Metagenomic analysis reveals the effect of revegetation types on the function of soil microorganisms in carbon and nitrogen metabolism in the open-cast mining area

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Abstract

Aims

Vegetation type is one of the most important factors contributing to the changes in soil properties during the rehabilitation process in mining areas. There is still insufficient awareness of the relationship between soil properties and the function of soil microbes in the carbon and nitrogen cycles under different revegetation type influences.

Methods

Seven reclaimed vegetation plots which has been restored for over 30 years in the Pingshuo opencast coal mine, China were established (RP: *Robinia pseudoacacia*, UP: *Ulmus pumila*, LG: *Larix gmelinii*, PC: *Populus simonii Carr.*, PT: *Pinus tabuliformis*, PA: *Picea asperata*, and PS: *Pinus sylvestris*). The soil properties and enzyme activity were detected, while the metagenomic approach was used to analyze the potential mechanisms of soil microorganisms work in carbon and nitrogen metabolism.

Results

Total N was obviously highest in the PC habitat (2.347 ± 0.017%), followed by RP (2.188 ± 0.059 %). The major facilitator Superfamily COG0477 and dehydrogenase the COG2133 were significantly enriched in the PR habitat. The acetolactate synthase COG0028 was significantly enriched in RP, followed by LG and PC ($P < 0.05$). β-glucosidase (EC3.2.1.21) were dominated in soil microbial communities among 7 vegetation types, and significantly richer in RP and LG, followed by PC and PT ($P < 0.05$). GTs and GHswere significantly richer in UP and LG, respectively.

Conclusion

Robinia pseudoacacia and *Larix gmelinii* may be good species choice for improving the productivity and stability of the soil ecosystem in mining areas.

Highlights

• The revegetation species affect soil nutrient conditions and enzyme activities.

• COG0477, COG2133 and COG0028 are abundant in *R. pseudoacacia* habits.

• β-glucosidase genes dominate among all of the revegetation habits.

• *R. pseudoacacia* and *L. gmelinii* are excellent species for reclamation in mining area.

Introduction

Opencast mining has contributed greatly to rapid economic development while causing incalculable losses to the ecological environment (Wong, 2003; Zhao et al., 2013). Most of the opencast mines in China are located in arid and semiarid regions on the Loess Plateau, with sparse vegetation and serious soil erosion, making ecological restoration more difficult and urgent (Bai et al., 1999). Vegetation reconstruction is considered one of the most effective methods for improving the productivity and stability of the soil ecosystem and prevails as a key method in ecological rehabilitation in mining areas (Jia et al., 2020; Liao et al., 2019). The application of revegetation can effectively alter soil physical properties, reduce soil and water loss, and even promote soil fertility (Zhang and Xu, 2015; Sun et al., 2019). However, there is still insufficient awareness of how the soil microbiological functions respond to revegetation types, and how these responses influence key functions of soil ecosystems, such as the carbon and nitrogen cycle.

Soil microorganisms have significant contributions to soil carbon and nitrogen cycling, the functions of which are sensitive to vegetational type and aboveground diversity (Shihan et al., 2017; Trivedi et al., 2013; Wu et al., 2019; Yuan et al., 2016, 2018). Some relevant research regarding the revegetation effects on soil microbial functions related to carbon/nitrogen cycling has been performed. These studies either addressed the diversity, abundance, and species composition of aboveground communities (Oelmann et al., 2021; Lange et al., 2021) or compared land use patterns Yuan et al., 2016, 2018). However, the comparison of soil microbial functions related to carbon and nitrogen cycling among different types of vegetation is lacking. Particularly, vegetation types have been widely acknowledged to influence functions of soil microbial carbon and nitrogen metabolism by altering the quality and quantity of soil organic matter inputs (Xie et al., 2022; Creamer et al., 2016; Zhou et al., 2019). Thus, selecting the appropriate type of revegetation is essential to maintain the soil microbial carbon and nitrogen cycling functions and enhance the stability of the ecosystem (Feng et al., 2019; Zhang et al., 2015).

Advanced sequencing technology provides technical support for studying the relationship of vegetation type and soil microbial functions. Fungal and bacterial abundance were found to be higher in mixed coniferous-broad forest compared to coniferous forest or broad forest, and the diversity of microbial communities was affected (Kubartová et al., 2009; Zhang et al., 2019a). However, concerns about microbial community structure or diversity are not sufficient to reflect the carbon and nitrogen metabolic functions of soil microorganisms since there are microbes or enzymes that perform the same function (Wagg et al., 2019). Metagenomics can identify diverse microbial activities including degradative enzymes and biosynthetic functions, which sufficiently reflect the metabolism or function of soil microbes (Handelsman et al., 2005; Riesenfeld et al., 2004). The cognitive deficiency of the effect of revegetation types on soil microbial function related to carbon and nitrogen cycling limits our ability to promote the potential for sustainable ecological restoration in mining areas. Thus, it is worth gaining a better comprehension of the microbial potential functions under vegetation dominated by different vegetation types, which may provide a scientific basis for the selection of plant species in the ecological restoration of open-pit coal mine dumps, thus potentially offering suggestions regarding achieving a sustainable ecological restoration in mining areas.

In order to elucidate how the revegetation type influences the potential function of soil microbes in the carbon and nitrogen cycles a metagenomic approach was used to analyze the function of soil microorganisms in carbon and nitrogen metabolism under 7 vegetation types in this study(RP: *Robinia pseudoacacia*, UP: *Ulmus pumila*, LG: *Larix gmelinii*, PC: *Populus simonii Carr.*, PT: *Pinus tabuliformis*, PA: *Picea asperata*, and PS: *Pinus sylvestris*). Soil samples were collected from the south dump of the Pingshuo opencast coal mine, which is one of the largest open pit mines in China and the south dump has been restored for over 30 years. The objectives of this study were as follows: (1) to analyze the variance in soil nutrients related to the carbon and nitrogen cycle influenced by seven revegetation types; (2) to explore the mechanism of the impact of revegetation types on the carbon and nitrogen metabolic capacity of soil microorganisms; and (3) to identify the appropriate and effective revegetation type for opencast coal mine reclamation.

Materials and methods

Study area and sample collection

The Pingshuo mine is one of the largest open pit mines in China, which is located in an ecologically fragile area with an arid to semi-arid continental monsoon climate. The average annual rainfall is about 450 mm, with most rain falling between June to September accounting for 65% of the annual rainfall. The terrain is loess hills with grass vegetation, but the primary vegetation has been damaged leading to soils with low levels of organic matter and poor structure. In 1994, the dump successively planted different plants for ecological restoration experiments, reclaiming 266.67 hm^2 of woodland.

In July 2021, we collected soil samples from *Robinia pseudoacacia* (RP), *Ulmus pumila* (UP), *Larix gmelinii* (LG), *Populus simonii Carr.* (PC), *Pinus tabuliformis* (PT), *Picea asperata* (PA), and *Pinus sylvestris* (PS). The characteristics of the seven stands are listed in Table 1. There were three sampling points in each plot on average, the area of each sampling point is 100 m^2 (10 m × 10 m). Removed surface vegetation and coverings of the soil, five sampling points were randomly collected at a soil depth of 0–20 cm in each plot and combined into one composite sample and sieved through a 2 mm screen. One portion of the composite sample was stored at 4°C until DNA extraction, and the other portion was air-dried to detect soil properties.

