutrients and specific functions that help improve the soil fertility, promote crop absorption, and enable element release [11–14]. Wang et al. [15] showed that the application of PGPM can change the soil microbial abundance, diversity, and community structure of organic gourd root zone, improve soil enzyme activity, and improve the quality of organic gourd. Wang et al. [16] reported that using appropriate organic fertilizer increased the diversity of soil actinomycetes/fungi, urease, catalase, sucrase, and alkaline phosphatase; furthermore, it increased the number of bolls per cotton plant, which promoted cotton growth and dry matter accumulation on the ground.

The alpine steppe ecosystem of the Qinghai–Tibet Plateau is subject to severe degradation. It is of great significance to study the use of PGPM to restore damaged alpine steppes. However, effective methods for promoting the growth of different PGPMs for fine pasture growth remain unclear. Therefore, based on the composition of plant community species, soil physical and chemical properties, and soil nutrition in the Qilian mountain open-pit coal mine in the Qinghai–Tibet Plateau, *E. dahuricus* was selected for this study in conjunction with fertilization and application of different ratios of PGPMs to perform indoor pot experiments to provide grassland restoration to the mining area. The study provides a theoretical basis and technical foundation for other applications and locations. Therefore, we aimed to determine (1) the difference in the characteristics of nutrient absorption of *E. dahuricus* using an organic fertilizer, (2) the difference in microbial communities and diversity using the organic fertilizer, and (3) the change of nutrient elements in the soil using the organic fertilizer.

2 Materials and methods

The soil in the mining area is a thin layer of alpine meadow soil. It is fertile and is mostly summer pasture. The original vegetation coverage rate was more than 50%. The soil is alkaline and contains calcium carbonate. The over-mining range of the mining area is mainly gray-black and dark gray gravel soil with small particle size and poor roundness. The mud content is 10–20%.

In 2018, we collected soil samples from the open-pit coal mine area. In the 0–20 cm soil layer, six soil samples (sampling sites at N 38°30′ 06.97″ to 38°29′ 46.38″, E 99°08′ 44.94″ to 99°09′ 45.43″) were collected from the research mining area; each sample was a mixture of four sub-samples, and the combined weight was about 2 kg. A sterile screw was used to load the sample into a plastic bag, and the samples were kept refrigerated during shipment to the laboratory. Some soil samples were placed in a 4°C refrigerator for analysis and measurement to determine their physical and chemical properties. Other samples were placed in the refrigerator for genome extraction and high-throughput sequencing.

Fluorescence spectrometry was performed per Chinese National Standard GB/T14506.1-2010 with a test environment of 26°C and 40% humidity to analyze the soil content of MgO, K_2O, P, CaO, Fe_2O_3, Cu, and Zn using an X-ray fluorescence spectrometer (Rigaku, Japan). Total organic carbon (TOC) was measured by an OI 1030 TOC analyzer (OI Analytical Co., College Station, TX, USA). pH was determined via a pH analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The soil N content was determined using the Kjeldhal analyzer (Hanon K1100F). Hydrolyzed N content (LY/T 1229-1999) [17], available K (LY/T 1236-1999) [18], and available P (LY/T 1233-1999) [19] in the soil were analyzed by chemical methods.

Elemental content of the plants was conducted via inductively coupled plasma emission spectrometer (6,300; Thermo Scientific, Waltham, MA, USA) at 26°C and 40% humidity for K, Ca, Mg, P, Fe, Cu, and Zn. Minimum detection concentrations (ω) for each compound were as follows: ω (Ca)/0.010×10^{-2}, ω (Mg)/0.05×10^{-2}, ω (P)/0.05×10^{-2}, ω (Fe)/0.05×10^{-6}, ω (Cu)/0.050×10^{-6}, and ω (Zn)/0.250×10^{-6}. The N content was determined using the Kjeldhal nitrogen analyzer.

The time of bacterial culture was 29 September to 31 October 2019. Preparation of liquid culture medium: liquid culture medium, solid culture medium, and the utensils used were sterilized for 25 min under high-pressure steam at 121°C.

A soil sample of 15 g was weighed using an analytical balance. The number was written on the weighing paper to avoid mixing samples. After the soil is weighed, the remaining soil samples were sealed, labeled, and put back to the original refrigerator storage location. Then, 15 g of the soil samples was put into the conical flask containing liquid medium, shaken well, and the bottle mouth is sealed with sealing membrane and rubber tendon. Four conical vials were placed on a water bath thermostatic oscillator at a speed of 150 rpm, and the liquid medium was changed after 7 days. After 2 days of continuous culture on the water bath thermostatic oscillator, the plate was coated on the solid medium in the petri dish on the super-clean workbench and cultured at room temperature around 15°C. Bacterial growth was not obvious within 2 days, and the temperature was raised to 27°C in the artificial climate chamber. After another day of continuous culture, bacterial colonies could be clearly seen in the petri dish. The solid medium was planked in the petri dish on the ultraclean table for further culture observation. After the colony growth was obvious, it was transferred to the liquid medium for enrichment culture and prepared to enter the pot experiment stage.

