Exploring the potential of Trichoderma asperellum TCS007 on growth promotion of pecan seedlings as well as rhizosphere soil nutrients and microbial community

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Abstract

Aims

Pecan (*Carya cathayensis* Sarg.) is an important forest trees in China, the application of chemical pesticides for disease control has caused severe damage to the soil, including reduced fertility and disruption of microbial communities. Although *Trichoderma* treatment has been shown to promote plant growth and improve soil quality, its effects on the growth promotion of pecan and the impact on soil microbial communities and physicochemical properties remained unclear.

Methods

In this study, we investigated the impact of *T. asperellum* TCS007 spore suspension and its fermented crude extract on the growth and development of pecan seedlings. We also explored the effects of TCS007 treatment on the nutrients, enzyme activities, and microbial diversity in the rhizosphere soil of pecan seedlings during their three main growth stages.

Results

Treatment with TCS007 spore suspension or crude extract promoted the growth of pecan seedlings, with significantly higher levels of leaf hormones and defense enzyme activity compared to the control (CK). Moreover, the content of soil organic matter and ammonium nitrogen, as well as the activity of soil enzymes such as catalase and urease, were all significantly higher than CK after treatment, and the soil pH shifted from slightly acidic to slightly alkaline. The results indicated that TCS007 treatment significantly increased the richness of beneficial fungi and bacteria in the soil.

Conclusion

The results demonstrated that TCS007 treatment significantly promoted the growth of pecan plants, increased enzyme activity and nutrient content in the soil, and improved the soil micro-ecological environment.

Introduction

Trichoderma spp., ubiquitously present in nature, are a group of fungi that significantly promote plant growth (Chacón et al. 2007; Tyśkiewicz et al. 2022; Chen et al. 2023). These fungi colonize the rhizosphere of plants, enhancing their tolerance to biotic and abiotic stresses (Vargas et al. 2009). They produce a variety of bioactive substances, including antibiotics and enzymes, which inhibit plant pathogens. By competing for nutrients and occupying space, they effectively suppress the growth of soil-borne pathogens, thereby protecting plants from disease (Garnica-Vergara et al. 2015; Shi et al. 2016).

Trichoderma harzianum has been shown to grow 2.0 to 4.2 times faster than *Botrytis cinerea*, thereby effectively inhibiting the latter's growth. This competitive exclusion not only deprives pathogens of essential resources but also physically blocks their access to plant tissues (Yao et al. 2023). Moreover, *Trichoderma* induces systemic resistance in plants, strengthening their resilience to adverse conditions (Kashyap et al. 2017; Gupta and Maya 2020). These functions make *Trichoderma* an important biocontrol agent, widely used in agriculture.

Trichoderma spp., have a significant positive impact on soil health. They promote the decomposition of organic matter in the soil, enhancing soil fertility and structure (Khan et al. 2017; Halifu et al. 2019). The metabolic activities of *Trichoderma* increase the availability of nutrients such as nitrogen and phosphorus to plants (Abdenaceur et al. 2022; Li et al. 2015). Furthermore, *Trichoderma* forms symbiotic relationships with plant roots, improving the efficiency of water and nutrient absorption by plants (Mehetre et al. 2015). They also improve the diversity of soil microbial communities, enhancing the ecological purification capabilities of soil (Mao and Jiang 2021). These functions of *Trichoderma* help maintain the balance of the soil ecosystem and improve its sustainable production capacity (Woo et al. 2023; Pinto et al. 2024). Studies had shown that the application of *Trichoderma* biofertilizers to the rhizosphere of corn could effectively increase the content of organic matter, nitrogen, phosphorus, and potassium in the soil. After spraying the suspension of *Trichoderma atroviride* on corn at 15 and 25 days after germination, the soil ammonium nitrogen content increased by 15% (Fu et al. 2019). Liu et al. (2020) found that after applying a microbial fertilizer containing *Trichoderma guizhouense* during the growth process of chili peppers, the soil urease activity significantly increased, and the transformation of soil organic nitrogen accelerated, increasing the available nitrogen content in the rhizosphere soil of chili peppers, promoting their nutrient absorption, and thus increasing the yield of chili peppers.

Pecan was primarily found in the Tianmu Mountain area, located at the border of Zhejiang and Anhui provinces. Thriving in the understory of mountain slopes or valleys that were rich in humus, it was an economically important forest tree in China (Guo et al. 2004). In recent years, the excessive use of chemical fertilizers and pesticides, coupled with the long-term overexploitation of pecan forests, led to soil fertility degradation, frequent soil-borne diseases, and reduced yields, causing economic losses for farmers (Fang et al. 2020). There was an urgent need for a biofertilizer product that could improve the fertility of pecan forest land and was also friendly to the micro-ecological environment. *Trichoderma* spp., as a green biological pesticide, had potential in environmental remediation and plant health, but their application in the pecan industry had been relatively understudied.

This study explored the effects of the *Trichoderma asperellum* TCS007 (isolated from Antarctic marine sediments) on the physiological and biochemical indicators of pecan plants, the physicochemical properties of rhizosphere soil, and soil enzymatic activity. High-throughput sequencing was used to analyze the diversity and structural composition of the microbial community in the rhizosphere soil of pecan, providing a theoretical basis for the development of TCS007 as a biofertilizer product, thereby contributing to the stable development of the pecan industry.

Materials and Methods

Experimental materials and equipment

Tested microbial strains and plants

The *T. asperellum* TCS007 was isolated from Antarctic marine sediments by our laboratory. The strain was preserved at the China General Microbiological Culture Collection Center (CGMCC), with the accession number CGMCC No.15677.

One-year-old pecan seedlings, provided by the Panmugang Modern Forestry Demonstration Base, located in Lin'an District, Hangzhou City, Zhejiang Province, were transplanted into plastic pots filled with a mixture of peat soil/vermiculite/sand (2:1:1, v/v/v) as the growth substrate. After transplanting, the seedlings were placed in a greenhouse under 12 h photoperiod at 26°C and allowed to acclimatize for 30 days before use.

Reagents and Culture Media

Plant hormone indole-3-acetic acid (IAA) and gibberellin (GA) assay kits were purchased from Nanjing Camilo Bioengineering Co., Ltd.; enzyme activity assay kits for soil catalase, urease, and β-glucosidase were purchased from Beijing Boxbio Science & Technology Co., Ltd., and soil genomic DNA extraction kits were purchased from Tiangen Biotech Co., Ltd.

PDA and PDB media (Li and He 2005) were used for cultivating the TCS007. Tryptone Beef Broth, Martin medium, and Modified Gause's No. 1 medium (Li et al. 1996) were used for the cultivation of bacteria, fungi, and actinomycetes from the soil samples.

Experimental Methods

Preparation of TCS007 spore suspension and liquid fermentation crude extract solution

For spore suspension preparation; two agar plugs were punched from the edge of a revived TCS007 colony on PDA using a 9 mm diameter borer and inoculated into 100 mL PDB medium in a 250 mL flask for cultivation at 28°C at 180 rpm for 7 days. The liquid culture was then filtered through sterile gauze three times to remove the mycelia and debrises, obtained the fermentation filtrate of TCS007. The concentration of the spore suspension was determined using a hemocytometer and adjusted to 1.0×10^6 and 1.0×10^8 spores/mL for subsequent use.

Preparation of TCS007 liquid fermentation crude extract solution: The TCS007 fermentation filtrate was acquired as aforementioned. According to the method described by Liu et al. (2019), the fermentation filtrate of TCS007 was extracted with an equal volume of ethyl acetate three times. The upper organic phase was collected and dried using a saturated sodium chloride solution and anhydrous sodium sulfate. After that, the extract was concentrated under reduced pressure using a rotary evaporator,

redissolved in acetone, and collected in a centrifuge tube. Once the solvent had evaporated, the TCS007 organic phase extract was obtained. It was then diluted with sterile water to a concentration of 50 mg/mL for subsequent use.

Experimental Design and Seedlings Inoculation with *T. asperellum* TCS007

The pot experiment was conducted using a single-factor completely randomized block design with a total of five treatments (Table 1). Each treatment consisted of 20 seedlings as replicates, and the treatments were initiated subsequent to the seedlings that had been acclimatized over a period of 30 days. Each treatment was applied three times, with a 30-day interval between successive applications.

