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Effects of Plant Growth-Promoting Rhizobacteria on the Growth and Soil Microbial Community of *Carya illinoinensis*

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are important members of soil microbial communities. In this study, the effects of several PGPR on the growth of *Carya illinoinensis* plants, the microbial community composition and soil nutrients were investigated by inoculation tests to identify excellent PGPR strains. The experiment showed that after PGPR application, the plant height, ground diameter, and dry weight of *C. illinoinensis* were significantly increased compared with those of the control group, and *Bacillus velezensis* YH20 had the most significant effect in promoting growth ($p < 0.05$). In addition, all the PGPRs used for inoculation promoted plant root growth, and the *Brevibacillus reuszeri* MPT17 strain had the most significant promoting effect on plant root growth ($p < 0.05$). The application of PGPRs also affected the nutrient levels in plants and plant rhizosphere soil. For example, compared with the control, the levels of available phosphorus and potassium in rhizosphere soil and the total potassium content in plant roots were significantly increased under *Br. reuszeri* MPT17 treatment ($p < 0.05$). The experiment showed that the relative abundance of *Mortierella*, *Dictyophora,* and *Bacillus* in the rhizosphere soil increased significantly after the application of PGPR ($p < 0.05$). These genera could effectively improve the rate of soil nutrient use, antagonize plant pathogenic bacteria, and promote plant growth. This study provides basic reference data regarding the use of PGPR to improve the microecological environment and promote the growth and development of *C. illinoinensis* plants.

Introduction

Carya illinoinensis, also known as American pecan, is native to the United States and northern Mexico [1]. It is a world-famous economically important tree species that produces superior quality nuts with a substantial oil content and is used for landscaping and the production of high-grade wood [2, 3]. There is a great market demand for *C. illinoinensis*, and it has considerable industrial value. China is exerting great efforts to develop its *C. illinoinensis* industry; the planting area in China is expanding annually, and the process of industrialization is accelerating [4]. To promote the growth of *C. illinoinensis* and control diseases that affect this species, numerous number of chemical fertilizers are applied to *C. illinoinensis* trees, leading to many problems, such as soil compaction and a high incidence of soil-borne diseases [5, 6].

Microbe-based biofertilizers have the combined advantages of various fertilizers and effectively improve soil properties and crop yield and quality. As the main component of microbial fertilizers, plant growth-promoting rhizobacteria (PGPR) are a popular research topic in ecological forestry [7–10]. PGPR are a group of beneficial bacterial strains that live in the rhizosphere or colonize the root surfaces of plants [11, 12]. By secreting organic acids and other substances, these microorganisms can transform some insoluble phosphate in the soil into a form that plants can absorb and utilize, ultimately increasing the available phosphorus (AP) content in the soil. PGPR can also secrete plant hormones, exopolysaccharides, and volatile organic compounds, which can affect the growth and development of plants. For

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example, *Bacillus* spp. can use the tryptophan secreted by plant roots to produce indoleacetic acid (IAA), which in turn, can promote the growth of plant roots and increase the root surface area (SA) [13]. In addition, by competing with pathogenic bacteria for niches, PGPR can improve the disease resistance of plants [14]. For example, myxobacteria have a chemotactic effect on maltose secreted by plant roots, enhancing their ability to colonize the root system; furthermore, myxobacteria feed on other harmful soil microorganisms and inhibit the growth of pathogenic bacteria [15, 16].

More than 20 genera of PGPR, including *Pseudomonas*, *Bacillus* and *Agrobacterium*, have been isolated from rhizosphere soil. *Bacillus* spp. are the most studied and important PGPR. Ijaz et al. applied *Bacillus* BTH-11 and *Pseudomonas* MTH-1 and THM-5 to wheat plants at planting and found that the application of these PGPR was effective in increasing the AP and alkali-hydrolysable nitrogen (AN) levels in the soil [17]. Gallart et al. applied *Paraburkholderia* SOS3 to macadamia plants and found that the application of *Paraburkholderia* SOS3 promoted the growth and development of *Macadamia integrifolia* and increased the nitrogen content in the soil [18]. Salvo et al. found that the application of *B. shortum* WP8 gradually affected the bacterial community structure in the interrhizosphere soil, especially that of the dominant populations, and the regulation of the soil bacterial community structure was an important mechanism by which *B. shortum* WP8 promoted plant growth [9].

We previously identified several PGPR with good characteristics, namely, *Bacillus pumilus* HR10, *Bacillus velezensis* YH20, and *Brevibacillus reuszeri* MPT17, which can effectively promote plant growth and prevent the occurrence of disease. For example, *B. velezensis* YH20 can effectively promote the growth and development of cherry saplings and control the occurrence and development of flowering cherry root gall [19], *B. pumilus* HR10 can effectively prevent the occurrence and inhibit the development of Fusarium wilt in pine, and *Br. reuszeri* MPT17 can promote the growth of *Pinus massoniana* [20]. Although some studies have investigated these PGPR-plant interactions in our laboratory, the growth-promoting effect of these bacteria on *C. illinoinensis* is unknown. To determine the effect of these PGPR on *C. illinoinensis*, we applied them to *C. illinoinensis* via irrigation water and measured the resulting changes in growth indexes, nutrient levels, and microbial community structure.

This study had three main objectives as follows: 1. investigate the effects of inoculation with several PGPR on the growth of *C. illinoinensis* plants; 2. determine the influence of PGPR inoculation on changes in soil nutrient levels and the microbial community structure; and 3.identify PGPR strains with excellent promoting effects on the growth and development of *C. illinoinensis*.

