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RESEARCH ARTICLE

Soil microbes-mediated enzymes promoted the secondary succession in post-mining plantations on the Loess Plateau, China

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HIGHLIGHTS

- Vegetation restoration of monoculture is not satisfactory in mining land.
- Native plants accelerated vegetation restoration and soil nutrient accumulation.
- Microbial enzymes boosted the initially slow nutritional metabolism of plantations.
- Soil microbial enzymes promoted positive succession of ecosystems.

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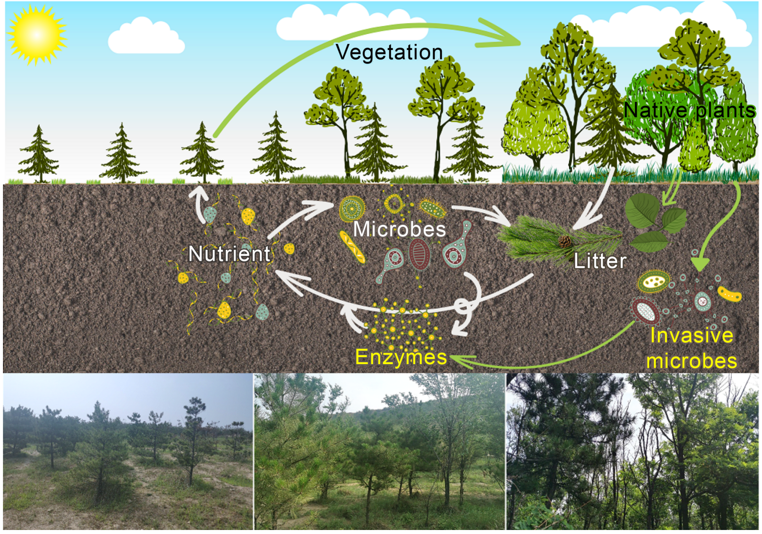
GRAPHICAL ABSTRACT



ABSTRACT

The diversity of vegetation configuration is the key to ecological restoration in open-pit coal mine dump. However, the recovery outcomes of different areas with the same vegetation assemblage pattern are completely different after long-term evolution. Therefore, understanding the causes of differential vegetation recovery and the mechanism of plant succession is of great significance to the ecological restoration of mines. Three *Pinus tabulaeformis* plantations with similar initial site conditions and restoration measures but with different secondary succession processes were selected from the open-pit coal mine dump that has been restored for 30 years. Soil physicochemical properties, enzyme activities, vegetation and microbial features were investigated, while the structural equation models were established to explore the interactions between plants, soil and microbes. The results showed that original vegetation configuration and soil nutrient conditions were altered due to secondary succession. With the advancement of the secondary succession process, the coverage of plants increased from 34.8% to 95.5% ($P < 0.05$), soil organic matter increased from 9.30 g kg^{-1} to 21.13 g kg^{-1} ($P < 0.05$), and total nitrogen increased from 0.38 g kg^{-1} to 1.01 g kg^{-1} ($P < 0.05$). The activities of soil urease and β-glucosidase were increased by 1.7-fold and 53.26%, respectively. Besides, the secondary succession also changed the soil microbial community structure and

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function. The relative abundance of *Nitrospira* genus which dominates the nitrification increased 5.2-fold. The results showed that urease and β-glucosidase promoted the increase of vegetation diversity and biomass by promoting the accumulation of soil organic matter and nitrate nitrogen, which promoted the ecological restoration of mine dumps.

1 Introduction

Open-pit coal mining causes severe damage to the surface ecosystem. Stripping of topsoil in open-pit mining not only destroys the original landscape, soil and plants, but also forms a mine dump that is extremely prone to geological disasters such as landslides and mudslides (Cheskidov and Bobyl'sky, 2017). Ecological restoration of the mine dumps requires urgent attention. Artificial ecological restoration is extensively adopted due to its rapidity and satisfactory short-term effect (Li and Liber, 2018; Patra et al., 2021), while the long-term effect of some artificial restoration projects is unsatisfactory, due to some technical and management reasons. Since the 1990s, *P. tabulaeformis* has been widely used in vegetation recovery of soil dumps in the mines in China's Loess Plateau, and is known for its excellent cold- and barren-resistant capability (Qi et al., 2020). After 30 years of growth, a small part of the *P. tabulaeformis* plantations is succeeded by more diversified native ecosystems (Wang et al., 2016). However, a greater proportion of the *P. tabulaeformis* plantations also undergo vegetation degeneration, as typified by the small, aged trees and even extensive death (Zhao et al., 2017). Therefore, studying the differentiation of vegetative succession and the mechanism behind it, as in the case of *P. tabulaeformis* plantations, might lay a foundation for ecological restoration of mines.

The secondary succession of the artificial ecosystem is extremely susceptible to the influence of introduced plants, and unreasonable vegetation configuration often poses a great potential threat to artificial ecosystem (Catford et al., 2019). Cultivated plants are often seen as invaders of an ecosystem, and artificial plants usually gain the competitive edge for sunlight, water, and nutrients due to their higher environmental tolerance and resources utilization efficiency (Whitney and Gabler, 2008), faster vegetative and reproductive growth (MacDougall et al., 2009), and broader niches (Schlaepfer et al., 2010; van Kleunen et al., 2011). Some artificial plant species may bring viral pathogens that lead to the death of the native plants (Borer et al., 2007). The artificial plant species may secrete allelochemicals (e.g., toxic glucosinolates) and disrupt the mutualism between the native plants and the soil microbes, leading to degeneration or death of the native plants (Stinson et al., 2006). In brief, the artificial pioneer plants usually suppress and expel the original plants to form monodominant communities. Consequently, this leads to the loss of biodiversity and damage to the diversity and stability of the ecosystem (Rodriguez et al., 2005). Some classical hypotheses have been proposed to explain the ecological threat due to non-native plant invasion such as the empty niche hypothesis and the evolution of increased competitive ability (Goldberg and Novoplansky, 1997; Goldberg et al., 2017). However, recent studies have shown that the effects of artificially guided ecological restoration in improving soil moisture content and nutrient conditions are very different (Guesewell and Kloetzli, 2012; Song et al., 2021). Some artificial plant species (e.g., *Medicago sativa*) could form a mutualistic association with the nitrogen(N)-fixing microorganisms in nature, thus increasing the soil N pool and promoting the growth of the native plants (Vitousek and Walker, 1989; Hughes and Denslow, 2005). Inversely, some artificial vegetation would strengthen the formation of dried soil layer thus causing ecological degradation (Zhang et al., 2018a). However, these phenomena cannot be adequately explained by the classical hypotheses on plant invasion or restoration ecology mainly because these hypotheses generally focus on the physiological and ecological features of the plant species while dismissing the plant-soil microbe interactions. Soil microbes have a non-negligible impact on artificial plants. While ammonium N and nitrate N in the soil can be directly absorbed by plants (Bloom, 2015), the assimilation mainly depends on N conversion by soil microbes through processes such as ammoniation, nitrification, and N fixation (Kuypers et al., 2018; Liu et al., 2018). Some artificial plants are native pioneer species themselves, therefore, microbes may be better able to assist them in nutrient acquirement (Yu et al., 2021), thus promoting ecological restoration.

