

Analysis of the variation characteristics of *Scutellaria baicalensis* rhizosphere soil microorganisms at different growth stages and their relationship with the accumulation of active components

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Abstract

In this study, *Scutellaria baicalensis* Gerog plants at different growth stages and their rhizosphere soil were used as experimental materials. The microbial diversity, physicochemical properties, and enzyme activities of the rhizosphere soil were systematically determined, while the active component contents of *Scutellaria baicalensis* shoots were analyzed. This research aims to provide a scientific basis for revealing the soil environment regulation mechanism underlying the accumulation of active components in *Scutellaria baicalensis* root. It revealed the association mechanisms between rhizosphere soil micro-ecological characteristics at different growth stages and active component accumulation of *Scutellaria baicalensis* root. The results showed that, compared with the flowering stage, the contents of total phosphorus (TP), alkali-hydrolyzable nitrogen (AN), soil organic carbon (SOC), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN), as well as the carbon/nitrogen (C/N) ratio, in *Scutellaria baicalensis* rhizosphere soil gradually increased during the fruiting and wilting stages. In contrast, the soil pH showed an opposite trend. Among the other soil nutrients, the contents of TP, available phosphorus (AP), and available potassium (AK) were the highest at the fruiting stage, followed by the wilting stage, the lowest at the wilting stage. Additionally, the TN content was the lowest at the flowering stage, and it was higher at the fruiting and wilting stages. Compared with the flowering stage, the activities of urease, sucrase, and alkaline phosphatase in *Scutellaria baicalensis* rhizosphere soil significantly increased at the fruiting and wilting stages. There are significant differences of bacterial community in *Scutellaria baicalensis* rhizosphere soil at different growth stages (flowering stage, fruiting stage, and withering stage). The relative abundance of bacteria at the genus level in *Scutellaria baicalensis* rhizosphere soil changes with the growth stages. The dominant genera in each stage is as follows: at the flowering stage, the dominant taxa are *Bacillus*, *Geodermatophilus*, and *Bryobacter*; at the fruiting stage, the dominant taxa are *Streptomyces*, *Sphingomonas*, *Blastococcuss*, *Haliangium*, and *Nocardioides*; At thewithering stage, the dominant taxa are *Gemmatimonas*, *Opitutus*, and *Lysobacter*. The relative abundance of fungi in *Scutellaria baicalensis* rhizosphere soil at the genus level changes with the growth stages. The dominant genera in each stage are as follows: at the flowering stage, the genera with the highest relative abundance are *Arthrographis*, *Penicillium* and *Preussia*; at the fruiting stage, the genera with the highest relative abundance are *Pseudogymnoascus*, *Aspergillus* and *Pseudallescheria*; at the withering stage, the genera with the highest relative abundance are *Mortierella*, *Alternaria*, *Mycochlamys* and *Fusarium*. Redundancy analysis indicated that AN and MBN were key environmental factors driving the differences of bacterial and fungal communities at the genus level in *Scutellaria baicalensis* rhizosphere soil at different growth stages. Pearson correlation analysis further showed that the main active components of *Scutellaria baicalensis* root, including baicalin, wogonoside, baicalein, and wogonin, were significantly positively correlated with the relative abundance of 10 bacterial genera such as *Gemmatimonas*, *Opitutus*, and *Lysobacter*, as well as with the relative abundance of 12 fungal genera such as *Mortierella*, *Alternaria*, and *Mycochlamys*. These results reveal that the rhizosphere microbial community plays an important role in the accumulation of root active components at different growth stages of *Scutellaria baicalensis*, providing a theoretical basis for the

development of precision fertilization strategies and scientific management in *Scutellaria baicalensis* cultivation.

Introduction

Scutellaria baicalensis Georg is an important medicinal plant resource with efficacies such as clearing heat and drying dampness, purging fire and detoxifying, and cooling blood and stabilizing pregnancy (Li et al. 2022). The annual demand for *Scutellaria baicalensis* is enormous, and after years of intensive harvesting, wild *Scutellaria baicalensis* resources are becoming increasingly depleted. Cultivated *Scutellaria baicalensis* has become the main source of *Scutellaria baicalensis* medicinal materials (Yuan et al. 2010). Germplasm sources, fertilization measures, planting years, soil microecological environment and so on are the key factors affecting the quality and yield of *Scutellaria baicalensis*. (Slusarczyk et al. 2024; Li et al. 2022a; Sun et al. 2024; Dong et al. 2022). Studies have found that the combined application of chemical fertilizer and bio-organic fertilizer not only significantly increases the bacterial diversity in *Scutellaria baicalensis* rhizosphere soil but also significantly increases the relative abundance of *Actinobacteria* and *Chloroflexi*, which is beneficial for improving the quality and yield of *Scutellaria baicalensis* (Sun et al. 2024). Additionally, the interaction between the rhizosphere microbiome and plants during plant growth also affects plant health status and productivity levels, a mechanism that is particularly critical in the medicinal plant cultivation field (Pshenichkina 2022; Dong et al. 2022).

The plant root system, rhizosphere microorganisms, and rhizosphere soil constitute the rhizosphere micro-ecosystem. In this micro-ecosystem, biological factors such as plant genotype, plant growth and development stages, and invading pathogenic microorganisms, as well as abiotic factors such as soil composition, soil management practices, and climate conditions, influence the composition and diversity of the rhizosphere microbial community (Liu et al. 2023). Conversely, rhizosphere microorganisms affect plant metabolism and the composition of plant root exudates, mainly reflected in changes in plant physiological characteristics and disease resistance function, thereby influencing plant growth and development (Bai et al. 2022; Xiong et al. 2021). Rhizosphere microorganisms also have a profound impact on soil evolution, among which beneficial microorganisms play a crucial role in the transformation process of barren soil into cultivable soil. (Zhang et al. 2024). Thus, the interactions among plants, microorganisms, and environmental factors form a complex symbiotic network within the rhizosphere micro-ecosystem. The dynamic changes in this symbiotic network not only affect the diversity of rhizosphere microorganisms but also alter the phenotypic characteristics of plants and the accumulation patterns of active components (Lu et al. 2018). Some beneficial rhizosphere microorganisms, such as bacteria *Gemmatimonas* spp., *Streptomyces* spp., *Nocardioides* spp., and fungi *Mortierella* spp., *Penicillium* spp., *Preussia* spp., can enhance plant root vitality, promote plant growth, and improve the yield and root active component content of medicinal plants by participating in soil nutrient cycling, secreting growth hormones, and antimicrobial substances (Oshiki et al. 2018; Wang et al. 2022; Youseif et al. 2023; Pak et al. 2020; Wani et al. 2027; Al-Hosni et al. 2018). Conversely, harmful rhizosphere microorganisms such as fungi *Alternaria* spp. and *Fusarium* spp. can induce plant

diseases, inhibit plant growth and development, and significantly reduce crop yields by infecting plant roots, secreting toxins, or competing for nutrients (Zhao et al. 2019; Wani et al. 2017). Studying the symbiotic relationships and interactions among medicinal plants, environmental factors, and microorganisms helps regulate the composition of microbial communities around the rhizosphere of medicinal plants, achieving high quality and yield of medicinal plants (Wang et al. 2022a; Gao et al. 2024).

