



Research on the Mechanisms Regulating Soil Microbial Community Structure and Functional Metabolism during Coloured Potato Cultivation with “Seed Rotation”

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Abstract

To address the continuous cropping obstacles associated with coloured potatoes (*Solanum tuberosum* L.) in the red soil region of Yunnan, in this study, two “seed rotation” cultivation regulation modes, namely cultivar rotation (red potato–purple potato) and crop rotation (red/purple potato–soybean), were innovatively proposed. Through pot experiments, the regulatory effects of different cultivation modes on soil nutrients, enzyme activities, microbial community structure, and functional metabolic networks were systematically analysed. Compared with the monocropping of a single potato cultivar, the cultivar switching treatment significantly improved the soil nutrient status, with the contents of soil available nitrogen, phosphorus, and potassium significantly increasing by 56.40–58.04%, 14.96–27.44%, and 58.59–62.21%, respectively, while the cultivar rotation treatment had a secondary effect. In addition, the interspecific rotation treatment enriched beneficial bacteria such as *Bradyrhizobium*; upregulated the abundance of secondary metabolites, including flavonoids; and activated biosynthetic pathways such as those involving phenylpropanoids, flavonoids, and various amino acids. Compared with the continuous cultivation of a single potato cultivar, the cultivar rotation treatment alleviated the homogenization trend of the soil microbial community, increased the abundance of organic acids and glycosides, and activated pathways such as the shikimate pathway and the biosynthetic pathways of aromatic amino acids (phenylalanine and tyrosine). In summary, both modes, especially the interspecific rotation mode, can improve the soil environment and have significant agronomic application value and ecological environmental benefits. Owing to the similar biological characteristics between the same family, the cultivar rotation mode results in relatively limited changes in the soil microbial community and metabolite composition, but it can delay the occurrence of continuous cropping obstacles. This study provides practical value for the sustainable soil management of potato cultivation in red soil regions.

Keywords Coloured potatoes · Continuous cropping obstacle · Functional metabolism · “Seed rotation” planting · Soil microbes

Introduction

Coloured potatoes, which are rich in anthocyanins, polyphenols, and active antioxidant ingredients, have both high nutritional value and market potential, and the scale of coloured potato cultivation has continued to expand in recent years (Sampaio et al. 2021). However, long-term intensive planting has aggravated continuous cropping obstacles, which have manifested mainly as soil nutrient imbalance (Zhou et al. 2018), decreased enzyme activity (Zhang et al. 2024a, b), deterioration of the microbial community structure (Chen et al. 2022), the accumulation of harmful metabolites, and the frequent occurrence of soil-borne diseases, severely constraining the sustainable development of the industry (Ma et al. 2025). Previous studies have shown that the continuous cropping of potatoes leads to an imbalance in the soil microbial community, with a reduction in the number of beneficial microorganisms, such as *Bacillus*, including *Bacillus xylosus*, and a strong enrichment of pathogenic microorganisms, such as *Fusarium oxysporum* and *Mycobacterium epidermidis* (Li et al. 2021a, b; Liu et al. 2014; Gao et al. 2019). Moreover, the accumulated phenolic acids, terpenoids, and other allelochemical substances in continuously cropped soil act as autotoxic substances, leading to disorders in soil carbon and nitrogen metabolism and a decrease in enzyme activity through a series of mechanisms, such as inhibition of the growth of the potato root system, inhibition of nutrient absorption, and alterations in soil metabolic pathways, thereby amplifying the negative effects of continuous cropping obstacles (Ding et al. 2024; Fu et al. 2023; Wang et al. 2022).

Although the application of chemical fertilizers and pesticides can mitigate continuous cropping obstacles in the short term (Huang et al. 2024; Ahsan et al. 2024), long-term application tends to induce secondary ecological issues, including soil acidification, degradation of microbial function, and the accumulation of pesticide residues (Zhang et al. 2022). As an eco-friendly agronomic measure, crop rotation with crops of different families has become a hotspot in current agroecological research because it improves soil health by regulating root secretions, optimizing soil nutrient cycling, and reshaping microbial community structure (Li et al. 2023). Wang et al. (2023a, b) demonstrated that crop rotation improved soil physicochemical properties and microbial community structure, optimized the abundance and function of soil rhizosphere metabolites, promoted soil nutrient cycling, and increased yield by 21.19–28.38%. Zhang et al. (2024a, b) reported that compared with continuous cropping, crop rotation increased the abundance of the microbial community, changed the structure of the bacterial and fungal communities, and enhanced the function of the microbial community; compared with continuous cropping, crop rotation increased the soil nitrogen and phosphorus metabolism capacity by 8.61–88.65%. Qin et al. (2022) reported that different crop rotation systems could increase the activity of soil enzymes and nutrient contents and maintain ecosystem health by recruiting beneficial microorganisms to maintain ecosystem health.

Therefore, crop rotation can effectively alleviate continuous cropping obstacles, improve soil fertility, and increase the stability and sustainability of soil ecosystems by improving the diversity and metabolic functions of soil microbial communities. Legume crops (e.g., soybean) have attracted much attention because of their nitrogen fixation ability and metabolite interactions (Ebede 2021), and the secondary metabolites secreted by the root system, such as flavonoids and phenolic acids, not only recruit beneficial microorganisms but also regulate the activity of enzymes related to carbon and nitrogen metabolism in the soil and promote the decomposition and transformation of soil organic matter to form many small-molecule metabolites, such as amino acids and sugars, which provide a source of carbon and energy for soil microbes, thus optimizing microbial community function (Lyu et al. 2022).

Currently, coloured potatoes have high added value and play important roles in increasing farmers' income and promoting the prosperity of southwestern mountainous areas. Therefore, in this study, a novel innovative “seed rotation” system was established with both “colored potato cultivar rotation” and “colored potato–soybean crop rotation” experimental frameworks. In this study, cultivar switching mode refers to a planting pattern in which two different cultivated potato varieties—red potato (*Solanum tuberosum* L. cv. Red) and purple potato (*Solanum tuberosum* L. cv. Purple)—are alternately grown in the same planting area over consecutive growing seasons. On the basis of soil physicochemical analysis, high-throughput sequencing and untargeted metabolomics, we investigated the regulation of soil functional microbial communities by different rotation modes, constructed an interaction network of key differentially abundant metabolites and microbial functional modules, compared the soil microecological structural and functional differences within the same family and among different crop rotation modes, and explored in depth the effects of continuous cropping obstacle mitigation, thereby increasing the cropping frequency of coloured potatoes in monoculture systems. The results of this study can provide data support for the construction of a green and high-efficiency production system for high value-added specialty crops and a technology for eliminating continuous cropping obstacles in Southwest China.

Materials and Methods

Overview of the Test Area

The experiments were conducted from April to December 2024 at the Teaching and Experimental Farm of Yunnan Agricultural University (25°22' N, 102°42' E). Located in a subtropical monsoon climate zone at an altitude of 1920 m, the area features distinct wet and dry seasons throughout the year. The soil type was red soil, and the basic soil conditions were as follows: pH, 6.34; organic matter, 9.90 g·kg⁻¹; total nitrogen, 0.55 g·kg⁻¹; total phosphorus, 0.75 g·kg⁻¹; total potassium, 3.80 g·kg⁻¹; available nitrogen, 61.44 mg·kg⁻¹; available phosphorus, 5.15 mg·kg⁻¹; and available potassium, 171.78 mg·kg⁻¹.