Soil bacteria medium (g/L) comprised peptone 2, NaCl 5, and ultrapure water 1,000 mL, sterilized at 121°C for 20 min. Isolation medium: 2% agar to the enrichment medium. Nitrifying bacteria medium (g/L) contained glucose 5.0, ammonium sulfate 2.0, NaCl 2.0, $FeSO_4{\cdot}7H_2O$ 0.4, K_2HPO_4 1.0, MgSO4·$7H_2O$ 0.5, and ultrapure water 1,000 mL, pH 7.2, sterilized at 121°C for 25 min. Ammonification bacteria enrichment medium (g/L) contained peptone 5, NaCl 0.25, KCl 0.3, K_2HPO_4 0.25, $MgSO_4{\cdot}7H_2O$ 0.5, $FeSO_4{\cdot}7H_2O$ 0.01, and ultrapure water 1,000 mL, pH 7.2, sterilized at 121°C for 25 min. Simultaneous nitrification and denitrification bacteria enrichment medium (g/L) had sodium citrate 3.7, ammonium sulfate 2.0, $CaCO_3$ 5.0, K_2HPO_4 1.0, $FeSO_4{\cdot}7H_2O$ 0.4, $MgSO_4{\cdot}7H_2O$ 0.5, NaCl 2.0, and ultrapure water 1,000 mL, sterilized at 121°C for 25 min. *Pleurotus* enrichment medium (g/L) contained glucose 15, peptone 2, LB broth 4, calcium carbonate 5, dipotassium hydrogen phosphate 5, magnesium sulfate 0.3, manganese sulfate 0.02, and ultrapure water 1,000 mL, pH 7.2–7.4, sterilized at 121°C for 25 min.

Sequencing and extraction of total DNA of amended mine soil microorganisms were conducted in Beijing. Extracted genomic DNA was detected using 1% agarose gel electrophoresis. Specific primers with barcodes were synthesized based on the designated sequencing regions.

First, the Fast DNA Spin Kit (MP Biomedicals) was used to extract the microbial DNA in the soil samples, and then NanoDrop200 UV–visible spectrophotometer was used (Thermo Scientific, Wilmington, USA) to determine the final DNA concentration and purity. Finally, 1% agar-gel electrophoresis was used to detect the extraction quality of DNA. The 16rRNA of microorganisms was amplified through polymerase chain reaction (PCR) using a bacteria-specific primer sequencing area 338F(ACTCCT ACGGGAGGCAGCAG) and 806R(GGACTACHVGGGTWTC-TAAT) in the high-variable region of V3–V4 (392BP) Polymerase; PCR instrument: ABI GeneAmp type 9700. All samples were carried out in accordance with official experimental conditions; each sample was repeated thrice. PCR products of the same sample were mixed and detected through 2% agarose gel electrophoresis. AxyPrepDNA gel recovery kit (AXYGEN) was used to cut glue to recover the PCR products, and Tris_HCl elution was used. 2% agarose electrophoresis quantitative results of preliminary reference electrophoresis, PCR products with QuantiFluor – ST blue fluorescence quantitative system (Promega) were used for testing, and conducted the appropriate proportional mixing according to the sequencing quantity requirements of each sample and then Miseq library construction and sequencing Miseq adopts Trimmomatic software quality control on the original DNA sequencing sequence, then use FLASH

software, get high-quality sequenceUPARSE software was used to perform OUT clustering of high-quality sequences under the condition of 97% similarity, and then UCHIME software was used to eliminate chimeras [20]. Finally, all OUT sequences were extracted according to a certain number of sequences, and the extracted samples were then analyzed later as follows:

PCR using TransGen AP221-02: TransStart Fastpfu DNA Polymerase, 20 μL Reaction system: 5× FastPfu Buffer 4 μL, 2.5 mM, dNTPs 2 μL, Forward Primer (5 μM) 0.8 μL, Reverse Primer (5 μM) 0.8 μL, FastPfu Polymerase 0.4 μL, BSA 0.2 μL, Template DNA 10 ng, Addition ddH_2O 20 μL. PCR (ABI GeneAmp® 9700) reaction parameter: (a) 1× (3 min at 95°C), (b) cycle number × (30 s at 95°C; 30 s at annealing temperature °C; 45 s at 72°C), (c) 10 min at 72°C, 10°C until halted by user.

Soil samples (15 g each) were cultivated in nutrient solution and subcultured after 8 days. After 2 days, samples were streaked onto solid medium and enriched via the streak-plate method with triple selection of single colonies. Enriched soil bacteria were cultured in liquid medium, and then *E. dahuricus* cultivation experiments were performed on the mine soil with added different bacteria as a standard application.

E. dahuricus seeds (0.2 g per pot) were planted at a sowing depth of 2 cm and 300 mL of soil. Nitrifying bacteria were a mixture of nitrifying bacteria, ammoniating bacteria, and simultaneous nitrifying and denitrifying bacteria.

