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Effects of plastic film mulching and legume rotation on soil nutrients and microbial communities in the Loess Plateau of China

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Abstract

Background Potato (*Solanum tuberosum* L.) continuous cropping causes the decrease of tuber yield, deterioration of quality and soil degradation in the semi-arid area. These negative effects can generally be mitigated by legume rotation and mulching. However, little is known about how can mulching and legume rotation alleviate the above damage through altering soil environment.

Methods A field experiment was conducted to investigate changes in soil properties and microbial community in response to legume rotation and mulching under six planting patterns: potato continuous cropping without film mulching (PC), potato continuous cropping with film mulching (PCF), potato–broad bean rotation without film mulching (R1), potato–broad bean rotation with film mulching (R1F), potato–pea rotation without film mulching (R2) and potato–pea rotation with film mulching (R2F).

Results Compared with the PC, the R1F and R2F had significantly enhanced the contents of alkaline nitrogen (AN), available phosphorus (AP), available potassium (AK), total carbon (TC) and total nitrogen (TN), but reduced soil pH and electrical conductivity (EC). The Shannon index of fungi in R1F and R2 was significantly higher than other treatments. The dominant bacterial and fungal phyla of each treatment was Proteobacteria and Ascomycota. R1, R1F, R2 and R2F enhanced the relative abundance of metabolic fungi and altered key differential microbial species. Soil EC, AN and AK were major factors influencing the soil bacterial and fungal communities.

Conclusion Overall, the study demonstrated that potato-broad bean/pea rotation with mulching can be adopted as the preferred cropping systems to alleviate potato continuous cropping obstacles through enhancing soil fertility and regulating soil microbial communities in the semi-arid of Loess Plateau, China.

Keywords Potato continuous cropping, Legume rotation, Soil properties, Soil microbial community

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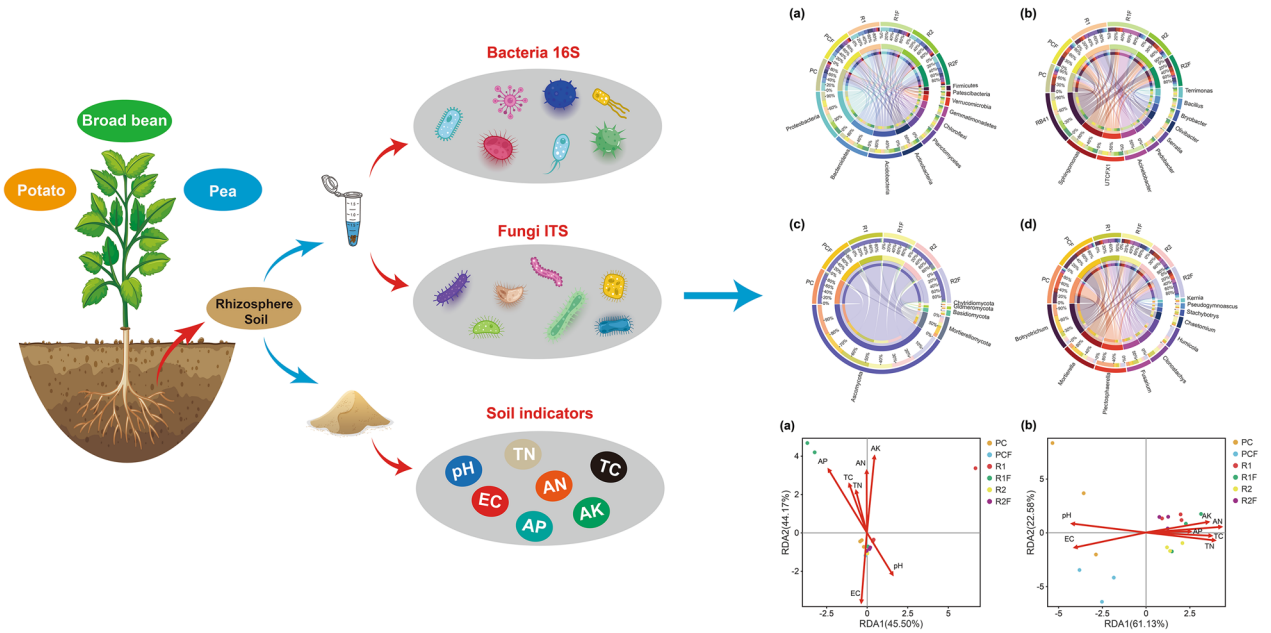
Graphical Abstract



Introduction

Potato (*Solanum tuberosum* L.) is a characteristic and dominant crop in the semi-arid region of the Western Loess Plateau, known for its excellent quality [1, 2]. In recent years, with the application of furrow and mulching technology and corresponding planting machinery, the potato cultivation area in the region has been expanding [2]. Potato cultivation has become the primary means for local farmers to increase their income. Continuous potato cultivation is the most common cropping system because of the constraints of arable land availability and the relatively small variety of crops that can be grown [3, 4]. Although continuous cropping benefits the full utilization of climatic resources and adaptation to market needs, the approach has a significant negative impact on crop growth and the soil environment [5]. Continuous cropping of potato leads to a significant reduction in tuber yield and quality [4, 6]. In addition, continuous cropping of potato causes soil nutrient imbalance, microbial community dysbiosis, self-toxicity, and crop disorders, which increase with the number of years of the continuous crop [4, 7].

