**RESEARCH**

# *Dicranopteris dichotoma* rhizosphere-derived *Bacillus* sp. MQB12 acts as an enhancer of plant growth via increasing phosphorus utilization, hormone synthesis, and rhizosphere microbial abundance

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## Abstract

In recent years, microbial inoculants have showed a great potential to replace chemical fertilizers as a new generation of soil amendment agents, however, the understanding of their effects on nutrient cycling within plants and rhizosphere microbial diversity are still limited. In this study, the rhizosphere growth-promoting bacteria MQB12 was used to inoculate *Vigna radiata* to evaluate the effects of external inoculants on plant transcriptomics and rhizosphere soil microbial diversity. Enrichment analysis using GO and KEGG revealed significant enrichment in DNA-binding transcription factor activity, transcriptional regulatory factor activity, and phenylpropanoid biosynthesis among the differentially expressed genes. MQB12 inoculation positively responded to phosphorus starvation response, increased the expression of phosphorus starvation response genes (PHT/PAP), enhanced the synthesis of ethylene and salicylic acid to cope with external stress, and improved the expression of plant disease resistance genes to strengthen the disease resistance of plants to pathogens. At the same time, microbial diversity analysis further revealed the positive effect of MQB12 inoculum. MQB12 inoculum enriched beneficial flora, improved flora abundance, changed the structure and diversity of *V. radiata* rhizosphere microbial community, enhanced the interconnections between the flora, and positively promoted growth. MQB12 was found to adjust the microflora of the rhizosphere, which subsequently changed the environment for plant colonization. This change led to the enrichment of beneficial bacteria and removal of pathogenic bacteria, which positively affected the internal pathways of plants. Additionally, changes in gene expression levels of plants resulted in the formation of different phenotypes and various metabolites, further influencing the formation of rhizosphere microbial communities through close contact between roots and soil. This study provides new insights into the effects of microbial agents on plant growth and root environment construction and is conducive to the further development and application of microbial agents.

**Keywords** Rhizosphere growth-promoting bacteria, Microbial diversity, Transcriptomics, Plant hormones

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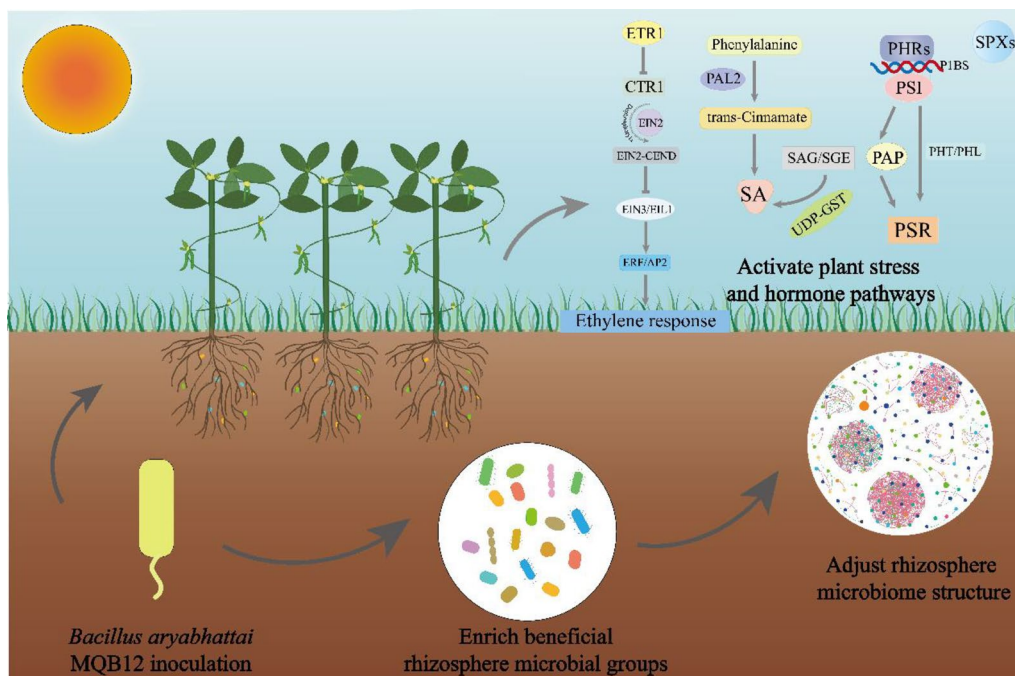†Rui Zhao and Fen He contributed equally to this work.

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**Graphical Abstract**



## Introduction

Soil is rich in a large number of nutrients, including macronutrients and micronutrients, among which nitrogen, phosphorus, and potassium are three essential elements for plant growth and development. A small part of the nutrients in the soil are free in the soil layer in the form of ions, which plants can quickly take up, and most of them are fixed in the soil in the form of binding. Due to the rapid increase in human activities, fast-acting ions can no longer supply the growth and development of plants. Demay et al. [1] conducted a simulation calculation of global soil phosphorus dynamics. The startling fact is that the level of agricultural development in different regions is related to the application of fertilizer to some extent, which may be partly attributed to large-scale agricultural production. This also brings a series of problems, such as the hardening of soil, eutrophication of water, and depletion of mineral resources. In recent years, scholars have conducted a series of studies to deal with this situation. Du et al. applied biological fertilizer, chemical fertilizer, and organic fertilizer to *Juglans regia* roots in different proportions [2]. The results showed that compared with the application of chemical fertilizer alone, the application of organic fertilizer and biological fertilizer changed the rhizosphere soil microbial community structure, and improved *Juglans regia* yield. A large number of studies have shown that organic fertilizer may replace chemical fertilizer as a key factor in improving the environment. However, with a booming human population, China's demand for food is increasing day by day, and the supply of nutritional elements through organic fertilizer alone cannot meet the needs of agricultural production. Therefore, microbial agents as a kind of pollution-free, large-scale commercialization, rapid-effect, and good-effect products appear in public view. It is easy to operate, have strong adaptability to crops, can resist a variety of biological and abiotic stresses, and have a good improvement effect on the soil environment. Commercial microbial agents are generally formulated with one or more growth-promoting bacteria, which can make up for each other's strengths and greatly improve the effect of promoting growth and increasing the production of plants.

Rhizosphere is an important area for plant growth, which refers to the contact surface between plant roots and soil (broadly defined to include plant roots). There are numerous microorganisms in rhizosphere soil, which contain many strains beneficial to plant growth and development. They are called plant growth-promoting rhizobacteria (PGPR). Diverse plant species and variability geographical environment have formed a complex and huge rhizosphere microflora. In recent years,

Zhao *et al. Chem. Biol. Technol. Agric.* (2024) 11:116

Page 3 of 17

many microorganisms have been found to be PGPR, including *Bacillus*, *Penicillium*, *Pseudomonas*, *Pantoea*, *Enterobacter*, *Streptomyces*, etc. PGPR has a variety of life-promoting properties, in which phosphorous solubilizing bacteria and potassium solubilizing bacteria can produce organic acids to mineralize insoluble metal complexes in soil and dissolve organometallic ions to improve soil nutrients. The siderophores produced by PGPR can also supplement the lack of iron ions in the soil and resist heavy metal stress. In addition, PGPR can also produce secondary metabolites such as antibiotics and flavonoids, resist crop diseases [3], and improve plant disease resistance as a biological control agent.

In plant rhizosphere, a variety of microorganisms together constitute the rhizosphere environment. In a relatively stable ecological environment, the root environment is dynamically balanced, and different bacteria play different functions. Therefore, the change in microbial community will greatly affect the growth of the upper-layer plants, and the diversity and abundance of microorganisms are important indicators of plant growth. Wu et al. introduced rhizosphere growth-promoting bacteria into *Hamamelidales* rhizosphere [4], which grew on soil contaminated with heavy metals and found that PGPR colonization in the rhizosphere significantly changed the diversity of the *Hamamelidales* microbial community, up-regulated the activity of genes such as antioxidant enzymes, and greatly reduced the toxicity of heavy metals, confirming the positive regulation of the rhizosphere grow-promoting bacteria on the microbial community. In addition, through the analysis of microbial diversity in the rhizosphere soil of soybeans with different genotypes, it was found that the microbial community structure of rhizosphere soil changed significantly with the changes of plant genotypes, reflecting the close relationship between microorganisms and plants [5]. How microbial agents balance the rhizosphere environment, affect plant growth, and regulate plant gene expression is a problem worthy of discussion.