Table 1
Sites information

Samples	Dominant species	Main herbs and shrubs under the forest
RP	*Robinia pseudoacacia*	*Artemisia ferruginea, Artemisia pigmenti, Artemisia altaicum, Rosa dioica*
LG	*Larix gmelinii*	*Lime, Artemisia ferruginea, Artemisia pigmenti, Artemisia altaica, Woodland Early Grass*
PA	*Picea asperata*	*Lime, Artemisia ferruginea, Artemisia pigmenti, Artemisia altaica, Woodland Early Grass*
PC	*Populus simonii Carr*	*Lime, Sea buckthorn, Woodland Early Grass, Altai Dogwood, Little Clover*
UP	*Ulmus pumila*	*Lime, Sea buckthorn, Woodland early grassland, Altai dogwood, Artemisia hogweed*
PT	*Pinus tabuliformis*	*Tuzhuang Hydrangea, Soya bean paste, small clover, Altai dogwort flower*,
PS	*Pinus sylvestris*	*Lime, Artemisia ferruginea, Artemisia pigmenti, Artemisia altaica, Woodland Early Grass*

RP: *Robinia pseudoacacia*; LG: *Larix gmelinii*; PA: *Picea asperata*; PC: *Populus simonii* Carr; UP: *Ulmus pumila*; PT: *Pinus tabuliformis*; PS: *Pinus sylvestris*

Soil physiochemical and enzyme activities analysis

Soil pH was determined with a glass electrode using a soil-to-water ratio of 1:2.5. NH_4^+-N and NO_3^--N in the supernatant were measured calorimetrically using a Lachat auto analysis system (Zellweger Analytics, Milwaukee, WI). Total N was determined by elemental analysis-stable isotope mass spectrometry (EA-IRMS, Iso Prime100, Germany). Soil organic carbon (SOC) was determined using the H_2SO_4-$K_2Cr_2O_7$ method.

6 enzyme activities involved in the soil C and N cycle were detected using an enzyme activity assay kit, colorimetric method. C cycle-related enzymes: dehydrogenase (Soil Dehydrogenase (S-DHA) Activity Assay Kit, Sangon Biotech, China), β-glucosidase (Soil β-glucosidase (S-β-GC) Activity Assay Kit, Sangon Biotech, China), and Cellulase (Soil Cellulase (S-CL) Activity Assay Kit, Sangon Biotech, China); N cycle-related enzymes: Urease (Soil Urease (S-UE) Activity Assay Kit; Sangon Biotech, China), Alkaline protease (Soil Alkaline Protease Activity Assay Kit; Sangon Biotech, China), and N-acetylglucosaminidase (Soil N-acetylglucosaminidase (S-NAG) Activity Assay Kit; Sangon Biotech, China).

DNA extraction, sequencing, and data processing

DNA for metagenomics was extracted from the soil samples by using the E.Z.N.A. DNA Kit for Soil (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. The concentration and

purity of extracted DNA were quantified with a TBS-380 mini-fluorometer (Turner BioSystems, Sunnyvale, CA, USA) and Nano-Drop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), respectively. Quality of extracted DNA was examined by 1% agarose gel electrophoresis, 5 V/cm, 20 min. Prepared DNA samples were sent to Shanghai Meiji Biomedical Technology Co., Ltd. (Shanghai, China) for metagenomics sequencing.

The 3' and 5' ends of the reads were sheared on the adapter sequences using Seqprep (https://github.com/OpenGene/fastp, version 0.20.0), and removed low-quality reads (sheared length of less than 50bp, the average base quality value is less than 20, and the reads containing N bases). Splicing and assembling the optimized sequence using MEGAHIT (https://github.com/voutcn/megahit, version 1.1.2) based on the principle of succinct de Bruijn graphs. ORF prediction of contigs in the splicing results was performed using MetaGene (http://metagene.cb.k.u-tokyo.ac.jp/). Genes with nucleic acid lengths ≥ 100 bp were selected and translated into amino acid sequences. The non-redundant gene set was constructed by CD-HIT (http://www.bioinformatics.org/cd-hit/, version 4.6.1), clustering the predicted gene sequences of all samples (parameters: 90% identity, 90% coverage), and the longest gene in each class was taken as the representative sequence. The high-quality reads of each sample were aligned with the non-redundant gene set (95% identity), and the genes were counted to obtain the abundance information in corresponding samples (SOAPaligner; http://soap.genomics.org.cn/, version 2.21).

COG, KEGG, and CAZyme annotations

Non-redundant coding sequence correspondent amino acid sequences were annotated to achieve the clusters of orthologous groups of proteins (COG) based on the eggNOG database using Diamond (http://www.diamondsearch.org/index.php, version 0.8.35). This catalog was also annotated to inform the metabolic pathway based on the Kyoto Encyclopedia of Genes and Genomes (KEGG). BLASTP alignment parameters set the expected value e-value 1e-5. Amino acid sequences of the non-redundant gene were annotated using hmm can (http://hmmer.janelia.org/search/hmmscan) of the CAZy database, which offers information on the carbohydrate-active enzyme. The alignment parameter sets the expected e-value to $1e^{-5}$.

Statistical analysis

Differences in the properties and enzyme activities of soil, abundances of functional enzymes genes, COG functional genes, and dominant genus in the 7 plant types were tested by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. $P < 0.05$ was considered to indicate a statistically significant difference. To identify the genes correlated with soil properties and enzyme activities, Spearman correlations were performed to find the relationships between gene abundance and soil properties and enzyme activities. All statistical analyses were conducted using R.

Results

Soil properties and enzyme activities

The content of SOC (9.595 ± 0.091g/kg) was significantly higher in the soil of PT than in other soils (P< 0.05; Table 2). NO_3^--N content differed significantly in 7 vegetation soils, which was highest in the soil of UP (60.425 ± 0.108 mg/kg; P< 0.05; Table 2). The soil of LG has the highest NH_4^+-N content (10.613 ± 0.061 mg/kg), but the NO_3^--N content is the lowest (22.6 ± 0.059 mg/kg; P< 0.05; Table 2).

Table 2
Physical and chemical properties of soil under 7 types of vegetation

	NH_4^+-N (mg / kg)	NO_3^--N (mg / kg)	pH	SOC (g / kg)	TN (%)	Bulk density(g/ml)
RP	9273 ± 0179abcd	56424 ± 0101c	7787 ± 0013bc	5227 ± 0303d	1094 ± 0059ab	04 ± 001b
LG	10613 ± 0061a	226 ± 0059g	779 ± 0008bc	6477 ± 0229c	0923 ± 0215b	042 ± 006ab
PA	8194 ± 0117d	4689 ± 0089d	786 ± 0011b	7719 ± 0136b	0489 ± 0112c	046 ± 002ab
PC	10146 ± 0187ab	32062 ± 0093f	7717 ± 0014c	7669 ± 0152b	1174 ± 0064a	043 ± 001ab
UP	8489 ± 0153cd	60425 ± 0108a	7997 ± 0003a	5307 ± 0198d	0930 ± 0036b	046 ± 004ab
PT	8883 ± 0146bcd	38575 ± 0125e	794 ± 0004a	9595 ± 0091a	0966 ± 0059b	05 ± 002a
PS	9789 ± 0212abc	58823 ± 0205b	786 ± 001b	6731 ± 0022c	0624 ± 0048c	044 ± 002ab

Data are means ± SD in parentheses and different letters in a column indicate significant differences (Turkey's test, P< 005) SOC, total soil organic carbon; TN, total nitrogen; RP: *Robinia pseudoacacia*; LG: *Larix gmelinii*; PA: *Picea asperata*; PC: *Populus simonii* Carr; UP: *Ulmus pumila*; PT: *Pinus tabuliformis*; PS: *Pinus sylvestris.*

The activity of N-acetylglucosaminidase was significantly different among 7 vegetation soils (P< 0.05; Table 3). The activities of β-glucosidase (170.776 ± 0.994 U/g) and alkaline protease (0.281 ± 0.005 U/g) were significantly highest in the soil of PC (P< 0.05; Table 3). Meanwhile, β-glucosidase and urease all showed significant minima (P< 0.05; Table 3) in the soil of RP, with a minimum of 56.799 ± 0.586, and 58.433 ± 1.868 U/g, respectively.