Experimental Design

The method of conducting experiments was by placing potted plants in the field. The planting spacing was 60 cm × 30 cm, and polyethylene pots (38 cm in diameter × 25 cm in height) were used as cultivation containers, with 12 kg of soil filled in each pot. To maintain consistency between the experimental environment and the field conditions, the flowerpots were buried in furrows, with the upper edges of the pots kept flush with the ridge tops. Six treatments were established, soil without potato planting as the control (CK); single-cultivar planting: red potato (RP_RP) and purple potato (PP_PP); cultivar switching: red potato followed by purple potato (RP_PP) and purple potato followed by red potato (PP_RP); and crop rotation: red potato followed by soybean (RP_SB) and purple potato followed by soybean (PP_SB). A schematic diagram of the experiment is shown in Fig. 1A. Each treatment was replicated in 15 pots. Prior to potato planting, the fertilizing amount according to compound fertilizer (N:P₂O₅:K₂O = 20:5:15) at 1200 kg ha⁻¹ made the conversion. Each pot received one virus-free pre-basic seed tuber weighing approximately 50 g, and the subsequent crop was planted in situ. Field management measures, including irrigation, fertilization, and pest control, were implemented in accordance with conventional field cultivation protocols. The test varieties were provided by the Potato Research Institute of Yunnan Agricultural University (Fig. 1B and C), with Dian shu 23 (D23) used as the red potato variety and Dian-coloured potato 104 (D104) used as the purple potato variety; all the varieties are commercial.

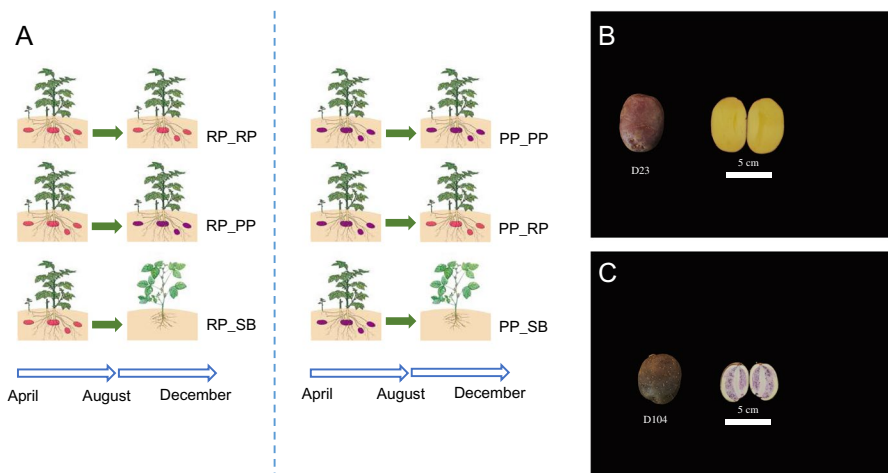


Fig. 1 Schematic diagram of planting patterns and phenotypes

Soil Sample Collection

Soil sampling was conducted after harvest in December 2024. Rhizosphere soil samples were collected from potato/soybean plants in 15 pots for each treatment. After the plant roots were removed, the soil adhering to the roots within 0–3 mm from the root surface was brushed off using a sterile brush and divided into two portions. One portion was dried naturally, ground to powder, and passed through a mesh sieve with a pore size of 0.15 mm for subsequent analysis of the soil physicochemical properties, and the other portion was placed at -80°C for measurement of the soil microbial diversity and for untargeted metabolomics. Six biological replicates were used for untargeted metabolomics, and three biological replicates were used for the remaining indicators.

Analysis of Soil Samples

Determination of Soil Physical and Chemical Properties and Enzyme Activities

The relevant indicators were determined following the standard procedures described by Zhang et al. (2018) and Bao (2000). Specifically, soil pH was measured using a Leici PHS-3E pH meter (Shanghai Yidian Scientific Instrument Co., Ltd.) at a soil-to-water ratio of 2.5:1; total soil nitrogen was determined by the Kjeldahl method with a K9860 Kjeldahl nitrogen analyser (Haineng Future Technology Group Co., Ltd.); total soil phosphorus was measured via the NaOH alkali fusion-molybdenum antimony anti-spectrophotometric method, using an SX4-7-12 ceramic fibre muffle furnace (Hebei Fissfu Instrument Co., Ltd.) and a Molecular Devices SpectraMax 190 full-wavelength microplate reader (Beijing Longyue Biotechnology Development Co., Ltd.); total soil potassium was determined by the NaOH fusion method with an FP640 flame photometer (Shanghai Jingke Instrument Co., Ltd.); alkali-hydrolysable nitrogen was measured using the alkali-hydrolysis diffusion method with an HJ-1 constant temperature incubator (Jiangsu Jintan Ronghua Instrument Manufacturing Co., Ltd.); available phosphorus was determined by spectrophotometry with a Molecular Devices SpectraMax 190 full-wavelength microplate reader (Beijing Longyue Biotechnology Development Co., Ltd.); available potassium was measured via the ammonium acetate extraction method with an FP640 flame photometer (Shanghai Jingke Instrument Co., Ltd.); and soil organic matter was determined by the potassium dichromate volumetric method.

Soil urease (URE) activity was determined by the sodium phenol–sodium hypochlorite colorimetric method; soil acid phosphatase (ACP) activity was determined by the disodium phosphate colorimetric method; soil catalase (CAT) activity was determined by the potassium permanganate titrimetric method; and soil polyphenol oxidase (PPO) activity was determined by using a reagent kit (Nanjing Ruiyuan Biotechnology Co., Ltd.) (Xu et al. 2021). Enzyme activity was defined as follows ($1\text{ U}\cdot\text{g}^{-1}$): one unit of URE activity was defined as the amount of enzyme that degraded 1 mg of $\text{NH}_3\text{-N}$ per gram of soil sample per day, one unit of soil

ACP activity was defined as the amount of enzyme that produced 1 nmol of phenol per gram of soil per day at 37 °C, one unit of soil CAT activity was defined as the amount of enzyme that degraded 1 mmol of H₂O₂ per gram of air-dried soil sample per day, and one unit of soil PPO activity was defined as the amount of enzyme that produced 1 mg of purple gallotannin per gram of soil sample per day.

High-Throughput Sequencing of Soils

The total genomic DNA of the microbial community was extracted according to the instructions of the E.Z.N.A. With a soil DNA kit, the quality was checked by 1% agarose gel electrophoresis, and the concentration and purity were determined by a NanoDrop2000. The DNA was used as a template for PCR amplification of the V3–V4 variable region of the bacterial 16S rRNA gene and the ITS-V2 region of the fungal ITS region using specific primers (Lu et al. 2021). The PCR products were recovered via a 2% agarose gel and DNA gel recovery kit and quantified with a Qubit 4.0 instrument. Sequencing was performed by Shanghai Major Biopharmaceutical Company (cloud.majorbio.com) using the Illumina Nextseq2000 platform. After sequencing, the raw sequences were subjected to quality control and spliced using fastp and FLASH (Fast Length Adjustment of Short Reads) software, and the number of sequences was decreased to 20,000 by removing the chloroplast and mitochondrial sequences with the DADA2 (divisive amplicon denoising algorithm 2) plug-in for noise reduction in the QIIME2 (Quantitative Insights Into Microbial Ecology 2) process. ASVs (amplicon sequence variants) were classified on the basis of the Silva 16S rRNA gene database (v 138) using naive Bayes classifiers in QIIME 2.

