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RESEARCH

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Legume-potato rotation affects soil physicochemical properties, enzyme activity, and rhizosphere metabolism in continuous potato cropping

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Abstract

Background Continuous cropping can reduce soil quality and affect rhizosphere metabolism, ultimately reducing crop yield. Crop rotation can mitigate the damage caused by continuous cropping, but different crop rotation patterns respond differently to soil quality and rhizosphere metabolism. We investigated the effects of different cropping patterns on soil physicochemical properties, enzyme activities, microbial quantity, and rhizosphere metabolism of continuous potato cropping based on a long-term field study from 2018 to 2022. The experiment was set up with the following three treatments: potato (*Solanum tuberosum* L.)-potato-potato-potato-potato (CK), potato-potato-potato-pea (*Pisum arvense* L.)-potato (T1), and potato-potato-potato-faba bean (*Vicia faba* L.)-potato (T2).

Results The results showed that pea-potato rotation (T1) and faba bean-potato rotation (T2) significantly improved soil physicochemical properties and microbial quantity, enhanced enzyme activity, and increased yield by 21.19% and 28.38%, respectively, compared with the continuous potato crop. Non-targeted metabolomics analysis showed that the differential metabolites of pea-potato and faba bean-potato rotation were mainly nucleotides, organic acids and derivatives, and flavonoids compared to continuous potato cropping. These differential metabolites are mainly enriched in the ABC transporter, purine metabolism, pyrimidine metabolism, and phenylalanine metabolism pathways. Combined analyses showed that legume-potato rotations improved soil physicochemical properties, enzyme activities, and microbial quantity of continuous potato cropping, ultimately increasing tuber yields. In addition, correlation analyses showed that differential metabolites significantly enriched in purine and phenylalanine metabolism (L-Tyrosine, Trans-Cinnamic acid, Guanine, and Adenine) were also strongly associated with these measurements.

Conclusions Therefore, we conclude that legume-potato rotations modulate the abundance and function of rhizosphere metabolites and significantly alter the low molecular metabolite profile of the soil under continuous potato conditions. Some of these important metabolites may play a part in the cycling of nutrients in the soil, making its physicochemical properties and microbial quantity better, raising the activity of soil enzymes, and ultimately increasing the yield of potato tubers. The above results indicate that legume-potato rotation has a positive effect on continuous potato soils. It lays a solid foundation for revealing the complex molecular network and metabolic pathways of microbial communities in soil after legume crop rotation.

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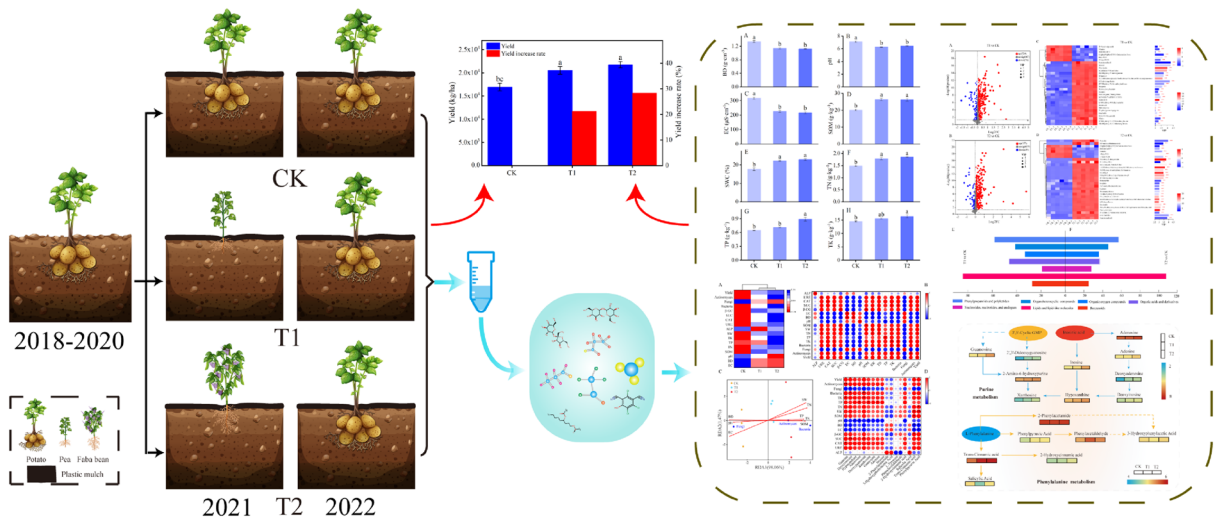
Keywords Potato, Legumes, Crop rotation, Soil physicochemical properties, Metabolomics

Graphical Abstract



Introduction

Potato (*Solanum tuberosum* L.) is an important food crop. Its production has increased dramatically in developing countries over the past two decades, where it is part of the daily diet of almost the entire world population [1]. Continuous crops can harm the soil microhabitat. Continuous potato crop succession has become one of the major threats leading to soil-borne diseases and reduced productivity, seriously affecting the sustainable and healthy development of the potato industry. Many fungicides have been used to mitigate continuous crop damage, but they disrupt agroecology and lead to various environmental problems [2]. Crop rotation attenuates the adverse effects of continuous cropping by improving soil physicochemical properties (BD, EC, SOM, TN) and increasing soil enzyme activities (SUC, URE) compared to continuous cropping. It is a safe and effective agricultural practice [3, 4].

Legume is a commonly used crop in crop rotation systems, and its main advantage is regulating soil fertility [5]. It positively affects soil microorganisms by increasing soil organic matter content and nutrient accumulation, which are utilized by the next stage of plant growth [6, 7]. Legume has a low carbon to nitrogen ratio, high proportion of metabolites and low molecular weight compounds. The turnover of particulate organic matter may fertilize legumes and efficiently form mineral-associated organic matter. They can maintain productivity while obtaining longer lasting soil organic C gains [8]. In addition, rotating legumes not only input nitrogen through biological nitrogen fixation and increase plant protein production. Rotation legumes reduce the need for nitrogen fertilizer and improves the overall nitrogen availability of the cropping system. It also reduces the cost of agrochemicals while increasing the potential yield of subsequent crops [9]. Varieties such as faba bean *(Vicia faba* L.) and pea (*Pisum sativum* L.) are highly adaptable and are essential members of the legume family. Their inclusion in the rotation sequence can increase soil organic matter content and nutrient accumulation and optimize the soil microbial community, with great ecological benefits [10]. For example, wheat in rotation with peas increased soil available N content [11]. Faba bean-potato-oat-faba bean rotation improves soil microbial community structure and reduces potato black scurf incidence [12]. The effects of different crop rotation patterns on microbial community diversity and soil ecosystem multifunctionality have been investigated [13]. However, little research has been reported on rhizosphere metabolism and its functions under different crop rotation patterns.