Soil bacteria (6.25 mL), *Pleurotus* (46.9 mL), and nitrifying bacteria (28 mL) were added to each corresponding pot, and water was added to a total volume of 75 mL for each pot to ensure soil saturation. *E. dahuricus* was planted and watered every 2 days. Plant heights (interval 10 cm) were measured and recorded after growth was observed. *E. dahuricus* pots were divided into 12 groups designated as NT1–12 and are summarized in Table 1. Nitrifying bacteria treatment included a mixture of nitrifying bacteria, ammoniating bacteria, and simultaneous nitrifying and denitrifying bacteria. After treatment, colonies from each sample were enriched and analyzed.

Organic fertilizer used herein contains peat, straw, perlite, mushroom, vermiculite, and wine residue, mixed in different proportions, with good fertility, water retention, and permeability. It provides a healthy environment for plant roots.

All data analysis was conducted using SPSS24.0 software (Table 3; Figure 1). The trend chart of the growth height of *Elymus dahuricus* from 14 November to 29 November was made using Excel 2016.

3 Results

3.1 G1 (NT1, NT2, and NT3)

3.1.1 Growth

The growth trends under different treatments are summarized in Figure 1a. *E. dahuricus* in the original soil NT1 did not grow, and the soil-like bacteria NT2 germinated slightly later than the combination of nitrifying bacteria and *Pleurotus* NT3.

The pots mixed with bacteria grew better than the pots without bacteria. Thus, bacteria may have an improvement effect on the soil. The nitrifying bacteria + *Pleurotus* group in NT1–NT2–NT3 was better than the soil in the soil group. The growth was slightly better, and the combination of nitrifying bacteria + *Pleurotus* may improve the soil than soil-like bacteria. This suggests that the use of microbial agents could replace chemical fertilizers. This treatment method is not harmful to the environment and can improve the nutritional effectiveness than chemical fertilizers [12].

3.1.2 Nutrient element concentration in plants

The NT1 potted original soil is not conducive to plant growth. Therefore, we only determined the concentration in NT2 and NT3. Comparisons revealed tiny content

Table 1: *E. dahuricus* pots were divided into 12 groups designated as NT1–12 in the pots

Pots	Soil addition ratio (%)	Organic fertilizer addition ratio (%)	Microbe
NT1	100	—	—
NT2	100	—	Soil bacteria
NT3	100	—	Nitrifying bacteria + *Pleurotus*
NT4	80	20	—
NT5	60	40	—
NT6	40	60	—
NT7	80	20	Soil bacteria
NT8	60	40	Soil bacteria
NT9	40	60	Soil bacteria
NT10	80	20	Nitrifying bacteria + *Pleurotus*
NT11	60	40	Nitrifying bacteria + *Pleurotus*
NT12	40	60	Nitrifying bacteria + *Pleurotus*

Note: Soil bacteria were extracted from original soil in coal mine.

difference in each of the elements in NT2 and NT3 pot experiments, except for Cu and P elements.

3.1.3 Soil chemistry

The soil chemistry data are summarized in Table 2, by comparison, showing that the nutrient elements content of NT1, NT2, and NT3 has tiny differences between them, such as MgO, K_2O, P, CaO, Fe_2O_3, Cu, Zn, N, and TOC. Only native soil NT1 available K, hydrolysable N, and available P were higher than NT2 and NT3.

3.2 G2 (NT4, NT7, and NT10)

3.2.1 Growth

The growth trend for a period is shown in Figure 1. In the G2, with the mixing of 20% organic fertilizer and soil bacteria, and nitrifying bacteria + *Pleurotus*, comparison revealed that the growth of soil bacteria (original soil) produced the most plant growth (NT7 > NT4 > NT10) using the mixing of 20% organic fertilizer (Figure 1b). These are used as inoculants to change the microbial diversity and interaction between soil and plants to promote plant growth [21,22].

3.2.2 Nutrient element concentration in plant

The concentration of nutrient elements in plants is shown in Table 3, wherein the Ca concentration in NT10 was shown to exhibit a significant decrease from NT4 0.315×10^{-2} to NT10 0.084×10^{-2}. Meanwhile, the Fe concentration in NT4 exhibited a significant decrease from NT4 168×10^{-6} to NT10 15.3×10^{-6}. Other element-concentration levels exhibited tiny changes in the group pot experiments.

3.2.3 Soil chemistry

The soil chemistry data of NT4, NT7, and NT10 are summarized in Table 2 by comparison, showing the nutrient