In most areas of southern China, the soil type is red soil, the content of nutrients in red soil is low, and the soil erosion is fast, which is not conducive to crop growth [6]. However, as a pioneer plant of community succession, *D. dichotoma* can show preferential growth in the barren red soil, suggesting that rhizosphere microorganisms play an important role in promoting plant growth. In this study, a strain of *Bacillus aryabhattai* MQB12 (Genbank accession: OR226572) was isolated from *D. dichotoma* rhizosphere soil in red soil area. MQB12 was used as experimental material, was identified as a multifunctional rhizosphere growth-promoting bacterium by the determination of *V. radiata* phenotype and yield. Meanwhile, the transcriptomic and microbial diversity results revealed the positive contribution of MQB12 in plant growth by inoculation on the rhizosphere soil microbial community and the gene transcription level in the plant through high-throughput sequencing.

## Material and methods

### PGPR function screening of strains

Detection of phosphate-solubilizing microorganisms: single colonies were inoculated on NBRIP medium [glucose 10 g, $Ca_3(PO_4)_2$ 5 g, $MgCl_2$ 5 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, KCl 0.2 g, $(NH_4)_2SO_4$ 1 g, pH 7.0], 30 °C inverted culture for 7 days. Observe the halo produced by the dissolution of tricalcium phosphate in the medium and record the ratio of PSI (the diameter of the halo to the colony) for the preliminary identification of phosphorus-solubilizing bacteria. 60 mL of NBRIP liquid culture medium was added into 250-mL triangular bottle, and 200 μL of MQB12 bacterial solution $OD_{600nm} = 0.8$ was added. The content of soluble phosphorus was determined by the Mo–Sb colorimetric method [7], soluble phosphorus content and pH value in the fermentation solution were recorded within 7 days.

Determination of cellulase production activity: the strains were inoculated on CMC-Na medium, cultured inversely for 3 d, stained with 1% Congo red solution for 1 h, and then rinsed with 1 M NaCl solution for 30 min to calculate the ratio of halo.

IAA activity detection: bacterial solution was cultured in incubator shaker to $OD_{600nm} = 0.8$, 200 μL bacterial solution was absorbed into 60 mL King's B medium, 500 mg/L L-tryptophan was added, and then cultured in incubator shaker at 160 r/min at 28 °C for 3 d. The fermentation solution was centrifuged for 15 min, the supernatant was mixed with PC colorimetric solution 1:2 and placed in the dark for color development for 30 min. The IAA standard solution was used as positive control and sterile water as negative control. The light absorption value was determined by $OD_{530nm}$ and the content was worked out by IAA standard curve. The IAA solution of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/L was prepared with the IAA standard material and the corresponding light absorption value was determined according to the above method to make the standard curve.

Detection of siderophores production: absorb 200 μL bacterial solution ($OD_{600nm} = 0.6$) into 50 mL MKB medium, culture at 150 r/min at 28 °C for 48 h, centrifuge the fermentation solution for 15 min, the ratio of supernatant and CAS test solution is 1:1, mixing uniformity, store in the dark for 1 h, take the blank medium as the reference ratio (Ar), determine the absorbance at $OD_{630nm}$, $pSU = (Ar - A)/Ar * 100\%$. The pSU value indicates the siderophores yield of the strain, and more than

Zhao *et al. Chem. Biol. Technol. Agric.*     (2024) 11:116

Page 4 of 17

60% is considered to be a high siderophores yielding strain.

### Plant experiment condition

Fresh, full, intact seed coat, high water content and undried seeds were selected and soaked in 75% ethanol solution for 1 min, followed by soaking in 5% NaClO solution for 5 min, rinsed with sterile water for 4 times, and the seeds were soaked for 12 h and then inoculated on autoclaved and cooled 1/2 MS solid medium at room temperature for 6 d, removed from the medium and transplanted into unsterilized peat soil (100 g/pot). The incubation conditions were 28 °C with a day/night ratio of 16 to 8, each *V. radiata* seedling was inoculated with 1.5 mL of bacterial solution ($OD_{600nm} = 0.6$) for a week and was watered every 2 days The growth of *V. radiata* seedlings was measured after 30 d of incubation, such as root length, plant length and fresh weight, etc.

### Microbial diversity analysis

The high-throughput sequencing data were first qualitatively filtered using Trimmomatic v0.33 [8], the primers were identified and removed by Cutadapt v1.9.1 [9]. Then USEARCH (v10.0) was used to cluster sequences at the 97% similarity level [10]. Species are annotated using joint databases such as Silva [11] and Unite [12]. After obtaining the annotated fungal and bacterial sequences, Chao1, Simpson, ACE and Shannon indices were used to evaluate the richness, evenness and diversity of the flora (ANOVA test). Principal component analysis is an arrangement analysis based on the original species composition, which mainly represents the differences in microbial community composition.

### Transcriptomic analysis

RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system, prepared transcriptome library, and sequencing, clean data (clean reads) were obtained by removing reads containing adapter. HISAT2 v2.0.5 was used for comparison with the reference genome, featureCounts (1.5.0-p3) is used to assess gene expression levels, DESeq2 software (1.20.0) was used for differential expression analysis, GO and KEGG enrichment analysis of differentially expressed genes was performed by clusterProfiler (3.8.1) software.

### Data analysis

Univariate analysis of variance and ANOVA test were used SPSS v27.0. The co-occurrence network analysis used Graph v4.4. Uniprot database was used to retrieve protein sequences and compare them. The full text uses Origin v.2022 for drawing.

## Results and discussion

### MQB12 has a variety of PGPR activities

In our study, a variety of physiological activities of MQB12 were measured, and the results showed that MQB12 can grow with CMC as the only carbon source and has a good cellulase production ability (EI = 1.7 ± 0.05). In addition, MQB12 can also be grown using calcium phosphate as the only phosphorus source (PSI = 1.5 ± 0.12). According to the phosphorus standard curve, it can be calculated that MQB12 shows the largest inorganic phosphorus dissolution ability during 4 d culture, and the soluble phosphorus content (6.66 mg/L) in the fermentation solution increases by about 16.9 times compared with the control, while the pH of the fermentation solution also decreases to the lowest (Fig. S1). In addition, MQB12 also can produce IAA (49.19 mg/L) and siderophore (60.7%), and the various physiological activities of MQB12 point to its excellent potential as a rhizosphere growth-promoting bacterium (Fig. 1A, B).

