Effects of Plant Growth Promoting Rhizobacteria Microbial Inoculants on the Growth, Rhizosphere Soil Properties, and Bacterial Community of *Pinus sylvestris* var. *mongolica* Annual Seedlings

Qian Song

Northeast Forestry University <https://orcid.org/0000-0003-1164-4816>

Xiaoshuang Song

Heilongjiang Forestry Academy

Xun Deng

Heilongjiang Forestry Academy

Jiayu Luo

Northeast Forestry University

Junkai Wang

Northeast Forestry University

Kai Min

Daqing Oilfield Logistic Administration

Ruiqing Song (✉ songruiqing@nefu.edu.cn)

Northeast Forestry University <https://orcid.org/0000-0001-6604-0281>

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Abstract

[Objective] Determine the ability of three plant growth promoting rhizobacteria (PGPR) strains (*Pseudomonas Mandelli* A36, *Serratia plymuthica* A13 and *Pseudomonas koreensis* A20) to promote plant growth, evaluate the effect of inoculation with PGPR strains on seedling biomass, root structure, nutrient index, and enzyme activity, and assess the effect of PGPR inoculation on soil nutrient index, enzyme activity, and the soil microecological environment.

[Method] The ability of the three PGPR strains to secrete indole-3-acetic acid (IAA), dissolve inorganic phosphorus, and produce siderophore and hydrolase was determined by the medium color change method, pot experiment to determine the effects of three PGPR strains on plant biomass, physicochemical properties, soil physicochemical properties and microbial diversity.

[Result] The three PGPR strains had the ability to secrete IAA, solubilize inorganic phosphorus, and produce siderophore, the results of the pot experiment showed that inoculation with PGPR strain had a significant effect on plant biomass, root index, nutrient index and enzyme activity, as well as soil nutrient index, enzyme activity and bacterial diversity.

[Conclusion] This study provides basic data references for PGPR strains to improve the soil microecological environment and promote the growth and development of *Pinus sylvestris* var. *Mongolica* seedlings.

1 Introduction

Soil microorganisms promote material cycling and energy flow in the ecosystem. They play important roles as both producers and decomposers in the ecosystem (Wu et al. 2013; Zhang and Yu, 1990). In addition, soil microorganisms perform processes such as oxidation, nitrogen fixation, nitrification, and ammoniation in the soil to promote the decomposition of soil organic matter and nutrient conversion. Soil microorganisms are widely distributed in the plant rhizosphere and are most speciose in a dynamically changing environment. In 1904, the German scientist Lorenz Hiltner first proposed the concept of the “rhizosphere” which refers to the soil around the root system. In the rhizosphere, plant root activity alters the physical and chemical properties of the soil, providing a special ecological environment for interaction between plants and soil microorganisms (Compant et al. 2010; Kloepper et al. 1980; Liu 2005). Since the concept of the “rhizosphere” was proposed, there have been increasing numbers of studies on the plant rhizosphere, mainly involving the physiological structure of the root system, rhizosphere soil nutrients, rhizosphere soil enzyme activities, and rhizosphere soil microorganisms, as well as the connection between them (Li 2002). There are a large number of active microorganisms, such as fungi, bacteria, and actinomycetes, in the rhizosphere soil, approximately 28–48 times higher than in non-rhizosphere soil. There are about 104–106 fungi genera in every 1 g of soil, and the main groups include filamentous fungi such as *Penicillium*, *Fusarium*, *Aspergillus*, and *Trichoderma* (Barreto et al. 2008; I. G et al. 2002; Sreevidya et al. 2016). There are about 104–108 actinomycetes genera in every 1 g of soil, primarily composed of *Streptomyces*, *Micromonospora*, and *Nocardia* (Cocking 2003). Bacteria are the most abundant rhizosphere soil microorganisms, with each 1 g of soil containing approximately 106–1010 bacteria genera. The main bacterial groups include *Bacillus*, *Pseudomonas*, *Flavobacterium*, *Serratia*, *Rhizobium*, and *Azotobacter*. Rhizosphere bacteria can promote plant growth, increase plant biomass, promote the absorption and utilization of soil nutrients by plants, improve the microecological environment of the rhizosphere soil, and inhibit or antagonize pathogenic bacteria (Vessey 2003). In addition, PGPR can degrade pollutants in the soil, improve soil fertility, control pests and diseases, and reduce the environmental pollution and soil compaction caused by the use of pesticides and fertilizers (Zhang et al. 2013). One study found that *Brevibacillus brevis* DZQ3 could significantly promote the growth of tobacco (Zhu et al. 2012), while another discovered that *Azospirillum* could promote the growth of corn (Zhu et al. 2012). A further study found that *Pseudomonas* CBT1, CBT6, and Cbt7 has a certain control effect on cucumber fusarium wilt (Yue and Zhang, 2009). Although PGPR has a significant impact on plant growth, the influence of PGPR on perennial tree species and their associated rhizosphere communities are still poorly investigated, and the number of related PGPR reported is also very limited. At present, *Bacillus* and *Pseudomonas* are the two most studied and most important plant growth-promoting bacteria (Myresiotis et al. 2012). In addition to these two genera, *Serratia* has also been reported on, though not extensively so. *Serratia* sp. sy5 was found to increase the biomass of corn (Koo and Cho 2009). *Serratia* sp. CDP-13 could enhance the induced resistance and salt tolerance of wheat (Singh and Jha 2016), while *Serratia* Sp. A21-4 could promote the growth and development of capsicum and also demonstrated strong rhizosphere colonization ability (Yayou et al. 2016).

Pinus sylvestris var. *mongolica* is a geographical variety of *Pinus sylvestris*. It has a developed root system and can fully absorb and utilize water. Due to its fast growth, cold resistance, and drought resistance, as well as its aesthetic qualities, it has been widely introduced into the three northern areas of China where it is the main tree species used in shelter and sand control engineering (Zhang and Li, 2003). However, due to the large-scale use of pesticides and chemical fertilizers in recent years, *P. sylvestris* var. *mongolica* populations have declined in many areas, causing great economic loss. The introduction of rhizosphere microorganisms to replace pesticides and chemical fertilizers could address this decline as well as promote the growth and development of *P. sylvestris* var. *mongolica*, improve its root structure, and enhance its resistance to stress. The application of PGPR is pollution-free, residue-free, and more conducive to human and animal safety (Fiorentino et al. 2018; Zhang et al. 2018). After the introduction of rhizosphere microorganisms, the population and distribution of soil microorganisms will change, the basic physical and chemical properties of the soil will be altered, and the growth and development of plants will also be affected. As different

rhizosphere microorganisms have different effects on the soil, it is essential that the impacts of rhizosphere microorganisms on the community structure, species composition, spatial distribution, and diversity of the soil microecological environment are explored.