Rhizosphere metabolites consist of four main components: root exudates, microbial metabolites, and plant and soil organic matter decomposition, which can reflect plant and soil microbial metabolic activities [14, 15]. The plant root system is a critical tissue that connects the plant to the soil and interacts with soil materials through

released root exudates, thereby modifying the soil environment [16, 17]. Soil metabolomics enable the identification of low molecular weight organic compounds in soils and provide an important means to clarify the complex molecular networks and metabolic pathways in soils [18]. Previously, pepper rhizosphere metabolites were studied by untargeted metabolomics. 11 differential metabolites, including organic acids and sugars, and the most significantly enriched metabolic pathway (starch and sucrose metabolism) were detected [19]. *Fusarium* has also been detected to improve the soil microenvironment by affecting the abundance of metabolites such as isoflavones, phenols, and terpenoids in the roots of successive soybean crops [20]. Therefore, analyzing changes in the soil microenvironment from the perspective of rhizosphere metabolism is vital to further reveal the effect of legumes added to the rotation sequence on continuous potato rhizosphere soil. In this study, we used a non-targeted metabolomics approach to analyze the changes in rhizosphere metabolites, reveal the critical differential metabolites and pathways of legume-potato rotation in response to continuous crop, and clarify the effects of rhizosphere metabolites on soil physicochemical properties, microbial quantity, and soil enzyme activities. This study provides a theoretical basis for explaining the mechanism of legume-potato rotation to mitigate continuous crop damage.

Materials and methods

Site description

The experiment was conducted from 2018 to 2022 at the Integrated Experiment Station of Gansu Agricultural University, Dingxi City, Gansu Province (35° 33′ N, 104° 35′ E, altitude 1920 m). Its soil is loess, and the average annual radiation, temperature, precipitation, and evaporation are 592.9 kJ/cm^2, 6.4 °C, 415.2 mm, and 1531 mm, respectively. Table 1 shows the basic soil properties of the experiment site.

Experimental design

This experiment was conducted in a randomized group design with three treatments: potato-potato-potato-potato-potato (CK), potato-potato-potato-pea-potato (T1), and potato-potato-potato-faba bean-potato (T2)

Table 1 Basic soil properties

Soil depth (cm)	pH	BD (g/cm^3)	EC (μS/cm)	SOM (g/kg)	TN (g/kg)	TP (g/kg)	TK (g/kg)
0–20	7.05	1.26	329	12.3	0.96	0.44	13.56

Bulk density (BD), electrical conductivity (EC), soil organic matter (SOM), total nitrogen (TN), total phosphorus (TP), total potassium (TK)

Fig. 1 Planting schematic

(Fig. 1). Each treatment was conducted in three plots measuring 4.5 m × 8 m for nine plots. Each plot was fully fertilized with a base fertilizer of 300 kg·ha^{-1} urea (N 46%), 250 kg·ha^{-1} calcium superphosphate (P_2O_5 16%), and 200 kg·ha^{-1} potassium sulfate (K_2O 52%) before planting. Potatoes and legumes (pea and faba bean) were grown on monopolies. Potatoes were planted in 2 rows per monopoly, with plant spacing and row spacing of 28 cm and 40 cm, respectively, and a planting density of 6.4965×10^4 plants·ha^{-1}. No watering or fertilizing during the whole reproductive period.

Potato tuber yield

All potato tubers were collected from each plot, and yield was calculated with fresh tuber weight on October 2, 2022.

Soil sample collection

Soil samples were collected on August 20, 2022. Sampling was carried out by the five-point sampling method. Soil samples were collected from different locations along the diagonal, and whole soil samples containing plant roots were collected. Soil loosely bound to the rhizosphere was shaken off using the "shaking method", and the soil samples from the same plot were then homogenized and mixed. A portion of the soil was collected in sterile tubes and stored at −80 °C for subsequent untargeted metabolomic analysis. The other part was returned to the laboratory to determine soil physicochemical properties and enzyme activity.

Soil physicochemical properties

BD and EC were determined concerning the method of [21]. SOM and SWC were determined concerning the method reported by Zhang et al. [22]. Soil pH was determined using a pH meter (PB-10, Sartorius, Germany) for aqueous extracts (soil samples: deionized water = 1:5, m/v). TN, TP, and TK were determined using an elemental analyzer (Vario MACRO cube, Elementar, Germany) [23].

Soil enzyme activities

The activities of sucrase, catalase, urease and alkaline phosphatase were determined by biochemical kits provided by Shanghai Tongwei Industrial Co., Ltd (Shanghai, China). Soil β-glucosidase activity was determined by a kit provided by Beijing Solarbio Science & Technology Co., Ltd (Beijing, China).

Soil microbial quantity

Beef extract-peptone medium (pH 7.0–7.2) was chosen for soil bacterial population determination, and the concentration of soil suspension was 10^{-4}. Martin Broth was chosen for soil fungal population determination, and the concentration of soil suspension was taken as 10^{-1}. Gause's Synthetic Medium No.1 (pH 7.2–7.3) was chosen for soil actinomycetes population determination, and the concentration of soil suspension was taken as 10^{-3} for all of them. Soil bacteria, fungi, and actinomycetes were inoculated by the plate coating method. Bacteria were incubated in a thermostat at 28 °C for 3 d. Fungi were incubated in a thermostat at 25 °C for 5 d. Actinomycetes were incubated in a thermostat at 28 °C for 7 d. The soil bacteria, fungi, and actinomycetes were inoculated by plate spreading. The calculation formula is as follows:

$$\text{Microbial quantity} = \text{Mean quantity of colonies} \times \text{dilution} \times 20 \times \text{fresh soil weight/dry soil weight.}$$

Nontarget metabolomics analysis of soil samples

Sample preparation

It was possible to get the metabolites out of the soil by mixing 50 mg of the sample with 400 μL of an extraction solution (4:1 methanol to water) that had 0.02 mg ·mL^{-1} of an internal standard (L-2-chlorophenylalanine) in a centrifuge tube. Sample solutions were first ground in a frozen tissue grinder for 6 min (−10 °C, 50 Hz) and extracted by low-temperature sonication for 30 min (5 °C, 40 kHz). The extracted samples were allowed to stand for 30 min at −20 °C and then centrifuged for 15 min (4 °C, 13,000*g*). The supernatant was finally transferred to an injection vial with an internal cannula for analysis [24]. Equal volumes of all samples were taken and mixed to prepare quality control (QC) samples. The samples were injected once every 6 samples during the instrumental analysis to examine the reproducibility of the whole analytical process.

LC–MS/MS analysis

The instrument platform for LC–MS/MS analysis was an ultra-high performance liquid chromatography–tandem Fourier transform mass spectrometry UHPLC -Q Exactive system from Thermo Fisher Scientific (Shanghai Meiji Biomedical Technology Co., Ltd.). The chromatographic and mass spectrometric parameters were set according to the study by Zeng et al. [25]. The separation was performed on an HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm) with an injection volume of 3 μL, a flow rate of 0.40 mL·min^{-1}, and a column temperature of 40 °C. The mobile phase A was 95% water + 5% acetonitrile (containing 0.1% formic acid), and the mobile phase B was 47.5% acetonitrile + 47.5% isopropanol + 5% water (containing 0.1% formic acid).

Identification and analysis of metabolites

The raw LC–MS/MS data obtained above were imported into Progenesis QI metabolomics processing software (Waters Corporation, Milford, USA) for pre-processing. The mass spectrometry information was subsequently matched and analyzed by the public metabolic database HMDB (http://www.hmdb.ca/), the KEGG (https://www.kegg.jp/kegg/pathway.html), and the Meguiar's library to obtain the final data matrix for subsequent analysis [24]. The ropls package (Version 1.6.2) in the R language was used for data analysis. The selection of significantly different metabolites was determined based on the variable weight values (VIP) obtained from the orthogonal partial least squares discriminant analysis (OPLS-DA) model and the p-values from the student's *t*-test. Pathway enrichment analysis was performed using the Python package scipy.stats, and the most relevant biological pathways to the experimental treatments were obtained by Fisher [26].

