Soil Microorganisms in Mixed Forest and Pure Forests of Ulmus pumila - Robinia pseudoacacia Based on Metagenomics

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Abstract

Aims

The present study aimed to explore the characteristics of the microbial community in *Ulmus pumila-Robinia pseudoacacia* mixed forest soils in the Yellow River Delta.

Methods

We used metagenomics to analyse the structure and functional characteristics of the soil microbial community in a pure forest of *Ulmus pumila,* a pure forest of *Robinia pseudoacacia*, and a mixed forest of *Ulmus pumila - Robinia pseudoacacia*.

Results

In total, 176 phyla and 21,760 species of microorganisms were identified. The proportion of bacteria (84.90% - 85.20%) was the highest among the microbial types in the soil of each forestland. Using the orthological database eggNOG, we identified the most dominant functional gene category is soil microbial metabolism. The active metabolism of carbohydrates in the KEGG pathway was prominent. In the carbon cycle, that soil microbial carbon metabolic activity in the mixed forests were greater than that in the pure forests. The gene abundance related to nitrogen metabolism was the highest in the *Ulmus pumila - Robinia pseudoacacia* mixed forest soil.

Conclusions

Mixed forests play specific roles in soil quality improvement by promoting the activity and functional metabolism of various soil microbiome communities.

Introduction

Mixed forests have functions such as water and soil conservation and soil environment improvement and play a very important role in maintaining the balance and stability of forest ecosystems (Bozali 2020). Most of the plantations in China and abroad are pure forests (Liu et al. 2018). The pure forest, single-species structure is uncomplicated, however, long-term, continuous single-species planting depletes forest benefits, causes serious loss of soil nutrients, and reduces microbial richness (Williams 2015). Zhou et al. (2019) showed that the pattern of long-term pure forest planting disrupts the soil microbiology of Xylopia rhizospheric interactions, significantly impacting the soil microbial community structure and causing soil degradation. By investigating the soil quality and soil microbial load under different vegetation restoration methods in the southwest karst region, Lu et al. (2015) concluded that single-species afforestation is not conducive to soil restoration. Multispecies mixed afforestation is an effective measure for improving the biodiversity and ecological stability of protected forests, and plays important roles in improving the resilience of forests and the prevention and control of ground failure (Pereira et al. 2018). Studies have demonstrated (Nunes et al. 2011, Wu 2019, Shen 2020) that mixed forests can improve the diversity of soil bacterial communities, increase metabolic functions of biological pathways and nitrogen cycling, promote the resistance and resilience of forests, and yield better soil improvement than pure forests. Research on mixed forests has become a hot spot in current research. Soil microorganisms are an important link between aboveground vegetation communities and belowground ecological processes (Waldrop 2004, You 2014). They participate in the decomposition of soil organic matter, regulate soil nutrient cycling processes (Wardle et al. 2004, Eisenhauer et al. 2010), are an important component in maintaining soil productivity (Tedersoo et al., 2020), and play a vital role in plant growth (Wiehe & Höflich, 1995; Young and Crawford, 2004, Morgan et al. 2005; Yang et al. 2015). Paul and Clark (1997) found from a study of mixed nitrogen-fixing tree species and Eucalyptus that mixed forests altered soil N effectiveness and soil microbial community structure, thereby positively affecting microbial processes that regulate soil carbon and N cycling in plantation forests. Zak et al. (2003) found that soil microbial biomass increased significantly with an increase in silvicultural tree species diversity. It has been shown that soil microorganisms are closely related to the structure, aeration, moisture status, and nutrient status of the soil (Fierer et al. 2003; Edwards et al. 2006; Liu and Han 2015), and the more suitable the soil environment is, the more stable the soil microbial community, the richer its diversity, and the greater the population density (Chen et al. 2016).

With the gradual advancement of research on soil microorganisms, methods and technologies are also constantly improving. At present, high-throughput sequencing technology is commonly used to study the structure and function of soil microbial communities

(Ma et al. 2021). However, few studies on the structure and function of microbial communities using metagenomics have been conducted. We made the hypothesis that the mixed forests are more conducive to improving soil microbial structure and functional activity, thereby changing the circulation of soil nutrients, promoting forests growth and the accumulation of mineral nutrients.In this study, metagenomics technology was used to analyse the physical and chemical properties of soils as well as their microbial community structure and functional genome characteristics and to clarify key relationships between the soil microbial community and the aboveground mixed forest to support the theoretical basis for the establishment of mixed forests and the informed utilization and improvement in soils throughout the region.

1. Materials And Methods

1.1 Study area

The present experimental study site is in Hekou District, Dongying city, northern Shandong Province (37°45' - 38°10'N, 118°10' - 119°05'E). This area has a warm temperate, semi-humid, continental monsoon climate. The average annual temperature is 12.7°C and the maximum annual temperature difference is 63.8°C. The frost-free period is 201 days, and the frozen soil period is 44 days. The annual average sunshine hours are 2,630-2,850 h, and the annual average precipitation is 550-600 mm. Seventy percent of the precipitation occurs in summer and often leads to flood disasters. The test area has flat terrain, a shallow groundwater level, and high mineralization. Soil salinity is high primarily in coastal saline areas. The plantation was built in 1985 with a row spacing of 2 m × 3 m. Artificial afforestation species mainly include *Robinia pseudocacia*, *Ulmus pumila*, *Populus euramericana*, *Tamarix chinensis*, etc.

1.2 Forest type selection and collection methods

The experimental forest was built in ten thousand mu of *Robinia pseudocacia* forest in Gudao town in April 1985. Mixed forest was planted in alternating rows of each species, and the plant spacing × row spacing was 2 m × 3 m for all species. In the early stage of afforestation thinning was carried out on each forestland, and the thinning intensity was 20% - 25%. The three forest types, including *Robinia pseudoacacia* pure forest, *Ulmus pumila* pure forest, and *Robinia pseudoacacia - Ulmus pumila* mixed forest, were planted with specimens of the same age, uniform growth, with consistent site conditions. Each forest type was set to a standard size of 20 m × 30 m. The random block test design was adopted, with three replicates of each treatment. The basic layout of tree height,diameter at breast height, canopy closure, and crown width in the sample plot are shown in table 1.

Table 1 Basic characteristics of mixed forests of different tree species

Forests		Stand age	Mixed way	Row spacing (m × m)	Canopy closure %	Height (m)	Diameter at breast height (cm)	Crown width	
								East and West	North and South
YS		34		2 × 3	83	9.21	16.37	3.67	3.96
CH		34		2 × 3	85	11.65	17.23	4.62	4.82
YSCH	YS	34	Inter-row Mixing	2 × 3	88	13.54	22.06	5.73	5.87
	CH					12.11	19.53	4.66	4.70

Note : YS : *Ulmus pumila* CH : *Robinia pseudoacacia* YSCH : *Robinia pseudoacacia* ×*Ulmus pumila* mixed forest

Samples were collected on the 28th of July 2019. The S-type sampling method with five plots of uniform area (2 m × 3 m) was randomly set in the test plot. Soil from the 5-15 cm depth was collected after the surface impurities were removed and stored at 4°C for the determination of soil enzyme activity and physicochemical properties. Additionally, 10 g soil samples were stored in the laboratory at -80°C for the determination of soil microbial species and gene composition.