Materials and Methods

Experimental Sites, Plant Materials, and Strains Used

The experiment was conducted from May 2021 to July 2021 in a *C. illinoinensis* cv. Pawnee plantation at a Jiangtao family farm in Lai'an County, Chuzhou city, Anhui Province, China (118° 38′ 73″ N, 32° 47′ 91″ E). The area has a northern subtropical monsoon climate, with an average annual temperature of 17 °C, an annual extreme high temperature of 39 °C, an annual extreme low temperature of − 10 °C, and an average annual precipitation of 1031 mm. The soil properties were as follows: pH 5.7; organic matter content, 18.27 g kg^{-1}; total nitrogen (TN) content, 2.24 g kg^{-1}; total phosphorus (TP) content, 0.7 g kg^{-1}; total potassium (TK) content, 28.2 g kg^{-1}; NH^{+4}-N content, 207.25 mg kg^{-1}; AP content, 18.74 mg kg^{-1}; and available potassium (AK) content, 54.31 mg kg^{-1}. Two-year-old *C. illinoinensis* seedlings were planted 40 cm apart; the seedlings had a root collar diameter of 36.27 ± 3.02 cm and a height of 9.77 ± 0.79 mm. The strains used in the experiments included *Br. reuszeri* MPT17 (isolated from the rhizosphere of *P. massoniana*) [20], *B. pumilus* HR10 (isolated from the rhizosphere of *Pinus thunbergii*) [21], and *B. velezensis* YH20 (isolated from the rhizosphere of *Cerasus serrulata*) [22]. The above-mentioned strains were maintained in the Forest Pathology Laboratory of Nanjing Forestry University.

Preparation and Application of Treatments

For the trials, the bacteria were grown on nutrient agar, and single colonies were transferred to flasks and then grown aerobically on a rotary shaker (150 rpm) for 48 h at 28 °C. The bacterial suspensions were resuspended in aseptic water to a final concentration of 10^7 CFU mL^{-1}, and the resulting suspensions were used as inocula. The seedlings were inoculated via the root irrigation method.

In this experiment, each plant was treated with one of the following four treatments: (a) 500 mL of *Br. reuszeri* MPT17 inoculant, (b) 500 mL of *B. velezensis* YH20 inoculant, (c) 500 mL *of B. pumilus* HR10 inoculant, and (d) 500 mL of aseptic water (as a blank control (CK) group). A total of thirty *C. illinoinensis* plants were included in each treatment group, and the conditions were the same except for the different treatments.

Measurements of Plant Growth Indicators

The seedling height (SH) and ground diameter (GD) of all *C. illinoinensis* plants were measured at 0 and 60 days after

inoculation, and five seedlings were randomly selected from each treatment group. The root structure, root length (RL), root surface area (SA), root average diameter (AD), number of root tips (TIPS), number of root forks (BN), and root volume (RV) were measured by scanning the root system with a root scanner (WinRHIZO STD4800 LA2400, Regent). Then, the different organs of the seedlings were placed in an oven at 105 °C for 30 min for sterilization. After sterilization, the temperature of the oven was adjusted to 85 °C. The samples of different organs of *C. illinoinensis* were dried to a constant weight, and the shoot dry weight (SDW) and root dry weight (RDW) were measured.

Soil and Plant Nutrient Content Determination

Five individual plants per pot in each treatment were collected, and the roots, stems, and leaves were dried to a constant weight, crushed into a powder and then sieved through a mesh screen (0.2 mm pore size). The powder was stored in a self-sealing bag at room temperature for the determination of the plant nutrient indexes. Regarding the collection of rhizosphere soil, the soil surrounding the roots was removed on the 60th day after inoculation, and the rhizosphere soil was collected and sieved through a mesh (a 0.2 mm pore size). Soil (15 g) was obtained from each treatment group and air-dried to determine the nutrient levels.

The TN content was determined by the indophenol blue colorimetric method [23], the TP content was determined by the Mo–Sb colorimetric method [24], and the TK content was determined by the flame photometry method [25]. The AN content in the soil was determined by the alkali–nitrogen diffusion method [26], the AP content in the soil was determined by the hydrochloric acid–ammonium fluoride method [26], and the AK content in the soil was determined by the ammonium acetate leaching method [26].

Soil Microbial Community Structure Determination

At 15 days after inoculation, the rhizosphere soil of 15 *C. illinoinensis* plants was obtained from each treatment group, the rhizosphere soil of every 3 *C. illinoinensis* plants was mixed thoroughly, and the soil from each treatment group was mixed together into five samples. After collection, the soil was immediately stored at − 80 °C.

A total of twenty DNA samples were extracted from the soils using a MagPure Soil DNA LQ Kit (Magen, Guangzhou, China) following the manufacturer's instructions. The DNA quantity and quality were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA); the DNA was stored at – 20 °C until it was used for PCR.

We performed targeted amplification of the bacterial 16S ribosomal RNA region and the fungal internal transcribed spacer (ITS) regions to characterize the composition of the soil microbial community. The primers 343F (5′-TACGGRAGGCAGCAG-3′) and 798R (5′-AGGGTATCTAATCCT-3′) were used to amplify the V3–V4 region of the bacterial 16S rRNA genes, and ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) were used to amplify the ITS 1 region of the fungal rRNA genes. The first PCR step involved the use of template-specific primers with a short adaptor sequence, and the second PCR step involved the use of primers containing Illumina barcodes. The composition of bacterial and fungal communities was analyzed by high-throughput sequencing using the Illumina NovaSeq 6000 platform by OE Biotech Technology Co., Ltd. (Shanghai, China). The raw sequences were deposited in the NCBI Short-Read Archive (BioProject PRJNA831643).

Raw amplicon sequencing data were obtained using QIIME [27]. Forward and reverse reads of the same sequence were merged such that there were more than 200 bp of overlap and < 0.25 mismatches using FLASH v1.2.5 [28]. Chimeric sequences were detected and removed with the UCHIME algorithm [29]. The sequences were clustered into operational taxonomic units (OTUs) with a 97% identity cut-off using VSEARCH 2.4.2, and their taxonomic affiliation was assigned using the RDP 16S rRNA reference database and UNITE ITS reference database [30, 31]. The alpha diversity was analyzed using QIIME, which included calculations of the Chao 1, Shannon, and Simpson indexes. Similarly, the beta diversity was estimated by computing the unweighted UniFrac distance and visualized via principal coordinate analysis (PCoA).

Data Processing

The experimental data were processed by Excel 2019. A one-way analysis of variance (ANOVA) was performed using IBM SPSS 25.0 software to compare the differences in the data among the different treatment groups ($p < 0.05$). The processing and visualization of the plant biomass data were performed with Prism software, and the Pearson method was used to analyze the correlations ($p < 0.05$).