Soil Untargeted Metabolomics Analysis

A 100-mg soil sample was placed in a 2-mL centrifuge tube, followed by the addition of one 6-mm grinding bead and 800 µL of extraction solution (methanol:water=4:1, v/v) containing four internal standards (including L-2-chlorophenylalanine at a concentration of 0.02 mg/mL). The mixture was ground in a frozen tissue grinder for 6 min (−10 °C, 50 Hz) and subjected to low-temperature ultrasonic extraction for 30 min (5 °C, 40 kHz). After incubation at −20 °C for 30 min, the mixture was centrifuged at 13,000×g for 15 min at 4 °C. A 3-µL aliquot of the resultant supernatant was taken and separated on a BEH C18 chromatographic column (100 mm×2.1 mm i.d., 1.7 µm) prior to mass spectrometry analysis. Mobile phase A was 2% acetonitrile in water (containing 0.1% formic acid), and mobile phase B was acetonitrile (containing 0.1% formic acid). Elution was performed with a specific gradient (0–0.5 min, 2% B; 0.5–7.5 min, to 35%; 7.5–13 min, to 95%; 13–14.4 min, maintain at 95%; 14.4–14.5 min, to 2%; 14.5–16 min, maintain at 2%) at a flow rate of 0.40 mL/min and a column temperature of 40 °C. The mass spectrum was acquired in positive and negative ion scanning modes (*m/z* 70–1050) with parameters set to a positive ion voltage of 3500 V and a negative ion voltage of −3000 V (Kong et al. 2022).

The raw LC–MS data were imported into Progenesis QI software for baseline filtering and peak identification to obtain the data matrix. After the metabolite information was matched through the Meiji MJDBPM database, the data were uploaded to the Meiji cloud platform for preprocessing: missing values were removed according to the 80% rule, the minimum value was filled, the sum was normalized, the variables with RSD > 30% of the QC samples were deleted, and the log₁₀ value was used to obtain the final analysis data matrix.

Data Analysis

Microsoft Excel 2024 was used to organize the data, and SPSS 26.0 was used for standardization and one-way analysis of variance (ANOVA), which was combined with the Majorbio cloud platform (<https://www.majorbio.com>) to assist the analysis. Microbial data were analysed via mothur software to calculate alpha diversity; the Wilcoxon rank sum test was used to analyse the differences between groups; principal coordinate analysis (PCoA), which is based on the Bray–Curtis distance combined with PERMANOVA, was used to test the differences in community structure; and linear regression was used to assess the effects of variables according to Spearman's correlation ($|r| > 0.6$, $p < 0.05$). Species association maps were drawn (annotation database: silva138/16S_bacteria; annotation method: classify-sklearn (Naive Bayes), confidence level 0.7; noise reduction: DADA2).

Metabolomic data were analysed by principal component analysis (PCA) and orthogonal projections to latent structures–discriminant analysis (OPLS-DA) using the R language ropls package (version 1.6.2), and differentially abundant metabolites were screened by the criteria $VIP > 1$ and $p < 0.05$; pathways were annotated by the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, and pathway enrichment analysis was accomplished by Python's scipy.stats package.

Results

Changes in Soil Physicochemical Properties after “Seed Rotation”

As shown in Table 1, compared with that in the CK, the soil organic matter content increased in all the treatments. The contents of total nitrogen, total phosphorus, and total potassium followed the order RP_SB, PP_SB > RP_PP, and PP_PP > RP_PP, PP_PP. Among these, the soil total nitrogen content slightly increased in the RP_SB and PP_SB treatments. In contrast to those in the CK, the total nitrogen, total phosphorus, and total potassium contents in the RP_PP, PP_PP, RP_PP, and PP_PP treatments decreased by 14.55–23.64%, 13.33–17.33%, and 13.95–18.94%, respectively.

As shown in Table 2, the contents of soil pH, available potassium, available nitrogen, and available phosphorus across all the treatments decreased in the following order: RP_SB, PP_SB treatments > RP_PP, PP_PP treatments > RP_PP, PP_PP treatments. Compared with those in the CK, the contents of available nitrogen,

Table 1 Changes in soil nutrient contents after “seed rotation” (g·kg⁻¹)

Treatment	SOM	TN	TP	TK
CK	9.90±0.320 d	0.55±0.012 b	0.75±0.034 a	3.80±0.121 a
RP_RP	11.10±0.272 b	0.42±0.021 d	0.62±0.031 c	3.11±0.030 d
RP_PP	10.98±0.355 b	0.44±0.010 cd	0.64±0.025 bc	3.27±0.082 c
PP_PP	10.45±0.094 c	0.44±0.020 d	0.64±0.013 bc	3.08±0.065 d
PP_RP	10.68±0.129 bc	0.47±0.015 c	0.65±0.004 bc	3.23±0.080 cd
RP_SB	12.13±0.377 a	0.58±0.011 a	0.67±0.012 b	3.43±0.060 b
PP_SB	11.98±0.266 a	0.60±0.014 a	0.68±0.010 b	3.33±0.109 bc

Different lowercase letters for data in the same column indicate significant differences between different treatments ($P < 0.05$)

Table 2 Changes in the contents of soil available nutrients after “seed rotation”

Treatment	pH	AN (mg·kg ⁻¹)	AP (mg·kg ⁻¹)	AK (mg·kg ⁻¹)
CK	6.34±0.015 a	61.44±1.864 a	5.24±0.113 a	171.78±0.154 a
RP_RP	5.90±0.015 e	40.68±1.842 c	4.30±0.124 b	99.09±0.599 e
RP_PP	6.11±0.012 c	45.89±1.601 b	4.51±0.132 b	118.86±0.189 c
PP_PP	5.77±0.010 f	40.76±1.109 c	4.48±0.437 b	98.67±0.418 e
PP_RP	6.01±0.032 d	44.34±1.866 b	4.52±0.178 b	107.11±5.100 d
RP_SB	6.27±0.010 b	64.29±2.963 a	5.48±0.226 a	157.15±4.889 b
PP_SB	6.13±0.015 c	63.45±2.107 a	5.15±0.106 a	160.05±5.218 b

Different lowercase letters for data in the same column indicate significant differences between different treatments ($P < 0.05$)

available phosphorus, and available potassium in the RP_RP, PP_PP, RP_PP, and PP_RP treatments decreased by 25.70–33.79%, 13.74–19.94%, and 30.81–42.56%, respectively. In comparison with those in the RP_RP and PP_PP treatments, the contents of available nitrogen, available phosphorus, and available potassium in the RP_SB and PP_SB treatments increased by 56.40–58.04%, 14.96–27.44%, and 58.59–62.21%, respectively, whereas those in the RP_PP and PP_RP treatments increased by 8.78–12.78%, 0.89–4.89%, and 8.55–19.95%, respectively.

As shown in Table 3, the URE, ACP, and CAT activities generally followed the same trend as follows: RP_SB, PP_SB treatments > control (CK) > RP_PP, PP_PP treatments > RP_RP, PP_PP treatments, whereas the opposite trend was observed for soil PPO activity.