Table 1
Treatments used in this study

Treatment	Test agent	Treatment concentration	Treatment method
A	sterile water	-	Root irrigation was performed with a volume of 50 mL
B	sterilized PDB	-	
C	TCS007 spore suspension	1.0×10^8 spores/mL	
D	TCS007 spore suspension	1.0×10^6 spores/mL	
E	TCS007 liquid fermentation crude extract	50 mg/mL	

Determination of Physiological and Biochemical Indices of Seedlings

Three months after treatment, ten seedlings were randomly selected from each treatment for further analysis. Plant height and ground diameter were measured using a millimeter scale ruler. Chlorophyll content was determined by UV-2801 spectrophotometry (Hitachi, Ltd., Japan.) (Pan et al. 2017). Leaf IAA and GA content were quantified using enzyme-linked immunosorbent assay kits, employing a double-antibody sandwich method (Chen et al. 2011). The activities of Superoxide dismutase (SOD) and Peroxidase (POD) enzymes were determined using the nitroblue tetrazolium method and the guaiacol method, respectively (Shahzad et al. 2018). With each treatment group replicated ten times.

Determination of soil physicochemical properties, enzyme activities, and microbial counts

During the leaf expansion stage (mid-April), the new shoot growth stage (mid-July), and the defoliation stage (early October) of the pecan seedlings, rhizosphere soil samples from each treatment group were collected. The 20 pecan seedlings in a treatment were randomly divided into 5 groups. For each seedling, 12.5 g of soil sample was taken from the region rich in fine roots approximately 1 mm in diameter in the soil layer at a depth of 10–30 cm (Zhang et al. 2021). The soil samples taken from the four seedlings

assigned in the same group in one treatment were mixed thoroughly to form a single soil sample. In total, five soil samples as five replicates were collected for each treatment for subsequent processing. The soil samples were air-dried at 25°C, passed through a 1 mm sieve, collected in sterile sample bags, and then stored at 4°C for future use.

The content of organic matter was determined using the dichromate volumetric method (Zhang and Fen 2023). The ammonium nitrogen content was determined using the titration method (Li et al. 2021b). The readily available phosphorus content was determined using the extraction-antimony molybdate colorimetric method (Li et al. 2021a). The content of ammonium nitrogen was determined using the titration method (Cheng, 2014). The available potassium content was determined using the atomic absorption spectrophotometer with a flame photometer method (Guo et al. 2012). The soil pH value was determined using the electrode method (Fu et al. 2012). With each treatment group replicated five times.

Enzyme activities of soil catalase, urease, and β-glucosidase were measured using assay kits from Beijing Boxbio Science & Technology Co., Ltd., following the manufacturer's instructions. Catalase activity was assessed by monitoring the decomposition of H_2O_2, which reduced absorbance at 240 nm. One unit of catalase activity was defined as the amount of enzyme degrading 1 mmol H_2O_2 per gram of air-dried soil. Urease activity was determined by the formation of indophenol blue (absorbance at 630 nm) from NH_3-N generated by urea hydrolysis. One unit of urease activity corresponded to the production of 1 μg NH_3-N per gram of soil. β-Glucosidase activity was measured by the hydrolysis of p-nitrophenyl-β-D-glucopyranoside to p-nitrophenol, with absorbance at 400 nm. One unit of β-glucosidase activity was defined as the amount of enzyme generating 1 μmol p-nitrophenol per gram of soil. With each treatment group replicated five times.

The numbers of bacteria, fungi, and actinomycetes in the soil samples from each treatment group were determined using the dilution plating method. Briefly, in a 250 mL Erlenmeyer flask, 50 mL of sterile water was added. Then, 5 g of fresh soil sample was introduced into the flask and agitated at room temperature for 10 min. Subsequently, 1 mL of the soil suspension was transferred to 9 mL of sterile water, and the resulting suspension was serially diluted by a factor of 10 up to 10^{-6}. Based on preliminary experiments, for this trial, fungi were diluted between 10^{-3} and 10^{-1}, while bacteria and actinomycetes were diluted between 10^{-5} and 10^{-3}. An aliquot of 50 μL of the diluted solution was spread evenly onto the culture medium with each dilution repeated three times (Li and He 2005). Tryptone Soya Broth agar, Martin medium, and Modified Gause's No. 1 medium (Li et al. 1996) were utilized for the isolation and enumeration of bacteria, fungi, and actinomycetes, respectively. With each treatment group replicated five times.

Construction of Internal Transcribed Spacer (ITS) and 16S rRNA Gene Libraries and Subsequent High-Throughput Sequencing Analysis

During the leaf expansion stage of the pecan seedlings, soil microbial DNA was extracted from the five soil samples in each treatment group. The quality of the extracted DNA was assessed using 1.5%

agarose gel electrophoresis and a spectrophotometer. The V3-V4 region of the bacterial 16S rRNA gene and the ITS1 region of the fungal ITS sequence were amplified using the universal primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), as well as ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'). PCR amplification was conducted in a 20 μL reaction system using TransStart FastPfu DNA Polymerase and TaKaRa rTaq DNA Polymerase. After amplification, the PCR products were recovered through purification, detection, and quantification steps. Subsequently, the amplified products were mixed in equimolar concentrations to form a single sequencing library. The constructed library was quality checked, and qualified libraries were sequenced using the Illumina MiSeq sequencing platform (PE300).

FLASH v1.2.7 software was used to assemble reads from each sample based on overlaps, yielding raw Tag data (Magoč et al. 2011). Trimmomatic v0.33 software was employed to filter the raw Tag data, resulting in high-quality Tag data (Bolger et al. 2014). UCHIME v4.2 software was utilized to identify and remove chimeric sequences, obtaining the final data.

For soil microbial genomic data, Operational Taxonomic Units (OTUs) clustering analysis was conducted using USEARCH software. Biostatistical analysis on OTUs at 97% similarity level was performed (Edgar et al. 2013). The clustered OTUs were then used for various analyses: alpha diversity analysis (ACE index, Chao1 index, Shannon index, Simpson index, and Coverage index), beta diversity analysis, and species composition and difference analysis. Alpha diversity indices were calculated under different random samplings using Mothur software, while beta diversity analysis was conducted with QIIME software. Non-metric multidimensional scaling (NMDS) analysis and heatmap analysis were executed using R software, and the ANOSIM test was applied for statistical comparisons. All tables were prepared with Excel 2021 software, and the analyses were performed on the Majorbio Cloud Platform (www.majorbio.com) in Shanghai.

Statistical Analysis and Data Interpretation

Physiological and biochemical data of plants and soil were statistically analyzed using Excel 2021 software. To determine differences in physiological and biochemical indices of pecan seedlings, soil physicochemical properties, and soil enzymatic activities, one-way analysis of variance (ANOVA) and Duncan's test within IBM SPSS 22.0 software (IBM Corporation, New York, NY, USA) were employed.

Results

The Impact of TCS007 on the Physiological and Biochemical Indicators of Pecan Plant

Plant height, chlorophyll content, and ground diameter were measured for each treatment group. Compared to the control group A, treatment group C showed a height increase of 31.8%, a chlorophyll content increase of 225.8%, and a ground diameter increase of 329.6%. Treatment group E exhibited increases of 19.3%, 56.3%, and 29.2%, respectively, in height, chlorophyll content, and groun d diameter, while treatment group B showed no significant differences in all three indicators. The experimental

results indicated that both the TCS007 spore suspension and crude extract promoted the growth of pecan plants, with the optimal growth-promoting effect observed at a concentration of 1.0×10^8 spores/mL for the TCS007 spore suspension (Table 2).

Table 2
The impact of *Trichoderma* inoculation on seedling biomass

Treatment groups	Plant Height (cm)	Ground diameter (cm)	Chlorophyll content ($mg \cdot g^{-1}$)
A	6.99 ± 0.19 c	0.27 ± 0.36 b	6.28 ± 0.19 d
B	7.28 ± 0.24 c	0.35 ± 0.82 b	6.94 ± 0.07 d
C	9.21 ± 0.23 a	1.16 ± 0.84 a	20.46 ± 0.96 a
D	8.71 ± 0.40 ab	1.02 ± 0.89 a	17.38 ± 1.03 b
E	8.34 ± 0.29 b	0.35 ± 0.40 e	9.82 ± 0.64 c

Note: Different letters in the columns indicate significant differences ($p < 0.05$), according to Duncan's new multiple range test.