Results

Effect of PGPR on the Growth of C. illinoinensis Seedlings

Compared with the CK plants, the growth of the *C. illinoinensis* seedlings increased in response to the three types

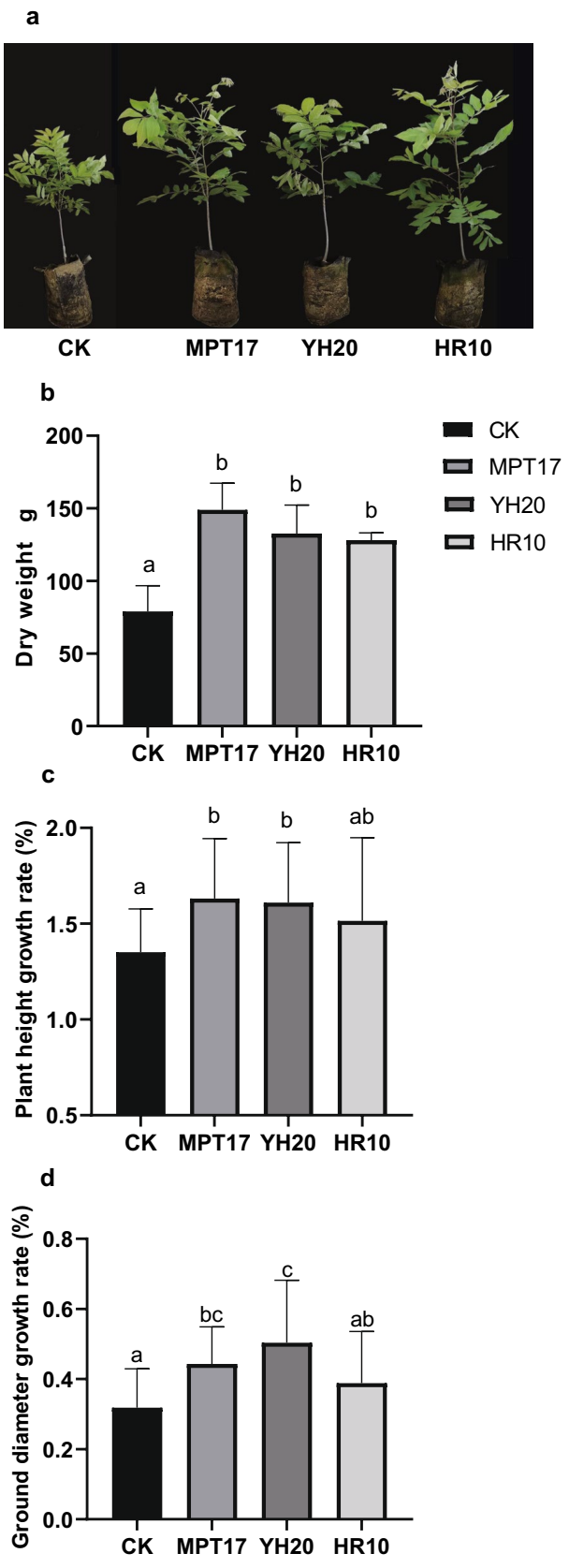

Fig. 1 Effects of different inoculation treatments on the biomass of 2-year-old *C. illinoinensis* seedlings. Effects of the plant growth-promoting rhizobacteria (PGPR) on *C. illinoinensis* cultivar Pawnee seedlings grown after 60 days (**a**), the dry weight (**b**), the growth rate of plant height (**c**), and ground diameter (**d**). Different lowercase letters indicate that there are significant differences between inoculation treatments ($p < 0.05$)

of PGPR (Fig. 1). Compared with the CK treatment, the MPT17 treatment significantly increased the dry weight of the *C. illinoinensis* seedlings by 91.20% ($p < 0.05$). Furthermore, the SH and GD increased by 30.31% and 14.12%, respectively ($p < 0.05$). Compared with the CK treatment, the YH20 treatment increased the dry weight of the *C. illinoinensis* seedlings by 69.63% ($p < 0.05$); in addition, the YH20 treatment significantly increased the SH and GD of the *C. illinoinensis* seedlings by 27.71% and 19.78%, respectively ($p < 0.05$). Compared with the plants inoculated with CK, the SH, GD and dry weight of *C. illinoinensis* inoculated with HR10 significantly increased by 19.37%, 8.36%, and 66.35%, respectively ($p < 0.05$).

Effect of PGPR on the Root Structure of C. illinoinensis Seedlings

RL, the total RV and SA can reflect the status and distribution of plant roots. Similarly, AD, TIPS, and BN can reflect the root absorption efficiency. The root structure parameters of *C. illinoinensis* plants were improved in response to the inoculation with the three types of PGPR (Figs. 2, 3). Compared with those inoculated with CK, the RL, RV, SA, TIPS, BN, and AD of the *C. illinoinensis* roots inoculated with MPT17 were significantly increased by 146.03%, 85.69%, 118.36%, 103.35%, 113.21%, and 54.58%, respectively ($p < 0.05$). Compared with the CK treatment, the YH20 treatment significantly increased the RL, RV, SA, TIPS, BN, and AD of the *C. illinoinensis* seedlings by 119.50%, 95.90%, 81.57%, 78.44%, 73.55%, and 159.51%, respectively ($p < 0.05$). Moreover, compared with the CK treatment, the HR10 treatment increased the RL, RV, SA, TIPS, BN, and AD of *C. illinoinensis* by 142.74%, 82.17%, 97.10%, 135.27%, 94.89%, and 113.66%, respectively ($p < 0.05$).

Effect of PGPR on the Nutrient Levels of C. illinoinensis Seedlings and Soil

Nitrogen, phosphorus, and potassium are essential nutrients for plant growth, and inoculation with the three PGPR strains had an effect on the nutrient levels in the plants and the soil (Table 1). At 60 days after the PGPR inoculation, compared with those treated with CK, the TP content in

