


Effects of *Trichoderma harzianum* biofertilizer on growth, yield, and quality of *Bupleurum chinense*

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Funding information

National Science and Technology to Boost Economy, Grant/Award Numbers: SQ2020YFF0426286, SQ2020YFF0426541

Abstract

The use of chemical fertilizers and pesticides led to a decline in the quality and yield of *Bupleurum chinense*. The aim of this study was to determine the effects of *Trichoderma harzianum* biofertilizer on the growth, yield, and quality of radix bupleuri and microbial responses. The results showed that *T. harzianum* biofertilizer promoted the growth of *B. chinense* and increased the yield and quality of radix bupleuri. In addition, it increased the contents of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, available K, and available P and increased the activities of sucrase and catalase in the rhizosphere soil. High-throughput analysis showed that the dominant bacteria in the rhizosphere were Proteobacteria (28%), Acidobacteria (23%), and Actinobacteria (17%), whereas the dominant fungi were Ascomycota (49%), Zygomycota (30%), and Basidiomycota (6%). After the application of *T. harzianum* biofertilizer, the abundance of Proteobacteria and Actinobacteria (relative to total bacteria) and Ascomycota and Basidiomycota (relative to total fungi) increased, but the relative abundance of Acidobacteria decreased. Canonical correlation analysis (CCA) showed that the relative abundance of *Pseudarthrobacter*, *Streptomyces*, *Rhizobium*, *Nocardioides*, *Minimedusa*, and *Chaetomium* were positively correlated with $\text{NO}_3^-\text{-N}$, $\text{NH}_4^+\text{-N}$, available K, available P, sucrase, and catalase in microbial communities, whereas *Aeromicrobium* and *Mortierella* were positively correlated with soil organic matter and urease. These results suggest that *T. harzianum* biofertilizer could significantly improve the yield and quality of radix bupleuri by changing the structure of soil microbial flora and soil enzyme activity. Therefore, it could be recommended for commercial scale production of *Bupleurum*.

KEYWORDS

Bupleurum chinense, enzyme activity, high-throughput sequencing, quality, *Trichoderma harzianum* biofertilizer

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1 | INTRODUCTION

Radix bupleuri is a commonly used Traditional Chinese medicine with many important properties such as antidepressant, antitumor, anti-inflammatory, and immunomodulatory activities (Yang et al., 2017). The cultivation of *Bupleurum chinense* in China has a long history, and the demand for radix bupleuri has increased rapidly during the last decade, especially after the new coronavirus outbreak (Zhong et al., 2020). However, the production of *Bupleurum* in China is still restricted by many factors, such as long-term excessive application of chemical fertilizers and pesticides and monoculture, which has caused soil acidification, salinization, nutrient imbalance, and degradation of other soil properties together with reduced crop yield and quality (Yan et al., 2018; Zhang, 2020).

Therefore, the amounts of chemical fertilizers should be appropriately reduced to improve the physical and chemical properties of soil as well as eliminate environmental pollution (Wang et al., 2020). In recent years, as society has paid more attention to protection of the agricultural environment, the research on replacing chemical fertilizers with biofertilizers has attracted widespread attention (Patel & Minocheherhomji, 2018).

Biofertilizer contains living microorganisms that can improve soil nutrients and physical and chemical properties, promote plant growth, and control pathogens (Kumar, 2016; Ren et al., 2020). Biofertilizers are considered to be a promising and nontoxic alternative to synthetic agrochemicals, making biofertilizers indispensable in improving the soil environment and plant growth (Suhag, 2016).

Trichoderma species are effective biological control agents able to inhibit soilborne pathogens and promote plant growth (Carro-Huerta et al., 2020; Sallam et al., 2019). *Trichoderma* species directly inhibit the growth of pathogenic bacteria by producing various secondary metabolites (Mironenka et al., 2021). On the other hand, they indirectly improve plant disease resistance and promote plant growth by changing soil microbial community structure and increasing the number of beneficial soil microorganisms (Zafra & Cortés-Espinosa, 2015). Therefore, *Trichoderma* strains have become a research focus for biological control in recent years. *Trichoderma harzianum* is one of the most commonly used biocontrol strains, which can antagonize pathogens and promote plant growth (Rubio et al., 2017). Poveda et al. (2019) found that the simultaneous application of *T. harzianum* strain and arbuscular mycorrhizal fungi inoculum significantly increased the colonization rate in the roots of arabidopsis and rapeseed and increased the yield of these two plants. The application of *T. harzianum* strain to soil from continuous cucumber culture effectively controlled cucumber *Fusarium* wilt, enriched soil microbial community, and restored soil biological functions (Chen et al., 2012).

However, to our knowledge, very little is known on the effects of *T. harzianum* on the soil environment and plant growth during the cultivation of *B. chinense*. In this study, the effects of *T. harzianum* biofertilizer on the growth, yield, quality of radix bupleuri, and microbial responses were investigated, which provided essential information for fertilizer management and sustainable development.

2 | MATERIALS AND METHODS

2.1 | Materials and reagents

T. harzianum biofertilizer was purchased from Shandong Tai'an Nong Bole Biological Fertilizer Company (China). It was composed of *T. harzianum* T22 strain and organic additives. The biofertilizer contained $\geq 10^9$ CFU g^{-1} . Organic fertilizer was provided by Shandong Kaoshan Biotechnology Company (China).

Saikosaponins A (P15S11F124709), saikosaponins C (M12O11S126866), and saikosaponins D (P15O10F100354) (with a purity of >98%) were purchased from Shanghai Yuanye Biotechnology (China). Other chromatographic grade reagents were purchased from Sinophenol Reagent Company (China).

2.2 | Field experiment design

The experimental work was conducted at Zhangqiu Planting Base in Shandong province from June 2018 to October 2020 (117°22'54" E 36°35'27" N, altitude 524 m), where *B. chinense* was cultivated for 2 years. Plant and row spacings were 5 and 10 cm, respectively. The predominant soil in this area was brown clayey soil.

Three treatments were tested: A, *T. harzianum* biofertilizer; B, organic fertilizers; and C, control without fertilizer. Each treatment consisted of three replicate blocks. The area of each block was 30 m². The fertilizer rate and application method were selected based on the manufacturers' instructions. Organic and biofertilizer were applied in the early and full blooming periods of *B. chinense*. Each fertilizer was applied weekly (four times). The first fertilization was conducted on 13th of July 2020. Samples of medicinal materials were collected on 15th of October 2020.