Table 3
Enzyme activity in soils under 7 types of vegetation

	C cycle-related enzymes (U/g)			N cycle-related enzymes (U/g)		
	Dehydrogenase	β-glucosidase	Cellulase	Urease	Alkaline protease	N-acetylglucosaminidase
RP	0767 ± 0018b	56799 ± 0586e	17355 ± 0514e	58433 ± 1868e	0236 ± 0001b	8633 ± 0096e
LG	1475 ± 0072a	101816 ± 12c	23055 ± 0388b	246773 ± 0626c	0196 ± 0001c	955 ± 0022d
PA	1425 ± 0048a	116024 ± 0951b	30486 ± 0239a	300372 ± 193b	0189 ± 0002c	16699 ± 0035a
PC	0108 ± 0011c	170776 ± 0994a	34524 ± 1756ab	252935 ± 0278c	0281 ± 0005a	12377 ± 0041b
UP	1483 ± 0026a	93618 ± 1998cd	2216 ± 0457b	178035 ± 1167d	0198 ± 0002c	7972 ± 0029f
PT	1283 ± 0016a	90864 ± 0685d	20495 ± 0125b	170969 ± 0146d	0177 ± 0007c	11081 ± 0012c
PS	0208 ± 0008c	113722 ± 1307b	23825 ± 048b	378592 ± 0226a	0232 ± 0b	10719 ± 0027c

Data are means ± SD in parentheses and different letters in a column indicate significant differences (Turkey's test, $P < 005$) RP: *Robinia pseudoacacia*; LG: *Larix gmelinii*; PA: *Picea asperata*; PC: *Populus simonii* Carr; UP: *Ulmus pumila*; PT: *Pinus tabuliformis*; PS: *Pinus sylvestris*.

COG annotation based on metagenome sequencing

To determine the potential roles of microbial communities in the carbon and nitrogen cycle, the specific COGs involved in carbohydrate transport and metabolism, amino acid transport and metabolism were analyzed. The total items of carbohydrate transport and metabolism, and amino acid transport and metabolism were 1371 (6.70%) and 551 (9.23%) in the COG function classification, respectively. The significant differences of related COGs abundance in 7 vegetation soils were tested (one-way ANOVA, Turkey's test, $P < 0.05$), and the top 10 abundant COGs are selected and shown in Fig. 1 and Table S1.

Several carbohydrate-related functions enriched in the 7 samples of soil microorganisms were the major facilitator Superfamily COG0477 (33561 average reads), Dehydrogenase COG2133 (26861 average reads), and transporter activity COG1653 (26300 average reads). Furthermore, the abundance of COG0477, COG2133, and COG1653 was significantly higher in sample RP than that in other samples ($P < 0.05$). Regarding amino acid-related function, acetolactate synthase COG0028 (35191 average reads), succinyl-diaminopimelate desuccinylase activity COG0624 (35071 average reads), and peptidase s9 prolyl oligopeptidase active site domain protein COG1506 (26841 average reads) were dominated in the 7 samples and significantly highest in the sample RP ($P < 0.05$; Fig. 1b; Table S1).

A potential link exists between microbial taxa and their function of carbohydrate transport and metabolism, amino acid transport, and metabolism (Fig. S1). Fig. S1 showed that carbohydrates and amino acids in the 7 plant soils are predominantly transported and metabolised by the *Solirubrobacter* (Actinobacteria), *Nocardioides* (Actinobacteria), *Bradyrhizobium* (Proteobacteria), and *Streptomyces* (Actinobacteria).

Functional enzymes and their presentations in KEGG

To further investigate the differences in carbon and nitrogen cycling function under different vegetation types, we annotated genes to the KEGG enzyme. There were 21 enzymes (14 involved in carbon cycling and 7 involved in nitrogen cycling) involved in 17 KEGG pathways that have significant differences among 7 types of plant soils ($P < 0.05$; Fig. 2, 3). The detailed information for enzymes and KEGG pathways are provided in Tables S2. As one of the dominant enzymes involved in carbon cycling, β-glucosidase (EC3.2.1.21; average reads 13625) was involved in multiple carbon cycling pathways (Fig. 2,3; Table S2). Cellulose could be hydrolyzed into cellodextrin with endo-1,4-b-glucanase (EC3.2.1.4), followed by hydrolysis into cellobiose with endo-1,4-b-glucanase (EC3.2.1.4) and β-Glucosidase (EC3.2.1.21; Fig. 2a). Subsequently, cellobiose could be glycosylated into D-glucose with b-glucosidase (EC3.2.1.21). Meanwhile, β-D-Glucoside could be directly glycosylated into D-glucose with EC3.2.1.21(Fig. 2a). Except for EC3.2.1.21, α-Amylase (EC3.2.1.1; average reads 10798) and α-Glucosidase (EC3.2.1.20; average reads 10048) also dominated in enzymes involved in carbon cycling (Fig. 3a; Table S2). Dextrin and maltodextrin could be hydrolyzed into starch and maltose, respectively, by α-Amylase (EC3.2.1.1; Fig. 2a). Subsequently, maltose could be glycosylated into D-glucose with α-Glucosidase (Fig. 2a).

Regarding the 7 enzymes involved in nitrogen cycling, argininosuccinate lyase (EC4.3.2.1; average reads 7309), aspartate aminotransferase (EC2.6.1.1; average reads 4517), and acetylornithine deacetylase (EC3.5.1.16; average reads 5072) were dominated (Fig. 3b; Table S2). N-(L-Arginino) succinate could be hydrolyzed into fumarate with argininosuccinate lyase (EC4.3.2.1; Fig. 2b). N-Acetyl-L-citrulline could be hydrolyzed into citrulline with acetylornithine deacetylase (EC3.5.1.16; Fig. 2b). Compared with argininosuccinate lyase and acetylornithine deacetylase, aspartate aminotransferase was involved in several metabolisms. 2-oxoglutarate, 3-(4-Hydroxyphenyl) pyruvate and L-erythro-4-Hydroxyglutamate could be hydrolyzed into L-Glutamate, L-Tyrosine, and (4R)-4-Hydroxy-2-oxoglutarate, respectively, by aspartate aminotransferase (Fig. 2b).

A potential link exists between microbial taxa and enzymes involved in carbon and nitrogen cycling (Fig. S2). Fig. S1 showed that β-Glucosidase (EC3.2.1.21) and α-Glucosidase (EC3.2.1.20) in the 7 plant soils were produced by the *Solirubrobacter* (Actinobacteria), *Nocardioides* (Actinobacteria), *Sphingomonas* (Proteobacteria), and *Streptomyces* (Actinobacteria). α-Amylase (EC3.2.1.1) was produced by *unclassified_c_Betaproteobacteria*, *Nocardioides* (Actinobacteria), and *Streptomyces* (Actinobacteria). Different from enzymes involved in carbon cycling, the potential link between microbial taxa and several enzymes involved in nitrogen cycling has differences. Argininosuccinate lyase (EC4.3.2.1) was produced by *unclassified_p_Acidobacteria*, *Nocardioides* (Actinobacteria), and *Streptomyces* (Actinobacteria).