The same restoration measures have produced completely different effects, reflecting the uncertainty of ecological restoration effects. There is limited knowledge on the influence and underlying mechanism of monoculture plantations on the secondary succession of mine ecosystem (Chen et al., 2020). This has restricted our understanding and use of ecological restoration methods in mines. We selected three plantations with the same site conditions and restoration measures but experienced different types of secondary succession to explore the mechanism of microbial action in the process of secondary succession in mine soil dump. Based on field survey and the existing knowledge on the substance cycling mechanism of the ecosystem (Dawson and Schrama, 2016; Jack et al., 2017), we proposed the following hypotheses: (1) secondary succession accompanied with proliferation and diffusion of native plants would promote the restoration of vegetation and soil fertility in *P. tabulaeformis* plantations, and (2) soil microbes could secrete more corresponding extracellular enzymes to improve the soil fertility. To test the hypotheses, we studied the *P. tabulaeformis* plantations established 30 years ago in Antaibao Mine Area in Shuozhou City, Shanxi Province, China. We investigated the interactions between plants and soil microbes during the secondary succession of the *P. tabulaeformis* plantations through structural equation models (SEM). The present study can provide us with information on the potential role of soil microbes in vegetation recovery in mines. The study findings illustrate the failure risk of using single tree species for mine ecological restoration, and provide support for the potential of using enzymes to improve soil in mining land. This study provides the possibility to use industrial enzyme preparations to improve soil fertility and promote ecological restoration. In addition, this study also provides ideas for selecting ecological restoration models such as vegetation allocation schemes according to the response of soil enzyme activities.

2 Materials and methods

2.1 Study area

The Antaibao open-pit coal mine is located in the eastern Loess Plateau at 112°11′−113°32′ E and 39°24′−39°38′ N. The annual average temperature in the Antaibao open-pit mine is 5.5 °C, with 117 days of frost-free period. The annual average precipitation is 426.7 mm, and the annual average evaporation is about 5 times the precipitation. The rainfall is concentrated from July to September, accounting for 75%−90% of the annual precipitation. The southern dump of Antaibao mine with an area of 180.5 hm^2 was selected as the study site. This dump is mainly produced from the stripping of topsoil at a depth of 10−15 m from an open pit mine 3 km away on the east side, which was opened in 1987. The soil dump has a stepped terrain where terraces and slopes alternate. The height of each step is about 15 meters, with a relative height of about 140 m, and the overall slope is about 20°. In 1992, the dump is divided into many small areas, and different plants were planted for ecological restoration experiments. According to the 1992 vegetation restoration plan, these three areas (Fig. 1A) were planned for *P. tabulaeformis* which was accompanied by the aerial sowing of *Stipa sareptana* and *Elymus dahuricus* for vegetation recovery. However, after 30 years of recovery, most of the *P. tabulaeformis* seedlings still grew at a slow rate due to adverse soil body configuration and nutrient deficiency. Some had turned into small, aged trees (unique tree phenomenon in the Loess Plateau), and some revealed extensive withering (Fig. 1B). The first area has been completely invaded by native pioneer plants and surrounding artificial plants, forming a tree-shrub-grass composite ecosystem (denoted as MP), consisting of *P. tabulaeformis*, *Robinia pseudoacacia*, *Ulmus pumila*, *Caragana korshinskii*,

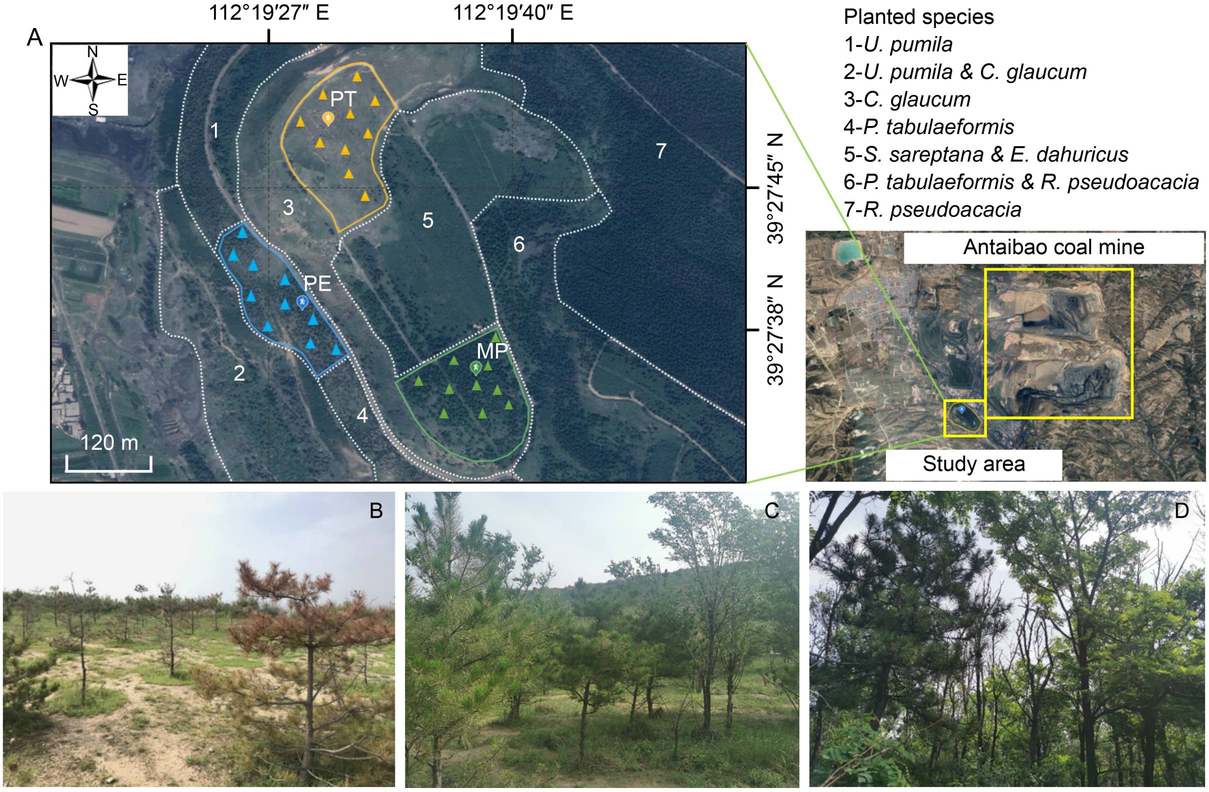

Fig. 1 The study area and sampling sites of mining reclamation area. (A) Relative location of Antaibao coal mine, and the original plant species used for restoration are shown in the legend. The photographs of the restoration status of these three areas: (B) PT, uninvaded *Pinus tabulaeformis* plantation basically, (C) PE, a mixed forest region, (D) MP, a tree-shrub-grass composite ecosystem.

and a variety of herbs. The second area has been invaded by *U. pumila*, a native pioneer tree species; this has led to the formation of a mixed forest (denoted as PE) consisting predominantly of *P. tabulaeformis* and *U. pumila*. The plant species composition in the third area primarily remains unchanged (denoted as PT), that is, and consists predominantly of *P. tabulaeformis* and gramineous species (Fig. 1). These three areas are located on platforms with close altitudes and have the same manual management measures. In these three areas with similar initial site conditions (Table S1), different types of secondary succession began after the planting of *P. tabulaeformis* on the barren dump. Therefore, we regard *P. tabulaeformis* as the initial species in these areas, and the native plants in the soil seed bank and the surrounding plants dominated the respective secondary succession types. In addition, during the succession process, some surrounding native plants would also enter the artificial *P. tabulaeformis* plantation during the spreading process (grazing, wind, animal carrying, etc.). In these three areas, *P. tabulaeformis*, *Stipa* and *Eldrine* are planted, while all other plants are native.