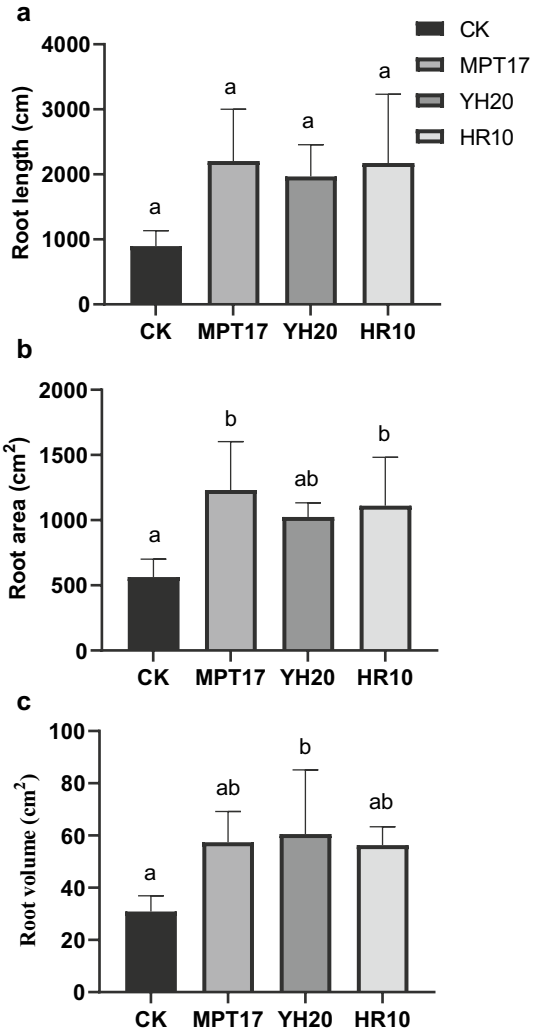

Fig. 2 Root indexes after inoculation of different treatment groups. **a** root length; **b** surface area; **c** root volume. Each bar chart represents the average "SE" of three independent experiments

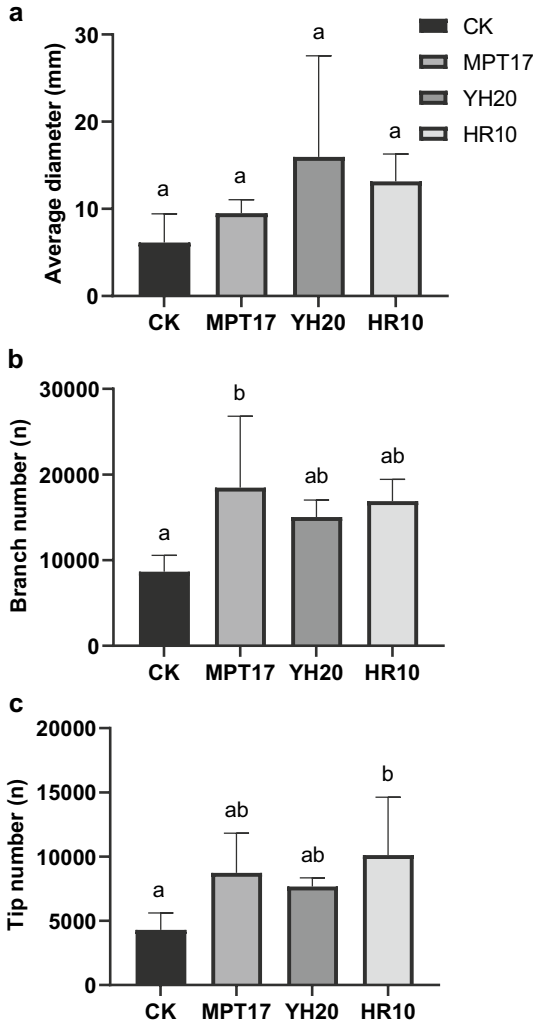

Fig. 3 Root indexes after inoculation of different treatment groups. **a** average diameter; **b** number of branches; **c** number of root tips. Each bar chart represents the average "SE" of three independent experiments

the roots and TK content in the stems of the plants treated with MPT17 were significantly increased (by 78% and 111.53%, respectively) ($p < 0.05$). The AN and AK levels in the roots of the plants treated with HR10 were significantly increased by 30.79% and 75%, respectively, compared with those in the plants treated with CK, and the AK content in the stems of the plants significantly increased by 79.48% ($p < 0.05$). Compared with the CK treatment, the YH20 treatment increased the AN and AK levels in the stems of *C. illinoinensis* by 32.92% and 139.74%, respectively ($p < 0.05$), and the TK content in the plant leaves was significantly increased (by 45.39%) ($p < 0.05$).

Regarding the rhizosphere soil nutrient contents, the AP and AK contents in the MPT17 treatment group were significantly increased (by 128.99% and 17.18%, respectively), compared with those in the CK group ($p < 0.05$). Moreover, compared with the CK control group, the levels of AN and AP in the rhizosphere soil in the YH20 treatment group were significantly increased (by 64.35% and 54.29%, respectively) ($p < 0.05$); the AP level in the rhizosphere soil in the HR10 treatment group was also increased (by 98.48%) ($p < 0.05$).

Correlation Analysis of the Soil Nutrient Levels and Growth Indexes of C. illinoinensis Plants

The Pearson's correlations between the seedling biomass indexes and changes in available nutrient content levels in the soil showed that both the root fresh weight (RFW) and stem dry weight (SDW) were significantly positively correlated with the soil TP content (Table 2).

High-Throughput Sequencing Analysis of Bacterial Microbial Diversity in the Rhizosphere Soil of C. illinoinensis

Diversity of Bacterial Communities

The coverage of the soil bank in the samples of the four treatment groups ranged from 99.02 to 99.14% (Table 3). The values were close to 1, indicating that the depth of this sequencing covered all species in the rhizosphere soil and that the sequencing results may reflect the real situation of soil bacteria in the samples. Regarding the Chao 1, Shannon and Simpson indexes, there were no significant differences among the four treatment groups, and the three PGPR strains did not cause major disturbances in the bacterial community diversity in the rhizosphere soil of *C. illinoinensis*.

Bacterial Community Structure

A Venn diagram was constructed showing the similarities and differences in the composition of bacterial communities among the different treatments. After clustering, isolation and elimination, a total of 5834 bacterial OTUs, with a similarity of 97% were obtained (Fig. 4a). There were 460, 217, 328 and 174 endemic OTUs in the CK, MPT17, YH20 and HR10 treatment groups, respectively. Moreover, there were 3515 overlapping OTUs between the CK treatment group and MPT17 treatment group, 3485 overlapping

Table 1 Means and standard errors of plant nutrient contents under PGPR treatments. Different lowercase letters show significant differences at $p < 0.05$ among treatments and the control.