1.3 Determination of soil physical and chemical properties

The soil bulk and soil porosity were determined using the ring knife method, and the soil moisture content was determined using the drying method.

soil moisture content = $\frac{m_1 - m_2}{m_2 - m} \times 100\%$

soil porosity=$(1 - \frac{soilbulkdensity}{soilproportion}) \times 100\%$

In the formula : m_1 represents the total mass of the fresh soil and aluminium box; m_2 is the total mass of the dried soil and aluminium box; m represents the aluminium box mass; and the soil specific weight was 2.65 g/cm3.

Determination of soil chemical composition was measured using the following methods: total nitrogen determined using the semimicro Kjeldahl method; total phosphorus determined using the perchloric acid-sulfuric acid method; ammonium nitrogen determined using indigo colorimetry; nitrate nitrogen determined using UV spectrophotometry; available potassium determined using flame atomic absorption spectrometry; fast-acting phosphorus determined using the $NaHCO_3$ leaching and the molybdenum antimony anti-spectrophotometric method.

1.4 Determination of soil enzyme activity

The urease activity in the soil was determined using a UV spectrophotometer (TU-1900) with the phenol sodium-sodium hypochlorite colorimetric method. The disodium phosphate method was used for colorimetric determination of soil phosphatase activity at 660 nm; the 3,5-dinitrosalicylic acid colorimetric method was used to determine soil invertase activity at 540 nm; and the soil catalase activity was determined by titration.

1.5 Soil microbial DNA extraction, processing and metagenomic assembly libraries

The total DNA of soil microbial genes was extracted by a PowerSoil ® DNA Isolation kit, and the experimental process was carried out according to the standard protocol provided by Illumina. After the genomic DNA of the sample was qualified, the DNA was fragmented by mechanical interruption (ultrasound), and then the fragmented DNA was purified, repaired, added to A at the 3’ end, and connected to the sequencing joint. Agarose gel electrophoresis was used to select the fragment size, and PCR amplification was performed to form the sequencing library. The constructed library was used for library quality inspection. The qualified library was sequenced by the Illumina sequencing platform. The quality of the original reads was controlled and filtered to obtain clean reads for subsequent bioinformatics analysis. The clean reads were assembled, the coding genes were predicted, and the functional annotations of the general database and special database were conducted. Simultaneously, the clean reads were subjected to taxonomic analysis, and the species composition and abundance were determined for each sample.

The metagenome library process consists mainly of genome interruption, end repair, splice ligation, PCR amplification and purification. The metagenome was assembled by MEGAHIT (Version 1.1.2), and contig sequences less than 300 bp were filtered by default parameters. The results were evaluated by QUAST (Version 2.3). MetaGene Mark software (Version 3.26) was used to predict the assembly results of each sample. Functional annotation uses Diamond software (Version 0.9.04) to compare protein sequences with the database. The above process was provided by Beijing BMK Biotechnology Co., Ltd.

1.6 Data analysis

Processing was conducted using a one-way analysis of variance (ANOVA) and significance tests using SPSS 25.0 software and plotting using Origin 2019b and Excel 2010 software. Principal components of species composition were analysed using R software (V.3.3.2).

2. Results

2.1 Analysis of soil physical and chemical properties

Table 2 shows that the soil water content of the *Ulmus pumila - Robinia pseudoacacia* mixed forest was significantly higher than that two kinds of the pure forests. The soil water content of the mixed forest was 1.6 times higher than that of the pure forest and 1.3 times higher than that of the *Robinia pseudoacacia* forest. The soil porosity of the mixed forest of *Robinia pseudoacacia - Ulmus pumila* showed no significant change compared with that of the pure forest of *Ulmus pumila* but increased 8.79% compared with that of the pure forest of *Robinia pseudoacacia*.

By analysing changes in nutrient content in soil samples from the mixed forest and pure forests (Table 2), it can be seen that compared with the pure forest of *Ulmus pumila*, the content of total nitrogen, nitrate nitrogen, and available phosphorus in the mixed forest of *Ulmus pumila - Robinia pseudoacacia* increased significantly ($P < 0.05$) by 23.91%, 178.76% and 49.85%, respectively, while changes in total phosphorus, ammonium nitrogen, and available potassium were not obvious. Compared with the *Robinia pseudoacacia* forest, the content of total nitrogen, nitrate nitrogen, and available phosphorus in the mixed forest soil was significantly increased ($P < 0.05$) by 32.56%, 115.24% and 7.08%, respectively, while the content of total phosphorus, ammonium nitrogen, and available potassium did not change significantly.

Table 2 Physical and chemical properties of soil in different forests

Forests	soil porosity (%)	Moisture content (%)	TP (mg/kg)	TN (g/kg)	N-NO^{3-} (mg/kg)	N-NH^{4+} (mg/kg)	AK (mg/kg)	AP (mg/kg)
YS	42.35± 1.82bc	8.01± 0.31c	602.22± 11.93ab	0.26± 0.05bc	3.39± 0.53c	9.15± 0.24a	285.95± 15.68ab	3.43± 0.05c
CH	41.61± 1.63cd	9.67± 0.89bc	597.33± 14.87ab	0.30± 0.02bc	8.20± 0.19b	9.19± 0.11a	284.74± 9.77ab	4.39± 0.02b
YSCH	45.26± 1.64ab	12.95± 0.88a	624.78± 17.00a	0.37± 0.02a	9.45± 0.76a	8.96± 0.39a	228.90± 15.52b	5.14± 0.02a

Note : YS : *Ulmus pumila* CH : *Robinia pseudoacacia* YSCH : *Robinia pseudoacacia - Ulmus pumila* mixed forest; data marked with different lowercase letters in the same row indicate significant differences between treatments ($P< 0.05$).

Analysis of the soil enzyme activity of the mixed forest and pure forests (Fig. 1) showed that there were significant differences in the soil enzyme activity. The urease and sucrase activities of mixed forest soils were significantly higher than those of the pure forests. The urease activity of the *Robinia pseudoacacia-Ulmus pumila* mixed forest was 120.72% and 150.13% higher than that of the *Ulmus pumila* pure forest and *Robinia pseudoacacia* pure forest, respectively. The sucrase activity in the *Ulmus pumila - Robinia pseudoacacia* mixed forest was 98.75% higher than that in the pure forest of *Ulmus pumila* and 40.64% higher than that in the pure forest of *Robinia pseudoacacia.* The phosphatase activity in the mixed forest of *Ulmus pumila - Robinia pseudoacacia* was 5.03% higher than that in the pure forest of *Robinia pseudoacacia* and 41.23% lower than that in the pure forest of *Ulmus pumila*. There was no significant difference in catalase activity between the mixed forest and pure forests.

2.2 Analysis of soil microbial community structure

2.2.1 Soil microbial composition at the phylum level

At the bacterial phylum level, there were 176 bacterial phyla in the soil of the *Robinia pseudoacacia* pure forest, *Ulmus pumila* pure forest and *Ulmus pumila-Robinia pseudoacacia* mixed forest. Among them, *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Candidatus _ Rokubacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Planctomycetes,* and *Verrucomicrobia* were the eight primary bacterial phyla. The sum of the relative abundances of the dominant bacterial phyla in all plots was 74.68%, 75.01% and 74.89%, respectively. The bacterial phyla with relative abundances less than 1% in the three forest types were expressed as “Others,” and their relative abundances were 2.89%, 2.82%. and 2.91%, respectively. The unclassified communities in the soil bacterial phyla of forestland were represented as “Unclassified,” and the relative abundances were 8.18%, 8.11%, and 8.13%, respectively. Unidentified bacterial communities in the soil were represented by “Unassigned.”