2.2 Soil samples collection and analysis

On August 15, 2020, 10 soil samples at a depth of 0−10 cm were collected from the representative plant communities in each of the three plantations, for a total of 30 soil samples. The plant species and their number around the sampling points were surveyed manually. The quadrats were delineated with the sampling points as the center and parallel to the direction of the trees in the plantation, and the quadrats for herbs and trees measured 1 m × 1 m and 10 m × 10 m, respectively. A total of 60 plant quadrats were investigated. Non-rhizosphere soils were collected to avoid of enzymes secreted by plant roots on soil microbial extracellular enzymes, and placed into special sterile bags, which were then sealed and transported back to the laboratory in a car refrigerator. A portion of fresh soil samples were frozen, sealed in a foam box, and mailed to Shanghai Majorbio Biopharm Technology Co., Ltd. for the genome sequencing. The remaining soil samples were air-dried indoors before removing the gravel and plant and animal residues. The soil samples were ground and passed through a 100-mesh sieve, and its physicochemical properties and enzyme activities were measured. The coordinates of the sampling points were recorded using a hand-held GPS device. The soil water content (SWC) and soil temperature (ST) were measured using a soil temperature and humidity speed measuring instrument (TR-6, Shunkeda, Guangdong). The vegetation canopy was measured according to the shadow cast by the canopy on the ground, from which the vegetation cover was estimated (Chmura et al., 2016).

pH and electrical conductivity (EC) were determined by the potential method (water:soil=1:2.5) (DDS-307A; Lei-ci, Shanghai, DDS-307A). Other physicochemical properties of the soil were measured following earlier published methods (Barahona and Iriarte, 2001). In brief, soil organic matter (SOM) level was determined by the colorimetric method using potassium dichromate (Kolar et al., 2017). Available phosphorus (AP) content was determined using the sodium hydrogen carbonate solution-Mo-Sb anti-spectrophotometric method (Matula, 2010). Ammonium nitrogen (AN) content was determined using the potassium chloride extraction-ultraviolet spectrophotometry (Beuters and Scherer, 2012). Nitrate nitrogen (NN) content was measured using the calcium chloride extraction-ultraviolet spectrophotometry (Dong et al., 2014). Total nitrogen (TN) content was measured using the Calvin-distillation-titration method (Avramidis et al., 2015). Soil enzyme activities were determined as described earlier. In brief, dehydrogenase activity (DHA) was measured using the triphenyl tetrazolium chloride method (TTC) (Ruggles et al., 2021). Urease (URE) activity was measured using the sodium phenolate-sodium hypochlorite colorimetric method (Nannipieri et al., 2012). Alkaline phosphatase (ALP) activity was measured by colorimetric method using the disodium phenyl phosphate (Kramer and Green, 2000). Fluorescein diacetate (FDA) activity was measured using fluorescein colorimetry (Adam and Duncan, 2001). Catalase (CAT) activity was measured by titration with potassium permanganate (Rodriguez-Kabana and Truelove, 1970). Beta glucosidase (BG) activity was measured by nitrophenol colorimetry using the S-β-GC Kit (Solarbio Science & Technology Co., Ltd., Beijing, China), and polyphenol oxidase (PPO) activity was measured by colorimetric method using pyrogallol (Perucci et al., 2000).

2.3 Soil microbial community analysis

DNA extraction was performed for 30 soil samples using the FastDNA™ SPIN Kit for Soil (MP Biomedicals Corp., USA) according to the instruction manual (Ma et al., 2021). PCR sequencing was carried out for the V4 and V5 zones of 16S rRNA in soil bacteria from three different plantations, using the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 907R (5'-CCGTCAATTCMTTTRAGTTT-3'). Concurrently, the fungal ITS1 zone was amplified using the primers ITS5F (5′-GGAAGTAAGTCGTAACAAGG-3′) and ITS1R (5′-GCTGCGTTCTTCTCGATGC-3′). The PCR procedure was as follows: predenaturation at 98 °C for 2 min, followed by 25 cycles of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, then a final extension at 72 °C for 5 min, followed by cooling to 10 °C. Next, the PCR-amplified products were separated by 2% agarose gel electrophoresis and the target fragments were eluted by using the Gel Extraction Kit (Axygen, USA). The elution products were further quantified using Quant-iT PicoGreen dsDNA Assay Kit and Microplate reader (BioTek, FLx800, USA). Then, the samples were mixed at an equal ratio. Sequencing libraries were constructed for soil bacteria and fungi from the three plantations using the TruSeq Nano DNA LT Library Prep Kit developed by Illumina (Illumina, USA). The constructed libraries were quantified by using

Qubit and Q-PCR, and sequencing was performed by Shanghai Majorbio Bio-pharm Technology Co., Ltd. on HiSeq2500 PE2500 (Illumina, USA).

Raw sequencing data for 16S rRNA gene were pretreated using FASTP v0.19.6 (Chen et al., 2018) and FLASH v1.2.7 (Magoč and Salzberg, 2011). Qualified sequences were denoised using DADA2 software (Callahan et al., 2016), which generates an error profile within samples and then infers true biological information based on the amplicon sequence variants (ASVs). The taxonomy of each 16S rRNA gene sequence was analyzed by VSEARCH consensus taxonomy classifier in QIIME 2 (Version 2020.6, https://qiime2.org/), and reference data sets from the Silva 16S rRNA database (138, https://www.arb-silva.de/). The taxonomy of each 18S rRNA gene sequence was analyzed by the RDP Classifier algorithm (version 2.11, https:// sourceforge.net/projects/rdp-classifier/) against the Silva rRNA database, UNITE fungal ITS database (version 8.0, https://unite.ut.ee/), and RDP rRNA database (version 11.5, http://rdp.cme.msu.edu/) using a confidence threshold of 70%. Microbial community composition was obtained by species annotation and classification based on ASV abundance. ASVs of samples were selected and matched with the enzyme classification in KEGG data library (Aoki-Kinoshita and Kanehisa, 2007) for the prediction of gene function. Gene functional prediction was made using PICRUSt2 (Douglas et al., 2020) to predict the abundances of enzyme function genes of soil bacteria and fungi. Microbial diversity was based on the calculation of the indices, e.g., ACE index, Shannon index, and Shannoneven index. Alpha diversity and beta diversity of microbes were analyzed on the free online platform of Majorbio Cloud Platform (www.majorbio.com).

2.4 Construction of structural equation models

In order to investigate the importance of soil microbes-mediated enzymes in ecological restoration, SEMs were constructed. According to the relevant research on the ecological functions of microorganisms and soil enzymes, two conceptual models have been established: (1) a specific model (recursive model) with enzymes as the first-order factors was used to verify the possibility of the pathway that enzymes affect plants by changing soil fertility, and (2) a cyclic framework model (nonrecursive structure) containing soil microbes, soil enzymes, soil fertility and plant factors was used to verify the possibility of enzymes stimulating positive feedback in the ecosystem. Nonrecursive structural models are difficult to identify, especially for a complex model. Therefore, we combine different categories of indicators (soil microbes, soil enzymes, soil fertility and plant) into composite indicators to simplify the model. These composite indicators do not represent latent variables, but are obtained through principal component analysis of constituent factors. The structural equation modeling analysis was conducted based on the robust maximum likelihood evaluation method using AMOS 21.0 software (AMOS IBM, USA). The SEM fitness were determined according to a non-significant χ^2 test ($P > 0.05$, $0 \leqslant \chi^2/\mathrm{d}f \leqslant 2$) (Rappaport et al., 2020), high comparative fit index (CFI > 0.95), the goodness-of-fit index (GFI > 0.95), and the root mean square error of approximation (RMSEA < 0.10) (Steiger, 1990). In the actual test, more than 8 pathways were provided for analysis to increase more possibilities. Finally, through existing studies and correction suggestions provided by AMOS software, the models with the best fit and the significant influence path were selected. Information about composite indicators is provided in Table S2. Information about our priori models is provided in Table S3.