There are few studies on the relationship between rhizosphere microbial communities and the accumulation of root active components of *Scutellaria baicalensis* at different growth stages under the combined application of organic and inorganic fertilizers. Given the important ecological functions and biological value of rhizosphere microorganisms, a systematic study was conducted on the variation characteristics the rhizosphere microbial communities of *Scutellaria baicalensis* at different growth stages. High-performance liquid chromatography (HPLC) was used to determine the content of root active components of *Scutellaria baicalensis* at different growth stages, and high-throughput sequencing technology was employed to analyze the rhizosphere microbial communities of *Scutellaria baicalensis*. This study aims to detect the microbial diversity, soil nutrient content, enzyme activity changes, and rhizosphere active component content in the rhizosphere soil of *Scutellaria baicalensis* at different developmental stages, exploring the associations among root active components, rhizosphere soil microorganisms, enzyme activity, and nutrients. Furthermore, it analyzes the dynamic variation characteristics of rhizosphere microorganisms during the growth and development of *Scutellaria baicalensis* and the accumulation patterns of active components at different growth stages. This study will provide new pathways for optimizing cultivation management measures of *Scutellaria baicalensis* and enhancing the yield scale and quality of medicinal plant.

Experimental design and methods

Description of the experimental site

The experimental field is located in Fenyang County, Shanxi Province (North Latitude: 37°93', East Longitude: 113°58'), at the experimental field of the Institute of the Industrial Crop, Shanxi Academy of Agricultural Sciences. The region has an annual average precipitation of 460 mm, an annual average temperature of $\geq 10\text{ }^{\circ}\text{C}$, and a frost-free period of 170 days. The soil in the experimental field is Yuanhuang cinnamon soil, with Malan loess as the parent material and a texture of sandy loam.

Experimental design.

The *Scutellaria baicalensis* experiment began in 2023. Chemical fertilizers were urea, triple superphosphate, and potassium sulfate, containing $\text{CO}(\text{NH}_2)_2$ 113 kg hm^{-2} , P_2O_5 140 kg hm^{-2} , and K_2O 15.8 60 kg hm^{-2} , respectively. The organic fertilizer was pig manure, containing H_2O , organic matter, total nitrogen (TN), P_2O_5 , and K_2O at 55%, 34.5 g kg^{-1} , 16.5 g kg^{-1} , 23.6 g kg^{-1} , and 15.8 g kg^{-1} , respectively. The fertilization treatment involved a combination of chemical fertilizers and organic fertilizer. *Scutellaria*

baicalensis seeds were randomly sown in experimental plots of 60 square meters, with each plot containing 1300 plants. The row spacing was 30 cm, and the plant spacing was 15 cm.

Soil sampling

At the flowering stage, fruiting stage, and wilting stage in 2024, fifteen *Scutellaria baicalensis* plants were randomly selected from each replicate plot. The *Scutellaria baicalensis* roots with soil were shaken to remove loose soil, and the soil adhering to the root surface was brushed off with a soft brush, which was considered rhizosphere soil. The rhizosphere soil from fifteen *Scutellaria baicalensis* roots was mixed to form one soil sample for each treatment. Each soil sample is divided into three portions, which are used for chemical composition analysis, enzyme activity determination, and microbial diversity analysis respectively.

Measurement of *Scutellaria baicalensis* growth indicators

After collecting rhizosphere soil samples at the fruiting stage, the *Scutellaria baicalensis* plants were used to determine shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) and root dry weight (RDW). Additionally, dry/fresh ratio (D/F) and root/shoot ratio (R/S), SDW/SFW and RDW/RFW were calculated. $R/S = RDW/SDW$.

Detection of active components in *Scutellaria baicalensis* root

High-performance liquid chromatography (HPLC) was used to determine baicalin, wogonoside, baicalein, and wogonin. Chromatographic column: kromasil eternity-5-C18 (250 mm×4.6 mm); mobile phase: acetonitrile solution (A)-0.4% acetic acid solution (B), gradient elution; column temperature: 30°C; detection wavelength: 280 nm; flow rate: 1.0 mL/min.

1.6 Measurement of soil nutrients, microbial biomass carbon, nitrogen ratio, and enzyme activity

The contents of soil organic carbon (SOC), total nitrogen (TN), alkali-hydrolyzable nitrogen (AN), total phosphorus (TP), available phosphorus (AP), total potassium (TK), and available potassium (AK) were determined using the method of Bao (Bao 2000). The contents of soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were also measured. The activities of urease (UR), alkaline phosphatase (PP), and sucrase (SUC) were determined using the method of Guan (Guan 1986).

Soil DNA extraction, Illumina sequencing, and data processing

Using the Soil DNA Extraction Kit from Omega Bio-Tec (Norcross, GA, US), microbial genomic DNA was extracted from soil samples. The bacterial 16S rDNA V3-V4 region was amplified using BacF:ACTCCTACGGGAGGCAGCA and Bac R:GGACTACHVGGGTWTCTAAT; the fungal ITS region was amplified using ITSF (5' GGAAGTAAAAGTCGTAACAAGG-3')/ITSR (5'-GCTGCG TTCTTCATCGATGC-3'). PCR conditions were 98°C for 5 min, 98°C for 30 s, 53°C for 30 s, 25 cycles, 72°C for 45 s, 72°C for 5 min,

and 12°C. The amplification results were subjected to 2% agarose gel electrophoresis, and the target fragments were excised and recovered using the Axygen Gel Extraction Kit. The DADA2 Method (Benjamin et al., 2016) was used to process the raw sequencing data, including primer removal, quality filtering to exclude low-quality sequences, denoising to correct noisy reads, automatic sequence merging for paired-end data, chimera detection to remove chimeric sequences, and deduplication. The deduplicated sequences generated after processing are called Amplicon Sequence Variants. Meanwhile, a feature table recording the abundance of these sequences in each sample is generated. These are the core data for subsequent microbiome analysis. Sequencing data accession numbers for bacteria and fungi in NCBI are PRJNA1303951 and PRJNA1303954, respectively.

Data analysis

Qime software was used to calculate Chao, Ace, and Shannon diversity index. R software was used to generate Venn Diagrams. Canoco 5.0 was used for Redundancy Analysis (RDA). Prism software was used to create bar charts. SPSS 16.0 software was used for One-Way ANOVA to analyze differences. Each experiment was repeated three times, and the measured values were expressed as Mean ± Standard Error (SE). Different letters show significant difference at $P < 0.05$ using Waller Duncan test.

Results

Changes in soil physicochemical properties of *Scutellaria baicalensis* at three different growth stages

Changes in the physicochemical properties of *Scutellaria baicalensis* rhizosphere soil at three different growth stages are shown in Table 1. Compared with the flowering stage, the contents of TK, AN, SOC, MBC, MBN, and the C/N ratio increased significantly by 6.53%, 23.86%, 31.93%, 49.01%, 31.01%, 19.52% at the fruiting stage and by 17.34%, 52.32%, 42.32%, 62.20%, 73.39%, 29.94% at the withering stage, with the order being withering stage > fruiting stage > flowering stage for all of them. The contents of TP and AP significantly increased by 38.83%, 50.68% and 27.18%, 36.59% at the fruiting stage and wilting stage compared with the flowering stage, respectively, with the order being fruiting stage > wilting stage > flowering stage. TP content was higher at the fruiting stage and wilting stage compared with the flowering stage, with little difference between the fruiting stage and wilting stage. Compared with the flowering stage, the gradual increase in contents of TN, TP, TK, AN, AP, AK, SOC, MBC, MBN, and the C/N ratio at the fruiting stage and wilting stage may be related to the gradual release of fertilizer effects after the application of organic fertilizer. pH value was the highest at the flowering stage (7.8), followed by the fruiting stage (7.5), and the lowest at the wilting stage (7.2). The gradual decrease in pH value may be related to the accumulation of rhizosphere exudates from *Scutellaria baicalensis*.