As a most important and effective planting pattern to relieve the occurrence of obstacles to continuous cropping, crop rotation is an essential biological measure to optimize land use, maintain soil health, and improve crop productivity [8]. Legumes form nitrogen-fixing symbioses with soil bacteria, are considered to be preferred for rotation with other crops [9, 10]. Rotation of legumes with other crops is beneficial for the regulation of soil fertility and effective improvement of the physicochemical properties of the soil [11, 12]. Such changes are vital for improvement of crop productivity and maintenance of soil health [6, 13]. The semi-arid region of Longzhong is located in the western portion of the Loess Plateau, which is arid and receives little rainfall, and has a fragile ecology subject to severe soil erosion [13]. In addition to potato, broad bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) are suitable crops in this region, and are commonly used in crop rotation with other crops to avoid the occurrence of continuous cropping [11, 14]. Therefore, the implementation of legume crop rotation significantly increases crop productivity and enhances the soil environment.

As an essential component of agroecosystems, soil microorganisms are sensitive to the soil environment [15]. Planting practices, soil aeration, hydrothermal conditions, and organic matter (OM) content can directly affect the species diversity and abundance of soil microorganisms [16–18]. In the semi-arid region of the western Loess Plateau, potato is mainly planted on ridges with a black plastic film mulch at present, which significantly improves the microecology of the root zone and the soil hydrothermal properties [1, 19]. Qin et al. [3] observed that mulching significantly affected the soil microbial community abundance, structure, and soil enzyme activity compared with non-mulching.

To date, studies on changes in soil nutrient status, enzyme activities, and soilborne diseases have been reported for the rotation of potato with non-leguminous crops [20–22]. The improvement of soil bulk structure and soil nutrient content under legumes in rotation with other crops has also been reported, for example, Oliveira et al. [23] found that legume crops in rotation improved soil aggregation and organic carbon in the short term. However, due to the complex composition of soil microbial communities, the mechanisms by which legume-potato rotations lead to changes in soil microbial communities are still poorly understood. Therefore, studying the changes in soil fertility and microorganisms under legume-potato rotation conditions provides a basis for sustainable potato production in the semi-arid region of the Loess Plateau in China.

In the present study, a field trial was conducted on a three-year potato continuous cropping field. Our objectives were (i) to investigate the response of soil microbial community composition to different rotation sequences; and (ii) to uncover the main soil parameters driving changes in soil microbial community composition in different cropping sequences.

Materials and methods

Study site

The experiment was conducted from April to October, 2021, at the Dingxi Experimental Station of Gansu Agricultural University (104.35′E, 35.33′N, 1920 m above sea level). Using the experimental field established in 2018, potato tubers were continuously planted for 3 years in 2018–2020 by ridge planting without film mulching. In 2021, some plots continued to produce potato, and the remainder were planted in rotation with broad bean or pea. The area is located in the western portion of the Loess Plateau, a typical semi-arid rain-fed agricultural area. The average annual radiation is 592.9 kJ/cm^2, the average annual temperature is 6.4 ℃, the average annual precipitation is 415.2 mm, the precipitation during the whole production period is 340.0 mm (April to October), and the annual evaporation is 1531 mm. The soil type is loess, which is suitable for potato cultivation. The primary nutrient contents at the study site in the 0–20 cm soil layer are summarized in Additional file 1: Table S1.

Experimental design

The study was conducted in a randomized group design with six treatments: potato continuous cropping without film mulching (PC), potato continuous cropping with film mulching (PCF), potato–broad bean rotation without film mulching (R1), potato–broad bean rotation with film mulching (R1F), potato–pea rotation without film mulching (R2) and potato–pea rotation with film mulching (R2F). Three replications were applied per treatment to a total of 18 plots. Each plot comprised an area of 36 m^2 (4.5 m × 8 m). The plots were spaced 40 cm apart with a 100 cm protective row around the experimental area.

The planting date was April 12, 2021, for broad bean and pea and April 28, 2021, for potato. All plants were planted on ridges of width 1.1 m using a dibbler. Broad bean plants were grown in three rows on each ridge, with row spacing of 23 cm and plant spacing of 15 cm, and a planting density of 10,106 plants/667 m^2. Pea plants were grown in four rows on each ridge, with row spacing of 17 cm and plant spacing of 4 cm, and a planting density of 60,636 plants/667 m^2. Potato plants were grown in two rows per ridge, with inter-row spacing of 40 cm and intra-row spacing of 28 cm, with a planting density of 4331 plants/667m^2 (Fig. 1). The broad bean and pea crops were harvested on July 23, and potato was harvested on October 15.

Each treatment was supplied with 300 kg ha^{-1} of urea (46% N), 250 kg ha^{-1} of calcium superphosphate (16% P_2O_5), and 200 kg ha^{-1} of potassium sulfate (52% K_2O) before planting. All fertilizers were applied before mulching, with no fertilizer and no irrigation supplied during the growth period. Other field management practices were consistent with the standard methods of the Academy of Agricultural Sciences. The cultivars used in the experiment were potato 'Dingshu No. 6', broad bean 'Qingcan No. 13', and pea 'Dingwan No. 10'.