Therefore, the objectives of this study were to

1. Determine the ability of three PGPR strains (*P. mandelii* A36, *P. koreensis* A20, and *S. plymuthica* A13) to promote plant growth;
2. Evaluate the effect of inoculation with PGPR strains on seedling biomass, root structure, nutrient index, and enzyme activity; and
3. Assess the effect of PGPR inoculation on soil nutrient index, enzyme activity, and the soil micro-ecological environment.

Our overall aim was to explore the use of rhizobacteria in changing the community structure of soil microorganisms, promoting the growth and development of *P. sylvestris* var. *mongolica*, and improving the stress resistance of *P. sylvestris* var. *mongolica*.

2 Materials And Methods

2.1 Identification of PGPR strains

The three PGPR strains A13, A36, and A20 were isolated from the rhizosphere soil collected from a *P. sylvestris* var. *mongolica* forest at the Zhanggutai Experimental Forest Farm in Liaoning Province (42°43'–42°51' N, 121°53'–122°22' E), China. These three strains are highly efficient strains with multiple growth-promoting characteristics. Analysis of 16S rRNA sequences was used to identify the three strains. The three strains were inoculated in nutrient broth (NB) (peptone 1%; beef extract 1%; sodium chloride 0.5%, Haibo Biotechnology, China) liquid medium and incubated at 37 °C with shaking (180 rpm) for 24 h, following which the genomic DNA was extracted from each strain using the bacterial genomic DNA isolation Kit DP302 (Beijing Tiangen Biochemical Technology Co., Ltd., China). The 16S rRNA gene primers 27F (5'-agagttgatctcggtcag-3') and 1541R (5'-aaggaggtgatccacgcca-3') were used for amplification (Galkiewicz and Kellogg, 2008). The polymerase chain reaction (PCR) was performed in 25-μL reactions containing 12.5 μL 2 × Taq PCR mix solution, 1 μL forward primer, 1 μL reverse primer, 1 μL template DNA, and 9.5 μL double-distilled H₂O. The reaction conditions were: 94 °C for 1 min, 30 cycles of 94 °C for 20 s, 56 °C for 20 s, and 72 °C for 2 min, followed by a final extension at 72 °C for 5 min. The reaction products were qualified by 0.8% agarose gel electrophoresis and then sent to Ruibo Biotech Co., Ltd (Harbin, China) for sequencing. The NCBI Blast server (<http://www.ncbi.nlm.nih.gov>) was used to compare the sequences with the registered sequences in the GenBank database. A phylogenetic tree was constructed using the maximum likelihood method in the software package MEGA (version 6.0), and the topology of the phylogenetic tree was evaluated using 1,000 bootstrap replicates.

2.2 Determination of the plant growth promotion characteristics of the PGPR strains

The molybdenum blue colorimetric method was used to determine the ability of the strains to dissolve inorganic phosphorus (Shekhar 1999). Specifically, the strain suspension was spot-inoculated on sterile National Botanical Research Institute phosphate (NBRIP) solid medium and cultured at 28 °C for 3 d, during which the formation of a transparent ring was observed. The strains producing transparent circles were inoculated in sterile NBRIP liquid medium and cultured at 28 °C with shaking (180 rpm) for 7 d. A non-inoculation control was set, and then the soluble phosphorus content of the strain culture solution was measured.

The ability of the strains to produce indole-3-acetic acid (IAA) was tested using the methods of Bric (1991) and (Bric et al. 1991). The strain was inoculated in R₂A liquid medium (0.5 g yeast, 0.5 g peptone, 0.5 g casein, 0.5 g glucose, 0.5 g soluble starch, 0.3 g K₂HPO₃, 0.05 g MgSO₄, 0.3 g sodium pyruvate, and 1000 mL H₂O) supplemented with 200 mg/L L-tryptophan and incubated at 28 °C with shaking (180 rpm) for 4 d, following which the strain culture was centrifuged at 12,850 × *g* for 10 min. One milliliter of the culture solution was used to determine the OD₆₀₀ value. The same volume of Salkawaski's reagent (50 mL 35% HClO₄ + 1 mL 0.5 M FeCl₃) was added, and the solution was placed in the dark for 30 min. A color change to red indicated that the strain had produced IAA. The IAA content of the sample was measured at Od₅₃₀, and the yield was calculated as (mg · L⁻¹ · OD₆₀₀⁻¹) = OD₆₀₀ / OD₅₃₀. All tests were conducted in triplicate.

The ability of the strain to act as an iron-producing carrier was assessed using the improved Chrome Azurol S (CAS) test plate (Shin et al. 2001). First, Mannitol Salt Agar (MSA) medium (0.5 g KCl, 0.5 gMgSO₄, 4 g glucose, 5 g casein peptone, 15 g agar, and 1 L distilled water), chromeazuroil S dye solution (1 mM CAS, 0.1 mM FeCl₃, and 4 mM cetyltrimethylammonium bromide), and phosphate solution (0.5905 g NaH₂PO₄ · 2H₂O, 2.427 g Na₂HPO₄ · 12H₂O, 0.25 g NH₄Cl, 0.075 g KH₂PO₄, 0.125 g NaCl, and 100 mL distilled water) were prepared, and then the three solutions were sterilized at 115 °C for 25 min. Before pouring the medium, 50 mL of CAS dye solution and 50 mL phosphate solution were added into 1000 mL MSA medium and mixed well to produce the MSA-CAS detection medium (Chen 2006; Pérez-Miranda et al. 2007; Xiang 2006). The strain suspension was inoculated on the MSA-CAS detection plate and cultured at 28 °C for 7 d, and a non-inoculation control was set. If an orange-yellow circle formed on the blue CAS plate, it indicated that the strain had produced siderophore.

The ability of the strain to produce HCN was measured using the improved method of Kloepper et al. (Kloepper et al. 1991). The strain was inoculated on King's B medium supplemented with 4.4 g/L glycine (20 g peptone, 1.5 g K_2HPO_4 , 1.5 g $MgSO_4 \cdot 7H_2O$, 10 g glycerol, 15 g agar, and 1000 mL distilled water). A filter paper strip soaked in picric acid solution (2.5 g picric acid, 12.5 g Na_2CO_3 , and 1000 mL distilled water) was placed on top of the plate cover and cultured at 28 °C for 3 d. The change in the color of the filter paper strip from yellow to brown to red indicated the generation of HCN. The intensity of the color was visually recorded.