Changes in plant mass accumulation of *Scutellaria baicalensis* at different growth stages

Table 1
Soil physicochemical properties of *Scutellaria baicalensis* at different growth stages

Sample	pH	TN g kg ⁻¹	TP g kg ⁻¹ (g/kg)	TK g kg ⁻¹ (g/kg)	AN mg kg ⁻¹	AP mg kg ⁻¹
Flowering stage	7.8 ± 0.1c	1.03 ± 0.02a	1.03 ± 0.02a	13.61 ± 0.03a	43.54 ± 0.59a	33.29 ± 0.16a
Fruiting stage	7.5 ± 0.1b	1.14 ± 0.01b	1.43 ± 0.03c	14.50 ± 0.02b	53.93 ± 0.99b	50.16 ± 0.12c
Wilting stage	7.2 ± 0.1a	1.13 ± 0.02b	1.31 ± 0.02b	15.97 ± 0.05c	66.32 ± 0.60c	45.47 ± 0.56b
Sample	AK mg kg ⁻¹	SOC g kg ⁻¹	MBC mg kg ⁻¹	MBN mg kg ⁻¹	C/N	--
Flowering stage	138.59 ± 1.04b	13.28 ± 0.03a	159.00 ± 0.79a	41.60 ± 0.21a	12.86 ± 0.23a	--
Fruiting stage	151.87 ± 1.10c	17.52 ± 0.01b	236.93 ± 1.11b	54.50 ± 1.28b	15.37 ± 0.14b	--
Wilting stage	134.28 ± 0.54a	18.90 ± 0.03c	257.90 ± 1.03c	72.13 ± 0.22c	16.71 ± 0.29c	--
Values are given as the means ± SD (n = 3); Values within a row followed by different lowercase letters are						
significantly different (n = 3, LSD, P < 0.05)						

The results are shown in Fig. 1. Compared with the flowering stage, RFW and RDW increased significantly by 60.00% and 50.99% respectively at the fruiting stage, and by 97.43% and 82.12% respectively at the withering stage. Compared with the flowering stage, SFW and RFW increased by 12.48% and 20.70% at the fruiting stage, and decreased by 41.767% and 32.26% at the wilting stage, respectively. Compared with the flowering stage, the R/S and SDW/SFW increased significantly by 25.09%, 7.31% at the fruiting stage and 168.86%, 16.32% at the withering stage, respectively; the RDW/RFW significantly decreased by 5.63% and 7.76% at the fruiting stage and wilting stage, respectively.

Accumulation of active components in *Scutellaria baicalensis* at different growth stages

As shown in Table 2, compared with the flowering stage, the contents of four components at the fruiting stage and wilting stage increased significantly, with the following percentage increases at the fruiting stage baicalin (83.87%), wogonoside (82.00%), baicalein (48.15%), and wogonin (61.54%) and at the wilting stage baicalin (114.52%), wogonoside (84.54%), baicalein (204.94%), and wogonin (210.26%).

Effects of fertilization on soil enzyme activity of *Scutellaria baicalensis*

The enzyme activities in the rhizosphere soil at the growth and development of *Scutellaria baicalensis* are shown in Fig. 2. Compared with the flowering stage, the activities of UR, PP, and SUC significantly increased by 100.67%, 29.30%, and 35.71% at the fruiting stage, and significantly increased significantly increased by 80.43%, 68.23%, and 59.07% at the wilting stage. This indicates that soil enzyme activity gradually increases with the growth stage and the gradual increase in rhizosphere soil nutrients

Table 2
Contents of active components in roots at different growth stages

Sample	Baicalin	Wogonoside	Baicalein	Wogonin
Flowering stage	8.27 ± 0.06a	1.70 ± 0.02a	0.27 ± 0.00a	0.13 ± 0.01a
Fruiting stage	15.20 ± 0.01b	3.10 ± 0.01b	0.40 ± 0.02b	0.21 ± 0.01a
Wilting stage	17.73 ± 0.02c	3.14 ± 0.01c	0.82 ± 0.01c	0.40 ± 0.01c
Values are given as the means ± SD (n = 3); Values within a row followed by different lowercase letters are				
significantly different (n = 3, LSD, P < 0.05)				

Effects of fertilization on soil microbial diversity of *Scutellaria baicalensis*

We used high-throughput sequencing technology to analyze the differences in bacterial and fungal community composition in *Scutellaria baicalensis* rhizosphere at the flowering stage, fruiting stage, and wilting stage. Table 3 shows that the mean values of high-quality sequences of bacteria at the flowering stage, fruiting stage, and wilting stage were 53113, 55521, and 59101, respectively. Compared with the flowering stage, the high-quality sequences and Shannon index significantly increased by 4.53%, 3.06% (at the fruiting stage) and 11.27%, 4.20% (at the wilting stage), respectively. Additionally, the Chao index significantly increased by 9.85% at the wilting stage compared with the flowering stage. For fungi, the values of high-quality sequences were 46564, 43068, and 42507, respectively. Compared with the flowering stage, the Chao index and Simpson index significantly increased by 7.95% and 4.54% at the fruiting stage, and by 8.46% and 6.10% at the wilting stage. Shannon index significantly increased by 9.09% at the wilting stage compared with the flowering stage. High-quality sequences significantly decreased by 7.51% and 8.71% at the fruiting stage and wilting stage compared with the flowering stage.

Table 3

Sequence numbers and diversity indices of rhizosphere soil of *Scutellaria baicalensis* at different growth stages

Sample	Chao	Shannon	Simpson	high-quality sequences
Flowering stage	3580.53 ± 80.21a	10.47 ± 0.03a	0.9982 ± 0.0001a	53113 ± 135a
Fruiting stage	3601.11 ± 16.12a	10.79 ± 0.01b	0.9987 ± 0.0000a	55521 ± 89b
Wilting stage	3856.21 ± 38.12c	10.91 ± 0.02c	0.9999 ± 0.0002b	59101 ± 98c
Sample	Chao	Shannon	Simpson	high-quality sequences
Flowering stage	288.67 ± 6.61b	5.28 ± 0.02a	0.9017 ± 0.0114a	46564 ± 70.15c
Fruiting stage	265.72 ± 1.96a	5.37 ± 0.01a	0.9426 ± 0.0027b	43068 ± 48.50b
Wilting stage	313.08 ± 0.93c	5.76 ± 0.06c	0.9567 ± 0.0026c	42507 ± 67.28a
Values are given as the means ± SD (n = 3); Values within a row followed by different lowercase letters are				
significantly different (n = 3, LSD, P < 0.05)				

Changes in bacterial composition at the phylum level in rhizosphere soil of *Scutellaria baicalensis* at different growth stages

In each sample, genera with relative abundance exceeding 0.5% were analyzed at the phylum level (Fig. 3). At the growth stage of *Scutellaria baicalensis*, the relative abundance of main bacterial communities in rhizosphere soil (including *Actinobacteriota*, *Bacteroidota*, *Proteobacteria*, and *Chloroflexi* significantly increased by 15.44%, 11.59%, 26.01%, and 10.88% respectively at the fruiting stage, and by 22.49%, 31.72%, 13.52%, and 22.76% respectively at the wilting stage, compared with the flowering stage. The relative abundance of *Acidobacteria*, *Firmicutes*, *Verrucomicrobiota*, and *Planctomycetota* significantly decreased by 8.43%, 13.36%, 35.75%, and 26.82% at the fruiting stage and 16.65%, 19.82%, 57.56%, and 50.85% at the wilting stage compared with the flowering stage. The relative abundance of *Gemmatimonadota* significantly increased by 15.59% at the fruiting stage compared with the flowering stage, with little difference between the wilting stage and the flowering stage.

