RESEARCH ARTICLE

# Study on the influence of various vegetation structures on soil microbial community in mine reclamation area

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**As the mining operation is completed, the key lies in how to rehabilitate and restore the soil ecosystem. In this study, properties of soil samples from the same soil layer of the reclamation area for different vegetation types including Oriental arborvitae, Chinese holly, Chinese littleleaf box, and Wheat were tested. The results showed that different vegetation types possessed varying abilities to restore the ecosystem of soil in coal mining areas. In addition, the land for oriental arborvitae also showed higher soil enzyme activity, fungal abundance, and diversity index. The utilization of oriental arborvitae in coal mine reclamation projects could enhance soil quality, promote soil fertility, enrich microbial diversity, and expedite the establishment of a sustainable ecosystem in polluted soils.**

**Keywords:** vegetation structure; soil microbial community; mine reclamation area; oriental arborvitae; soil enzyme activity.

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## Introduction

The restoration of the ecological environment in mine reclamation areas depends on the interaction between vegetation and the soil microbial community. The structure of vegetation reflects the spatial distribution and organization of plants within a given community, while the soil microbial community includes all kinds of microorganisms inhabiting the soil [1]. There is a close interaction between them. The alternation of vegetation structure will directly affect the elements of ecological cycle in the soil such as water, oxygen, *etc*. These factors will further affect the living environment of soil microorganisms. It is also important to establish a symbiotic relationship between microorganisms and vegetation in the rhizosphere, fostering a close interaction that facilitates vegetation growth and promotes nutrient cycling in the soil. They share nutrients, promote mutual growth, and work together to maintain plant and soil health [2]. However, in mine reclamation areas, the soil often suffers from long-term damage and contamination, leading to a significant impact on the diversity and structure of microbial communities. Through the restoration of vegetation, particularly by selecting plant species that are adaptable and tolerant to pollution, the soil environment can be improved, providing better habitat conditions, and promoting the recovery and diversity of microbial communities. At the same time, plants supply nutrients and energy to soil microorganisms through root material input and symbiotic relationships, thereby further stimulating and enhancing their activities and promoting them to better play their functions in the soil ecosystem [3]. At present, a lot of research has been done on the vegetation structure and microbial community within reclamation areas. He *et al*. reviewed the publication status, international collaborations, and current research focal points concerning soil microorganisms and vegetation studies and found that the regulation mechanism of soil microorganisms and vegetation, along with related topics, had emerged as a prominent area for future research, providing a reliable reference for individuals interested in exploring the characteristics of the relationship between soil microorganisms and vegetation. Furthermore, it also provides direction and guidance for future innovative research endeavors [4]. Chen *et al*. conducted a comprehensive study on the status of vegetation restoration in landfills for controlling gas pollution and found that specific indicator plants exhibited significant tolerance and methane oxidation capacity in landfill soil, thereby providing a valuable research direction for future control of landfill gas pollution through vegetation restoration and microbial interventions [5]. Guan *et al*. studied microbial nitrogen limits under different vegetation restoration methods in karst landforms based on soil-specific enzyme stoichiometry sizes and proposed optimization strategies for plant nutrients and improvement strategies for the soil carbon pool [6]. Han *et al*. studied the effects of different vegetation combinations on soil nutrients, soil enzyme activity, and microbial structure diversity under artificial restoration and artificial simulated natural restoration modes in a quarry in Sanhe, Hebei Province, China [7]. Despite previous investigations into the relationship between vegetation structure and soil microbial communities, relatively few studies have been conducted in the context of mine reclamation. For the purpose of effective promotion of mining area reclamation and fostering sustainable development of land ecosystems, this study investigated the vegetation structure in the mine reclamation area of Jiangsu Province, China by collecting soil samples from different vegetation types, measuring their microbial diversity index, studying the influence of different vegetation types on soil properties [8], and exploring the interaction between vegetation and soil ecosystems, especially microorganisms.