2.3 | Collection of soil samples

The five-point mixing method was used to collect and process *B. chinense* radix and the corresponding rhizosphere soil in different fertilization treatments (He et al., 2020). The rhizosphere soil samples were collected into sterile bags, cooled in a portable refrigerator, and brought back to the laboratory where they were screened (2 mm) and stored at -20°C for microbial and soil analyses.

2.4 | Growth parameters and yield

At harvest, 10 healthy *B. chinense* plants were randomly collected from each treatment and used to determine growth index by measuring radix/stem length and diameter. Radix and whole plant weights were determined by an electronic balance. The results were expressed as means \pm standard deviation. The average value of bupleurum radix from five quadrats was taken as the yield per treatment, which was then scaled up to yield per hectare.



2.5 | Quality analysis

The radices of 10 plants were collected from each treatment, dried at 50°C, mixed, ground with a powder beater, and passed through an 80-mesh sieve. The contents of saponins A, saponins C, and saponins D in *B. chinense* were determined by HPLC (National Pharmacopoeia Commission et al., 2020).

2.6 | Soil physicochemical properties

After air-drying, the contents of soil organic matter (SOM), available phosphorus (Ava-P), and available potassium (Ava-K) were determined as described by Qu et al. (2019). The contents of NO₃⁻-N and NH₄⁺-N in soil were determined as described by Dang et al. (2021).

2.7 | Soil enzyme activities

The activities of sucrase, urease, and catalase were determined by using colorimetric method, sodium phenol-sodium hypochlorite colorimetric method, and potassium permanganate titration method, respectively (Guan et al., 1986; Hu et al., 2014; Huang et al., 2016).

2.8 | DNA extraction, PCR amplification, and Illumina MiSeq sequencing

Three replicates (0.5 g each) of soil samples were extracted using a Soil DNA Kit (Mo Bio Laboratories, Carlsbad, CA, USA). The V4-V5 region of soil bacteria 16S rDNA and soil fungi ITS1 were amplified by PCR (94°C for 2 min, followed by 22 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 10 min), using the primers 515F 5'-GTGCCAGCMGCCGCGGTAA-3', 926R 5'-CCGTC AATTCMTTGGAGTTT-3', ITS1F 5'-CTTGGTCATTTAGAG-GAAGTA, A-3', and ITS1R 5'-GCTGCGTTCTTCATCGATGC-3'. The target bands were detected by running the PCR products on 2% agarose gels and recovered by a QIAamp DNA Micro Kit (Qiagen, Valencia, CA, USA). Finally, paired-end sequencing of the amplicon library was performed on an Illumina MiSeq platform (TinyGene Bio-Tech Co., Ltd., China), according to the standard protocol.

2.9 | Processing and analysis of the sequencing data

The raw fastq files were demultiplexed based on the barcodes. Paired-end reads for all samples were run through Trimmomatic (Version 0.35) to remove the low-quality base pairs. Flash (Version 1.2.11) software was used to splice the paired reads into a sequence. Mothur soft (Version 1.33.3) was used to control the sequence quality to obtain the optimized sequences. Then, UPARSE software (usearch Version V8.1.1756, <http://drive5.com/uparse/>) was used for OTU (the operational taxonomic unit) clustering. Based on taxonomic information, statistical analysis of community structure was carried out at the phylum and genus levels.

Diversity analyses were performed using Mothur soft (Version 1.33.3). On the basis of the above analyses, Venn, NMDS, community structure histograms, canonical correlation analysis, and LDA effect size were calculated and visualized by R software (Version 3.6.0).

3 | RESULTS

3.1 | Effect of *T. harzianum* biofertilizer on plant growth

The results showed that application of *T. harzianum* biofertilizer and organic fertilizer both improved the growth and yield of *B. chinense* (Table 1). However, compared with the organic fertilizer, *T. harzianum* biofertilizer significantly increased the stem length and thickness, root thickness and whole plant weight.

3.2 | Effect of *T. harzianum* biofertilizer on main active components

Compared with the control, *T. harzianum* biofertilizer significantly increased the contents of saikosaponins A, C, and D in radix bupleuri. However, in the treatment with organic fertilizer the content of saikosaponins A and D were significantly increased (Figure 1).

TABLE 1 Growth parameters and yield of *Bupleurum chinense* under different fertilization treatments

Treatment	Stem length/cm	Root length/cm	Whole plant weight/g	Root weight/g	Stem diameter/cm	Root diameter/cm	Yield/kg
A	100.38 ± 9.10 ^a	12.21 ± 2.48 ^a	8.66 ± 3.86 ^a	1.07 ± .40 ^a	1.34 ± .34 ^a	1.01 ± .22 ^a	.27 ± .07 ^a
B	90.08 ± 11.84 ^b	12.94 ± 3.29 ^a	5.49 ± 3.04 ^b	1.05 ± .56 ^a	1.15 ± .32 ^b	.76 ± .20 ^b	.25 ± .03 ^a
C	83.29 ± 8.30 ^c	11.34 ± 2.54 ^a	3.82 ± 2.37 ^c	.79 ± .46 ^b	1.10 ± .23 ^b	.73 ± 0.18 ^b	.17 ± .04 ^b

Notes: A, *Trichoderma harzianum* biofertilizer. B, Organic fertilizer. C, Control. Different lowercase letters in a column indicate significant differences among treatments ($P < .05$, $n = 30$).