At different growth stages of *Scutellaria baicalensis*, the relative abundance of main fungal communities in rhizosphere soil, such as *Ascomycota*, was 93.67%, 95.52%, and 91.41% at the flowering stage, fruiting stage, and wilting stage, respectively. The relative abundance of *Mortierellomycota*, *Aphelidiomycota*, and *Chytridiomycota* significantly decreased by 13.46%, 52.38%, and 25.66% at the fruiting stage and 49.20%, 63.18%, and 61.03% at the wilting stage compared with the flowering stage. The relative abundance of *Basidiomycota* significantly increased by 50.41% at the fruiting stage and by 207.91% at the wilting stage compared with the flowering stage.

Changes in bacterial composition at the genus level in *Scutellaria baicalensis* rhizosphere soil at different growth stage

In each sample, genera with relative abundance exceeding 0.5% were analyzed at the genus level (Fig. 4). Among 30 bacterial genera, *Sphingomonas* had the highest relative abundance, with values of 1.52%, 2.34%, and 2.07% at the flowering stage, fruiting stage, and wilting stage, respectively. Compared with the flowering stage, the relative abundance of 9 bacterial genera (*Haliangium*, *Blastococcus*, *Solirubrobacter*, *Streptomyces*, *Cellulomonas*, *Rubrobacter*, *Ensifer*, *Georgenia*, and *Iamia*) exhibited a significant increase, specifically rising by 22.74–73.58% at the fruiting stage and by 9.46–42.43% at the wilting stage. The relative abundance of these 9 genera increased from the flowering stage to the fruiting stage and decreased from the fruiting stage to the wilting stage, with the fruiting stage being higher than the flowering stage. The relative abundance of *Bradyrhizobium* increased by 50.23% at the fruiting stage compared with the flowering stage, with little difference between the wilting stage and the flowering stage. Compared with the flowering stage, the relative abundance of *Bacillus*, *Nocardioides*, *Geodermatophilus*, *Mycobacterium*, *Bryobacter*, *Pseudomonas*, *Skermanella*, *Agromyces*, and *Devosia* significantly decreased by 8.08%~31.32% at the fruiting stage and by 23.40%~57.59% at the wilting stage. Compared with the flowering stage, the relative abundance of *Gemmatimonas*, *Lysobacter*, *Ramlibacter*, *Pedomicrobium*, *Rhodomicrobium*, *Aeromicrobium*, *Phenyllobacterium*, *Opitutus*, *Arenimonas*, and *Candidatus Solibacter* significantly increased by 14.76%~48.60% at the fruiting stage and by 16.34%~175.98% at the wilting stage. The relative abundance of these 10 genera gradually increased at the flowering stage, fruiting stage, and wilting stage.

Among 30 fungal genera, *Mycochlamys* had the highest relative abundance, with values of 4.41%, 6.78%, and 7.82% at the flowering stage, fruiting stage, and wilting stage, respectively. Compared with the flowering stage, the relative abundance of *Trichocladium*, *Fusarium*, *Plectosphaerella*, *Cladosporium*, *Scopulariopsis*, *Sodiomyces*, *Mortierella*, *Podospora*, *Metarhizium*, and *Myrmecridium* significantly increased by 24.22%~195.57% at the fruiting stage and by 43.66%~418.83% at the wilting stage. The relative abundance of these 11 genera gradually increased in rhizosphere soil during the growth stage of *Scutellaria baicalensis*. The relative abundance of *Alternaria* significantly increased by 90.67% at the wilting stage compared with the flowering stage and fruiting stage, with little difference between the flowering stage and fruiting stage. Compared with the flowering stage, the relative abundance of *Preussia*, *Arthrographis*, *Eremomyces*, *Corynascella*, *Zopfella*, *Bisifusarium*, *Penicillium*, *Beauveria*, *Solicoccozyma*, and significantly decreased by 4.05%~82.83% at the fruiting stage and by 24.88%~90.58% at the wilting stage. The relative abundance of these 10 genera gradually decreased in rhizosphere soil at the growth stage of *Scutellaria baicalensis*. The relative abundance of *Botryotrichum*, *Pseudogymnoascus*, *Aspergillus*, *Xenodidymella*, and *Subramaniula* significantly increased by 28.55%~234.19% at the fruiting stage compared with the flowering stage and significantly decreased by 14.03%~47.47% at the wilting stage compared with the flowering stage. The relative abundance of these 6 genera showed a trend of first increasing and then decreasing at the three growth stages of *Scutellaria baicalensis*, with the relative abundance at the wilting stage being lower than that of the flowering stage. The relative abundance of *Acaulium* and *Stolonocarpus* significantly decreased by 66.07% and 75.90% at

the fruiting stage compared with the flowering stage, with little difference between the flowering stage and wilting stage.

Compared with the flowering stage, the relative abundance of *Wardomyces* and *Pseudallescheria* significantly increased by 43.47% and 6.53% at the fruiting stage, and by 262.99% and 64.06% at the wilting stage, respectively.

Relationship between rhizosphere soil nutrients, microbial groups, and enzyme activity of *Scutellaria baicalensis*

RDA revealed the relationship between soil chemical properties, microbial community, and enzyme activity, with soil properties as environmental factors and microbial groups as variables (Fig. 5a, b, c, d). Axis 1 and Axis 2 explain 75.48% and 20.29% of the total variation between the community composition at the bacterial phyla level and soil properties (Fig. 5a). The contents of AN, AP, AK, SOC, MBC, MBN, and C/N ratio were significantly positively correlated with the relative abundance of *Bacteroidetes*, *Gemmatimonadetes*, *Proteobacteria*, *Chloroflexi*, *Actinobacteria*, and *Cyanobacteria*; The pH value was significantly positively correlated with the relative abundance of *Verrucomicrobia*, *Firmicutes*, *Acidobacteria*, *Nitrospirae*, *Planctomycetes*, and *Armatimonadetes*. The most significant factor affecting bacteria at the phyla level was AP ($F = 19.8$, $P = 0.002$), followed by MBC ($F = 14.9$, $P = 0.014$). Axis 1 and Axis 2 explain 86.70% and 4.84% of the total variation between the community composition at the bacterial genus level and soil properties (Fig. 5b). The contents of AN, AP, AK, MBN, MBC, and C/N ratio were positively correlated with the relative abundance of *Gemmatimonas*, *Pedomicrobium*, *Aeromicrobium*, *Arenimonas*, *Lysobacter*, *Ramlibacter*, *Rhodomicrobium*, *Phenylobacterium*, *Opitutus*, and *Candidatus Solibacter*. The most significant factor affecting bacteria at the genus level was AN ($F = 35.8$, $P = 0.002$), followed by MBN ($F = 30.0$, $P = 0.004$). Axis 1 and Axis 2 explain 83.45% and 15.02% of the total variation between the community composition at the fungal phyla level and soil properties (Fig. 5c). RDA showed that the pH value and AK content were positively correlated with the relative abundance of *Aphelidiomycota*, *Mortierellomycota*, *Ascomycota*, and *Chytridiomycota*; the relative abundance of *Basidiomycota* was positively correlated with the contents of AN, AP, SOC, MBC, MBN, and C/N ratio. The most significant factor affecting fungi at the phyla level was MB ($F = 9.7$, $P = 0.018$), followed by AN ($F = 9.0$, $P = 0.018$). Axis 1 and Axis 2 explain 97.28% and 2.34% of the total variation between the community composition at the fungal phyla level and soil properties (Fig. 5d). RDA showed that the contents of AN, AP, AK, MBN, MBC, and C/N ratio were positively correlated with the relative abundance of *Mycoclhamys*, *Trichocladium*, *Fusarium*, *Plectosphaerella*, *Cladosporium*, *Alternaria*, *Scopulariopsis*, *Sodiomyces*, *Mortierella*, *Podospora*, *Metarhizium*, and *Myrmecridium*. The most significant factor affecting fungi at the genus level was MBN ($F = 117$, $P = 0.004$), followed by AN ($F = 152$, $P = 0.004$). Axis 1 and Axis 2 explain 95.23% and 4.63% of soil enzyme activities and soil properties (Fig. 5e). PP activity was positively correlated with the contents of AN, AP, MBC, MBN, SOC, and C/N ratio; UR activity was positively correlated with the contents of AN, AP, AK, MBC, MBN, SOC, and C/N ratio; SUC activity was positively correlated with the contents of AN, AP, MBC, MBN, SOC, and C/N ratio. The activities of the three enzymes significantly influenced soil physicochemical properties.