The ability of the strain to produce hydrolase was measured using the method of Cappuccino and Sherman (Cappuccino 2010). The strain suspension was inoculated on skim milk agar medium (100 g skim milk, 5 g peptone, 15 g agar, and 1000 mL distilled water) and starch agar medium (10 g soluble starch, 5 g peptone, 3 g beef extract, 15 g agar, and 1000 mL distilled water) and cultured at 28 °C for 3 d to observe any transparent areas around the spots. The medium was prepared by adding 1% cellulose, 0.5% chitin, and 1% pectin to the basic medium (1 g glucose, 0.5 g yeast extract, 0.5 g $MgSO_4$, 1 g KCl, 1 g $NaNO_3$, 1 g K_2HPO_4 , 15 g agar, and 1000 mL distilled water), and the strain suspension was inoculated into cellulose medium, chitin medium, and pectin medium at 28 °C for 5 d. The cellulose medium was soaked with 0.01% Congo red solution for 15 min, following which the solution was poured out and decolorized with 1% NaCl solution for 5 min to observe whether any transparent areas appeared on the red background. Gram iodine liquid was then poured into the chitin medium and pectin medium, and the appearance of clear areas on the dark blue background was observed.

2.3 Pot experiment

The seeds of *P. sylvestris* var. *mongolica* (purchased from the Zhanggutai Experimental Forest Farm in Zhangwu County, Liaoning Province, China) were surface-sterilized with potassium permanganate (0.5%, v/v) for 30 min and then washed five times with sterile distilled water. They were then germinated on sterile moistened gauze at 25 °C for 5 d. After germination, the seedlings were transferred to plastic pots (15 × 15 cm, 20 seeds per pot) filled with a sterile culture substrate of peat soil/vermiculite/sand (2:1:1, v/v/v). The pots were kept under greenhouse conditions (day/night thermal regime of 22/30 ± 3 °C, and 14 h light/10 h dark photoperiod) and watered every 2 d for 14 d, after which the seedlings were inoculated with the bacterial inoculum (Qi et al. 2019).

Before plant inoculation, the three strains were separately inoculated into 250 mL Erlenmeyer flasks containing 100 mL of NB liquid medium and maintained in 28 °C with shaking (180 rpm) for 48 h. The bacterial cultures were centrifuged at 8000 *g* for 10 min at 4 °C, and the collected bacteria were repeatedly centrifuged, washed with sterilized water three times, and then diluted with sterilized water to achieve an OD_{600} of 0.6, which was the final volume ratio used as the inoculum.

For all treatments, including the control, 10 pots (15 seedlings per pot) were prepared, giving a total of 150 seedlings per treatment. There were four treatments: (1) inoculation with sterile water (CK); (2) inoculation with A13; (3) inoculation with A20; and (4) inoculation with A36. The inoculations were performed by transferring 100 mL of the bacterial inoculum into the planting hole (Yang et al. 2019), where it was introduced at the root system level. The control plants were inoculated with 100 mL of sterile water. All treatments were arranged at random under the greenhouse conditions given above.

2.4 Sampling and biomass analysis of the seedlings

Samples were taken at three months after seedling inoculation. Fifty seedlings from each treatment group were randomly selected, of which 30 were used to measure biomass index, including seedling height (SH), ground diameter (GD), fresh weight of seedling (SFW), dry weight of seedling (SDW), fresh weight of root (RFW), and dry weight of root (RDW). During sampling, damage to the root system of the seedlings was minimized. The root system was washed to remove the soil, following which 10 randomly selected seedlings were used for scanning and grading the root system using an Epson v 700 root scanner. The indexes of root length, surface area, average diameter, number of root tips, bifurcation number, and root volume were analyzed.

2.5 Nutrient and physiological parameter analysis of the seedlings

Ten seedlings of *P. sylvestris* var. *mongolica* were randomly selected for drying. After drying, the roots, stems, and leaves were ground separately and stored in test tubes at room temperature for future use. Total nitrogen (TN) was determined using the Kjeldahl method, and available nitrogen (AN) was determined using the alkaline hydrolysis diffusion method (LY/T 1228–2015). Total phosphorus (TP) was determined by Mo-sb anti-colorimetry, and available phosphorus (AP) was determined using the sodium bicarbonate extraction method (LY/T 1232–2015). Total potassium (TK) was determined by flame photometry, and available potassium (AK) was determined using the NH_4OAc leaching flame photometer method (LY/T 1234–2015). Organic matter (OM) was determined using the potassium dichromate oxidation-external heating method (LY/T 1237–1999). Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malondialdehyde (MDA), plant-soluble sugar (PSS) and proline (PRO) were determined using a kit from the Nanjing Jiancheng Bioengineering Company (Patterson et al. 1984).

2.6 Soil enzyme activities and physiochemical properties analysis

Upon collection of the plants, the topsoil was removed, and the rhizosphere soil was collected and sieved with a 20-mesh screen. Three replicates were tested in each treatment group. Ten grams of rhizosphere soil was obtained from each treatment group and stored at 4 °C for the determination of rhizosphere soil enzyme activity. Fifteen grams of soil from each treatment group was divided into three test tubes on average and stored at -80 °C for measuring soil microbial diversity. The rest of the rhizosphere soil was air-dried and stored at room temperature for measuring rhizosphere soil nutrients. The determination methods of soil TN, AN, TP, AP, TK, AK, and OM were the same as those described in 2.5. The soil acid phosphatase (APA), catalase (CA), sucrase (SA), and urease (UA) activities were measured using the Nanjing Jiancheng Biological Engineering Company kit.

2.7 Bacterial diversity analysis

Three replicate rhizosphere soil samples of the *P. sylvestris* var. *mongolica* seedlings from each treatment group were sieved with a 10-mesh screen and stored in a refrigerator at -80 °C for high-throughput sequencing. High-throughput sequencing was performed by Majorbio Bio-Pharm Technology Co., Ltd (Shanghai, China) on the Illumina Miseq Sequencer platform. FLASH (version 1.2.11) software was used to splice the paired-end sequences (<https://ccb.jhu.edu/software/FLASH/index.shtml>) (Magoc and Salzberg, 2011). Usearch (version 7.0) was used to count operational taxonomic unit (OTU) (<http://www.drive5.com/usearch/>). At the same time, Qiime (version 9.1) was used to duplicate the filters and classify the sequences. The β diversity distance was also calculated (<http://qiime.org/install/index.html>) (Caporaso et al. 2010). Uparse (version 7.0.1090) was used to cluster the sequences with a threshold of 97% similarity (<http://www.drive5.com/uparse/>) (Edgar 2013). RDP Classifier (version 2.2) was used to annotate the sequences using the default confidence threshold of 0.7 (<http://sourceforge.net/projects/rdp-classifier/>).

2.8 Data processing and analysis

Data were processed using Excel 2013 software, one way analysis of variance (ANOVA) of IBM spss19.0 software was used to test the significant differences of plant biomass, root structure, nutrient index, enzyme activity, soil nutrient index, enzyme activity, and relative abundance of different bacterial taxonomic levels. The Duncan test separates the mean when $p < 0.05$, expressed as the mean $\pm$ standard deviation. At the same time, Pearson correlation analysis of spss19.0 software was used to test the relationship between environmental factors, α diversity index and dominant bacteria. Mothur (version 1.30.2) software was used to analyze the α diversity index (https://www.mothur.org/wiki/Download_mothur) (Uroz et al. 2016), and the R 'language' software package was used to produce the Venn diagram, bar diagram, Heatmap diagram and PCoA diagram. Circos-0.67-7 software was used to analyze the proportion of dominant species in each sample. The Vegan package in the R statistical program was used to for the redundancy analysis (RDA).