Statistical analysis

Potato tuber yield, soil physicochemical properties, and enzyme activity were counted by Microsoft Excel 2019 and analyzed by ANOVA by Duncan's new complex polar difference method ($p \leq 0.05$) via SPSS 25.0 (IBM 2017, Armonk, USA) software and plotted by Origin Lab 2022 (Origin Lab Corporation, Northampton, USA). The OmicShare platform was used for RDA (Redundancy analysis) (https://www.omicshare.com/tools/).

Results

Effect of legume-potato crop rotation on yield

The legume-potato rotation significantly increased potato tuber yields. Potato yields in continuous potato (CK), pea-potato rotation (T1), and faba bean-potato rotation (T2) are shown in Fig. 2. Compared to CK, T1 and T2 yields were 21.19% and 28.38% higher, respectively.

Effect of legume-potato crop rotation on soil physicochemical properties

T1 and T2 significantly reduced BD, pH, and EC compared to CK treatment. T1 and T2 treatments SOM significantly increased by 31.09% and 30.26%, and TN significantly increased by 20.23% and 24.72%, respectively. In T2, TP and TK were significantly increased by 37.23% and 12.80%, respectively, compared to CK. In T1, TP and TK were increased, but the difference was insignificant (Fig. 3).

Effect of legume-potato crop rotation on soil enzyme activity

Legume-potato crop rotation (T1, T2) could alter soil enzyme activity in potato continuous crop soil.

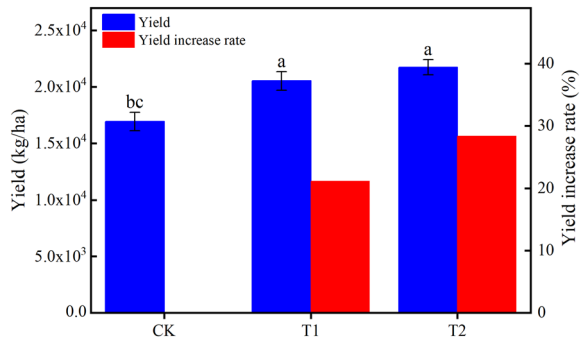


Fig. 2 Effect of legume-potato crop rotation on tuber yield. Blue represents yield, and red represents yield increase rate. Mean ($n = 3$) ± standard error of the mean, and different letters indicated differences among treatments at the statistical ($p \leq 0.05$). CK, potato-potato-potato-potato-potato; T1, potato-potato-potato-pea-potato; T2, potato-potato-potato-faba bean-potato

Compared with CK, URE activity was significantly increased by 20.53% and 25.74% in T1 and T2 treatments, respectively. SUC activity was significantly enhanced by 16.33% in T2 treatment. There was no significant change in ALP and CAT activities, while β-GC activity was significantly increased by 71.41% and 70.33% in T1 and T2 treatments, respectively (Fig. 4).

Effect of legume-potato crop rotation on microbial quantity

Legume-potato rotation had a significant effect on rhizosphere soil microorganisms. Compared to CK, the T1 treatment significantly increased the quantities of bacteria and actinomyces by 23.72% and 28.39%, respectively, and fungi were decreased by 12.72% in the T2 treatment. Bacteria and actinomyces quantities were significantly increased by 38.18% and 52.64%, respectively, and fungi were decreased by 18.1% in T2 treatment.

Effect of legume-potato crop rotation on rhizosphere metabolism

Classification of rhizosphere metabolites

To understand the taxonomy of rhizosphere metabolites under different crop rotation patterns, rhizosphere metabolites were annotated by the KEGG database and the HMDB database. The rhizosphere metabolites in the KEGG database were annotated to Lipids, Nucleic acids, Organic acids, Carbohydrates, Peptides, Hormones and transmitters, Vitamins and Cofactors, and Steroids in 8 major groups (Fig. 5A, Additional file 1: Table S1). The top 3 metabolites were Lipids, Nucleic acids, and Organic acids. Results of the HMDB database annotation showed

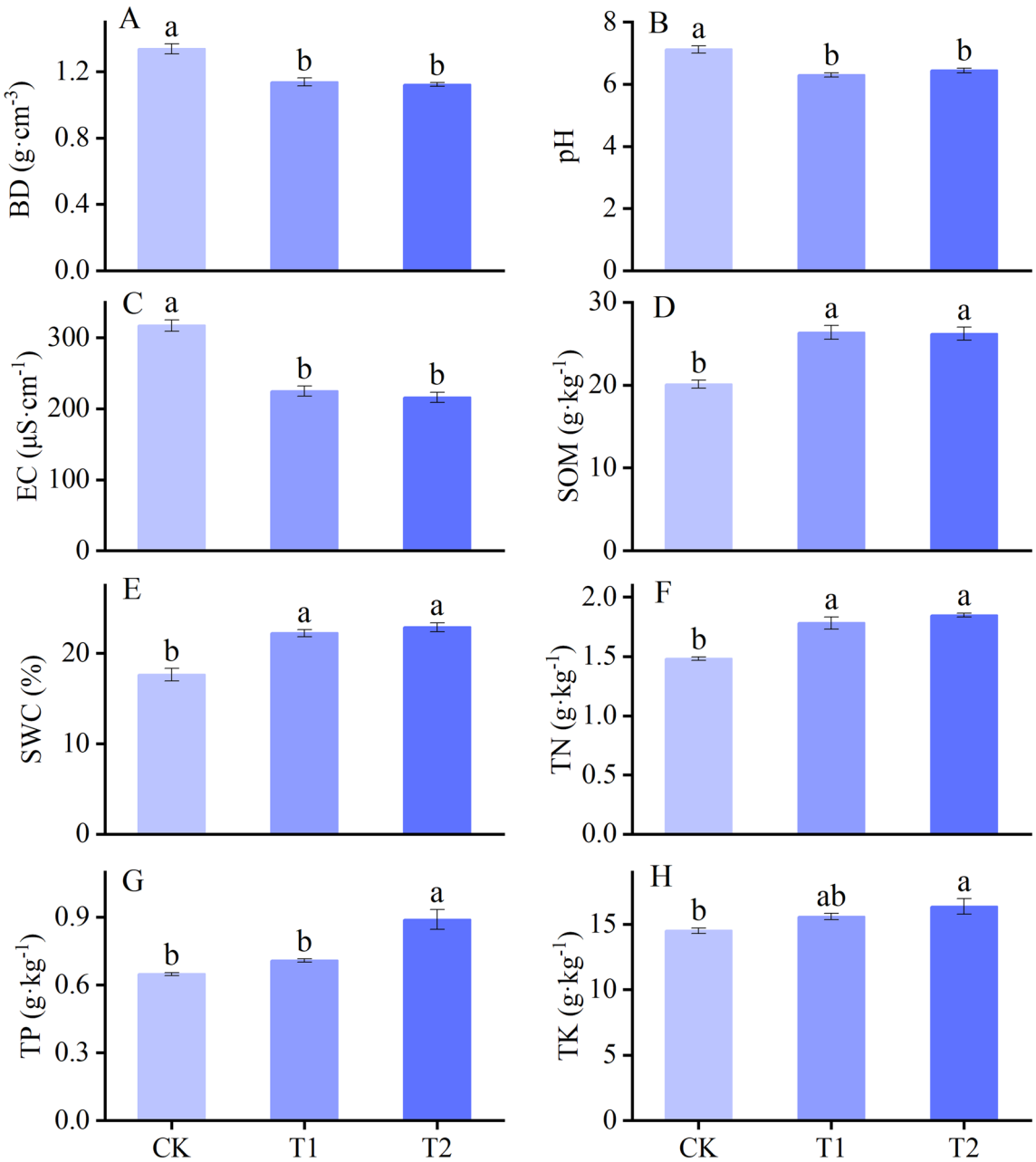


Fig. 3 Effect of legume-potato rotation on soil physicochemical properties. **A**–**H** indicate histograms of BD, pH, SOM, SWC, TN, TP, and TK, respectively. Bulk density (BD), electrical conductivity (EC), soil organic matter (SOM), soil water content (SWC), total nitrogen (TN), total phosphorus (TP), and total potassium (TK). Mean ($n = 3$) ± standard error of the mean, and different letters indicated differences among treatments at the statistical ($p \leq 0.05$). CK, potato-potato-potato-potato-potato; T1, potato-potato-potato-pea-potato; T2, potato-potato-potato-faba bean-potato

that 870 metabolites were annotated to the database in 14 major categories (Fig. 5B). Lipids and lipid-like molecules accounted for 33.91% of the total metabolites, Organo-heterocyclic compounds for 14.14%, and Organic acids and derivatives for 12.53%.