Among the discovered phosphorus-solubilizing bacteria, *Bacillus* sp. strains are numerous and capable of dissolving insoluble inorganic phosphorus, such as tricalcium phosphate and phosphate rock powder. In previous studies, the isolated strain with the strongest phosphorus solubilizing ability is the strain that can release 125.88 mg/L soluble phosphorus [13], compared with the MQB12 strain in this study, which has a moderate ability to dissolve inorganic phosphorus. The mechanism of phosphorus-solubilizing bacteria dissolving inorganic phosphorus has been preliminarily proved. Other studies have found that inorganic acid produced by phosphorus-solubilizing bacteria can change the pH of fermentation environment to solubilize inorganic phosphorus. This phenomenon is also confirmed in this experiment. Cellulose is the main component of plant's somatic cell wall. In soil, especially in soil with more humus, there exists a class of strains that can produce cellulase to hydrolyze cellulose, which is called cellulose-degrading strains. In recent studies, most of the cellulose-degrading strains are fungi, mainly *Penicillium* sp. and *Aspergillus* sp. However, the cellulose-degrading bacteria genera are also very diverse. Such as *Bacillus* sp. [14], *Mycobacterium* sp., *Chryseobacterium* sp.. In this study, MQB12 strain can grow on CMC-Na medium and produce a halo, and its cellulase function was preliminarily determined. In addition, IAA and siderophores activity are also key indicators of rhizosphere growth-promoting bacteria. IAA secreted by bacteria can promote plant root development and affect plant growth at low concentrations [15], siderophores regulate iron availability in iron-deficient plants and reduce heavy metal toxicity plays a role in phytoremediation [16, 17].
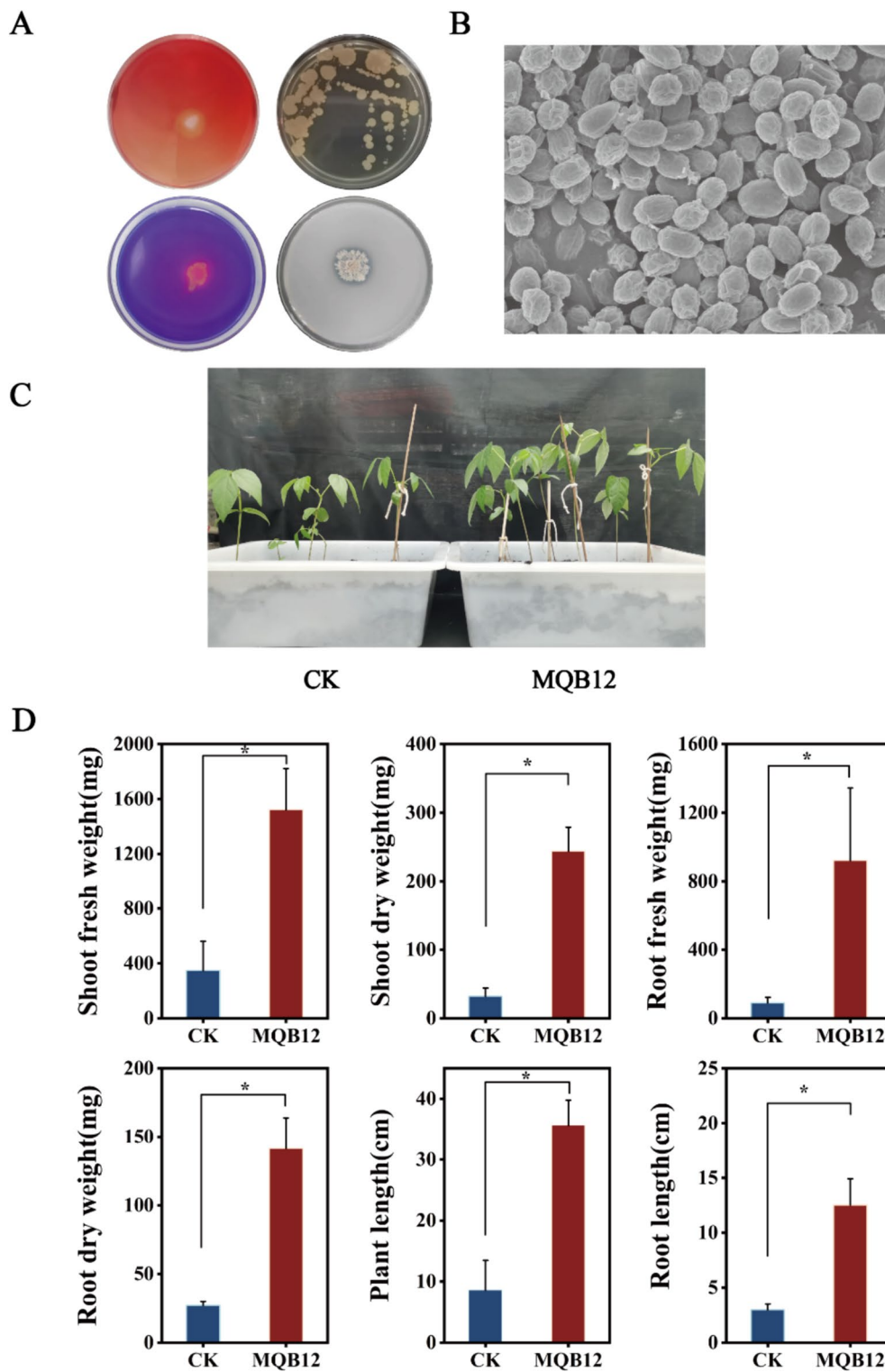
**Fig. 1** MQB12 promotes plant growth properties. **A** MQB12 colony plate and PGPR traits, from left to right, from top to bottom are: CMC-Na medium plate, MQB12 colony plate, siderophore plate, phosphorus soluble plate; **B** scanning electron microscope morphology of MQB12; **C** MQB12 promoting growth phenotype of *V. radiata*; **D** physiological indexes of MQB12 on *V. radiata* growth, ANOVA test, $p < 0.05$

Zhao *et al. Chem. Biol. Technol. Agric.* (2024) 11:116

Page 6 of 17

Compared with the control group, the shoot fresh and dry weight, the root fresh and dry weight, plant length, and root length (Fig. 1C, D) of above and underground *V. radiata* after inoculating MQB12 strain were significantly increased ($p < 0.01$). There was no difference in the number of leaves. Our results were similar to those of Shameem et al. They isolated a nitrogen-fixing bacterium with various life-promoting properties from rhizosphere soil, and its inoculation with *V. radiata* and *Eleusine coracana* greatly increased the dry weight and plant height of the plant [18]. The study of Bai et al. also showed that growth-promoting bacteria could increase dry and fresh weight and root length of above-ground and underground crops under salt stress to promote plant growth [19]. In addition, we also determined the soil total phosphorus content of MQB12 group and CK group, and the total phosphorus content of CK group was 0.9 g/kg, while the total phosphorus content of MQB12 inoculation group was 1.01 g/kg. MQB12 inoculation not only promoted the growth of *V. radiata*, but also increased the phosphorus content of soil.

## Effects of MQB12 treatment groups on soil microbial community

The diversity of rhizosphere soil microflora in MQB12 and CK groups was evaluated. Raw reads and clean reads were 85,754 and 49,426, respectively, and 41,200 and 22,859, respectively, with a coverage rate of more than 99%. The diversity index analysis of samples from two groups showed that the Chao1 index, ACE index, Simpson index and Shannon index of MQB12 treatment group were significantly higher than those of the control group ($p < 0.01$), indicating that the bacteria abundance of MQB12 treatment group was higher than that of the CK group (Fig. 2A–D). Likewise, Simpson and Shannon indices of fungi in the MQB12 inoculation group were higher than those in the control group (Fig. 2E–H), but Chao1 and ACE index decreased ($p < 0.05$), indicating that the diversity and richness of fungi in soil rhizosphere microorganisms increased and decreased.

Weighted Unifrac algorithm was used to analyze the species diversity distribution, and PCA analysis presented the cluster results of bacterial and fungal communities under different treatments. The CK treatment of bacterial (Fig. 3A) and fungal (Fig. 3B) communities was significantly different from the MQB12 treatment,
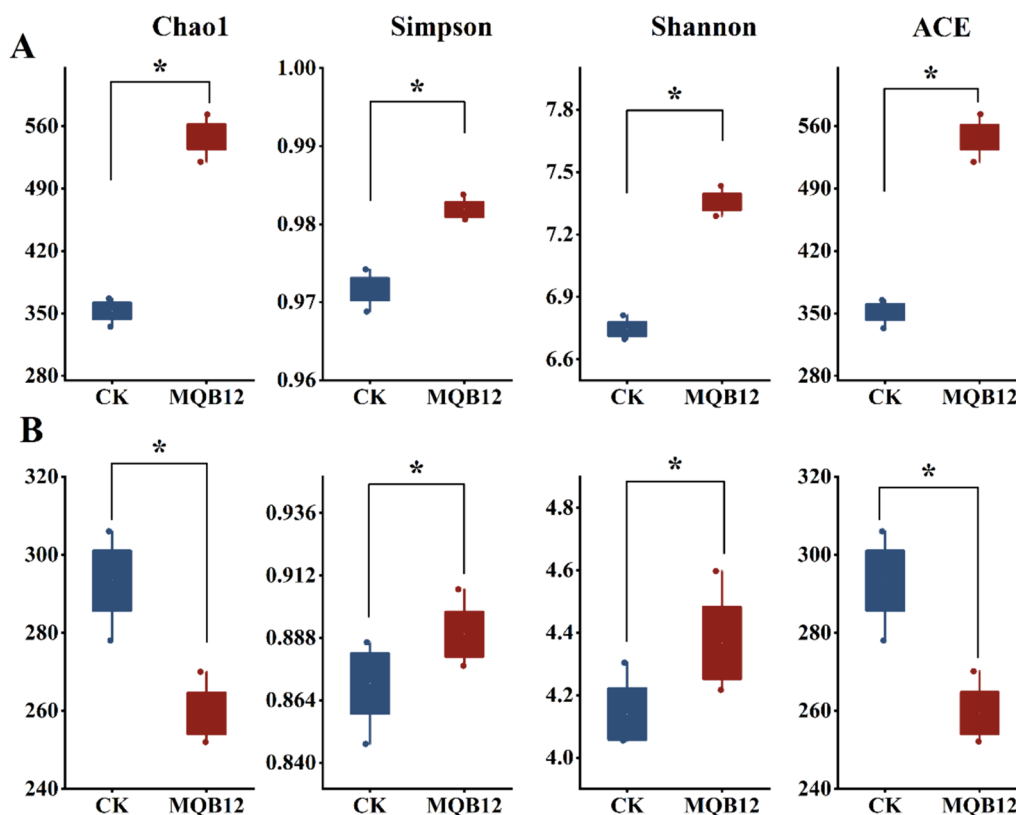