2.5 Statistical analysis

Intergroup comparison of the soil physicochemical properties, enzyme activities, and microbial diversity was measured using one-way analysis of variance (ANOVA) under SPSS v21.0 (IBM, USA). The differences of microbial enzyme functional genes were calculated based on the enzyme abundance matched with KEGG library, and standardized by Z-Score method using SPSS v21.0 (IBM, USA). The data were processed and plotted using Excel 2019 (Microsoft, USA) and R-project (MathSoft, USA).

3 Results

3.1 Vegetation characteristics of different secondary succession types

The field survey of plantations indicated that the restoration of vegetation communities of the three secondary succession types showed obvious differences, including the number of regional vegetation, vegetation coverage and diversity (Table 1 and Table S4). In PT, *P. tabulaeformis* remained the constructive species, and some common herbs were also observed. In PE, *U. pumila* and *Poa annua* were identified as the native pioneer species, and succession into a plant community predominantly consisting of *P. tabulaeformis* and *U. pumila* was complete. In MP, the plant community consisted predominantly of native species and the surrounding planted species, including *R. pseudoacacia*, *C. korshinskii*, *C. aristatum*, and *M. sativa* and secondary succession had dramatically changed the vegetation configuration in the plantations. In PT, the herbs had a distribution pattern that centered around *P. tabulaeformis*, but vertically, only the herbaceous layer and the *P. tabulaeformis* layer were observed. In PE, *U. pumila* was also the focal point of the distribution pattern of herbaceous plants and the main species in the arborous layer. In MP, the invasion by *R. pseudoacacia* and *U. pumila* greatly enriched the arborous layer; their young seedlings together with *C. korshinskii* occupied the shrub layer, and the herbs had nearly covered the entire ground.

Table 1 Vegetation characteristics of plantations with different types of secondary succession

Habitat	PT	PE	MP
Coverage (%)	34.8 ± 3.3a	64.7 ± 3.3b	95.5 ± 2.6c
DBH (cm)	7.3 ± 0.3a	9.1 ± 0.2b	11.3 ± 0.2c
Arbors number	14.7 ± 2.2a	24.0 ± 2.6b	35.2 ± 3.0c
Species number	5	9	16
Ligneous plants	*Pinus tabulaeformis*	*Pinus tabulaeformis, Ulmus pumila*	*Pinus tabulaeformis, Ulmus pumila, Robinia pseudoacacia, Salix cheilophila, Caragana korshinskii*
Herbaceous plants	*Stipa sareptana, Elymus dahuricus, Chenopodium glaucum, Plantago asiatica*	*Stipa sareptana, Elymus dahuricus, Chenopodium glaucum, Plantago asiatica, Setaria viridis, Artemisia annua, Poa annua*	*Stipa sareptana, Elymus dahuricus, Setaria viridis, Chenopodium glaucum, Artemisia annua, Medicago sativa, Astragalus adsurgens, Lespedeza davurica, Melilotus officinalis, Cirsium setosum, Chenopodium aristatum*

Coverage represents the average vegetation coverage. DBH (diameter at breast height) represents the diameter at the breast height (1.3 m) of all tree species. Arbor represents the number of trees in the survey square, including *Pinus tabulaeformis*, *Ulmus pumila*, *Robinia pseudoacacia*, and *Salix cheilophila*. Species represents the number of plant species in all survey plots in the area. Data are presented as mean ± standard deviation. Values in the same row with the same letter(s) do not differ significantly as per the Duncan analysis at $P < 0.05$ between different treatments.

3.2 Influence of secondary succession on soil physicochemical properties and enzyme activities

Different types of secondary succession lead to significant differences in soil properties (Fig. 2). First, SWC in PT was 8.69%, while that in MP increased by 32.71% ($P < 0.05$). Besides, ST in MP decreased by about 5°C compared with that in PT ($P < 0.05$). Second, an obvious differentiation was observed in soil fertility under different types of secondary succession. NN and TN increased consistently with the advancement of the succession stage. The soil TN in MP was approximately 2.03-fold that in PT (0.38 g kg^{-1}) ($P < 0.05$). Likewise, SOM exhibited the same variation pattern as TN. Compared with PT (9.3 g kg^{-1}), SOM in PE and MP increased by 47% and 1.27-fold ($P < 0.05$), respectively. Furthermore, the types of secondary succession also resulted in the differentiation of soil enzyme activities. With the advancement of the succession stage, both URE and BG increased significantly. Compared with PT, URE in the soil of PE and MP increased by 1.32- and 1.71-fold ($P < 0.05$), respectively, and BG increased by 20.06% and 53.62%, respectively ($P < 0.05$) (Table S5).

3.3 Influence of secondary succession types on soil microbial communities

3.3.1 Influence of secondary succession types on soil microbial community diversity

The ASV number and diversity of soil microbes increased significantly as the advancement of the succession stage

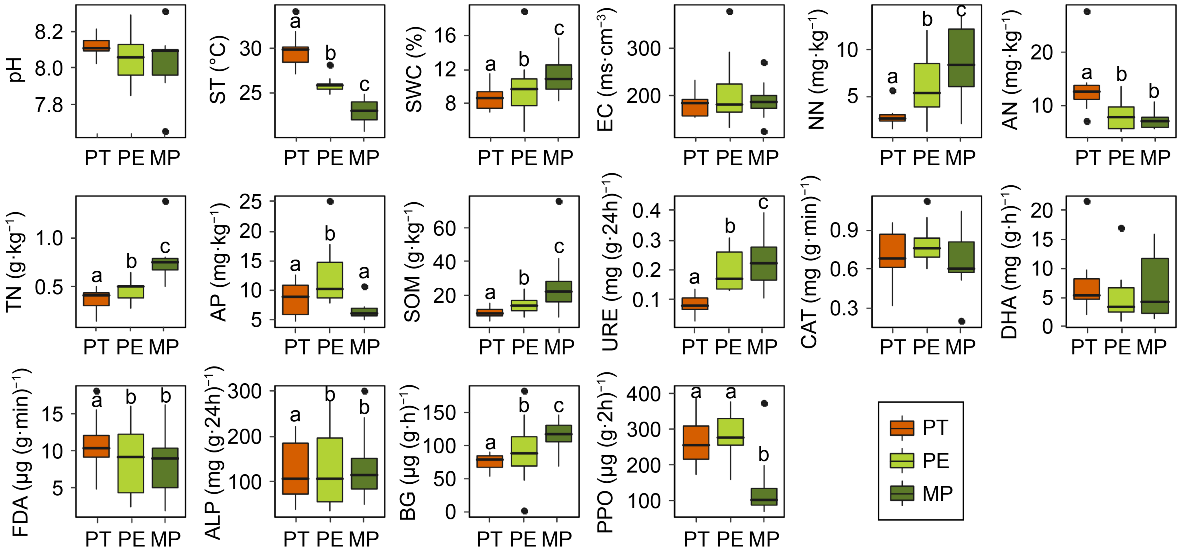

Fig. 2 Soil physicochemical properties and enzyme activities of plantations with different secondary succession types. Bars with different lowercase letters are significantly different ($P < 0.05$) as per Duncan analysis. ST, soil temperature; SWC, soil water content; EC, electrical conductivity; NN, nitrate-nitrogen; AN, ammonium nitrogen; TN, total nitrogen; AP, available P; SOM, soil organic matter; URE, urease; CAT, catalase; DHA, dehydrogenase; FDA, fluorescein diacetate; ALP, alkaline phosphatase; BG, beta glucosidase; PPO, polyphenol oxidase.

became higher (Table 2). In PT, PE and MP, 744 926, 774 558, and 853 069 valid genes sequences were detected, respectively. Among them, 424 945, 446 212, and 501 999 sequences belonged to the fungi, respectively. There was no significant difference in the ASV number of microbes between PE and PT. Compared with PT, the ASV number of soil bacteria and fungi in MP increased by 6.92% and 12.50%, respectively ($P < 0.05$). In the *P. tabulaeformis* plantations with faster secondary succession (PE and MP), both the Shannon diversity index and the Shannoneven index increased significantly. Meanwhile, the abundance-based coverage estimator (ACE) of soil fungi increased considerably, while the richness of bacterial community barely changed. On the species level, the amplicon sequence variants (ASV) were selected for principal coordinates analysis (PCoA) (Fig. 3). According to PCoA, no obvious differentiation was found between PT and PE after 30 years of secondary succession. Nevertheless, the clustering effect of PE was superior to that of PT, and a particular evolutionary trend was noted. By contrast, an apparent differentiation was found in the soil microbial community in MP compared with that in PT and PE.