In terms of phytohormone content, the C treatment group showed an increase of 60.0% in IAA and 60.8% in GA in the leaf tissues, compared to the A treatment group. The E treatment group exhibited increases of 26.2% in IAA and 31.4% in GA, respectively (Fig. 1a-b). Regarding defense enzymes, the C treatment group had a 53.5% increase in superoxide dismutase (SOD) and a 140.0% increase in peroxidase (POD) enzyme activities in the leaf tissues. The treatment of group D showed even higher increases by 69.5% and 166.1% for SOD and POD, respectively (Fig. 1c-d). The B treatment group did not exhibit significant differences in either phytohormone content or defense enzyme activities. The experimental results suggested that both the TCS007 spore suspension and crude extract could enhance hormone content and defense enzyme activities in the leaf tissues of pecan plants, with the most pronounced effects observed at a TCS007 spore suspension concentration of 1.0×10^8 spores/mL.

The Impact of TCS007 on the Physicochemical Properties of Soil in the Rhizosphere of pecan

During the leaf expansion, shoot growth, and defoliation stages of pecan seedlings, the organic matter content in the treatment of group C increased by 19.4%, 17.0%, and 10.9%, respectively, the ammonium nitrogen content increased by 20.0%, 21.3%, and 10.3%, respectively, and the available phosphorus content increased by 23.7%, 16.7%, and 17.7%, respectively. The soil pH values were elevated to 7.53, 6.96, and 6.76, respectively. In the treatment of group D, the organic matter content increased by 12.7%, 13.2%, and 6.3%, the ammonium nitrogen content increased by 17.3%, 15.3%, and 8.0%, and the available phosphorus content increased by 13.7%, 14.4%, and 15.2%, with soil pH values elevated to 7.32, 6.84, and 6.62, respectively. However, there were no significant differences in available potassium (Table 3). The experimental results indicated that the TCS007 spore suspension significantly increased the organic matter content, ammonium nitrogen content, available phosphorus content, and soil pH in the rhizosphere soil of pecan, while the crude extract treatment group showed no significant differences.

Table 3
The influence of different treatments on the physicochemical properties of the rhizosphere soil during various growth stages of pecan

Pecan growth stages	Treatment groups	Organic matter content/%	Ammonium Nitrogen /mg·kg^{-1}	Available Phosphorus /mg·k^{-1}	Available Potassium /mg·kg^{-1}	pH value
Leaf expansion stage	A	2.48 ± 0.73 c	44.57 ± 2.38 b	22.92 ± 2.19 c	54.63 ± 3.25 a	6.32 ± 5.36 b
	B	2.63 ± 1.83 b	46.76 ± 2.03 b	23.33 ± 0.97 c	53.96 ± 1.64 a	6.35 ± 0.89 b
	C	2.96 ± 0.82 a	53.49 ± 1.97 a	28.36 ± 1.87 a	54.38 ± 2.33 a	7.53 ± 3.28 a
	D	2.84 ± 0.62 a	52.28 ± 1.23 a	26.06 ± 0.93 b	54.20 ± 1.47 a	7.32 ± 2.08 a
	E	2.52 ± 1.32 c	43.66 ± 1.28 b	22.67 ± 1.65 c	54.32 ± 1.48 a	6.49 ± 1.38 b
Shoot growth stage	A	2.42 ± 1.48 b	45.23 ± 0.77 b	23.64 ± 1.93 b	55.49 ± 2.39 a	6.44 ± 2.01 b
	B	2.45 ± 0.56 b	46.18 ± 1.63 b	24.11 ± 0.78 b	55.29 ± 1.47 a	6.37 ± 1.84 b
	C	2.83 ± 1.04 a	54.88 ± 2.31 a	27.58 ± 1.27 a	54.18 ± 0.48 a	6.96 ± 0.49 a
	D	2.74 ± 1.23 a	52.16 ± 1.71 a	27.05 ± 0.85 a	53.26 ± 1.39 a	6.84 ± 1.63 a
	E	2.43 ± 0.73 b	45.87 ± 1.02 b	23.17 ± 1.38 b	52.93 ± 3.19 a	6.53 ±

Note: Different letters in the columns indicate significant differences ($p < 0.05$), according to Duncan's new multiple range test.

Pecan growth stages	Treatment groups	Organic matter content/%	Ammonium Nitrogen /mg·kg^{-1}	Available Phosphorus /mg·k^{-1}	Available Potassium /mg·kg^{-1}	pH value
						0.76 b
Defoliation stage	A	2.39 ± 1.76 b	42.33 ± 1.74 b	21.95 ± 1.47 b	50.88 ± 2.38 a	6.27 ± 1.68 b
	B	2.41 ± 1.27 b	42.89 ± 2.18 b	22.16 ± 1.29 b	48.87 ± 0.38 a	6.33 ± 3.94 b
	C	2.65 ± 0.93 a	46.71 ± 0.87 a	25.83 ± 0.48 a	49.73 ± 1.20 a	6.76 ± 2.06 a
	D	2.54 ± 1.32 a	45.71 ± 0.97 a	25.29 ± 1.94 a	49.15 ± 0.68 a	6.62 ± 1.83 a
	E	2.31 ± 1.76 b	41.97 ± 1.22 b	21.37 ± 0.99 b	51.28 ± 0.77 a	6.19 ± 6.93 b

Note: Different letters in the columns indicate significant differences ($p < 0.05$), according to Duncan's new multiple range test.

The Impact of TCS007 on the Enzymatic Activity of Rhizosphere Soil in Pecan

During the leaf expansion stage of the pecan, the activities of soil catalase, urease, and β-glucosidase in the C treatment group significantly increased by 55.2%, 78.4%, and 62.0%, respectively, compared to the A treatment group. The E treatment group also showed increases in soil enzyme activities of 32.8%, 22.4%, and 96.3%. During the shoot growth stage, the C treatment group showed an increase in soil enzyme activities of 46.1%, 37.6%, and 44.1%, while the E treatment group's increases were 36.5%, 9.0%, and 65.8%. By the time of defoliation stage, the C treatment group's soil enzyme activities had further increased by 30.5%, 20.8%, and 23.2%, the E treatment group's activities increased the soil enzymatic activity by 25.5%, 15.9%, and 22.7%, and the B treatment group showed no significant difference. The experimental results indicated that the spore suspension and crude extract of TCS007 could effectively enhance soil enzyme activity, with the crude extract showing the most pronounced effect (Fig. 2).

The Influence of TCS007 on the Culturable Microbial Population in the Rhizosphere Soil of Pecan

During the leaf expansion stage, compared to the A treatment group, the microbial population in the rhizosphere soil of the C treatment group significantly increased, while in the E treatment group, the

number of fungi decreased by 69.2%, bacteria by 57.8%, and actinomycetes by 66.7%. During the shoot growth stage, compared to the A treatment group, the microbial population in the rhizosphere soil of the C treatment group significantly increased again, with a 49.6% reduction in fungal numbers, a 21.1% increase in bacterial numbers, and a significant 79.3% decrease in actinomycetes in the E treatment group. In the defoliation stage, compared to the A treatment group, the number of fungi and bacteria in the rhizosphere soil of the C treatment group significantly increased, while the actinomycetes decreased by 7.5%. In the E treatment group, the numbers of fungi and actinomycetes decreased by 39.2% and 16.4%. Respectively. In the B treatment group, the numbers of fungi and bacteria only significantly increased during the leaf expansion stage, with no significant changes in the microbial population in the rhizosphere soil during other stages. The experimental results indicated that during the growth process of pecan plants, the number of fungi in the rhizosphere soil first decreased and then increased after treatment with TCS007 spore suspension (Fig. 3a), bacteria decreased and then stabilized (Fig. 3b), and actinomycetes significantly decreased (Fig. 3c). After treatment with TCS007 crude extract, the number of fungi in the rhizosphere soil significantly decreased (Fig. 3a), the number of bacteria significantly increased after the leaf expansion stage (Fig. 3b), and the number of actinomycetes did not significantly change (Fig. 3c). The spore suspension and crude extract of TCS007 both significantly affected the number of soil microorganisms, with the spore suspension of TCS007 having the most pronounced effect (Fig. 3).