**Fig. 2** Effects of MQB12 inoculation treatment on Alpha diversity (Chao1, Simpson, Shannon, ACE) indices of bacteria (**A**) and fungi (**B**). Different lowercase letters indicate significant differences among treatments, *$p < 0.05$
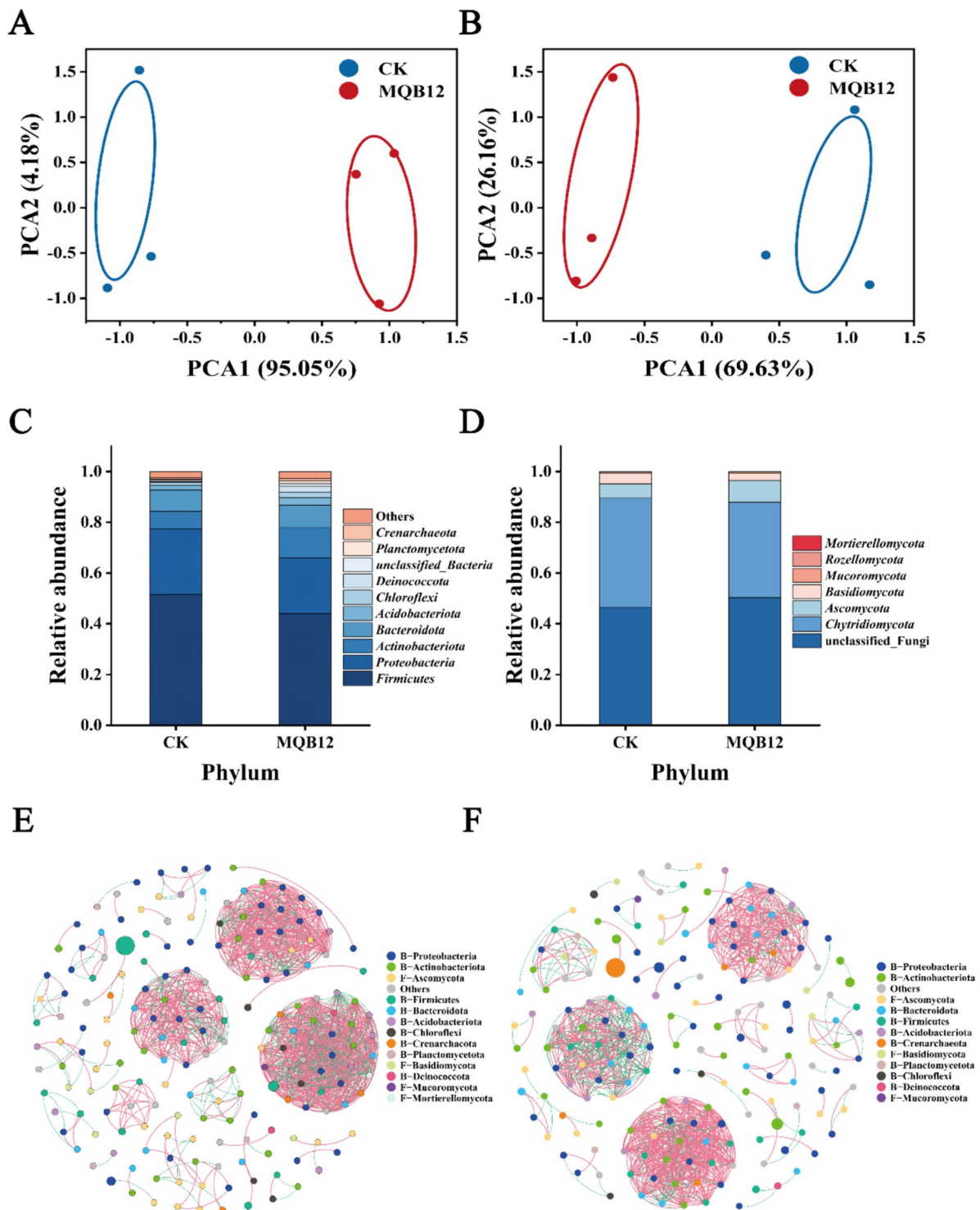
**Fig. 3** **A**, **B** Principal component analysis (PCA) with weighted UniFrac distance showing effects of MQB12 inoculation treatment on beta diversity of soil bacteria (**A**) and fungi (**B**); **C**, **D** effects of MQB12 inoculation treatment on the composition of bacterial (**C**) and fungal (**D**) communities; **E**, **F** soil microbial co-occurrence network in CK (**E**) and MQB12 (**F**) treatment groups were divided (Spearman test, $p < 0.05$)

Zhao *et al. Chem. Biol. Technol. Agric.*      (2024) 11:116

Page 8 of 17

and the bacterial and fungal communities could be well separated in PCA analysis. Bacteria PCA1 accounted for 69.63% of the results, fungi PCA1 accounted for 69.63% of the results, which showed that the distribution of bacteria and fungi in rhizosphere soil changed greatly after MQB12 inoculation.

The sequencing results revealed that the abundance of fungi in the rhizosphere soil decreased, while the abundance of bacteria increased after treatment with MQB12. In the MQB12 group, a total of 778 fungal OTUs and 1642 bacterial OTUs were obtained. Furthermore, 6 phyla, 14 classes, 22 orders, 34 families, 48 genera, and 60 species were identified for fungi, whereas 23 phyla, 45 classes, 106 orders, 160 families, 250 genera, and 272 species were identified for bacteria. In the CK group, a total of 880 fungi and 1051 bacteria were identified, including 7 phyla, 17 classes, 29 orders, 38 families, 54 genera, and 64 species for fungi, and 21 phyla, 41 classes, 87 orders, 132 families, 199 genera, and 210 species for bacteria.

At the bacteria phylum level (Fig. 3C), CK shares the same dominant phylum as MQB12. *Firmicutes* sp. and *Proteobacteria* sp. are the first and second dominant phyla, respectively, accounting for more than 60% of the total abundance. MQB12 treatment reduced the relative abundance of the two dominant phyla. In the treatment group, the relative abundance of *Actinobacteriota* sp. is higher than that of *Bacteroidota* sp. Ranked as the third dominant phylum, this is the opposite of the situation in CK. *Actinobacteriota* sp. is widely distributed in nature. The secondary metabolites produced by *Actinobacteriota* sp. can inhibit soil pathogens and enhance plant resistance [20], and the hydrolase produced by *Actinobacteriota* sp. can promote the transformation of organic matter in the soil, playing an important role in maintaining the ecological cycle of the soil system [21]. Wolikanska et al. [22] showed that the relative abundance of *Bacteroidota* sp. in cultivated soil was lower than that in uncultivated soil, and *Bacteroidota* sp. could be used as a sensitivity index of agricultural soil. In addition, inoculation of MQB12 also increased the relative abundance of *Acidobacteriota* sp. ($p < 0.01$), *Deinococcota* sp. ($p < 0.01$), *Chloroflexi* sp. ($p < 0.01$), *Planctomycetota* sp. ($p < 0.01$) and *Crenarchaeota* sp. ($p < 0.05$), both of them have a certain regulation effect on soil N cycle [23, 24], and *Chloroflexi* sp. also participates in the cycling and coupling of C, S and other elements through the photosynthetic oxidation process [25, 26], which plays an important role in promoting the cycling of elements in rhizosphere soil. Moreover, Kalam et al. also showed that *Acidobacteria* sp. was found in rhizosphere soil after inoculation with PGPR and there was a significant increase in the abundance of *Crenarchaeota* sp. [27], which is consistent with our study. At the same time, combined with previous

studies, Liu et al. also found a relationship between the abundance of *Acidobacteria* sp. and soil pH and carbon–nitrogen ratio, the latter two factors drive the community distribution of *Acidobacteria* sp. [28]. In addition, the relative abundance of *Deinococcota* sp. in MQB12 group was higher than that of CK ($p < 0.01$), *Deinococcota* sp. also known as extreme particles, it is a phylum that is extremely tolerant to the environment. Compared with CK, MQB12 inoculation also enriched some special bacteria, such as *Hadarchaeota* sp., *Methylomirabilota* sp., *Nitrospirota* sp. In summary, MQB12 inoculation enriched more soil element cycling and environmental tolerance related phyla at the phyla level.