3.3.2 Influence of secondary succession types on soil microbial community composition

The soil microbial communities differed significantly in the three plantations with different secondary succession types (Fig. 4). At the ASV level, the number of shared bacteria decreased by 25.5%, with the order of PT>PE>MP, while the shared fungi presented the same trend with a 24.4% decline (Fig. 4B and D). Among the dominant bacteria (relative abundance above 1%), the largest number of endemic species were present in MP, but in PE and PT, shared species accounted for the majority of the microbial communities (Fig. 4A). Compared with PT, the ASV number of *Gemmatimonadetes* bacteria in PE and MP increased by 19% and 47%, respectively, and the ASV number of *Nitrospirota* bacteria increased by 1.06- and 5.16-fold, respectively (Tables S6 and S7). In PE, the ASV number of *Latescibacteria* and *Entotheonella* bacteria decreased by 22% and 47.9%, respectively, and in MP, the increase was 1.34- and 6.8-fold, respectively. At the genus level, the differentiation in the ASV number of bacterial became even more pronounced. Among the genera with a relative abundance above 1%, the ASV number of *Solirubrobacter*,

Table 2 Soil microbial alpha diversity of three plantations with different types of secondary succession.

Types of microorganisms	Diversity indexes	PT	PE	MP
Bacteria	ACE	1441 ± 212a	1599 ± 167a	1517 ± 140a
	Shannoneven	0.911 ± 0.01a	0.923 ± 0.01b	0.925 ± 0.01b
	Shannon	6.62 ± 0.17a	6.81 ± 0.09b	6.78 ± 0.13ab
Fungi	ACE	194.6 ± 76.2a	260.1 ± 42.8b	265.9 ± 38.1b
	Shannoneven	0.583 ± 0.11a	0.604 ± 0.11b	0.592 ± 0.08b
	Shannon	3.06 ± 0.82a	3.36 ± 0.67b	3.31 ± 0.52b

Data are presented as mean ± standard deviation. Values in the same row with the same letter(s) are not significantly different between different treatments as per the Duncan analysis at $P < 0.05$. ACE index, Shannoneven index, and Shannon index reflect the species richness, evenness and overall diversity of the microbial community, respectively.

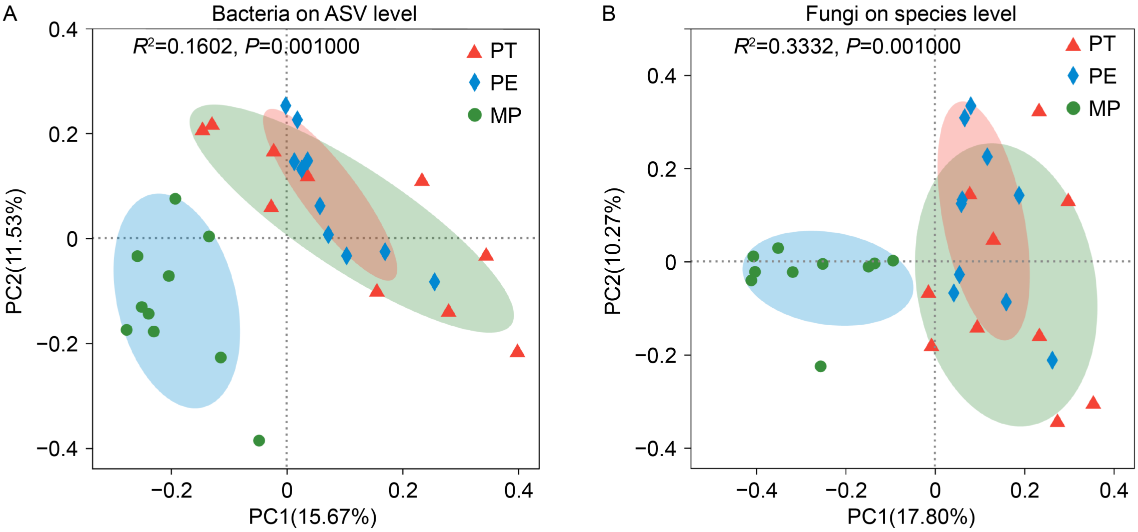

Fig. 3 The principal coordinate analysis of soil bacteria (A) and fungi (B) of three different secondary succession types of plantations at the species level.