Soil sample collection and processing

On July 28, 2021 (the harvest period for broad bean and pea and the tuber expansion period of potato), five plants were randomly selected from each plot. The plant roots were excavated intact with a shovel and large clumps of soil around the roots were removed. The soil in the rhizosphere was collected by shaking the roots. The five samples per plot were thoroughly mixed and passed through a 2 mm sieve, and any impurities (e.g. plant residues and stones) were removed. The soil samples were divided into

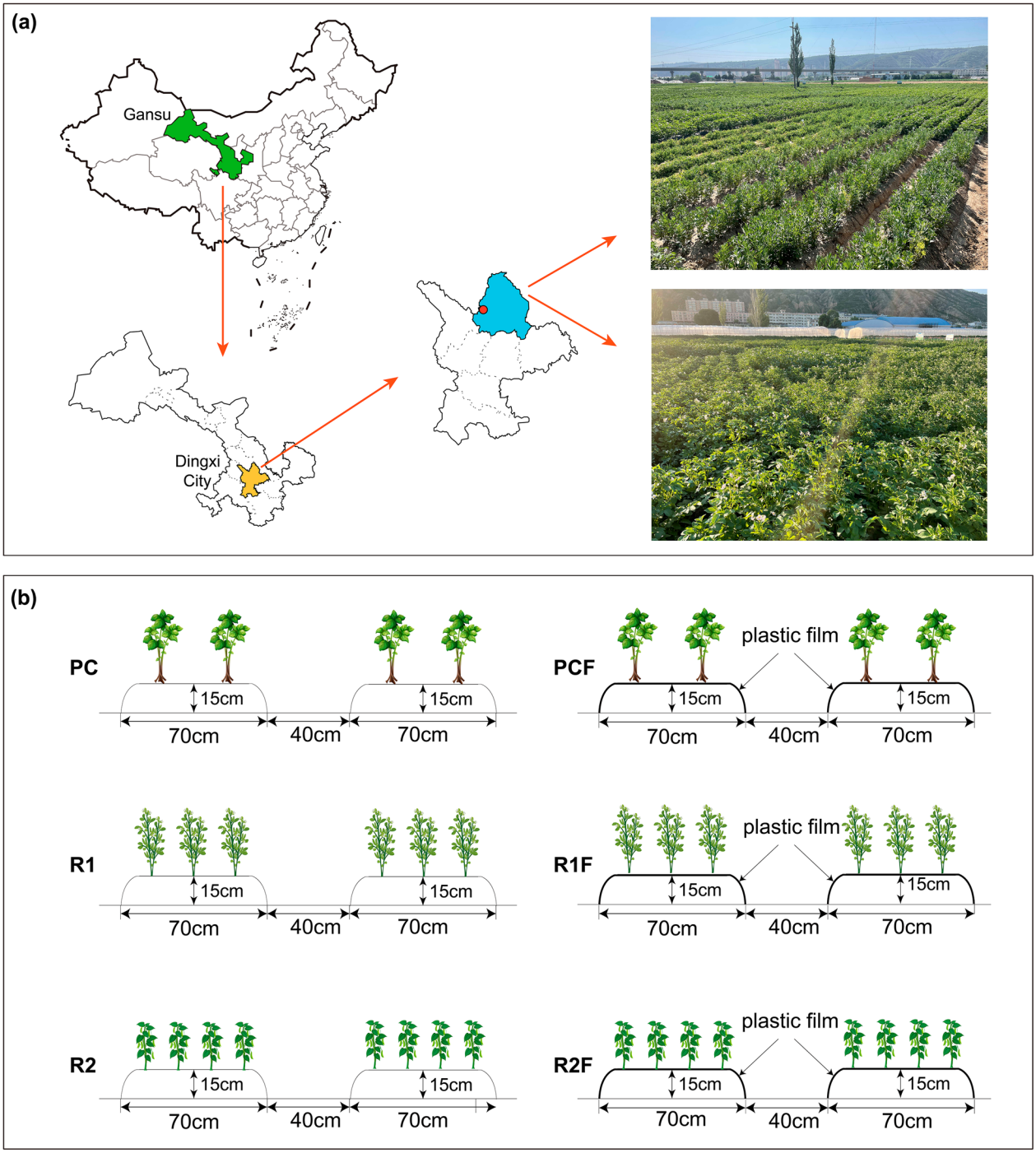


Fig. 1 Geographic location of the study site **a** and experimental design **b**. PC, potato continuous cropping without film mulching; PCF, potato continuous cropping with film mulching; R1, potato–broad bean rotation without film mulching; R1F, potato–broad bean rotation with film mulching; R2, potato–pea rotation without film mulching; R2F, potato–pea rotation with film mulching

two portions: one portion was immediately snap-frozen with liquid nitrogen, placed in an ice box, transported to the laboratory, and stored in a − 80 ℃ refrigerator for determination of the diversity of the soil microbial community. The other portion was stored at room temperature in a sterilization bag and transported to the laboratory. After air-drying, the soil was ground and passed through a 1 mm sieve for determination of the soil

properties. During the sampling process, the shovel was sterilized after collection of each sample to avoid contamination between treatments.