At the class, genus, order and species levels, the microorganism abundance of MQB12 treatment group was significantly higher than that of CK treatment group, such as *Deinococci* sp., *Vicinamibacteria* sp., *Acidimicrobiia* sp. The results showed that MQB12 inoculation promoted the enrichment of beneficial microorganisms in the rhizosphere to a certain extent. MQB12 is molecularly annotated as *Bacillus* sp., and the inoculation of MQB12 increases *Bacilli* sp. in the soil. MQB12 inoculation increased the abundance of *Bacilli* sp., but decreased the relative abundance of *Bacilli* sp. with the increase of others.

At the fungal phylum level (Fig. 3D), except for fungal that could not be classified, *Chytridiomycota* sp., *Ascomycota* sp., *Basidiomycota* sp. are the three dominant fungal phylum. In the CK group, the relative abundance of *Chytridiomycota* sp. and *Basidiomycota* sp. was higher but *Ascomycota* sp. ($p < 0.01$) was lower than inoculated group. The relative abundance of *Mucoromycota* sp. and *Rozellomycota* sp. were low, accounting for 0.1% and 0.49% of the total fungal abundance. In addition, *Mortierellomycota* sp. was not found in the MQB12 group.

At the fungal class level, in the MQB12 treatment group, a total of 6 classes (unclassified_Fungi, *Sordariomycetes* sp., *Dothideomycetes* sp., *Eurotiomycetes* sp., unclassified_Ascomycota, *Mucoromycetes* sp.), the abundance were significantly higher than that of CK group, and except for unclassified fungal. In MQB12 treated group, the first three dominant bacteria class were *Dothideomycetes* sp., *Sordariomycetes* sp., and unclassified_*Basidiomycota*, which were the same as the dominant class in group CK, but in CK, unclassified_*Basidiomycota* exists as the first dominant class. MQB12 inoculation enhanced the resistance of plants to external diseases, and reduced recruitment of disease-associated fungi, such as *Laboulbeniomycetes* sp., which was easy to parasite in worms, and *Microbotryomycetes* sp. (one of the most important modes of survival is parasite survival) and *Oiliomycetes* sp. have not been detected in MQB12 group [29, 30]. Our results are

consistent with some studies. Xiong et al. evaluated the invasion of microbial inoculants and found that most of them would cause changes in the ecological niche of soil microbial communities, including antagonism to pathogenic bacteria [31] and enrichment of beneficial bacteria [32].

At the level of phyla, Pearson analysis showed that *Firmicutes* sp. was significantly negatively correlated with the biomass and plant height of *V. radiata*, indicating that the increase in the abundance of this bacterium was unfavorable to the growth of *V. radiata* (Fig. S2). In addition, *Crenarchaeota*, unclassified_Bacteria, *Actinobacteriota*, *Planctomycetota*, *Deinococcota*, *Chloroflexi* and *Acidobacteriota* are all significantly correlated with plant growth. At the genus level, unclassified fungi, *Eurotiomycetes* and unclassified_*Ascomycota* were significantly correlated with plant growth. *Agaricomycetes*, unclassified_*Basidiomycota*, unidentified were negatively correlated with biomass, and increasing their abundance had no positive effect on plant growth. In general, the access of MQB12 changes the structure of rhizosphere microorganisms in a direction conducive to biological growth.

Co-occurrence network analysis (Spearman test, $r > 0.9$, $p < 0.001$) was performed on rhizosphere microflora of MQB12 treatment group (Fig. 3F) and CK group (Fig. 3E) at the genus level, involving 9 bacterial genera and 6 fungal genera. The results show that the network of the MQB12 treatment group is more complex, with 355 nodes and 14,570 edges, while CK has 310 nodes and 9742 edges. MQB12 shares 1925 edges with CK, which has 7817 unique edges, and MQB12 has 12,645 unique edges. Through the co-occurrence network, it can be seen that *Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, and *Ascomycota* are closely related to other bacteria genera in the rhizosphere microbial community of *V. radiata* and play a major role in them.

### Effects of MQB12 treatment on plant genes transcription levels

The quality of MQB12 samples in the treatment group and CK samples in the control group were evaluated

and compared, and three biological replicates were performed in each group (Table 1). The average raw reads of MQB12 group and CK group were 433,879,987 and 43,165,911, and the average clean reads were 41,619,997 and 41,562,280, respectively. After sequencing quality control, the percentage of Q20 base was more than 96%, and the percentage of Q30 base was more than 90%. The filtered reads of the two samples were sequentially compared with the specified reference genome. The efficiency range of comparison was 94.56% to 95.21%, and the GC content of both samples was above 43%. The above data results indicate that the sequencing results are of reliable quality and can be further analyzed for biological information.

To further understand the transcriptome characteristics of the effects of external inoculants on plant growth, the genes of CK plants in the control group and MQB12 plants in the treatment group were compared (Fig. S5). Compared with the control group, a total of 519 DEGs (different enrichment genes) were detected by MQB12, among which 393 differential genes were up-regulated and 126 DEGs were down-regulated. The results showed that the up-regulated expression genes were significantly higher than down-regulated expression genes in *V. radiata* plants treated with external inoculants, and the number of up-regulated DEGs was 3.1 times that of down-regulated DEGs.

To explore the function of DEGs in *V. radiata* growth process, GO functional enrichment analysis was performed on differential genes under this treatment. The results showed that a total of 475 DEGs were annotated into 39 branches of biological process (BP), cell component (CC) and molecular function (MF) by GO analysis, among which 328 DEGs were up-regulated and 147 DEGs were down-regulated. The genes of BP were mainly concentrated in metabolic processes (6043), cellular processes (5480) and single biological processes (3821). CC are mainly enriched in Cell (1287), Cell part (1287) and membrane (953). Catalytic activity (6080), transporter activity (707) and binding (8223) are the main function involving MF in catalytic activity (Fig. S3A). The 30 most enriched GO differential genes were screened, and the

**Table 1** Statistics of RNA-seq data of all samples

| Groups | Raw reads | Clean reads | Total map (%) | Q20 (%) | Q30 (%) | GC (%) |
|--------|-----------|-------------|---------------|---------|---------|--------|
| CK | 43,961,134 | 42,435,150 | 95.04 | 96.68 | 91.03 | 43.5 |
| CK | 43,344,852 | 41,647,830 | 94.80 | 96.37 | 90.34 | 43.4 |
| CK | 42,191,748 | 40,603,860 | 94.79 | 96.38 | 90.37 | 43.5 |
| MQB12 | 41,658,914 | 41,800,492 | 94.56 | 96.5 | 90.68 | 44.08 |
| MQB12 | 43,980,082 | 40,537,272 | 95.21 | 96.55 | 90.69 | 43.63 |
| MQB12 | 44,524,994 | 42,522,226 | 94.89 | 96.37 | 90.39 | 43.56 |

Zhao *et al. Chem. Biol. Technol. Agric.*     (2024) 11:116

Page 10 of 17

biological process differential genes were mainly enriched in response to stress and response to oxidative stress (Fig. S3B). DEGs was mainly concentrated in the anchored component of membrane and peroxisome. Molecular functional differential genes are mainly concentrated in DNA-binding transcription factor activity and transcription regulator activity. Under MQB12 treatment, upregulated differentially expressed genes were more than down-regulated genes in the top 30 go-enriched DEGs, and the above-regulated genes were the main ones. It is mainly concentrated in DNA-binding transcription factor activity, transcription regulator activity, transferase activity, transferring glycosyl groups, heme binding, response to stress, among which DNA-binding transcription factor activity upregulates the most genes. There were 16 more up-regulated genes than down-regulated genes.

A total of 85 KEGG differential genes were annotated, of which 69 up-regulated DEGs and 16 down-regulated DEGs. The main pathways of significant enrichment to KEGG are phenylpropanoid biosynthesis (12), biosynthesis of amino acids (11), carbon metabolism (9), and linoleic acid metabolism (4), etc. (Fig. S4).