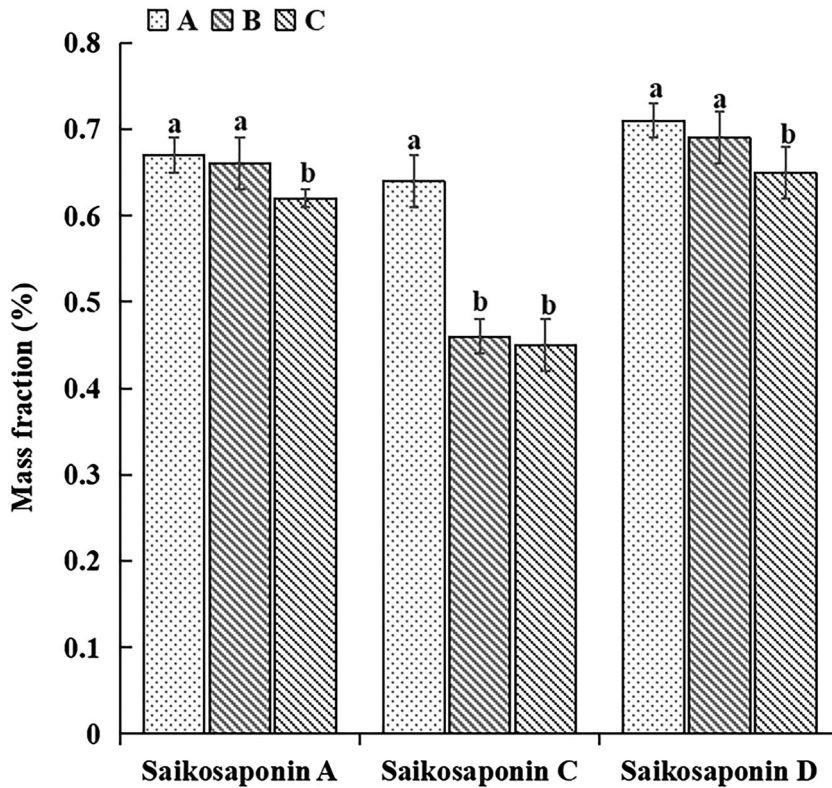


FIGURE 1 Determination of saikosaponins A, saikosaponins C, and saikosaponins D in *Bupleurum chinense* samples. A, *Trichoderma harzianum* biofertilizer. B, organic fertilizer. C, control. Different lowercase letters indicate significant differences among treatments ($P < .05$, $n = 3$).

3.3 | Effect of *T. harzianum* biofertilizer on soil physicochemical properties

Compared with the control, the contents of NH_4^+-N , NO_3^--N , Ava-K, and Ava-P, but not the soil organic matter content, significantly increased after application of *T. harzianum* biofertilizer (Figure 2).

3.4 | Effect of *T. harzianum* biofertilizer on soil enzyme activities

Compared with the control, the activities of sucrase and catalase significantly increased, but the activity of urease significantly decreased after applying *T. harzianum* biofertilizer. After application of the organic fertilizer, the activities of urease and catalase increased, but the activity of sucrase decreased significantly (Figure 3).

3.5 | Analysis of sequencing data

The dilution curves of rhizosphere soil samples are shown in Figure S1. The number of OTUs increased sharply and then gradually leveled off, indicating that the sequencing library has reached saturation. Therefore, the sequencing results can be used for subsequent analysis.

Through classification analysis, the common or unique OTUs of each sample were determined with 97% sequence identity and were

displayed in the Venn diagram (Figure 4). The number of soil bacteria-specific OTUs to which *T. harzianum* biofertilizer and organic fertilizer were applied increased significantly (Figure 4). Similarly, the number of soil fungi-specific OTUs with *T. harzianum* biofertilizer showed a similar trend. However, the number of OTUs specific to soil fungi decreased significantly after applying the organic fertilizer.

3.6 | Alpha diversity of bacterial and fungal communities

The microbial richness (Ace) and community diversity (Shannon and Simpson indices) were measured in different fertilization regimes. The results (Table 2) showed that the bacterial richness in the rhizosphere soil of *B. chinense* decreased after applying *T. harzianum* biofertilizer and organic fertilizer. Bacterial diversity showed an upward trend after application of *T. harzianum* biofertilizer and organic fertilizer. After application of *T. harzianum* biofertilizer, the abundance of fungi increased, but the diversity decreased. However, the abundance and diversity of fungi decreased after application of organic fertilizer.

3.7 | Beta diversity of bacterial and fungal communities

To further investigate the differences in microbial community structure, we performed NMDS analysis based on the weighted UniFrac distance (Figure 5). The NMDS results showed that the soil samples

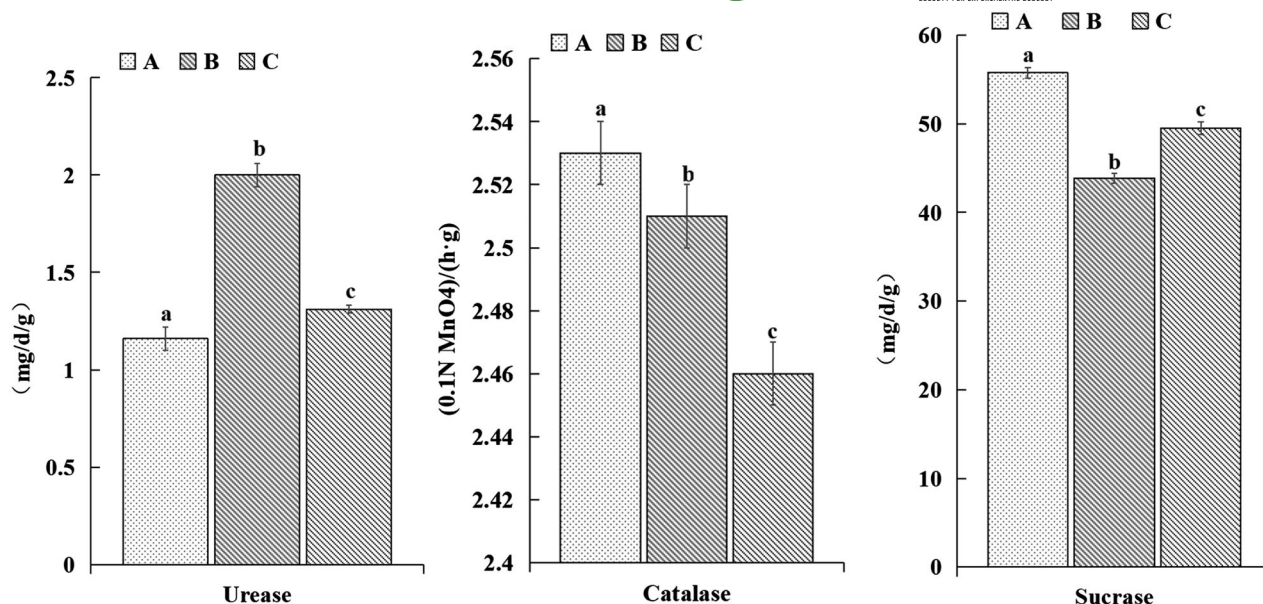


FIGURE 2 Effects of different fertilization treatments on physicochemical properties of the *Bupleurum chinense* rhizosphere soil. Different lowercase letters indicate significant differences among treatments ($P < .05$, $n = 3$). Ava-K, available potassium; Ava-P, available phosphorus; SOM, soil organic matter.