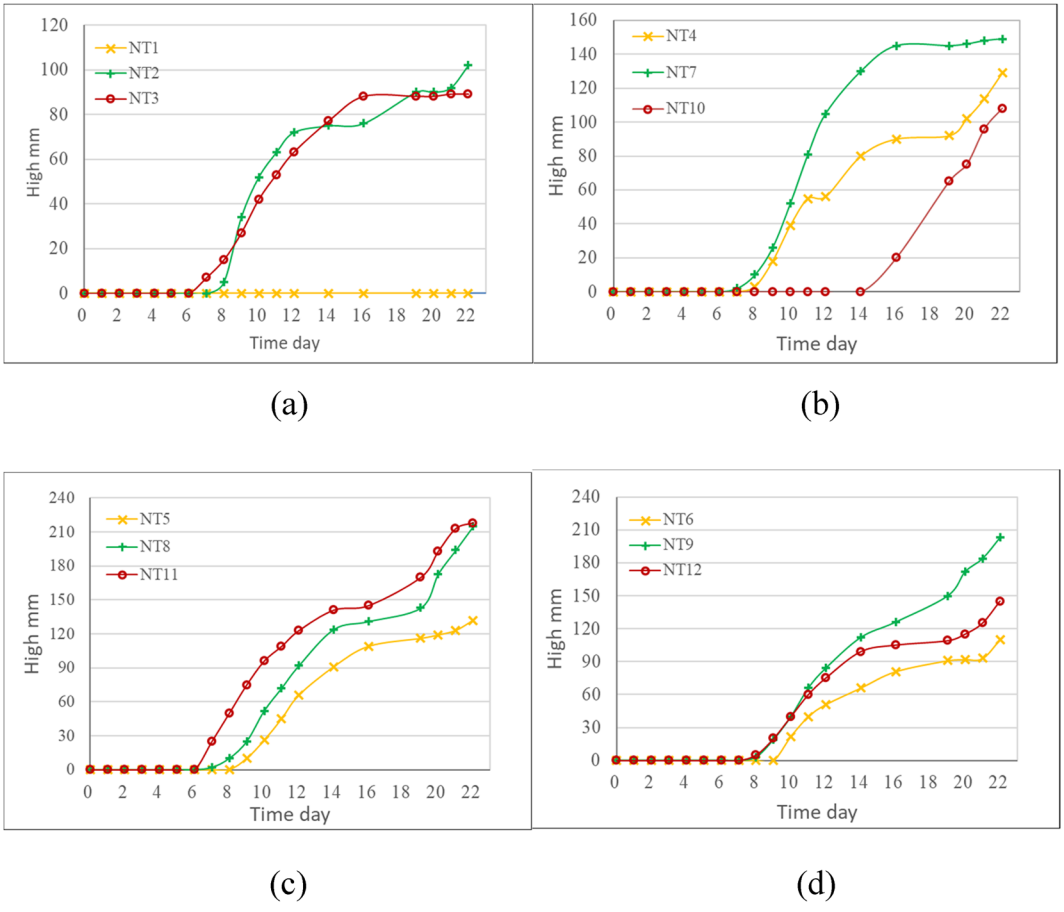

Figure 1: Growth (in height) of *E. dahuricus* from 14 November to 29 November. (a) NT1, NT2, and NT3. (b) NT4, NT7, and NT10. (c) NT5, NT8, and NT11. (d) NT6, NT9, and NT12.

Table 2: Concentration level of nutrient elements of coal mine soil from Qilian area

Treatments	Sample	MgO	K_2O	P	CaO	Fe_2O_3	Cu	Zn	N	TOC	Available K	Hydrolysable N	Available P	pH
		%	%	μg/g	%	%	μg/g	μg/g	%	%	μg/g	μg/g	μg/g	
CK	NT1	2.03	1.93	737	2.68	8.05	41.5	108	0.218	0.060	1,197	12 7	0.753	7.388
T1	NT2	2.21	1.88	775	2.66	7.85	35.8	105	0.211	0.080	993	116	<0.5	7.553
T2	NT3	2.07	2.04	931	2.75	7.91	36.2	113	0.137	0.055	951	126	<0.5	7.597
T3	NT4	1.95	1.84	1,419	2.53	8.00	38.5	122	0.255	0.078	991	253	0.806	7.622
T4	NT7	1.85	1.81	1,730	2.71	8.70	41.1	159	0.323	0.203	132	268	0.957	7.594
T5	NT10	1.98	1.92	1,895	2.70	8.64	46.1	158	0.301	0.065	1,129	265	1.40	7.880
T6	NT5	1.83	1.77	2,249	2.59	8.02	44.2	156	0.395	0.084	1,051	370	1.60	7.689
T7	NT8	1.80	1.82	2,188	2.34	8.18	48.7	145	0.401	0.058	1,053	303	1.69	7.810
T8	NT11	1.80	1.75	2,592	2.69	7.78	40.5	137	0.432	0.065	146	383	2.27	7.860
T9	NT6	1.90	1.87	3,020	2.97	7.87	35.5	178	0.450	0.031	865	437	2.29	7.875
T10	NT9	1.72	1.84	3,110	2.77	7.66	48.2	145	0.464	0.082	954	525	2.36	7.665
T11	NT12	1.66	1.69	5,005	3.16	8.11	37.9	186	0.656	0.088	175	578	5.93	7.918

Note: CK, blank; T1, soil bacteria + 100% soil; T2, nitrifying bacteria + *Pleurotus* + 100% soil; T3, 20% organic fertilizer + 80% soil; T4, 40% organic fertilizer + 60% soil; T5, 60% organic fertilizer + 40% soil; T6, 20% organic fertilizer + soil bacteria + 80% soil; T7, 40% organic fertilizer + soil bacteria + 60% soil; T8, 60% organic fertilizer + soil bacteria + 40% soil; T9, 20% organic fertilizer + nitrifying bacteria + *Pleurotus* + 80% soil; T10, 40% organic fertilizer + nitrifying bacteria + *Pleurotus* + 60% soil; T11, 60% organic fertilizer + nitrifying bacteria + *Pleurotus* + 40% soil.

elements content yielded by mixing 20% organic fertilizer NT1, NT2, and NT3 have moderate differences between them. For example, the concentration level of NT3 K_2O, P, CaO, Cu, P, and pH is higher than other NT4 and NT10. Only native soil TOC was observed to be smaller than NT2 and NT3.