### Changes of phosphorous starvation regulatory pathway

Pi starvation responses (PSR) is a response mechanism plants evolve to resist stress when external phosphorus concentration is low in plant growth and development [33]. In the process of phosphorus regulation, the SPX gene and phosphate starvation response (PHR) domain strongly respond to phosphorus starvation. The main effect of the SPX domain on plant growth is that it participates in the regulation of plant phosphorus. In *Arabidopsis thaliana*, rice, and other plants, the mechanism involved in PSR at different phosphorus concentrations has been identified: at low phosphorus concentration, a sequence of P1BS on the promoter element of Pi starvation-induced gene (PSI) competes with SPX for the PHR binding site and activates the PHR gene to induce the expression of downstream phosphorus response genes [34]. The plant tolerance to low phosphorus stress was improved through the secretion of acid phosphatase and phosphorus redistribution [35, 36]. In the case of high phosphorus, SPX combines with PHR to inhibit the transcriptional activity of downstream genes, to avoid the excessive absorption of phosphorus by plants and the excessive accumulation of phosphorus in plants, leading to phosphorus poisoning [37]. Compared with the control group, four SPX genes were up-regulated in the inoculated group MQB12 transcriptome (Fig. 4A). They are *VraSPX1* (LOC106756795), *VraSPX2* (LOC106771555), *VraSPX3* (LOC106773836), and *VraSPX1-like* (LOC106766822). The above four SPX genes were

compared with AtSPXs gene sequences in NCBI, and the similarity between *VraSPX2* and *AtSPX2*, *VraSPX1* and *AtSPX1*, *VraSPX3* and *AtSPX3* sequences was 100, indicating that they were homologs (Fig. 4C). VraSPX1/2/3 is predicted to play a major role in regulating phosphorus in *V. radiata*. SPX domain proteins can also bind to the major facilitator superfamily (MFS) domain to form SPX-MFS, which mediates phosphorous transport from cells to vacuoles. Previous studies found that the large accumulation of glycerol-3-phosphate transporter (GlpT) on phosphorus starvation may be due to its belonging to the MFS family [38], which can promote G3P uptake and simultaneously promote phosphorus inflow into the cytoplasm, suggesting that the large expression of GlpT in this study is similar.

In the process of phosphorus regulation, the SPX-PHR domain plays an important role, and PHR is even more important as a regulatory core transcription factor [39]. Its homolog PHL (PHR-like) belongs to the MYB-CC family, and several PHRS have been identified in *Arabidopsis thaliana*, all of which can regulate PSR [40–42]. In this experiment, several *V. radiata* PHR genes –Vra PHR and Vra PHR-like were annotated, and their gene expression levels increased but did not change significantly. PHR regulates many downstream transcription factors and target genes, such as *IPS1/AT4* [43], miRNA827/399 [44], and various phosphatases (PLA/PLC/PLD/PAP) [45]. Phosphate transporter (PHT) is the gene most associated with phosphorus uptake in plants. Multiple transporters of PHT1-4 have been found in *Arabidopsis*, rice, soybean, etc. (Fig. S6A), which are responsible for phosphorus transport in plants and are important for above-ground phosphorus transfer in plants [46, 47]. In this experiment, two inorganic phosphate transporter genes (LOC106757893 and LOC106779138) were found to have significantly higher expression levels in the MQB12 group than in the CK group (log2FoldChange > 5), and LOC106779138 was only expressed in the MQB12 treated group. Moreover, through protein sequence comparison, it was found that the sequence similarity with *AtPHT1,4* was 100. It was preliminarily identified as a downstream phosphate transporter regulated by PHR, and a P1BS promoter sequence (GNATATNC) was found in the promoter region of LOC106779138 gene. It is further determined that it can bind to the PHR domain and participate in the SPX-PHR-mediated phosphorous regulation pathway. Combined with the soil test data of *V. radiata* planting, it can be determined that the plant is in a low phosphorus concentration environment which is lower than the required nutrients for plant growth. The upregulated and down-regulated differential expressions
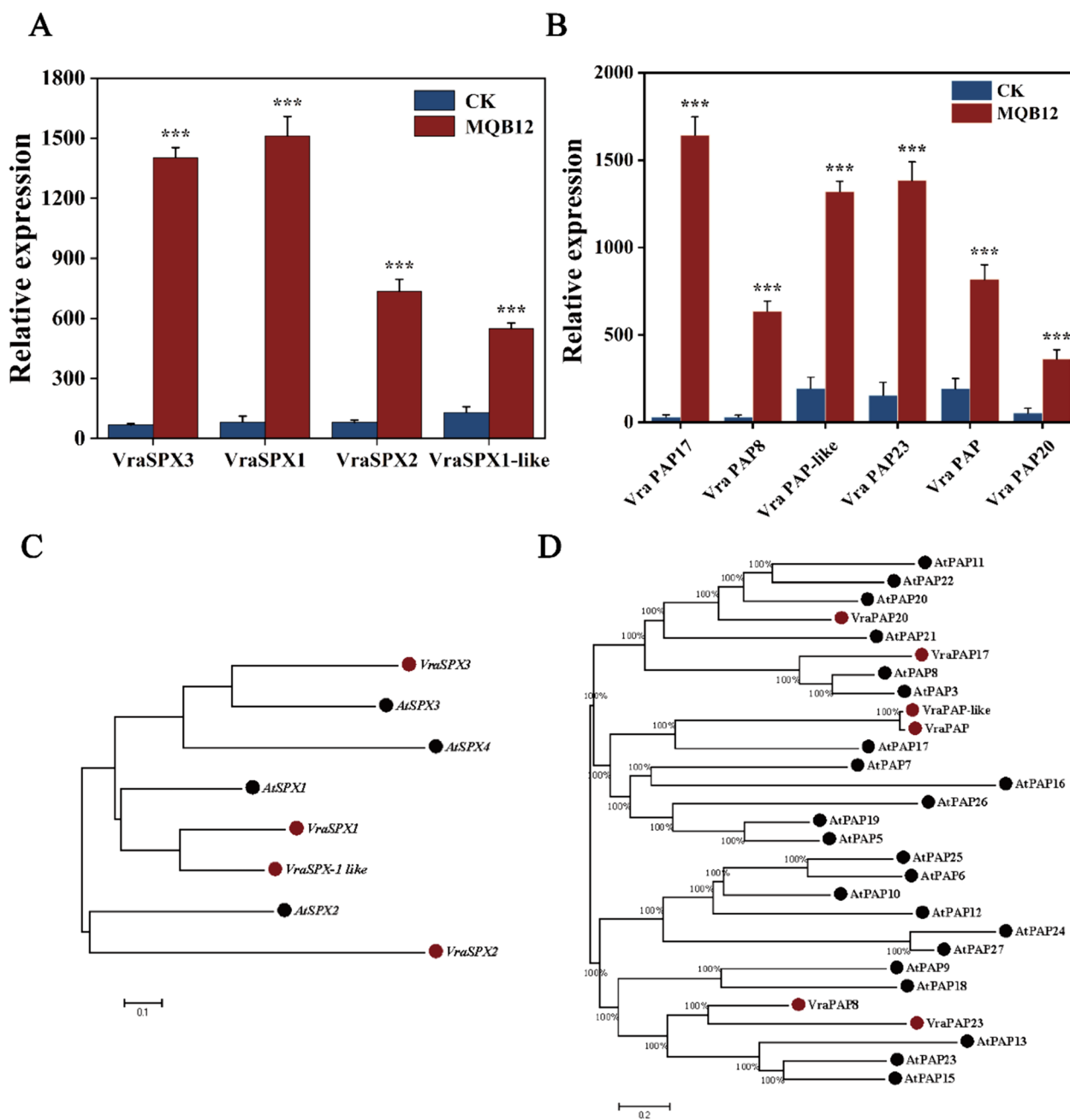
**Fig. 4** SPX and PAP gene expression (**A**, **B**) and phylogenetic tree (**C**, **D**) in CK and MQB12 treatment groups. The bar chart represents the relative expression levels of SPX (**A**) and PAP (**B**) genes in the two groups, with *** representing the extremely significant, ANOVA test ($p < 0.01$). Each gene label's first two or three letters represent the abbreviated species name: At, *Arabidopsis thaliana*; Vra, *Vigna radiata*. SPX: SYG1/Pho81/XPR1; PAP: phosphatidic acid phosphatase. All mentioned gene names are indicated in Table S1

of SPX1/2/3, PHR, and PHT genes screened in differential genes with highly similar sequences to *Arabidopsis thaliana* are consistent with the findings of previous studies: under the condition of low phosphorus, SPX1/3 positively regulates the phosphorus starvation process, and PHR regulates the internal phosphorus cycle by binding to the P1BS site on the PHT gene to avoid low phosphorus stress and maintain the physiological process of plants.