Relationship between effective components and growth condition of *Scutellaria baicalensis* root and microbial community.

Figures 7a and 7b are correlation heatmaps of bacterial and fungal communities with plant effective components and growth traits, respectively. The heatmaps visually present the Pearson correlation between plant indicators and microbial communities through color. Figure a shows that baicalin, wogonoside, baicalein, wogonin, SDW/SDF, and R/S were significantly positively correlated with 10 bacterial genera such as *Gemmatimonas* and *Pedobacter*, while negatively correlated with 9 bacterial genera such as *Bacillus* and *Nocardioides*; the RDW/RFW showed the opposite trend. Figure b shows that baicalin, wogonoside, baicalein, wogonin, SDW/SFW, and R/S were significantly positively correlated with 12 fungal genera such as *Mycochlamys* and *Trichocladium*, while negatively correlated with 9 fungal genera such as *Preussia* and *Arthrographis*; the RDW/RFW showed the opposite trend.

Discussion

During the cultivation of *Scutellaria baicalensis*, soil nutrients play an important role in promoting the formation of yield and quality. Studies have shown that nutrient cycling is closely related to soil bacterial diversity and function, especially the changes in soil nutrients at different growth stages after *Scutellaria baicalensis* planting, which affect the bacterial diversity in rhizosphere soil. Meanwhile, soil enzyme activity plays an important role in soil nutrient transformation. This study found that from the flowering stage to the wilting stage, urease activity in the soil gradually increased, and AN content correspondingly increased, indicating that the increase in urease activity promoted the decomposition of organic nitrogen in the soil, increasing the AN content (Table 1). This is consistent with the findings of Wu in *Fructus aurantii* (Wu et al. 2023). At the wilting stage, soil PP activity was higher than in the fruiting stage, while the total AP content in the soil was lower (Table 1, Fig. 2). This may be because the demand for phosphorus element increases with the growth of *Scutellaria baicalensis*, reducing the AP content in the soil. Previous studies on *Rehmannia glutinosa* found that appropriate application of phosphate fertilizer increased the phosphorus content in *Rehmannia glutinosa* roots (Gao et al. 2021), supporting our result.

In our previous experiments on different fertilization measures for *Scutellaria baicalensis*, we found that increasing organic fertilizer could improve the content of soil organic matter. Therefore, this experiment adopted the measure of combining organic fertilizer and inorganic fertilizer, which is consistent with the findings of Sun in *Scutellaria baicalensis* fertilization experiments (Sun et al. 2024). Additionally, changes in soil nutrients can alter the bacterial diversity and function in rhizosphere soil. In this study, compared with the flowering stage, the Shannon index and Chao index of the soil increased at the fruiting and wilting stages (Table 3), indicating that soil organic matter facilitates the formation of soil bacterial diversity.

To confirm our hypothesis, we further analyzed the functions of bacteria and fungi at the phyla level during different growth stages of *Scutellaria baicalensis*. From the flowering stage to the withering stage, the relative abundance of *Actinobacteria* and *Bacteroidetes* gradually increased, and the contents

of AN, SOC, MBC, MBN, and C/N ratio in *Scutellaria baicalensis* rhizosphere soil showed a gradual increasing trend (Table 1). The reasons are, firstly, the continuous release of fertilizer efficiency from organic fertilizer in the soil during the growth and development of *Scutellaria baicalensis* promotes the reproduction of nutrient-rich bacteria such as *Actinobacteria* and *Bacteroidetes*, and *Actinobacteria* contributes to the degradation and utilization of organic matter; secondly, from the flowering stage to the fruiting stage, the vigorous growth of *Scutellaria baicalensis* roots produces a large amount of rhizosphere exudates, promoting the formation of organic matter in the soil, and the rich organic matter benefits the growth of *Actinobacteria*. This study found that with the growth and development of *Scutellaria baicalensis*, the abundances of *Proteobacteria* and *Chloroflexi* in the soil both showed a trend of first increasing and then decreasing (Fig. 3). This variation pattern was consistent with the content dynamics of AK and AP in the soil, and also aligned with the conclusion from previous research that *Proteobacteria* are eutrophic bacteria (Fierer et al., 2012). However, this trend was not entirely consistent with the existing finding that *Chloroflexi* are oligotrophic bacteria (Xian et al., 2020). We speculate that this may vary depending on plant species. At the genus level, the study found that *Bacillus* spp. in the rhizosphere participates in the cycling of proteins and cellulose, and *Bacillus velezensis* YH-18 and *Bacillus velezensis* YH-20 can promote the growth of stems and leaves of peach plants and the cultivation of high-quality tree species (Shi et al. 2022). *Geodermatophilus obscurus*, isolated from dolomite marble, can tolerate harsh environments such as dryness, mitomycin C, hydrogen peroxide, ionizing radiation, and ultraviolet radiation (Montero-Calasanz et al. 2014). *Bryobacter* spp. can decompose and utilize polysaccharides, various sugars, and organic acids in carbon cycling (Li et al. 2023). *Nocardioides* spp. not only has phosphorus-solubilizing functions but also promotes the degradation of various hard-to-degrade organic compounds such as aromatic compounds, hydrocarbons, halogenated alkanes, nitrogen-containing heterocyclic compounds, and polymeric polyesters (Wang et al. 2022b); *Sphingomonas* spp. can degrade 3-(4-isopropylphenyl)-1,1-dimethylurea in soils with pH above 7.0 (Bending et al. 2003). *Streptomyces* spp. participates in the decomposition of organic matter and can promote the activation of plant disease resistance mechanisms; compared with normal nitrogen fertilizer application, reducing nitrogen fertilizer by 50% combined with *Streptomyces* sp. NGB-Act4 and NGB-Act6 significantly promoted wheat growth and increased yield (Youseif et al. 2023); further demonstrating the growth-promoting effect of *Streptomyces* spp. on plants. Our study found that *Streptomyces* spp. first increased and then decreased (Fig. 4), indicating that this genus played an active role during the vigorous growth stage of *Scutellaria baicalensis*. *Blastococcus* spp. is widely present in soils with extreme cold, extreme heat, salinization, toxic amendments, and significant heavy metal pollution, and can be used for soil improvement (Sbissi et al. 2025); *Haliangium* can be used as a plant bactericide (Kumar et al. 2023). *Opitutus* has nitrogen fixation and nitrogen preservation functions (Hu et al. 2024). *Lysobacter enzymogenes* is a non-pathogenic strain that inhibits various crop fungal diseases by synthesizing antifungal factors (Zhao et al. 2019). *Gemmatimonas* spp. participates in the reduction of N₂O, reducing nitrogen loss in the form of N₂O, allowing more nitrogen to remain in the soil for crop absorption, thereby improving nitrogen fertilizer utilization efficiency and reducing agricultural production costs (Oshiki et al. 2022). The study of bacterial functions shows that the above 11 genera are related to nutrient cycling, organic matter degradation, or antagonism against soil pathogens. 4 genera had the