As shown in Figure 2, the relative abundance of Acidobacteria in the *Ulmus pumila* pure forest, *Robinia pseudoacacia* pure forest, and the mixed forest was the highest, which was 31.4%, 32.2%, and 32.6%, respectively. The relative abundances of *Proteobacteria*, *Actinobacteria*, *Candidatus _ Rokubacteria,* and *Chloroflexi* in the mixed forest soil were higher than those in the *Ulmus pumila* pure forest, and the relative abundances of the four bacterial phyla in the mixed forest soil were lower than those in the *Ulmus pumila* pure forest. The relative abundances of other bacterial phyla in the *Ulmus pumila - Robinia pseudoacacia* mixed forest were higher than those in the *Robinia pseudoacacia* pure forest except for *Chloroflexi* and *Gemmatimonadetes.*

2.2.2 Soil microbial composition at the species level

Figure 3 shows the principal component analysis of soil microorganisms in the three treatments at the species level. The contribution of PC1 was 64.30% and that of PC2 was 20.95%. Due to the low contribution and the variation of other principal components, principally from the first and second principal components, only the first and second principal components were analysed.

The mixed forest of *Ulmus pumila* and *Robinia pseudoacacia* is concentrated in the fourth quadrant, the *Ulmus pumila* pure forest is concentrated in the second quadrant, and the *Robinia pseudoacacia* pure forest is concentrated in the third quadrant. The distribution distance of the three treatments was large, indicating that the composition of the three treatments was substantially different at the level of microbial species.

2.2.3 Bacterial diversity index analysis

The Shannon index, Simpson index, and Chao1 index of the *Ulmus pumila* - *Robinia pseudoacacia* mixed forest were 5.02, 0.90 and 22950.80, respectively. The Shannon index of the mixed forest was significantly higher than that of the *Ulmus pumila* pure forest and the *Robinia pseudoacacia* pure forest, and the Shannon index also showed a significant difference between the two pure forests ($P < 0.05$). There was no significant difference in the Simpson index and Chao1 index among the three forest soils.

Table 3 Analysis of bacterial community diversity in the soils under different forests

Index	Forests		
	YS	CH	YSCH
Shannon index	4.99±0.0057b	4.96±0.0033c	5.02±0.0021a
Simpson index	0.90±0.0004a	0.90±0.0004a	0.90±0.0007a
Chao1 index	23119.35±48.5996a	23199.50±34.1691a	22950.80±135.1587a

Note : There are significant differences in data representation processing of different lowercase letters marked on the same row ($P < 0.05$).

2.3 Functional analysis of the soil microbial community

2.3.1 eggNOG annotation library analysis

By comparing the soil microbial genes of three different treatments with the eggNOG database, the function of eggNOG was annotated into four categories: metabolism, cellular processes and signalling, information storage and processing, and poor characteristics. The main function of the three different treatments was soil microbial metabolism. Compared to *Ulmus pumila* pure forest (Fig. 4), a significant increase in metabolic functional genes in the Orthologous Group category of *Ulmus pumila* - *Robinia pseudoacacia* mixed forest was observed in the transport and metabolism of amino acids (E) and carbohydrates (G); a significant decrease in the transport and metabolism of lipids (I); compared with the *Robinia pseudoacacia* pure forest, the transport and metabolism of carbohydrates (G) in the *Ulmus pumila* - *Robinia pseudoacacia* mixed forest increased significantly. Lipid transport and metabolism were significantly reduced (I).

2.3.2 KEGG annotation library analysis

A total of 8,548,899 genes were annotated by KEGG pathway in the three treatments. According to the types of pathways in KEGG, 21 signalling pathways were classified into 4 functional categories in the KEGG database (Fig. 5). The four major functional categories included metabolism (5,793,230 71.12%), genetic information processing (1,044,205 12.82%), environmental information processing (863,618 10.60%) and cellular processes (444,465 5.46%). Carbohydrate metabolism (1,309,242) was the most abundant gene among the metabolic pathways, and biosynthesis of other secondary metabolites (1,24,959) was the least. Regarding metabolic pathways, 1,951,661 genes were annotated in *Ulmus pumila* - *Robinia pseudoacacia* mixed forest, accounting for 33.69% of the total number of metabolic pathway genes. The pure forests of *Ulmus pumila* and *Robinia pseudoacacia* accounted for 33.19% and 33.12%, respectively.

2.3.3 CAZy annotation library analysis

CAZy is a database of carbohydrate-active enzymes. The results showed that CAZy divided carbohydrate-active enzymes into six components: glycosyltransferase (GT), glycosidase (GH), carbohydrate esterase (CE), carbohydrate-binding module (CBM), pseudosaccharide lyase (PL), and auxiliary oxidoreductase (AA) (Fig. 6). A total of 450,104 carbohydrate-active enzymes were identified in soil samples from the three treatments, with glycogen transferases (GT, 145,085) and glycoside hydrolases (GH, 137,008) being the most abundant, accounting for 62.67% of the total number of enzymes detected, followed by carbohydrate esterases (CE, 80,786), carbohydrate-binding modules (CBM, 56,689), and polysaccharide lyase (PL, 18,707). Auxiliary oxidoreductase (AA, 11,829) accounted for the lowest proportion, only 2.63%. A total of 152,792 carbohydrate-active enzymes were annotated in *Ulmus pumila* - *Robinia pseudoacacia* mixed forest, accounting for 33.95% of the total. *Ulmus pumila* pure forest (149,345) and *Robinia pseudoacacia* pure forest (147,967) accounted for 33.18% and 32.87%, respectively.

2.3.4 Soil Microbial Community Metabolism - Carbon Metabolism

Through the analysis of functional genes related to carbon metabolism (Fig. 7), the results showed that the abundance of acyltransferase (K00626) involved in amino acid metabolism, glucose metabolism, lipid metabolism, pyruvate metabolism, carbon fixation, degradation and metabolism of foreign substances, and acetyl coenzyme A (K01895) involved in antibiotic synthesis, methane metabolism, glycolysis, pyruvate metabolism, and propionic acid lipid metabolism were at an elevated level in the mixed forest.

Compared with the Ulmus pumila pure forest, the abundances of glycine dehydrogenase (K00281), aminomethyltransferase (K00605), and fumaric acid hydratase (K01676) in the tricarboxylic acid cycle, D-3–phosphate glyceryl dehydrogenase (K00058) in glycolysis, and formate dehydrogenase (K00122) in the methane metabolism pathway in the mixed forest of Ulmus pumila and Robinia pseudoacacia increased significantly. Compared with the Robinia pseudoacacia pure forest, the Ulmus pumila - Robinia pseudoacacia mixed forest significantly increased K01808 and K00122 in terms of methane metabolism.

2.3.5 Soil microbial community metabolism - nitrogen metabolism

By comparing the relative abundance of genes related to nitrogen metabolism KO (Fig. 8), the abundances of NO reductase (K04561), N2O reductase (K00376), and nitrite reductase (NO) (K00368) in the Ulmus pumila - Robinia pseudoacacia mixed forest were significantly higher than those in the Ulmus pumila pure forest. Glutamate dehydrogenase (K15371) was significantly lower than that in the pure elm forest. The abundance of glutamate synthase (K00284), K04561, K00376 and K00368 in Ulmus pumila × Robinia pseudoacacia mixed forest was significantly higher than that in Robinia pseudoacacia pure forest.