Identification and analysis of differential metabolites

OPLS-DA analyzed similarities and differences in metabolic profiles. The OPLS-DA model was subjected to 200 permutation tests (Additional file 2: Figure S1). Based on the results of the above analysis, a different volcano map was drawn. Moreover, the differential metabolites

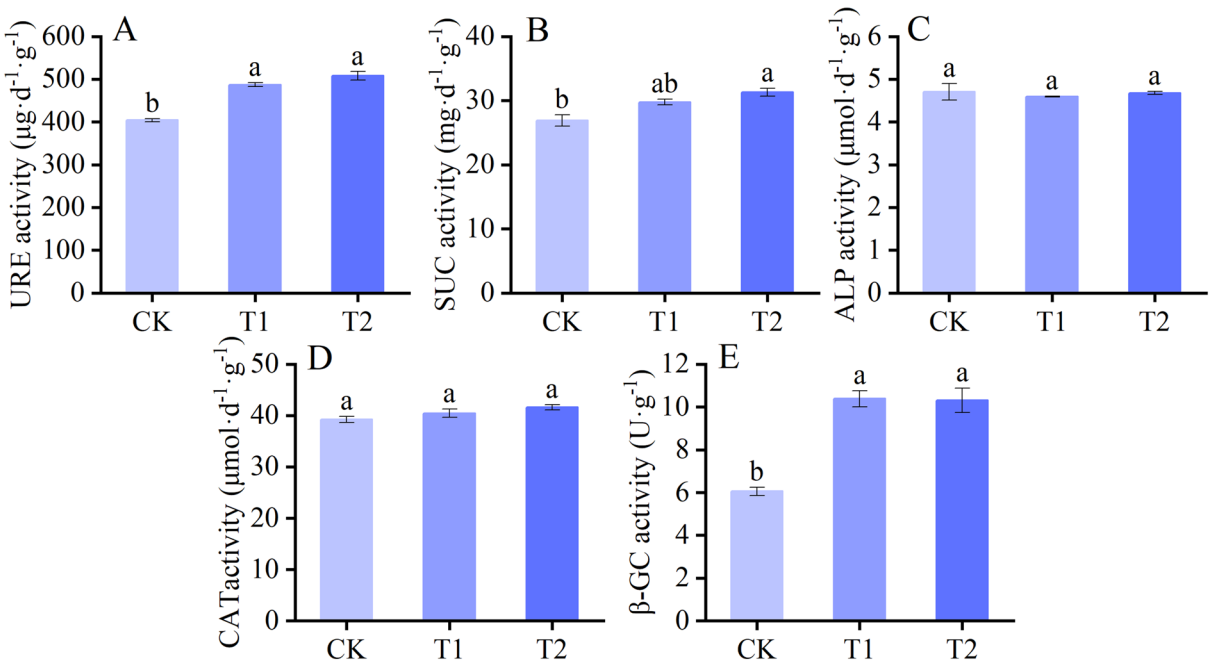


Fig. 4 Effect of legume-potato crop rotation on soil enzyme activities. **A**–**E** shows the histograms of URE, SUC, ALP, CAT, and β-GC activities. Alkaline phosphatase (ALP), Urease (URE), Catalase (CAT), Sucrase (SUC), and β-Glucosidase (β-GC). Mean ($n = 3$) ± standard error of the mean, and different letters indicated differences among treatments at the statistical ($p \leq 0.05$). CK, potato-potato-potato-potato-potato; T1, potato-potato-potato-pea-potato; T2, potato-potato-potato-faba bean-potato

were classified. A total of 423 differential metabolites were identified by T2 treatment, mainly Phenylpropanoids and polyketides (Trans-Cinnamic acid); Organoheterocyclic compounds (Cytosine); Organic acids and derivatives (L-Serine), Nucleosides, nucleotides, and analogues (Inosine). Trans-Cinnamic acid, Adenine, and 2′,3′-Dideoxyguanosine were 375 differential metabolites up-regulated, and Petasinine and Kainic acid were 48 differential metabolites down-regulated (Fig. 6A, B, E, F Additional file 1: Table S1 and Additional file 3: Table S2). VIPs in the OPLS-DA model were calculated, and metabolites were clustered and analyzed for VIP values (Top30). The results showed that 24 differential metabolites were significantly up-regulated, and 6 differential metabolitedown-regulated in abundance in T1 compared to CK. 25 differential metabolites were significantly up-regulated, and 5 differential metabolites were down-regulated in abundance in T2 (Fig. 6C and D).

Differential metabolite KEGG pathway enrichment and key metabolic pathways

Pathway enrichment analysis was performed by KEGG ID of differential metabolites to explore the potential impact of differential metabolites. Differential metabolites were significantly enriched in ABC transporters, nucleotide metabolism (Purine metabolism), amino acid metabolism (Phenylalanine metabolism), sugar metabolism (Galactose metabolism), and the synthesis of secondary metabolites (Flavone and flavonol biosynthesis, Biosynthesis of phenylpropanoids) and other metabolic pathways in T1 Compared to CK ($p \leq 0.05$) (Fig. 7A). Differential metabolites were significantly enriched in ABC transporters, nucleotide metabolism (Purine metabolism, Pyrimidine metabolism), secondary metabolite synthesis (Flavonoid biosynthesis, Flavone and flavonol biosynthesis), and other metabolic pathways in T2 ($p \leq 0.05$) (Fig. 7B). Based on the above analysis results, we identified 2 key metabolic pathways (purine metabolism, and phenylalanine metabolism) associated with continuous and rotational cropping. The expression abundance of metabolites such as 2′,3′-Dideoxyguanosine, Guanosine, Inosine, Deoxyadenosine, and Xanthosine in Purine metabolism was significantly upregulated in T1 and T2 treatments, and T2 was higher than T1. Metabolites such as Trans-Cinnamic acid, 2-Phenylacetamide, Phenylpyruvic Acid, and 2-Hydroxycinnamic acid significantly increased the Phenylalanine metabolism pathway (Fig. 7C).