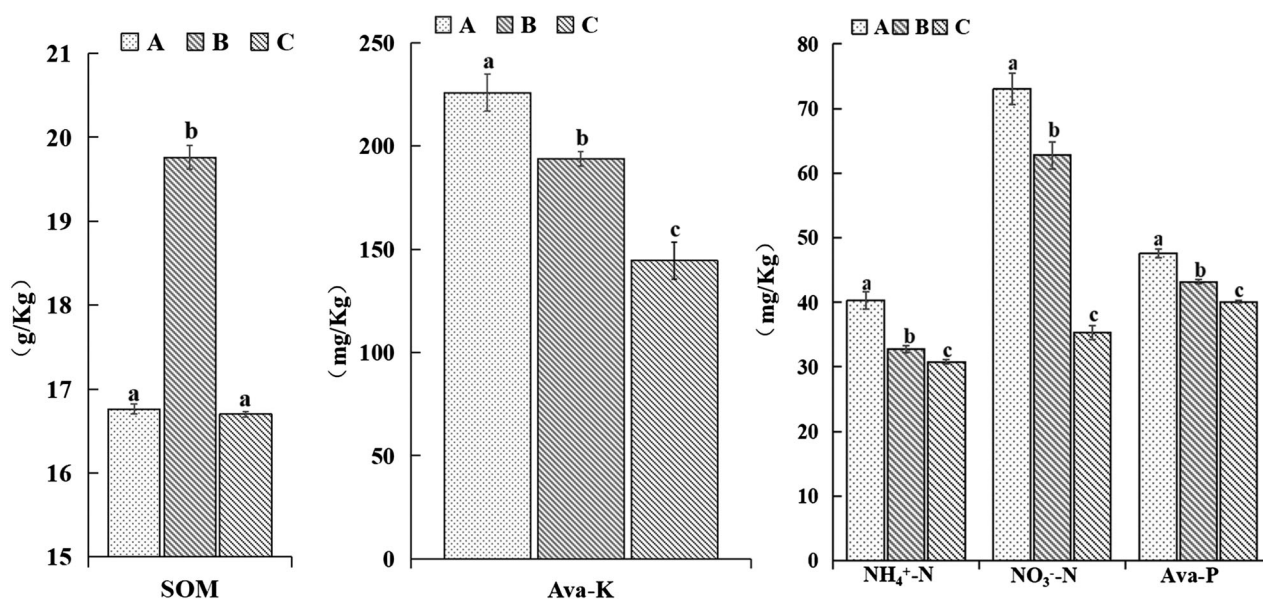


FIGURE 3 Effects of different fertilization treatments on the activity of enzymes in the *Bupleurum chinense* rhizosphere soil. Different lowercase letters indicate significant differences among treatments ($P < .05$, $n = 3$).

to which *T. harzianum* biofertilizer was applied were clearly separated from the soils of the other treatments. In the heat map, when the sample distance was greater than 0.2, the samples could be divided into three groups (Figure 6). The microbial community structure within groups was similar, but the differences among the groups were significant, indicating that fertilization regimes have a great influence on the microbial community composition in the rhizosphere soil of *B. chinense*.

3.8 | Composition and structure of the bacterial community

To clarify the microbial community structure in the *B. chinense* rhizosphere after fertilization, two taxonomic levels (phyla and genera) were analyzed. As shown in Figure 7a, 13 bacterial phyla were detected in the soil from different fertilization treatments. The dominant bacterial phyla in the *B. chinense* rhizosphere were

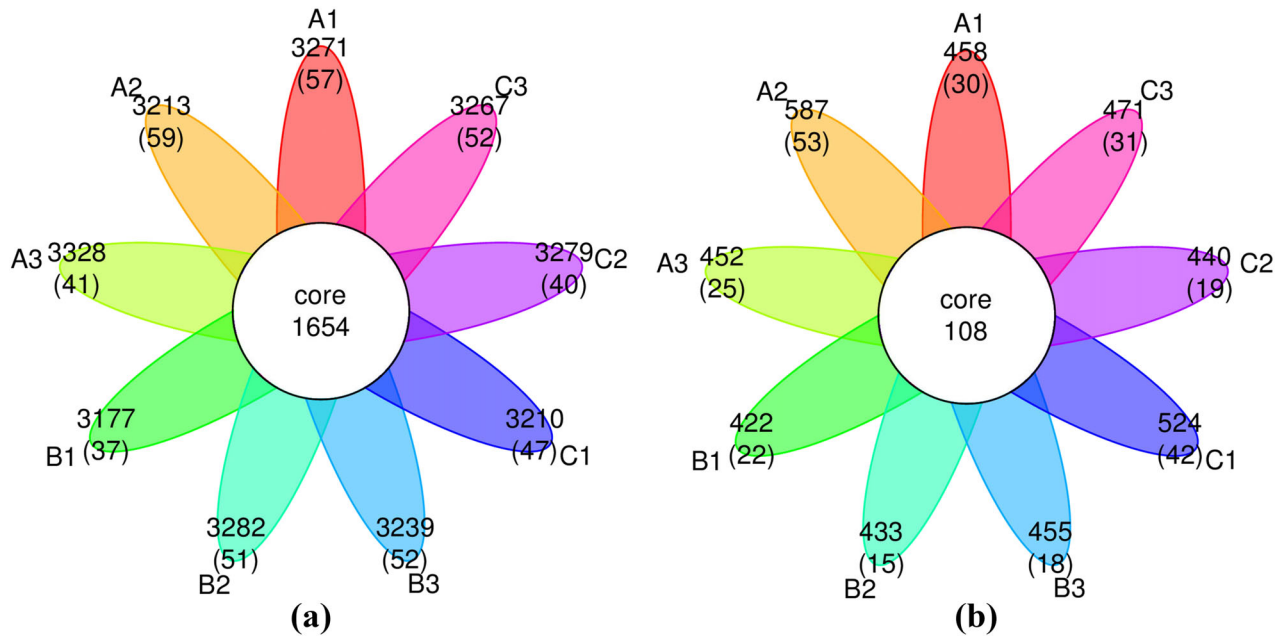


FIGURE 4 Venn diagram of the similarity and overlap of microbiome operational taxonomic unit (OTU) from different fertilization regimes. (a) Bacteria, (b) fungi