Argininosuccinate lyase (EC4.3.2.1) was produced by *unclassified_p_Acidobacteria*, *Nocardioides* (Actinobacteria), and *Streptomyces* (Actinobacteria). Aspartate aminotransferase (EC2.6.1.1) was produced by *Bradyrhizobium* (Proteobacteria) and *unclassified_p_Acidobacteria*. Acetylornithine deacetylase (EC3.5.1.16) was produced by *Bradyrhizobium* (Proteobacteria), *unclassified_p_Acidobacteria*, *Solirubrobacter* (Actinobacteria), *Streptomyces* (Actinobacteria), and *unclassified_p_Chloroflexi*.

CAZyme annotation based on metagenome sequencing

To better understand the carbohydrate metabolism in the soil ecosystem with 7 vegetation types, we annotated our metagenome for genes encoding carbohydrate-active enzymes (Fig. 4). The relative abundance of glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), carbohydrate-binding modules (CBMs), and auxiliary activities (AAs) showed significant difference among 7 vegetation soils ($P < 0.05$; Fig. 4). GTs and GHs which involve in soil organic C decomposition and biosynthesis respectively had higher relative abundance (Relative abundance > 30%; Fig. 4e, f).

The relationship between microbial community composition and CAZyme-encoding genes was shown in Fig. 5. The abundance of CAZyme-encoding genes varied across the bacterial phyla. As shown in Fig. 5, most of these genes in the soil microorganisms under 7 vegetation types were from *Nocardioides* (Actinobacteria), *Solirubrobacter* (Actinobacteria), *Bradyrhizobium* (Proteobacteria), *Streptomyces* (Actinobacteria), *Sphingomonas* (Proteobacteria), *Conexibacter* (Actinobacteria), and *Mycobacterium* (Actinobacteria). Members of Actinobacteria, Acidobacteria, and Proteobacteria were dominant in carbohydrate-active modules of GHs, GTs, CEs, and CBMs, whereas those from Acidobacteria were dominant in PLs (Fig. 5).

Relationships between the abundance of microbial genes, soil properties and enzyme activities

The relationship between the soil properties and enzyme activities and the abundance of six microbial gene groups for C cycling showed that TN was significantly correlated with GHs, GTs, PLs, CEs, CBMs, and AAs ($P < 0.05$; Fig. 6b). In addition, NO_3^--N showed a significant correlation with GHs which were involved in soil organic C decomposition ($P < 0.05$; Fig. 6b). Urease and dehydrogenase were significantly correlated with TN ($P < 0.05$; Fig. 6a). Meanwhile, N-acetylglucosaminidase showed a significant relationship with SOC and NO_3^--N ($P < 0.05$; Fig. 6a). The relationship between the soil properties and enzyme genes involved in carbon-nitrogen cycling was shown in Fig. 6c. Interestingly, NO_3^--N and pH showed a significant correlation ($P < 0.05$) with several enzymes involved in carbon cycling but have no significant relationship with enzymes involved in nitrogen cycling ($P > 0.05$; Fig. 6c).

Discussion

Vegetation may affect the physical and chemical properties of soil owing to differences in growth conditions, surface coverage, the chemical composition of root exudates, and root turnover rates (Shahzad et al., 2015). The present study found that the soil total N was significantly highest in the PC habitat (2.347 ± 0.017%), followed by RP (2.188 ± 0.059%; Table 2). *Robinia pseudoacacia* has been widely used in the restoration of degraded ecosystems due to its drought tolerance and nitrogen fixation characteristics (Roth et al., 2017; Lian et al., 2015; Sheoran et al., 2010). The reason for the high total N concentration in PC soils may be due to *Populus simonii Carr*. can increase the content of total C and total N in soils by promoting herbal restoration and litter decomposition, within a certain density range shade provision (Zhang et al., 2019b).

Soil enzyme activities were significantly related to soil organic carbon fractions and microbial biomass carbon determinations (Wang et al., 2013). Soil organic carbons act as substrates and stimulate enzyme release to improve soil enzyme activities (Allison et al., 2006). The activity of urease, β-glucosidase, cellulase, and N-acetylglucosaminidase was significantly lowest in RP which has a lower SOC content ($P < 0.05$; Table 3). Contrary to RP, the activity of β-glucosidase, cellulase, and alkaline protease was significantly higher in PC which has a high SOC content ($P < 0.05$; Table 3). The cellulase enzyme complex consists of three major components: β-glucosidase (EC3.2.1.21), endo-1,4-β-glucanase (EC3.2.1.4), and exo-1,4-β-D-glucanase (EC3.2.1.91) (Geiger et al., 1998). Therefore, cellulase activity was closely related to the β-glucosidase activity and together carried out the hydrolytic conversion of cellulose. In addition, the activity of urease significantly correlated with TN ($P < 0.05$; Fig. 6b). The synthesis of urease was closely related to nitrogen content meanwhile nitrogen control of urease synthesis occurs in a number of bacterial species, and urease is not synthesized when the cells are grown in the presence of high-quality nitrogen sources such as ammonia (Friedrich and Magasanik, 1977).

The major facilitator Superfamily COG0477 was significantly enriched in the sample PR ($P < 0.05$; Fig. 1a), related to carbohydrate transport and metabolism, is a diverse group of secondary transporters found in all kingdoms of life, that can catalyze the transport of ions, carbohydrates, lipids, amino acids, peptides, nucleosides et al. substrates (Saier, 2000; Reddy et al., 2012; Madej et al., 2014). Again, the dehydrogenase COG2133 which was involved in both glycolytic and pentose phosphate pathways was significantly highest in sample PR (Wang et al., 2016). Furthermore, the ATP-binding cassette (ABC) -type sugar transporters COG1653 and COG0395, which may be involved in the uptake of carbohydrates, were significantly higher in sample RP and LG than in other samples ($P < 0.05$; Fig. 1a) (Nanavati et al., 2006). Except for the function related to carbohydrate transport and metabolism, several predicted genes related to the amino acid transport and metabolism system were detected in 7 vegetation soils. The acetolactate synthase COG0028 was significantly enriched in RP, followed by LG and PC ($P < 0.05$, Fig. 1b). SOC bacteria contain the acetolactate synthase COG0028, which has been characterized either in cellular extracts or as purified protein from both native and recombinant sources (Mccourt and Duggleby; 2006). Furthermore, succinyl-diaminopimelate desuccinylase (COG0624) was significantly higher in RP, followed by LG ($P < 0.05$, Fig. 1b). As the product of the *dap*E gene, succinyl-diaminopimelate desuccinylase was involved in the diaminopimelate pathway for lysine biosynthesis. Altogether, the abundant function genes

of carbohydrate and amino acid metabolism reveal a high potential for carbohydrates and amino acids metabolism in the *Robinia pseudoacacia* and *Larix gmelinii* habitats.