3.3 G3 (NT5, NT8, and NT11)

3.3.1 Growth

After mixing 40% organic fertilizer, the growth of nitrifying bacteria + *Pleurotus* was the best (NT11 > NT8 > NT5) (Figure 1c). After being mixed with 40% organic fertilizer, the growth without bacterium is the best, followed by NT8 with soil-like bacteria (NT5 > NT8 > NT11).

3.3.2 Nutrient element concentration in plants

Table 2 shows that the nutrient concentration in NT8 was significantly lower than that in NT5 and NT11, excluding N. Meanwhile, the Fe concentration in NT5 (80.1×10^{-6}) was significantly higher than the NT8 (33.8×10^{-6}) and NT11 (30.7×10^{-6}) in the group pot experiments.

3.3.3 Soil chemistry

The soil chemistry data of 40% organic fertilizer additions of NT5, NT8, and NT11 are summarized in Table 1, showing that the nutrient element content of NT5, NT8, and NT11 exhibits minor differences between them. However, Zn and available K of NT11 were lower than that in other pot experiments, wherein available K was more

Table 3: Concentration level of nutrient elements of *E. dahuricus* under treatment pathways

Treatments	Sample	ω (N)/10^{-2}	ω (K)/10^{-2}	ω (Ca)/10^{-2}	ω (Mg)/10^{-2}	ω (P)/10^{-2}	ω (Fe)/10^{-6}	ω (Cu)/10^{-6}	ω (Zn)/10^{-6}
CK	NT1	—	—	—	—	—	—	—	—
T1	NT2	1.060	0.147	0.280	0.066	0.032	115.00	1.720	7.980
T2	NT3	0.776	0.190	0.242	0.067	0.073	108.00	0.90	6.950
T3	NT4	0.907	0.181	0.315	0.073	0.036	168.00	0.956	13.40
T4	NT5	0.926	0.185	0.183	0.068	0.066	80.10	1.040	16.60
T5	NT6	0.854	0.180	0.285	0.112	0.103	78.60	1.710	33.70
T6	NT7	1.290	0.121	0.082	0.057	0.040	3.310	0.450	3.38
T7	NT8	1.010	0.069	0.054	0.035	0.015	33.80	0.446	7.72
T8	NT9	1.210	0.124	0.107	0.033	0.031	25.80	0.546	10.80
T9	NT10	0.908	0.068	0.084	0.03	0.020	15.30	0.286	9.01
T10	NT11	0.912	0.193	0.177	0.071	0.071	30.70	0.864	18.80
T11	NT12	1.150	0.234	0.167	0.063	0.076	24.60	0.816	12.60

obvious. By comparison, the group nutrient elements and pH were observed to be higher than those in other first group and second group.

3.4 G4 (NT6, NT9, and NT12)

3.4.1 Growth

After mixing 60% organic fertilizer, the growth of soil-like bacteria is the best (NT9 > NT12 > NT6) (Figure 1d). After being mixed with 60% organic fertilizer, the growth without bacterium was the best, followed by NT9 with soil-like bacteria (NT6 > NT9 > NT12).

3.4.2 Nutrient element concentration in plant

The concentration of nutrient elements in plants of the group pot experiments is summarized in Table 3, showing that the element concentration in NT6 was significantly higher than that in NT9 and NT12, particularly of Zn, Cu, Fe, P, and Mg. It was identical to the above group (NT5, NT8, and NT11).

3.4.3 Soil chemistry

The soil chemistry data of 60% organic fertilizer additions of NT6, NT9, and NT12 are summarized in Table 1, showing that the nutrient element content of NT6, NT9, and NT12 exhibits moderate differences between them. By comparison, MgO, K, and available K of NT11 were observed to be lower than that in other pot experiments of NT6 and NT9, respectively. Other element levels and pH were higher than those in NT6 and NT9.

3.5 Comparison

In comparison to NT4–NT7–NT10, when 20% organic fertilizer was mixed, the combination of nitrifying bacteria and *Pleurotus* is more beneficial to the growth of *E. dahuricus*. The microbial load of soil bacteria in NT7 was observed to be higher than that in NT4 and NT10 (Table 4).

In comparison to NT5–NT8–NT11, when 40% organic fertilizer was mixed, no bacteria were mixed, but better growth was observed. The microbial load in NT11 with nitrifying bacterial + *Pleurotus* was higher than that in NT5 and NT8 (Table 4).

In comparison to NT6–NT9–NT12, there was no obvious concentration of *E. dahuricus*. This was probably because when the organic fertilizer was mixed in a large amount of 60%, it was not sensitive to the added bacteria, and the added bacteria showed no obvious effects on soil improvement.