highest abundance at the flowering stage, 4 genera had the highest relative abundance at the fruiting stage, and 3 genera had the highest relative abundance at the wilting stage. This indicates that the communities and abundance of beneficial bacteria in *Scutellaria baicalensis* rhizosphere at the three different growth stages show regular changes. Correlation analysis showed that *Opitutus*, *Lysobacter*, and *Gemmatimonas* were significantly positively correlated with the accumulation of effective components (Fig. 6), further supporting our results.

Research on fungal functions has found that *Ascomycota* has higher abundance in soils with lower organic matter than in soils with higher organic matter. This phylum can participate in the formation of soil aggregates and serves as an important source of various toxins. (Li et al. 2014; Fu et al. 2020); *Basidiomycota* is saprophytic fungi suitable for survival in environments rich in organic matter (Fu et al. 2020). The results of this study showed that compared with the flowering stage, the relative abundance of *Ascomycota* increased at the fruiting stage and decreased at the wilting stage, while the relative abundance of *Basidiomycota* gradually increased at the fruiting and wilting stages; this is related to the gradual increase in soil organic matter with the growth and development of *Scutellaria baicalensis*. *Arthrographis* spp. has the ability to produce cellulase and is commonly found in compost (Eida et al. 2011; Okeke et al. 1993). Some species of *Penicillium* spp. can produce soluble phosphorus, iron carriers, and plant hormones (such as indole-3-acetic acid and gibberellin), which play an important role in plant health (Park et al. 2020). *Preussia* spp. can produce plant growth hormones to promote plant growth (Al-Hosni et al. 2018). *Aspergillus brunneoviolaceus* HZ23 is a plant growth-promoting bacterium that changes the physicochemical properties of rhizosphere soil of pak choi and can be used as an amendment for newly reclaimed soil (Li et al. 2023). *Pseudogymnoascus* spp. is the most abundant fungal genus in the rhizosphere, and its members are usually involved in cellulose degradation (Sigler et al. 2000). *Pseudallescheria* spp. is a potential pathogen found in soil and on humans and animals (Rainer et al. 2020). *Mycochlamys* can participate in chitin degradation (Debode et al. 2016). A large amount of *Mortierella alpina* in the rhizosphere not only participates in the decomposition of plant residues and organic matter but also promotes plant growth by facilitating the biosynthesis of carotenoid derivatives and enhancing stress tolerance (Wani et al., 2017); some genera of *Mortierella* spp. can convert insoluble phosphorus in the soil into soluble phosphorus, improving plant phosphorus absorption capacity and promoting plant growth and development (Sang et al. 2022). *Alternaria* spp. is a potential plant pathogenic fungus that can cause potato early blight, potato brown spot, and soybean black spot (Xu et al. 2022). *Fusarium* spp. can cause crop diseases such as potato dry rot and potato *Fusarium wilt* (Zhang et al. 2017). The study of fungal functions shows that the above 10 genera are related to nutrient cycling, organic matter degradation, and plant pathogenicity. Two genera had the highest abundance at the flowering stage, 2 genera had the highest relative abundance at the fruiting stage, and 4 genera had the highest relative abundance at the wilting stage. The highest relative abundance of *Mycochlamys*, *Mortierella*, *Alternaria*, and *Fusarium* at the wilting stage was significantly positively correlated with effective components such as baicalin (Fig. 6), further supporting our results.

Conclusion

The research results show that influenced by the growth stage, the soil physicochemical properties of *Scutellaria baicalensis* at different growth stages exhibit various changes. The diversity of fungi and bacteria is the highest at the wilting stage compared with fruiting stage and wilting stage. The groups of functional microorganisms among fungi and bacteria exhibit distinct characteristics at different growth stages of *Scutellaria baicalensis*. The association patterns between bacterial and fungal diversity and the accumulation of active components in *Scutellaria baicalensis* root provide key data for in-depth analysis of the ecological interaction network between plants and microorganisms. This helps explore the bidirectional mechanisms of plant metabolites regulating microbial communities and microorganisms influencing plant growth and development. It also provides a theoretical basis for optimizing plant growth and enhancing the accumulation of secondary metabolites through microbial community regulation.

Declarations

Author Contribution

Lixia Xu and Minna Guo Wrote the main manuscript text and Meishan Yue and Chunyan Guo prepared figures 1-3. All authors reviewed the manuscript.