2.9 Accession number(s)

The 16S rRNA gene sequences of the three strains were submitted and stored in the GenBank database with the accession numbers MT280201 (*Pseudomonas koreensis* A20), MT280202 (*Serratia plymuthica* A13), and MT280203 (*Pseudomonas mandelii* A36).

3 Results

3.1 Identification of PGPR strains and plant growth-promoting characteristics

The 16S rRNA gene sequences of the three strains were analyzed, and phylogenetic trees were constructed (Fig. 1). The similarity between strain A13 and *Serratia plymuthica* DSM 4540 (AJ233433) was 99.00%, and thus the strain was preliminarily identified as *Serratia plymuthica*. The similarity between strain A36 and *Pseudomonas mandelii* (AF058286) was 99.72%, and thus the strain was preliminarily identified as *Pseudomonas mandelii*. The similarity between strain A20 and *Pseudomonas koreensis* Ps 9–14 (AF468452) was 98.80%, and thus the strain was preliminarily identified as *Pseudomonas koreensis*.

The three strains had various plant growth-promoting properties (Table 1). Strains A36, A13, and A20 were able to dissolve inorganic phosphorus, and the solubility of the inorganic phosphorus was 189.50 $\mu\text{g/mL}$, 211.00 $\mu\text{g/mL}$, and 429.50 $\mu\text{g/mL}$, respectively, and thus strain A13 exhibited the highest inorganic phosphorus solubility of 429.50 $\mu\text{g/mL}$. Strains A36, A13, and A20 all had the ability to secrete IAA. The IAA content of the three strains was 18.88 $\mu\text{g/mL}$, 4.59 $\mu\text{g/mL}$, and 22.66 $\mu\text{g/mL}$, respectively. All three strains could produce siderophore. Strains A36 and A20 produced light yellow circles on the CAS detection plate, while strain A13 produced orange-yellow circles. The filter paper strips of strains A13 and A20 changed from yellow to brown after 24 h of inoculation, and from brown to red after 60 h of inoculation. The filter paper strip of strain A36 remained unchanged, indicating that strains A13 and A20 had the ability to produce HCN, while A36 did not.

As indicated in Table 1, the three strains produced transparent areas on skim milk agar medium and starch agar medium, indicating that all three strains could produce protease and amylase. Strains A36 and A20 produced transparent areas on the cellulose medium, indicating that these two strains had the ability to produce cellulase. With the exception of strain A36, which was negative on the chitin medium, the remaining strains were positive and could produce chitinases. All three strains produced clear areas against the dark blue background of the pectin medium, indicating that all three strains had the ability to produce pectinase.

Table 1
Plant growth-promoting characteristics of the three strains

Strains	Hydrolytic enzyme production			Growth promoting traits					HCN production
	Protease	α -amylase	Cellulase	Chitinase	Pectinase	IAA Production	P solubilized	Siderophores production	
A36	+	+	+	-	+	+	+	+	-
A13	+	+	-	+	+	+	+	+	+
A20	+	+	+	+	+	+	+	+	+
(-), not detected; (+), production									

3.2 Effects of PGPR on seedlings biomass and root structure

Inoculation with PGPR microbial inoculum influenced the growth characteristics of the *P. sylvestris* var. *mongolica* seedlings (Fig. 2a). Compared with CK, after inoculation with A13, SH, SFW, RFW and SDW increased the most, increasing by 27.60%, 76.19%, 46.55% and 22.86% respectively ($p < 0.05$). After inoculation with A20, GD increased by up to 22.99% ($p < 0.05$), after inoculation with A36, RDW increased by up to 30.23% ($p < 0.05$).

Root length (RL), root volume (RV) and surface area (SA) are important parameters for plant root distribution. The average diameter (AD), the number of root tips (TIPS) and the number of branches (BN) are important parameters for measuring root absorption efficiency. Inoculation with PGPR had a certain effect on the root structure of *P. sylvestris* var. *mongolica* (Fig. 2b,c). Inoculation with A20 had the greatest effect on the root system, with the greatest increase in RL, SA, AD, and BN, increasing by 47.88%, 63.21%, 10.81% and 57.20% respectively ($p < 0.05$), inoculation of A13 increased BN by up to 30.61%, and inoculation of A36 increased RV by up to 75.00%.

3.3 Effects of PGPR inoculation on seedlings and soil nutrients.

OM and N, P, and K are essential nutrients for plant growth. Inoculation with PGPR had a certain effect on the nutrient content of roots, stems and leaves of *P. sylvestris* var. *mongolica* seedlings (Fig. 3a). The content of OM and TN in the roots of inoculation with A36 increased the most, 52.79% and 21.36%, respectively ($p < 0.05$), the TP content of roots inoculation with A13 increased the most by 28.94% ($p < 0.05$); The content of OM and TN in the stems of inoculation with A36 increased the most, 21.16% and 89.87%, respectively ($p < 0.05$), the TK content of stems inoculation with A13 increased the most by 37.75%; The TN and TK content of leaves inoculation with A20 increased the most, 61.07% and 14.04%, respectively ($p < 0.05$), and inoculation with A20 increased the TP content of leaves by up to 26.85% ($p < 0.05$).

Inoculation with PGPR increased soil nutrient content (Fig. 3b). Inoculation with A13 increased the soil OM, TN and AN content the most, 39.58%, 49.18% and 47.62%, respectively ($p < 0.05$); Inoculation with A20 increased soil TP content by 97.44%; Inoculation with A36 increased the soil AP and TK content the most, 83.32% and 20.16%, respectively ($p < 0.05$); Inoculation of three PGPRs increased the soil AK content significantly. Among them, the inoculation of A36 had the best effect, increasing by 98.10% ($p < 0.05$).

3.4 Effects of PGPR inoculation on seedlings and soil enzyme activities

Inoculation with PGPR has a certain effect on plant enzyme activities (Fig. 4a, b, c, d, e, f). Inoculation with three PGPRs significantly increases POD and PSS by 23.15%-53.70% and 12.66%-27.29%, respectively; Inoculation with A20 increased SOD activity by 16.76% ($p < 0.05$); Inoculation with three PGPRs had a huge increase in CAT and PRO enzyme activities, the increase was 98.35%-128.69% and 283.67%-684.31% ($p < 0.05$), among them, inoculation with A13 had the largest increase in CAT activity, while inoculation with A20 had the largest increase in PRO activity; inoculation with A36, A13 and A20 treatments reduced the MDA content by 36.22%, 56.12% and 13.78% respectively ($p < 0.05$).