Combined analysis

Clustering analysis showed that the T1 and T2 treatments clustered into one category and CK into a

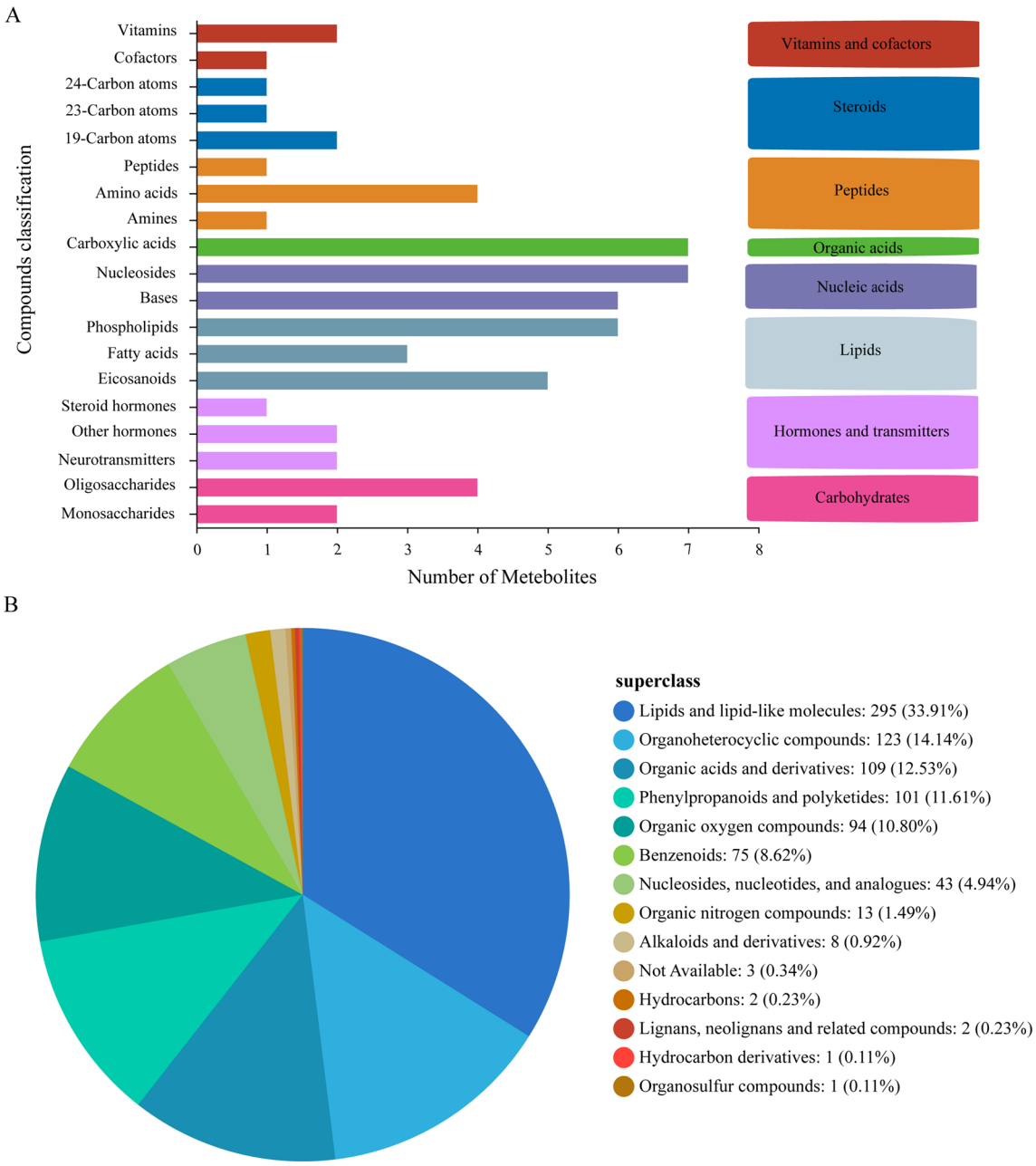


Fig. 5 Taxonomic annotation of metabolites in the rhizosphere. **A** indicates KEGG taxonomic annotation, and **B** is HMDB taxonomic annotation

separate category (Fig. 8A). Correlation and redundancy analyses showed that tuber yield was significantly positively correlated with URE and TN, but it was significantly negatively correlated with fungi. SW, TN, TP, TK, and SOM were positively correlated with bacteria and actinomycetes, while BD, pH, and EC were negatively correlated with fungi. Moreover, the explanatory rates of RDA1 and RDA2 were 98.06% and 1.47%, respectively, and the cumulative explanatory rate was 99.53% (Fig. 8B and C). We correlated the differential metabolites in the purine and phenylalanine metabolism pathways with soil physicochemical properties, enzyme activity, microbial quantity, and tuber yield. The results showed that most differential metabolites in these two pathways were negatively correlated with pH, BD, EC, and fungi. Among them, Trans-Cinnamic acid showed a highly significant negative correlation