The Influence of TCS007 on the Microbial Composition of the Rhizosphere Soil in Pecan

Microbial Sequencing Data Analysis

The statistics of the sequencing data after quality control, filtering, and assembly for the five treatment groups are presented in Table 4. The effective bacterial sequences were 28089, 21237, 26931, 25779, and 24914; and the effective fungal sequences were 49472, 49101, 45903, 39691, and 42696. The sequencing quality, measured by the Q30 score, ranged from 97–99%, while the Q20 score ranged from 99–99.5% (Table 4).

Table 4
Summary of basic sequencing data for each sample

Microorganisms	Treatment	Shortest tags	Longest tags	Mean tags	Effective tags	Total bases
Bacteria	A	301	504	419	28089	21438243
	B	317	527	417	21237	21312593
	C	317	497	418	26931	21395076
	D	319	456	419	25779	19405297
	E	270	469	418	24914	19781631
Fungi	A	141	526	237	49472	12587811
	B	141	533	232	49101	12441487
	C	142	384	245	45903	12569760
	D	143	522	237	39691	10209814
	E	142	514	236	42696	11074245

Microbial Community Diversity Analysis

Alpha diversity indices analysis revealed that the coverage indices for all treatment groups were close to 1, indicating that the sequencing results could accurately reflect the actual conditions of the soil samples tested. The ACE and Chao indices were indicative of the richness of soil microbial communities, while the Simpson and Shannon indices reflected the diversity of these communities. Among bacterial samples, the analysis of Chao1 and ACE indices across treatment groups revealed that the bacterial community richness in treatment groups C and D was significantly higher than in groups A and B ($p < 0.05$), with the bacterial community richness in pecan rhizosphere soil increasing as the concentration of TCS007 spores increased. Analysis of the Simpson and Shannon indices showed that the bacterial community diversity in treatment group C was significantly higher than in other groups ($p < 0.05$), with no significant difference in diversity between groups B and D compared to group A. After treatment with group E, the bacterial community richness and diversity in the pecan rhizosphere were significantly reduced compared to other treatment groups ($p < 0.05$). In the case of fungal samples, the analysis of Chao1 and ACE indices demonstrated that the richness of fungal communities in treatment groups C and D was significantly higher than in other groups ($p < 0.05$). Analysis of the Shannon and Simpson indices indicated that the fungal community diversity in groups C and D was significantly higher than in group A ($p < 0.05$). However, the richness and diversity of fungal communities in group E were significantly lower than in other treatment groups ($p < 0.05$) (Table 5).

Table 5
The impact of various treatments on the diversity indices of the microbial community

Microorganisms	Treatment	Shannon Index	Simpson Index	Chao Index	ACE Index	Coverage %
Bacteria	A	6.79 ± 0.02a	0.23 ± 0.01b	1579.79 ± 0.40b	1591.28 ± 0.34c	0.99 ± 0.01a
	B	6.79 ± 0.01a	0.24 ± 0.15b	1634.00 ± 0.14b	1666.65 ± 0.36b	0.99 ± 0.01a
	C	6.83 ± 0.02a	0.59 ± 0.03a	1708.34 ± 0.36a	1725.41 ± 0.56a	1.00 ± 0.00a
	D	6.80 ± 0.11a	0.24 ± 0.01b	1699.44 ± 0.28a	1714.37 ± 0.19a	0.99 ± 0.01a
	E	6.49 ± 0.19b	0.20 ± 0.01c	1315.56 ± 0.60c	1337.70 ± 0.20d	0.99 ± 0.01a
Fungi	A	4.35 ± 0.01c	0.07 ± 0.01c	515.00 ± 0.48d	515.00 ± 0.18b	1.00 ± 0.00a
	B	4.42 ± 0.02b	0.53 ± 0.03a	551.67 ± 0.44c	553.87 ± 0.43b	1.00 ± 0.00a
	C	4.75 ± 0.05a	0.12 ± 0.01b	630.91 ± 0.08a	632.87 ± 0.19a	1.00 ± 0.00a
	D	4.73 ± 0.03a	0.12 ± 0.01b	568.41 ± 0.18b	569.46 ± 0.57a	1.00 ± 0.00a
	E	3.14 ± 0.03d	0.05 ± 0.02c	414.17e	416.38 ± 0.35c	1.00 ± 0.00a

Note: Different letters in the columns indicate significant differences ($p < 0.05$), according to Duncan's new multiple range test.

Microbial Community Compositional Differences Analysis

Beta diversity analysis revealed that compared to the treatment group A, the soil bacterial community compositions of the other treatment groups showed significant differences, with the most pronounced difference observed between treatment group C and group A, while treatment groups D and E exhibited a higher similarity in their bacterial community compositions (Fig. 4a). In comparison to treatment group A, the soil fungal community compositions of treatment groups C and D were significantly different, whereas the fungal community composition of treatment group E showed a higher similarity to that of treatment group A (Fig. 4b).

The analysis of soil microbial community compositional differences revealed that the structure of the community in different samples was known at various taxonomic levels, such as domain, kingdom, phylum, class, order, family, genus, and species. The bacterial community in the rhizosphere soil of pecan was primarily composed of the phyla *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, and

Chloroflexi, with *Actinobacteria*, *Proteobacteria*, and *Acidobacteria* being the dominant. Compared to treatment A, treatment C increased the abundance of *Actinobacteria* by 36.19% and significantly increased the abundance of *Firmicutes* by 301.57%. In contrast, the abundance of *Acidobacteria* and *Chloroflexi* in the rhizosphere soil bacterial community decreased by 45.49% and 34.92%, respectively (Fig. 5a). Additionally, treatment C reduced the abundance of the *Acidobacteriaceae* and *RB41* genera in the bacterial community by 46.25% and 61.03%, respectively, while the abundance of the genera *Nocardioides*, *Bacillus*, *Micrococcaceae*, and *Agromyces* increased, with *Bacillus* showing a significant increase of 377.65%. However, treatments B, D, and E did not show significant differences in the abundance of the bacterial community in the rhizosphere soil compared to treatment A (Fig. 5b, Fig. 6).

The fungal community in the rhizosphere soil of pecan was predominantly composed of the phyla *Ascomycota*, *Mortierellomycota*, and *Basidiomycota*, with *Ascomycota* and *Mortierellomycota* being the dominant. Compared to the A treatment group, the abundance of *Ascomycota* in the C treatment group increased by 23.72%, while the abundance of *Mortierellomycota* and *Basidiomycota* decreased by 41.18% and 21.87%, respectively (Fig. 7a). Furthermore, the abundance of the genera *Trichoderma* and *Apiotrichum* in the C treatment group significantly increased, while other fungi were generally inhibited. Notably, the genera *Mortierella*, *Neocosmospora*, *Fusarium*, *Chaetomium*, *Talaromyces*, *Aspergillus*, *Cladosporium*, and *Acremonium* showed the most pronounced inhibition. Specifically, the abundance of *Mortierella*, *Neocosmospora*, *Fusarium*, and *Cladosporium* decreased by 41.10%, 45.77%, 55.71%, and 46.15%, respectively. However, the B, D, and E treatment groups showed no significant differences (Figs. 7b and 8).

Discussion

As a commonly used biocontrol agent, *Trichoderma* species exhibit a range of efficacy. Upon application, *Trichoderma* played a crucial role in the rhizosphere by competing against other microbes, particularly plant pathogens (Ferreira and Musumeci 2021). This competition was multifaceted and included direct antagonism, nutrient and space competition, and the production of antifungal compounds (Manzar et al 2022). Additionally, *Trichoderma* could outcompete pathogens for nutrients and space by rapidly colonizing plant roots and the surrounding soil (Tyśkiewicz et al. 2022). This competition not only suppressed the growth of pathogens but also promoted plant health by enhancing nutrient uptake and inducing systemic resistance in plants.(Dutta et al. 2022). Then, it produces plant growth hormones that enhance the solubility of nutrients in the soil, improves rhizosphere microecology, and thereby facilitates nutrient uptake by plants, promoting growth and increasing yield (Contreras-Cornejo et al. 2024; Li et al. 2024; Wei et al. 2024). Additionally, *Trichoderma* induces the production of defense enzymes in plants, such as peroxidase (POD) and superoxide dismutase (SOD), which play a crucial role in the plant resistance to stress and diseases (Ahmad et al. 2015; Zhang et al. 2016; Pacheco-Trejo et al. 2022). *Trichoderma* elicits plant immune responses through multiple mechanisms. It secretes microbe-associated molecular patterns (MAMPs) recognized by plant cell surface pattern recognition receptors (PRRs), triggering basal immunity (Alfiky and Weisskopf 2021). Our study revealed that the TCS007 spore suspension and crude extracts significantly promoted the growth of pecan seedlings and