In the process of maintaining phosphorus homeostasis in plants, phosphorus concentration can be maintained through cell membrane lipid degradation and remodeling

Zhao *et al. Chem. Biol. Technol. Agric.*     (2024) 11:116

Page 12 of 17

in addition to phosphorus transfer in different parts of plants by PHT gene. In MQB12 and CK group, another PHR of downstream genes sulfoquinovosyltransferase2 (SQD2) (LOC106772910) is found, which are the essential genes of sulfur fat synthesis [48], and similar in *Arabidopsis thaliana*, have PHR binding sites. It is speculated that PHR regulates its expression, and it is involved in the reconstruction process of plant membrane lipids by regulating the accumulation or transfer of glucose and lipids, thus balancing the internal homeostasis of phosphorus. In addition to the regulation of phosphorus-related genes in plants in response to low phosphorus, plants can also decompose organic phosphorus in the environment by promoting the secretion of acid phosphatase and increasing the uptake of phosphorus by plants, among which the most important phosphatase is purple acid phosphatase (PAP). Compared with the control group, the expression of six PAP enzymes in the MQB12 group was significantly increased ($p < 0.05$) (Fig. 4B). They are *VraPAP17* (LOC106764973), *VraPAP8* (LOC106768896), LOC106756075, *VraPAP23* (LOC106764520), LOC106756073, *VraPAP20* (LOC106756937) (Fig. 4D). The inoculation of MQB12 promoted the production of acid phosphatase and thus promoted the uptake of phosphorus in soil and the growth of plants. No more downstream phosphorus response genes were found in the differential genes, and the single expression difference can only provide a hypothesis for the internal phosphorus regulation mechanism of *V. radiata*, which needs to be further explored by knocking out the corresponding genes.

In the above SPX and downstream phosphorous related pathways, we predicted multiple genes affected by MQB12 inoculation, and conducted correlation analysis with *V. radiata* biomass (person test, $p < 0.05$). The correlation heat map (Fig. S7) showed that the predicted SPX1 and SPX1-like genes were significantly correlated with ($R > 0.8$) the biomass, root length, and plant height of *V. radiata.* It may indicate that this gene is actively involved in phosphorous transport pathway transduction. It is worth mentioning that in the above results, all the six phosphatase genes up-regulated were significantly positively correlated with the expression level of SPX gene, and all except PAP20 were significantly positively correlated with *V. radiata* biomass, which further verified the changes of phosphorus transport in plants under MQB12 inoculation.

## Changes in plant hormone and disease resistance regulatory pathways

In the ethylene pathway, ethylene responsive factor (*AP2*/*ERF*) can directly bind to the PHR promoter to synergistically regulate the expression of plant PHR response to phosphorus hunger and participate in the secretion of plant hormone ethylene to promote plant growth [49] (Fig. 5). In this study, one ETR1 receptor gene (LOC106754771) was found to be significantly reduced. Seven ethylene response factors were up-regulated (LOC106757455, LOC106768471, LOC106775532, LOC106771073, LOC106771568, LOC106759144, LOC106752743). In the ethylene regulatory pathway, the ethylene receptor 1 (ETR1) is insensitive to low ethylene concentration. Binding to the constitutive triple response 1 (CTR1) N-terminal, ethylene insensitive 2 (EIN2) is phosphorylated, and the EIN2-CNED is degraded by E3 ubiquitin ligase, resulting in ethylene pathway closure. When stimulated by high concentrations of ethylene, the ETR1 receptor binds to ethylene molecules, resulting in the inactivation of CTR1, which regulates downstream ethylene response factors through EIN3/EIL1 [50]. Although the expression levels of CTR1 and EIN2 are not changed, it can be inferred that the regulation of their pathway is consistent with the change mechanism of high ethylene concentration, that is, after inoculating the MQB12 strain, the strain produces a large amount of ethylene, the ethylene receptor is closed, and the level of ETR1 is reduced, so the change of CTR1 level is not detected. At the same time, the phosphorylation process of EIN2 was inhibited, so EIN2 was not activated, but the expression of ethylene response factor was affected. E3 ubiquitin ligase genes also play an important role in hormone response. In this study, three U-box E3 ligase genes were strongly expressed in the strain treatment group (LOC106779891, LOC106774444, LOC 106770381). Gene expression of one Ring domain family is down-regulated (LOC 106766717), which may respond to stress by regulating hormone levels such as ABA.

Auxin, also known as indole acetic acid, plays different roles in different stages of plant growth and development and can differentiate plant tissues by changing the concentration of various parts of plants. Its transport in plants requires two pathways to regulate: passive transport and polar transport [51]. Auxin polar transport requires the participation of carrier proteins, among which three types of membrane proteins have been proven to transport auxin polar transport: auxin input vector AUX/LAX (AUXIN1/LIKE-AUX1) [52], auxin output vector PIN (Pin-formed) [53], ABCB/PGP (ATP-Binding Cassette subfamily) B/P-glycoprotein transporters [54]. The concentration difference of auxin in plants is mainly related to auxin output vectors, among which PIN family members are the most widely studied [55]. In this study, transcriptome analysis of MQB12 and CK groups showed significant differences in the expression levels of two PIN family members (LOC106767916 and LOC106758994) The gene expression levels were
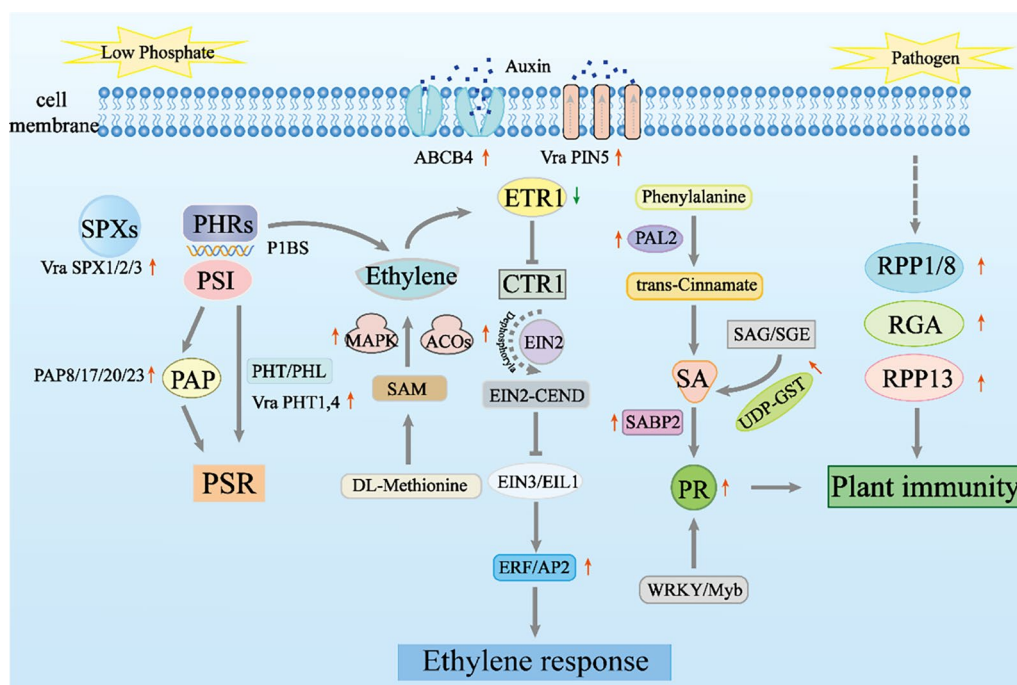
**Fig. 5** Changes of gene transcription levels and pathways in *V. radiata* treated with MQB12. Under the pressure of low phosphorus stress, plants in MQB12 inoculation group responded more quickly to phosphorus starvation stress. The P1BS sequence on the PHT gene promoter competed with SPX for the binding site on the PHR domain, resulting in a large amount of SPX gene accumulation, while inducing a large number of downstream PHT and PAP genes to express in response to phosphorus starvation stress. When plants are subjected to external stress, plant hormones can be mobilized to participate in the stress response. Through the action of MAPK and ACOs enzymes, ethylene precursor SAM is converted into ethylene, ETR1 is turned off, and CTR dephosphorylates EIN2, disactivates transcription inhibition of EIN3/EIL1, and a large amount of ERF/AP2 is expressed. Resist plant stress while promoting plant growth. In addition, salicylic acid can also be produced under the action of PAL2 or UDP-GST, and through the expression of SABP2, regulate the expression of plant disease resistance gene PR and participate in the plant disease resistance pathway. Under the stimulation of external pathogens, PR gene collaborates with RPP1/8, RPP13 and RGA to improve plant disease resistance. All the gene names mentioned above are listed in Table S1

log2FoldChange = 2.6 and 5.22, respectively. The annotation of LOC106767916 was PIN-like3, and the annotation of LOC106758994 was PIN5. In *Arabidopsis thaliana*, PIN1-4 and PIN7 are located on the plasma membrane and mediate the polar transport of auxin between cells [56]. PIN5 and PIN8 are located in the endoplasmic retina and mainly respond to the intracellular auxin. Multiple sequences of PIN member sequences in *Arabidopsis thaliana* were compared, and the evolutionary tree was obtained (Fig. S6B). VraPIN5 was more than 90% similar to PIN5 sequences in *Arabidopsis thaliana* and rice and was predicted to be a potential auxin export vector in *V. radiata*. At the same time, another output vector ABCB family member was also found in the differential gene, annotation as ABCB21-like, gene number LOC106766519. BLAST comparison with the ABCB member with prominent functions in *Arabidopsis thaliana* showed that the similarity with ABCB4 reached 100. As a homology of ABCB4, it has been reported that the intracellular characteristics and functions of ABCB4 have been investigated [57, 58], and it has been confirmed that

ABCB4 is an output vector that regulates intracellular auxin homeostasis and effervescent function, different from the characteristics of PIN family.