Rhizosphere soil enzymes promote soil metabolism, change the form of soil nutrients, improve soil characteristics, and help increase the productivity of plant rhizosphere soil. PGPR inoculation had a certain effect on soil enzyme activity (Fig. 4g, h, i, j). Inoculation of A36, A13 and A20 significantly increased soil SA, increasing by 38.97%, 48.98% and 37.98% respectively ($p < 0.05$); At the same time, inoculation of the three PGPRs significantly increased soil APA by 22.48%, 38.95%, and 21.48% ($p < 0.05$); While only inoculation with A20 significantly increased CA and UA, by 10.45% and 23.00%, respectively ($p < 0.05$).

3.5 High-throughput sequencing analysis of microbial diversity in the rhizosphere soil

3.5.1 Effect of PGPR on the composition of the soil bacterial community

The Venn diagram reflected the similarity and overlap of the samples. Following OTU clustering, separation, and elimination, a total of 3191 bacterial OTUs were obtained with a similarity of 97% (Fig. 5a). The unique bacterial OTUs of the A36, A13, A20, and CK groups were 174, 252, 192, and 200, respectively. There were more unique bacteria in the soil treated with A13, indicating that inoculation with A13 provided a more favorable environment for microbial diversity. CK and A36 shared 1,550 OTUs, CK and A13 shared 1,731 OTUs, and CK and A20 shared 1,550 OTUs, indicating that the bacterial communities of the CK and A13 groups were more similar than those of the other groups.

Analysis of the sequencing results indicated that soil samples from the four treatment groups comprised a total of 27 phyla, 68 classes, 186 orders, 343 families, 641 genera, and 1310 species of soil bacteria. The bar chart shows the species composition of different groups at the phylum level (Fig. 5b). The most abundant bacterial phyla were Acidobacteria, Patescibacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes, Firmicutes, Actinobacteria, and Proteobacteria, which contributed almost 97.2% of the bacterial sequences. In the A36, A13, and A20 treatment groups, the relative abundance of Acidobacteria and Firmicutes was significantly lower than that of the CK group ($p < 0.01$), while the opposite was observed regarding Patescibacteria ($p < 0.01$), the relative abundance of which after PGPR treatment was significantly higher than that of the CK group. There was no significant difference in the relative abundance of Proteobacteria. The relative abundance of Bacteroidetes in the A20 group was not significantly different from that of the CK group, but was significantly higher than that of the A36 and A13 groups ($p < 0.01$). The relative abundance of Gemmatimonadetes in the A36 group was not significantly different from that of the CK group, but it was significantly higher than that of A20 and A13 groups ($p < 0.01$). The relative abundance of Actinobacteria in the A36 and A20 groups was significantly higher than that of CK and A13 groups ($p < 0.01$).

The cluster analysis by heat map showed the community composition among the samples (Fig. 5c). The sample clustering results showed that the A13 group and the CK group were relatively similar, and the A36 group and the A20 group were relatively similar. The A36, A20, and CK groups were clearly separated, indicating that the soil bacterial community changed after the addition of PGPR. The relative abundance of *Arthrobacter* in the CK group was 3.48%, while the relative abundance of *Arthrobacter* was the highest in the A36 and A20 groups at 9.61% and 12.15%, respectively, showing a significant increase ($p < 0.05$). In the A13 group, the relative abundance of *Serratia* was the highest at 5.42%, while in the CK group, the relative abundance of *Serratia* was 0, indicating that the relative abundance of *Serratia* increased significantly in the A13 group ($p < 0.05$).

3.5.2 Relationship between soil bacterial community composition and soil environmental factors

The relative abundance of bacterial dominant genera in 12 soil samples (Table 2), a total of 28 bacterial genera accounted for more than 1% of the total community of all treatment groups, and the bacterial dominant genera of each treatment group showed differences ($p < 0.01$). Compared with CK, inoculation with PGPR significantly reduced the relative abundance of *Burkholderia-Caballeronia-Paraburkhol*, *Nocardioides* and *Blastococcus* ($p < 0.01$), and significantly increased the relative abundance of *Mesorhizobium*, *Tumebacillus*, *Conexibacter* and *Caenimonas* ($p < 0.01$). There were also differences in the dominant genera of bacteria.

Table 2
The community composition of soil bacteria after inoculation of different treatment groups

Genera	A36	A13	A20	CK
<i>Arthrobacter</i>	10.67 ± 0.58 B	5.03 ± 0.73 C	14.00 ± 1.00 A	3.87 ± 1.50 C
<i>Ramlibacter</i>	6.47 ± 0.49 B	9.30 ± 0.35 A	6.20 ± 0.20 B	11.47 ± 1.86 A
<i>Gemmatimonas</i>	9.53 ± 0.57 AB	7.97 ± 0.40 B	5.87 ± 0.21 C	9.93 ± 1.10 A
<i>Bacillus</i>	6.63 ± 0.31 A	6.87 ± 0.32 A	9.70 ± 2.25 A	9.37 ± 0.71 A
<i>Massilia</i>	8.10 ± 0.40 B	6.73 ± 0.25 C	10.67 ± 0.58 A	7.97 ± 0.23 B
<i>Burkholderia-Caballeronia-Paraburkhol</i>	12.30 ± 2.56 A	7.73 ± 0.50 BC	8.87 ± 1.47 AB	3.73 ± 0.83 C
<i>Sphingomonas</i>	11.67 ± 1.15 A	3.77 ± 0.61 B	12.00 ± 0.00 A	5.80 ± 0.75 B
<i>Pseudolabrys</i>	7.27 ± 0.06 B	8.50 ± 1.30 AB	9.87 ± 0.23 A	7.40 ± 0.43 B
<i>Nocardioides</i>	12.33 ± 1.53 A	7.30 ± 1.08 AB	4.37 ± 0.67 B	9.53 ± 4.84 AB
<i>Tumebacillus</i>	0.42 ± 0.08 D	13.33 ± 1.15 B	3.17 ± 0.23 C	16.33 ± 0.58 A
<i>Serratia</i>	7.00 ± 3.56 B	22.33 ± 0.58 A	3.77 ± 0.98 BC	0.01 ± 0.00 C
<i>Candidatus_Solibacter</i>	5.67 ± 0.25 B	9.43 ± 1.43 A	6.70 ± 0.72 B	11.67 ± 0.58 A
<i>Flavisolibacter</i>	8.23 ± 0.35 AB	7.20 ± 0.66 B	8.73 ± 0.61 AB	9.70 ± 1.15 A
<i>Streptomyces</i>	9.20 ± 4.85 A	11.00 ± 1.00 A	3.80 ± 0.20 A	6.83 ± 0.25 A
<i>Mesorhizobium</i>	6.00 ± 0.44 C	7.77 ± 0.60 B	8.07 ± 0.31 B	11.33 ± 0.58 A
<i>Devosia</i>	8.37 ± 0.06 AB	9.30 ± 0.20 A	7.87 ± 0.29 B	7.77 ± 0.67 B
<i>Conexibacter</i>	4.43 ± 0.72 C	10.63 ± 0.63 A	8.37 ± 0.57 B	10.00 ± 0.91 AB
<i>Terrabacter</i>	13.33 ± 1.53 A	2.63 ± 0.31 B	13.67 ± 1.53 A	3.87 ± 1.07 B
<i>Bryobacter</i>	5.63 ± 0.06 A	33.27 ± 43.08 A	7.80 ± 0.44 A	8.63 ± 0.40 A
<i>Lysobacter</i>	6.20 ± 0.75 B	9.87 ± 1.20 A	8.63 ± 0.40 AB	8.50 ± 1.21 AB
<i>Rhodanobacter</i>	7.57 ± 1.42 B	10.93 ± 1.10 A	8.60 ± 0.40 AB	6.40 ± 1.06 B
<i>Micropepsis</i>	8.90 ± 0.72 A	9.40 ± 0.56 A	8.77 ± 0.72 A	6.10 ± 0.62 B
<i>Caenimonas</i>	0.97 ± 0.14 C	9.03 ± 0.25 B	9.23 ± 0.35 B	14.33 ± 1.53 A
<i>Pullulanibacillus</i>	5.97 ± 0.55 B	2.77 ± 0.25 B	19.67 ± 3.21 A	4.60 ± 0.56 B
<i>Pseudomonas</i>	9.87 ± 7.22 A	10.17 ± 0.76 A	11.83 ± 2.84 A	1.42 ± 0.93 A
<i>Blastococcus</i>	13.67 ± 3.05 A	6.73 ± 1.70 B	4.70 ± 1.24 B	8.10 ± 0.69 B
<i>Dyella</i>	3.50 ± 0.90 B	3.30 ± 0.36 B	25.00 ± 1.73 A	2.17 ± 0.35 B
<i>Pantoea</i>	0.04 ± 0.07 A	32.93 ± 42.50 A	0.00 ± 0.00 A	0.40 ± 0.43 A