enhanced chlorophyll content in leaves. Concurrently, there was a notable increase in the levels of plant hormones (IAA, GA) and the activity of defense enzymes (POD, SOD) in the leaves, with the optimal effect observed at a spore suspension concentration of 1×10^8 spores/mL. Studies showed that *Trichoderma* strains isolated from various regions could secrete IAA, enhancing the photosynthetic rate and promoting the growth of plants such as cucumber and corn (Vinale et al. 2008a; Vargas et al. 2009). Zhang et al. (2021) found that *Trichoderma harzianum* significantly increased the plant height, basal diameter, and fresh weight of *Malus hupehensis* Rehd. Moreover, *Trichoderma* species were capable of producing a variety of secondary metabolites, including 6-pentyl-2H-pyran-2-one (6PP), Koninginin A5, gliotoxin, and viridin, which played significant signaling roles in plant-microbe interactions (Forde et al. 2014; Garnica-Vergara et al. 2015; Moisan et al. 2021). It was demonstrated that *Trichoderma atroviride* could produce at least 25 types of volatile organic compounds, encompassing alcohols, ketones, alkanes, ethylene, monoterpenes, and sesquiterpenes, among which gaseous ethylene acted as a core signaling molecule in *Trichoderma*-plant interactions (Lee et al. 2016; Estrada-Rivera et al. 2019). Vinale et al. (2008b) observed growth promotion in etiolated pea stems treated with the main secondary metabolites produced by different *Trichoderma* strains, harzianic acid, and 6PP.

Soil nutrients, often existing in sparingly soluble or insoluble forms, impeded nutrient cycling, highlighting the importance of enhancing nutrient availability. *Trichoderma* species, through the secretion of organic acids, activated soil nutrients and promoted plant absorption, playing a crucial role in rehabilitating degraded soils (Sindhu et al. 2022; Singh et al. 2024). Our study demonstrated that TCS007 spore suspension significantly increased organic matter, ammonium nitrogen, available phosphorus content, and pH value in the rhizosphere soil of pecan, with the optimal effect at a concentration of 1×10^8 spores/mL. Asghar and Kataoka. (2021) found that soil phosphatase activity had increased by more than 10% after inoculation with *Trichoderma* RW309 for two months, accelerating the transformation of organic phosphorus compounds or inorganic phosphates in the soil. The physiological metabolism of different microbial communities was closely related to the production of soil enzymes, which could reflect the presence and activity of corresponding functional microbes (Mao and Jiang. 2021; Jia et al. 2024; Aljeddani et al. 2024). *Trichoderma* species rapid colonization capability enhanced rhizosphere-soil contact, promoting the secretion of extracellular enzymes such as catalase, urease, and β-glucosidase, as well as organic acids (Solomon et al. 2024). Catalase served as an indicator of soil microecology, urease reflected the soil's nitrogen supply capacity, and β-glucosidase provided a carbon source for soil microbes (Woese et al. 1987; Stott et al. 2010; Su et al. 2016). Our results indicated that TCS007 spore suspension and crude extracts effectively enhanced soil enzyme activity, with the most significant effect observed at a concentration of 50 mg/L for the crude extracts. In a cabbage pot experiment, the treatment with *Trichoderma* increased soil transformation enzyme activity by 45%, effectively promoting nutrient transformation and improving the soil ecological environment (Shi et al. 2021).

Forest soil microbes played a pivotal role in ecosystems, encompassing soil formation, development, and the maintenance of ecological balance. The ratio of soil bacteria, fungi, and actinomycetes served

as a vital indicator of soil fertility, with their quantities showing a significant positive correlation with soil nutrients and crop yields (Hang et al. 2022). Increasing the populations of rhizosphere bacteria and actinomycetes aided in enhancing soil nitrogen and phosphorus content, promoting plant root development and nutrient absorption (Mäkipää et al. 2017; Xiong et al. 2017; Harsonowati et al. 2020; Chen et al. 2021). Soil microbial diversity and richness are considered critical for the integrity, function, and long-term sustainability of soil ecosystems. The latter are usually reduced by agricultural perturbations (Fu et al. 2019). Our study found that TCS007 spore suspension significantly increased the number of culturable bacteria and actinomycetes in the rhizosphere soil of pecan during the leaf expansion and shoot growth stage, particularly the beneficial microbes *Nocardia* and *Bacillus*, enhanced the abundance of bacterial community richness and bacterial community diversity in the soil. Concurrently, TCS007 spore suspension increased the abundance of *Trichoderma* and *Apiotrichum* fungi, suppressing the growth of plant pathogens such as *Fusarium*, *Colletotrichum*, and *Magnaporthe oryzae*, thus promoting the growth of beneficial fungal communities and inhibiting pathogenic fungi. Consequently treating soil with TCS007 spore suspension would altering the uniformity and richness of soil species, and altering the soil's microbial diversity. Fu et al. (2019) study indicated that *Trichoderma* treatment impacts bacterial diversity in the rhizosphere soil of maize. The diversity of the bacterial community in soil following *Trichoderma* treatment was lower than that in the control, untreated, soil. This might be associated with the fact that Trichoderma promotes plant growth and plant nutrient absorption from the soil. Hou et al. (2021) demonstrated that *Trichoderma harzianum* TH62 effectively inhibited soil-borne pathogens such as *Fusarium oxysporum* and *Alternaria alternata*. In addition, *T. harzianum* ESALQ-1306 and *T. asperellum* BRM-29104 had a minor impact on soil fungal abundance, they shifted the soil microbial community from fungi to bacteria, maintaining microbial balance and reducing crop diseases and pests (Cordier and Alabouvette. 2009; Zhang et al. 2019; Silva et al. 2021). These results indicated that TCS007 spore suspension could regulate soil microbial communities, enhancing soil fertility and crop health. Future research should focus on elucidating the specific mechanisms underlying the beneficial effects of *T. asperellum* TCS007 on pecan plants and their rhizosphere environment. Long-term field trials are needed to assess the sustained impact of TCS007 on soil health and plant growth under varying environmental conditions. Additionally, studies should explore the potential synergistic effects of TCS007 with other soil amendments or agricultural practices. Investigating the genetic and biochemical pathways involved in the interactions between TCS007 and pecan plants could provide valuable insights into optimizing its application as a biological fungal fertilizer.

Conclusions

In a pioneering effort, our research team has introduced the marine-derived strain *T. asperellum* TCS007 to the economically important tree species pecan. This study demonstrated that treatment with TCS007 spore suspension or crude extract promoted the growth of pecan seedlings, with significant increases in plant height, ground diameter, and chlorophyll content compared to the CK. Additionally, the contents of IAA and GA in the leaves, as well as the activities of SOD and POD enzymes, were significantly enhanced.

The best growth-promoting effect was observed when the TCS007 spore suspension was applied at a concentration of 1.0×10^8 spores/mL.

In addition, the levels of organic matter, ammonium nitrogen, and available phosphorus content in the rhizosphere soil, as well as the activities of soil catalase, urease, and β-glucosidase, were significantly higher in the treatment groups than those in the CK, and the soil pH shifted from slightly acidic to slightly alkaline after treatment. The soil microbial communities of pecan seedlings treated with TCS007 were analyzed during the leaf expansion stage, high-throughput sequencing revealed that the abundance of beneficial microbes, such as *Nocardia*, *Bacillus*, *Micrococcus*, and *Mycobacterium* in the bacterial community and *Trichoderma* and *Penicillium* in the fungal community, significantly increased, while the abundance of *Acidobacterium* and *RB41* in bacterial community and other fungal genera decreased in the group treated with TCS007 spore suspension.

In summary, the TCS007 spore suspension could effectively promote the growth and development of pecan plants, improved the stability of the rhizosphere soil microecology of pecan, and increased the content of soil nutrients. It had a positive effect on the prevention and control of soil-borne diseases in pecan and the regulation of soil pH. These results indicated that *T. asperellum* TCS007 had great potential in being developed into a biological fungal fertilizer product for pecan.

Declarations

Declarations

The authors have no relevant financial or non-financial interests to disclose.