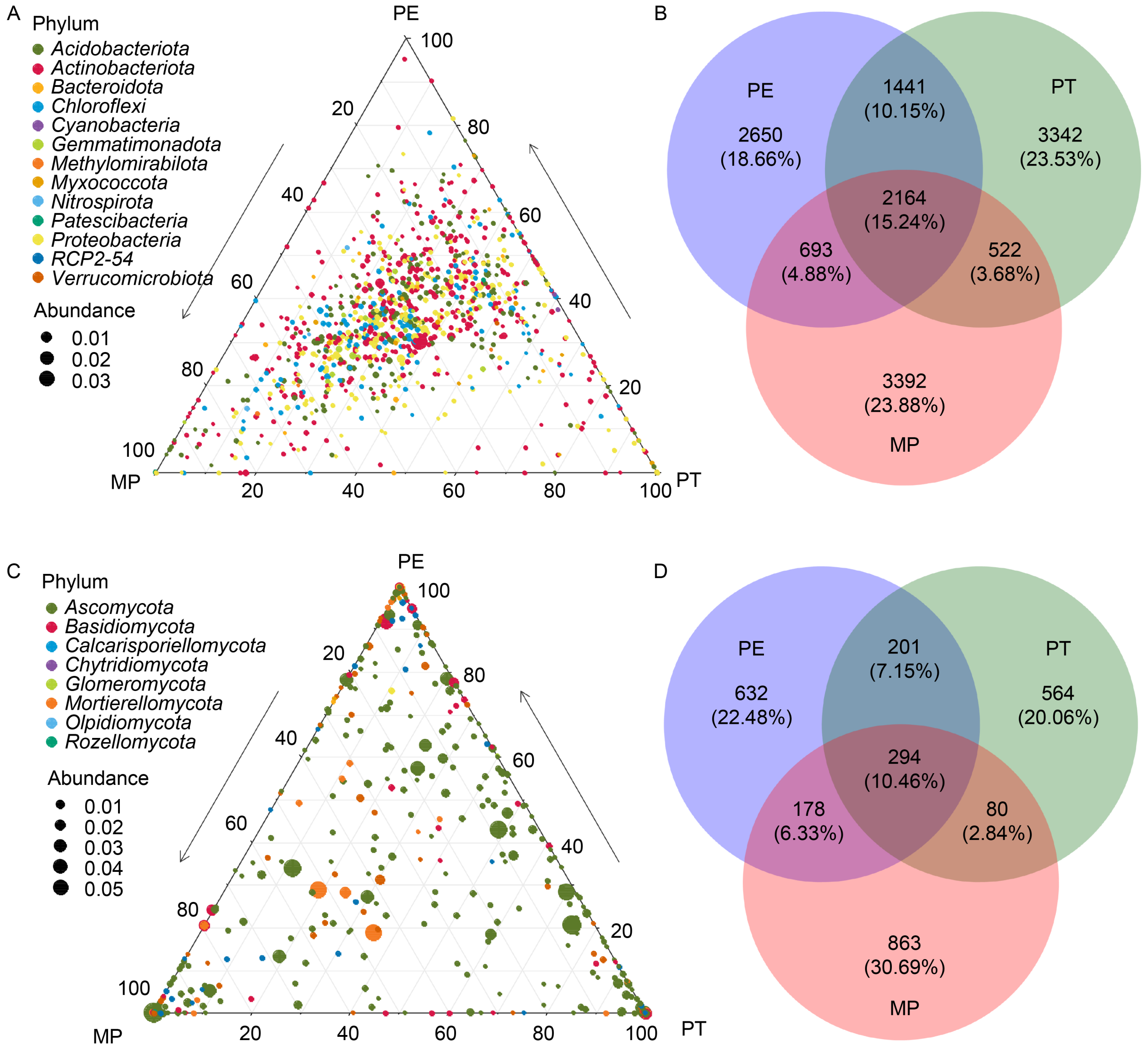

Fig. 4 Composition of soil bacteria (A-B) and fungi (C-D) of three plantations with different types of secondary succession.

Blastococcus, *Gaiella*, and *Streptomyces* bacteria increased significantly in PE and MP by 23%−94%, with the ASV number of *Gaiella* increasing most significantly. In MP, the increase amplitude was 93.76%. Other genera with very significant differentiation across the plantations included *Rhizobium*, *Hyphomicrobium*, and *Nitrospira*. The ASV number of *Nitrospira* in MP increased by 5.2-fold compared to that in PT.

In studied plantations, fungi accounted for 57.88% of all microbes. Among fungi, 25 species, for about 78% of all fungi, were dominant (with a relative abundance above 1%). Compared with bacteria, the type and number of fungi changed more intensely. Several endemic species were found in PE and MP (Fig. 4A and C), that had a very high abundance in the fungal community. For example, ASV1302 (*Leptosphaeria*) were nearly absent in PT and PE but accounted for 6.65% of the fungal abundance in MP. Besides, there was several-fold increase in the ASV number of *Gibberella* and *Mycocentrospora*, among which *Gibberella* increased by 29.9- and 10.67-fold, respectively in PE and MP.

3.4 The role of microbes-mediated enzymes in the process of secondary succession

The role of soil microbes in secondary succession was explored using SEM. Plants can change soil fertility by influencing soil microbes and regulating the activities of the microbial extracellular enzymes (Fig. 5A). To further represent the possible action pathways of soil enzymes in substance cycling, structural equation modeling with soil enzymes as first-order variables was performed (Fig. 5B). The model results showed that the activities of several soil enzymes had a significantly positive impact on soil carbon, N, and P. Among the six enzymes examined, URE exhibited the strongest and most extensive influence on soil fertility, with the greatest influence on SOM, AN, and NN. Soil AP, AN, NN, and SOM had a significant and widespread impact on the biomass and diversity of aboveground vegetation. In

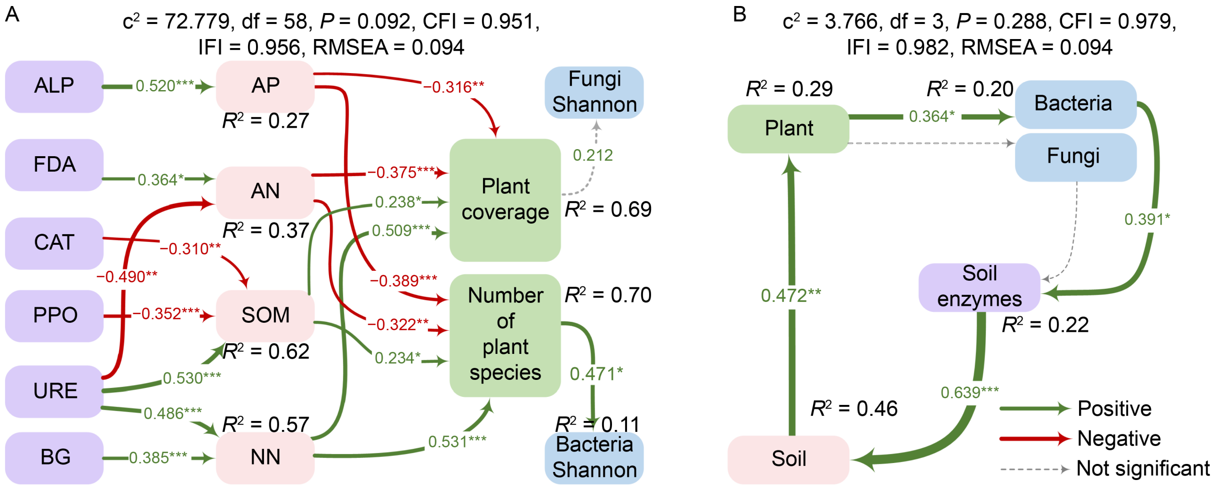

Fig. 5 Structural equation modes of the interaction of vegetation, soil, and microbes. Both the framework model (A) and the specific model (B) are credible ($0 \leqslant \chi^2/\mathrm{df} \leqslant 2$ and $0.05 < P \leqslant 1.00$) and the fitting effect is acceptable ($0.95 < \mathrm{CFI/IFI} \leqslant 1.00$; $0 \leqslant \mathrm{RMSEA} \leqslant 0.10$). The composite indicator Plant (B) is represented by the Coverage index and Species indicator of vegetation. Fungi and Bacteria (B) are represented by their abundance and Shannon indexes. Soil (B) is represented by soil organic matter (SOM), total nitrogen (TN), and available phosphorus (AP). Soil enzymes (B) are represented by urease (URE) and alkaline phosphatase (ALP). Red lines indicate negative relationships, while green lines indicate positive relationships. Numbers on arrows are standardized path coefficients. The width of arrows indicates the strength of significant standardized path coefficients. Paths with non-significant coefficients are presented as gray lines. R^2 denotes the proportion of variance explained. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

addition, the influence of SOM and NN on vegetation biomass and diversity was positive. Furthermore, vegetation biomass and diversity of the plantations could strongly influence soil bacteria. On either scale, microbes played a positive role in facilitating plant and soil substance cycling. In order to illustrate the mediating role of soil enzymes in ecosystem cycling, we simplified the ecosystem biogeochemical cycle process. The model (Fig. 5B) results show that soil microbes can mediate the positive feedback between soil and plants through enzymes, and promote the positive succession of the ecosystem.