β-glucosidase (EC3.2.1.21) genes were dominated in soil microbial communities among 7 vegetation types, and significantly richer in RP and LG, followed by PC and PT ($P < 0.05$, Fig. 3a). As another class of classical hydrolases involved in cellulose degradation, endo-1,4-β-glucanases (EC3.2.1.4) were also detected in soils of 7 vegetation types. β-glucosidase activity is well-known as the key factor in the cellulolytic process, with particular importance for the efficient functioning of the cellulase system (Berlemont and Martiny, 2013; Sternberg, 1976; Sternberg and Vuayakumar, 1997). Meanwhile, among the many microorganisms that produce cellulolytic enzymes β-glucosidase generally occurred intracellularly and in comparatively small quantities (Shewale, 1982). These suggest that the microbe in *Robinia pseudoacacia* and *Larix gmelinii* habitats have a greater potential for cellulose degradation than that in other plant habitats in this study. Except for β-glucosidase (EC3.2.1.21) genes, the abundance of α-Amylase (EC 3.2.1.1) and α-glucosidase (EC 3.2.1.20) is also higher in RP and LG than that in other samples. α-Amylase (EC 3.2.1.1) is a well-known endoamylase, which can cleave α,1–4 glycosidic bonds present in the inner part (endo-) of the amylose or amylopectin chain. It is found in a wide variety of microorganisms, belonging to the Archaea as well as the Bacteria (Pandey et al., 2000). α-glucosidase (EC 3.2.1.20) belonging to the exoamylases, cleaves both α,1–4 and α,1–6 glycosidic bonds and produces only glucose (glucoamylase and α glucosidase), or maltose and β-limit dextrin (β-amylase) (Xie and Gross, 1998). These suggest that the microbe in *Robinia pseudoacacia* and *Larix gmelinii* habitats have a greater potential for cellulose degradation than that in other plants habitats in this study. More so, the abundance of α-glucosidase and β-glucosidase genes was significantly related to pH and NO_3^--N, indicating pH and NO_3^--N might affect the abundance of α-glucosidase and β-glucosidase genes via influence the microbe with α-glucosidase and β-glucosidase genes. Both argininosuccinate synthetase (EC 6.3.4.5) and argininosuccinate lyase (EC 4.3.2.1) was detected in samples, the former has significant differences among 7 vegetation habitats, but the latter hasn't ($P < 0.05$; Fig. 3b). Argininosuccinate synthetase (EC 6.3.4.5) and argininosuccinate lyase (EC 4.3.2.1) represent the last two steps in the arginine biosynthetic pathway, involved in the detoxification of ammonia through the production of urea in ureotelic species. Meanwhile, the two enzymes participate in the citrulline-nitric oxide (NO) cycle in which argininosuccinate synthetase was found to have a rate-limiting role in high output NO synthesis (Nussler et al., 1994; Hattori et al., 1994; Xie and Gross, 1998)

Carbohydrate-active enzymes (CAZy) function in degradation, modification, and glycosidic bond formation, which can reveal the metabolism of microbial carbohydrates (Yan et al., 2020). GTs and GHs had a higher relative abundance, and were significantly richer in UP and LG, respectively ($P < 0.05$; Fig. 4e, f). Microbial genes for GHs and GTs are the key gene groups involved in soil organic matter (e.g., cellulose, hemicellulose, and starch) decomposition and biosynthesis, respectively. The close relationship between GHs and GTs abundance and TN in soils with 7 vegetations indicates the content of nitrogen might influence the decomposition and biosynthesis of soil organic matter (Fig. 6b). Nitrogen in the soil significantly affected microbial carbon metabolism. The decrease of nitrogen content in soils may

accelerate the mineralization of soil organic matter by changing the composition of the soil microbial community. Additionally, insufficient nitrogen required for microbial fixation and mineralization is unfavorable to microbial respiration, thereby increasing soil carbon accumulation.

The involved genera included but were not limited to, Nocardioides (Actinobacteria), *Solirubrobacter* (Actinobacteria), *Bradyrhizobium* (Proteobacteria), *Streptomyces* (Actinobacteria), *Sphingomonas* (Proteobacteria), *Conexibacter* (Actinobacteria), and *Mycobacterium* (Actinobacteria). *Streptomyces* are noted for lignocellulose degradation (de Gannes et al., 2013; Zhang et al., 2016; Ventorino et al., 2016). In addition, both modulating and free-living soil N_2 fixing bacteria—*Sphingomonas* identified in the soils may play an important role in nitrogen-fixing (Sithole et al., 2021). Although *Solirubrobacterales* has not been extensively studied, recent studies have shown its members to be adaptive in their ability to colonize different ecosystems (Ishak et al., 2011; Lopez-Velasco et al., 2011; Saul-Tcherkas et al., 2011; Chong et al., 2012).

Conclusion

Overall, this study provides insight into how soil microbiomes play in the carbon and nitrogen cycle under different vegetation types during the ecological restoration process in the mining areas. The results showed that the soil nutrient conditions, and the soil enzyme activities was obviously different among the revegetation types. The soil total N was significantly highest in the PC habitat (2.347 ± 0.017%), followed by RP (2.188 ± 0.059%; Table 2). Meanwhile, the SOC was significantly higher in the PC habitat than in RP, UP, LG, and PS habitats ($P < 0.05$; Table 2). Vegetation species affect the carbon and nitrogen metabolising capacity of soil microorganisms. The major facilitator Superfamily COG0477 and dehydrogenase the COG2133, which related to carbohydrate transport and metabolism, were significantly enriched in the PR habitat. The acetolactate synthase COG0028, which related to the amino acid transport and metabolism, was significantly enriched in RP, followed by LG and PC ($P < 0.05$). β-glucosidase (EC3.2.1.21) is class of classical hydrolases involved in cellulose degradation, which genes were dominated in soil microbial communities among 7 vegetation types, and significantly richer in RP and LG, followed by PC and PT ($P < 0.05$). GTs (involved in soil organic matter decomposition) and GHs (involved in soil organic matter biosynthesis) were significantly richer in UP and LG, respectively. The metagenomic approach analyze the potential mechanisms of soil microorganisms work in carbon and nitrogen metabolism among different revegation species, proposed that *Robinia pseudoacacia* and *Larix gmelinii* may be excellent species choice for improving the productivity and stability of the soil ecosystem and achieving ecological rehabilitation in mining areas.

Declarations

Acknowledgments

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Data availability statement

The data that support the findings of this study are available upon request from the authors.

All authors have read and approved the final manuscript. There is no conflict of interest on this manuscript. This manuscript has not been previously published, is not currently submitted for review to any other journal and will not be submitted elsewhere before a decision is made by this journal.