Soil physical and chemical properties

The soil pH was determined at the soil-to-water ratio of 1:2.5 using a PHS-3E acidity meter (Leici, Shanghai, China). Electrical conductivity (EC) was measured directly using a DDSJ-308A meter (Leici). The alkali nitrogen (AN) was detected using the alkali hydrolyzed diffusion method [13]. The method of Han et al. [24] was used for determination of available phosphorus (AP) and available potassium (AK). Soil total carbon (TC) and total nitrogen (TN) contents were determined using an elemental analyzer (Vario EL, Elementar, Hanau, Germany).

Diversity of the soil microbial community

Total soil DNA was extracted from the frozen samples using HiPure Soil DNA Kits (Magen, Guangzhou, China). The *16S* rRNA gene of bacteria was amplified using the primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACHVGGGTATCTAAT-3′) in the V3–V4 region. The amplification steps were as follows: predenaturation at 95 °C for 5 min, followed by 12 cycles of denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. The internal transcribed spacer 1 (ITS1) rDNA region of fungi was amplified using the primers ITS1-F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′). The amplification procedure was identical to that used for *16S* rRNA. All PCR reactions were performed in a triplicate in a 50 μL mixture containing 10 μL of 5 × Q5® Reaction Buffer, 10 μL of 5 × Q5® High GC Enhancer, 1.5 μL of 2.5 mM dNTPs, 1.5 μL of each primer (10 μM), 0.2 μL of Q5® High-Fidelity DNA Polymerase, and 50 ng template DNA. Relevan PCR reagents were obtained from New England Biolabs (Ipswich, MA, USA).

Amplicons were purified from 2% agarose gels using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) in accordance with the manufacturer's instructions and quantified using an ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, CA, USA). The purified amplicons were pooled in equimolar ratios and paired-end sequencing (PE250) was performed on an Illumina platform in accordance with standard protocols.

The raw reads were transformed by base-calling analysis into sequenced reads, which contained the sequence information of the reads and their corresponding sequencing quality information. Using FLASH (version 1.2.11) software, the reads from each sample were spliced by overlap and the resulting spliced sequences were the raw tags. The spliced raw tags were filtered using QIIME (version 1.9.1) to obtain clean tags. Chimeric sequences were identified and removed using UPARSE (version 7.1) software to obtain effective tags. The optimized clean tags were clustered into operational taxonomic units (OTUs) with 97% sequence similarity. Representative OTU sequences were classified as organisms based on public databases (bacteria: SILVA database, fungi: UNITE database) with the RDP classifier (version 2.2) using a plain Bayesian model with a confidence threshold value of 0.8. The raw data for the bacterial and fungi sequences were deposited in the National Center for Biotechnology Information (NCBI) database under the accession number PRJNA869187.

Statistical analysis

The effects of film mulching, crop rotation, and film mulching × rotation on soil properties were analyzed by two-way analysis of variance (ANOVA) using IBM SPSS Statistics 19.0 (IBM Corporation, Armonk, NY, USA). Significant differences ($p < 0.05$) between individual means were analyzed using Duncan's multiple comparison method. One-way ANOVA and Duncan's multiple extreme difference test ($p < 0.05$) were used to assess the effect of mulching and crop rotation on soil properties using SPSS 19.0. Data are expressed as the mean ± standard error.

A Venn diagram was generated with the 'Venn' R package (version 1.6.16) and an upset plot was generated with the 'UpSetR' R package (version 1.3.3) to identify unique and common OTUs. Alpha diversity analysis was conducted using QIIME (version 1.9.1) with calculation of the Chao1, abundance-based coverage estimator (ACE), and Shannon indices [25]. Tukey's honestly significant difference test was used for alpha-diversity index comparison among groups with the 'Vegan' R package (version 2.5.3).

Differences in beta diversity among samples were assessed at the OTU level by conducting a principal coordinate analysis (PCoA) based on Bray–Curtis distances using R software [24]. The Anosim test was performed with the 'Vegan' R package (version 2.5.3). Circular layout representations of species abundance were generated using Circos (version 0.69-3). Species differences among treatments were analyzed using the linear discriminant analysis effect size (LefSe) software, with the LDA threshold set at 4.0 [26]. Bacterial function was inferred using Tax4fun (version 1.0) [24], and fungal function was inferred using FUNGuild (version 1.0) [27].

Redundancy analysis (RDA) of soil microbial communities and environmental factors was performed using the 'Vegan' R package (version 2.5.3). Correlations between community structure and soil properties

were tested with the Mantel test [27]. Pearson correlation analysis were was to analyze the relationship between soil properties and microbial communities with IBM SPSS Statistics 22.0. Correlation heatmaps were produced using Omicsmart (http://www.omicsmart.com). Graphic retouching and layout adjustment were performed with Adobe Illustrator CC 2018 (Adobe Inc., San Jose, CA, USA).

Results

Soil properties

Film mulching (F) significantly affected soil pH, EC, AP, AK, TC and TN. Rotation (R) had a significant effect on pH, EC, AN, AP, AK, TC and TN. The interaction of film mulching and rotation (F × R) significantly affected soil pH, EC, AP and TN (Table 1).

