

Distribution characteristics of endophytic bacteria and endophytic nitrogen fixing bacteria in *Vicia faba* root nodules in rocky desertification areas of Southwest China

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Abstract

We conducted an experiment to explore the community structure and distribution characteristics of endophytic bacteria and endophytic nitrogen fixing bacteria in wild *Vicia faba* root nodules in rocky desertification area of Honghe Prefecture Southwest China, determine the related soil physical and chemical properties, and explore the key influencing factors of microbial groups, so as to provide a scientific basis for the screening and utilization of functional microorganisms in *Vicia faba* root nodules. Wild *Vicia faba* nodule samples were collected from four different rocky desertification areas in Honghe. The 16S rDNA gene and *nifH* gene of *Vicia faba* nodule endophytic bacteria were sequenced based on high-throughput sequencing technology. The diversity and community structure of endophytic bacteria and endophytic nitrogen fixing bacteria were analyzed by bioinformatics. The results show that: (1) A total of 451 endophytic bacteria OTU were obtained after annotation and classification of four groups of samples of *Vicia faba* root nodules, and the dominant phylum of endophytic bacteria was Proteobacteria; The dominant bacteria were *Pseudomonas* and *Rhizobium*. The composition of bacterial community in the four rocky desertification areas based on species classification was significantly different ($R = 0.577$, $P = 0.001$). *Rhizobium* was significantly positively correlated with soil available phosphorus content and soil rocky desertification degree, and negatively correlated with soil water content, indicating that soil available phosphorus content, soil rocky desertification degree and soil water content were the main physical and chemical factors affecting the composition of endophytic bacterial community in *Vicia faba* nodules; (2) A total of 309 endophytic nitrogen fixing bacteria OTU were obtained from four groups of samples of *Vicia faba* nodules after annotation and classification. The dominant bacteria of endophytic nitrogen fixing bacteria was Proteobacteria; The dominant bacteria are *Bradyrhizobium* and *Rhizobium*. There were significant differences in the composition of nitrogen fixing bacteria community among the four groups ($R = 0.194$, $P = 0.054$). Soil total phosphorus, total nitrogen content and soil pH were the main physical and chemical factors affecting the composition of endophytic nitrogen fixing bacteria community in *Vicia faba* nodules. The diversity study can reveal the differences in the community structure of endophytic bacteria and the relationship with related species, so as to reveal the diversity of endophytic bacteria in rocky desertification legume nodules, and analyze the relationship between soil physical and chemical properties and the composition and nutrients of endophytic microbial community in nodules, so as to provide a theoretical basis for the governance and restoration of rocky desertification areas.

Introduction

The main rock type of Karst areas in Southwest China is carbonate rock, which has strong weathering resistance and slow soil formation rate (Lin JY et al. 2022). Yunnan Province is one of the main provinces with the most serious rocky desertification in China (Zhu MZ 2012). Due to the pure thickness of carbonate rock stratum, low soil forming ability and serious water leakage, this field has become the world's largest exposed and semi exposed Karst area and a fragile ecological area with widely developed rocky desertification (Li S et al. 2007). Honghe Prefecture of Yunnan Province is a key area for the implementation of rocky desertification control, with a total karst area accounting for more than 53% of the state's land area, and is listed in the rocky desertification control and monitoring list of China (Min YP 2015). The sensitivity and vulnerability of karst ecological environment in Honghe have become the main constraints on the development of the region, and accelerating the process of rocky desertification control has become one of the important work of the local government (Zhai LL et al. 2019). The effect of rocky desertification control often takes a long time to show, which is not visible in the short term (Luo DH et al. 2010). The key points of rocky desertification control are to improve the vegetation coverage, conserve water and soil, thereby increasing the content of carbon, nitrogen and phosphorus in the soil (Guan LT et al. 2010).

The symbiotic nitrogen fixation of legumes and rhizobia accounts for about 80% of the total biological nitrogen fixation (Werner Gijssbert DA 2015; Chen LY et al. 2021). The most important nitrogen fixation group in nature is leguminous nitrogen fixation plants, which are also the most important nitrogen source in biological nitrogen fixation system (Mhamdi R et al. 2014; Tang MD et al. 2011). Rhizobia can coexist with legumes, form nodules in their roots or stems, and fix nitrogen in the air to provide nutrition for plants (Werner Gijssbert DA 2015). *Vicia faba* and other leguminous plants, through symbiotic nitrogen fixation with rhizobia, convert molecular nitrogen into ammonia nitrogen. Therefore, the improvement effect of legumes on total nitrogen, available nitrogen and organic carbon in soil is significantly higher than that of non legumes (Guan LT et al. 2010). Legumes can effectively reduce the loss of runoff, sediment and nutrients (Ye N et al. 2023), which has always been the focus of nitrogen fixing microorganisms research, so they are considered to be the most effective pioneer plants for adverse ecological restoration and remediation of poor degraded soil (Liu Y et al. 2022).

Endophytic bacteria in plants have rich biodiversity and are distributed in different classes and phyla (Rosenblueth et al. 2006). In recent years, the research hotspots of plant endophytic bacteria mainly focus on finding new bioactive substances from plant endophytic bacteria. It can not only regulate the growth and stress-related plant hormones, promote the absorption of nutrients by plants, but also indirectly control the infection of pests and pathogenic microorganisms through secretory substances such as antibiotics or activate plant defense, so as to improve plant health. Endophytes induce plants to produce induced resistance, which is more efficient and residue free in plant disease control, and has less impact on the environment (Zhang JY et al. 2014). In the long-term coevolution, rhizobia and host legumes recognize each other to establish a symbiotic relationship (Yan J et al. 2014), so as to increase the symbiotic nitrogen fixation efficiency, reduce nitrogen fertilizer application, and develop green and sustainable agriculture (He C et al. 2021). Biological nitrogen fixation refers to the process in which molecular nitrogen in the atmosphere is reduced to ammonia by nitrogenase in microorganisms (nitrogen fixing organisms). It is one of the important sources of soil nitrogen. Nitrogen fixing microorganisms contain *nifH* gene encoding nitrogenase ferritin, and the phylogeny of *nifH* gene is significantly consistent with 16S rRNA (Young JPW 1992). Therefore, *nifH* gene has become an ideal target for studying nitrogen fixing microorganisms (Raymond J et al. 2004).

To sum up, this experiment took *Vicia faba* nodule and its rhizosphere soil in the rocky desertification area of Honghe Prefecture, Yunnan Province as the research object, determined the soil nutrients, and explored the relationship between the diversity of endophytic bacteria in nodule and the environment. Taking 16S rDNA gene and *nifH* gene as molecular targets, high-throughput sequencing and data analysis were carried out to evaluate the diversity of endophytic bacteria in *Vicia faba* nodules in rocky desertification area of Honghe Prefecture, and reveal the relationship between the diversity of endophytic bacteria and nitrogen fixation efficiency. The diversity study can reveal the differences in community structure of endophytic bacteria in rocky desertification legume nodules and the relationship with related species, thus revealing the diversity of endophytic bacteria in rocky desertification legume nodules, and providing a reference for the correlation between endophytic bacteria, endophytic nitrogen fixing bacteria and environmental factors, and providing a scientific basis for biological control of rocky desertification.

Materials and methods

Test sample

The tested *Vicia faba* nodules were collected from Jianshui County, Gejiu City and Kaiyuan City in Honghe Prefecture of Yunnan Province with different degrees of rocky desertification (Zhong WCH et al. 2013). The information of sample collection place is shown in Table 1. Each group of samples was set with 3 biological replicates, a total of 12 samples.