Simultaneously, in the group not mixed with organic fertilizer and bacteria, *E. dahuricus* did not grow at all, indicating that the original soil was not suitable for the growth of *E. dahuricus*.

In the *E. dahuricus* mixed only with organic fertilizer and without bacteria, the *E. dahuricus* mixed with 40% organic fertilizer experienced the best growth in comparison with NT4–NT5–NT6, and the suitable proportion of mixed organic fertilizer for soil improvement was close to 40%. The soil volume ratio was random.

In comparison to NT7–NT8–NT9, soil-like bacteria were added. When comparing the growth of *E. dahuricus*,

Table 4: Soil microbial load of post-harvest soil sampling

Sample name	Target name	C_T mean	Quantity mean	Dilution ratio	Elution volume	Weight (g)	Quantity mean (copies/g)
1	16S	21.07	6.37×10^3	100	100	0.16	3.98×10^8
2	16S	20.65	8.36×10^3	100	100	0.18	4.64×10^8
3	16S	19.27	2.04×10^4	100	100	0.28	7.28×10^8
4	16S	18.60	3.14×10^4	100	100	0.19	1.65×10^9
5	16S	18.28	3.86×10^4	100	100	0.20	1.93×10^9
6	16S	17.29	7.33×10^4	100	100	0.18	4.07×10^9
7	16S	17.68	5.70×10^4	100	100	0.37	1.54×10^9
8	16S	17.94	4.82×10^4	100	100	0.13	3.70×10^9
9	16S	18.64	3.06×10^4	100	100	0.27	1.13×10^9
10	16S	18.00	4.63×10^4	100	100	0.21	2.20×10^9
11	16S	17.36	7.01×10^4	100	100	0.19	3.69×10^9
12	16S	17.33	7.15×10^4	100	100	0.24	2.97×10^9

it was found that when soil-like bacteria was added and mixed with organic fertilizer, the appropriate proportion was still close to 40%. The soil's microbial load data of NT1 and NT12 are summarized in Table 4, showing that microbial load level of soil was lower than that in other plots.

In comparison to NT10–NT11–NT12, when mixed with nitrifying bacteria and *Pleurotus* and mixed with different proportions of organic fertilizer, there is no obvious concentration phenomenon. It may be that nitrifying bacteria and *Pleurotus* are unstable when mixed with organic fertilizers [23]. Table 3 shows that NT11–NT12 soil microbial load level higher than NT10.

In addition, mixed organic manure + nitrifying bacteria + *Pleurotus* had a good growth trend (NT10, NT11, and NT12). The second was mixed with organic fertilizer and soil-like bacteria (NT7, NT8, and NT9). The organic fertilizer (NT4, NT5, and NT6) was worse than the above two groups. The results showed that the combined use of fertilizers and fungi was significantly better than the individual usage [24,25]. However, the soil microbial load of NT4, NT5 and NT8, NT9 was identical as presented in Table 4.

Strengthening the amount of bacteria could regulate the degradation of organic matter and increase the concentration of soil nutrients [26]. In addition, fungi usually form a symbiotic relationship with plant species to provide nutrients for plant growth [27,28]. Therefore, organic fertilizers and microbial agents have different regulatory effects on the local soil to promote the growth and development of plants.

4 Discussion

4.1 The changes of soil microbial under different treatment strategies

The Qinghai–Tibet Plateau is a treasure house of natural resources with the most peculiar ecological environment and richest biological resources on the roof of the world. It is also the most sensitive area to global climate change. Frequent human activities in recent years have severely damaged the ecological environment of the Qinghai–Tibet Plateau. As a recovery method that is both economical and free of secondary pollution, plant restoration is favored by many scholars. Plants and underground microorganisms are closely related and altogether promote various biochemical reactions and energy flows in the soil environment. Microbes are one of the important components of soil. Soil microbes participate in various biochemical reactions in soil and are sensitive indicators for detecting changes in climate and soil environment.

The community analysis bar chart on phylum level% for each sample is summarized in Figure 2, respectively. To further clarify the effect of fertilization measures on soil microbial communities, the changes of microbial communities in each sample were analyzed. As a whole, information including abundance, coverage, and diversity of species in the community can be obtained through diversity index analysis, based on the comparative analysis of Silva, RDP, Greengene, and Unite databases. Microbial community structure of soil samples at the phylogenetic level is as follows: Patescibacteria 2.28%; Gemmatimonadetes 2.18%; Bacteroidetes 4.36%; Firmicutes 5.27%; Acidobacteria 9.43%; Chloroflexi 18.28%; Actinobacteria 26.54%; Proteobacteria 27.53%; others 4.13%. According to the analysis, proteobatoncteria, actinobacteria, and chloroflexi are the main bacterial groups in soil samples; in general, the more the bacteria in the soil, the higher the fertility level [29].