Treatments	CK	MPT17	YH20	HR10
Root				
TN (mg·kg^{-1})	4.17 ± 0.1^{a}	1.88 ± 0.14^{ab}	1.93 ± 0.26^{ab}	2.06 ± 0.24^{b}
TK (mg·kg^{-1})	3.57 ± 0.55^{a}	7.83 ± 1.27^{b}	3.27 ± 0.25^{a}	7.7 ± 0.26^{b}
TP (mg·kg^{-1})	4.81 ± 0.3^{a}	4.81 ± 0.75^{a}	3.32 ± 0.68^{a}	3.9 ± 1.08^{a}
Stem				
TN (mg·kg^{-1})	0.74 ± 0.12^{ab}	1.98 ± 0.21^{ab}	2.31 ± 0.41^{b}	2.21 ± 0.02^{ab}
TK (mg·kg^{-1})	1.91 ± 1.77^{a}	11 ± 1.65^{bc}	12.47 ± 1.46^{c}	9.33 ± 1.55^{b}
TP (mg·kg^{-1})	0.61 ± 0.18^{a}	1.17 ± 0.35^{a}	1.03 ± 0.47^{a}	1.13 ± 0.54^{a}
Leaf				
TN (mg·kg^{-1})	4.4 ± 0.85^{a}	2.91 ± 0.31^{a}	2.71 ± 0.24^{a}	2.7 ± 0.18^{a}
TK (mg·kg^{-1})	3.03 ± 1.42^{a}	6.57 ± 0.61^{ab}	7.37 ± 1.52^{b}	6.47 ± 1.53^{ab}
TP (mg·kg^{-1})	5.07 ± 0.47^{a}	2.62 ± 2.41^{a}	1.21 ± 0.21^{a}	1.93 ± 1.44^{a}
Soil				
TN (mg·kg^{-1})	213.11 ± 74.86^{a}	203.78 ± 28.51^{a}	294 ± 45^{b}	230.22 ± 16.39^{ab}
TK (mg·kg^{-1})	1.98 ± 0.55^{a}	45.34 ± 2.46^{c}	30.55 ± 6.78^{b}	39.3 ± 3.09^{c}
TP (mg·kg^{-1})	55.1 ± 2.91^{b}	64.57 ± 1.63^{c}	56.6 ± 1.93^{b}	47.77 ± 2.66^{a}

Table 2 Each correlation analysis value between seedling biomass and available nutrient contents in soil represents the average selenium content in three independent experiments

Index	SH	GD	SFW	RFW	SDW	RDW
TN	–	–	–	–	–	–
TP	–	–	–	0.971*	0.991*	–
TK	–	–	–	–	–	–

SH Seedling height, *GD* ground diameter, *SFW* stem dry weight, *RFW* root fresh weight, *SDW* stem dry weight, *RDW* root dry weight

Table 3 Soil bacterial α diversity index values in different treatment groups after inoculation

Treatments	Coverage	Chao 1	Simpson	Shannon
CK	0.9902 ± 0.00053^{a}	$2921.8963 \pm 278.06325^{a}$	0.9855 ± 0.00858^{a}	8.3341 ± 0.74537^{a}
MPT17	0.9906 ± 0.00181^{a}	$2631.7698 \pm 573.18828^{a}$	0.9678 ± 0.0315^{a}	7.74 ± 0.98575^{a}
YH20	0.9906 ± 0.00127^{a}	$2765.1425 \pm 414.91316^{a}$	0.9881 ± 0.00421^{a}	8.3096 ± 0.48254^{a}
HR10	0.9914 ± 0.00134^{a}	$2391.9679 \pm 605.54564^{a}$	0.9539 ± 0.0595^{a}	7.1796 ± 1.56561^{a}

Different lowercase letters show significant differences at $p < 0.05$ among treatments and the control

OTUs between the CK treatment group and YH20 treatment group, and 3257 overlapping OTUs between the CK treatment group and HR10 treatment group, indicating that the bacterial communities in the CK treatment group and MPT17 treatment group were more similar than those in the other groups, while the similarity between the CK treatment group and HR10 treatment group was lower than that of the other two groups.

A bar chart was constructed showing the species composition of the rhizosphere soil bacteria at the phylum level in the different treatment groups (Fig. 4b). Proteus (60.28%), Actinomycetes (14.37%), Lactobacillus acidophilus (7.11%), Bacteroides (6.28%) and gram-negative bacteria (5.79%) were the main flora, accounting for 98.05% of the total organisms that corresponded to them. Firmicutes (4.21%), Nitrospirae (0.70%), Patescibacteria (0.57%), Elusimicrobia (0.13%), Verrucomicrobia (0.11%), Chloroflexi (0.10%), Dependentiae (0.08%), Fusobacteria (0.05%), Fibrobacteres (0.03%), and Chlamydiae (0.03%) were also detected but were not abundant (0.1% < relative abundance < 5%).

At the phylum level, the relative abundance of Bacteroides in the rhizosphere soil in the YH20 and HR10 treatment groups was significantly lower than that in the CK treatment group ($p < 0.05$). Moreover, the relative abundance of Actinomycetes in the rhizosphere soil in the MPT17 treatment group was significantly lower than that in the CK, YH20 and HR10 treatment groups ($p < 0.05$). The HR10 treatment significantly decreased the relative abundance of bacteria, which were increased by 71.38%, 20.31%, and 6.06%, and the relative abundance of bacteria in the YH20 group was significantly lower than that in both the CK and HR10 groups ($p < 0.05$).