Table 1
Information on sample collection locations

Group	Nodule host	Collection site	Longitude and latitude	Altitude(m)	Rocky desertification degree/RD
GJ3	<i>Vicia faba</i>	Gejiu City tin town	Longitude 23°21'2" Latitude 103°9'1"	1792.99	Moderate
GJ8	<i>Vicia faba</i>	The old city of old factory town selected mining area	Longitude 23°18'28" Latitude 103°12'24"	2341.36	Moderate
JS6	<i>Vicia faba</i>	Jianshui County mian dian town village	Longitude 23°37'47" Latitude 103°1'47"	1263.02	Mild
KY4	<i>Vicia faba</i>	Big iron section, Dazhuang Township, Kaiyuan City	Longitude 23°37'20" Latitude 103°18'31"	1307.15	Moderate

Experimental method

Sample collection and processing

The whole plant of wild *Vicia faba* was dug out with a sterile shovel, and the roots of different plants were packed in sterile preservation tubes, stored in 0°C ice box and brought back to the laboratory. Select *Vicia faba* nodules with full shape and large volume, rinse the soil attached to the nodules with running water (Zhang JY et al. 2014), and then rinse them with sterile water for 5 times. The cleaned root nodules were dried with filter paper, soaked in 95% ethanol for 5min, taken out and then put into 2% sodium hypochlorite solution for surface sterilization for 3min, and then put into sterile water for multiple cleaning. The last cleaned sterile water was cultured on LB medium to record that there was no microbial growth, so as to ensure complete disinfection of the surface. The samples were put into sterile preservation tubes for standby.

Shake off the soil attached to the root of *Vicia faba*, collect the soil 0 ~ 2mm attached to the root as the rhizosphere soil (Bao SD 2000), and package the rhizosphere soil with a sterile bag for the determination of soil physical and chemical properties (Li H et al. 2020).

Determination of soil physical and chemical properties in the sample collection area

Use pH meter (geomagnetic phs-3c) (water: soil = 2.5:1) to measure soil pH; The total nitrogen(TN) content was determined by Kjeldahl method; The content of alkali hydrolyzable nitrogen(AN) was determined by diffusion absorption method; The total phosphorus (TP) content was determined by molybdenum antimony anti colorimetric UV spectrophotometry (Cui WF et al. 2023), and the available phosphorus (AP) content was determined by extraction method; The soil water/moisture content(SWC) was determined by drying method.

Extraction of total DNA of endophytic bacteria from *Vicia faba* root nodules

Microbial DNA was extracted using hipure soil DNA Extraction Kit (Magen, Guangzhou, China) in accordance with the operation guide.

High throughput sequencing of bacterial 16S rDNA

PCR amplification:

The primers for the target region of 16S rRNA gene (v5-v7 region: 799F 5'-aacmgttacacckg-3', 1193R 5'-acgtcatccccaccttcc-3') were used.

PCR amplification conditions:

The samples were pre denatured at 95°C for 5min, then denatured at 95°C for 1min, annealed at 60°C for 1min, and extended at 72°C for 1min for 30 cycles. Finally, the samples were extended at 72°C for 7min, and then kept at 4°C for 2% agarose gel electrophoresis detection.

Illumina sequencing:

PCR products were purified using axyprep DNA gel extraction kit (axygen Biosciences, Union City, CA, USA) according to the instructions. ABI steponeplus real-time PCR system (life technologies, Foster City, USA) was used for quantification. The purified amplicon was sequenced on the Illumina platform of Shanghai Meiji Biomedical Technology Co., Ltd. (PE250) according to the standard operation.

High throughput sequencing of *nifH* gene of endophytic nitrogen fixing bacteria

PCR amplification:

nifH gene primers (*nifHF* 5'-aaaggyggwatcgaaartccaccac-3'; *nifHR* 5'-ttgttsgcsgcrtacatccatcat-3') were used.

PCR amplification conditions:

The samples were pre denatured at 95°C for 3min, then denatured at 95°C for 30s, annealed at 60°C for 30s, and extended at 72°C for 42s for 30 cycles, and finally extended at 72°C for 10min. after that, the samples were kept at 4°C for 2% agarose gel electrophoresis detection.

Illumina sequencing: Method as above.

Bioinformatics analysis

Quality control was performed on the data, and clustering and dechimeras were obtained to obtain the OTU (Operational Taxonomic Units) representative sequence and OTU abundance information. Species annotation was carried out based on OTU sequence, and species abundance information of each level was obtained based on OTU abundance information statistics. Based on OTU and species abundance table, biological information such as species composition, indicator species, Alpha diversity, Beta diversity, functional prediction, and environmental factor association were analyzed.

The Sob index is a direct statistic of the total number of detected OUT or species, reflecting only the abundance.

Chao1 index is an index used to estimate the total number of sample species, calculated by the following formula:

$$\text{Chao1} = S_{obs} + \frac{F1(F1 - 1)}{2F2(F2 + 1)}$$

Note

Sobs is the number of species detected, F1 is the number of species with abundance of 1 in the sample, F2 is the number of species with abundance of 2 in the sample.

Shannon index reflects species richness and evenness. The greater the value, the higher the species diversity. The calculation formula is as follows:

$$H = - \sum (Pi) (\log_2 Pi)$$

Note

Pi is the proportion of the number of individuals of the I-th species to the total number of individuals in the sample.

Simpson index comprehensively considers the richness and evenness of species. The greater the value, the higher the species diversity. The calculation formula is as follows:

$$Ds = 1 - \sum_{i=1}^s Pi^2$$

Note

s represents the total number of species, and Simpson's value ranges from 0 to 1.

The ACE index is the index used to estimate the total number of species in the sample, calculated by the following formula:

$$S_{ace} = S_{common} + \frac{Srare}{C_{ace}} + \frac{F1}{C_{ace}} r_{ace}^2$$

Data analysis

The physical and chemical indexes of each sample were determined three times. The experimental data were processed by Excel 2003, origin 2018, SPSS statistics 24 and other software. The statistical data are expressed by mean and standard error, P < 0.05 is a significant difference, P < 0.01 is a very significant difference.

Results

Physical and chemical properties of soil in *Vicia faba* collecting area

The pH, TN, TP and other soil physical and chemical properties of the collected rhizosphere soil of *Vicia faba* are determined, and the results are shown in Table 2. There were significant differences in soil physical and chemical properties among the four plots, (1) The SWC in mild rocky desertification area (JS6) was slightly higher than that in

moderate rocky desertification area (GJ3, GJ8, KY4); (2) The soil AP content in Gejiu rocky desertification area (GJ3) was significantly higher than that in Jianshui and Kaiyuan; The soil TP content in Gejiu rocky desertification area (GJ8) was higher than that in Jianshui and Kaiyuan; (3) The contents of TN and AN in rhizosphere soil of *Vicia faba* in Gejiu area (GJ8) were significantly higher than those in Jianshui and Kaiyuan areas; (4) The soil pH in Kaiyuan rocky desertification area (KY4) was significantly higher than that in Jianshui and Gejiu rocky desertification areas. In conclusion, the SWC in mild rocky desertification area is higher than that in moderate rocky desertification area; The soil pH in moderate rocky desertification area was higher than that in mild rocky desertification area, indicating that there was a positive correlation between the pH of *Vicia faba* rhizosphere soil and the degree of rocky desertification.

Table 2 Analysis of soil physical and chemical properties in different degrees of rocky desertification areas						
Group	pH	Total nitrogen(g/kg)	Total phosphorus(g/kg)	Alkali-hydrolyzed nitrogen(mg/kg)	Available phosphorus(mg/kg)	Soil water content(%)
GJ3	6.67 ± 0.07d	0.58 ± 0.13b	0.38 ± 0.02a	213.27 ± 23.86b	12.49 ± 9.58a	15.28 ± 0.59ab
GJ8	7.80 ± 0.06b	1.19 ± 0.11a	0.43 ± 0.70a	260.40 ± 9.18c	0.48 ± 0.06b	15.53 ± 0.43ab
JS6	7.53 ± 0.10c	0.40 ± 0.60d	0.10 ± 0.01b	120.40 ± 8.74a	2.14 ± 0.93b	16.46 ± 0.33a
KY4	8.14 ± 0.22a	0.29 ± 0.06d	0.12 ± 0.02b	104.07 ± 37.21a	1.92 ± 0.31b	14.55 ± 1.47b
Note: Different lowercase letters in the same column indicate significant differences (p < 0.05).						

Diversity analysis of endophytic bacteria in *Vicia faba* root nodules

Endophytic bacteria in *Vicia faba* root nodules α Diversity analysis

The α diversity index (Table 3) shows that the coverage of endophytic bacteria in *Vicia faba* root nodules is above 0.998, indicating that the coverage of the sample library is high, and the sequencing analysis is representative. The Sobs index, Shannon index, Chao index and Ace index of JS6 were slightly higher than those of the other groups, and there was no significant difference in diversity index among the other groups, indicating that the species richness in mild rocky desertification areas was slightly higher than that in moderate rocky desertification areas.