The Pearson rank correlation was used to evaluate the relationship between the dominant bacterial genera and environmental factors (Table 3). There was a Pearson correlation between the dominant bacterial genera (RA > 1%) and environmental factors. The relative abundances of *Arthrobacter*, *Bacillus*, *Massilia*, *Burkholderia-Caballeronia-Paraburkhol*, *Pseudolabrys*, *Streptomyces*, *Devosia*, *Bryobacter*, *Rhodanobacter*, *Micropepsis*, *Pseudomonas*, *Blastococcus* and *Pantoea* were positively correlated with environmental factors, on the contrary, *Ramlibacter*, *Sphingomonas*, *Nocardioides*, *Tumebacillus*, *Candidatus_Solibacter*, *Flavisolibacter*, *Mesorhizobium*, *Conexibacter* and *Caenimonas* were significantly negatively correlated with environmental factors ($P < 0.05$), and other genera did not have any significant correlations (Table 2). The content of OM, TP, AP, TK and AK in soil may be the main driving force affecting the composition of bacterial communities.

Table 3

Pearson correlation data between dominant bacterial genera and soil environmental factors after inoculation in different treatment groups

Genera	OM	TN	AN	TP	AP	TK	AK	SA	CA	UA
<i>Arthrobacter</i>	-	-	-	0.951*	-	-	-	-	-	-
<i>Ramlibacter</i>	-	-	-	-0.993**	-	-	-	-	-	-
<i>Bacillus</i>	-	-	-	-	-	-	-	-	0.944**	-
<i>Massilia</i>	-	0.911*	-	-	-	-	-	-	-	-
<i>Burkholderia-Caballeronia-Paraburkhol</i>	-	-	-	-	0.907*	0.903*	-	-	-	-
<i>Sphingomonas</i>	-0.920*	-	-	-	-	-	-	-	-	-
<i>Pseudolabrys</i>	-	-	-	-	-	-	-	-	-	0.935*
<i>Nocardioide</i>	-	-	-	-	-	-	-	-	-	-0.949**
<i>Tumebacillus</i>	-	-	-	-0.926*	-	-	-	-	-	-
<i>Candidatus_Solibacter</i>	-	-	-	-0.949**	-	-	-	-	-	-
<i>Flavisolibacter</i>	-	-	-	-	-	-	-	-0.906*	-	-0.988**
<i>Streptomyces</i>	-	0.967**	-	-	-	-	-	-	-	-
<i>Mesorhizobium</i>	-	-	-	-	-	-	-0.950**	-	-	-
<i>Devosia</i>	-	0.913*	-	-	-	-	-	-	-	-
<i>Conexibacter</i>	-	-	-	-	-0.982**	-0.945**	-	-	-	-
<i>Bryobacter</i>	0.982**	-	-	-	-	-	-	-	-	-
<i>Rhodanobacter</i>	-	-	0.972**	-	-	-	-	-	-	-
<i>Micropepsis</i>	-	-	-	-	-	-	0.972**	0.999**	-	-
<i>Caenimonas</i>	-	-	-	-	-0.950**	-0.972**	-	-	-	-
<i>Pseudomonas</i>	-	-	-	-	-	-	0.965**	0.937*	-	-
<i>Blastococcus</i>	-	-	-	-	-	-	-	-	-	0.911*
<i>Pantoea</i>	0.936*	-	0.916*	-	-	-	-	-	-	-

3.5.3 Diversity of bacterial community

The coverage of the four soil libraries ranged from 0.987 to 0.990 (Fig. 6a), which was close to 1, indicating that the sequencing depth covered all the species of the soil sample, suggesting that the sequencing results represent the true situation of the soil bacteria. The order of the Chao index was A13 > CK > A20 > A36 (Fig. 6b), and except for the A13 group, there was no significant difference between the other groups. This indicated that the total number of soil bacterial species in the A13 group was higher than the other groups, and the community richness was also higher than the other groups. The Shannon index performance was: A13 > CK > A36 > A20 (Fig. 6c). The larger the Shannon value, the higher the community diversity. The Simpson index was as follows: A20 > A36 > CK > A13 (Fig. 6d). The larger the Simpson value, the lower the community diversity. The A13 group index was significantly higher than other groups, indicating that the A13 group community diversity was higher.

Pearson analysis was used to analyze the correlation between the α diversity index and environmental factors (Fig. 6e). Chao and Shannon indexes were significantly positively correlated with TN and AN content ($p < 0.05$), and were extremely significantly positively correlated with OM content ($p < 0.01$). The Coverage index was significantly positively correlated with TP and AK content ($p < 0.01$). The Simpson index was positively correlated with TP content, but was negatively correlated with OM content. These findings indicate that PGPR has a crucial effect on soil nutrient cycling.