TABLE 2 α -Diversity index of microorganisms in the rhizosphere soil of *Bupleurum chinense* under different fertilization treatments

Treatment	Bacteria			Fungi		
	Ace	Shannon	Simpson	Ace	Shannon	Simpson
A	3698.54 \pm 58.69	6.51 \pm .04	.0045 \pm .0002	544.88 \pm 70.60	3.27 \pm .45	.10 \pm .05
B	3686.55 \pm 37.31	6.54 \pm .03	.0045 \pm .0001	494.48 \pm 18.58	3.07 \pm .34	.14 \pm .04
C	3722.48 \pm 70.72	6.51 \pm .02	.0048 \pm .0001	519.40 \pm 28.40	3.46 \pm .46	.09 \pm .03

Notes: A, *Trichoderma harzianum* biofertilizer. B, Organic fertilizer. C, Control. Ace represents the abundance of bacteria and fungi. Shannon and Simpson indices represent the diversity of bacteria and fungi.

Proteobacteria (28%), followed by Acidobacteria (23%) and Actinobacteria (17%). The relative abundance of Proteobacteria and Actinobacteria increased after application of *T. harzianum* biofertilizer. The relative abundance of Acidobacteria decreased after organic fertilizer addition but increased when no fertilization was applied.

Thirty bacterial genera (Figure 8a) were detected in the soil from different fertilization treatments. The dominant genera were *Pseudarthrobacter* (2%), *Streptomyces* (2%), *Rhizobium* (1%), *Nocardioides* (1%), *Aeromicrobium* (1%), and *Pirellula* (1%). The relative abundance of other dominant genera (except *Aeromicrobium* and *Opiritutus*) increased after application of *T. harzianum* biofertilizer. The relative abundance of *Aeromicrobium* increased only after application of organic fertilizer, whereas the relative abundance of *Opiritutus* increased only after application of *T. harzianum* biofertilizer.

3.9 | Composition and structure of the fungal community

As shown in Figure 7b, eight fungal phyla were detected in the soil samples from different fertilization treatments. The dominant fungal phyla were Ascomycota (49%), Zygomycota (30%), and Basidiomycota (6%). The relative abundance of Ascomycota and Basidiomycota increased after application of *T. harzianum* biofertilizer, whereas the relative abundance of Ascomycota and Basidiomycota decreased after application of organic manure. The relative abundance of Zygomycota increased after application of the organic fertilizer but decreased after application of *T. harzianum* biofertilizer.

Thirty-three fungal genera (Figure 8b) were detected in the soil from different fertilization treatments. The dominant fungal genera in the rhizosphere were *Mortierella* (30%), *Gibberella* (8%), *Chaetomium*

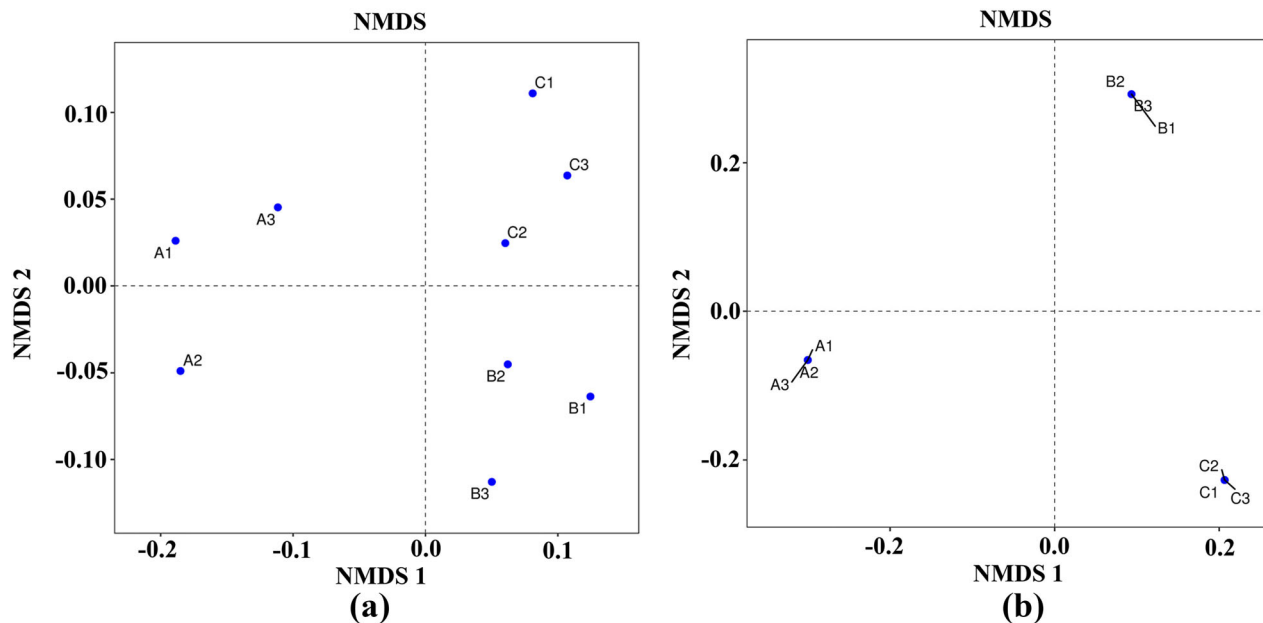


FIGURE 5 The non-metric multidimensional scale (NMDS) map of the unweighted UniFrac distance represents the microbial community structure. (a) Bacteria, (b) fungi