Table 3
The α diversity analysis of endophytic bacteria in *Vicia faba* root nodules

Group	Sobs index	Shannon index	Simpson index	Chao index	Ace index	Coverage
GJ3	146.67 $\pm$ 15.14bc	2.37 $\pm$ 0.21b	0.70 $\pm$ 0.05a	203.93 $\pm$ 9.35b	205.82 $\pm$ 9.92ab	0.9983
GJ8	171.67 $\pm$ 20.84ab	2.74 $\pm$ 0.37ab	0.75 $\pm$ 0.05a	213.90 $\pm$ 23.80a	222.18 $\pm$ 24.58a	0.9995
JS6	190.00 $\pm$ 28.36a	2.94 $\pm$ 0.42a	0.76 $\pm$ 0.07a	241.40 $\pm$ 36.27a	244.61 $\pm$ 42.19a	0.9993
KY4	127.67 $\pm$ 14.01c	1.24 $\pm$ 0.18c	0.48 $\pm$ 0.09b	157.43 $\pm$ 18.22a	162.05 $\pm$ 8.72b	0.9996
Note: Different lowercase letters in the same column indicate significant difference (P < 0.05).						

Endophytic bacteria in *Vicia faba* root nodules α Correlation between diversity analysis and soil physical and chemical properties

Endophytic bacteria in *Vicia faba* root nodules α The correlation between diversity analysis and soil physical and chemical properties is shown in Table 4. Simpson index was negatively correlated with soil pH, and positively correlated with TN and AN content, indicating that soil pH, TN and AN content affected the diversity of endophytic bacteria in root nodules. The higher the soil pH, the lower the Simpson index, the richer the diversity of endophytic bacteria in *Vicia faba* nodules; The higher the contents of TN and AN in soil, the higher the Simpson index, and the richer the diversity of endophytic bacteria in *Vicia faba* nodules.

Table 4
Correlation between the α diversity of endophytic bacteria in *Vicia faba* root nodules and soil physical and chemical properties

α Diversity index	pH	TN(g/kg)	TP(g/kg)	AN(mg/kg)	AP(mg/kg)	SWC(%)
sobs	-0.035	0.163	0.021	0.12	-0.326	0.341
shannon	-0.446	0.486	0.334	0.46	0.01	0.407
simpson	-0.518*	0.534*	0.407	0.521*	0.152	0.394
chao	-0.154	0.057	0.052	0.061	-0.123	0.151
ace	-0.19	0.147	0.102	0.14	-0.148	0.271
Note: The same column of data * indicates significant difference (p < 0.05).						

Composition analysis of endophytic bacteria in *Vicia faba* root nodules

After 12 samples of *Vicia faba* nodules were annotated and classified, 451 OTUs were obtained, which were annotated to 8 phyla, 14 classes, 38 orders, 63 families and 101 genera. Through Venn diagram analysis, we can see the number of common and unique OTUs of the four groups of samples in different places. As shown in Fig. 1(a), there are 62 OTUs in *Vicia faba* samples, accounting for 13.75% of bacterial OTUs. The number of unique OTUs of sample JS6 is the largest (45), and the number of GJ3 is the least (21). The results showed that the

distribution of endophytic bacteria in *Vicia faba* root nodules had certain degree of similarity and specificity under different degrees of rocky desertification. The lower the degree of rocky desertification, the more the endemic species; Under the same degree of rocky desertification, the distribution of endophytic bacteria in *Vicia faba* root nodules in different regions also had differences and similarities.

At the phylum level, as shown in Fig. 1(b), endophytic bacteria are annotated to 11 phyla, including Proteobacteria and Bacteroidetes, of which Proteobacteria is the dominant phylum of endophytic bacteria in *Vicia faba* root nodules. The proportion of Proteus in GJ3, GJ8, JS6 and KY4 were 99.38%, 94.01%, 95.90% and 99.63%, respectively.

At the genus level, as shown in Fig. 1(c), the endophytic bacteria in *Vicia faba* root nodules are annotated with *Pseudomonas*, *Rhizobium*, *Oligotrophomonas*, *Ensifer* and other genera. *Pseudomonas* is the dominant genus in the four groups of samples, with a relative abundance of 40.48%, 36.45%, 41.70% and 62.06%; *Rhizobium* was the secondary dominant genus of GJ3 (32.90%) and KY4 (34.28%); In GJ8 and JS6, *Oligotrophomonas* was the second dominant genus, accounting for 28.14% and 16.16%, while *Rhizobium* accounted for 16.31% and 9.78%.

In order to further analyze the differences of endophytic bacteria in *Vicia faba* root nodules in different degrees of rocky desertification, the samples were analyzed by lefse. Figure 1(d) shows the results of LDA distribution of different species. The color represents different groups, and the length represents the contribution of different species, that is LDA score (Yang LJ et al. 2023). Figure 1(e) shows the evolutionary branch tree of different species. It is set that there is a significant difference in LDA score > 2 from domain to gene (only the species with significant difference are shown). There are 47 distinct units, 16 distinct units in GJ3, 10 distinct units in GJ8, 6 distinct units in JS6, and 15 distinct units in KY4. As shown in Fig. 1(d), Rhizobiaceae showed significant differences among groups, and its abundance was the highest in KY4; The LDA value of Rhizobiaceae was higher than that of other taxa, indicating that it had the greatest impact on the differences between groups; *Variovorax* showed significant differences among groups, and the highest abundance was found in JS6; Xanthobacteraceae showed significant differences among groups, and the highest abundance was found in GJ8; There were significant differences in the abundance of Hyphomycetes between groups, and the highest abundance was found in GJ3. At the phylum level, the phyla with significant differences include Proteobacteria and so on. At the genus level, there are 22 bacterial genera with significant differences, including *Rhizobium*, *Pseudomonas*, etc.

The Heatmap diagram shows the composition of endophytic bacteria in *Vicia faba* root nodules at the genus level, as shown in Fig. 1(f), it can be clearly seen that the four groups of samples are divided into two categories; GJ3 and KY4 were first grouped into one sample; JS6 and GJ8 were collected together; From the clustering results of the four groups of samples, it can be seen that the bacterial similarity of GJ3 and KY4 is high, and the bacterial composition of JS6 and GJ8 is different from that of the previous two groups of samples.

Endophytic bacteria in *Vicia faba* root nodules α Correlation between diversity analysis and soil physical and chemical properties physicochemical properties

The β diversity principal coordinates (PCoA) analysis showed that the bacterial composition of different *Vicia faba* samples was similar or different. As shown in Fig. 2(a), the bacterial communities of samples GJ8 and JS6 are quite similar, indicating that the bacterial community structures of the two samples are similar, but there are some differences with the bacterial community composition of GJ3 and KY4. At the same time, the nonparametric test for the similarity of bacterial communities among the four groups of samples was carried out by combining the Anosim analysis based on the Barry-Curtis distance algorithm (Fig. 2(b)), which showed that the composition of

endophytic bacterial communities in the four groups of *Vicia faba* nodule samples showed a very significant difference on the whole at the OTU level (R = 0.577, P = 0.001).

Difference analysis of endophytic bacterial community composition and environmental factors in *Vicia faba* root nodules

At the genus level, Pearson correlation coefficient was used to analyze the correlation between the relative content of endophytic bacteria in the top 20 of abundance and soil physical and chemical factors. The correlation heat map is shown in Fig. 3(a), and the most bacteria (11) were significantly related to soil SWC. Among them, the secondary dominant genus *Rhizobium* was significantly positively correlated with soil AP content and RD, and negatively correlated with soil SWC; The results showed that SWC determined the degree of rocky desertification, and the lower the SWC, the higher the degree of rocky desertification.

RDA analysis was used to explore the physical and chemical factors affecting the composition of nitrogen fixing bacteria community. RDA analysis was conducted at the genus level by combining soil physical and chemical factors with OTUs table, as shown in Fig. 3(b). According to the display of each physical and chemical factor in the figure, soil AP, TN and SWC were the main physical and chemical factors affecting the composition of endophytic bacterial community in *Vicia faba* nodules, followed by soil an, RD, TP and pH.