2.4 Correlation analysis between soil flora and soil environmental factors

The correlation between soil nutrient content, soil enzyme activity, and soil dominant bacterial phylum (relative abundance > 1%) abundance in different treatments was analysed (Table 4, Table 5). At the bacterial phylum level, Acidobacteria was extremely significantly positively correlated with soil total nitrogen and significantly negatively correlated with total phosphorus, nitrate nitrogen, and available potassium.

Proteobacteria were extremely significantly positively correlated with total phosphorus and significantly positively correlated with available potassium content; actinobacteria was significantly positively correlated with soil ammonium nitrogen and available potassium; phylum Candidatus - Rokubacteria was significantly positively correlated with ammonium nitrogen; Chloroflexi was significantly positively correlated with total nitrogen and ammonium nitrogen; Gemmatimonadetes was significantly negatively correlated with total nitrogen and ammonium nitrogen content; planctomycetes was significantly negatively correlated with nitrate nitrogen content; verrucomicrobia was significantly positively correlated with ammonium nitrogen. In summary, mixed forests can affect the growth of various soil microorganisms by affecting the soil nutrient content.

Acidobacteria was significantly negatively correlated with soil urease and invertase activities; Proteobacteria was significantly positively correlated with catalase activity; Candida _ Rokubacteria phylum was significantly positively correlated with urease and invertase activities; Chloroflexi was significantly positively correlated with urease and invertase activities; Gemmatimonadetes was significantly negatively correlated with invertase activity; Verrucomicrobia was significantly positively correlated with urease activity; actinobacteria and Planctomycetes had no significant correlation with soil enzyme activity.

Table 4 Correlation analysis of soil environmental factors with soil flora and enzyme activity

Index	TN	TP	N-NH_4^+	N-NO_3^-	AK	AP	Urease activity	Sucrase activity	Phosphatase activity	Catalase activity
Acidobacteria	0.65**	-0.46*	0.35	-0.53*	-0.49*	-0.07	-0.65**	-0.62**	0.37	0.17
Proteobacteria	0.21	0.56**	-0.18	0.37	0.50*	0.29	0.15	0.34	-0.04	0.47**
Actinobacteria	-0.09	-0.10	0.79**	0.15	0.80**	0.14	0.20	0.31	0.23	-0.06
Candidatus_ Rokubacteria	-0.26	0.17	0.65**	-0.12	0.10	0.13	0.65**	0.67**	-0.01	0.34
Chloroflexi	0.700*	0.19	0.52*	-0.19	0.16	0.17	0.48*	0.47*	0.18	0.30
Gemmatimonadetes	-0.58**	-0.12	-0.66**	-0.25	-0.12	-0.34	0.11	-0.46*	0.13	-0.13
Planctomycetes	0.31	-0.04	-0.23	-0.51*	0.24	0.03	0.17	0.20	0.20	-0.30
Verrucomicrobia	0.05	-0.36	0.53**	0.15	0.11	0.37	0.44*	0.32	0.02	-0.06

Note : * showed significant correlation ($P < 0.05$); * * showed highly significant correlation ($P < 0.01$).

3. Discussion

Forest types affect soil nutrient content and soil enzyme activity and play a vital role in soil microbial activities (Chen et al. 2018). Xue (2003) studied the soil nutrient and microbial and enzyme activity of Pinus elliottii mixed forests and reported that the soil nutrient content of mixed forests was higher than that of the larch pure forest, which promoted the soil enzyme activity of the mixed forests to be higher than that of pure forests. In this study, compared with the pure forests, the content of total nitrogen, nitrate nitrogen, available phosphorus, urease and invertase activities in the mixed forest were significantly increased, indicating that the *Ulmus pumila - Robinia pseudoacacia* mixed forest was helpful to the improvement in the soil nutrient content and enzyme activity. The increase in soil nutrient content provides abundant resources to soil microorganisms in the *Ulmus pumila - Robinia pseudoacacia* mixed forest, enhances microbial metabolic activity and, thus, improves soil enzyme activity (Han et al. 2020).

Soil microorganisms are collectively the most active component in soils. This study showed that Acidobacteria, Proteobacteria, and Actinobacteria were the dominant members of the soil microbiome communities of the three treatment plantations, which is consistent with Hui et al. (2012). Studies have shown that (Klimek et al. 2015, Carvalho et al. 2016) varied forest stands affect the soil microbial community structure. Kennedy et al. (2004) used T-RFLP technology to study soil microorganisms in soils of seven different vegetation types and found that they significantly affected soil microbial diversity and microbiome community structure. In this study, 176 bacterial phyla were detected in the soil of *Ulmus pumila - Robinia pseudoacacia* mixed forest, while only 174 and 175 bacterial phyla were detected in the soil of *Ulmus pumila* pure forest and *Robinia pseudoacacia* pure forest, respectively, indicating that the *Ulmus pumila - Robinia pseudoacacia* mixed forest made the soil bacterial composition richer. Analysis of the correlation between soil nutrients, soil enzyme activity and flora showed that ammonium nitrogen, total nitrogen, soil urease, invertase, and catalase activity were important factors affecting soil flora. This may be due to the increased activities of soil ammonium nitrogen, total nitrogen, urease, invertase, and catalase in the *Ulmus pumila - Robinia pseudoacacia* mixed forest, which improved the soil microenvironment and increased the abundance of the soil microbial species.

Soil microbial functional genes are important markers of soil microbes and the microbial functional genes in mixed forests are primarily metabolic. Waid (1999) studied the mixture of Larix olgensis and Juglans mandshurica and found that the metabolic activity of the soil microbial community was significantly higher than that of pure forests. It was concluded that vegetation types and composition promoted changes in soil microbial activity and functional groups. In this study, the soil microbial metabolic activity of the mixed forest was higher than that of the pure forests, which may be because the mixed forest has more plant species and more individuals than the single-species pure forests, so the soil microbial community metabolic activity is more vigorous. Soil microbes mediate the transformation of soil carbon and are the “key” and “driving force” of the carbon cycle. Analysis of functional genes related to carbon metabolism showed that compared with the pure forests, the processes of glycolysis, amino acid metabolism, and carbon fixation in the soil of the *Ulmus pumila-Robinia pseudoacacia* mixed forest were promoted. This was like the characteristics

reported for soil microbial carbon metabolism in coniferous and broad-leaved mixed forests (Zhang et al. 2018). This may be because the diversity of mixed forests provide a wider array substances to the soil, promoting microorganisms in decomposing organic matter into carbon dioxide and energy, thereby accelerating carbon metabolism (Tao et al. 2013). Soil nitrogen cycling is principally mediated by soil microorganisms (Kuypers et al. 2018). The higher the richness of the soil microbiome, the more conducive it is to the migration and transformation of nitrogen and other substances. The nitrogen cycle was positively correlated with the synthesis and metabolism of carbohydrates in tree species. By studying changes in functional genes related to the nitrogen cycle and their abundances, we found that genes, such as the nitrogen monoxide reductase and the glutamate synthase genes, were significantly more abundant than those in the control pure forests, thereby promoting the process of soil nitrification and denitrification, as was seen by Li (2020). In addition, Proteobacteria endow soils with a nitrogen fixation capacity, which converts organic nitrogen into NH_3 by ammoniation, promoting the nitrogen cycle.

Soil microbiome community structure is affected by many factors, including vegetation type, climatic conditions, and soil type, as the primary external factors, and soil organic matter, as the principal internal factor. Tree litter is the central source of soil organic matter input, which can provide nutrients for the growth of microorganisms and is the material basis for microbial metabolic activities. The quality and quantity of litter input affects the abundance and diversity of the soil microbiome community composition (Yang et al. 2015). As a common forest management type, mixed forests improve stable community structure, enhance forest ecological service functions, positively affect soil nutrient content and soil enzyme activity, and enhance soil microbial diversity, thereby promoting soil synergistic microbiome community activities. The enhancement of soil microbial metabolic activity plays a crucial role in improving the quality of mixed forest soils.