The different treatments significantly affected soil properties (Table 2). Compared with PC, PCF significantly increased EC, AP and TC. Except for AP, R1 and R2 significantly reduced pH and EC, enhanced instead the contents of AN, AK, TC and TN. Similarly, R1F and R2F significantly decreased pH and EC, enhanced AN, AP, AK, TC and TN contents.

Sequencing quality analysis and microbial community abundance

A total of 2,182,770 bacterial and 2,273,689 fungal tags were obtained as raw tags for subsequent analysis. Under PC, PCF, R1, R1F, R2 and R2F, the number of bacterial OTUs was 3258, 3364, 3100, 3334, 3348, and 3499, the number of total tags was 108,194, 98,452, 99,608, 113,344, 108,653, and 110,899, and the number of taxon tags was 54,328, 47,319, 49,419, 55,925, 53,062, and 52,024, respectively. The number of fungal OTUs was 327, 389, 455, 448, 392, and 301, with total tags of 122,206, 126,028, 120,029, 122,397, 125,784, and 123,202, and the number of taxon tags was 121,625, 125,346, 118,320, 121,057, 124,004, and 122,369, respectively (Additional file 1: Fig. S1). The unique and shared OTUs were visualized in Venn diagrams and upset plots (Fig. 2, Additional file 1: Fig. S2). The number of unique bacterial OTUs was higher in R2F than other treatments, whereas the number of unique fungal OTUs in treatment R1F was higher than other treatments (Fig. 2). The number of shared bacterial and fungal OTUs was 1642 and 188, respectively (Additional file 1: Fig. S2).

Alpha diversity of soil microbial community

Two-way ANOVA revealed that F significantly affected the Chao1 index for soil bacteria (Fig. 3a). R had a

Table 1 Two-way analysis of variance results for the effects of film mulching, crop rotation, and their interaction on soil properties

Factor	pH	EC	AN	AP	AK	TC	TN
F	5.08*	89.95***	4.38	91.36***	6.25*	45.11***	5.66*
R	33.25***	7122.91***	88.42***	41.22***	85.76***	47.51***	29.04***
F*R	2.52*	32.25***	0.87	8.67***	0.36	1.76	7.19**

Film mulching (F), crop rotation (R), interaction of film mulching and crop rotation (F × R)

AN alkaline nitrogen, *AP* available phosphorus, *AK* available potassium, *TC* total carbon, *TN* total nitrogen

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 2 Effect of film mulching and crop rotation treatments on soil properties

Treatment	pH	EC (us/cm)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)	TC (g/kg)	TN (g/kg)
PC	7.72 ± 0.06a	391.25 ± 0.74b	13.22 ± 0.02c	11.32 ± 0.37d	166.94 ± 7.38d	14.11 ± 0.14d	0.89 ± 0.01c
PCF	7.60 ± 0.08a	403.08 ± 1.92a	14.34 ± 0.05c	12.30 ± 0.18c	177.28 ± 4.24 cd	14.73 ± 0.14c	0.93 ± 0.01c
R1	7.36 ± 0.06b	279.54 ± 0.45e	20.02 ± 0.57ab	12.44 ± 0.24c	227.87 ± 5.09a	15.11 ± 0.07c	0.98 ± 0.01b
R1F	7.16 ± 0.03c	278.04 ± 1.18e	21.53 ± 0.06a	15.25 ± 0.23a	232.80 ± 1.18a	16.04 ± 0.15a	1.08 ± 0.03a
R2	7.32 ± 0.02b	334.04 ± 0.27d	18.62 ± 0.92b	11.78 ± 0.13 cd	185.83 ± 3.46bc	15.06 ± 0.15c	1.03 ± 0.01ab
R2F	7.36 ± 0.04b	346.75 ± 0.10c	18.75 ± 0.75b	13.3 ± 0.13b	198.24 ± 3.30b	15.54 ± 0.05b	1.00 ± 0.02b

PC, potato continuous cropping without film mulching; PCF, potato continuous cropping with film mulching; R1, potato–broad bean rotation without film mulching; R1F, potato–broad bean rotation with film mulching; R2, potato–pea rotation without film mulching; R2F, potato–pea rotation with film mulching. Values are means of three replicates ± standard error (SE). Different lowercase letters within a column indicates a significant difference (Duncan's test, $p < 0.05$)

AN alkaline nitrogen, *AP* available phosphorus, *AK* available potassium, *TC* total carbon, *TN* total nitrogen