Specifically, compared with the RP_RP and PP_PP treatments, the RP_SB and PP_SB treatments increased the activities of URE, ACP, and CAT by 40.00–55.56%, 13.20–14.49%, and 37.05–37.60%, respectively, while decreasing the PPO activity by 23.06–28.57%. In contrast, compared with the RP_RP and PP_PP treatments, the RP_PP and PP_RP treatments led to increases of 0–11.11% in URE activity, 2.77–4.35% in ACP activity, and 5.22–11.07% in CAT activity, respectively, as well as a reduction of 8.20–9.60% in PPO activity.

Table 3 Changes in soil enzyme activities after “seed rotation” planting

Treatment	URE (U·g ⁻¹)	ACP (U·g ⁻¹)	CAT (U·g ⁻¹)	PPO (U·g ⁻¹)
CK	0.12 ± 0.004 b	10,049.08 ± 81.392 b	6.45 ± 0.240 c	3.70 ± 0.206 e
RP_RP	0.09 ± 0.006 c	9186.25 ± 124.266 d	5.56 ± 0.155 de	4.48 ± 0.046 a
RP_PP	0.10 ± 0.007 c	9585.86 ± 178.771 c	5.85 ± 0.118 d	4.05 ± 0.167 b
PP_PP	0.10 ± 0.005 c	9201.20 ± 47.802 d	5.24 ± 0.296 e	4.51 ± 0.124 a
PP_RP	0.10 ± 0.005 c	9456.20 ± 79.006 c	5.82 ± 0.118 d	4.14 ± 0.160 b
RP_SB	0.14 ± 0.011 a	10,517.75 ± 259.815 a	7.62 ± 0.136 a	3.20 ± 0.181 f
PP_SB	0.14 ± 0.006 a	10,415.68 ± 105.081 a	7.21 ± 0.303 b	3.47 ± 0.228 ef

Different lowercase letters for data in the same column indicate significant differences between different treatments ($P < 0.05$)

Changes in the Structure of the Soil Microbial Community after “Seed Rotation”

The results of the soil microbial community alpha diversity analysis at the ASV level (Figs. 2 and 3) revealed that compared with those in the CK, the Shannon index, Pielou_e index, and Sobs value of the rhizosphere soil bacteria in the RP_RP and PP_PP treatments generally tended to decrease, whereas those in the RP_PP, PP_PP, RP_SB, and PP_SB treatments tended to increase, with the most significant increase observed in the RP_SB and PP_SB treatments (Fig. 2). These findings indicated that, compared with the CK, the RP_PP, PP_PP, RP_SB, and PP_SB treatments were conducive to improving the diversity, evenness, and richness of the soil bacterial community, whereas the RP_SB and PP_SB treatments had better effects.

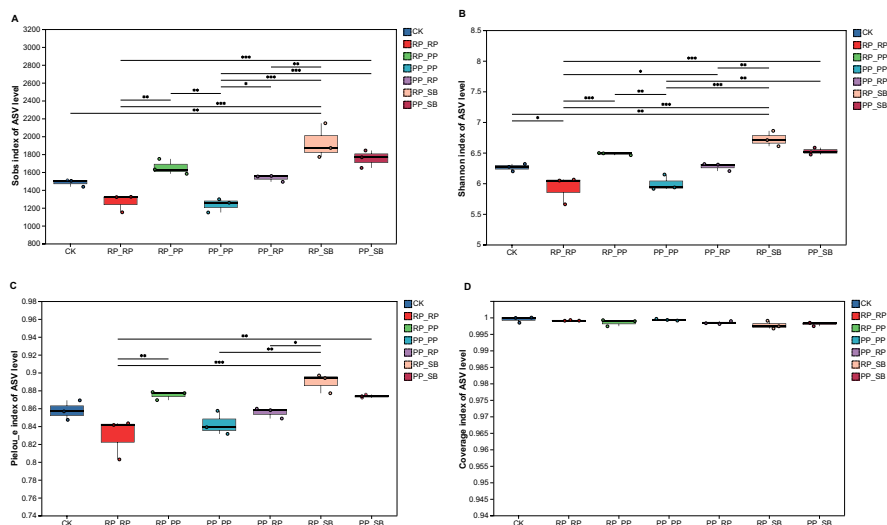


Fig. 2 Inter-group difference test for alpha diversity indices of soil bacterial communities at the ASV level Note: A, B, C, D = sobs, Shannon, Pielou_e and Coverage indices of bacterial communities, respectively

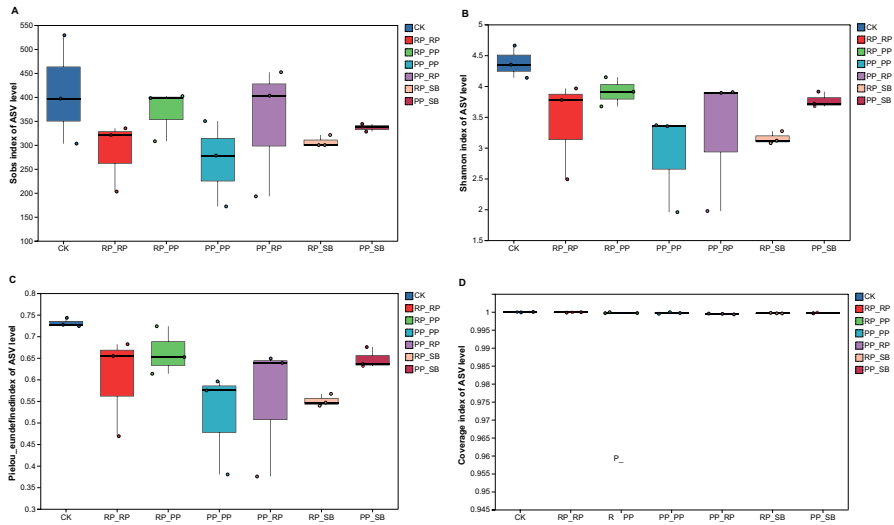


Fig. 3 Inter-group difference test for alpha diversity indices of soil fungal communities at the ASV level. Note: **A, B, C, D** = sobs, Shannon, Pielou_e and Coverage indices of fungal communities, respectively

In contrast, the diversity, evenness, and richness of the soil bacterial community tended to decrease in the RP_RP and PP_PP treatments.

With respect to the fungal community, the Shannon index, Pielou_e index, and Sobs value of the rhizosphere soil fungi in all the treatments generally tended to decrease, suggesting that the diversity, evenness, and richness of the soil fungal community decreased after crop planting (Fig. 3). The coverage index of all the treatments (Figs. 2D and 3D) was greater than 0.995, indicating that the sequencing data had high coverage and that the results were highly reliable.