Declarations

Author contributions: Conceptualization: Rui Chen, Haoran Cui,; Methodology: Rui Chen, Haoran Cui; Formal analysis and investigation: Rui Chen, Zhiqiang Zhou, Haoran Cui and Qian Xu; Writing-original draft preparation: Rui Chen, Haoran Cui; Writing—review and editing: Rui Chen, Haoran Cui, Fengyun Ma, Zhiqiang Zhou, and Qian Xu; Obtaining funding: Fengyun Ma.

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Data Availability:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Table 5

Table 5 is not available with this version

Figures

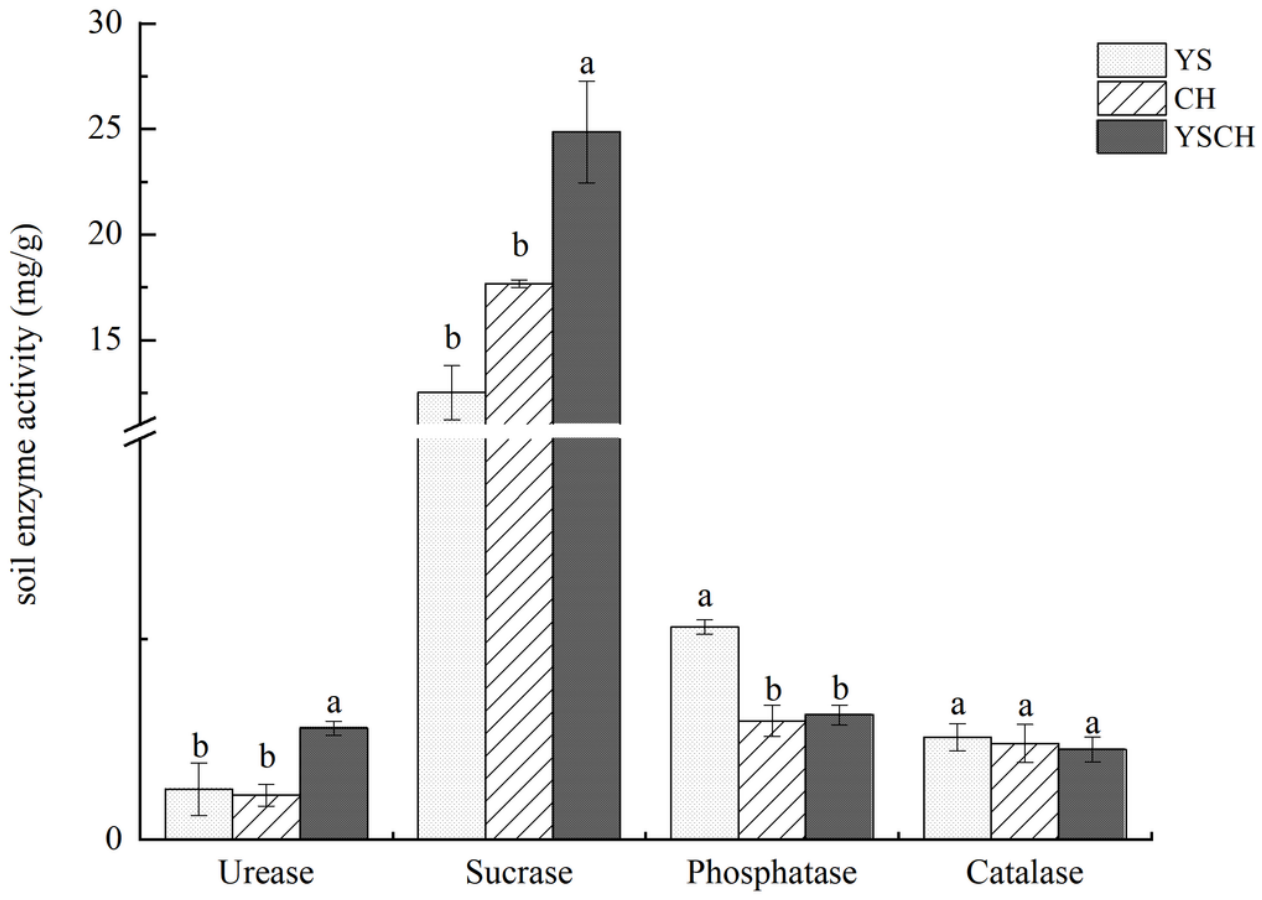

Figure 1

Differences enzyme activity of soil in different forests

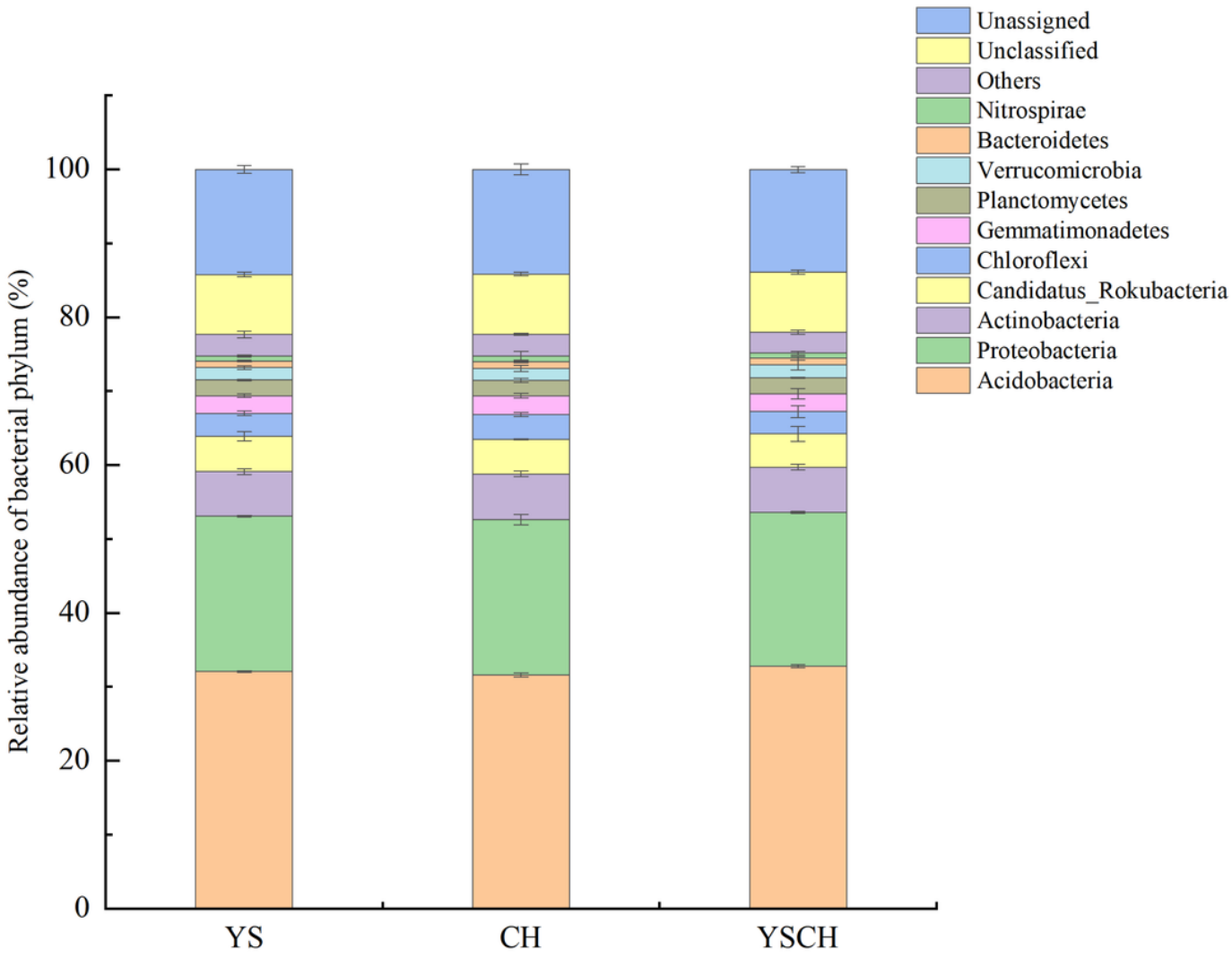

Figure 2

Relative abundance of soil bacterial phylum

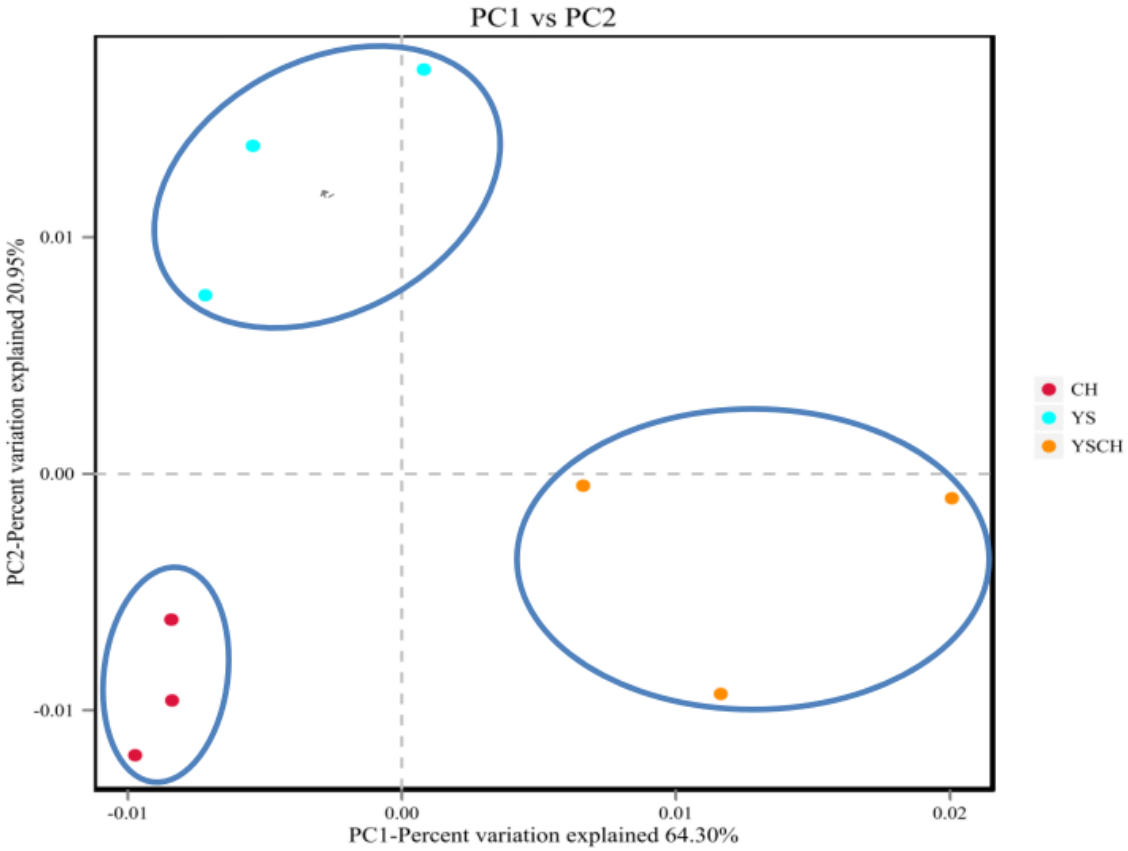

Figure 3

Principal component analysis of soil microbial species composition in each forest

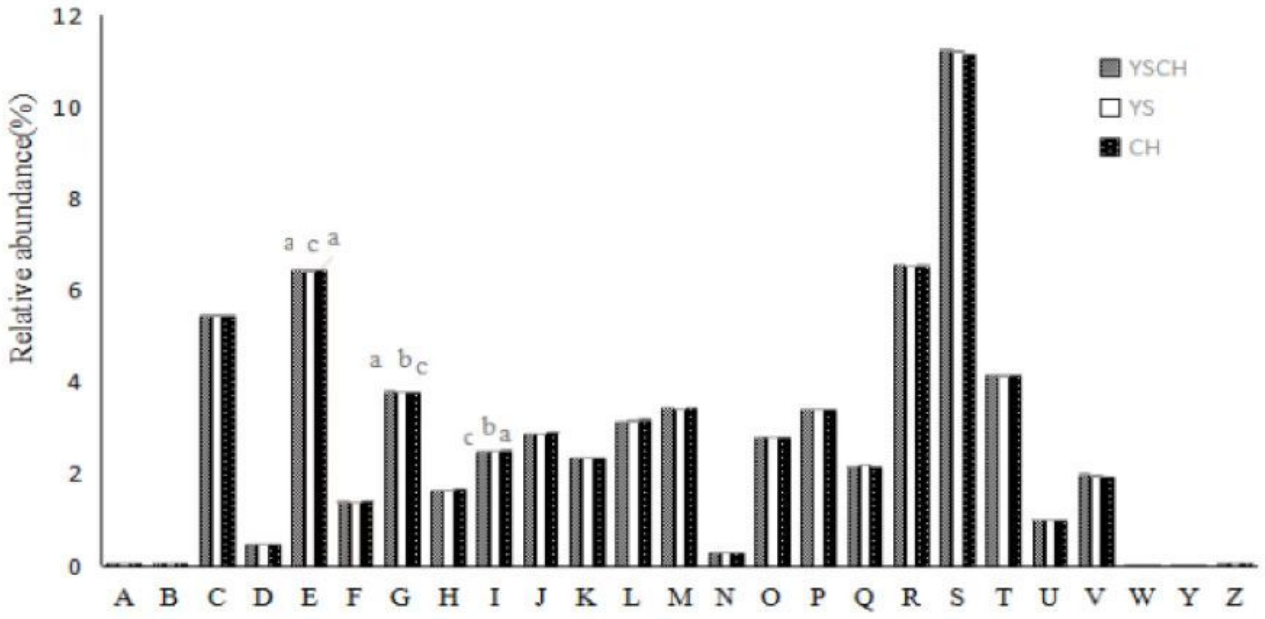

Figure 4

The functional gene differences of eggNOG in the soil of *Ulmus pumila - Robinia pseudoacacia* mixed forest, *Ulmus pumila* pure forest and *Robinia pseudoacacia* pure forest.