3.5 Predictive analysis of microbial enzyme functional genes

In this work, ASV of samples were selected and matched with the Kyoto Encyclopedia of Genes and Genomes (KEGG) data library for the prediction of gene function using PICRUSt. The results (Fig. 6) showed that secondary succession in PE and MP substantially increased the abundance of microbial hydrolase and oxidoreductase genes in the soil. The genes detected in the bacterial community encoded 2262 enzymes, while those in the fungal community encoded 888 enzymes (Table S8). We chose 39 genes with amplitude above 20% among all 268 enzyme-encoding genes having a relative abundance above 0.1%. These genes mostly encoded hydrolases, oxidoreductases, and transferases. The increase in amplitude of the gene encoding EC 3.1.1.1 (carboxylesterase) in PE and MP was as high as 36.68% and 71.21%, respectively. The abundance of genes encoding oxidoreductases increased in both PE and MP compared with those in PT. Further, the amplitude of the gene encoding EC 1.1.1.91 (oxidoreductase) increased by 24.14% in PE. In PE and MP, the variation of the amplitude of the abundance of bacterial enzyme-encoding genes was within 10%, while that of the abundance of fungal enzyme-encoding genes was higher. Besides, 88.54% of the enzyme-encoding genes were of fungal origin.

4 Discussion

4.1 The impact of secondary succession types on the vegetation-soil system of plantations

As to the outcome of vegetation restoration in PT, pure *P. tabulaeformis* plantations were found to be non-conducive to rapid ecosystem recovery in the mine lands. Instead, native plants accelerated the positive succession of the plant system of the mine land. In the present study, the initial physicochemical properties of soil in the three study areas were similar without significant differences. According to the vegetation survey, the diffusion and invasion of native plants and surrounding artificial plants may be the main reasons for the difference in secondary succession. The surrounding area of PT is mainly grassland, and it is far away from woody plant restoration areas, so there is little disturbance. The deep root system of *P. tabulaeformis* has a good water-retaining capacity (Khasanova et al., 2013) but its litter may inhibit the growth of herbal plants near the root system (Xiong and Nilsson, 1999; Zhang et al., 2018b). Therefore, the sparse distribution of plants in the pure *P. tabulaeformis* plantations gives rise to many empty niches, which creates a favorable condition for native plants to form new vegetation

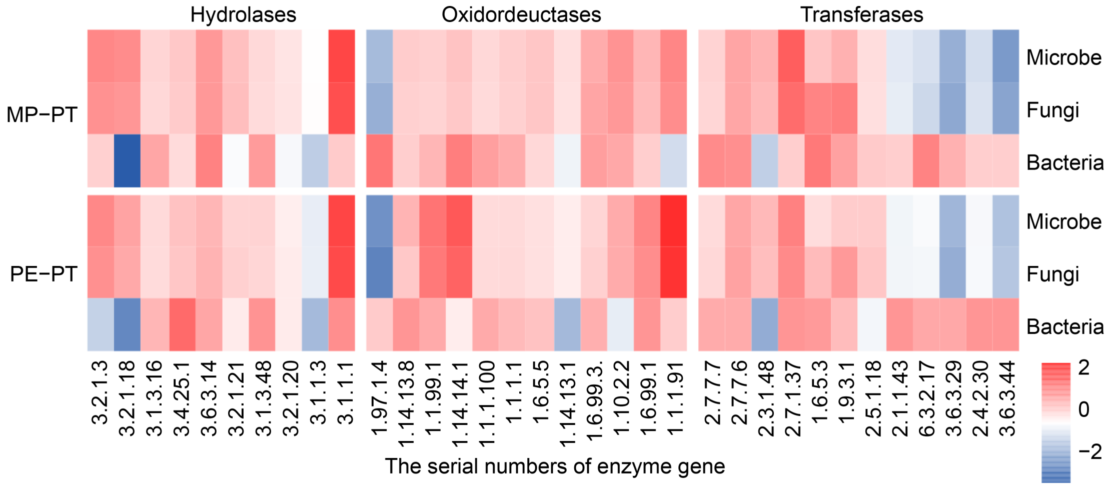

Fig. 6 Abundance changes of soil microbial enzyme genes in PE and MP compared with those in PT. Z-Score scale function in R was used to standardize the data. Microbe means the sum of fungi and bacteria.

configurations (Guerra-Coss et al., 2021; Salles and Mallon, 2014). In PE, the invasive *U. pumila* plants usually stood adjacent to *P. tabulaeformis* plants. Besides, in the plantations with rapid succession (PE and MP), the diameter at breast height of *P. tabulaeformis* was significantly higher than that in the pure plantations (PT). This phenomenon can be explained by the mutualism between *U. pumila* and *P. tabulaeformis*. The water-retaining capacity of *P. tabulaeformis* deep root system and litter promotes the coordinated growth of the plant system. The invasion of *U. pumila* resulted in the improved activity of decomposition enzymes to promote litter decomposition and nutrient cycling. Such mutualism was already demonstrated in a previous study (Tang et al., 2019). Secondary succession led to the formation of a tree-shrub-grass composite ecosystem formed in MP. This is probably because *R. pseudoacacia* and *U. pumila* have larger and thinner crowns compared with *P. tabulaeformis*, which helps reduce surface evaporation and creates more favorable lighting and water conditions for the growth of understory herbs. As a result, the herb cover increased. With the increase in amount of vegetation, problems such as water loss and soil erosion (Burylo et al., 2012), and surface evaporation (Liste and White, 2008) are improved, which further promotes the spread of vegetation. Therefore, empty niches in pure *P. tabulaeformis* plantations (Dong et al., 2020) coupled with the gain effect of native species might be the primary reason for faster vegetation recovery in plantation PE.

The secondary succession led by native plants accelerated substance cycling in the *P. tabulaeformis* plantations, thus improving the fertility of understory soil. In the process of secondary succession, soil microbes that have undergone selective and adaptive changes can rapidly decompose *P. tabulaeformis* litter (Li et al., 2020), facilitating N cycle. The genera *Hyphomicrobium* (Fan et al., 2018) and *Nitrospira* (Zhuang et al., 2020) are widely recognized as closely related to soil N fixation and N cycle. A significant increase in the abundance of these two genera contributed to the rise in soil TN in PE and MP to a certain degree (Fig. 2). An increase in soil nutrient content promotes the growth and development of native plants (Fig. 5). With the increase in vegetation biomass, a richer source of nutrients is available to the soil microbes. The microbe-mediated decomposition further increases the nutrients released into the soil, including SOM and NN (Fig. 5) (Castro-Diez et al., 2019). Therefore, in the *P. tabulaeformis* plantations, native plants accelerated substance metabolism and cycling of the ecosystem and improved the nutrient conditions of the soil.

4.2 Adaptation of soil microbial communities to secondary succession in plantations

Secondary succession of plantations imposed significant selective pressure on soil microbes. In the early stage of secondary succession, some alien microbes may colonize along with the invasion of certain surrounding plant species (Mallon et al., 2015). We observed an increase in the ASV number of heterotrophic fungi *Gibberella* (Gaffoor et al., 2005) and *Mycocentrospora* (Louarn et al., 2012) parasitizing the plants (Table S7), possibly due to the diffusion and colonization of host plants. Besides, plant litter also directly impacts the microbial community structure (Guo et al., 2013). Along with the germination of the original soil seed bank and the spread of surrounding plants, abundant plants inhabiting MP bring a richer and more diversified source of nutrients. The moist humus layer on the earth surface offers favorable survival space and a source of nutrients for the growth of saprotrophic fungi (Setala and McLean, 2004). Given the above, the increase in vegetation may be the reason for the greater number and diversity of microbes inhabiting MP (Fig. 5B) (Huang et al., 2014). Besides, the invasive surrounding species can also recruit microbes beneficial to them (Bahram et al., 2020). For example, the abundance of root nodule bacteria *Rhizobium* and *Leptosphaeria* show a consistent trend as the vegetation cover, which may be due to their mutualistic association with plants (Chen et al., 2021; Vega-Lopez and Ritchie, 2014).