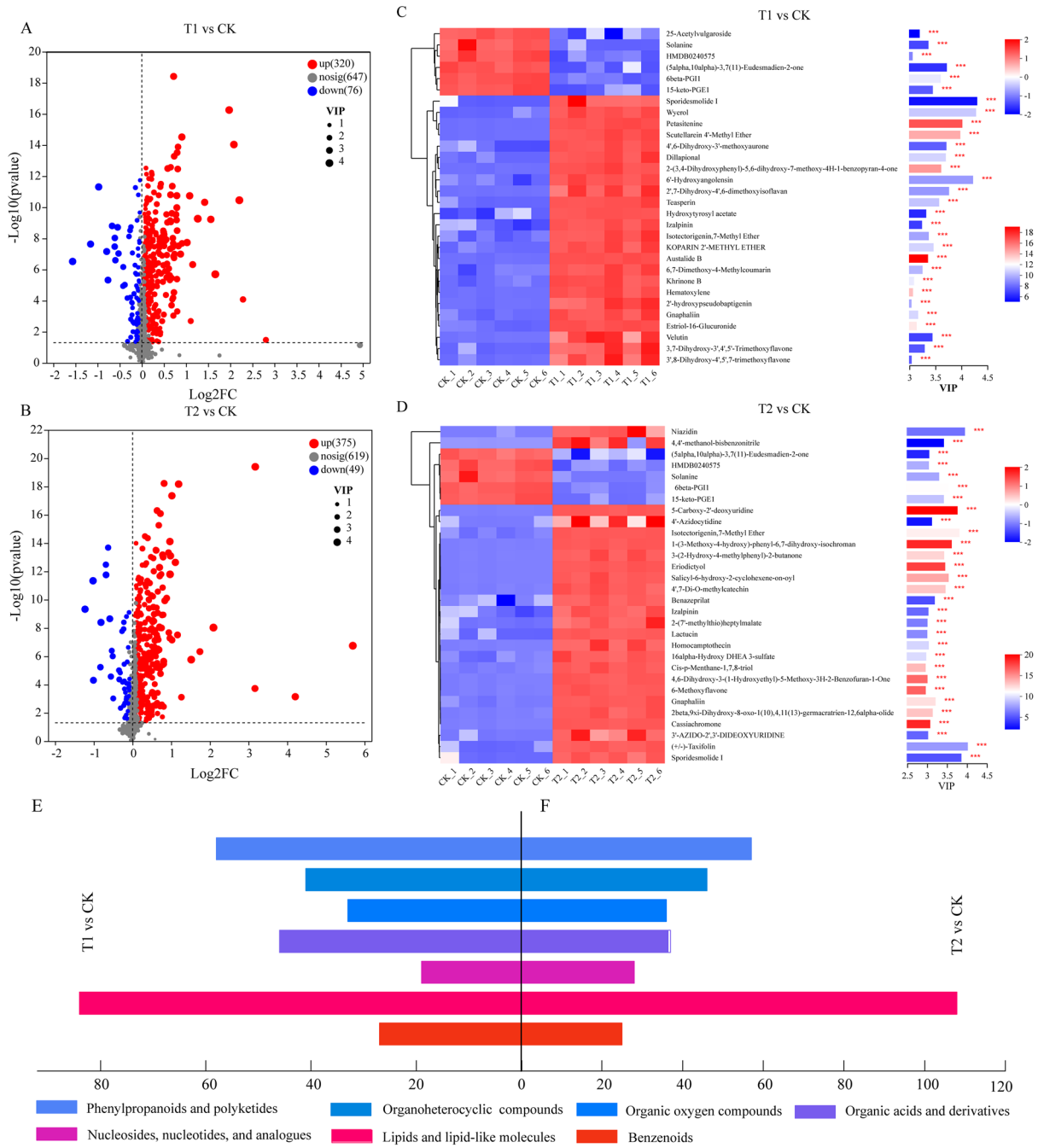


Fig. 6 Identification and analysis of differential metabolites. **A** and **B** are up- and down-regulated differential volcano plots of T1vsCK and T2vsCK differential metabolites, respectively. **C** and **D** are the T1vsCK and T2vsCK differential metabolite cluster analysis and VIP worth score plots (Top30), respectively. On the left side is the plot of differential metabolite cluster analysis, and the color indicates the size of the relative expression of that metabolite in that group of samples. On the right side is the VIP bar graph of differential metabolites. Bar lengths indicate the contribution of that metabolite to the difference between the two groups, and values indicate the mean ± standard deviation of six individual tests. * Represents $p \leq 0.05$, ** represents $p \leq 0.01$, and *** represents $p \leq 0.001$. **E** and **F** represent classification maps for T1vsCK and T2vsCK differential metabolites, respectively. CK, potato-potato-potato-potato-potato; T1, potato-potato-potato-pea-potato; T2, potato-potato-potato-faba bean-potato

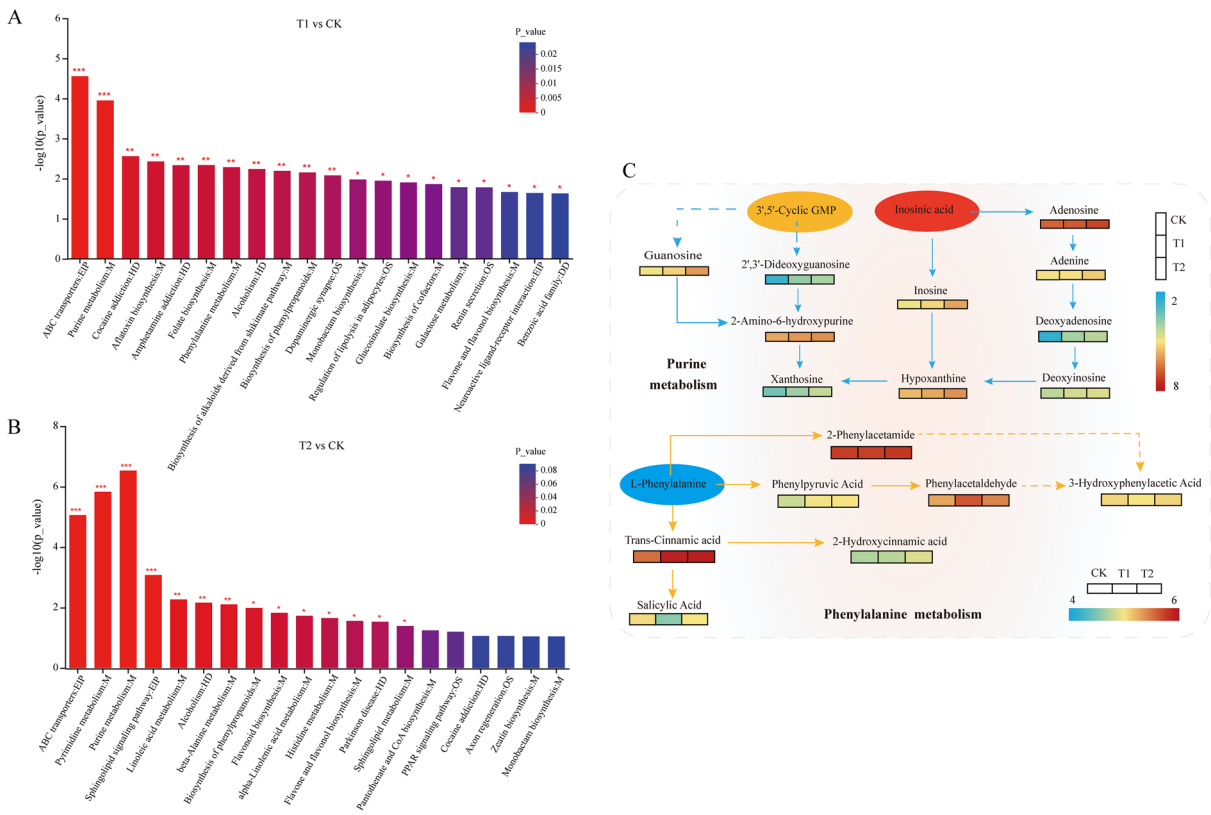


Fig. 7 KEGG pathway enrichment and important metabolic pathways for differential metabolites. **A** and **B** are KEGG pathway enrichment maps of T1vsCK and T2vsCK differential metabolites, respectively. **C** shows the screened key enrichment pathways (purine metabolism, phenylalanine metabolism). CK, potato-potato-potato-potato-potato; T1, potato-potato-potato-pea-potato; T2, potato-potato-potato-faba bean-potato

with BD. Positive correlations were found with Yield, TN, TP, TK, SWC, and SUC. Deoxyinosine showed a highly significant positive correlation with SUC, and Phenylpyruvic acid showed a highly significant positive correlation with SWC. Guanine showed a highly significant positive correlation with actinomycetes (Fig. 8D).

Discussion

Continuous cropping leads to the deterioration of the soil environment and a decrease in plant yield, while crop rotation can increase crop yield [27]. In the present study, legume-potato rotation significantly increased potato tuber yield (Fig. 2), consistent with the previous findings. Including legumes in crop rotation sequences increases maize and wheat yields [28, 29]. Changes in soil physicochemical properties in agricultural production can directly affect crop growth conditions [30]. Crop rotation improves soil fertility and nutrient use efficiency due to increased soil microbial biomass, soil carbon and nitrogen availability, and plant uptake capacity [31]. The legume-potato rotation significantly reduced EC and pH, effectively delaying soil salinization. It also reduced BD and increased SWC and SOM, which may further trigger the transfer and accumulation of soil nutrients, thereby enhancing organic carbon accumulation and nutrient bioavailability. The broad bean-potato rotation (T2) also significantly increased the soil total N, total P, and total K content. They increase root biomass N return to the soil and provide sufficient nutrients for subsequent crops (Fig. 3) [32]. Crop rotation can increase soil enzyme activity to alter nutrient cycling and energy metabolism [33]. In this study, legume-potato rotation significantly increased URE activity and SUC activity (Fig. 4), and URE can rapidly catalyze urea hydrolysis, promote N cycling, and increase soil N content [34]. SUC catalyzes the release of fructose and glucose from sucrose in the soil, improving soil organic matter content and enriching the soil carbon pool. Adequate carbon sources are provided for later crops and microorganisms [35]. And the increase in β-GC activity can be directly reflected in crop yield [36]. In conclusion, our results demonstrate that legume-potato rotation can improve the physicochemical properties, soil enzyme activity, and tuber yield of potato continuous cropping soils.