3.5.4 Redundancy analysis of soil bacteria (RDA)

The relationship between soil physicochemical properties and the relative abundances of dominant bacteria was assessed using RDA at the genus level (Fig. 7). The A20 sample was positively correlated with urease activity, but negatively correlated with TN and OM. A36 was positively correlated with TK and AP, and negatively correlated with AN, acid phosphatase activity, and CAT activity. A13 was positively correlated with OM and TN, and negatively correlated with TP. The RDA showed that the relative abundance of *Arthrobacter*, *Sphingomonas* was positively correlated with AP; the relative abundance of *Arthrobacter* and *Sphingomonas* was positively correlated with TP; the relative abundance of *Arthrobacter* was positively correlated with AK; and the relative abundance of *Serratia* and *Tumebacillus* was positively correlated with OM. The Monte-Carlo permutation test indicated that the AP, TP, AK, and OM indicators of the soil were significantly related to the bacterial community composition at the genus level (AP: pseudo-F = 14.35, $p = 0.004$; TP: pseudo-F = 7.96, $p = 0.02$; AK: pseudo-F = 6.13, $p = 0.03$; OM: pseudo-F = 10.30, $p = 0.009$), indicating that soil AP, TP, AK, and OM were the main environmental factors influencing the composition of the microbial communities (at the genus level).

4 Discussion

Current agricultural production practices need to prioritize environmental sustainability, and thus the use of soil microorganisms has been suggested as a promising alternative to harmful pesticides and fertilizers, as well as for increasing crop yield (Wertz et al. 2007). PGPRs not only promote plant growth, control plant disease, and increase crop yield, but also exhibit strong rhizosphere colonization ability and specific microecological functions, and are thus potentially important biocontrol microorganisms (Schippers et al. 2003). Currently, the most representative PGPR genera that have been discovered are *Pseudomonas*, *Enterobacter*, *Clostridium*, *Arthrobacter*, *Achromobacter*, *Micrococcus*, *Flavobacterium*, *Azospirillum*, *Azotobacter*, and *Bacillus* (Cristiana et al. 2008; Swain and Ray, 2009). The three types of PGPR used in this study were *P. mandelii*, *S. plymuthica*, and *P. koreensis*. *Pseudomonas* rhizosphere bacteria have been extensively studied, though the two species used in the present experiment have been studied comparatively less. For instance, Ródenas et al. found that *P. mandelii* strain 29 could significantly increase mycorrhizal colonization (Navarro-Ródenas et al. 2016), while another study found that *P. mandelii* was associated with rice plant nitrogen fixation. *Pseudomonas koreensis* has not been reported on much, though *P. koreensis* Ps 9-14T has been isolated from the soil (Kwon et al. 2003), and another study discovered that *P. koreensis* could enhance the drought resistance of *Helianthus annuus* (Macleod et al. 2015). A *P. koreensis* JDM-2 strain was isolated from *Eucommia ulmoides* roots and exhibited ACC deaminase activity, and found that it had significant antibacterial effect on *Bacillus subtilis* (Gong 2011). Additionally, the genome sequence of *P. koreensis* CRS05-R5 strain has been analyzed (Lin et al. 2016). One study found that *S. plymuthica* BU09 had an obvious control effect on potato scab (Zhang and Liu, 2017), while another discovered that *S. plymuthica* A21-4 could improve cucumber quality and regulate the micro-ecological environment of cucumber rhizosphere soil (Ding et al. 2018). In this study, three treatment methods, including inoculation, compound inoculation, and non-inoculation, were used. The bacterial suspension was perforated and injected into the rhizosphere soil. The results showed that the seedling height, ground diameter, fresh weight, and dry weight of the *P. sylvestris* var. *mongolica* seedlings increased significantly after PGPR inoculation, which was consistent with previous results whereby the PGPRs *Serratia proteamaculans* 1-102 and *Serratia liquefaciens* 2-68 could promote the emergence and growth of corn (Pan et al. 1999). Thiery et al. found that the PGPR *Bacillus amyloliquefaciens* FZB 42 could promote cotton growth (Alavo et al. 2015), indicating that PGPRs can effectively promote plant growth.

In this study, the root length, surface area, number of root tips, and root volume of the seedlings increased significantly after PGPR inoculation, which may be related to the secretion of IAA by the three PGPR strains. A previous study showed that the effect of PGPR on root development is related to the secretion of IAA, as IAA can promote root growth and development, increase root surface area, and promote root metabolism (Glick 2014). Additionally, the inoculation of IAA-secreting PGPR on canola significantly increased the number of stems and branches (Asghar et al. 2004). The PGPR strains CA1001 and CA2004, which can secrete IAA, were inoculated into crops to enhance root and stem biomass (Chandra et al. 2018). Therefore, the increase in the root structure index of *P. sylvestris* var. *mongolica* seedlings may be mainly due to the IAA secreted by the PGPR. There is further evidence to illustrate this point; that is, among the three PGPR strains, A20 secreted the highest amount of IAA (22.66 µg/mL), and inoculation with strain A20 increased the root length, root surface area, root average diameter, and the number of root tips of the seedlings most significantly.

Plant enzymes such as SOD, POD, and CAT can effectively remove the reactive oxygen generated during metabolism, thus balancing the production and removal of active oxygen in the plant and preventing reactive oxygen from causing membrane peroxidation and other damage (Gururani and Upadhyaya, 2013; Habib et al. 2016). In this study, compared with CK, inoculation of A36, A20, and A13 significantly increased the POD, SOD, and CAT activity by 23.15–53.70%, 16.05–16.76%, and 98.35–128.69%, respectively ($p < 0.05$). This showed that the antioxidant enzyme activity of the *P. sylvestris* var. *mongolica* seedlings increased after inoculation with PGPR, and previous reports have suggested that SOD, POD, and CAT are important indicators of plant resistance induced by PGPR (Hahm et al. 2017; Uddin et al. 2015). This was confirmed by another study (Jha and Subramanian, 2013) that showed that after PGPR inoculation, the antioxidant enzyme activity of rice increased, thereby increasing plant resistance to salt stress.