The microbial community was also found to have undergone an adaptive succession in response to changes in the soil environment and vegetation. Fungi decompose hydrophobic organic matters (*P. tabulaeformis* secretions and litter) more efficiently than bacteria (Deng et al., 2021). Compared with PT, the increase in the ASV number of soil fungi inhabiting MP was 1.8 times that of the bacteria, indicating that the microbial community adapted to the improvement of soil nutrient conditions during secondary succession. *Gemmatimonadota* can secrete highly active proteases that decompose nitrogenous compounds (Zeng et al., 2017). *Entotheonella* can utilize a wide variety of substrates and have the potential to generate massive amounts of coenzymes and transferases (Lackner et al., 2017). *Candidatus latescibacteria* carries similar genes encoding extracellular hydrolases as do cellulolytic bacteria (Rinke et al., 2013). The higher efficiency of utilizing a greater variety of substrate allowed these bacteria to better adapt to the soil nutrient conditions in MP. As a result, the ASV number of these bacteria increased dramatically in MP (Fig. 4). A large amount of plant-derived SOM was released into the soil through litter decomposition mediated by the above bacteria (Fig. 2). Thus, the strictly heterotrophic microbes such as *Solirubrobacter* (Zhang et al., 2014), *Blastococcus* (Hezbri et al., 2017), *Gaiella* (Albuquerque et al., 2011), and *Streptomyces* (Zhu et al., 2020) have easy and adequate access to the nutrient source (Guo et al., 2013). An increase in the nutrient source may be the primary reason for the dominant presence of heterotrophic microbes in MP (Fig. 4, Table S6 and Table S7) (Setala and McLean, 2004). In addition, the carbon-to-nitrogen (C/N) ratio in the leaves of *U. pumila*, *R. pseudoacacia*, and many gramineous plants is much higher than in the pine needles. To decompose the litter from these plants, the microbes need to take up more available N from the soil (Knops et al., 2002). *Hyphomicrobium* and *Nitrospira* have been shown to be the primary bacteria involved in N cycling (Sun et al., 2020), and fungi usually have a faster N cycling rate (Nicolas et al., 2019). Therefore, a higher ratio of fungi to bacteria and the multiplication of *Hyphomicrobium* and *Nitrospira*in in PE and PT can be deemed a response of the microbial community to a growing N demand.

4.3 The action mode of soil microbes in the process of secondary succession in plantations

In the substance and energy cycling between soil and plants, the effect of plants on soil is largely mediated by soil microbes (Zhang et al., 2017), in which enzymes are important drivers for soil microbes to fulfill their ecological functions (Fig. 5) (Li et al., 2020). There was a consistent and apparent increasing trend in the abundance of genes encoding 10 hydrolases and 11 oxidoreductases (except EC 1.97.1.4) in MP (Fig.6). These genes are the fundamental reasons for the enhanced activities of URE and BG in the soil (Chen and Sinsabaugh, 2021; Trivedi et al., 2016). Plant litter serves as the primary substrate for microbes; its decomposition by URE can increase SOM generation (Fig. 5) (Cetin et al., 2009). URE is widely present in fungi to decompose the N-containing organic matter into readily available soluble amino acids and ammonium salt to plants (Schnurer and Rosswall, 1982). These inorganic nitrogen can be directly absorbed and utilized by plants (Cetin et al., 2009). Besides, URE and BG can work synergistically to facilitate the mineralization of N in SOM, further promoting plant growth by increasing the input of NN into the soil (Fig. 5) (Cetin et al., 2009; Kunito et al., 2009). This increase in NN content also promotes the increase of the diversity of plants (Fig. 5, $P < 0.001$), probably because the increase of available N facilitates the diffusion of the plant species that are initially restricted by limited N availability (Yelenik et al., 2004). While the microbes influence the plants through the soil enzymes, plants, in turn, also affect the microbes (Fig. 5). A higher vegetation cover significantly promotes bacterial diversity, as was observed in the present study (Fig. 5, $P < 0.05$). This may be attributed to the dramatic increase in substrate availability (Fig. 2) (Guo et al., 2013). However, the increase in the number of plant species had an opposite effect, which may be due to the increase in the relative abundance of *Streptomyces* caused by the spread of other plants in the surrounding area. *Streptomyces* is the source of 90% of the antibiotics produced by *Actinomyces*. The anti-bacterial metabolites, including glucanases, thus produced can inhibit the colonization of other microbes (Zhu et al., 2020). Soil microbes produce enzymes to improve soil fertility, which further promotes plant recovery. The elements of an ecosystem and their interactions are highly complex. During pathway analysis (Fig. 5), our models focused on the key roles played by soil microbes and enzymes, but to describe the internal interactions within a real ecosystem, we need to introduce more transcriptomic or metabolomic indicators.

The initially slow substance metabolism in *P. tabulaeformis* plantations was facilitated by microbes. This was also evidenced by the functional genes of microbial enzymes. In MP, the soil microbes showed great potential in producing enzymes related to the metabolism of complex lipids and cellulose (e.g., EC 3.1.1.1 and EC 1.1.1.91, respectively) (Fig. 6). Among them, carboxylesterase (EC 3.1.1.1) is considered an essential enzyme in lipid degradation and is specific to various organisms (Glogauer et al., 2011). EC 1.1.1.91 is an oxidoreductase that catalyzes coniferyl alcohol dehydrogenase (Sloane, 1973). Coniferyl alcohol proves to be an important precursor for lignin, which is hard to degrade (Tramontina et al., 2020). The accelerated metabolism of coniferyl alcohol can effectively mitigate the problem of the accumulation of complex carbon sources. Thus, in MP, an abundance of *P. tabulaeformis* litter dramatically boosted the growth and multiplication of microbes. As the organic matters, such as lipids and cellulose, that are otherwise difficult to degrade, undergo an accelerated metabolism in the presence of microbial enzymes, boosting the initially slow nutritional metabolism of the *P. tabulaeformis* plantations.

The functional genes of soil microbial enzymes and soil enzyme activities are not able to establish a strong connection through correlation analysis, mainly because we can divide enzymes into many types according to genes, but in soil, we can usually only classify them into a few categories. Therefore, there is no accurate one-to-one correspondence between enzyme genes and soil enzymes. Therefore, we can only reflect the potential of soil enzyme activity changes through the changes of enzyme genes, without introducing it into SEMs.

5 Conclusions

The secondary succession of plants is extremely important for mine ecological restoration, which determines the restoration of soil nutrients, plant and microbial biomass and diversity. This study investigated the interaction between soil, microbes and vegetation in plantations with different secondary succession types, and draw the following conclusions:

(1) The successional direction of the *P. tabulaeformis* plantations significantly shifted due to the invasion of surrounding plants, which resulted in an obvious increase in species number and vegetation coverage (increased by 60.7%).

(2) With the advancement of the secondary succession process, the functional gene encoding carboxylesterase (EC 3.1.1.1) enhanced by 71.21%, with the activities of enzymes URE and BG increased 1.71-fold and 53.26%, respectively. The metabolism in MP was accelerated, and the accumulations of SOC and TN were promoted.

(3) For pure *P. tabulaeformis* plantations, the surrounding plant species combined with soil microbes have triggered a positive feedback, which pushed the positive succession towards a more perfect ecosystem (MP), with higher biodiversity and better soil environment.

The soil microbes fulfill their functions by expressing the genes that encode specific enzymes, so we can study the intra-ecosystem interactions by introducing other immediate factors related to the transcriptome or metabolome in the future. This study opens up a new perspective for understanding the mechanism of the plant-soil-microbe system in ecological restoration and tapping the potential of microbial enzymes utilization, so as to formulate more efficient ecological restoration schemes for mining land.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Electronic supplementary material

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