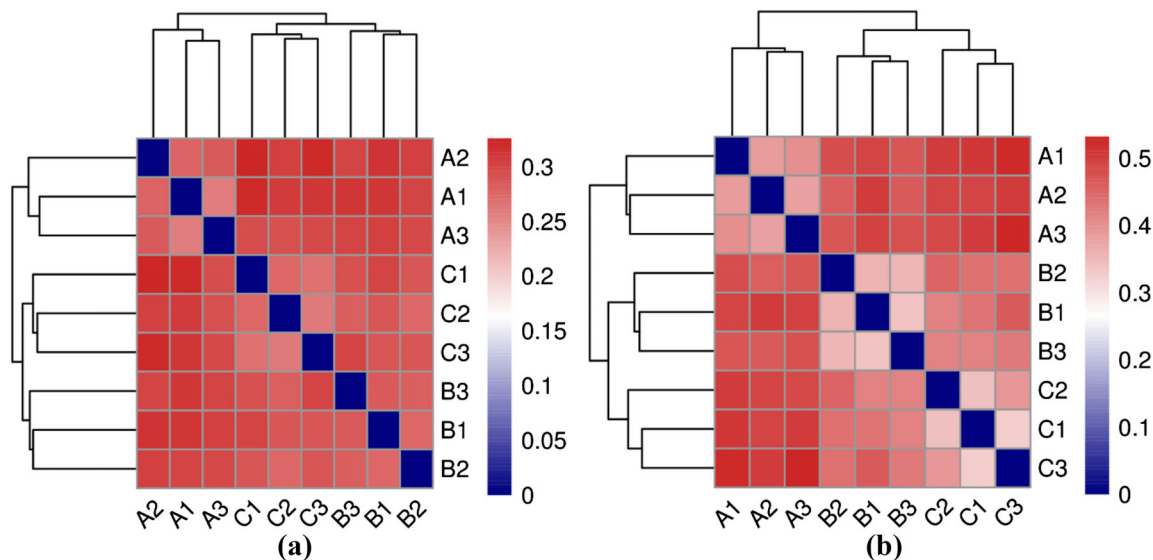


FIGURE 6 Based on the unweighted UniFrac distance, the heat map analysis of the microbiome community structure in the rhizosphere soil of *Bupleurum chinense* from different fertilization regimes. (a) Bacteria, (b) fungi

(3%), *Paraphoma* (3%), *Minimedusa* (2%), *Alternaria* (2%), *Tetracladium* (1%), and *Cladosporium* (1%). Among them, after the application of *T. harzianum* biofertilizer, the relative abundance of *Gibberella*, *Chaetomium*, and *Paraphoma* decreased, but that of *Alternaria*, *Tetracladium*, and *Cladosporium* increased. Other fungi showed different responses to fertilization treatments. For example, the relative abundance of *Mortierella* increased when the organic fertilizer was applied but decreased in response to *T. harzianum* biofertilizer. The relative abundance of *Minimedusa* increased significantly when *T. harzianum*

biofertilizer was applied but decreased significantly in the organic fertilizer treatment.

3.10 | Correlation analysis of dominant bacteria and soil properties

To study the correlation between the *B. chinense* rhizosphere microorganisms, soil nutrients, and enzyme activities under different

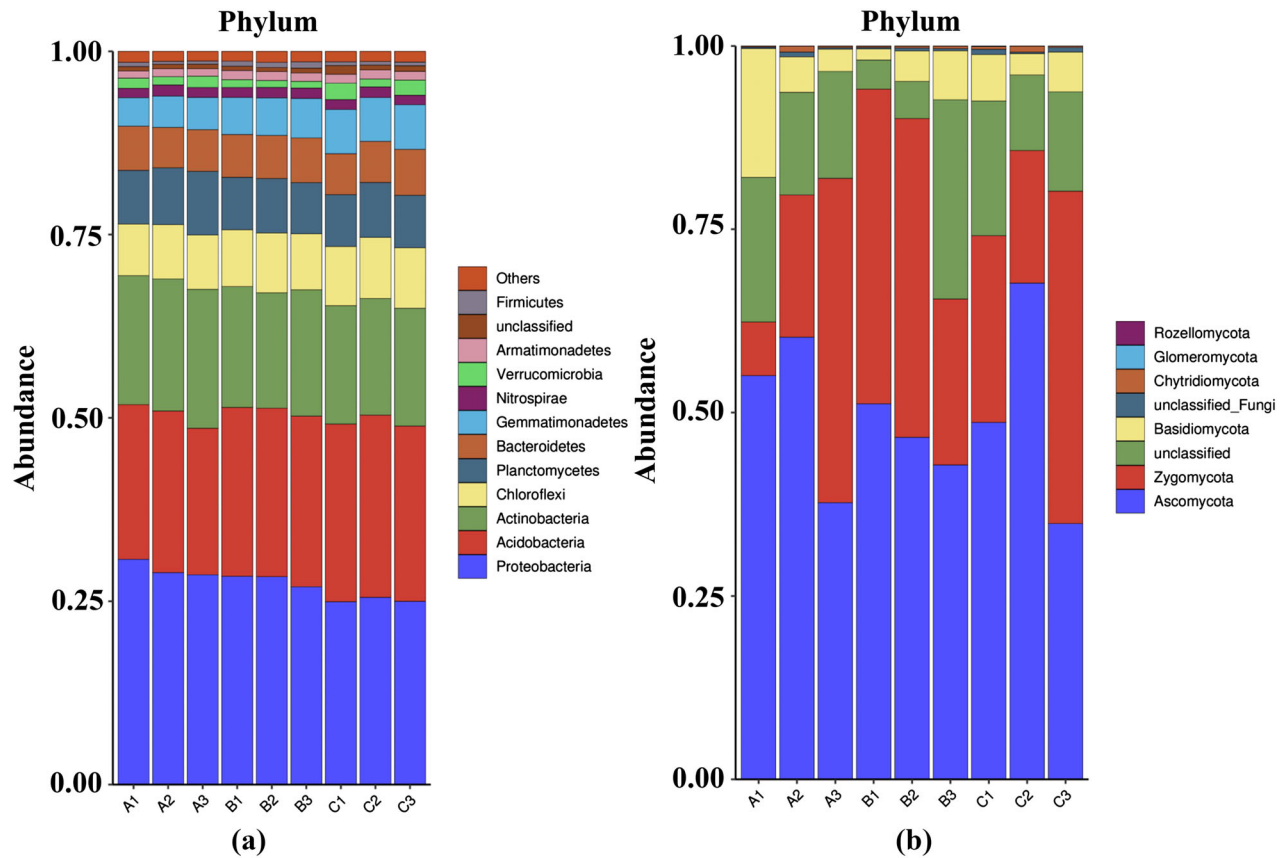


FIGURE 7 Relative abundance of microbial phyla under different fertilization regimes. (a) Bacteria, (b) fungi