PGPRs not only promote plant growth but also improve the rhizosphere environment of the plants. Soil enzyme activity can reflect the transformation ability of soil nutrients and the health of soil to a certain extent (Zhang et al. 2014). Inoculation with PGPRs was previously

found to significantly increase soil enzyme activity, total nitrogen, available nitrogen, and soil organic matter content (Ju et al. 2019). In this study, the same trend was observed. After inoculation with PGPR, the nutrient content of TP, AP, AK, and other nutrients in the rhizosphere soil of *P. sylvestris* var. *mongolica* increased significantly, and enzyme activities such as invertase and acid phosphatase were also increased. Some studies have demonstrated a close relationship between soil enzyme activity and soil available nutrients (Lian et al. 2011). For example, Li et al. found that soil CAT had a significant negative correlation with available phosphorus, and that urease had a significant positive correlation with available potassium (Ning et al. 2014). Another study found that soil CAT and phosphatase were significantly positively correlated with available nitrogen (Chen et al. 2014). In this study, after inoculation with the PGPRs, the enzyme activity of the rhizosphere soil increased, following which the content of available nutrients increased. The content of available nitrogen, phosphorus, and potassium increased the most, namely by 109.52%, 143.50%, and 98.10%, respectively. To some extent, the content of nitrogen, phosphorus, potassium, and other nutrients in the soil represents the potential fertility of the soil (Wang et al. 2015). Inoculation with PGPRs effectively improved the nutrient content of the rhizosphere soil of *P. sylvestris* var. *mongolica* and improved the ability of the plant to tolerate its external environment. In this study, it was found that after PGPR inoculation, in addition to the significant increase in biomass, the nutrient content and enzyme activity of the seedlings also increased. Many reports have suggested that PGPRs improve the biomass and the nutrient and enzyme activity of plants, thereby promoting plant growth and metabolism, such as in wheat and spinach (Akmal et al. 2007), pea (Akhtar 2014), and organically-grown raspberry (Orhan et al. 2006).

Inoculation with rhizosphere bacteria enriches some dominant bacterial groups, including some beneficial bacteria that participate in soil nutrient cycling, improves soil texture, and prevents and controls plant diseases. In this study, after inoculation with PGPR, some growth-promoting bacterial genera in the rhizosphere soil increased significantly, such as *Arthrobacter*, *Ramlibacter*, *Gemmatimonas*, *Bacillus*, and *Serratia*. Of these, *Arthrobacter* is a beneficial functional bacteria that can improve the IAA content and salt tolerance of plants (Velázquez-Becerra et al. 2011). One of the important characteristics of the genus *Bacillus* is that it can produce spores with special resistance under adverse conditions, thus playing an active role in preventing plant diseases, improving plant resistance, and promoting plant growth (Oliveira et al. 2010; Probanza et al. 2002). *Serratia* is a beneficial bacteria that can tolerate heavy metals and repair plants, playing an important role in protecting the environment (76). *Actinobacteria* is a Gram-positive bacteria that can degrade cellulose and chitin as the main resource for soil nutrient supply. A recent study found that after applying nitrogen fertilizer, the abundance of *Actinobacteria* in the Gurbantünggüt Desert soil increased significantly (Huang et al. 2018), which is similar to the results of this study. After inoculation with PGPRs, the relative abundance of *Actinobacteria* in the rhizosphere soil was significantly positively correlated with soil AN content ($p < 0.05$). *Chloroflexi* is a Gram-negative bacteria that can potentially autotrophically metabolize through photosynthesis. This study found that after inoculation with PGPR, the relative abundance of *Chloroflexi* in the rhizosphere soil was significantly negatively correlated with soil AN content ($p < 0.05$), which is similar to the results of Ren et al. (Ren et al. 2020) who found that after inoculation with biochar + PGPR, the soil nitrogen content increased, while the abundance of *Chloroflexi* decreased. The cluster analysis results showed that the bacterial communities treated by strains A20 and A36 differed significantly from those treated with strains A13 and CK at the genus level, indicating that inoculation with strains A20 and A36 altered the soil bacterial community. The RDA results showed that soil AP, TP, AK, and OM indicators significantly affected the bacterial community composition, suggesting that there are the main environmental factors that contribute to the differences in microbial community composition.

List Of Abbreviations

AK, Available potassium; AN, Available nitrogen; AP, Available phosphorus; CAS, Chrome Azurol S; CAT, Catalase; IAA, Indole-3-acetic acid; MDA, Malondialdehyde; MSA, Mannitol Salt Agar; NB, Nutrient broth; OM, Organic matter; NBRIP, National Botanical Research Institute Phosphate; PCR, Polymerase Chain Reaction; PGPR, Plant Growth Promoting Rhizobacteria; POD, Peroxidase; PSS, Plant-soluble Sugar; PRO, Proline; PTP, Plant total protein; SOD, Superoxide dismutase; TK, Total Potassium; TN, Total Nitrogen; TP, Total Phosphorus.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

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Availability of data and materials

Not applicable

Competing interests

All the authors declare no conflict of interest.

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Authors' contributions

“Conceptualization, X-S. S. and X.D.; methodology, Q.S. and X.D.; software, Q.S., J-Y.L., J-K.W. and K.M.; resources, R-Q.S.; data curation, Q.S.; writing—original draft preparation, Q.S.; writing—review and editing, X.D. and X-S. S.; project administration, R-Q.S.; funding acquisition, R-Q.S. All authors have read and agreed to the published version of the manuscript.”

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Figures

Figure 1

Phylogenetic tree of bacterial 16S rRNA gene sequences. Bootstrap values are indicated at tree branching points.

Figure 2

Biomass and root system indexes after inoculation in different treatment groups (a), biomass index (b), root system index (c), root system.

Figure 3

Nutrient index of seedlings and soil after inoculation in different treatment groups. (a), The nutrient index of seedling (b), the nutrient index of soil.

Figure 4

The enzyme activity indexes of seedlings and soil after inoculation in different treatment groups. (a) POD index of seedling, (b) SOD nutrient index of seedling, (c) CAT index of seedling, (d) MDA index of seedling, (e) PRO index of seedling, (f) PSS index of seedling, (g) SA index of soil, (h) CA index of soil, (i) UA index of soil, (j) APA index of soil.

Figure 5

The species composition of soil after inoculation in different treatment groups (a) Species venn diagram, (b) Community Bar diagram (c) Community Heatmap diagram, the species cluster relationship is shown on the left tree. The sample cluster relationship is displayed on the top tree.

(e)

Index	Coverage	Chao	Simpson	Shannon
OM		0.937**	0.873*	0.993**
TN	-	0.855*	-	0.847*
AN	-	0.922*	-	0.828*
TP	0.964**	-	0.848*	-
AK	0.955**	-	-	-

Figure 6

The relationship between soil bacterial alpha diversity index and environmental factors after inoculation of different treatment groups. (A) Coverage index, (b) Chao index, (c) Simpson index, (d) Shannon index, (e) α diversity index and its Pearson correlation with environmental factors, * Significantly correlated at the 0.05 level, ** Significantly correlated at the 0.01 level.

RDA on Genus level

Figure 7

Redundancy analysis of the composition of the soil bacterial community and soil physiochemical properties at the genus level. Bacterial genera are represented by blue lines, and soil physiochemical properties (environmental factors) are represented by red lines (1: Tumebacillus, 2: Ramlibacter, 3: Candidatus, 4: Brevundimonas, 5: Bacillus, 6: Serratia, 7: Gemmatimonas, 8: Nocardioides, 9: norank_f_67-14, 10: Pseudolabrys, 11: Massilia, 12: Sphingomonas, 13: Arthrobacter, 14: Paraburkholderia, and 15: Saccharimonadales).