Founding

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Figures

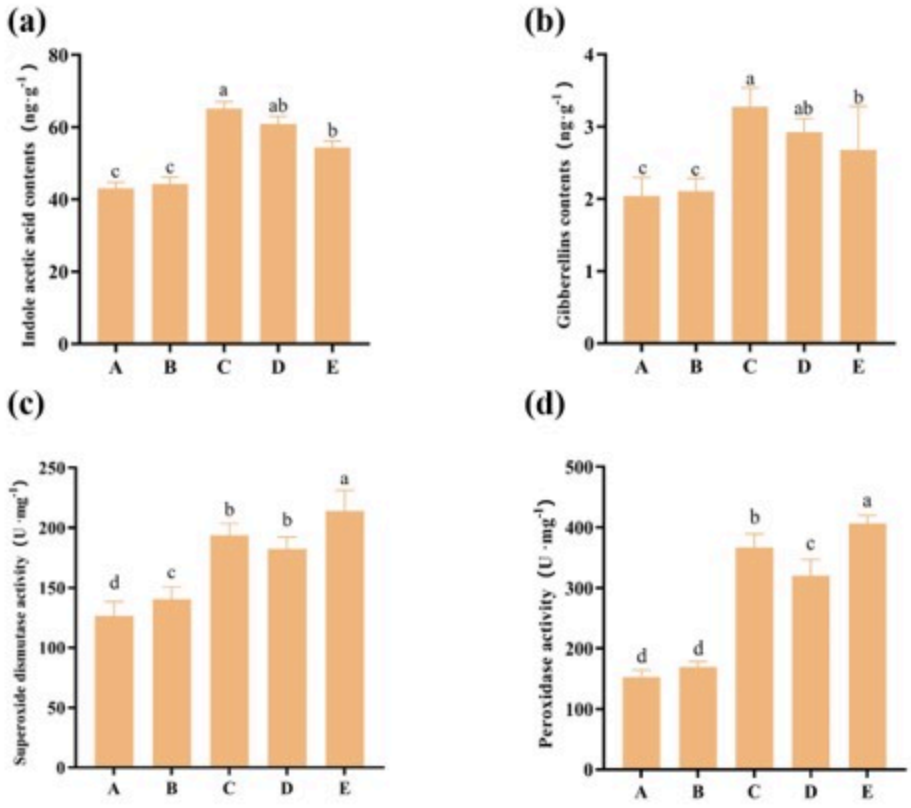

Figure 1

The impact of different treatments on hormone levels and defensive enzyme activities in pecan leaf tissues. (a): IAA content, (b): GA content, (c): SOD activity, (d): POD activity. Note: Different letters in the columns indicate significant differences ($p < 0.05$), according to Duncan's new multiple range test.

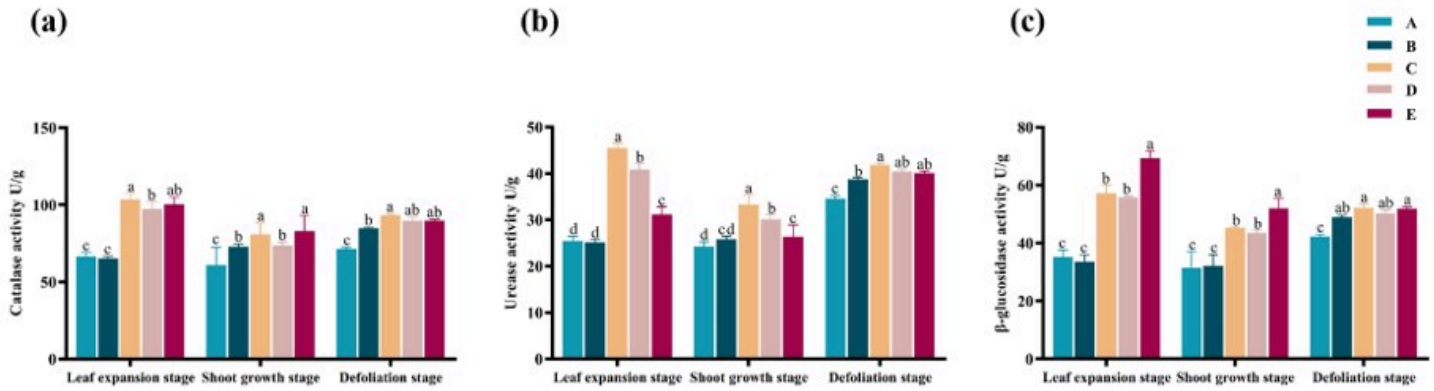

Figure 2

The effect of different treatments on the enzymatic activity in the rhizosphere soil of pecan. (a): Catalase activity, (b): Urease activity, (c): β-glucosidase activity. Note: Different letters in the columns indicate significant differences ($p < 0.05$), according to Duncan's new multiple range test.

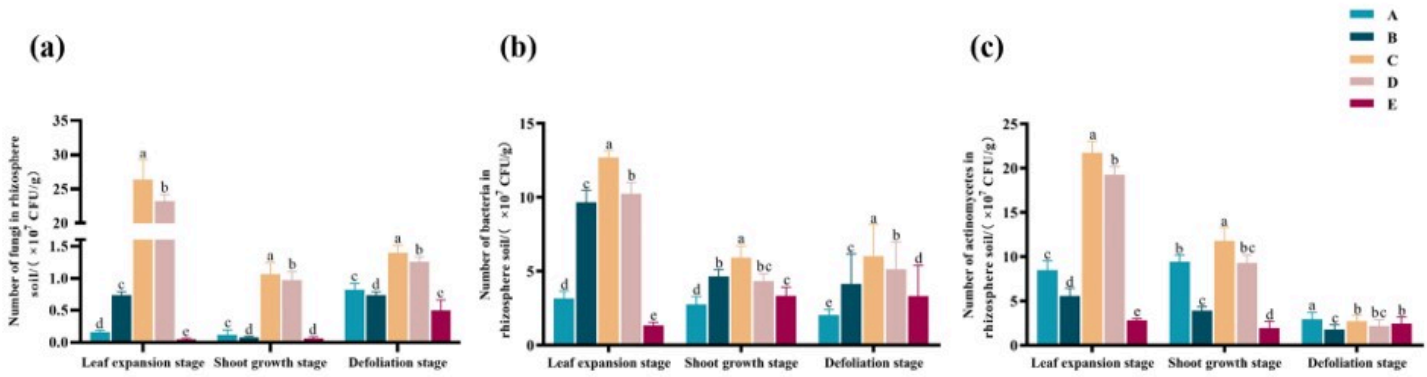

Figure 3

The impact of different treatments on the microbial abundance in the rhizosphere soil of pecan. (a): Number of fungi, (b): Number of bacteria, (c): Number of actinomycetes. Note: Different letters in the columns for each plant growth stage indicate significant differences ($p < 0.05$), according to Duncan's new multiple range test.

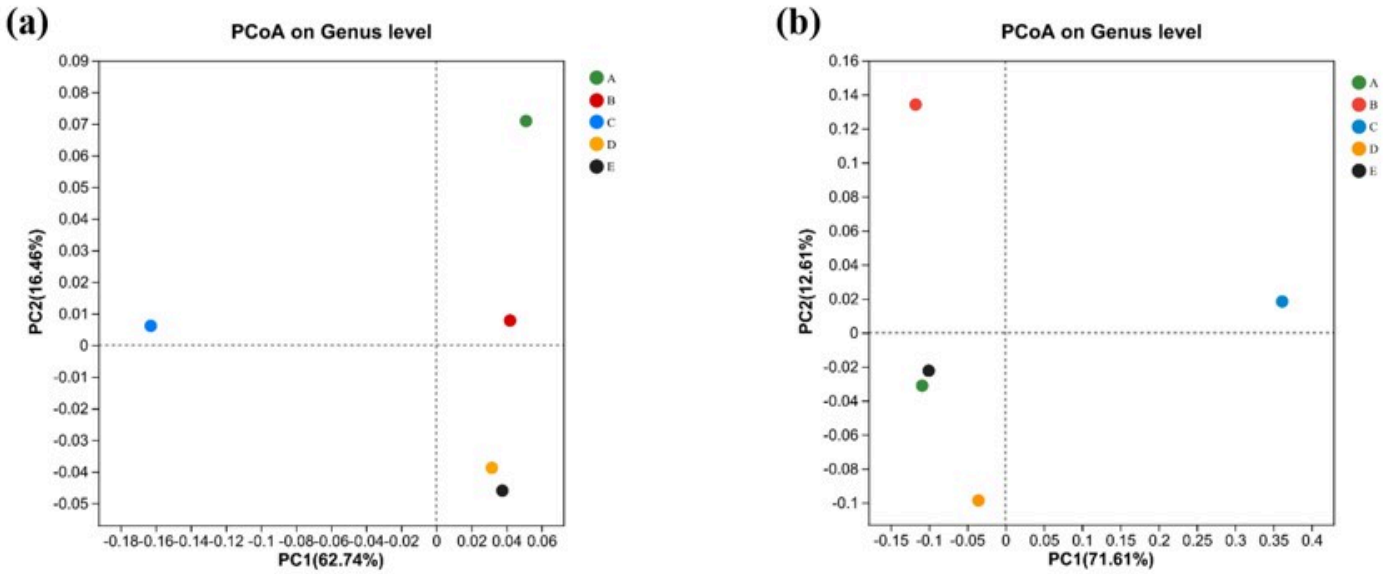

Figure 4

Principal coordinate analysis (PCoA) of soil bacterial (a) and fungal (b) communities in the rhizosphere soil samples of pecan from different treatment groups.