The results of PCoA of soil microbial communities at the ASV level (Fig. 4A and B) revealed a clear separation of bacterial and fungal communities among the CK, the RP_RP, PP_PP, RP_PP and PP_PP treatments, and the RP_SB and PP_SB

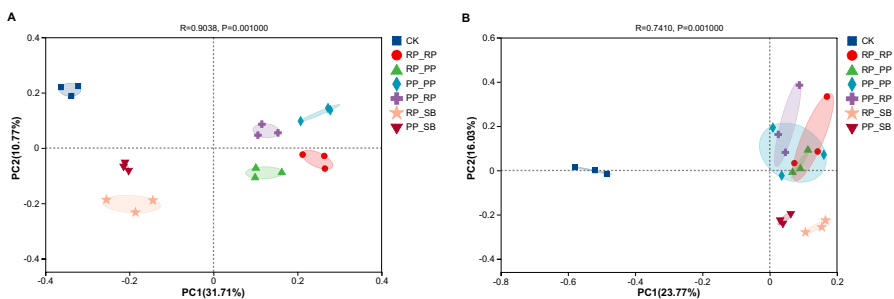


Fig. 4 PCoA of soil microbial communities at the ASV level. Note: **A** the bacterial community; **B** the fungal community

order of *biosynthesis of phenylpropanoids*, *isoflavonoid biosynthesis*, and *flavonoid biosynthesis*. The differential abundance score plot of metabolite KEGG pathways (Fig. 6) revealed that, compared with those in the RP_RP and PP_PP treatments, the abundances of all annotated differentially abundant metabolites in pathways such as *biosynthesis of alkaloids derived from the shikimate pathway* were upregulated in the RP_PP and PP_PP treatments, whereas those in pathways such as *galactose metabolism*, *starch and sucrose metabolism*, *pyrimidine metabolism*, *ABC transporters*, and *nucleotide metabolism* were generally downregulated (Fig. 6A). Moreover, the differentially abundant metabolites between these two groups were enriched in the phenylpropanoid biosynthesis pathway (Supplementary Fig. 4). In contrast, in the RP_SB and PP_SB treatments, all annotated differentially abundant metabolites in pathways such as *isoflavonoid/flavonoid biosynthesis*, *biosynthesis of phenylpropanoids*, *amino acid metabolism*, *biosynthesis of various other secondary metabolites*, and *ABC transporters* exhibited a significant upregulation trend (Fig. 6B). Most of these metabolites were significantly enriched in the biosynthesis pathways of phenylpropanoids, flavonoids, and various amino acids (Supplementary Fig. 5).

The results of differentially abundant metabolite clustering analysis (Fig. 7) revealed that in the RP_RP, PP_PP, RP_PP, and PP_PP treatments, the expression levels of myristyl sulphate, flupicolide, soyasapogenol E, methyl 4-methoxy-1h-indole-2-carboxylate, difenoconazole, fluroxastrobil acid, violacein, azoxystrobin acid, azoxystrobin, and compound classes such as *lipids and lipid-like molecules*, *organoheterocyclic compounds*, *benzenoids*, and other unclassified compounds were all upregulated. In addition, the expression levels of diplodiatoxin, 16-hydroxy-10-oxohexadecanoic acid, 1-kestose, citisinine C, soyasapogenol E, and other metabolites, as well as compound classes such as *lipids and lipid-like molecules*, *organic acids and derivatives*, *organic oxygen compounds*, and *organoheterocyclic compounds*, were generally upregulated in the soils of the RP_SB, PP_SB, RP_PP, and PP_PP treatments.

Integrated Analysis of Related Indicators after “Seed Rotation” Planting

On the basis of the results of the Spearman correlation coefficient analysis of the top 20 dominant microbial community genera in terms of abundance with environmental factors (Fig. 8), among the bacterial communities (Fig. 8A), the abundances of *Bradyrhizobium*, *norank_c_AD3*, *HSB_OF53-F07*, *norank_o_Gaiellales*, *JG30a-KF-32*, *Ramlibacter*, *norank_o_Acidobacteriales*, *norank_f_Chitinophagaceae*, and *Bryobacter* were significantly correlated with soil pH; the TN, TP, TK, AN, AP, AK, and SOM levels and URE, AOP, and CAT activities were positively correlated with soil pH; and the TN, TP, TK, AN, AP, AK, and SOM levels and URE, AOP, and CAT activities were significantly negatively correlated with PPO activity. On the other hand, the abundances of *Sphingomonas*, *Rhodanobacter*, *norank_f_Roseiflexaceae*, *Pseudomonas*, *Lysobacter*, *norank_f_Chitinophagaceae*, and *Castellaniella* were negatively correlated with PPO activity. Among the fungal communities (Fig. 8B), the abundances of *Cumuliphoma* and *Plectosphaerella* were significantly positively correlated with overall soil PPO activity and significantly negatively

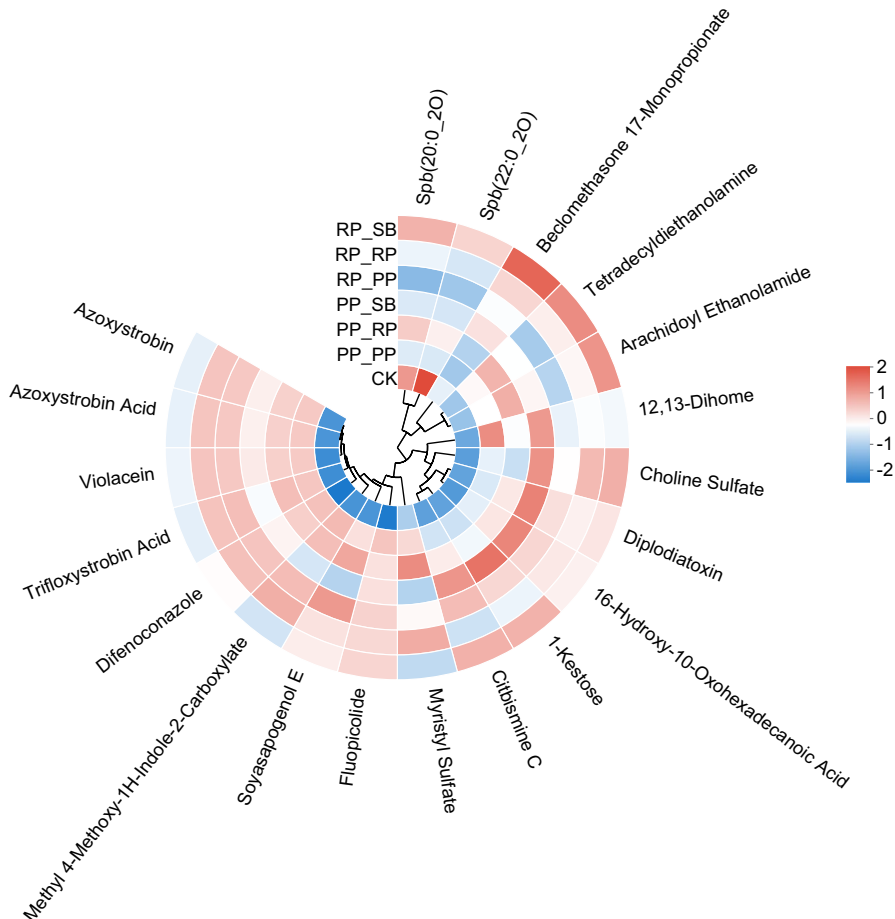


Fig. 7 Cluster analysis of differential soil metabolites

correlated with the other environmental factors. *Powellomycetaceae_gen_Incertae_sedis*, *Fusarium*, etc. presented the opposite trend.