Soil microorganisms play a crucial role in agroecosystems and are important determinants of plant health and

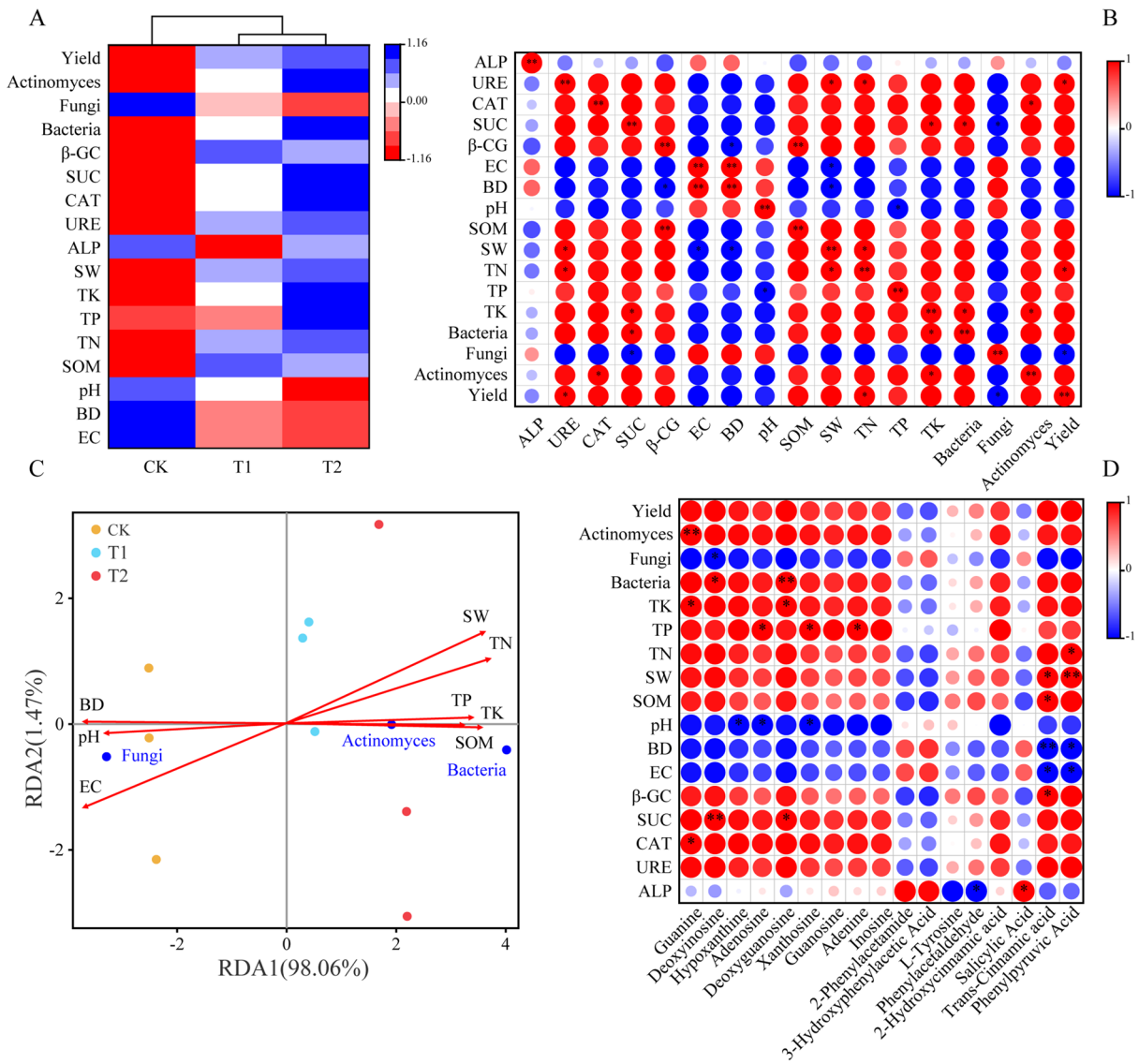


Fig. 8 Combined analysis. **A** is the cluster analysis of different treatments with soil physicochemical properties, enzyme activity, microbial quantity, and yield. **B** is the correlation among the index. **C** is the redundancy analysis of soil microorganisms with soil environmental indicators. **D** is the correlation analysis of soil physicochemical properties, enzyme activity, and yield with differential metabolites enriched in important pathways. * Represents $p \leq 0.05$, ** represents $p \leq 0.01$. CK, potato-potato-potato-potato-potato; T1, potato-potato-potato-pea-potato; T2, potato-potato-potato-faba bean-potato

Table 2 Effect of legume-potato rotation on microbial quantity

Treatment	Bacteria ($\times 10^5$ CFU·g^{-1})	Fungi ($\times 10^5$ CFU·g^{-1})	Actinomyces ($\times 10^5$ CFU·g^{-1})
CK	47.19 ± 0.42c	81.28 ± 0.42a	16.85 ± 0.52c
T1	58.39 ± 0.57b	70.94 ± 0.19b	21.63 ± 0.23b
T2	65.21 ± 1.56a	66.57 ± 0.39c	25.72 ± 0.22a

Mean ($n = 3$) ± standard error of the mean, and different letters indicated differences among treatments at the statistical ($p \leq 0.05$). CK, potato-potato-potato-potato-potato; T1, potato-potato-potato-pea-potato; T2, potato-potato-potato- faba bean-potato

productivity [37]. Previous studies have shown that soil improvement by crop rotation is associated with changes in soil microbial community structure [38]. In the present study, we found that legume-potato rotation was able to increase the quantity of bacteria and actinomycetes and decrease the quantity of fungi (Table 2). It is consistent with the results of previous studies that the quantity of bacteria in the soil of crop rotation was higher than that in the soil of continuous cropping. The diversity of soil bacterial communities increased under the crop rotation system, while the diversity of fungal communities decreased significantly, possibly related to reduced soil acidity [39–41]. Changes in bacterial and fungal communities may also be due to differences in the accumulation of crop rhizosphere metabolites and residues in the soil environment under different cropping patterns. This is because rhizosphere metabolites and residues can influence the structure of microbial communities by providing different nutrients to microorganisms [13]. In addition, the abundance of actinomycetes in the soybean-corn rotation was significantly higher than that in the continuous corn crop, reducing plant pests and diseases [42, 43]. It has also been found that rotating legumes at least once can directly affect soil microbial communities. Such systems also tend to have higher microbial biomass and activity [44]. And the specific role of legume incorporation in determining soil microbial diversity and function requires further research in the future.

Metabolomics can reveal changes in substances in soil and is an essential tool for exploring soil quality [45]. In this study, 870 metabolites were detected, among which Lipids and lipid-like molecules accounted for the highest percentage (33.91%), followed by Organic acids and derivatives (14.14%), Organoheterocyclic compounds (12.53%) and Organic oxygen compounds (11.61%), etc. (Fig. 5), which is similar to previous findings that rhizosphere metabolites mainly include sugars, organic acids, and amino acids [46, 47]. The composition of soil metabolites is the same under different soil conditions, but the abundance of each component is different.