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Figures

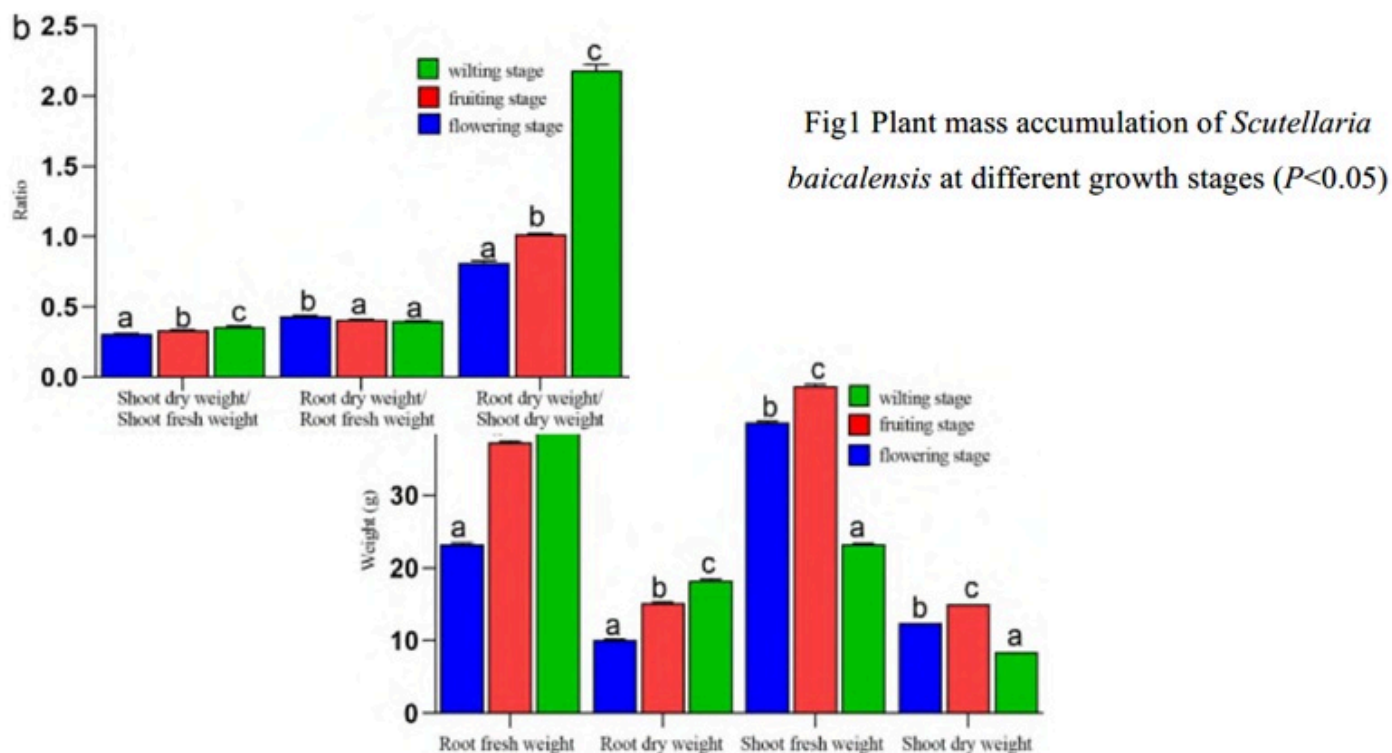


Figure 1

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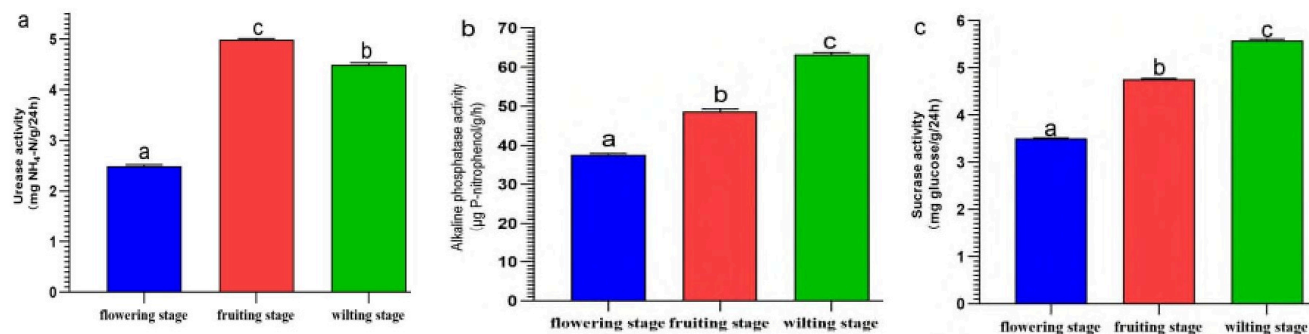


Fig 2 Rhizosphere soil enzyme activities of *Scutellaria baicalensis* at different growth stages ($P<0.05$)

Figure 2

See image above for figure legend.

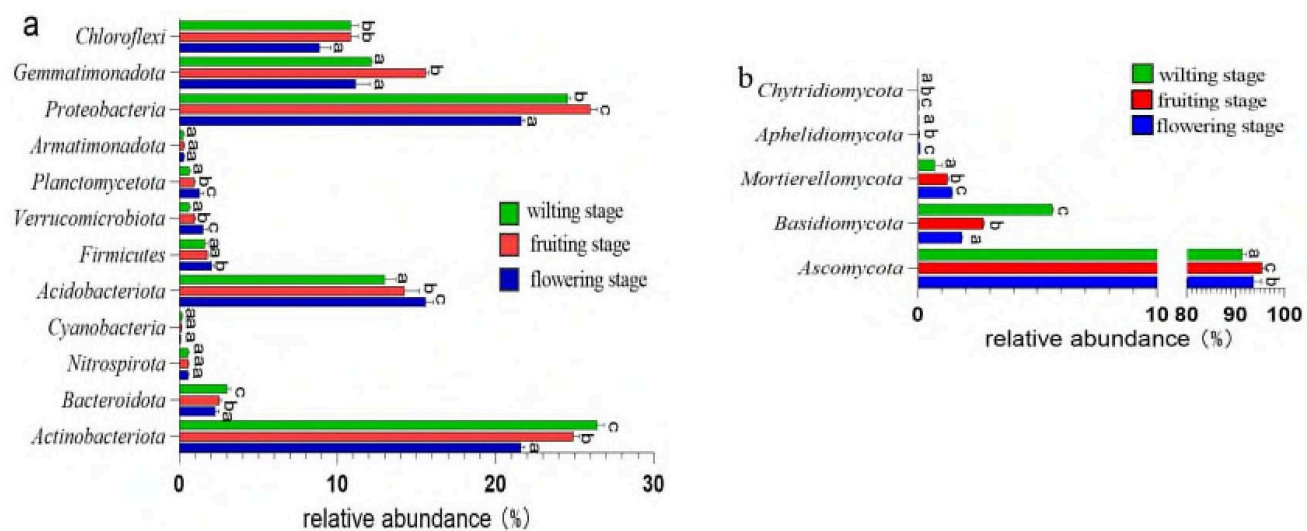


Fig 3 Diversity of rhizosphere soil microorganisms at the phylum level in *Scutellaria baicalensis* at different growth stages (a Bacteria; b Fungi)

Figure 3

See image above for figure legend.

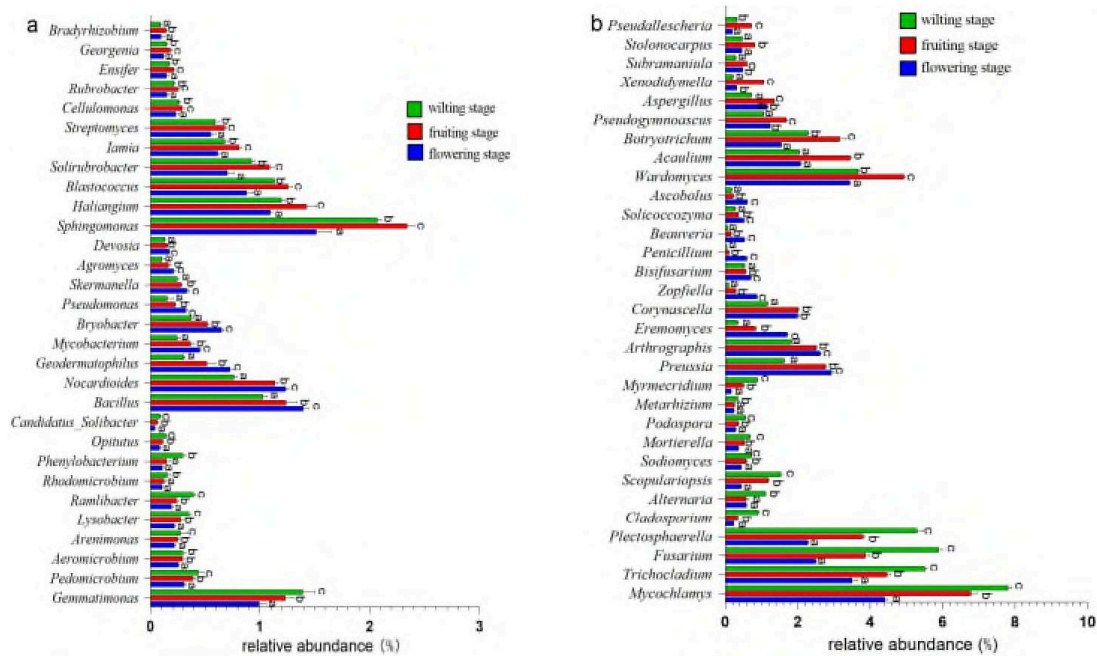


Fig 4 Diversity of rhizosphere soil microorganisms at the genus level in *Scutellaria baicalensis* at different growth stages (a Bacteria; b Fungi)