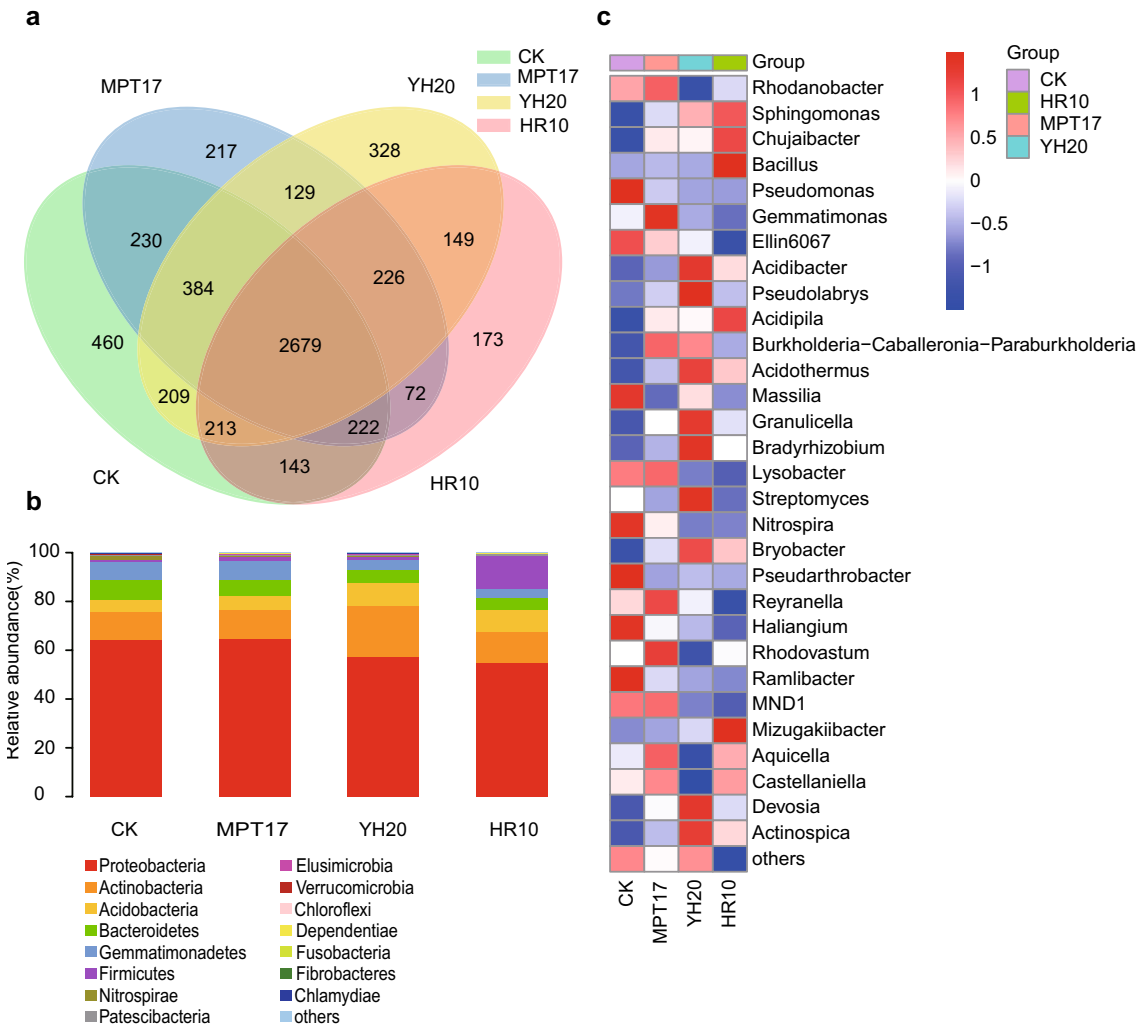

Fig. 4 Venn diagram, heatmap and bar diagram of soil communities in different treatment groups after inoculation. **a** Species Venn diagram; **b** horizontal community histogram of bacterial phylum; **c** heatmap

Heatmap clustering was performed to determine the community composition of the different genera under the treatments (Fig. 4c). The relative abundance of *Bacillus* in the YH20 and HR10 treatment groups was significantly greater than in the CK treatment group, while the relative abundances of *Nitrospira* and *Pseudomonas* in the YH20 and HR10 treatment groups were significantly lower than those in the CK treatment group. The relative abundances of *Bryobacter* and *Granulicella* in the YH20 and HR10 treatment groups were significantly greater than those in the CK treatment group ($p < 0.05$). The relative abundance of *Massilia* in the rhizosphere soil of the MPT17 and HR10 treatment groups was significantly lower than that in the CK treatment group ($p < 0.05$). The relative abundance of *Massilia* in the rhizosphere soil of the MPT17 and HR10 treatment groups was significantly lower than that in the CK treatment group ($p < 0.05$), and the relative abundances of *Nitrospirillum* and *Pseudomonas* in the rhizosphere soil of the HR10 treatment group were significantly lower than those in the CK treatment group ($p < 0.05$).

High-Throughput Sequencing Analysis of Fungal Microbial Diversity in the Rhizosphere Soil of *C. illinoinensis*

Diversity of Fungal Communities

The coverage of the four soil banks was between 0.9985 and 0.9995 (Table 4). The values were close to 1, indicating that the sequencing depth of the rhizosphere soil fungal community structure covered all species in the soil samples. Thus, the sequencing results may reflect the real situation of the structure of the soil fungal community in the rhizosphere of *C. illinoinensis*. The order of the Chao 1 index was HR10 > YH20 > CK > MPT17 (Table 4). The Chao 1 index in the YH20 and HR10 treatment groups was significantly greater than that in the CK and MPT17 treatment groups, indicating that the total number of fungal species and community richness in the rhizosphere soil of the YH20 and HR10 groups were greater than those in the other two groups, but there was no significant difference in the Shannon index and Simpson index among the different treatment groups.

Fungal Community Structure

A Venn diagram was constructed showing the similarities and differences in the composition of fungal communities among the different treatment groups. After OTU clustering, isolation, and elimination, a total of 1130 fungal OTUs, with a similarity of 97% were obtained (Fig. 5a). There were 113, 57, 74, and 133 OTUs of endemic fungi in the CK, MPT17, YH20, and HR10 treatment groups, respectively. There were 415 fungal OTUs overlapping between the CK treatment group and MPT17 treatment group, 438 fungal OTUs overlapping between the CK treatment group and YH20 treatment group, and 454 fungal OTUs overlapping between the CK treatment group and HR10 treatment group, indicating that the fungal communities in the CK treatment group and HR10 treatment group were more similar than those in the other groups and that the similarity between the CK treatment group and the MPT17 treatment group was lower than that in the other two groups.

A histogram was constructed showing the species composition at the phylum level in the different treatment groups (Fig. 5b). In the rhizosphere soil of the different treatment groups, Ascomycota (61.56%) and Basidiomycota (33.73%) were the main fungal communities at the phylum level, accounting for 95.29% of the organisms that corresponded to the total sequences. Zygomycota (4.42%), Chytridiomycota (0.23%), Glomeromycota (0.06%), Cercozoa (0.01%), and Rozellomycota were detected but not abundant (0.01% < relative abundance < 5%). The relative abundance of Rozellomycota in the rhizosphere soil of the MPT17 treatment group was significantly greater than that in the CK treatment group and the YH20 treatment group. The relative abundance of Cercozoa in the rhizosphere soil of the MPT17 treatment group and YH20 treatment group was significantly lower than that in the CK treatment group.