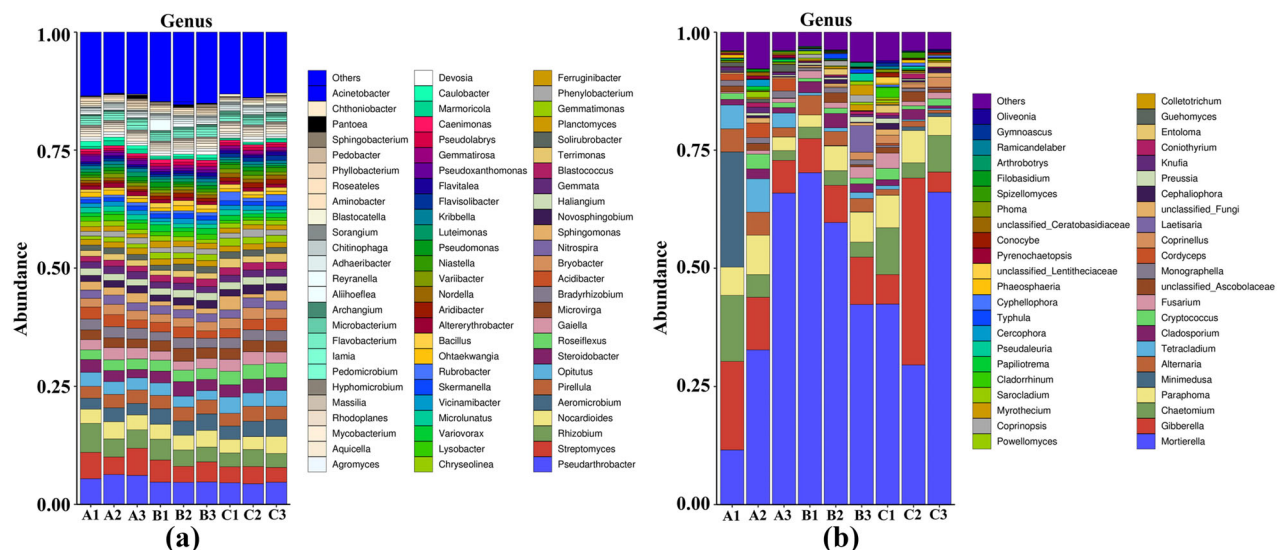


FIGURE 8 Relative abundance of microbial genera with significant differences under different fertilization regimes. (a) Bacteria, (b) fungi

fertilization treatments, we used canonical correlation analysis (CCA). As shown in (Figure 9), the correlations between rhizosphere microorganisms and soil nutrients and enzyme activities were different under

different fertilization treatments. The relative abundance of *Pseudarthrobacter*, *Streptomyces*, *Rhizobium*, and *Nocardioidea* was positively correlated with NO_3^- -N, NH_4^+ -N, Ava-K, Ava-P, sucrose, and

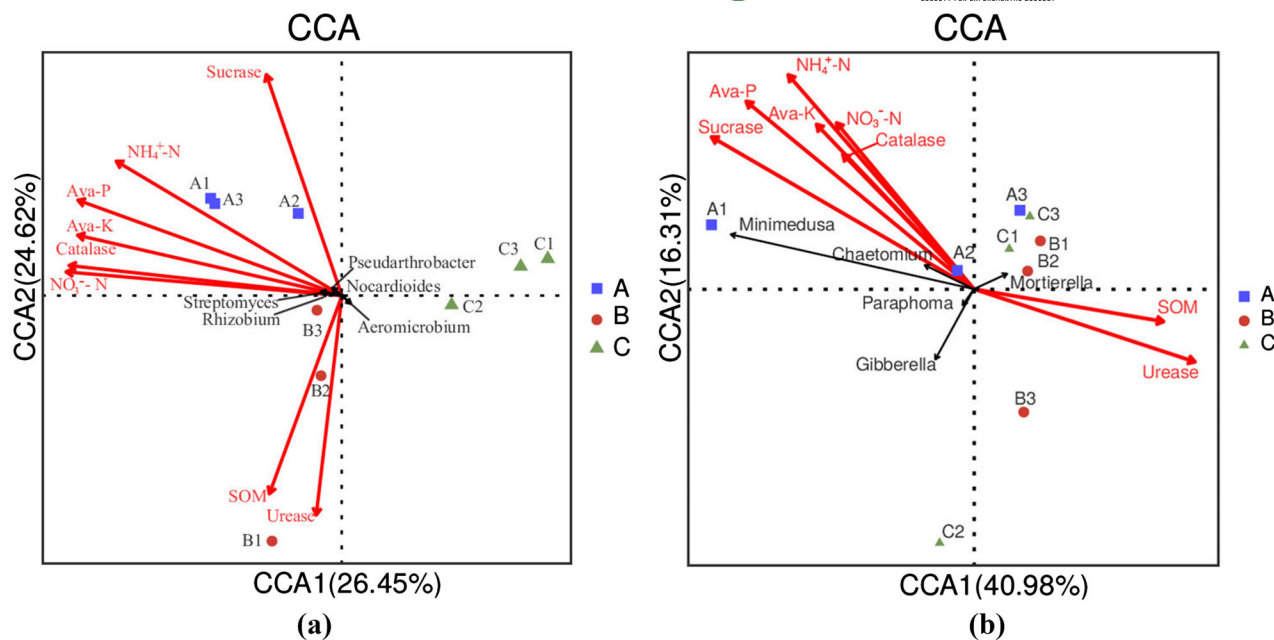


FIGURE 9 Canonical correlation analysis (CCA) of soil chemical properties and dominant bacterial (a) and fungal genera (b)

catalase, whereas the relative abundance of *Aeromicrobium* was positively correlated with SOM and urease. For fungi, the relative abundance of *Minimedusa* and *Chaetomium* were positively correlated with NO_3^- -N, NH_4^+ -N, Ava-K, Ava-P, sucrase, and catalase, whereas that of *Mortierella* was positively correlated with SOM and urease. After the application of *T. harzianum* biofertilizer, NO_3^- -N, NH_4^+ -N, Ava-K, Ava-P, sucrase, and catalase had a large influence on the structure of rhizosphere microbial communities, whereas after the application of organic fertilizer, the most influential factories were SOM and urease.