## Materials and Methods

### Study area

The area of this study is the Jiawang ore mining area located in the southwest part of Jiawang District, Xuzhou City, Jiangsu Province, China (34.2N, 117.1E). It falls within the mid-latitude zone and belongs to a warm temperate monsoon climate area with four distinct seasons, abundant sunshine, and long winter and summer seasons. The vegetation in the surrounding hills is distributed at different altitudes and slopes, forming a multi-level vegetation structure. This area is dominated by hilly terrain, and the soil is primarily consisting of leucobrown soil that is relatively shallow in depth but abundant in coal resources. The Jiawang coal mine boasts a century-long history. Prolonged and excessive exploitation has led to surface subsidence, serious water accumulation, farmland settlement, and other problems in the Jiawang area, causing significant damage to the ecological environment. Since 2009, the Xuzhou city government has implemented the coal gangue backfilling afforestation and pulverized coal filling afforestation modes to transform the mining subsidence area and restore the ecological environment of the mining area. After the reclamation of the mining area, a portion of the reclamation area is transformed into an artificial reconstruction forest, while another portion is converted into cultivated land. At present, there are 48 different species of trees and plants within the reformed forest land, encompassing 24 families and 37 genera. Among them, conifer species such as oriental arborvitae, dragon savin, and cedar are the main species. At the same time, the introduction of glossy privet, Chinese holly, and other evergreen broad-leaved tree species forms a denser understory vegetation. Additionally, there are also 12 kinds of exotic evergreen shrubs from 11 families and 12 genera, mainly including Chinese littleleaf box, Japan fatsia, and Seatung. Among the herbaceous vegetation within the forest, the area of ophiopogon is the largest [9]. Sampling areas were designated in restored planting sites of oriental arborvitae, Chinese holly, and Chinese littleleaf box established in 2020 with a soil depth of 20 cm. The distance between adjacent plots was more than 6 m. Four replicate plots were established for each vegetation type with each plot measuring 5 m × 5 m.

**Sample collection**

The snake method was adopted for sampling in June 2022. A starting point was chosen within the selected vegetation sampling areas of oriental arborvitae, Chinese holly, Chinese littleleaf box, and wheat. Soil samples were collected at intervals of 5 m along the snake path using a sterile soil drill with a dep range of 0 to 20 cm. Any impurities present in the samples were removed [10]. The thoroughly mixed soil samples were put into sterile hermetically sealed bags, which were subsequently partitioned into multiple portions with each weighing 100 g. Each individual sample was labeled for convenient identification and tracking purposes. In order to maintain the samples at low temperatures, the bags were carefully positioned within dry ice containers. Following transportation to the laboratory, the samples underwent air-drying procedures to eliminate any excess moisture. A series of physical and chemical properties of the sample were then determined to understand the trace element composition of the sample. The remaining soil samples were stored at a constant temperature of -20℃ to ensure their optimal preservation for further testing and research in the future.

**Testing soil physical and chemical properties**

The soil samples were tested in accordance with various national standards for the determination of soil physical and chemical properties [11]. The soil was mixed with water in a ratio of 1:2.5 and shaken for 30 minutes. Then, the pH value of the mixed suspension was measured by using Orion Star portable pH meter (Thermo Fisher Scientific, Waltham, MA, USA) to determine the acidity or alkalinity level of the soil. The total nitrogen content in the soil was determined by using the Kjeltec™ 9 Analyser (FOSS, Hillerod, Denmark) following manufacturer's instructions. Briefly, 2 g of soil sample was transferred into a digestion tube and moistened with a small amount of deionized water. 2 g of mixed accelerator ($K_2SO_4$:$CuSO_4$ = 10:1) and 10 mL of concentrated $H_2SO_4$ solution were added and shaken until homogeneous. The mixture was placed in Kjeltec™ 9 automatic distillation apparatus (FOSS, Hillerod, Denmark) and boiled at a temperature of 180℃ for 2 hours. After cooling down, the sample was subjected to alkaline distillation for 3 minutes using Kjeltec™ 9 automatic distillation apparatus. The distilled ammonia gas was condensed and absorbed by boric acid. The total nitrogen content of the soil was then calculated by titrating the boric acid absorption solution with a standard hydrochloric acid solution. To ensure the accuracy of the experiment, the experiment was repeated seven times, and the corresponding control groups were set up.

The contents of organic matter were determined by complete wet combustion of chromic acid-phosphoric acid. The available phosphorus content (AP) was determined using a sulphate-perchloric acid deboiling method, which facilitated the release and quantification of phosphorus readily accessible in the soil. Briefly, 0.5 g of soil sample was mixed with 25 mL of oxidizing solution (200 g of $CrO_3$, 2.5 g of $CuSO_4 \cdot 2H_2O$, 800 mL of concentrated $H_3PO_4$, 150 mL of water) before heating at 160 ℃ in an automatic distillation apparatus for 3 hours. The product was rinsed and added with 20 mL of $BaCl_2$ and 3 drops of phenolphthalein indicator. Excess KOH was titrated with HCl. All experiments were repeated seven times.