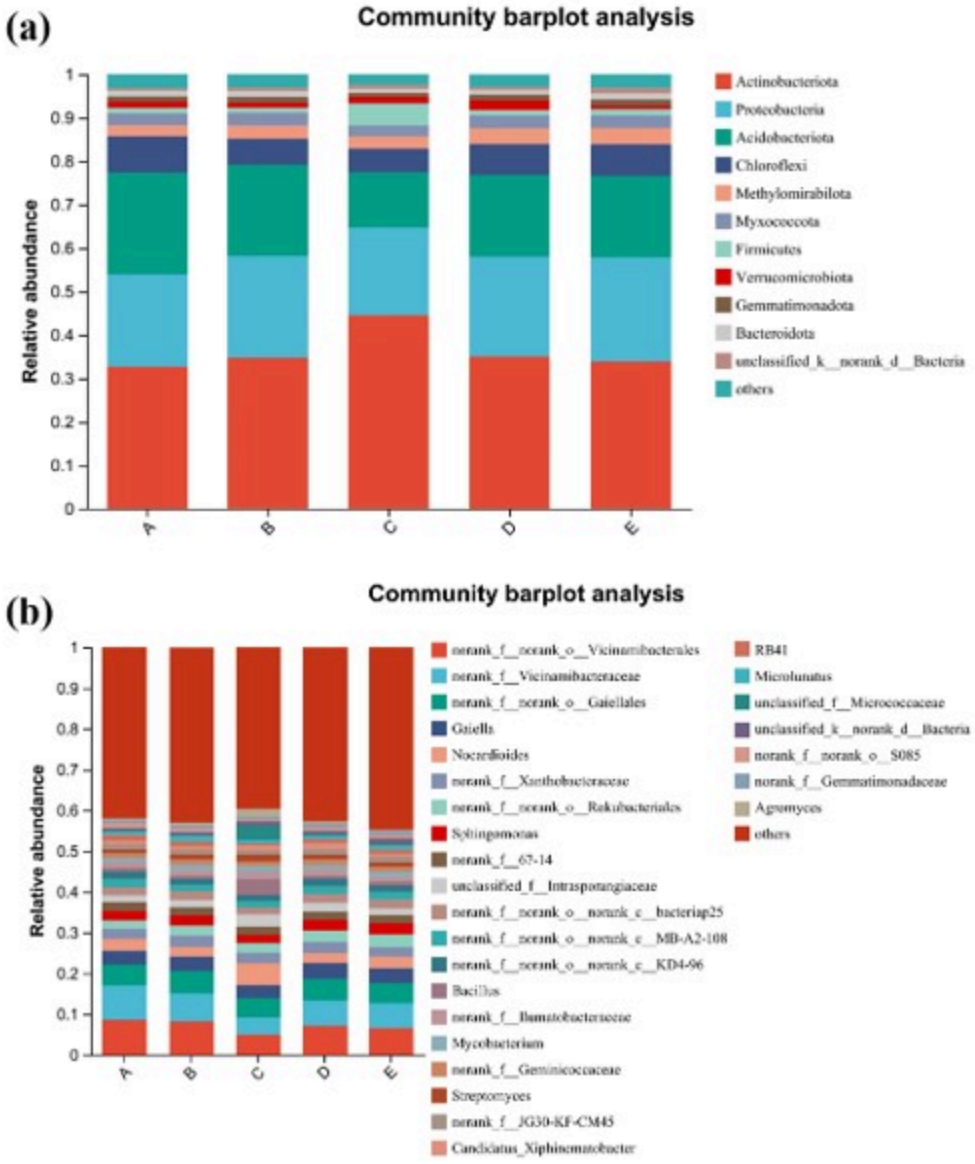

Figure 5

Distribution of bacterial community abundance at the phylum level (a) and genus level (b) in rhizosphere soil of different treatment groups.

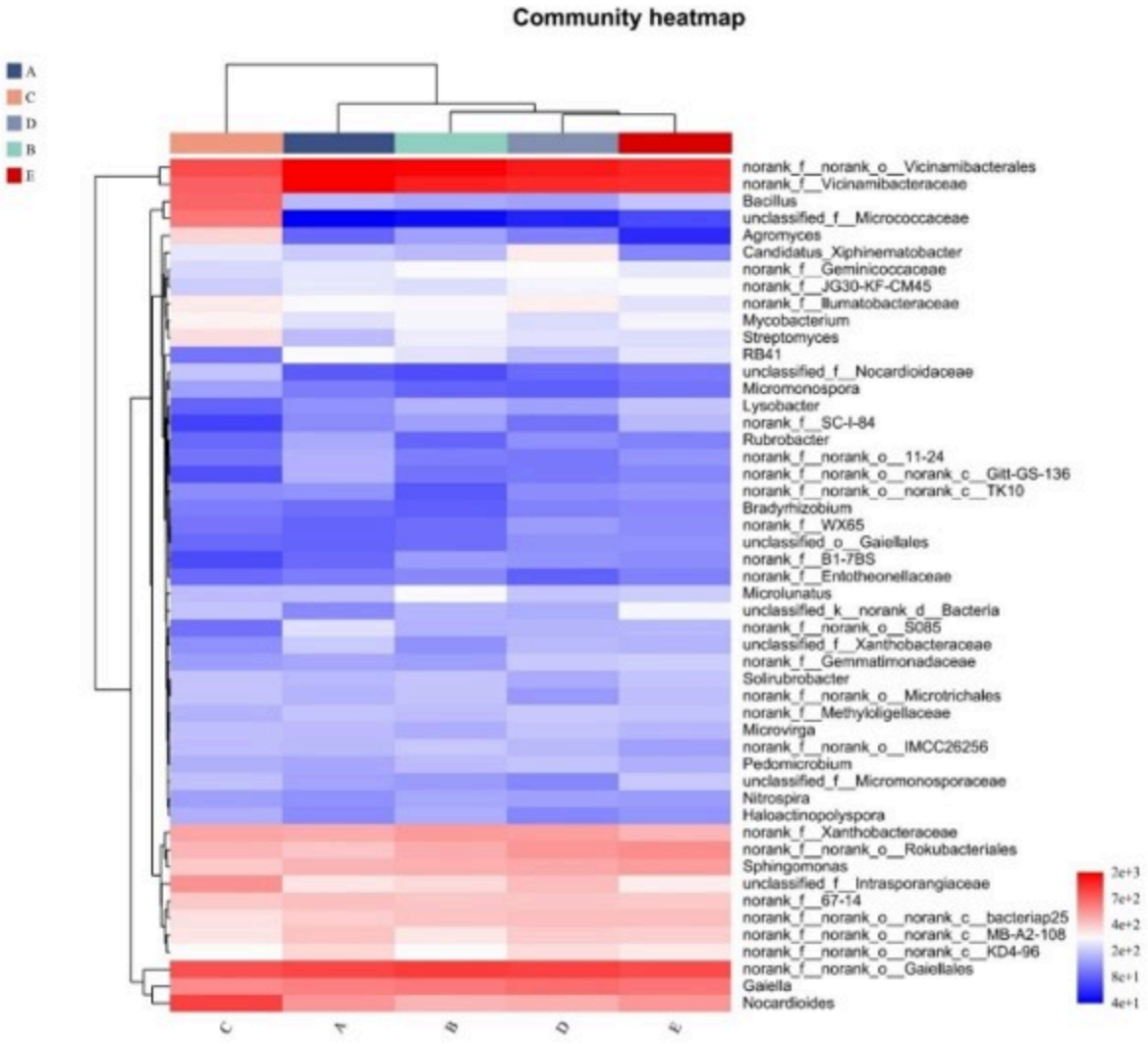

Figure 6

Cluster heatmap analysis of bacterial community abundance at the genus level in the rhizosphere soil of different treatment groups.