Diversity analysis of nifH gene of endophytic nitrogen fixing bacteria in *Vicia faba* root nodules

Endophytic nitrogen fixing bacteria in *Vicia faba* root nodules α Diversity analysis

The α diversity index (Table 5) shows that the coverage index of endophytic nitrogen fixing bacteria in *Vicia faba* root nodules is above 0.999, indicating that the coverage rate of the sample library is high. The sobs index, Shannon index, Chao index and Ace index of JS6 were slightly higher than those of the other groups, and there was no significant difference in diversity index among the other groups, indicating that the abundance of endophytic nitrogen fixing bacteria in *Vicia faba* nodules in mild rocky desertification areas was slightly higher than that in moderate rocky desertification areas.

Table 5
Diversity index of endophytic nitrogen fixing bacteria in *Vicia faba* nodules

Group	Sobs index	Shannon index	Simpson index	Chao index	Ace index	Coverage
GJ3	16.67 ± 8.74c	0.72 ± 0.43b	0.66 ± 0.25a	32.34 ± 5.37b	38.61 ± 8.45b	0.9995
GJ8	57.67 ± 19.63ab	1.25 ± 0.60ab	0.49 ± 0.21a	94.40 ± 9.76a	97.18 ± 6.24a	0.9990
JS6	85.33 ± 14.64a	1.73 ± 0.16a	0.42 ± 0.11a	106.86 ± 5.65a	104.56 ± 6.24a	0.9990
KY4	31.00 ± 14.73bc	0.90 ± 0.16b	0.65 ± 0.13a	60.83 ± 53.17ab	49.20 ± 22.21b	0.9995

Note: Different lowercase letters in the same column indicate significant difference (P < 0.05).

Composition analysis of endophytic nitrogen fixing bacteria in *Vicia faba* root nodules

After sequencing the *nifH* gene of nitrogen fixing bacteria in *Vicia faba* root nodules by Illumina MiSeq, a total of 137064 valid sequences were obtained from the four groups of samples. After the sample sequences were split and de redundant, the OTU was divided and clustered under 97% similarity. A total of 331 nitrogen fixing bacteria OTUs were obtained, which were annotated to 7 phyla, 11 classes, 16 orders, 19 families and 24 genera.

At the phylum level, as shown in Fig. 4(a), endophytic nitrogen fixing bacteria are annotated to seven phyla, including Proteobacteria and Verrucomicrobia. Verrucomicrobia is the dominant phyla of sample JS6, with a relative abundance of 19.74%, and the nitrogen fixing bacteria that are not annotated in JS6 account for the majority (43.80%); Proteobacteria is the dominant phylum of GJ3, GJ8 and KY4, with relative abundance of 97.17%, 96.59% and 98.29%. The results showed that the species abundance of endophytic nitrogen fixing bacteria in *Vicia faba* nodules was related to the degree of soil rocky desertification, and the endophytic bacteria abundance in *Vicia faba* nodules in mild rocky desertification areas was higher than that in moderate rocky desertification areas.

At the genus level, as shown in Fig. 4(b), 24 genera including *Rhizobium*, *Bradyrhizobium*, *Klebsiella*, and *Opitus* are annotated with endophytic nitrogen fixing bacteria in *Vicia faba* nodules, of which the slow *Rhizobium* is the dominant genus of GJ8, with a relative abundance of 44.85%; *Klebsiella* is the dominant genus of GJ3, with a relative abundance of 39.61%; *Rhizobium* is the dominant genus of KY4 and the secondary dominant genus of JS6, with relative abundance of 82.48% and 26.46%, and most of the nitrogen fixing bacteria in js6 are not clearly annotated (unclassified_k_norank_d_bacteria:43.80%, norank_f_opitutaceae:19.74%). The results showed that there were similarities and specificities in the distribution of endophytic nitrogen fixing bacteria in *Vicia faba* root nodules under different degrees of rocky desertification. The lower the degree of rocky desertification, the more the endemic species.

Analysis of differences in composition of endophytic nitrogen fixing bacteria Community in *Vicia faba* nodules

The β diversity principal coordinates (PCoA) analysis showed that the composition of nitrogen fixing bacteria in different *Vicia faba* samples was similar or different. PCoA analysis based on Bray-Curtis distance algorithm was carried out for the endophytic nitrogen fixing bacteria Community in *Vicia faba* root nodules at the OTU level, as shown in Fig. 5, the differences between groups were greater than those within groups, and there were significant differences in samples between groups. It can be seen from the figure that GJ8 and JS6 are relatively scattered. The distribution range of samples GJ3 and GJ8 partly coincided, indicating that the bacterial community structure of the two samples was similar, but different from that of JS6 and KY4. ($R = 0.194$, $P = 0.054$)

Analysis of environmental factors of endophytic nitrogen fixing bacteria in *Vicia faba* root nodules

At the genus level, Spearman correlation coefficient was used to analyze the correlation between the genus pair content of nitrogen fixing bacteria in the top 20 and soil physical and chemical factors. The correlation heat map is shown in Fig. 6(a): The results showed that the most bacteria (3) were significantly correlated with soil total phosphorus content, and the dominant bacteria (*Rhizobium*) were significantly negatively correlated with TN and AN, and significantly positively correlated with pH; The secondary dominant bacteria, *Bradyrhizobium*, were significantly positively correlated with TP and RD.

RDA analysis was used to explore the physical and chemical factors affecting the composition of nitrogen fixing bacteria community. The soil physical and chemical factors were combined with OTUs table for RDA analysis at the genus level. As shown in Fig. 6(b), TP ($P = 0.02$) is significantly correlated with the composition of nitrogen fixing bacteria community ($P < 0.05$). At the same time, according to the physical and chemical factors shown in the figure, soil TP, TN and pH are the main physical and chemical factors affecting the composition of endophytic nitrogen fixing bacteria Community in *Vicia faba* nodules, followed by soil AN, RD, SWC and AP.

Discussion

There are differences in the structure of endophytic bacteria flora in different plant hosts or different plant parts. For example, there are differences in the diversity of endophytic bacteria in different medicinal plants, with the conservation of the host (Zheng H 2009); The endophytic bacteria from *Hippophae rhamnoides* root nodules in Tibet adapt to different geographical conditions (Zhang AM et al. 2018) and show significant differences at different altitudes. Important factors affecting the community structure of endophytic bacteria in plants include: host plant species, genotype, physiological and biochemical characteristics and plant growth environment (Lan JL et al. 2009; Li QH et al. 2021). The bacterial community composition of the same leguminous plant in different regions was similar, but the proportion of each taxon was different. Studies on the diversity of nitrogen fixing bacteria are also found in different habitats (Luo R 2019; Zhang M 2021; Li YY 2018). *Pseudomonas* and *Bacteroides* belong to associated nitrogen-fixing bacteria in the nitrogen fixing bacteria group, which are widely distributed in different habitats to strengthen the utilization of nitrogen by plants. Liu QG et al. (2018) and others' research results on nitrogen fixing microorganisms in paddy soil found that among the 12 categories annotated, the abundance of Proteobacteria in samples at different periods was higher than 80%, which was similar to the results of this study. The distribution of bacteria in root nodules was affected by the synergy of spatial geographical factors, soil physical and chemical factors, local topography and rhizosphere soil microorganisms.

The contents of soil total nitrogen, total potassium, total phosphorus and organic matter have an impact on the community structure of endophytic bacteria in jasmine plants (Chen MCH et al. 2018). Previous studies have found that the endosymbiotic nitrogen-fixing rhizobium mainly comes from *Rhizobium*, *Ensifer*, *Bradyrhizobium*, *Mesorhizobium*, *Mesobradyrhizobium* and *Azorhizobium* of α -Proteobacteria class. (Chen ZB et al. 2018; MH Ramírez Bahena et al. 2014; Gao SHK 2017); The endophytes of non nitrogen fixing rhizobia mainly come from *Pseudomonas* and *Sphingobacterium*. The endophytic bacteria detected in this study are partly the same as the endosymbiotic bacteria of other plants, which indicates that *Vicia faba* plants are similar to the dominant bacteria of endophytic bacteria in other plants. The composition of endophytic bacterial community in *Vicia faba* was mostly related to the contents of TN and TP in soil. Redundancy analysis of nitrogen fixing bacteria and soil physical and chemical factors showed that TN, TP and pH values may be the main physical and chemical factors affecting the composition of nitrogen fixing bacteria (Jiao BJ et al. 2023).