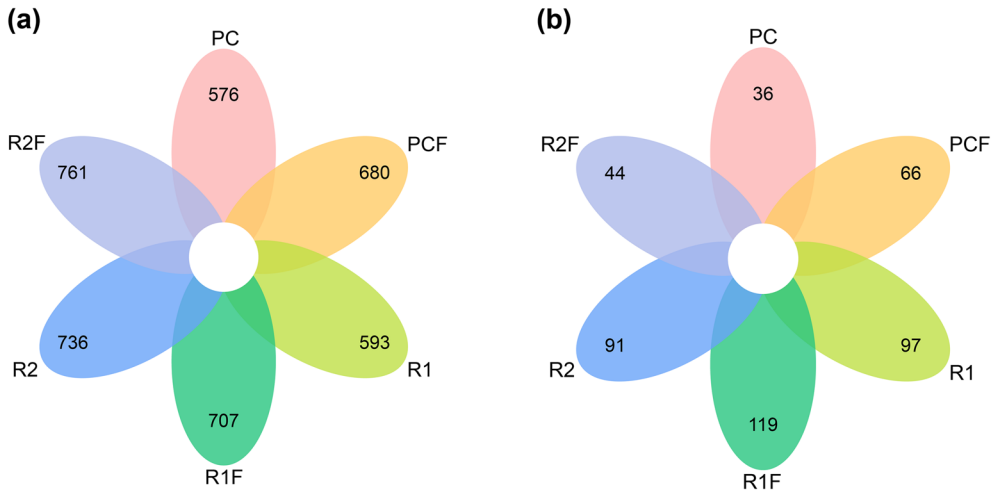


Fig. 2 Venn diagram of operational taxonomic units (OTUs) for **a** bacteria and **b** fungi under different film mulching and crop rotation treatments. PC, potato continuous cropping without film mulching; PCF, potato continuous cropping with film mulching; R1, potato–broad bean rotation without film mulching; R1F, potato–broad bean rotation with film mulching; R2, potato–pea rotation without film mulching; R2F, potato–pea rotation with film mulching

significant effect on the Shannon index for soil bacteria (Fig. 3e) and the ACE, Chao1, and Shannon indices for soil fungi (Fig. 3b, d, f). The interaction of F and R significantly affected the ACE index for soil fungi (Fig. 3d).

Analysis of alpha diversity in the rhizosphere soil samples showed that the ACE, Chao1, and Shannon indices for soil bacteria did not differ significantly among different treatments (Fig. 3a, c, e). For the fungi, PCF, R1, R1F, R2 and R2F had no significant effect on Chao1 index and ACE index compared to PC (Fig. 3b, d). R1F and R2 significantly enhanced the fungal Shannon index (Fig. 3f).

Beta diversity of the soil microbial community

A PCoA analysis was conducted for the different treatment soil microbial communities based on the Bray–Curtis algorithm (Fig. 4). PCo1 and PCo2 explained 29.21% and 14.55% of the soil bacterial community structure. PC, PCF, R2 and R2F clustered together and were distinctly separated from R1 on the PCo2 axis. Anosim analysis revealed significant differences in soil bacterial community composition among the treatments (Adonis $R^2=0.6018$, $P=0.001$) (Fig. 4a). PCo1 and PCo2 axes explained 30.62% and 18.34% of the variation in soil fungal community structure, respectively. The distribution of PC and PCF was relatively concentrated and distinctly separated from the other four treatments on the PCo2 axis. The distribution of R1, R1F and R2 was relatively concentrated and distinctly separated from R2F on the PCo1 axis. Anosim analysis detected significant differences in soil fungal community composition among the treatments (Adonis $R^2=0.6523$, $P=0.001$) (Fig. 4b).

Soil microbial community structure

The relative abundance of the top 10 bacteria and top 5 fungi at the phylum level, and the top 10 bacteria and fungi at the genus level were compared among treatments (Fig. 5). The community structure of bacteria at the phylum level was generally consistent across treatments. The top 10 phyla for each treatment were Proteobacteria, Bacteroidetes, Acidobacteria, Actinobacteria, Planctomycetes, Chloroflexi, Gemmatimonadetes, Verrucomicrobia, Patescibacteria and Firmicutes. Notably, the relative abundance of Proteobacteria, Bacteroidetes, and Firmicutes was higher in R1, R1F, R2 and R2F than PCF. The relative abundance of Acidobacteria, Chloroflex, Gemmatimonadetes, and Patescibacteria was lower in R1, R1F, R2 and R2F than PCF (Fig. 5 a). The community composition at the bacterial genus level is shown in Fig. 5b. The top 10 genera in relative abundance were RB41, *Sphingomonas*, UTCFX1, *Acinetobacter*, *Pedobacter*, *Serratia*, *Olivibacter*, *Bryobacter*, *Bacillus*, and *Terrimonas*. The relative abundance of *Olivibacter* was increased in R1, R1F, R2 and R2F compared with PC, whereas the relative abundance of *Sphingomonas* was decreased.

For fungi (Fig. 5c, d), Ascomycota was the dominant taxon at the phylum level, with a relative abundance of 71.76–91.64%, followed by Mortierellomycota (4.01–22.88%), Basidiomycota (0.35–3.16%), Glomeromycota (0.04–3.00%), Chytridiomycota (0.02–1.00%), and Mortierellomycota (0.59–2.99%). Unclassified Fungi accounted for 1.61–8.66% of the fungal community. R1, R1F, R2 and R2F increased the relative abundance of Basidiomycota and Glomeromycota (Fig. 5c). At the