Figure 4

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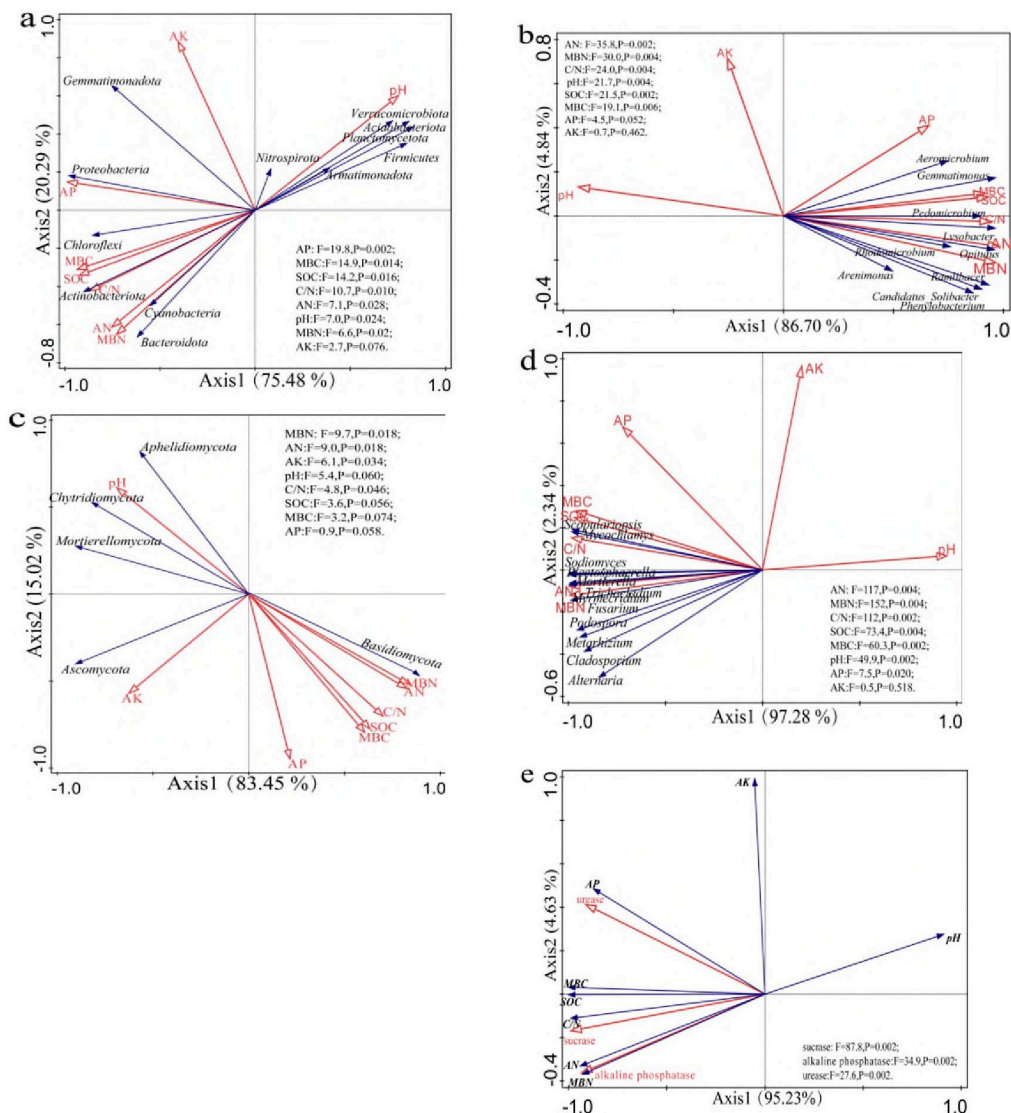


Fig. 5 Relationship between the soil properties, soil enzyme activities and the abundances of bacteria and fungi. a Soil properties and the the bacterial community composition at the phylum level. b Soil enzyme activities and the bacterial community composition at the genus level. c Soil properties and the fungal community composition at the phylum level. d Soil enzyme activities and the fungal community composition at the genus level. e Soil enzyme activities and soil properties

Figure 5

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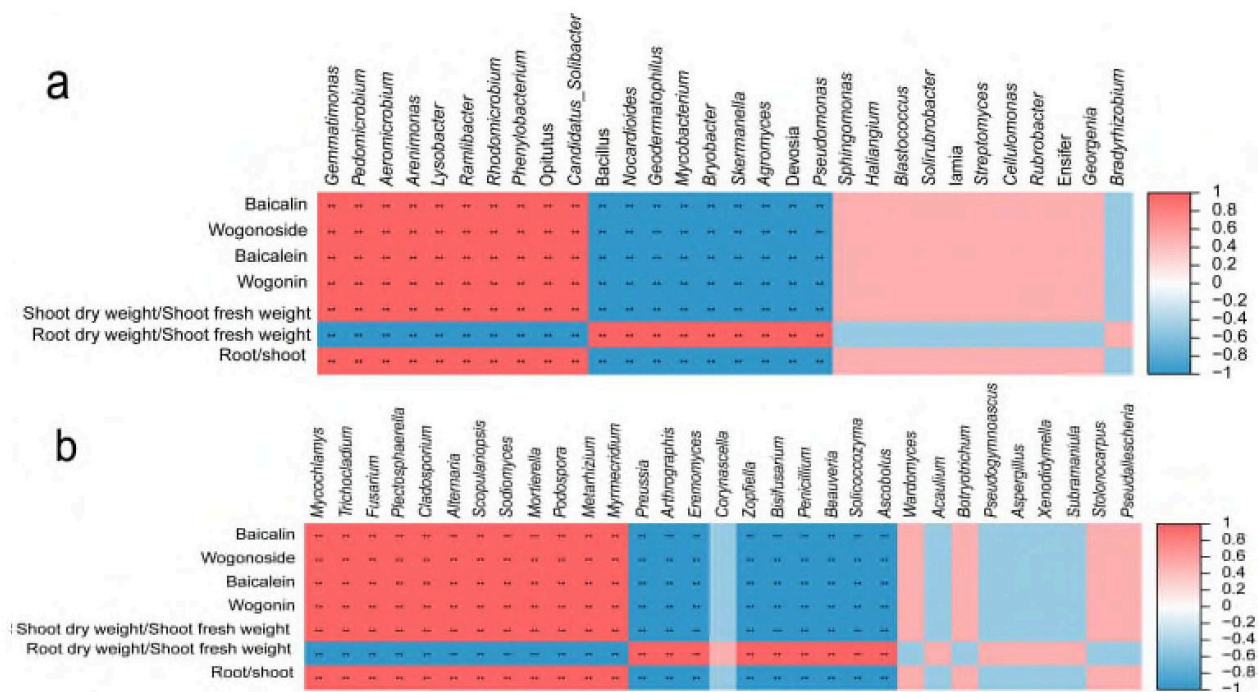


Figure 6 Heatmap of correlations between active components, characteristic indicators of *Scutellaria baicalensis* and microbial community composition at the genus level

a: Heatmap of correlations with bacteria; b: Heatmap of correlations with fungi. The color gradient indicates the strength of correlation: red represents positive correlation, blue represents negative correlation, and the darker the color, the higher the correlation

Figure 6

See image above for figure legend.