Schleuss PM et al. (2020) showed that biological nitrogen fixation was not controlled by N or P alone, but by the ratio of nitrogen to phosphorus. When nitrogen content is rich, the *nifH* gene in legumes has better diversity and specificity, which indicates that host plants may play an important role in the process of nitrogen cycle in the ecosystem (Mingshu L et al. 2023). Xu GQ et al. (2020) found in the correlation analysis of environmental factors that the content of TP and AN in soil may be the main physical and chemical factors affecting the composition of nitrogen fixing bacteria community in *Vicia faba* samples. In addition to the influence of host plant species, genotypes and their own physiological and biochemical characteristics, the growth environment of plants is also an important factor affecting the structure of plant endophytic bacteria community, Biological nitrogen fixation is an

essential source of nitrogen. The deep understanding of biological nitrogen fixation in rocky desertification ecosystem is conducive to the mitigation of rocky desertification.

In the future, the cultivation method and non cultivation method can be used to more comprehensively understand and compare the endophytic microorganisms in the dominant vegetation of rocky desertification and the soil microbial community structure in rocky desertification area, and the nitrogen fixation function or other stress resistance function of the isolated endophytic bacteria can be further studied to provide reference for the construction of artificial vegetation restoration ecology, so as to provide reference for the development and utilization of microbial resources in soil remediation.

Conclusion

This study took *Vicia faba* nodules as the research object to explore the community structure and diversity of endophytic bacteria in *Vicia faba* nodules, and to understand the similarities and differences of endophytic bacteria and endophytic nitrogen fixing bacteria in *Vicia faba* nodules in different areas of rocky desertification. Microbial biogeography research based on species classification can not well explain the mechanism of species coexistence and predict the response mechanism of microbial community to the environment (Nelson MB et al. 2016). Based on the determination of soil physical and chemical properties in different sample collection areas, using bacterial 16S rDNA as marker gene, IlluminaMiSeq high-throughput sequencing and bioinformatics analysis technology were used to compare and analyze the community structure, diversity characteristics and relationship with soil physical and chemical properties of four groups of endophytic bacteria in *Vicia faba* nodules in different rocky desertification areas. The results show that:

1. There were significant differences in soil physical and chemical properties among the four sample plots. The SWC in mild rocky desertification area was higher than that in moderate rocky desertification area, indicating that the rhizosphere SWC of *Vicia faba* was negatively correlated with the degree of rocky desertification; The soil pH in moderate rocky desertification area was higher than that in mild rocky desertification area, indicating that there was a positive correlation between the pH of *Vicia faba* rhizosphere soil and the degree of rocky desertification.
2. In the α diversity analysis of the bacterial community, Simpson index was significantly negatively correlated with soil pH, and significantly positively correlated with TN and AN content, indicating that soil pH, TN and AN content affected the diversity of endophytic bacteria in nodules. The higher the soil pH, the lower the Simpson index, the richer the diversity of endophytic bacteria in *Vicia faba* nodules; The higher the contents of TN and AN in soil, the higher the Simpson index, and the richer the diversity of endophytic bacteria in *Vicia faba* nodules.
3. In the composition of bacterial community, *Pseudomonas* is the dominant genus in the four groups of samples, *Rhizobium* and *Oligotrophomonas* are the secondary dominant genera. The bacterial community composition of the four groups of samples based on species classification was significantly different ($R = 0.577$, $P = 0.001$). *Rhizobia* was the dominant genus in the four groups of samples. There were significant differences in the composition of nitrogen fixing bacteria community among the four groups ($R = 0.194$, $P = 0.054$).
4. The correlation analysis between bacterial community and soil physical and chemical properties of four groups of samples showed that soil pH, TN and AN content affected the diversity of endophytic bacteria in root nodules. The higher the soil pH, the lower the Simpson index, and the richer the diversity of endophytic bacteria in *Vicia faba* root nodules; The higher the contents of TN and AN in soil, the higher the Simpson index, and the

richer the diversity of endophytic bacteria in *Vicia faba* nodules. The correlation between a diversity of endophytic nitrogen-fixing bacteria and soil physicochemical properties was similar to that of bacteria; Soil TP, TN content and soil pH were the main physical and chemical factors affecting the composition of *Vicia faba* rhizobium nitrogen fixing bacteria community, followed by soil AN, soil RD, SWC and soil AP content.

In conclusion, there are a large number of nitrogen fixing microorganisms in *Vicia faba* root nodules in rocky desertification areas. The abundance of endophyte community in nodules was mainly affected by the degree of soil rocky desertification, soil water content and soil nitrogen content. In the correlation analysis between dominant bacteria and the degree of rocky desertification, it was found that nitrogen fixing bacteria such as *Pseudomonas*, *Rhizobia* and *Bradyrhizobium* were significantly positively correlated with the degree of rocky desertification. With the increase of rocky desertification degree, the host plants are in a harsh environment, the abundance of endophytic nitrogen fixing bacteria increases, and the nitrogen fixation efficiency increases.

Declarations

Data availability statement

All data that support the findings of the present study are included in the manuscript.

Author Contribution Statement

All authors participated in the study conception and design. The research and data collection and analysis were performed by Zichang Liu under supervision and validation of Prof. Jinhua Wang and Wuxian Zhang. The original draft of the manuscript was prepared by Zichang Liu, Bo Yang, Ziyun Yang and all authors commented on previous versions of the manuscript. All authors read and approved the finally manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

1. Bao SD (2000) Agrochemical analysis of soils. 3rd Ed. China Agriculture Press, China. ISBN:978-7-109-06644-1
2. Chen LY, Wang ZHB, Hu LP (2021) Effects of Interplanting Six Leguminous Green Manure Plants in Apple Orchard on Soil Carbon and Nitrogen Characteristics. *Acta Agrestia Sinica* 29(4):6. <https://doi.org/10.11733/j.issn.1007-0435.2021.04.005>
3. Chen MCH, Zhu YQ, Liu B et al (2018) Analysis of Endophytes Diversity in *Jasminum sambac* (L.) Ait by Metagenomic Technology. *Journal of Tropical and Subtropical Botany* 26(6):11. <https://doi.org/10.11926/jtsb.3925>
4. Chen ZB, Gao X, Hu Y et al (2018) Analysis on Diversity of Endophytic Bacteria in Peanut Kernel with Illumina MiSeq High-throughput Sequencing Technology. *Journal of Kunming University* 40(03):86-89. <https://doi.org/10.14091/j.cnki.kmxyxb.2018.03.017>
5. Cui WF, Yu XF, Wang ZHG et al (2023) Effects of straw returning and tillage methods on soil quality of corn field in Inner Mongolia plain irrigation area. *Jiangsu Agricultural Sciences* 51(02):217-224. <https://doi.org/10.15889/j.issn.1002-1302.2023.02.032>
6. Gao SHK (2017) Study on characteristics of soil microbial community and response mechanism under different silvicultural measures in *Pinus massoniana* Plantations. Dissertation, Chinese Academy of Forestry
7. Guan LT, Sun KN, Zhao XY (2010) Discussion on natural ecological environment management of mines in Chengde City. *Silviculture & Management* No.105(09):14-15. <https://doi.org/10.13456/j.cnki.lykt.2010.09.003>
8. He C, Gao H, Wang H et al (2021) GSK3-mediated stress signaling inhibits legume-rhizobium symbiosis by phosphorylating GmNSP1 in soybean. *Mol Plant Mar* 1;14(3):488-502. <https://doi.org/10.1016/j.molp.2020.12.015>
9. Jiao BJ, Xu L, Li XZ et al (2023) Diazotrophic community diversity in the succession development of biological soil crusts in water-wind erosion crisscross region of the Loess Plateau. *Acta Ecologica Sinica* 43(23):9662-9673. <https://doi.org/10.20103/j.stxb.202210072842>
10. Lan JL, Liu B, Zhu YQ et al (2009) Community and diversity of endophytic bacteria inside eggplant. *Ecology and Environmental Sciences* 18(04):1433-1442. <https://doi.org/10.16258/j.cnki.1674-5906.2009.04.056>
11. Li S, Wei XH, Huang JG et al (2007) Cause and Processes of Rocky Desertification Lands in Karst Areas of South China. *Journal of Desert Research* 27(6):918-926. <https://doi.org/CNKI:SUN:ZGSS.0.2007-06-004>
12. Li H, Wang Q, Sun W et al (2020) Remediation of vanadium contaminated soil with rhamnolipid assisted by *Amorpha fruticosa* L.. *Nonferrous Metals(Mine Section)*. <https://doi.org/10.3969/j.issn.1671-4172.2020.04.020>
13. Li QH, Yin M, Pei Y et al (2023) Analysis of endophytic bacterial diversity in seven pear varieties based on high-throughput sequencing. *Southwest China Journal of Agricultural Sciences* 51(02):217-224. 2021. <https://doi.org/10.7540/j.ynu.20200114>