For a single sample, the single microbial population of the original soil sample No. 1 was not conducive to vegetation restoration. In samples 1–3, the proportion of Patescibacteria was extremely low, whereas the proportion of Patescibacteria mixed with organic fertilizers and bacterial agents was relatively high. However, the ratio of Cyanobacteria in samples 1–3 was higher than that in the samples mixed with organic fertilizer, and the proportion of sample 1 in the original soil was higher than that in samples 2 and 3 with the addition of bacteria. Organic fertilizer increased the amount of Patescibacteria in the soil and decreased the amount of Cyanobacteria. Gemmatimonadetes in samples 1–6 accounted for a higher proportion than that in the samples of organic fertilizers. 60% organic fertilizer samples 6 and 12 were higher in Firmicutes than in other samples. It is worth noting that the proportion of Spirochaetes and Fusobacteria in sample 6 was higher than that in other samples. Sample 6 was treated with 60% organic fertilizer. Too much organic fertilizer will increase the number of two bacteria.

Zhong et al. [30] found that soil bacterial community structure was significantly changed in the combination treatment of microbial agent and organic fertilizer compared with the single application of chemical fertilizer and organic fertilizer. Some studies also showed that [31] the combination of microbial agents and organic fertilizers had no significant effect on the number of soil bacteria and actinomycetes. In this study, the results indicate that organic fertilizer blending agent reduced

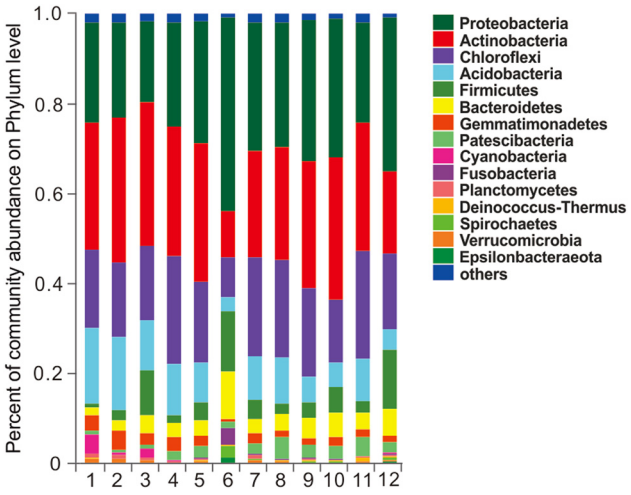

Figure 2: Community analysis bar plot on phylum level% for each sample.

the number of gemmatimonadetes but increased the proportion of Spirochaetes, Proteobacteria, and Firmicutes. Therefore, this study found that the application of organic fertilizers could improve the diversity and richness of soil bacterial and fungal communities.

Li et al. [32] also showed that organic fertilizer contains a large amount of organic materials and nutrients, which can provide sufficient nutrients for microorganisms, can promote the growth of soil microorganisms, and increase the abundance of microorganisms. O'Dell et al. [33] confirmed that the application of organic fertilizer can effectively reduce plant absorption concentration of some heavy metals and can reduce the toxic effect of heavy metals on microorganisms, promote the reproduction of microorganisms, increase the number of flora, and provide the biomass of soil microorganisms.

4.2 The effect of different treatments on soil nutrient elements

Most of the nutrients contained in organic fertilizers are in an organic state, and it is difficult for crops to directly use them. Through the action of microorganisms, a variety of nutrients are slowly released, and nutrients are continuously supplied to crops. Application of organic fertilizers can improve soil structure, coordinate water, fertilizer, gas, and heat in the soil, and improve soil fertility and land productivity. Therefore, we suggest that these treatments may have different durations of effectiveness and need further research to investigate possible long-term differences in these treatments.

The concentration level of nutrient elements of coal mine soil from Qilian area is summarized in Table 1. This study found that as a greater proportion of organic fertilizer is mixed into the soil, Zn, hydrolyzed N, quick-acting P, and P increased, whereas MgO content decreased. Other elements did not change much, and there was no obvious pattern.

For each individual group (NT1, NT2, and NT3), adding soil samples alone and blank samples could increase MgO, P, and TOC, and reduce Cu, quick-acting K, and hydrolyzable N. The components added to the complex cells were better than adding soil samples. The original soil samples increased the content of K, P, Ca, Zn, and decreased the level of N, TOC, available K, and available P.

(NT4, NT7, and NT10): Mixing 20% organic fertilizer + soil-like bacteria and mixing 20% organic fertilizer + nitrifying bacteria + *Pleurotus* significantly increased the levels of various nutrients compared to applying organic fertilizer alone. Adesemoye and Kloepper [34] and Bolan et al. [35] indicated that microorganisms could increase the usage rate of fertilizers and improve the level of trace elements in the soil. However, mixing 20% organic fertilizer + nitrifying bacteria + *Pleurotus* reduced the TOC content, possibly because the compound bacteria could further accelerate the decomposition of organic matter [26].

(NT5, NT8, and NT11): In comparison with the previous group, this group of soil nutrient elements continued to increase, whereas the organic carbon content continued to decline. It is worth noting that the content of quick-acting K mixed with 40% organic fertilizer + nitrifying bacteria + *Pleurotus* had reduced from 1,129 to 146 μg/g. In combination with the upward trend of *E. dahuricus*, the upward trend would not be affected by the content of quick-acting K, but the upward trend of NT11 was better.