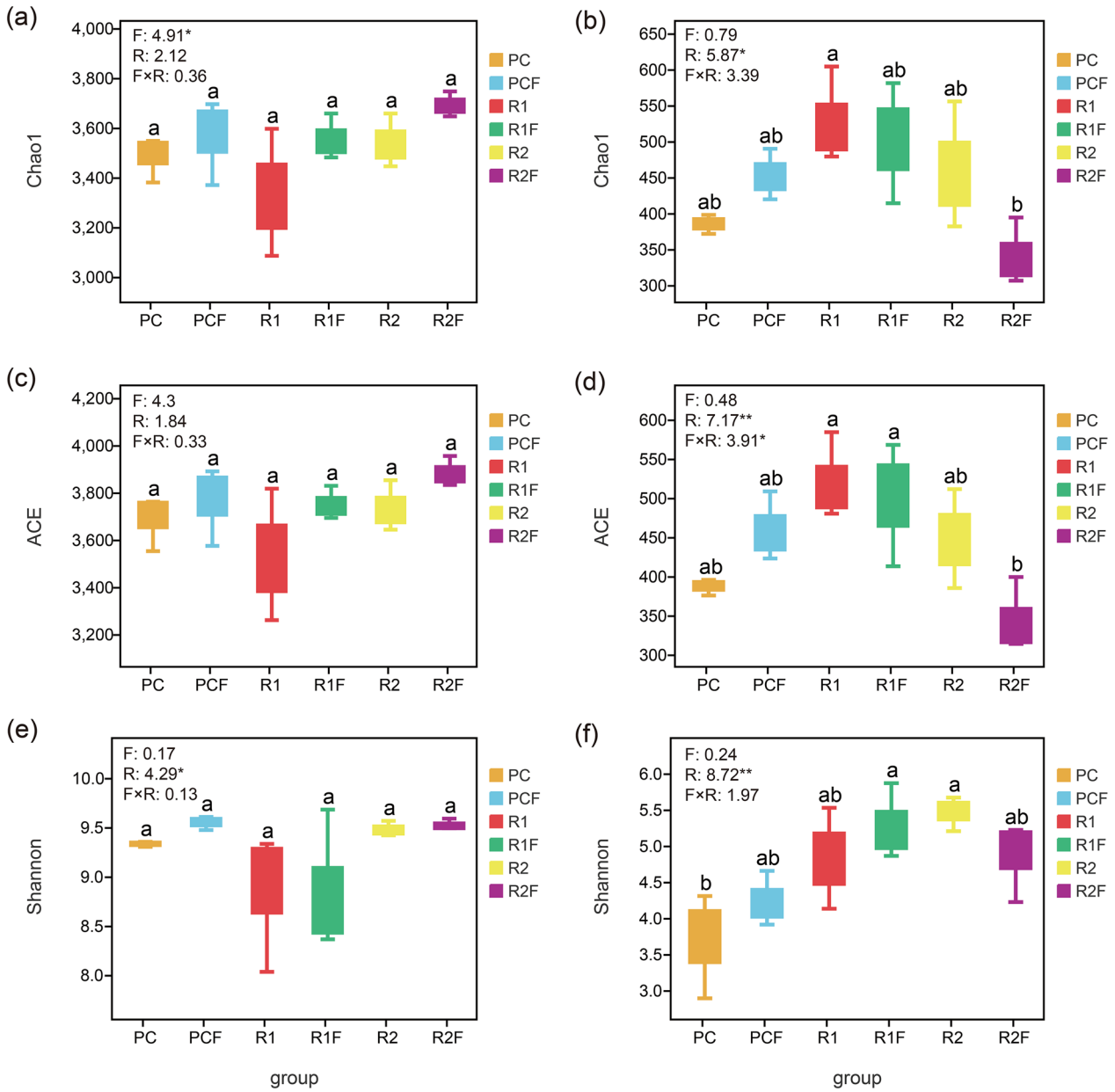


Fig. 3 Effect of different film mulching and crop rotation treatments on bacterial and fungal alpha diversity. **a**, **c**, **e** Chao1, ACE, and Shannon indices for bacteria. **b**, **d**, **f** Chao1, ACE, and Shannon indices for fungi. Different lowercase letters indicate a significant difference between treatments ($p < 0.05$). Film mulching (F), crop rotation (R), interaction of film mulching and crop rotation (F × R). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. PC, potato continuous cropping without film mulching; PCF, potato continuous cropping with film mulching; R1, potato–broad bean rotation without film mulching; R1F, potato–broad bean rotation with film mulching; R2, potato–pea rotation without film mulching; R2F, potato–pea rotation with film mulching

genus level, the soil fungal communities of the different treatments were dominated by *Botryotrichum*, *Mortierella*, *Plectosphaerella*, and *Fusarium*, with relative abundances of 4.75–34.86%, 3.82–22.37%, 2.03–34.13% and 4.95–11.24% (Fig. 5d). R1, R1F, R2 and R2F increased the relative abundance of *Clonostachys*, *Humicola*, *Stachybotrys*, and *Pseudogymnoascus*, whereas decreased the relative abundance of *Botryotrichum*.