14. Li YY (2018) Microbial diversity, nitrogen utilization strategy and environmental change responses in the Northwest Pacific Ocean and the basin of the South China Sea. Dissertation, Xiamen University
15. Lin JY, Cheng YN (2022) Identification of Bare Rock and Evaluation of Early Rocky Desertification Using UAV Remote Sensing Imag. *Journal of Southwest University(Natural Science Edition)* 44(12):125-135. <https://doi.org/10.13718/j.cnki.xdzk.2022.12.013>
16. Liu QG, Li F, Luo H et al (2018) The influence of introducing soft-shelled turtle on soil nitrogen-fixing microorganisms in paddy field*. *Chinese Journal of Applied and Environmental Biology* 24(03):483-492. <https://doi.org/10.19675/j.cnki.1006-687x.2017.06041>
17. Liu Y, Zhang JL, Li XG et al (2022) Research progress on nitrogen efficient utilization mechanism of leguminous crops. *Chinese Journal of Oil Crop Sciences* 44(03):476-482. <https://doi.org/10.19802/j.issn.1007-9084.2021123>
18. Luo DH, Xia J, Yuan JW et al (2010) Root biomass of karst vegetation in a mountainous area of southwestern China. *Chinese Journal of Plant Ecology* 34(05):611-618. <https://doi.org/10.3773/j.issn.1005-264x.2010.05.015>
19. Luo R (2019) Study on microbial community structure of *Pinus tabulaeformis* Plantation participating in soil nitrogen cycle function on the Loess Plateau. Dissertation, Northwest A&F University
20. MH Ramírez Bahena, MJ Cuesta Sánchez, Tejedor C et al (2014) Biotecnología microbiana para la agricultura en Producción Integrada: Buscando nuevos biofertilizantes para patata. <http://www.irnasa.csic.es/2desarrollo-sostenible-de-sistemas-agroforestales>
21. Mhamdi R, Nouairi I, Hammouda TB et al (2014) Growth capacity and biochemical mechanisms involved in rhizobia tolerance to salinity and water deficit. *Journal of Basic Microbiology*. <https://doi.org/10.1002/jobm.201400451>
22. Min YP (2015) Present situation of rock desertification in karst area of Yunnan province and its prevention and control measures. *Modern Horticulture* No.286(10):229-230. <https://doi.org/10.14051/j.cnki.xddy.2015.10.196>
23. Mingshu L , Li G , White JC et al (2023) Nano-enabled strategies to enhance biological nitrogen fixation. *Nature nanotechnology*. <https://doi.org/10.1038/s41565-023-01392-5>
24. Nelson MB, Martiny AC, Martiny JBH (2016) Global biogeography of microbial nitrogen-cycling traits in soil. *Proc Natl Acad Sci USA* 113(29):8033-8040. <https://doi.org/10.1073/pnas.1601070113>
25. Raymond J, Siefert JL, Staples CR et al (2004) The natural history of nitrogen fixation. *Molecular biology and evolution* 21(3): 541-554. <https://doi.org/10.1093/molbev/msh047>
26. Rosenblueth, Mónica, Martínez-Romero et al (2006) Bacterial Endophytes and Their Interactions with Hosts. *Mol Plant Microbe Interact* 19(8):827-837. <https://doi.org/10.1094/MPMI-19-0827>
27. Schleuss PM , Widdig M , Heintz-Buschart A et al (2020) Interactions of nitrogen and phosphorus cycling promote P acquisition and explain synergistic plant-growth responses. *Literature Cited* 101(5):e03003. https://doi.org/10.15495/EPub_UBT_00005057
28. Tang MD, Ai SHY, Li MJ et al (2011) Effect of *Astragalus sinicus* on the growth, Cd and Pb concentration of leafy vegetables. *China Environmental Science* 31(3):5. <https://doi.org/CNKI:SUN:ZGHJ.0.2011-03-028>
29. Werner Gijbert DA, Cornwell William K, Cornelissen Johannes HC et al (2015) Evolutionary signals of symbiotic persistence in the legume-rhizobia mutualism. *Proceedings of the National Academy of Sciences of the United States of America* 112(33). <https://doi.org/10.1073/pnas.1424030112>

30. Xu GQ, Liu YX, Chao PX et al (2020) Study on endophyte diversity of *Oxytropis glacialis* in different ecological environments in Qinghai-Tibet Plateau. *Plateau Science Research* 4(01):20-29.
<https://doi.org/10.16249/j.cnki.2096-4617.2020.01.004>
31. Yan J, Han XZ, Ji ZJ et al (2014) Abundance and Diversity of Soybean-Nodulating Rhizobia in Black Soil Are Impacted by Land Use and Crop Management. *Applied & Environmental Microbiology* 80(17).
<https://doi.org/10.1128/AEM.01135-14>
32. Yang L J, Xu Y, Gao H et al (2023) Diversity and function prediction of endophytic bacteria in different tissues of *Stephania tetrandra* based on high throughput technology. *Jiangsu Agricultural Sciences* 51(17):19-28.
<https://doi.org/10.15889/j.issn.1002-1302.2023.17.003>
33. Ye N, Liu H, Luo Q et al (2023) Different Leaf Nutrient Use Strategies of Nitrogen-fixing and Non-nitrogen-fixing Leguminous Trees in South China. *Journal of Tropical and Subtropical Botany* 31(03):334-340.
<https://doi.org/10.11926/jtsb.4583>
34. Young JPW (1992) Phylogenetic classification of nitrogen-fixing organisms. *Biological nitrogen fixation* 1544: 43-86.
35. Zhai LL, Lei JY, Chen JS et al (2019) Evolution process and comprehensive control of rocky desertification in Honghe state, Yunnan Province. *Carsologica Sinica* 38(05):704-712. <https://doi.org/10.11932/karst20190506>
36. Zhang AM, Han XY, Zhang SHH et al (2018) Diversity of root nodule endophytic bacteria in *Hippophae thibetana* at different altitudes in Gannan area. *Biotic Resources* 40(02):114-119.
<https://doi.org/10.14188/j.ajsh.2018.02.004>
37. Zhang JY, Wang JH, Xiong ZH et al (2014) 16S rDNA sequence analysis of rhizobia isolated from leguminous plants in Wenshan Rocky Desertification Area. *Jiangsu Agricultural Sciences* 42(12):44-46.
<https://doi.org/10.15889/j.issn.1002-1302.2014.12.012>
38. Zhang M (2021) Composition, diversity and isolation of nitrogen fixation bacteria in three types of grasslands. Dissertation, Inner Mongolia University. <https://doi.org/10.27224/d.cnki.gnmdu.2021.000561>
39. Zheng H (2009) The effect of UV-B radiation on the endophytic bacteria in plants of Qinghai-Tibet plateau. Dissertation, Lanzhou University
40. Zhong WCH, Wang JP, Luo FY et al (2013) Relationship of Carbonate rocks insoluble residues and rocky desertification study in Dian Dong Plateau. *Jilin Water Resources* No.368(01):1-3+21.
<https://doi.org/10.15920/j.cnki.22-1179/tv.2013.01.006>
41. Zhu MZ (2012) Resource of Symbiotic Nitrogen-Fixation of Legume and Diversity of Rhizobia in Karst Shrub Communities. Dissertation, Journal of Central South University of Forestry & Technology.
<https://doi.org/10.7666/d.y2096953>