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Figures

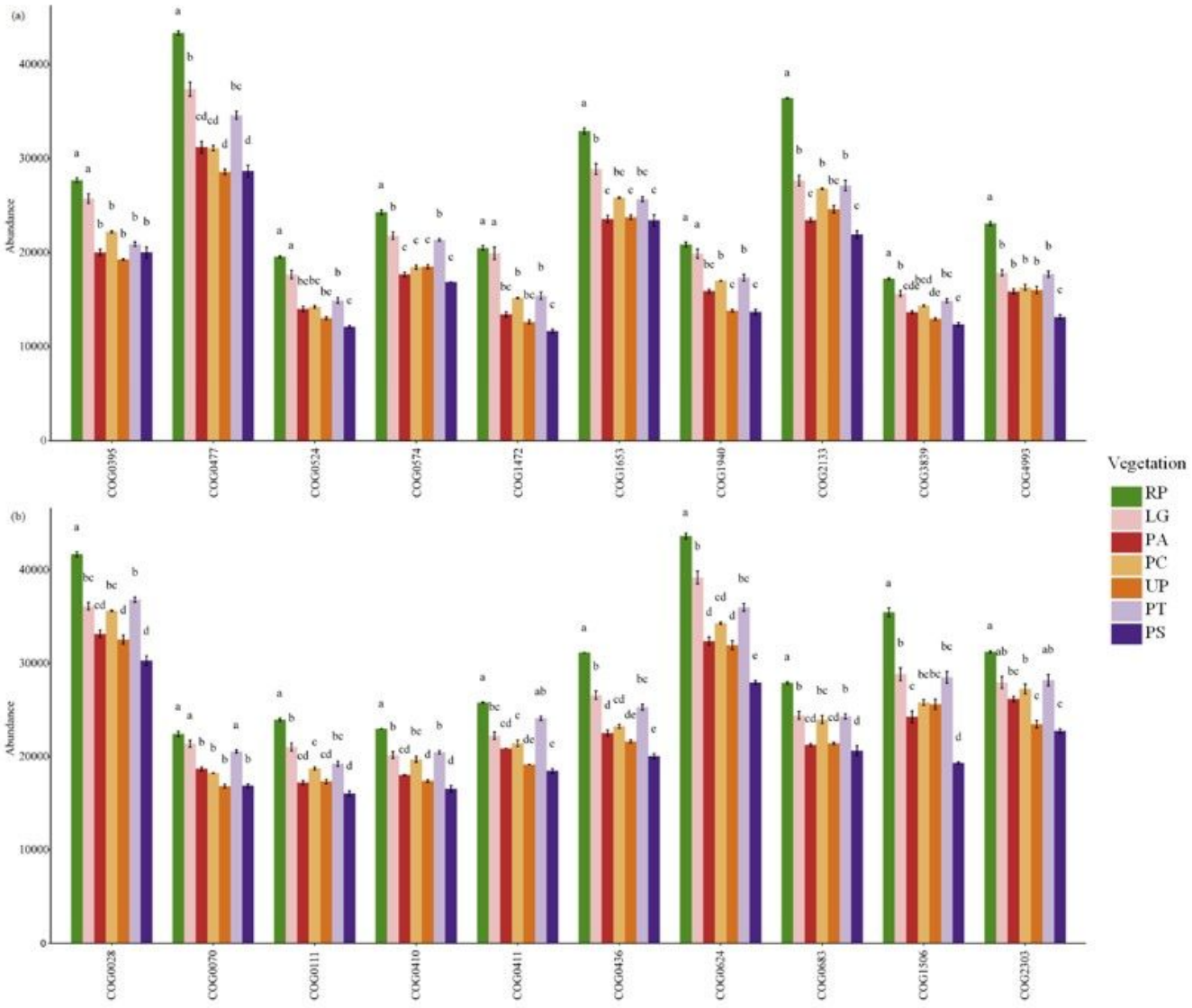

Figure 1

Clusters of Orthologous Group (COG) annotations of samples under 7 types of vegetation (a) Comparison of carbohydrate transport and metabolism functional genes (b) Comparison of amino acid transport and metabolism functional genes Dominant COG functions (top 10 abundant COGs) with significant differences in 7 vegetation soils were selected Bars with different letters indicate significant differences (one-way ANOVA, $P < 005$) RP: *Robinia pseudoacacia*; LG: *Larix gmelinii*; PA: *Picea asperata*; PC: *Populus simonii* Carr; UP: *Ulmus pumila*; PT: *Pinus tabuliformis*; PS: *Pinus sylvestris var dmongolica Litv*

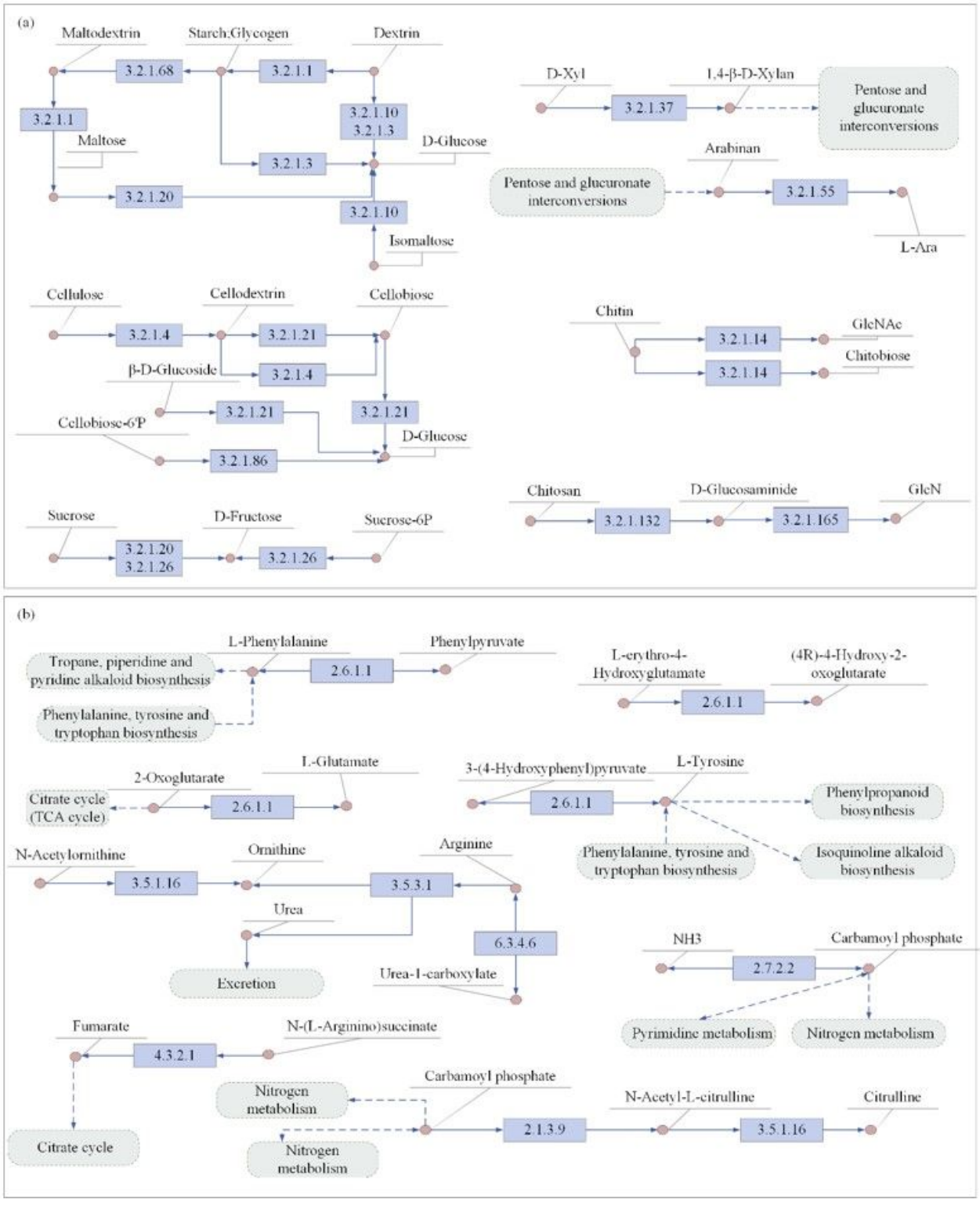

Figure 2

Summarized Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways involved in the carbon cycle (a) and nitrogen cycle (b) Substrates and products are annotated with red circles; enzymes are annotated using enzyme commission numbers with blue rectangles Other connected pathways are annotated with green chamfered rectangles