Salicylic acid, like auxin and ethylene, is an important hormone affecting plant growth. It plays a positive role in plant stress resistance (such as salt stress and drought stress, etc.), and can also activate plant allergic reactions and systemic acquired resistance under the invasion of plant pathogens and activate plant defense mechanisms. In this study, significant upregulation of two SA binding protein 2 (SABP2) genes was detected in MQB12 group (log2FoldChange = 4.5), as a member of the α/β hydrolytic superfamily, SABP2 has a high affinity for salicylic acid and can catalyze the formation of salicylic acid by methyl salicylic acid (MeSA) and regulate the salicylic acid pathway [59]. Indicating that after inoculating the bacterial solution, SABP2 activity increased, catalyzed the increase of salicylic acid formation of methyl salicylate, and the experimental plants thus obtained higher resistance to pathogens.

Zhao *et al. Chem. Biol. Technol. Agric.* (2024) 11:116

Page 14 of 17

There are two ways for plants to cope with external pathogens. First, under the stimulation of external pathogens, plants prevent the penetration of pathogens by increasing lignin synthesis, thickening the cork layer, and other physical barriers; Plants, on the other hand, rely on two regulatory mechanisms: pathogen-associated molecular pattern (PGAMP)-triggered immunity, PTI) and effector triggered immunity (ETI). The NBS-LRR (NLR) family plays a major role in the ETI process, which consists of multiple components [60], among which CC and TIR are signal domains, which can induce defense response when expressed separately. The NBD and LRR domains perform regulatory and sensor functions. An NLR that contains the cc domain is called CNL, and an NLR that contains the tir domain is called TNL. Nine NB-ARC (generalized NBS-LRR) genes can be found in this transcript. Includes 3 Resistance Gene (RGA) analogs genes (LOC106770073, LOC106778373, LOC106770069), six RPP13-like genes (LOC106779084, LOC106779070, LOC106763150, LOC106763525, LOC106778114, LOC106779105). The expression levels of C106778114 and LOC106779105 were significantly up-regulated compared with the control. Cooley et al. found that Arabidopsis RPP13, RPP1 and RPP8 could activate Enhanced Disease Susceptibility 1 (EDS1) and Nonrace-specific disease resistance 1 (NDR1) independently, a separate pathway that regulates the disease defense process in plants [61]. Bittner-Eddy et al. proved that although the amino acid sequence of RPP13 gene varied greatly in LRR structure [62], the same amino acid sequence (LLRVLDL) in LRR was conserved in other LZ NBS-LRR R-type proteins. Pathogenesis-related (PR) protein has 17 families in its pathogenesis [63]. However, PR protein also occurs in its pathogenesis by exogenous abiotic stress. In this analysis, one PR gene was also detected to be up-regulated in the treatment group. LOC106760535 was identified as *VraPR4* with up-regulated expression log2FoldChange=5.229. Phylogenetic tree analysis showed that this gene was highly similar to the PR4 gene sequence in Arabidopsis. The PR4 protein contains a C-terminal Barwin domain, and the PR4 protein identified in multiple species has a certain resistance to fungal pathogens, stimulating membrane signal transduction and triggering the second messenger to amplify the signal step by step under external stress. Moreover, downstream transcription factors (WRKY, Myb, etc.) are activated by salicylic acid pathway to regulate PR4 gene expression [64]. Combined with salicylic acid pathway analysis, it can be inferred that under the condition of low phosphorus, plants in the group supplemented with bacteria have higher stress perception intensity, can significantly improve PR4 gene activity in the early stage of stress, and rely on salicylic acid and other plant hormone pathways to fight stress in a timelier manner, to minimize the impact of stress. In the process of plant growth and development, hormones can not only directly regulate the development of various tissues, but also regulate the disease resistance process of plants by regulating downstream signal molecules. In soybean, ERF, a transcription factor related to ethylene regulation, was found to interact with bHLH to improve plant tolerance to pathogens. Two bHLH genes, bHLH162 (LOC106754207) and bHLH14 (LOC106774715), were also found in *V. radiata*, suggesting that they have the same role as in soybean [65].

## Conclusions

In our study, the physiological activity of MQB12 strain was tested. MQB12 has the activity of phosphorus-dissolving, IAA production, iron carrier production and cellulase production. After inoculating MQB12 into the rhizosphere soil of *V. radiata*, the biomass, root length, and plant height of *V. radiata* were significantly increased. Transcriptome analysis showed that after MQB12 inoculation, related genes in the phosphorus pathway, plant hormone metabolism pathway and disease resistance pathway were significantly up-regulated and down-regulated, and it was predicted that there was a significant positive correlation between phosphorus-related genes and *V. radiata* biomass. The results of microbial diversity analysis showed that MQB12 inoculation increased the diversity and abundance of bacterial community, changed the structure of plant rhizosphere bacterial community, and enriched more bacteria beneficial to *V. radiata* growth, but MQB12 inoculation had little effect on fungal community. These experiments preliminarily explained the mechanism of microbial inoculants promoting plant growth and provided a theoretical basis for the wide application of microbial inoculants in agriculture.

**Abbreviations**

| | |
|---|---|
| DEGs | Different enrichment genes |
| *V. radiata* | *Vigna radiata* |
| PHT | Phosphate transporter |
| PGPR | Plant growth-promoting rhizobacteria |
| *D. dichotoma* | *Dicranopteris dichotoma* |
| MF | Molecular function |
| CC | Cell component |
| BP | Biological process |
| PSR | Pi starvation responses |
| PHR | Phosphate starvation response |
| SPX | SYG1/Pho81/XPR1 |
| PSI | Pi starvation-induced gene |
| MFS | Major facilitator superfamily |
| GlpT | Glycerol-3-phosphate transporter |
| PHL | Phosphate starvation response-like |
| SQD2 | Sulfoquinovosyltransferase2 |
| PAP | Purple acid phosphatase |
| *ERF* | Ethylene responsive factor |
| ETR1 | Ethylene receptor1 |

Zhao *et al. Chem. Biol. Technol. Agric.*    (2024) 11:116

Page 15 of 17

| CTR1 | Constitutive triple response1 |
| EIN2 | Ethylene insensitive 2 |
| AUX | Auxin input vector |
| ABCB | ATP-binding cassette subfamily |
| SABP2 | SA binding protein 2 |
| MeSA | Methyl salicylic acid |
| PTI | Pathogen-associated molecular pattern triggered immunity |
| ETI | Effector triggered immunity |
| RGA | Resistance gene |
| EDS1 | Enhanced Disease Susceptibility 1 |
| NDR1 | Nonrace-specific disease resistance |
| PR | Pathogenesis-related |

## Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

## Author contributions

R. Z.: writing—original draft; writing—review and editing, formal analysis, software, visualization, data curation, conceptualization, methodology, project administration. F. H.: conceptualization, formal analysis, methodology, project administration, writing—review and editing. Wf. H.: experimental design and analysis. Yf. Z.: conceptualization, software, formal analysis. Jl. Z: investigation, data curation, visualization. Qy. C.: writing—review and editing, software. Fq. W.: project administration, formal analysis. X. C.: data curation. B. H.: writing—review and editing. Y. W.: experimental design and analysis, funding acquisition, validation, supervision, writing—review and editing. All authors reviewed the manuscript.

## Data availability

No datasets were generated or analyzed during the current study.

## Declarations

## Competing interests

The authors declare no competing interests.

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Zhao *et al. Chem. Biol. Technol. Agric.*     (2024) 11:116

Page 17 of 17

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