Figures

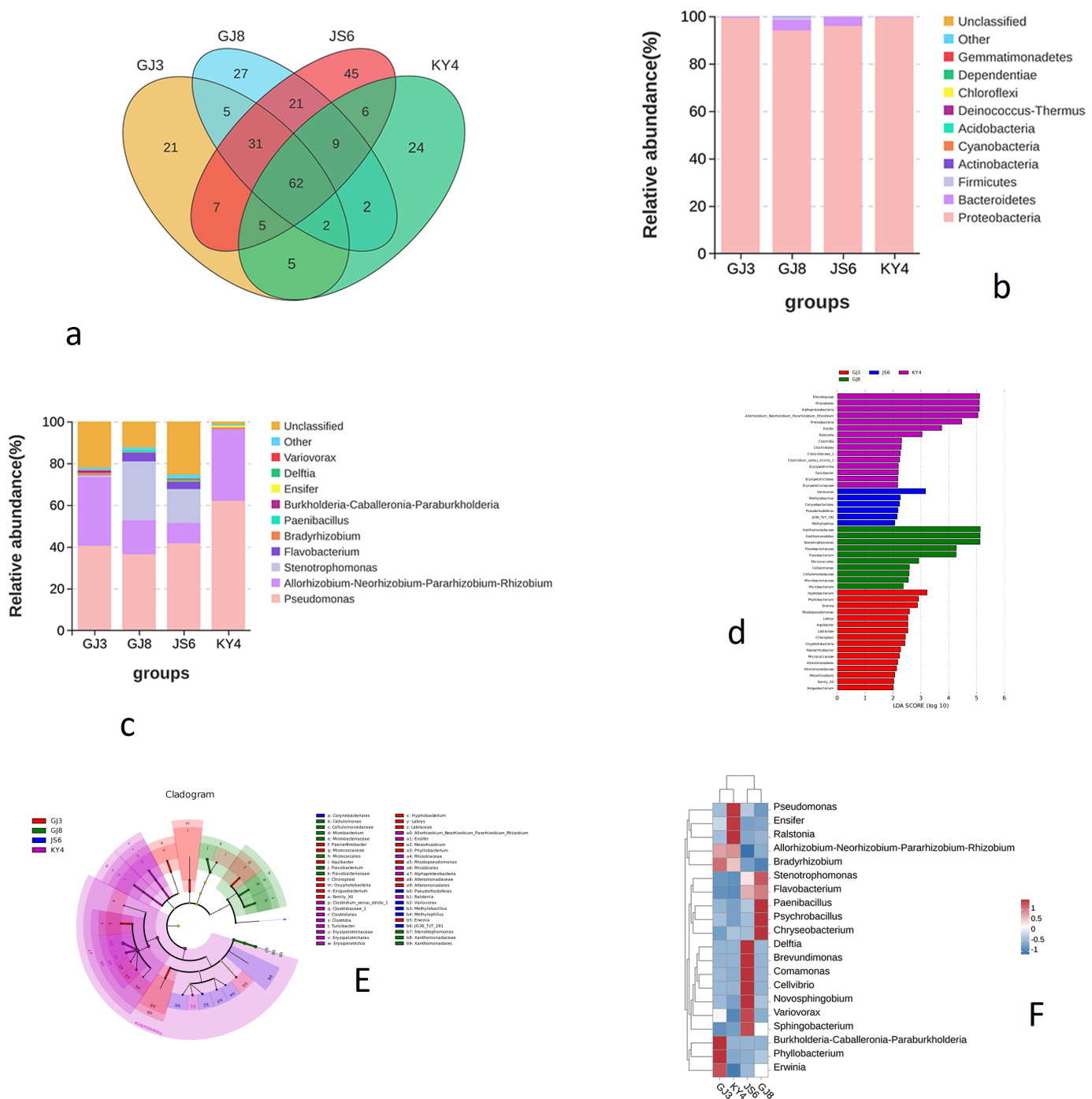


Figure 1

Composition analysis of endophytic bacteria in *Vicia faba* root nodules

(a) Venn diagram of OTU level of endophytic bacteria in *Vicia faba* nodules, (b) Stacking diagram of horizontal species distribution of endophytic bacteria in *Vicia faba* nodules, (c) Horizontal species distribution of endophytic bacteria in *Vicia faba* nodules, (d) LDA bar chart of endophytic bacteria in *Vicia faba* nodules in different degrees of rocky desertification areas, (e) LEfse tree of endophytic bacteria in *Vicia faba* root nodules in different degrees of rocky desertification areas, (f) Horizontal species of endophytic bacteria in *Vicia faba* root nodules Heatmap map

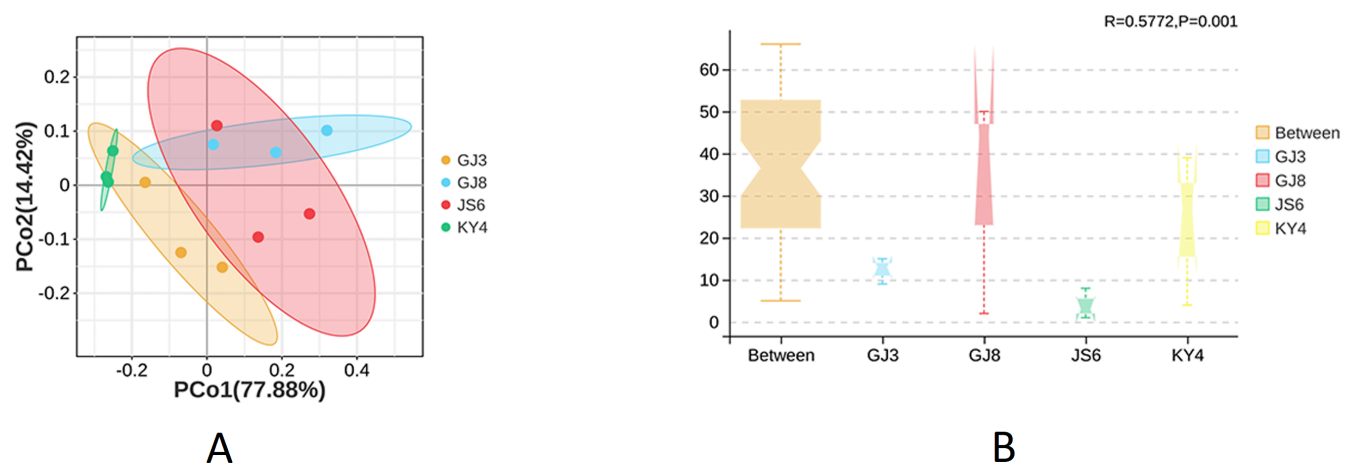


Figure 2

(a) Analysis of endophytic bacteria PCoA in root nodules of *Vicia faba*, (b) Analysis of endophytic bacteria anosim in *Vicia faba* root nodules

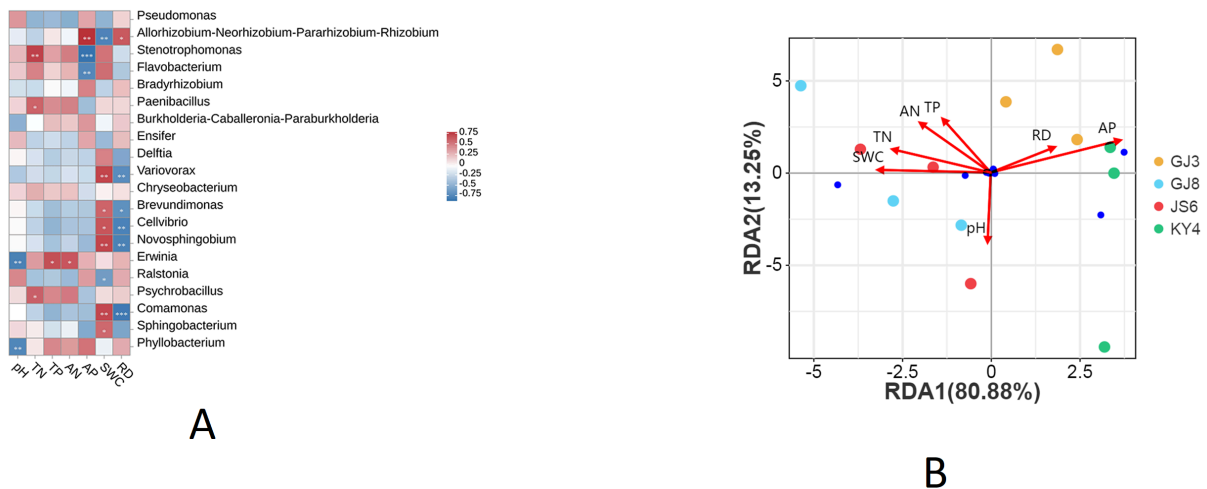


Figure 3

Difference analysis of endophytic bacterial community composition and environmental factors in *Vicia faba* root nodules