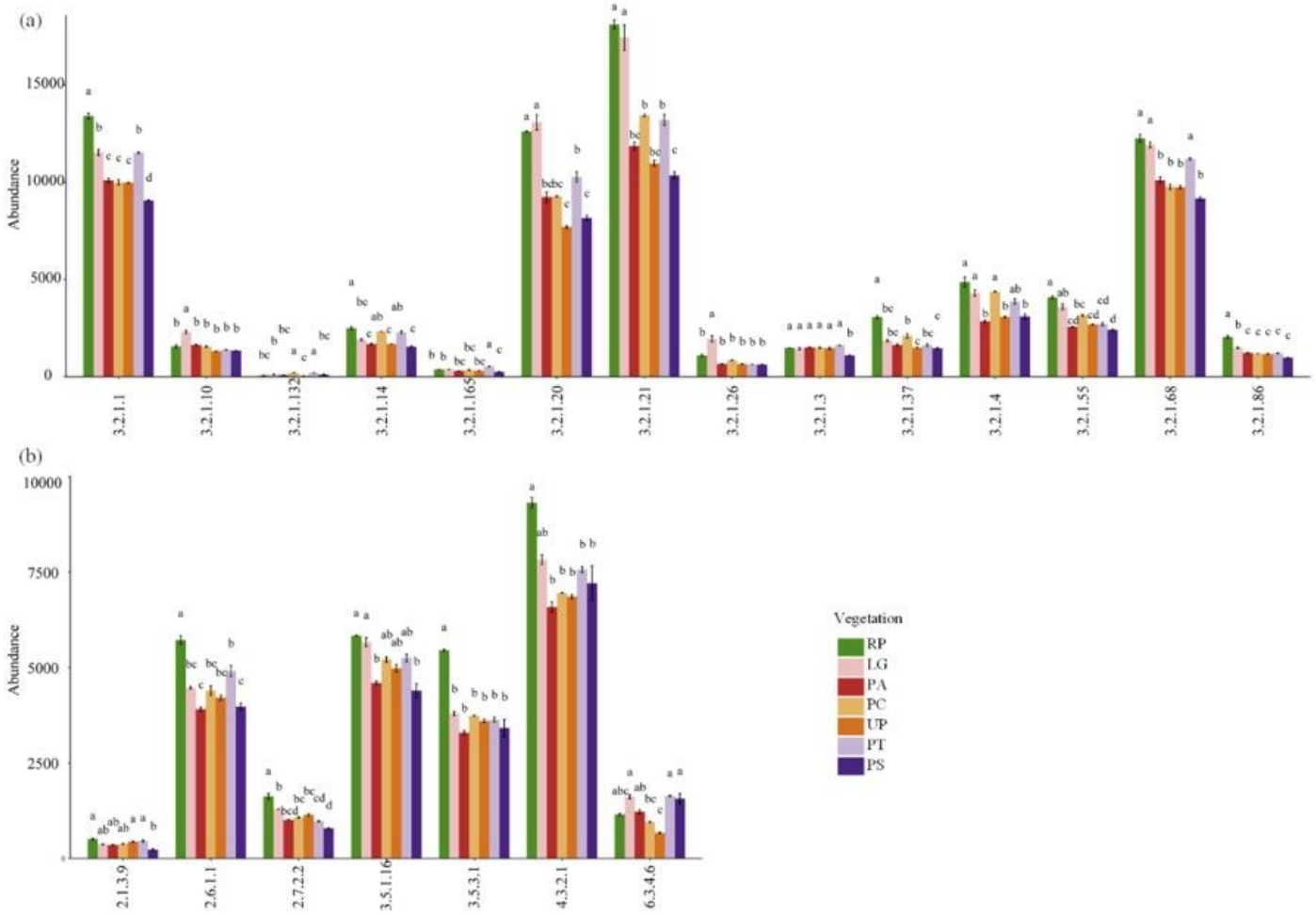

Figure 3

The abundance of enzymes involved in the carbon cycle (a) and nitrogen cycle (b) under 7 types of vegetation Dominant enzymes (top 14 and 7 abundant involved in the carbon cycle and nitrogen cycle, respectively) with significant differences in 7 vegetation soils were selected Bars with different letters indicate significant differences (one-way ANOVA, $P < 005$) RP: *Robinia pseudoacacia*; LG: *Larix gmelinii*; PA: *Picea asperata*; PC: *Populus simonii* Carr; UP: *Ulmus pumila*; PT: *Pinus tabuliformis*; PS: *Pinus sylvestris*

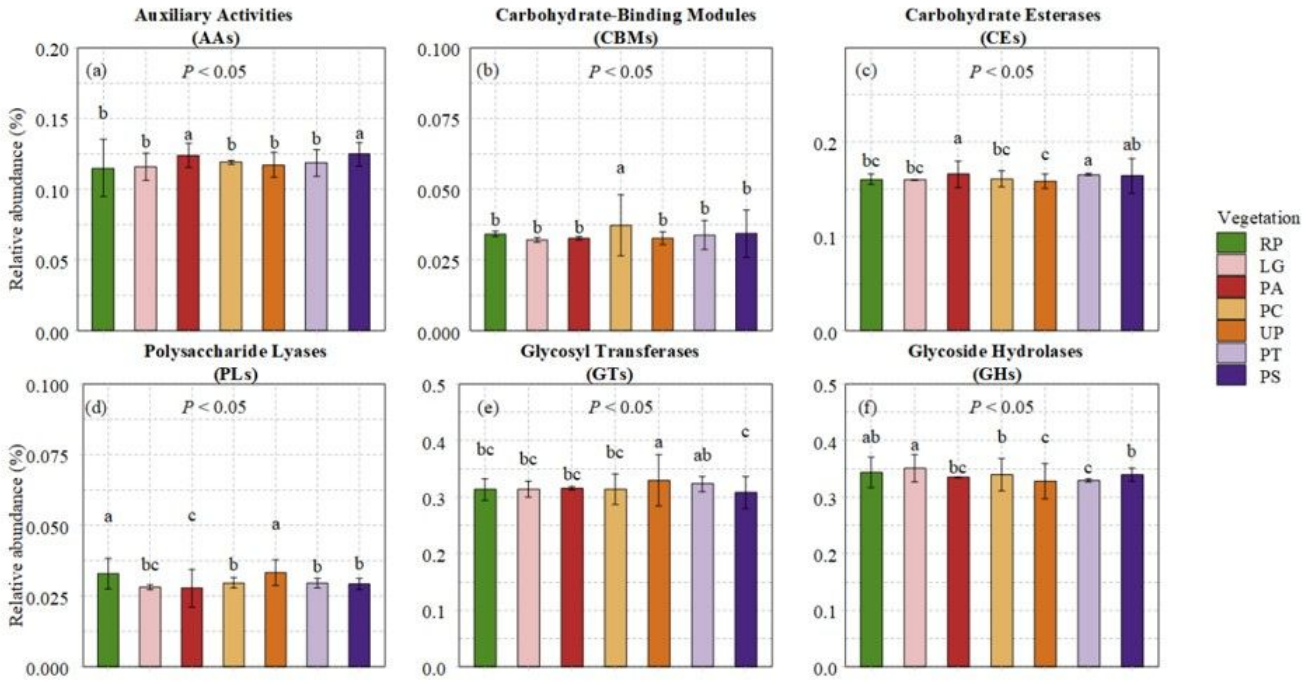

Figure 4

Differences in the relative abundance of microbial gene groups encoding glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), carbohydrate-binding modules (CBMs), and auxiliary activities (AAs) under 7 types of vegetation The GHs and GTs involve in soil organic C decomposition and biosynthesis respectively The CBMs facilitate the binding of enzymes to substrates The PLs, CEs, and AAs involve in microbial decompositions of polysaccharides, carbohydrate ester, and lignin respectively Bars with different letters indicate significant differences (one-way ANOVA, *P*< 005) RP: *Robinia pseudoacacia*; LG: *Larix gmelinii*; PA: *Picea asperata*; PC: *Populus simonii* Carr; UP: *Ulmus pumila*; PT: *Pinus tabuliformis*; PS: *Pinus sylvestris*