A heatmap of the clustering results was constructed showing the community composition differences between the samples (Fig. 5c). The relative abundance of *Guehomyces* in the rhizosphere soil of the YH20 treatment group was significantly lower than that in the CK treatment group ($p < 0.05$). The relative abundance of *Mortierella* in the HR10 treatment group was significantly greater than that in the rhizosphere soil of the CK treatment group. Moreover, the relative abundances of *Candida* and *Mortierella* in the

Table 4 Alpha diversity index of soil fungi in different treatment groups after inoculation

Treatments	Coverage	Chao 1	Simpson	Shannon
CK	0.9994 ± 0.0002^{a}	$326.4955 \pm 37.88038^{a}$	0.8289 ± 0.11342^{b}	3.9184 ± 1.19978^{b}
MPT17	0.999 ± 0.00021^{a}	$311.0145 \pm 54.35433^{a}$	0.6118 ± 0.17844^{a}	2.4192 ± 0.65474^{a}
YH20	0.9987 ± 0.00015^{a}	$389.2259 \pm 37.32349^{b}$	0.7228 ± 0.0721^{ab}	3.0493 ± 0.36329^{ab}
HR10	0.999 ± 0.00026^{a}	392.5782 ± 52.5865^{b}	0.7535 ± 0.14452^{ab}	3.5818 ± 1.21047^{ab}

Different lowercase letters show significant differences at $p < 0.05$ among treatments and the control

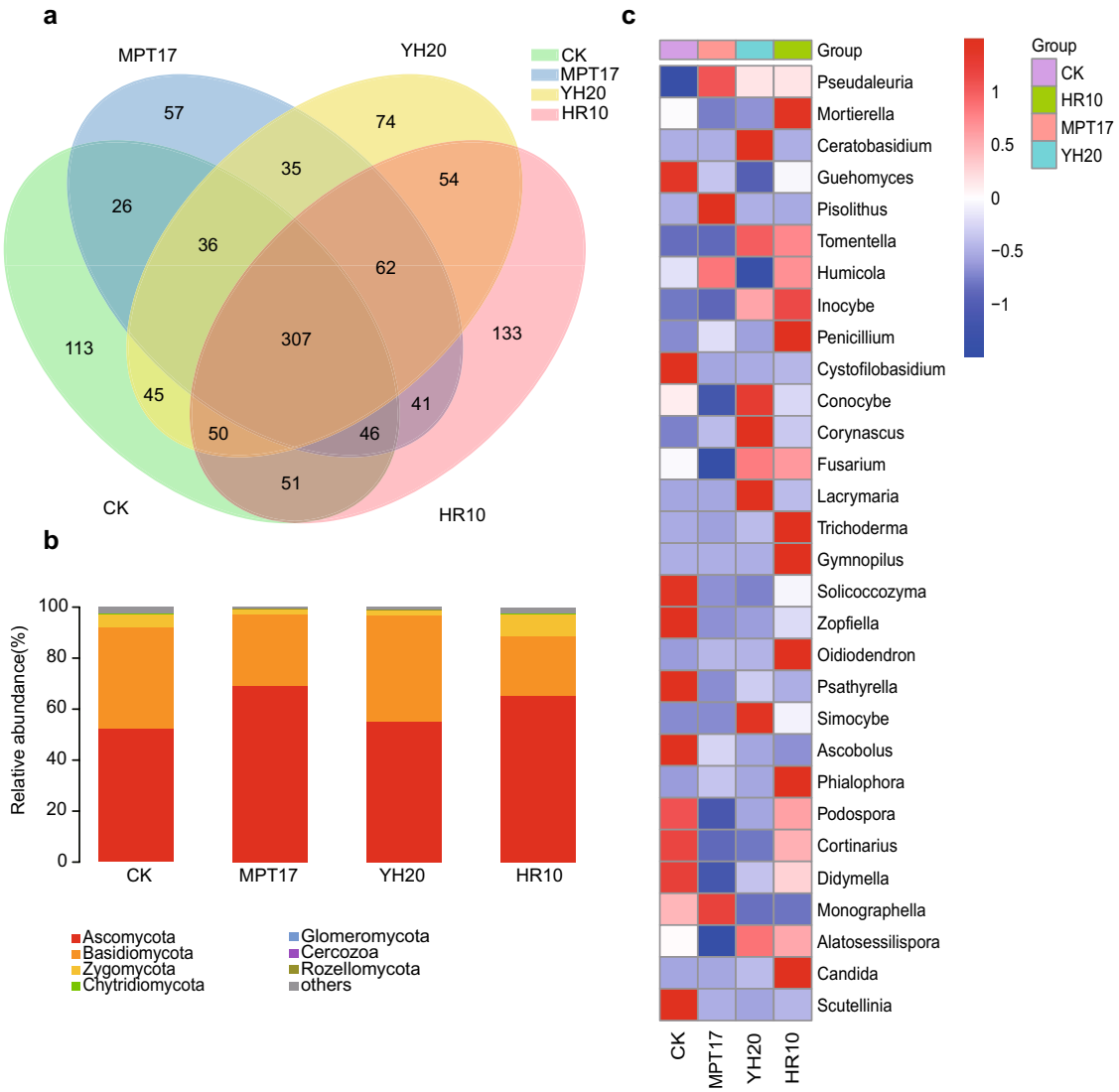

Fig. 5 Venn diagram and bar diagram of the soil microbial community in different treatment groups after inoculation. **a** Species Venn diagram; **b** horizontal community histogram of fungal phylum; **c** heatmap

HR10 treatment group were significantly greater than those in the CK treatment group, and the relative abundances of *Candida*, *Oidiodendron,* and *Phialophora* were significantly greater than those in the CK treatment group ($p < 0.05$). The relative abundance of *Pseudaleuria* in the rhizosphere soil of the MPT17 treatment group was significantly greater than that in the CK treatment group ($p < 0.05$).