(NT6, NT9, and NT12): In comparison with the above components, the content of N, P, hydrolyzed N, and quick-acting P would continue to increase.

Combined with the gains, the two components (NT5, NT8, and NT11) and (NT6, NT9, and NT12) were better than the other two groups. NT11 was the best, followed by NT12. Thus, we analyzed that the content of applying 40% organic fertilizer was better than that of applying 20 or 60% organic fertilizer. Fertilization increased the growth and gas exchange capacity of rhizosphere microorganisms, enhanced the activity of rhizosphere microorganisms, and increased the concentration of zinc, magnesium, and nitrogen in leaves [36].

Research by Marcote et al. [37] believes that increased application of microbial agents could improve soil

physicochemical properties and microflora and increase soil enzyme activities. Other studies [38] have shown that in poor soil, the effect of single application of inoculants is not obvious, and the combined application of organic and inorganic fertilizers and inoculants can better improve soil fertility. Zhang et al. [39] also showed that chemical fertilizers combined with bio-organic fertilizer could significantly increase the content of soil organic matter, available phosphorus, total nitrogen, and total phosphorus. This is mainly because the combined application of organic and inorganic fertilizers can provide not only nutrients but also rich carbon and energy sources for the growth of microorganisms, thus improving soil fertility. Organic fertilizers combined with microbial agents could improve soil nutrients and was a fertilization measure to maintain soil fertility. Meanwhile, the pH value of the organic fertilizer content varied from 7.388 to 7.918. This is contrary to Guo Jinli's research [40] and may be related to the nature of the soil and the different bacterial agents added.

4.3 The effect of different treatment strategies on plant nutrient elements

The concentration levels of nutrient elements of *E. dahuricus* under treatment strategies are summarized in Table 2.

(NT1, NT2, and NT3): Except for K and P, NT2 was lower than NT3 with added bacteria, and the remaining elements were more abundant than NT3.

(NT4, NT7, and NT10): NT4 applied with organic fertilizer alone was more abundant than NT7 and NT10 mixed with bacteria. Hamnér and Kirchmann [41] reported that the use of organic residues could increase the concentration of Zn in cereals. Tang et al. [42] claimed that organic fertilizer can significantly improve the concentration of Fe, Mn, Cu, and Zn in soil and plants. The Fe, Cu, and Zn added to the sample of the inoculant significantly decreased.

(NT5, NT8, and NT11): In comparison with the previous component, the content of Fe, Cu, Zn, Ca, and P had increased, but the richness of plant nutrient elements of the sample added with NT11 mixed fungicide was significantly better than that of NT8 alone added to the soil samples of bacteria, and the sample plants showed the best uptrend.

(NT6, NT9, and NT12): In comparison with the previous group, the content of nutrient elements continued to increase, but the richness of Fe, Cu, Zn, P, and MgO in the samples of the bacterial fertilizer NT9 and NT12 was lower than that of the organic fertilizer alone, sample NT6. From the perspective of plant uptrend, NT9 uptrend was higher than NT6 and NT12.

Overall, Fe, Cu, Zn, Ca, and MgO were higher in (NT1–NT6) plants. In the 6–12 samples of organic fertilizers combined with bacterial agents, Fe, Cu, Zn, Ca, and MgO in plants decreased, whereas the content of element N increased. The results showed that the bacteria agent played a great role in this by promoting the absorption of nutrient elements, thereby reducing the absorption rate of excessively high elements of plants. Li et al. [43] considered that microbial agents contain a large number of functional bacteria, which optimize the soil microbial population structure, accelerate the decomposition of soil organic matter, promote the transformation of fixed nutrients to effective nutrients, and then promote plant growth.

In conclusion, the addition of complex microbial agents to organic fertilizers plays an important role in improving soil fertility and soil microecological environment. However, there are some limitations in this experiment. First, the soil flora is sensitive to the soil environment, which may change with the increase in time; Second, excessive application of organic fertilizer will cause a lot of nitrogen loss and nutrient utilization rate decreased significantly [44]; in the case of long-term fertilization, it is necessary to clarify the threshold of soil organic fertilizer input. Therefore, long-term fertilization experiments should be carried out to analyze the long-term dynamic change process of soil nutrients and microecological environment, systematically summarize the law of soil evolution, and explore the improvement effect and influence mechanism of different treatment strategies.

5 Conclusion

The study showed that because of different fertilization treatments, there was a significant difference in the upward trend of *E. dahuricus*. The 40% organic fertilizer in conjunction with the application of the compound bacterial agent had the most optimal effect on growth. The second was the application of 40% organic fertilizer with soil bacteria. In addition, the richness of nutrient elements in the soil-plant system was superior to the samples of other components. Too much organic fertilizer would have a negative impact on the growth of the plant. Meanwhile, under different treatment strategies, the soil microbial community structure had a certain difference.

The organic fertilizer blending agent reduced the number of gemmatimonadetes but increased the proportion of Spirochaetes, Proteobacteria, and Firmicutes and the diversity and richness of soil bacterial and fungal communities. Subsequent studies should perform long-term continuous observations on the spot and monitor microbial community characteristics, soil chemical properties, and enzyme activities.

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