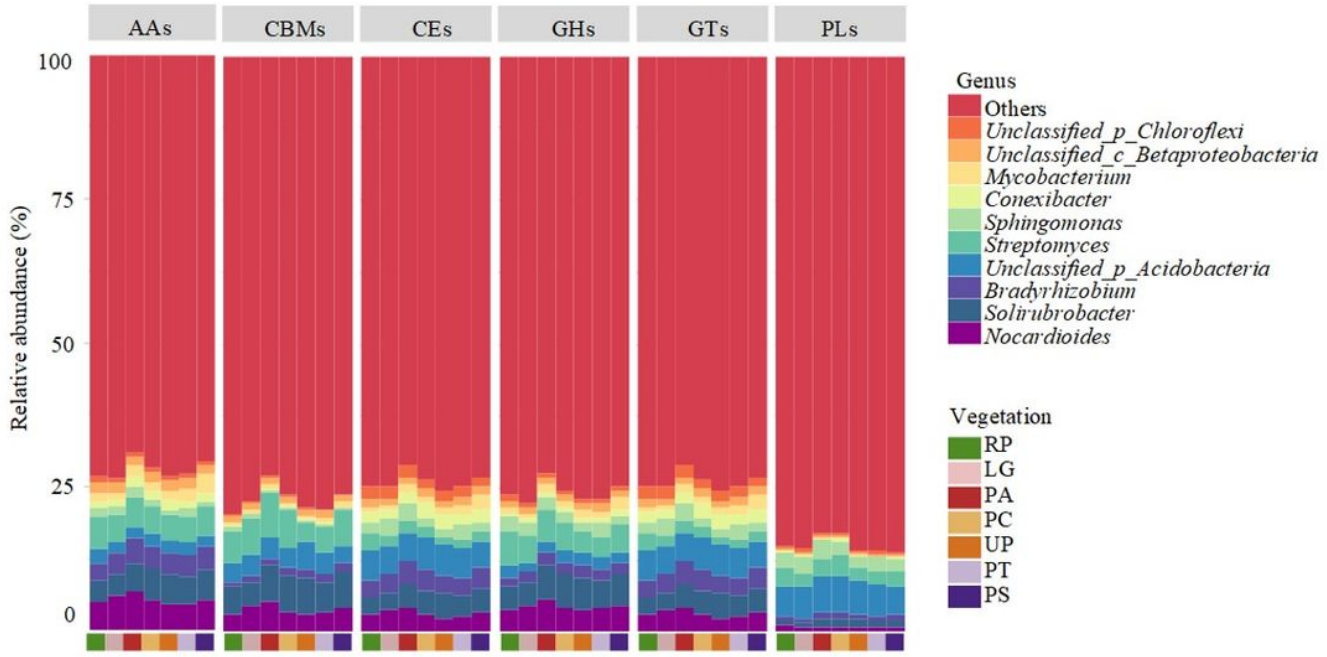

Figure 5

Distribution of genera enriched with carbohydrate-active enzyme (CAZyme) genes in soils under 7 types of vegetation RP: *Robinia pseudoacacia*; LG: *Larix gmelinii*; PA: *Picea asperata*; PC: *Populus simonii* Carr; UP: *Ulmus pumila*; PT: *Pinus tabuliformis*; PS: *Pinus sylvestris*

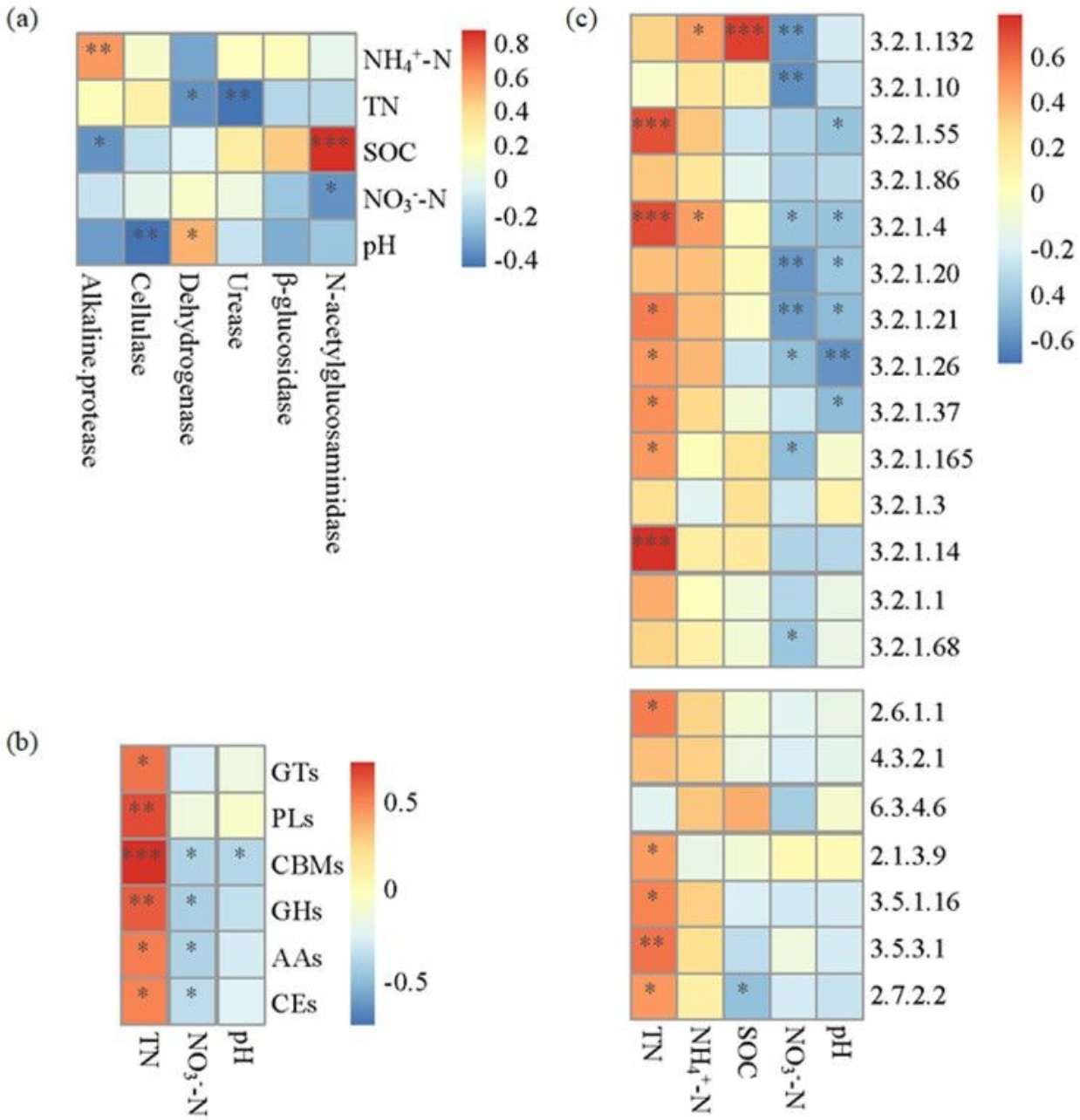

Figure 6

Relationship between the soil properties and, the abundance of (a)six microbial gene groups for C cycling (CAZyme annotation), (b)enzyme activities, (c)the abundance of enzymes involved in carbon-nitrogen metabolism (KEGG enzyme annotation) in soils under 7 vegetation types SOC, total soil organic carbon; TN, total nitrogen The *P*-values of <005 adjusted by FDR Correction were presented "*" *P* < 005; "**" *P* < 001; "***" *P* < 0001

Supplementary Files

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