(a) Correlation heat map of soil physicochemical properties and endophytic bacteria in faba root nodules, (b) Soil physical and chemical properties and redundancy analysis of endophytic bacteria in *Vicia faba* nodules

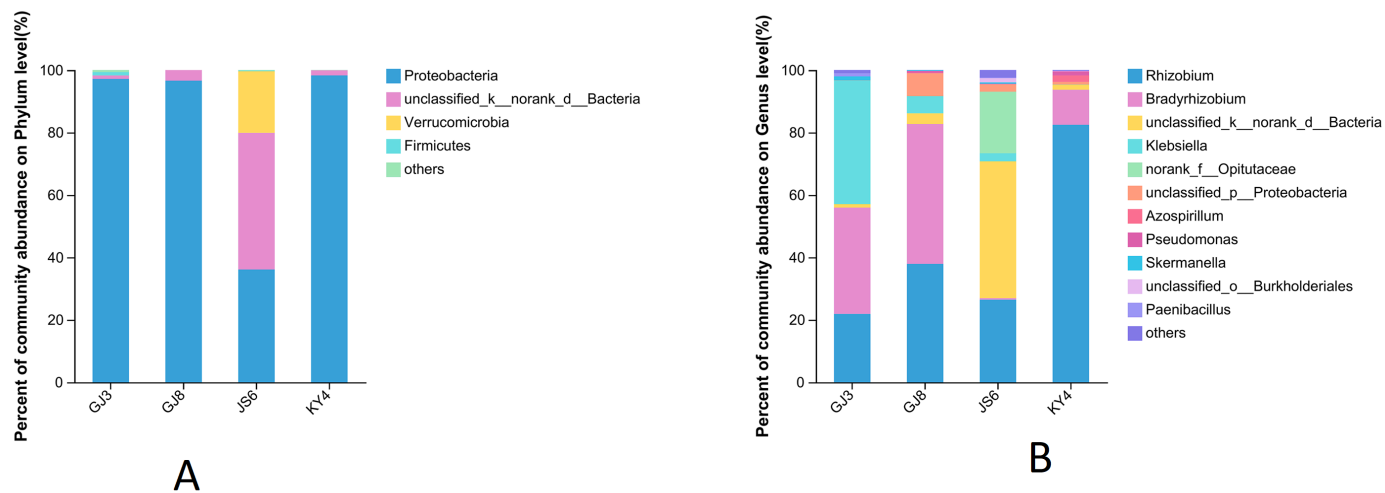


Figure 4

Diversity analysis of *nifH* gene of endophytic nitrogen fixing bacteria in *Vicia faba* root nodules

(a) Stacking diagram of horizontal species distribution of endophytic bacteria in *Vicia faba* nodules, (b) Stacking diagram of horizontal species distribution of endophytic bacteria in *Vicia faba* nodules

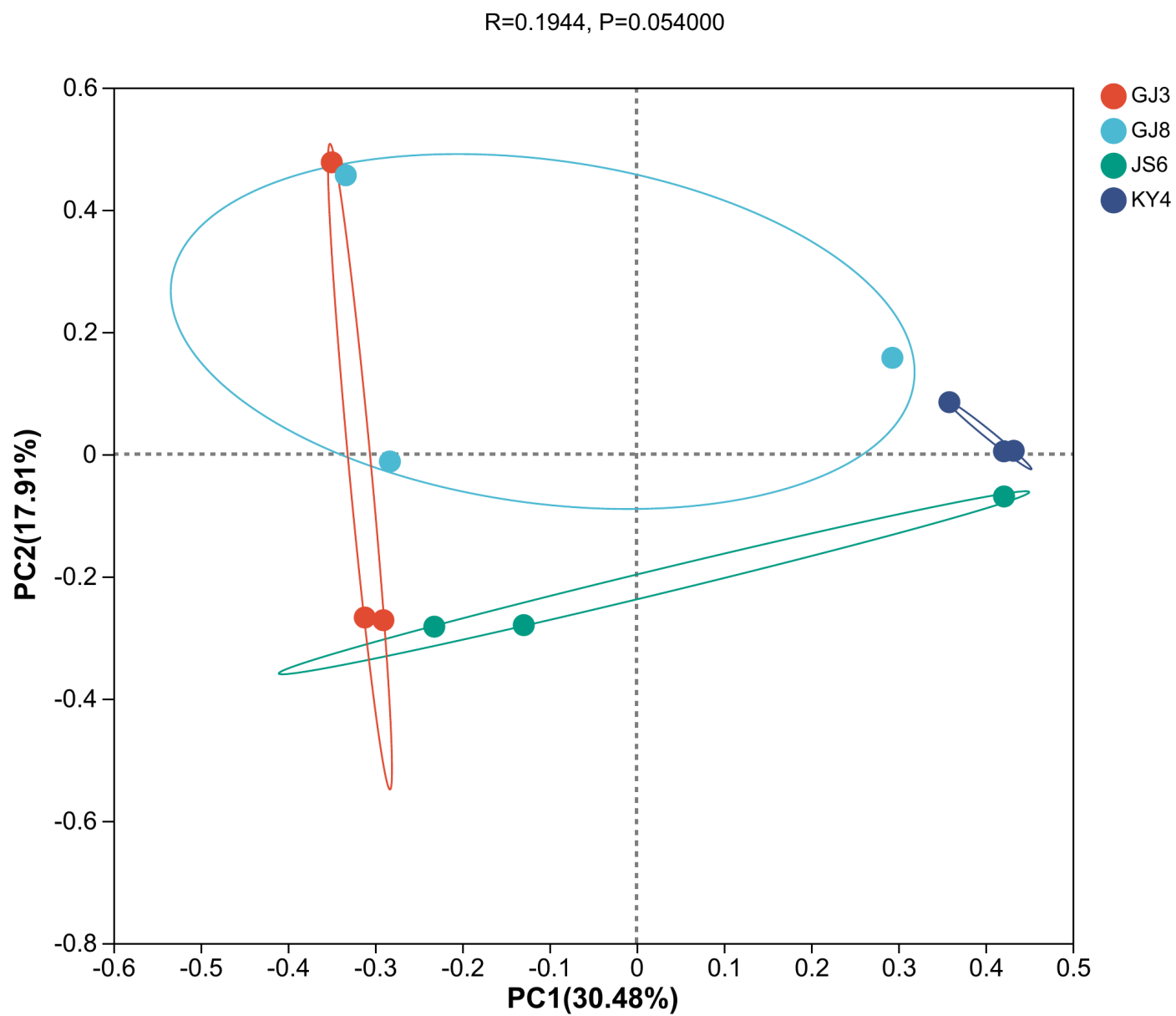
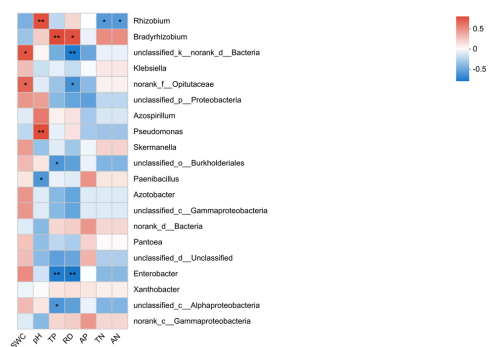
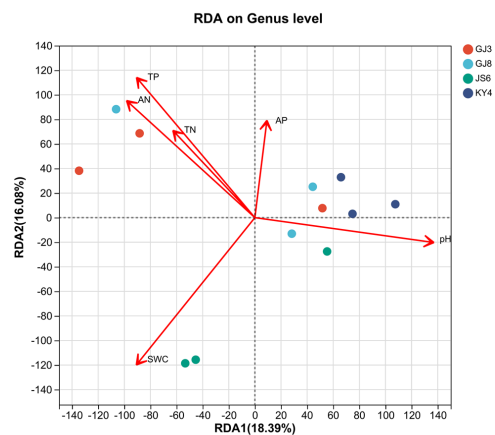


Figure 5

PCoA diagram of endophytic bacteria in *Vicia faba* nodules



A



B

Figure 6

Analysis of environmental factors of endophytic nitrogen fixing bacteria in *Vicia faba* root nodules

(a) Thermogram of correlation between soil physical and chemical properties and endophytic nitrogen fixing bacteria in *Vicia faba* nodules, (b) Soil physical and chemical properties and RDA analysis of endophytic nitrogen fixing bacteria in *Vicia faba* nodules