The correlations between the top 20 differentially abundant metabolites and the 12 environmental factors were analysed by Spearman's analysis (Fig. 9) to clarify the relationships between the soil metabolites and the environmental factors. The results revealed that the trifloxystrobin acid, difenoconazole, methyl 4-methoxy-1H-indole-2-carboxylate, soyasapogenol E, myristyl sulphate, fluopicolide, violacein, azoxystrobin acid, and azoxystrobin levels were generally positively correlated with the SOM content and PPO activity and negatively correlated with the remaining environmental factors. The Sph(14:0_20), tetradecyldiethanolamine, arachidoyl ethanolamine, choline sulphate, 1-kestose, and 16-hydroxy-10-oxohexadecanoic acid levels were positively correlated with the AN, AP, and TN levels and URE, CAT, and ACP activities; highly significantly positively correlated with

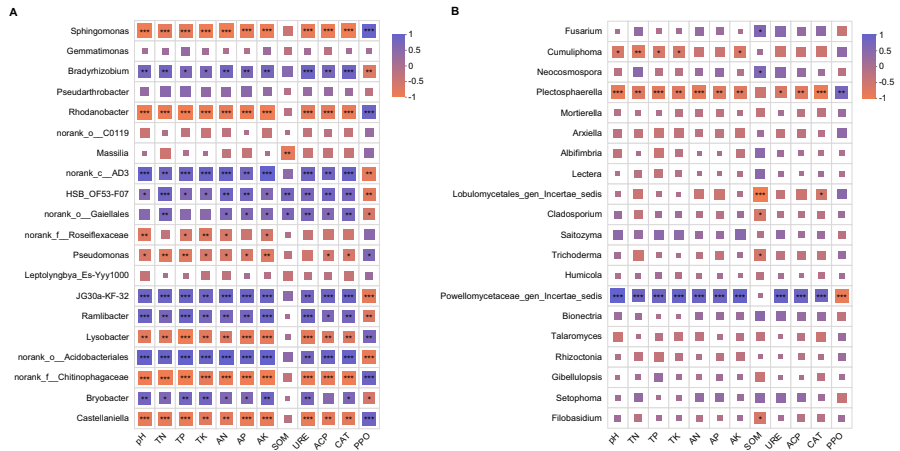


Fig. 8 Correlation heatmap of soil microbial communities at the genus level. Note: **A** the bacterial community; **B** the fungal community

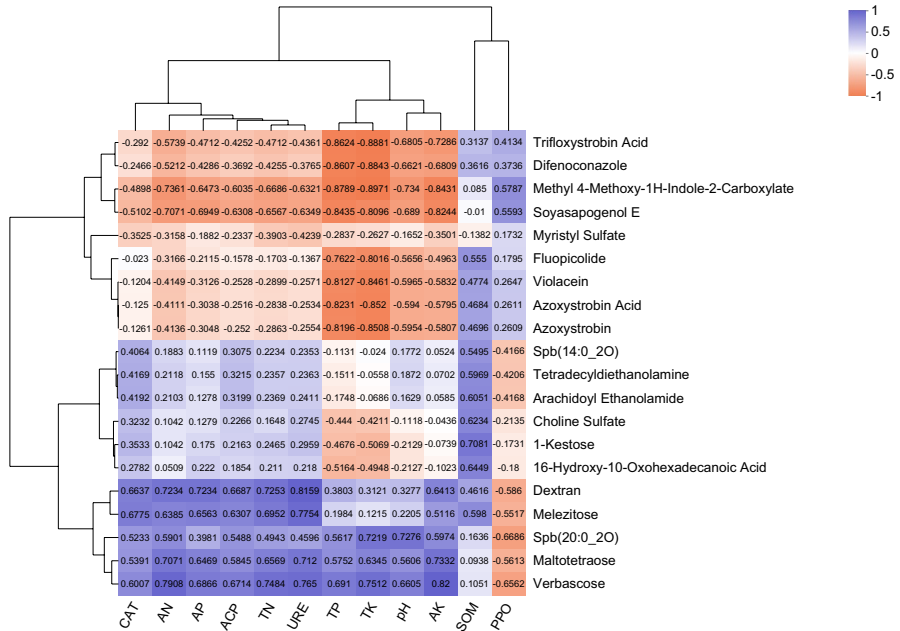


Fig. 9 Correlations between differential soil metabolites and environmental factors

the SOM content; and negatively correlated with the TP and TK contents and PPO activity in general. The dextran, melezitose, Sph(20:0_20), maltotetraose, and verbascose levels were significantly negatively correlated with PPO activity and significantly negatively correlated with the remaining environmental factors overall.

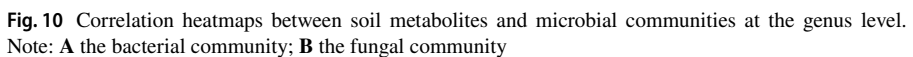
Correlations between the top 20 ranked differentially abundant metabolites and the abundances of microbial genera were analysed via Spearman analysis (Fig. 10). Among the bacterial communities (Fig. 10A), the abundances of *Pseudomonas*, *norank_f_Chitinophagaceae*, Ophagaceae, *Sphingomonas*, *Rhodanobacter*, and *Lysobacter* were significantly positively correlated overall with the levels of myristyl sulphate, dihydroxyacetone, 1,11-hexadecanediylsulphate, thiocyanic acid, soyaapogenol E, azoacridine, azoxystrobin acid, violacein, and others, whereas the abundances of *norank_c_AD3*, *Ramlibacter*, *JG30a-KF-32*, *norank_o_Acidobacteriales*, *HSB_OF53-F07*, and *Bradyrhizobium* presented the opposite trends. The abundance of *Massilia* was significantly negatively correlated with cafestol, 1-kestose bacillithiolone, 11-methoxystreptopolic acid, dipropionate, fumagillol, and choline sulphate levels. The abundances of *Pseudarthrobacter* and *unclassified_f_Micrococcaceae* were negatively correlated with the levels of almost all the metabolites. Moreover, the correlation network revealed (Supplementary Figure S6A) that *Sphingobium*, *Geothrix*, *Chujaibacter*, and TN were strongly positively correlated with various factors, whereas *Anaeromyxobacter*, *Segetubacter*, *Ramlibacter*, *Kibdelosporangium*, AK, and pH were strongly negatively correlated; the metabolites cyclopassifloic acid E, spriostane + 10, -2H, O-hex-Dhex, polysaccharide A, and 4-hydroxyindole also strongly interacted with the other types of factors.

In the fungal community (Fig. 10B), the abundances of *Lectera*, *Fusarium*, *Neocosmospora*, and *Albifimbria* were positively correlated with the beclomethasone 17-monopropionate, violacein, azoxystrobin, azoxystrobin acid, tetradecyldiethanolamine, arachidoyl ethanolamide, 12,13-dihome, citbismine C, 1-kestose, 16-hydroxy-10-oxohexadecanoic acid, diprotocatechuic acid, furopyrimidone, and choline sulphate levels, whereas the abundances of *unclassified_f_Plectosphaerellaceae*, *Lobulomycetales_gen_Incertae_sedis*, *Cladosporium*, and *Trichoderma* were negatively correlated. The abundances of *unclassified_o_Pleosporales*, *Saitozyma*, and *Powellomycetaceae_gen_Incertae_sedis* were significantly negatively correlated with the abundances of myristyl sulphate, difenoconazole, methyl 4-methoxy-1H-Indole-2-carboxylate, 1,1-dimethylurea, trifloxystrobin acid, soyaapogenol E, and Spb (2 0:0_2O), whereas the abundances of *Cumuliphoma*, *Arxiella*, *unclassified_f_Nectriaceae*, *unclassified_f_Didymellaceae*, and *Plectosphaerella* were positively correlated. The correlation network (Supplementary Figure S6B) revealed that *Neocosmospora*, *Rhizophagus*, TN, and CAT were significantly positively correlated with most of the factors, whereas Typhula, Bjerkandera, pH, TP, and AK were mainly negatively correlated.