Rhizosphere metabolites play a crucial role in the interroot soil environment. They can influence soil physicochemical properties and soil microorganisms [48]. We found that differential metabolites in the legume-potato rotation treatments (T1 and T2) were mainly nucleotides, lipids, and organic acids (Fig. 6E and F), indicating that carbon and nitrogen metabolism were altered in the rotation treatments. Similar metabolites have been found in previous studies on crop rotation and mulching patterns [49, 50]. Carbohydrates are closely related to carbon metabolism, and pea-potato rotation increased Sucrose content and decreased Lactose and Stachyose content in the soil metabolites of potato continuous crop fields. In contrast, faba bean-potato rotation increased Lactose and Stachyose content and decreased Sucrose content (Additional file 3: Table S2). This differs from previous findings and could mean that plants recruit inter-root-specific microbiota by secreting metabolites. And there are differences in carbon source preferences among different microbiota, which affect sugar metabolism [51, 52]. Lipids, as an essential compound, assimilate many chemicals (e.g., organic acids) when synthesized, which may be responsible for the significant decrease in soil organic acids in rice under crop rotation treatment [53]. In contrast, the increased abundance of soil organic acids under the crop rotation treatment in this study contradicts this, which may be due to the different crop species grown. Flavonoids are a class of secondary metabolites produced from small molecules. These flavonoids can be recognized by receptors on the cell surface of the rhizobium, thereby inducing the rhizobium to synthesize factors [54]. In the present study, a large number of flavonoids were found. The levels of Flavone and flavonol biosynthesis intermediate metabolites such as Kaempferol-3-*O*-rutinoside, Chrysoeriol, and Laricitrin were up-regulated in the T2 treatment (faba bean-potato rotation) (Additional file 3: Table S2). They may act as modulation signals for rhizobia, influence rhizome development and nitrogen fixation, and participate in legume-microbe interactions [55]. Taxifolin, Liquiritigenin, and Sakuranetin were up-regulated in Flavonoid biosynthesis (Additional file 3: Table S2). Flavonoid compounds were also found to play an essential role in rhizosphere microbial nitrogen fixation in studies on maize [56] and alfalfa [57]. Organic acids are a class of acidic compounds secreted by plants through the root system into the interroot soil [58]. Pea-potato rotation (T1) enhanced the content of organic acids such as *P*-Anisic Acid, 2,4,6-Trihydroxybenzoic acid, and 2-(8-hydroxyethyl)-6-Methoxybenzoic Acid (Additional file 3: Table S2). Previous studies have found that the content of organic acids in plant rhizosphere metabolites increases under abiotic stress conditions, and plant stress tolerance is enhanced [59, 60]. Therefore, we hypothesize that legume-potato crop rotation mitigates the adverse effects of continuous crops by altering the abundance of some metabolites.

In recent years, it has been found that plants can resist stress through some specific mechanisms [61]. In the present study, pea-potato rotation (T1) significantly affected ABC transporters, Purine metabolism, Phenylalanine metabolism, Flavone and flavonol biosynthesis, and other metabolic pathways. Faba bean-potato rotation (T2) significantly affected the metabolic pathways such as ABC transporters, Purine metabolism, Pyrimidine metabolism, Flavonoid biosynthesis, Flavone and flavonol biosynthesis (Fig. 7A and B). ABC transporter proteins

have been found to be involved in the biodegradation and transport of a variety of secondary metabolites [62, 63]. Our study found that the abundance of rhizosphere metabolites of ABC transporter metabolites such as L-Serine, Guanosine, Inosine, and deoxyadenosine was upregulated in T1 and T2 treatments (Additional file 3: Table S2). It indicates that legume-potato rotation may enhance the transport of secondary metabolites by ABC transporters and stimulate plants to adapt to the soil environment through specific plant cell membrane export or import of metabolites [64]. Phenylalanine is an aromatic amino acid that is involved in the synthesis of a variety of proteins and secondary metabolites. Legume-potato rotation affected phenylalanine biosynthesis by up-regulating Phenylalanine metabolism intermediates (Fig. 7C, Additional file 3: Table S2), while changes in phenylalanine metabolic pathways indicated that its defense was activated [26]. Purine metabolism products such as Xanthosine may promote plant growth. Elevated levels of Adenine nucleotide acid (AMP) and Guanine nucleotide (GMP) can accelerate metabolism and produce more Adenosine triphosphate (ATP) and GTP [65, 66]. In the present study, 10 differential metabolites were significantly enriched in Purine metabolism in the faba bean-potato rotation treatment. Guanine, Adenosine, and Xanthosine metabolites were significantly up-regulated (Fig. 7, Additional file 3: Table S2). Purine metabolism is promoted to increase the amount of adenine and adenosine excreted by the root system into the inter-root soil, which may be a strategy for the plant to reallocate energy and resources to other related processes [67].

Cluster analysis and correlation analysis were carried out for soil physicochemical properties, soil enzyme activities, microbial quantity, and tuber yield of different treatments. The results showed that legume-potato rotation could improve the soil physicochemical properties, microbial quantity, and enzyme activity of continuous potato cropping, ultimately increasing the yield (Fig. 8A and B). Redundancy analysis showed that soil physicochemical properties can affect soil microorganisms, and SW, TN, TP, TK, and SOM were positively correlated with bacteria and actinomycetes. BD, pH, and EC were positively correlated with fungi (Fig. 8C). Correlations were analyzed between soil physicochemical properties, soil enzyme activity, and tuber yield, with differential metabolites significantly enriched in purine and phenylalanine metabolism (Fig. 8D). The results demonstrate that differential metabolites correlate with soil physicochemical properties, enzyme activity, and tuber yield. It is suggested that legume-potato crop rotation improves soil quality and microorganism microflora by optimizing the abundance and function of rhizosphere metabolites, attenuating the harmful effects of continuous potato cropping on the soil environment.

Conclusion

In conclusion, pea-potato and faba bean-potato rotations significantly increased SOM, SWC, and TN and decreased BD, pH, and EC. SUC, URE, and β-GC activities were also significantly increased. It also significantly increased the quantity of bacteria and actinomycetes and decreased the quantity of fungi. In addition, the legume-potato rotation optimized the composition and abundance of rhizosphere metabolites, altered the content of organic acids and derivatives, flavonoids, and lipids in the soil, and improved nucleotide, amino acid, and energy metabolism. Ultimately, potato tuber yield was increased.

Abbreviations

BD	Bulk density
EC	Electrical conductivity
SOM	Soil organic matter
TN	Total nitrogen
TP	Total phosphorus
TK	Total potassium
QC	Quality control
SUC	Sucrase
CAT	Catalase
URE	Urease
ALP	Alkaline phosphatase
β-GC	β-Glucosidase
LC–MS/MS	Liquid chromatography-tandem mass spectrometry
HMDB	Human Metabolome Database
KEGG	Kyoto Encyclopedia of Genes and Genomes
OPLS-DA	Orthogonal Partial Least Squares-Discriminant Analysis
VIP	Variable weight values
p	*P*-Value
ANOVA	One-way analysis of variance
RDA	Redundancy analysis

Supplementary Information

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Additional file 1: Table S1. Notes on the classification of soil metabolites (part).

Additional file 2: Figure S1. A-D are OPLS-DA analysis and permutation testing plots for positive and negative ions in the T1vsCK group, respectively. E–H are OPLS-DA analysis and permutation testing plots for positive and negative ions in the T2vsCK group, respectively.

Additional file 3: Table S2. Differential metabolites (part).

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Author contributions

YW contributed to the design of experiments and manuscript writing. MS and RZ reviewed data curation and writing. YK was involved in revising the manuscript. WZ and YL contributed to the investigation. DS and XW prepared Figs. 1, 2, 3, 4. SQ provided fund acquisition. All authors read and approved the final manuscript.

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Availability of data and materials
All data obtained from the current study are available from the corresponding author on a reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All co-authors have seen and agreed on the contents of the manuscript, and there is no financial interest to report.

Competing interests
The authors declare there are no conflicts of interests.

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