Note : A, B, J, K, L are microbial information storage and processing functions; O, D, M, N, T, U, V, W, Y, Z belong to cell signal transduction function; R, S function unknown; C : Energy production and conversion E Amino acid transport and metabolism F Nucleotide transport and metabolism G : Carbohydrate transport and metabolism H : Coenzyme transport and metabolism I : Lipid transport and metabolism P: Inorganic ion transport and metabolism Q: Secondary metabolites biosynthesis, transport and catabolism. There were significant differences between the groups with different lowercase letters marked in the column ($P < 0.05$).

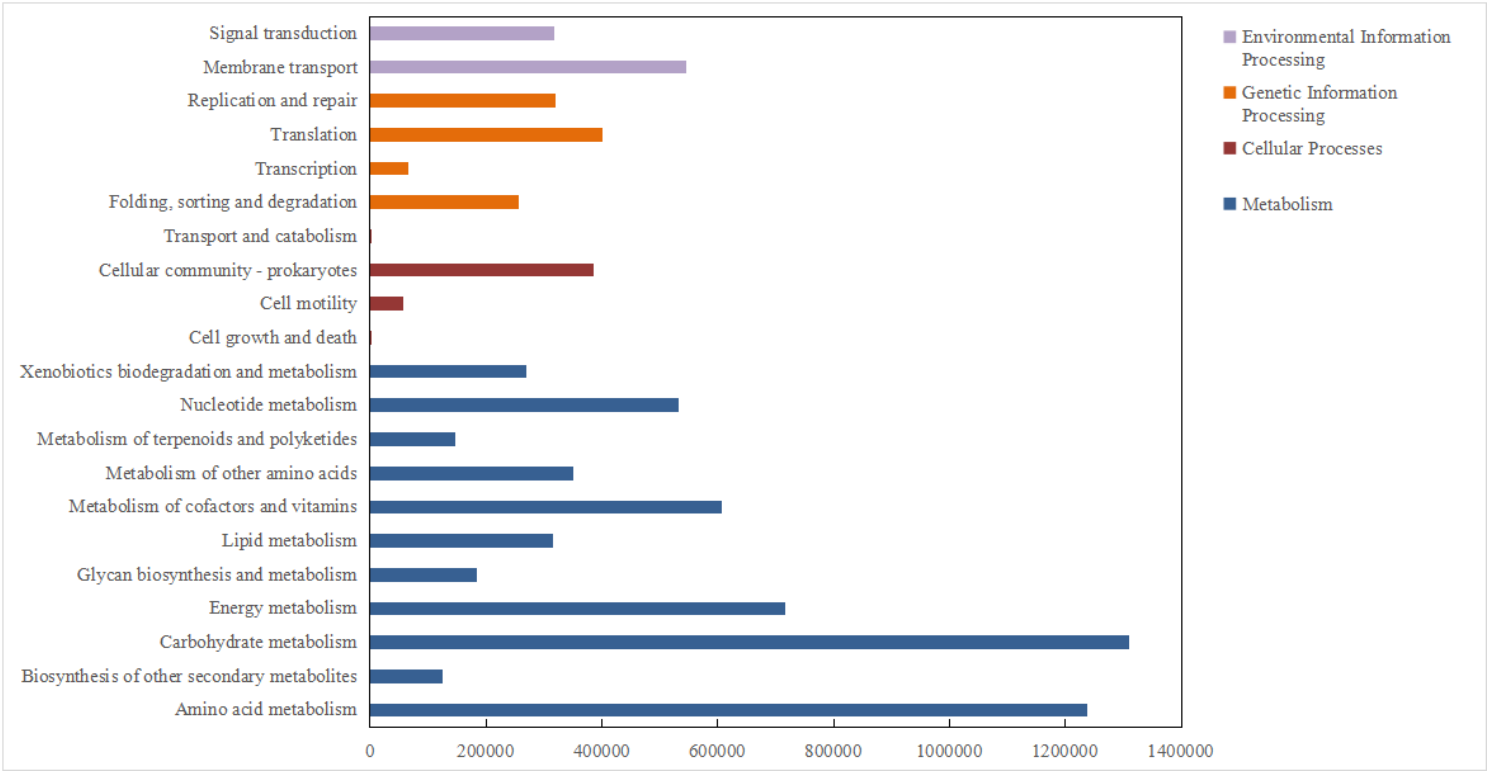

Figure 5

Statistics of metabolic pathway of soil microbial gene KEGG in three different stands