**Detection of soil enzyme activity**

The state of microbial activity in the soil was assessed by measuring the activity levels of

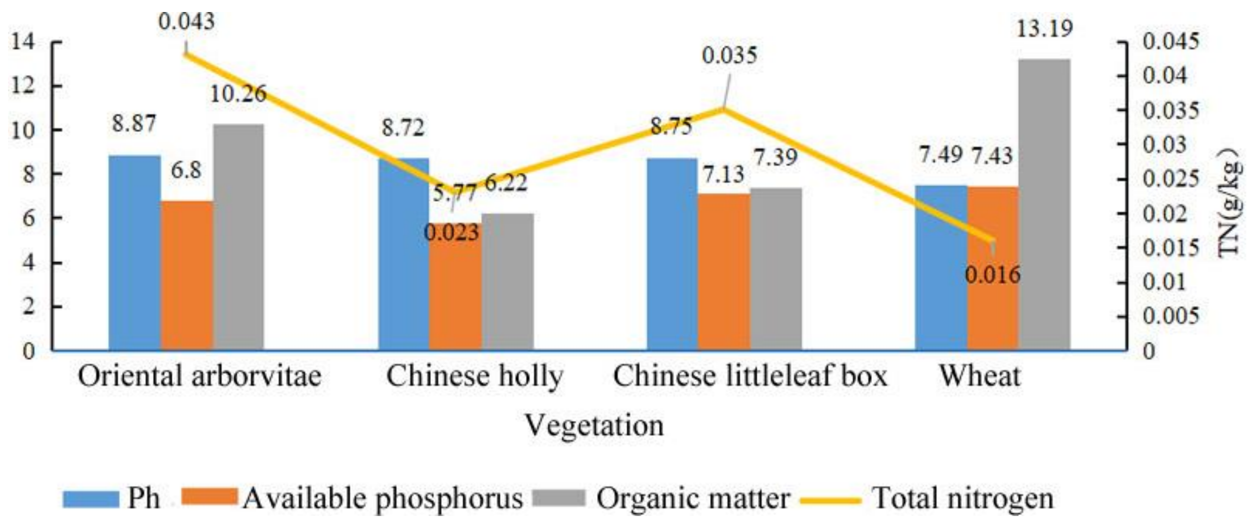


**Figure 1.** Soil physical and chemical properties of the four plants.

specific enzymes presented within soil [12]. The activities of catalase, urease, acid phosphatase, and acid invertase in the soil were measured by using the soil enzyme activity kits of catalase (S-CAT), urease (UE), acid phosphatase (ACP), and acid invertase (AI) (Adanti Biotechnology Co., Ltd., Wuhan, Hubei, China) following the manufacturer's instructions.

**Determination of soil microbial diversity**

Changes in the ecosystem of the natural environment can be reflected in the characteristics of the microbial community. Commonly used measures of microbial abundance and diversity were employed, including the Chao1 richness estimator, Ace richness estimator, Shannon Wiener diversity index (a population diversity index), and Simpson diversity index (a species evenness index). Data analysis was conducted using QIIME2 software (https://qiime2.org/).

**Statistical analysis**

SPSS 25.0 software (IBM, Armonk, New York, USA) was employed for the analysis of the differences in phenolic acid content among different soil samples. One-way analysis of variance (ANOVA) and Duncan's multiple range test were performed with $P < 0.05$ as significant difference. Principal component analysis (PCA) was implemented to analyze the physicochemical indicators and microbial characteristics of the soil.

## Results and discussion

**Vegetation types and physical and chemical properties of soil**

In the reclamation area, different types of vegetation exhibited distinct variations in soil characteristics within the same soil layer depth [13]. Oriental arborvitae displayed the highest soil pH value of 8.87, whereas the wheat cultivation zone demonstrated the lowest soil pH level (Figure 1). Except in the wheat-growing areas, the soil pH was found to be above 8.7, showing a generally weak alkalinity nature, which suggested that different vegetation types could contribute to different soil salinity levels. In addition, the study found that the planted area of oriental arborvitae exhibited the highest content of organic matter, followed by the wheat area, while there was little disparity in organic matter content between the other two planted areas. On the other hand, the total nitrogen content of the soil sample of oriental arborvitae was 0.043 g/kg, which was much higher than that of the other three vegetations, which might be because the abundant organic matter in the soil of