Discussion

Effects of Soil Nutrient and Enzyme Activities after “Seed Rotation”

Soil pH (hydrogen ion concentration index, an indicator reflecting the acidity or alkalinity of soil solution) is closely correlated with the accumulation of



functional compounds in potatoes. It modulates nutrient availability, root growth, and metabolic pathways such as phenylpropanoid biosynthesis. The optimal pH range of 6.0–7.0 facilitates the synthesis of functional compounds, whereas acidic or alkaline stress inhibits nutrient uptake and impairs enzyme activity, thereby reducing the accumulation of these compounds. Furthermore, intercultural differences in pH adaptability regulate this correlation via root exudates (Tafesse et al. 2021). Soil moisture affects pH by mediating ion migration and transformation: drought increases soil solution concentration and suppresses microbial activity, leading to elevated pH; humid conditions induce leaching of base cations and release of organic acids, resulting in decreased pH; and excessive moisture modulates acid–base balance through the activity of anaerobic microorganisms (Ma et al. 2017). In this study, soil pH in the RP_RP and PP_PP treatments declined to below 6.0, which may inhibit nutrient uptake and impair enzyme activity.

Crop rotation (RP_SB, PP_SB) significantly increased the soil organic matter, total nitrogen, and available nutrient (nitrogen, phosphorus and potassium) contents. This could be attributed to two aspects: on the one hand, the yield of potato was higher than that of soybean, leading to greater nutrient uptake and lower nutrient residue in the soil after potato harvest (Zhou et al. 2018). On the other hand, the symbiotic rhizobia in soybean roots could convert atmospheric nitrogen into plant-available nitrogen, thereby increasing soil nitrogen content (Ebede 2021). Moreover, soybean root secretions contain a variety of organic compounds, such as amino acids, organic acids, carbohydrates, and nitrogen compounds (Wang et al. 2023a, b), that can significantly influence the structure of the soil microbial community and its function, thereby improving soil fertility. The beneficial bacteria identified in this study included mainly *Bradyrhizobium* and *Pseudomonas*. Previous studies have shown that organic acids secreted by soybean roots may recruit beneficial bacterial communities such as *Pseudomonas*, thereby promoting the activation and uptake of nutrient elements, including phosphorus, iron, and sulphur, in the rhizosphere (Damo et al. 2023). In contrast, the results of this study revealed that planting a single potato variety (RP_RP or PP_PP) resulted in a decrease in the soil total nitrogen, total phosphorus, and total potassium contents, which was attributed to the continuous uptake of soil nutrients during the growth of potato plants. On the other hand, the imbalance in the structure of the soil microbial community and the deterioration of soil physicochemical properties caused by continuous cropping obstacles inhibited the effective supply of soil nutrients and ultimately aggravated soil nutrient depletion (Zhou et al. 2018). This is consistent with the gradual homogenization of the microbial community structure in the RP_RP and PP_PP treatments in this study. In contrast, cultivar switching within the same family of potatoes briefly alleviated the continuous consumption of specific nutrients through varietal turnover but failed to reverse the persistent depletion of total nitrogen, phosphorus, and potassium. These findings indicate that crops within the same family share a highly overlapping spectrum of soil nutrient requirements, making the cycle of specific nutrient depletion caused by continuous potato cropping a persistent challenge.

Additionally, URE, ACP, and CAT activities significantly increased in the crop rotation groups, and the increased activities of these enzymes, which are involved in carbon, nitrogen, and phosphorus metabolism, respectively, indicated that the soil biological activity improved, which was conducive to the decomposition of organic matter and nutrient transformation in the soil (Qin et al. 2017). The decrease in PPO activity may be attributed to the decrease in the accumulation of phenolics in the soil by soybean root secretions. Phenolic acids secreted by the root system of potato plants during continuous cropping may disrupt the soil microecological balance by inhibiting the growth and function of beneficial microorganisms (Zhao et al. 2025).

Effects on the Structure and Function of Soil Microbial Communities after “Seed Rotation”

Crop rotation with soybean significantly increased bacterial diversity and abundance (e.g. of *Bradyrhizobium*). Soybean root secretions contain a variety of sugars, amino acids, and organic acids and serve as rich sources of nutrients for microorganisms. These secretions attract a variety of beneficial microorganisms to colonize plants and promote symbiotic relationships among them (Chen and Liu 2024; Sarao et al. 2025). In the present study, we reported that the abundances of *Sphingomonas*, *Rhodanobacter*, and *Pseudomonas* increased in soils with the continuous cultivation of potatoes, similar to the findings of Agegnehu et al. (2016) and Zhang et al. (2021). *Sphingomonas* maintains its carbon metabolism dominance by degrading autotoxic substances such as phenolic acids and aromatic compounds accumulated by continuous cropping (Ding et al. 2024), whereas *Rhodanobacter*, which prefers acidifying environments and relies on nitrifying/denitrifying metabolism, can tolerate continuous cropping-induced decreases in soil pH and changes in nitrogen patterns (Rodrigo et al. 2011). *Pseudomonas* species occupy ecological niches by competitively utilizing carbon sources in root secretions, secreting iron carriers or antibiotics to suppress pathogenic bacteria, and forming biofilms under nutrient-poor or oxidative stress conditions (Rumberger et al. 2007); all three species respond to the combined accumulation of self-toxic substances, soil acidification and microbial imbalance induced by continuous cropping and gain competitive advantages in the rhizosphere microenvironment through metabolic function differentiation and stress tolerance strategies.

With respect to the fungal community, all the treatments reduced fungal diversity. The relative abundance of *Ascomycota* was greater under crop rotation with soybeans, and some *Ascomycota* species can produce antibiotics or compete with pathogenic fungi for nutrients and survival space, inhibiting the growth of pathogenic fungi such as *Fusarium* through antagonism and thus contributing to the maintenance of soil health (Todorović et al. 2023). The continuous planting of potato significantly enriched *Ascomycota*. Some species of *Ascomycota* can participate in the degradation of phenolic acids, but over-enrichment may lead to its dominant position in the soil fungal community, which in turn inhibits the growth of other ecologically important fungi and destroys the structure and function of the soil fungal community. Additionally, anthracnose fungi, *Fusarium*, and *Verticillium*

in Ascomycota, among others, are important plant pathogenic fungi. These fungi may trigger plant disease (Qin et al. 2022; Xing et al. 2024). Notably, the relative abundance of *Fusarium* decreased in the soils in the RP_RP and PP_PP treatments, whereas the opposite trend was observed in the RP_SB and PP_SB treatments. This phenomenon could be attributed to two aspects: On the one hand, continuous potato cropping may induce a shift in the soil type from a ‘bacteria-dominated’ state to a ‘fungi-dominated’ state (Wang et al. 2023a, b; Qin et al. 2022), thereby reducing the relative abundance of *Fusarium*; on the other hand, rotation with soybean might provide a new host for the ubiquitous *Fusarium* species (Shi et al. 2024), leading to a short-term increase in its relative abundance.