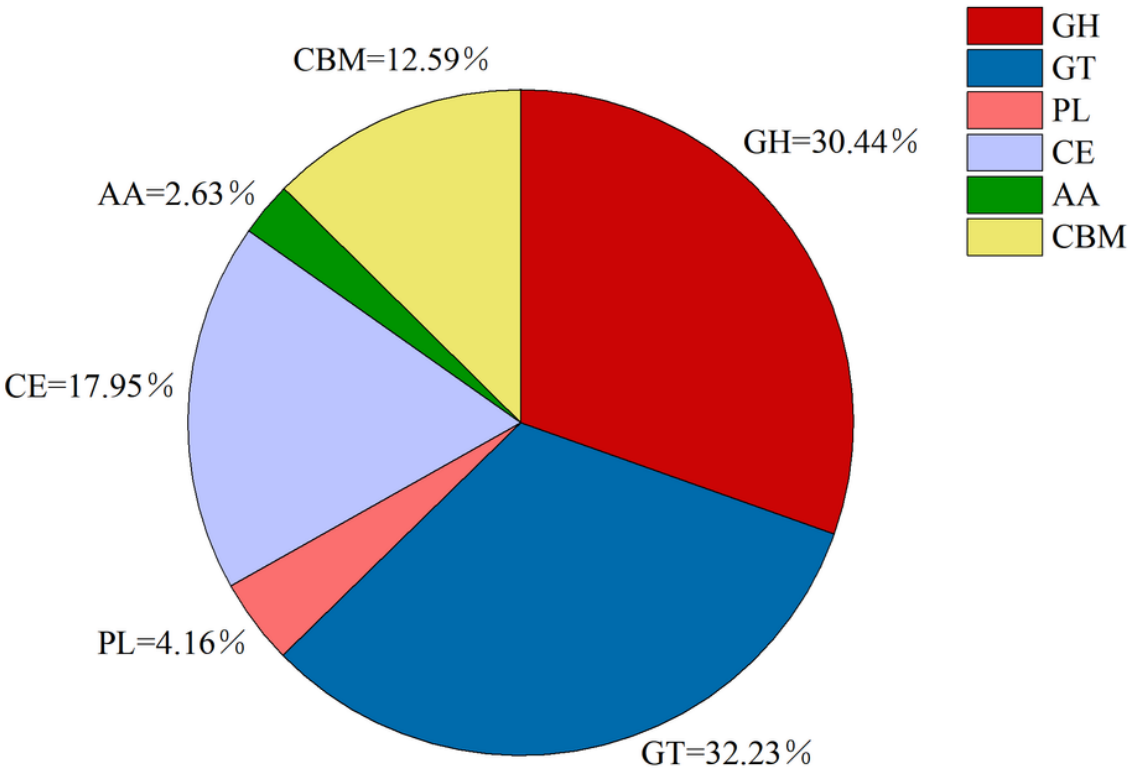

Figure 6

Statistics of CAZy of soil microbial in three different stands

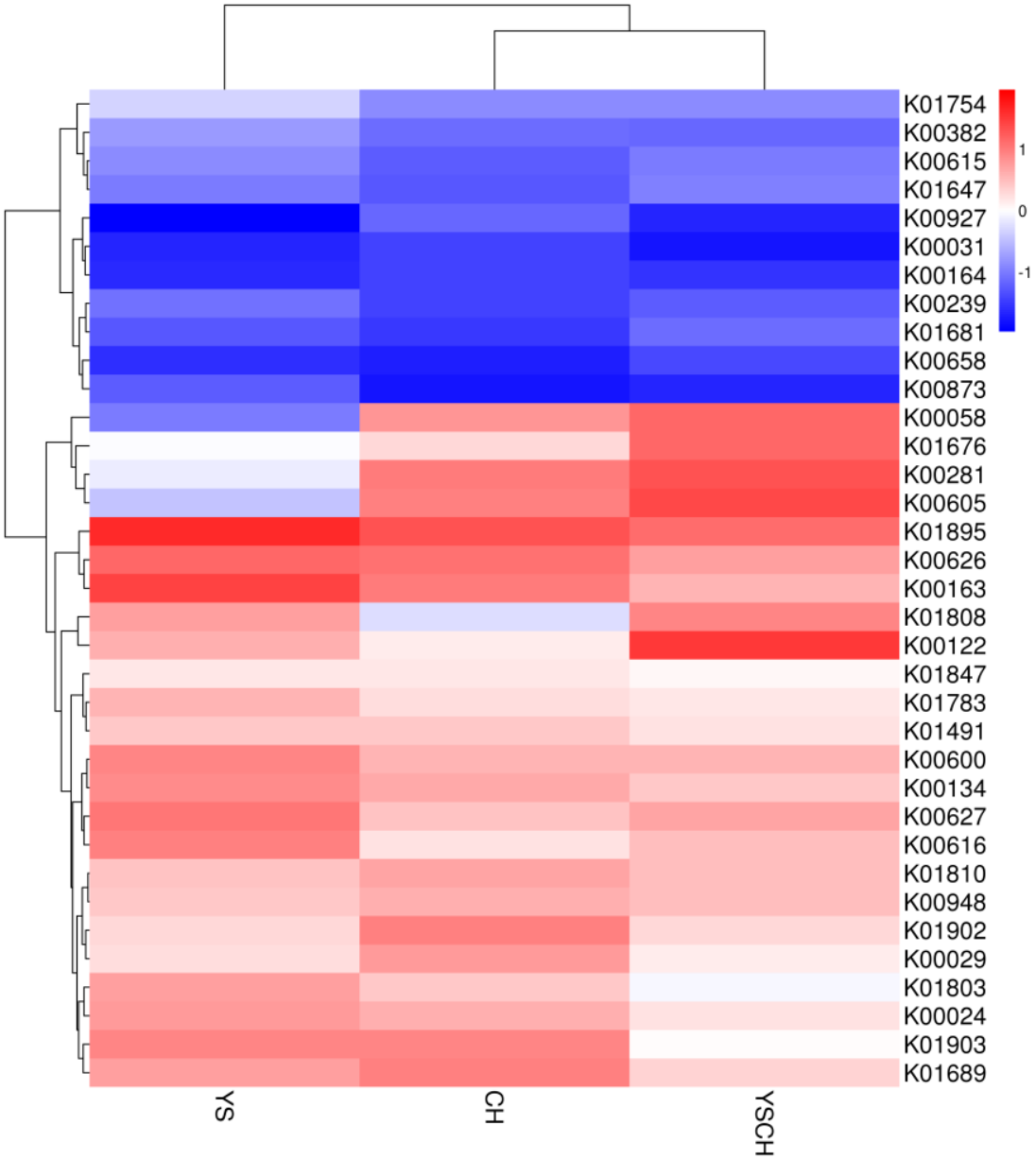

Figure 7

Heat map of relative abundance difference of genes related to C metabolism

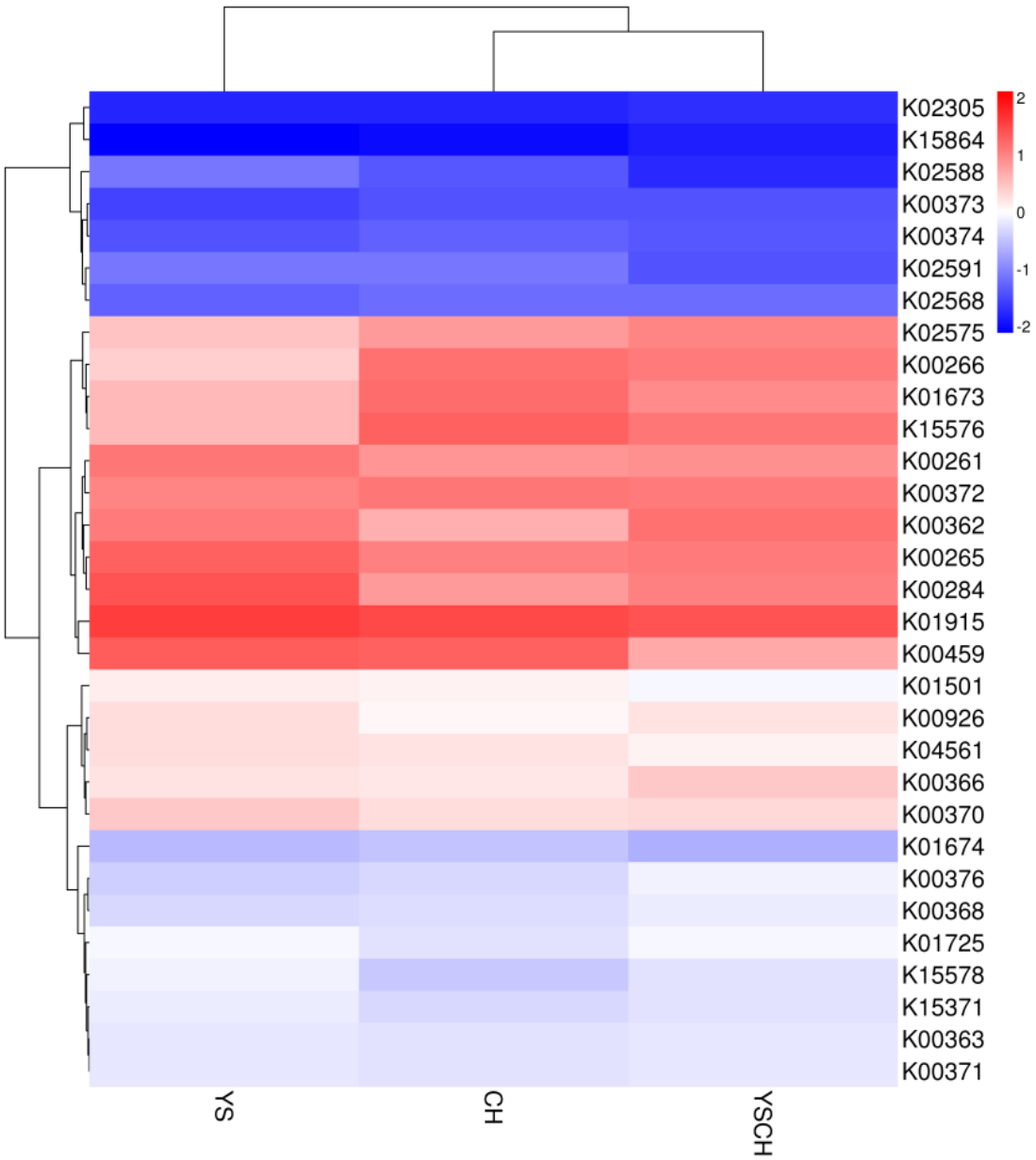

Figure 8

Functional genes related to N metabolism annotated by KEGG