**Table 1.** Soil enzyme activities of different vegetation.

| Vegetation | Catalase | Urease | Acid phosphatase | Acid invertase |
|---|---|---|---|---|
| Oriental arborvitae | 98.77 ± 2.04$^a$ | 515.41 ± 9.47$^a$ | 11.86 ± 0.32$^a$ | 55.77 ± 2.14$^a$ |
| Chinese holly | 88.26 ± 2.35$^b$ | 151.2 ± 2.03$^b$ | 8.35 ± 0.10$^b$ | 21.44 ± 0.16$^b$ |
| Chinese littleleaf box | 89.84 ± 1.46$^b$ | 76.71 ± 2.06$^b$ | 8.08 ± 0.06$^b$ | 35.27 ± 0.58$^b$ |
| Wheat | 97.83 ± 2.14$^a$ | 109.22 ± 3.41$^b$ | 11.16 ± 0.08$^a$ | 40.03 ± 0.23$^b$ |

**Notes:** Different lowercase letters in the same column indicate a significant difference ($P < 0.05$).

**Table 2.** Soil flora index of different vegetation.

| Vegetation | Flora abundance index | | Flora diversity index | | Coverage rate% |
|---|---|---|---|---|---|
| | ACE | Chao1 | Simpson | Shannon | |
| Oriental arborvitae | 577.7845$^a$ | 558.0123$^a$ | 0.9591$^a$ | 5.9277$^a$ | 99.98$^a$ |
| Chinese holly | 554.2650$^a$ | 565.2389$^a$ | 0.9261$^a$ | 5.1354$^a$ | 99.87$^a$ |
| Chinese littleleaf box | 416.1290$^b$ | 399.8569$^b$ | 0.5677$^b$ | 2.8946$^b$ | 99.84$^a$ |
| Wheat | 563.7854$^a$ | 506.5577$^a$ | 0.8793$^a$ | 4.7211$^a$ | 99.81$^a$ |

**Notes:** Different lowercase letters in the same column indicate a significant difference ($P < 0.05$).

oriental arborvitae produced a lot of nitrogen.

**Soil enzyme activity**

Different types of vegetation in the same soil layer show different soil enzyme activities, and these enzymes also play their unique functions [14]. For example, catalase can help remove harmful hydrogen peroxide from cells, while urease is involved in nitrogen metabolism, acid phosphatase is involved in the phosphorus cycle in soil, and acid invertase decomposes and transforms organic matter, thereby affecting the organic matter cycle within soil. The catalase activity of oriental arborvitae was the highest one of 98.77 followed by wheat and Chinese littleleaf box, while Chinese holly was the lowest one (Table 1). However, overall, there was minimal variation in catalase activity across different vegetation types. Furthermore, the urease activity of oriental arborvitae was observed to be the highest one, while that of Chinese littleleaf box was the lowest one, thereby confirming the presence of a high total nitrogen content in oriental arborvitae soil. The acid phosphatase activity of oriental arborvitae and wheat exhibited higher levels, and the other two demonstrated lower levels. This observation also indicated that the soil associated with oriental arborvitae was enriched with trace elements, while the phosphorus content in wheat soil may be related to human cultivation practices and fertilization techniques. The acid invertase activity of oriental arborvitae was 55.77, significantly surpassing that of the other three vegetation types, which suggested a higher organic matter content in the soil of the oriental arborvitae planting area, thereby facilitating enhanced microbial nutrient availability. Overall, this study demonstrated that oriental arborvitae exhibited greater resilience to coal mining-induced soil contamination and damage in this coal mine reclamation area compared to the other three vegetation types.

**Detection of soil samples**

The coverage rate of the four vegetations was above 99%, showing no significant difference (Table 2). Through the analysis of Shannon and Chao1 indices, a higher index indicated greater species diversity in the sample. Similarly, larger values for Shannon and Simpson indices reflected greater fungal community diversity. It was found that the flora abundance index and flora diversity index were the highest in the oriental arborvitae area, followed by Chinese holly and wheat areas, while the fungal community diversity was the lowest in the Chinese littleleaf box area at the same soil depth.

## Conclusion

Research has shown that complex vegetation structures help to maintain the diversity of the ecosystem, and the influence of vegetation type and microbial community on the soil is huge. Within the reclamation area of Jiawang coal mine, oriental arborvitae exhibited remarkable efficacy in soil physical and chemical properties, such as soil pH value and available phosphorus. Moreover, the soil demonstrated higher levels of organic matter content, enzyme activity, and fungal diversity, thereby facilitating the accumulation of soil organic matter as well as enhancing microbial activity and diversity. Therefore, the large-scale cultivation of oriental arborvitae was essential for the purpose of ecological restoration and sustainable development. However, it should be noted that this study was limited to these four specific vegetation types, and the potential of other plants or combinations should be further explored in the futureto maximize the advantages of vegetation in coal mine reclamation areas and achieve better ecological restoration effects.

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