3.11 | Biomarker analysis

To identify the dominant microbial biomarkers in the rhizosphere soil of *B. chinense* under different fertilization treatments, LDA effect size (LEfSe) was used for analysis (Figure 10). The LDA results identified 42, 26, and 25 bacterial biomarkers in the *T. harzianum* biofertilizer, organic manure, and the unfertilized treatments, respectively (Figure 10a). Alpha-Proteobacteria and Proteobacteria were the most prevailing types of bacteria in the soil in the *T. harzianum* biofertilizer treatment. Burkholderiales and Comamonadaceae were abundant in the rhizosphere in the organic fertilizer treatment, and Acidobacteria were significantly abundant in the rhizosphere in the control without fertilization.

In the fungal community, 35, 20, and 34 fungal biomarkers were identified in the *T. harzianum* biofertilizer, organic fertilizer, and unfertilized treatments, respectively (Figure 10b). The relatively abundant biomarker fungal groups included *Mortierella fimbriicystis* and Pleosporales in the *T. harzianum* biofertilizer treatment; *Coprinopsis vermiculifer*,

Myrothecium gramineum, *Mortierella*, and Zygomycota in the organic fertilizer; and *Coprinellus radians* and *Coprinellus* sp. in the unfertilized control.

4 | DISCUSSION

Reducing the use of chemical fertilizers and pesticides without loss of productivity is feasible, but there are huge challenges. Numerous studies have shown that application of biofertilizers can effectively reduce the use of chemical fertilizers and pesticides. Jia et al. (2020) found that biofertilizers can replace 25% of chemical fertilizers without a detectable impact on the yield and quality of Chinese cabbage. Other studies have shown that inoculation with *T. harzianum* strains significantly increased the biomass and nutrient uptake of tomato seedlings grown in low-nutrient soils (Li et al., 2015). Our study showed that application of *T. harzianum* biofertilizer increased the yield of *B. chinense*. However, application of organic fertilizer alone reduced the yield of *B. chinense*, which is consistent with the results of Ye et al. (2020).

The biofertilizer industry is developing rapidly in China. Biofertilizers are widely recognized by most crop growers for their ability to improve crop growth parameters, increase yield, and control soilborne diseases (Zhou et al., 2020). Our study showed that *T. harzianum* biofertilizer could improve the growth parameters, quality, and yield of radix bupleuri, mainly due to improvement of soil fertility and abundance of beneficial bacteria (Wang et al., 2017).

Fertilization is one of the main factors affecting soil nutrient content and enzyme activity in the rhizosphere soil of *B. chinense*. The contents of available nutrients reflect the dynamic balance between



FIGURE 10 LDA effect size analysis of microbial community differences in the *Bupleurum chinense* rhizosphere soil in different fertilization treatments. (a) Bacteria, (b) fungi



soil mineralization and plant absorption (Ling et al., 2014). Soil enzyme activity can reflect the nutrient absorption, utilization, and growth of crops to a certain extent and is also an important index of soil fertility, soil quality, and soil health (Yu et al., 2019). This study showed that *T. harzianum* biofertilizer significantly improved soil available nutrient contents and sucrase and catalase activities, but did not increase the activities of urease and organic matter in soil. However, an appropriate amount of organic fertilizer can be used to increase the content of soil organic matter (Liu et al., 2021).

Soil microbial diversity, abundance, and community structure are related to the functions of microbial community and soil health (Ruiz Gómez et al., 2019). Microbial communities participate in soil functions including nutrient cycling, formation of soil aggregates, and suppression of soilborne diseases. This study showed that *T. harzianum* biofertilizer significantly improved the diversity of bacteria and the abundance of fungi. The relative abundance of beneficial bacteria increased significantly, which included *Streptomyces* (Heinsch et al., 2019), *Rhizobium* (Naamala et al., 2016), *Steroidobacter* (Alcaraz et al., 2018), and *Gaiella* (Lazcano et al., 2021). By contrast, the abundance of harmful fungi was significantly reduced, including *Gibberella* (Zhao et al., 2021), *Paraphoma* (Marin-Felix et al., 2019), and *Fusarium* (Tong et al., 2021). The microbial changes were more obvious in soil bacteria than fungi, suggesting that soil bacterial communities are more sensitive to planting systems (Zhao et al., 2017).

The dominant bacterial groups in rhizosphere mainly included Proteobacteria, Acidobacteria, and Actinomycetes, most of which are involved in decomposition of organic matter. This study showed that the rhizosphere microbial community structure dominated by these bacterial groups was significantly correlated with the contents of soil available nutrients and the activities of urease, catalase, and invertase, indicating that the rhizosphere environment had an important effect on the bacterial and fungal community structures (Lauber et al., 2009).

5 | CONCLUSION

This study demonstrated that *T. harzianum* biofertilizer promoted the growth of *B. chinense*, increased yield, improved its quality, and increased the contents of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, Ava-K, and Ava-P and the activities of sucrase and catalase in the rhizosphere soil. Moreover, these parameters showed positive correlation with the relative abundance of beneficial bacteria in soil, such as *Pseudarthrobacter*, *Streptomyces*, *Rhizobia*, and *Nocardia*. Therefore, *T. harzianum* biofertilizer can be used in *Bupleurum* production. Further research will be conducted to investigate the effects of *T. harzianum* biofertilizer on dynamics of various functional bacteria during *bupleuri* production.

ACKNOWLEDGMENTS

This work was supported by grants from the National Science and Technology to Boost Economy 2020 (SQ2020YFF0426286 and SQ2020YFF0426541). We especially thank EditSprings (<https://www.editsprings.cn/>) for the expert linguistic services provided.

CONFLICT OF INTEREST

The authors declare they have no competing interests.

AUTHOR CONTRIBUTIONS

Professor Gao Demin designed the experiments and revised the manuscript. Liu Li conducted field experiments, analyzed data, and wrote manuscripts. Professor Cao Hailu, Fan Ya, and Du Kan collected the rhizosphere soil of *Bupleurum chinense*. Professor Yuansong Xu and Bu Xun assisted in microbial sequencing. All authors read the final manuscript.

DATA AVAILABILITY STATEMENT

The data generated for this study are available in the NCBI Sequence Read Archive database under accession number SRP34470.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Liu, L., Xu, Y., Cao, H., Fan, Y., Du, K., Bu, X., & Gao, D. (2022). Effects of *Trichoderma harzianum* biofertilizer on growth, yield, and quality of *Bupleurum chinense*. *Plant Direct*, 6(11), e461. <https://doi.org/10.1002/pld3.461>