Effects of Differentially Abundant Metabolite Function in Soil after “Seed Rotation”

Crop rotation with soybean significantly increased the levels of beneficial metabolites such as flavonoids, glycosides, organic acids, and peptides. Organic acids can perform antioxidant functions through iron chelation and can effectively inhibit lipid-derived peroxyl radical- and alkoxyl radical-mediated oxidative damage (Genaro-Mattos et al. 2015); amino acid derivatives such as soybean saponins and flavonoids can promote microbial interactions and increase the stability of the soil microbial community. Moreover, these compounds have a certain degree of antimicrobial activity and inhibit the growth of pathogenic bacteria, thereby enhancing soil function (Fujimatsu et al. 2020; Tava et al. 2025). Single-cultivar/cultivar switching causes the enrichment of lipids (e.g. pyrimidic acid) and heterocyclic compounds (e.g. phenyl ether metronidazole), which may be secondary metabolites produced via plant–microbe interactions. Moreover, the accumulation of hazardous substances among these metabolites may have a toxic effect on the soil microbial community, interfering with the normal functioning of the soil ecosystem and negatively impacting soil health (Xing et al. 2024).

Metabolic pathway enrichment analysis revealed that the activation of biosynthetic pathways for phenylpropanoids, flavonoids, and various amino acids in soybean plants under switched cropping was closely associated with increased plant resistance and microbial signaling (Deng and Lu 2017). The phenylpropanoid metabolic pathway and the downstream flavonoid/isoflavonoid synthesis pathway are key pathways in the plant defence system, and their enrichment may increase soybean resistance to soil-borne diseases caused by continuous cropping-related obstacles (e.g. antimicrobial activity of isoflavones) (Sharma et al. 2019). Phenylpropanoids and flavonoids have antioxidant and antimicrobial properties that help plants resist external stresses, and they also provide signaling molecules for microorganisms, which may regulate microbial community structure and function (Naoumkina et al. 2010). In addition, active amino acid metabolism may promote nitrogen metabolism and help plants adapt to soil nutrient changes after seed rotation (e.g. via increased symbiotic efficiency of nitrogen-fixing bacteria). Crop rotation of potato with soybean plants activated the biosynthesis of alkaloids derived from the shikimate pathway to some extent. As the upstream pathway for secondary metabolites

such as phenylpropanoids and flavonoids, the activation of alkaloid biosynthesis may increase plant stress resistance through mechanisms such as antioxidation and antimicrobial activity (El-Azaz and Maeda 2024; Maeda and Dudareva 2012). ABC transporter protein, galactose metabolism, starch and sucrose metabolism, and pyrimidine/nucleotide metabolism pathways were also downregulated, possibly because switching shifted the energy allocation of the plant to secondary metabolism instead of primary metabolism or altered the efficiency of carbon source utilization (Liu et al. 2023). The continuous cultivation of potatoes may help the plants adapt to environmental stresses caused by continuous cropping-related obstacles by activating the ABC transporter protein pathway, which increases the transmembrane transport of metabolites (e.g. via elimination of harmful substances or uptake of nutrients) (Do et al. 2018; Dahuja et al. 2021).

Soil Nutrient Factor—Microbe—Metabolite Interaction Network

In potato–soybean rotation cropping, symbiotic rhizobacteria (e.g. *Bradyrhizobium*) in the soybean root system improve the structure and function of the soil microbial community through nitrogen fixation and through root secretions containing amino acids and organic acids; increase the contents of soil organic matter, total nitrogen, and available nutrients; and increase the activities of enzymes involved in carbon, nitrogen, and phosphorus metabolism, such as urease (Lindström et al. 2020). The abundance of *Bradyrhizobium* was positively correlated with soil nutrient levels and enzyme activities, and in addition to nitrogen fixation, it may also be involved in the decomposition of soil organic matter, promoting the cycling of carbon, phosphorus and other nutrients (Jabborova et al. 2020). The abundances of *Pseudomonas* and *Sphingomonas* were positively correlated with the levels of lipid metabolites, flavonoids, etc., and these species are presumed to be involved in the biosynthesis of antimicrobial substances and play a role in plant immune defence (Li et al. 2021a, b). Synergistic interactions between *Bradyrhizobium* and *Pseudomonas* increase the efficiency of nutrient uptake by soybeans from the soil (Tu et al. 2021; Kumawat et al. 2019). A synergistic mechanism of ‘nutrient increase–beneficial microbial enrichment–beneficial metabolite enhancement’ is formed. In contrast, when a single variety of potato is continuously planted, the plants continuously absorb nutrients, and continuous cropping triggers an imbalance in the microbial community and a deterioration of physicochemical properties, resulting in a decrease in the total nitrogen, phosphorus, and potassium contents of the soil; the enrichment of phytopathogens belonging to the phyla Proteobacteria and Ascomycota; and the accumulation of harmful metabolites, such as lipids and heterocyclic compounds (Arie et al. 2019). The abundance of *Fusarium* was positively correlated with lipid metabolite levels; these fungi may adapt to the accumulation of harmful substances in soil by altering lipid metabolism pathways. However, their proliferation can cause plant diseases, thus intensifying continuous cropping-related obstacles (Ha et al. 2023) and forming a vicious cycle of ‘nutrient depletion–pathogenic microbial enrichment–accumulation of harmful metabolites’. Owing to the similarity of root secretions and the overlapping nutrient demand spectrum of the same family of crops,

potato varietal switching only slightly slowed the consumption of available nutrients, but the total nitrogen, phosphorus, and potassium losses could not be reversed, and the fungal community and function did not significantly change, making it difficult to disrupt the pathogenic microbial chemosensory interaction network.

Conclusions

In the potato cropping system, potato–soybean crop rotation establishes a favourable soil environment for potato growth by improving soil nutrient status, optimizing microbial communities (e.g., enriching *Bradyrhizobium*), upregulating beneficial metabolites (e.g. caffeic acid and soyasapogenol E), and activating relevant biosynthetic pathways, thereby effectively alleviating continuous cropping obstacles. In contrast, switching potato cultivar, a cropping regime more aligned with the demands of monoculture potato production, somewhat alleviates the soil shift from a *bacteria-dominated* to a *fungi-dominated* state. It also activates the shikimate pathway, together with the biosynthetic pathways of aromatic amino acids (phenylalanine and tyrosine) and phenylpropanoids, which contributes to increasing the stress resistance, metabolism and quality of potatoes themselves. This cultivar rotation model does not require modifying the potato-dominated cropping structure, meaning that it is simple to operate and strongly adaptable. Although its ability to improve soil microecology is limited because of the similar biological characteristics of the rotated cultivars, it can delay the occurrence of continuous cropping obstacles while ensuring the continuity of potato cultivation. In conclusion, this provides a more practically valuable technical approach for the green, efficient, and sustainable production of potatoes in southwestern China.

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Data Availability All the data generated and analysed during this study are included in this article (and the Supplementary File).

Declarations

Institutional Review Board Not applicable.

Consent to Participate Informed consent was obtained from all the subjects involved in the study.

Conflict of Interest The authors declare no competing interests.

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