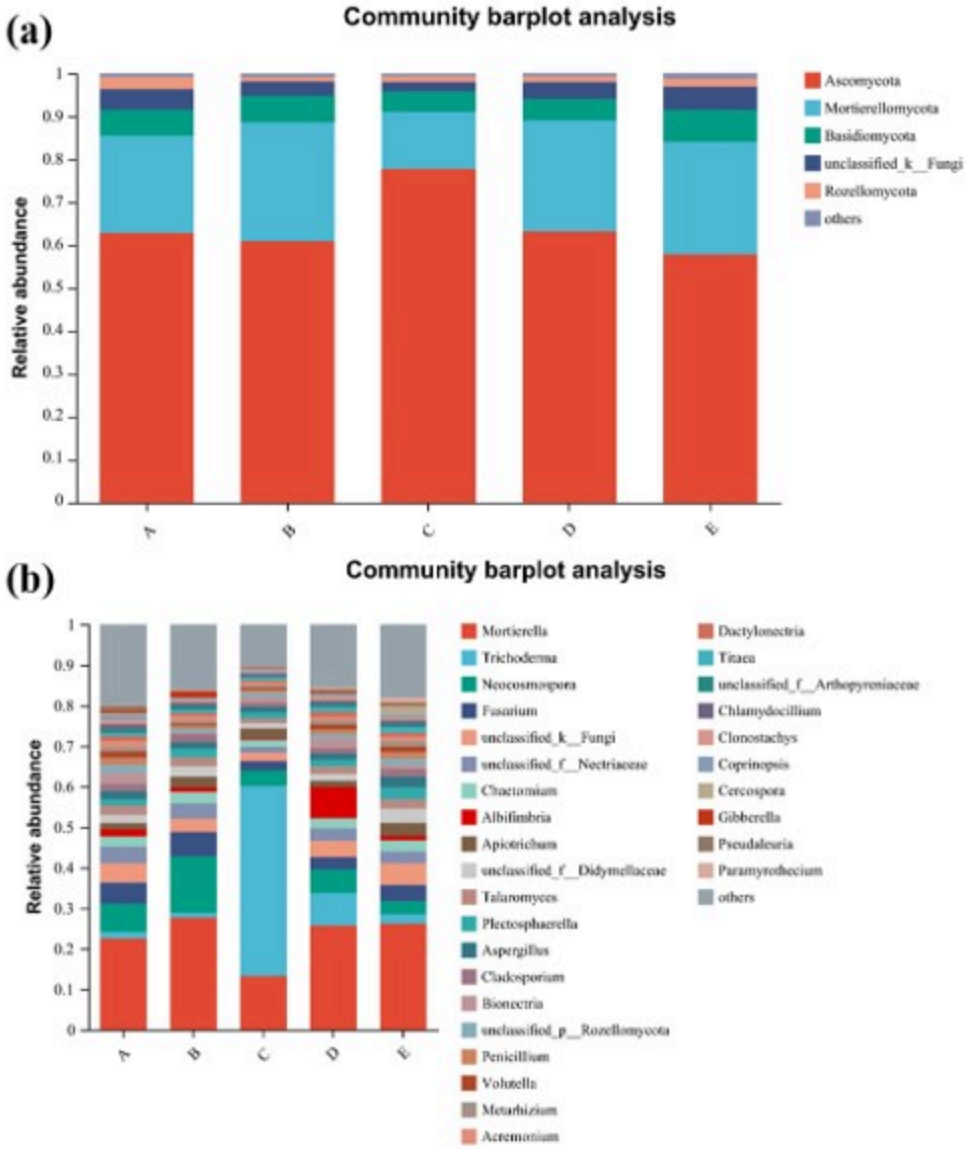

Figure 7

Distribution of fungal community abundance at the phylum level (A) and genus level (B) in the rhizosphere soil of different treatment groups.

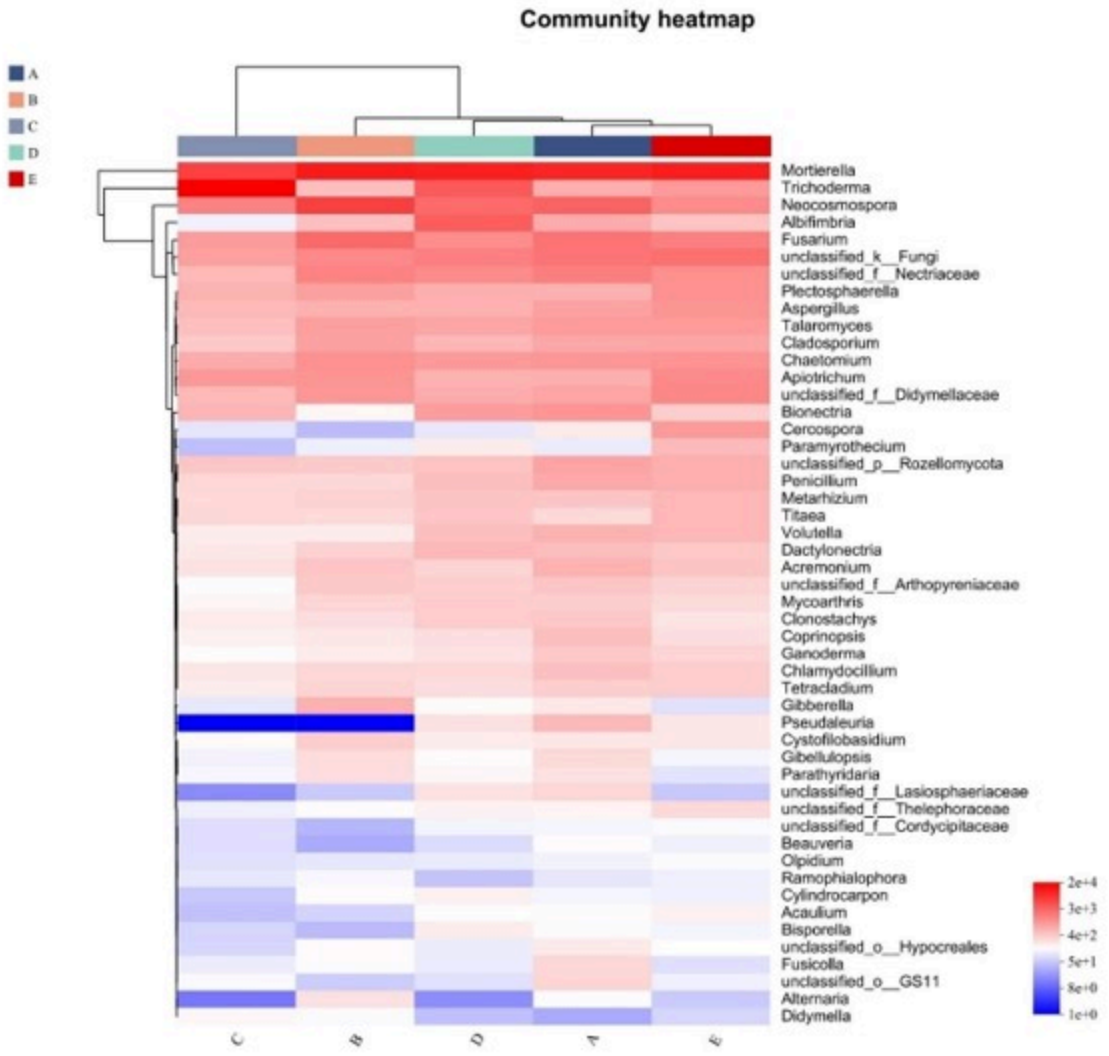

Figure 8

Cluster heatmap analysis of fungal community abundance at the genus level in the rhizosphere soil of different treatment groups.