Discussion

PGPR provide many benefits to plants; in return, plants provide microbes with reduced carbon and other metabolites. PGPR play key roles in nutrient acquisition and assimilation, improvements in the soil texture, secretion, and modulation of extracellular molecules such as hormones and various signal compounds, all of which lead to enhanced plant growth; thus, PGPR are potentially important biocontrol microorganisms [32]. The three PGPR strains used in this study were *Brevibacillus reuszeri* MPT17, *Bacillus pumilus* HR10, and *B. velezensis* YH20, and the experimental results showed that all applied PGPR strongly promoted the growth and development of *Carya illinoinensis* plants.

In this study, the application of all three PGPR strains significantly increased the seedling height, ground diameter and biomass of *C. illinoinensis*. The root surface area, root average diameter, root length, number of root forks, root volume, and number of root tips of the *C. illinoinensis* plants significantly increased in response to the different treatments compared with those in the CK treatment group. PGPR affect the

structure and growth of the plant root system through direct or indirect action, increasing the surface area of the plant root system and increasing nutrient uptake [33]. A previous study showed that the effects of PGPR on plant root growth and development are related to the secretion of IAA [34]. Whether the mechanism underlying the growth-promoting effect of the three PGPR strains on the plant root system in this study was the same as that in previous studies needs to be further investigated. HR10 and MPT17 are mycorrhizal helper bacteria that promote the formation of mycorrhizal symbiosis [35]. Mycorrhizal fungi can promote plant growth because they can absorb mineral nutrients such as nitrogen and phosphorus from the soil and transport them to host plants, improving plant growth [36]. Our results suggest that HR10 and MPT17 may improve the plant root structure by promoting the growth of mycorrhizal fungi in the soil. In previous studies, it has been shown that YH20 bacteria have the ability to secrete IAA, which is essential for improving plant development given that IAA alter a plant's auxin pool to optimal or supraoptimal levels and can directly improve plant growth [22, 37]. Our results suggest that IAA secretion is a possible mechanism by which YH20 improves the plant root architecture.

Nitrogen, phosphorus and potassium are the main essential nutrients of plants, and their amounts in the soil directly affect plant growth and development. In forest soils, the availability of nitrogen, phosphorus and potassium is low; thus, so increasing the availability of these nitrogen, phosphorus and potassium nutrients in the soil is an important way to promote tree growth [38–40]. Song et al. applied PGPR to sphagnum pine and found a significant increase in sphagnum pine biomass and a significant positive correlation between the nutrient content in the soil and biomass [41]. Ekinci et al. applied *B. subtilis* UG-41 to pepper seedlings, and the bacteria ultimately improved the ability of pepper seedlings to absorb minerals [42]. In the present study, the results were similar, as the application of MPT17 significantly increased the TK content in the soil and the TK content in both the roots and stems of the plants. The Pearson correlation analysis revealed that there was a correlation between the soil nutrient content and seedling biomass, the TP content in the soil affected the accumulation of plant biomass and the significant increase in *C. illinoinensis* biomass after inoculation with PGPR might be related to the changes in the soil nutrient levels.

Moreover, regarding the alpha diversity index, there was no significant difference in the rhizosphere soil bacteria between the treatment group and the CK group, but there was some difference in fungal abundance. The abundance of microflora related to nutrient cycling and metabolism, such as *Bacillus*, *Pseudomonas* and *Devosia*, in the rhizosphere soil of *C. illinoinensis* treated with the PGPR strains was significantly increased. When these bacteria are applied to other plant species, they can significantly enhance growth and development. For example, Ahmad et al. applied two PGPR strains, *B. aryabhattai* S10 and *B. subtilis* ZM63, to mung bean and maize and showed that both strains significantly promoted plant growth and improved the plant nutrient status [15]. Sandheep et al. applied *P. fluorescens* to herbs; the bacteria significantly increased the biomass of plants and improved their nutrient absorption capacity [43]. Many studies have shown that *Bacillus* alters the microbial community structure by becoming the dominant member of the flora or secreting bacteriostatic substances, helping plants obtain nutrients, regulating endogenous hormones and promoting plant growth in the soil [44]. Indeed, the effects on plants may be the result of synergistic/antagonistic interactions between inoculated strains, and the native microbiota may be related to the induction or repression of indigenous microbial populations [45]. In previous studies, it has been shown that both HR10 and YH20 can produce antagonistic substances and reduce the relative abundance of pathogenic bacteria [46, 47]. The application of these exogenous flora can increase the abundance of beneficial microorganisms in the soil, decrease the abundance of pathogenic microorganisms, and steer the structure of the bacterial community in the soil in a healthy direction, all of which promote plant growth and development.

Regarding the structure of the fungal community in the rhizosphere soil, after the application of PGPR, the relative abundance of some genera that can promote plant growth was significantly increased, while the relative abundance of genera that hinder plant growth was significantly decreased. For example, the abundance of *Trichoderma*, the fungi of which are extremely important biocontrol organisms, was increased in rhizosphere soil in response to applications of PGPR [48]. By studying the colonization characteristics and diversity of dark septate endophytes in Pb–Zn slag heap plants, Zhang et al. found that *Phialophora* plays an important role in promoting plant growth and enhancing the host plant response to environmental stress [49]. Similarly, *Mortierella* can improve the bioavailability of phosphorus and iron in the soil and improve the nutrient use efficiency of plants. Moreover, the relative abundance of pathogenic fungi such as *Didymella* and *Fusarium*, was significantly decreased in rhizosphere soil where PGPR were applied [50]. MPT17 and HR10 are mycorrhizal auxotrophs that can promote the growth of fungi in soil and increase their relative abundance [36]. Therefore, the application of these strains can steer the composition of soil fungal communities in a healthy direction.

Conclusion

The application of PGPR to *C. illinoinensis* plants promoted increases in root growth and development, soil nutrient levels, plant nutrient uptake and use efficiency,

and overall plant growth and development. In addition, the application of PGPR significantly increased the relative abundance of beneficial microorganisms and decreased the relative abundance of pathogenic microorganisms in the soil, promoting a healthy microbial community structure in the soil and providing a favorable soil environment for the growth and development of *C. illinoinensis* plants. The three PGPR strains tested in this study could promote the growth of *C. illinoinensis* seedlings. Thus, this study shows that the three PGPR strains have certain application potential for the development of the *C. illinoinensis* industry and provides basic reference information regarding these strains.

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Data Availability The data presented in this study are available upon request from the corresponding author.

Code Availability Not applicable.

Declarations

Conflict of interest There are no financial/personal interest or belief that could affect the objectivity of the study.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

Consent for Publication Not applicable.

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