Differential species analysis

Based on the LEfse analysis, 20 biomarkers were identified at all taxonomic levels of the soil bacterial

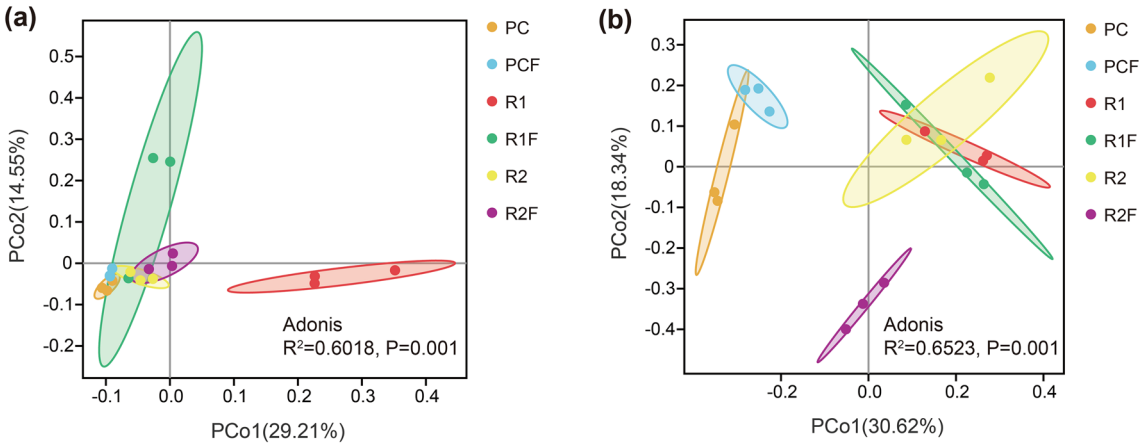


Fig. 4 Principal coordinate analysis of **a** bacteria and **b** fungi (operational taxonomic units > 0.01%) under different film mulching and crop rotation treatments. The sample groups show 95% confidence ellipses for each treatment. PC, potato continuous cropping without film mulching; PCF, potato continuous cropping with film mulching; R1, potato–broad bean rotation without film mulching; R1F, potato–broad bean rotation with film mulching; R2, potato–pea rotation without film mulching; R2F, potato–pea rotation with film mulching

community (LDA threshold of 4.0), including five PC-enhanced organisms, six PCF-enhanced organisms, seven R1-enhanced organisms, and two R2-enhanced organisms. In contrast, no differential species were identified for bacterial populations in R1F and R2F at the LDA threshold of 4.0 (Fig. 6a, b). For example, *Sphingobacteriaceae* was the most dominant biomarkers in soils of the R1, and Acidobacteria and Cytophagales were the most dominant biomarkers for PCF and R2. In addition, nine biomarkers were identified in the soil fungal community (LDA threshold of 4.0), including three PC-enhanced organisms, one R1-enhanced organism, one R1F-enhanced organism, and four R2F-enhanced organisms. No differential species were identified in the fungal populations of PCF and R2 at the LDA threshold of 4.0 (Fig. 6c, d). Nectriaceae and Eurotiomycetes were biomarkers for R1 and R2F. The abundance of Plectosphaerellaceae was higher in PC than other treatments.

Prediction of microbial metabolic pathways in rhizosphere soil

Metabolism, Environmental Information Processing and Genetic Information Processing were the primary metabolic pathways, accounting for 61.40–63.26%, 17.73–18.49% and 11.70–12.17%, respectively (Additional file 1: Fig. S3). A total of 41 functional features were annotated on the secondary metabolic pathways. The relative abundance of the top 10 bacterial functional groups for functions such as Amino Acid Metabolism, Carbohydrate Metabolism, Membrane Transport, Signal Transduction, Metabolism of Cofactors and Vitamins, and Energy Metabolism is shown in Additional file 1: Fig. S3. R1 significantly differed from the other treatments with regard to the Carbohydrate Metabolism, Signal Transduction and Translation functions.

Functional annotation of the fungi using FUNGuild revealed nine types: Pathotroph-Saprotroph-Symbiotroph, Saprotroph, Unassigned, Saprotroph-Symbiotroph, Pathotroph-Saprotroph, Pathotroph, Symbiotroph, and Pathotroph-Symbiotroph (Additional file 1: Fig. S4). The relative abundances of Pathotroph-Saprotroph-Symbiotroph and Saprotroph-Symbiotroph were higher, accounting for 20.32–47.20% and 12.51–38.58% of the soil fungal community, respectively. R1, R1F, R2 and R2F increased the relative abundance of Pathotroph-Saprotroph, Pathotroph, Symbiotroph, Pathotroph-Symbiotroph and Unassigned fungi, whereas decreased the relative abundance of Saprotroph.

Relationship between soil microorganisms and soil factors

Redundancy (RDA) analysis revealed that RDA1 and RDA2 explained 45.50% and 44.17%, respectively, of the variance at the soil bacterial genus level, and thus reflected 89.67% of the influence of soil environmental factors on the soil bacterial community structure (Fig. 7a). A total of 83.71% of the effect of soil environmental factors on communities at the fungal genus level was explained by RDA1 and RDA2 together (Fig. 7b). The Mantel test revealed that, at the genus level, soil EC ($R=0.469$, $P=0.001$), AN ($R=0.207$, $P=0.026$), and AK ($R=0.471$, $P=0.001$) were the crucial environmental factors that dominated differences in soil bacterial communities. The fungal communities were mainly affected by soil pH, EC, AN, AP, AK, TC and TN (Table 3).

Pearson correlation analysis was performed between the top 10 microorganisms in relative abundance at

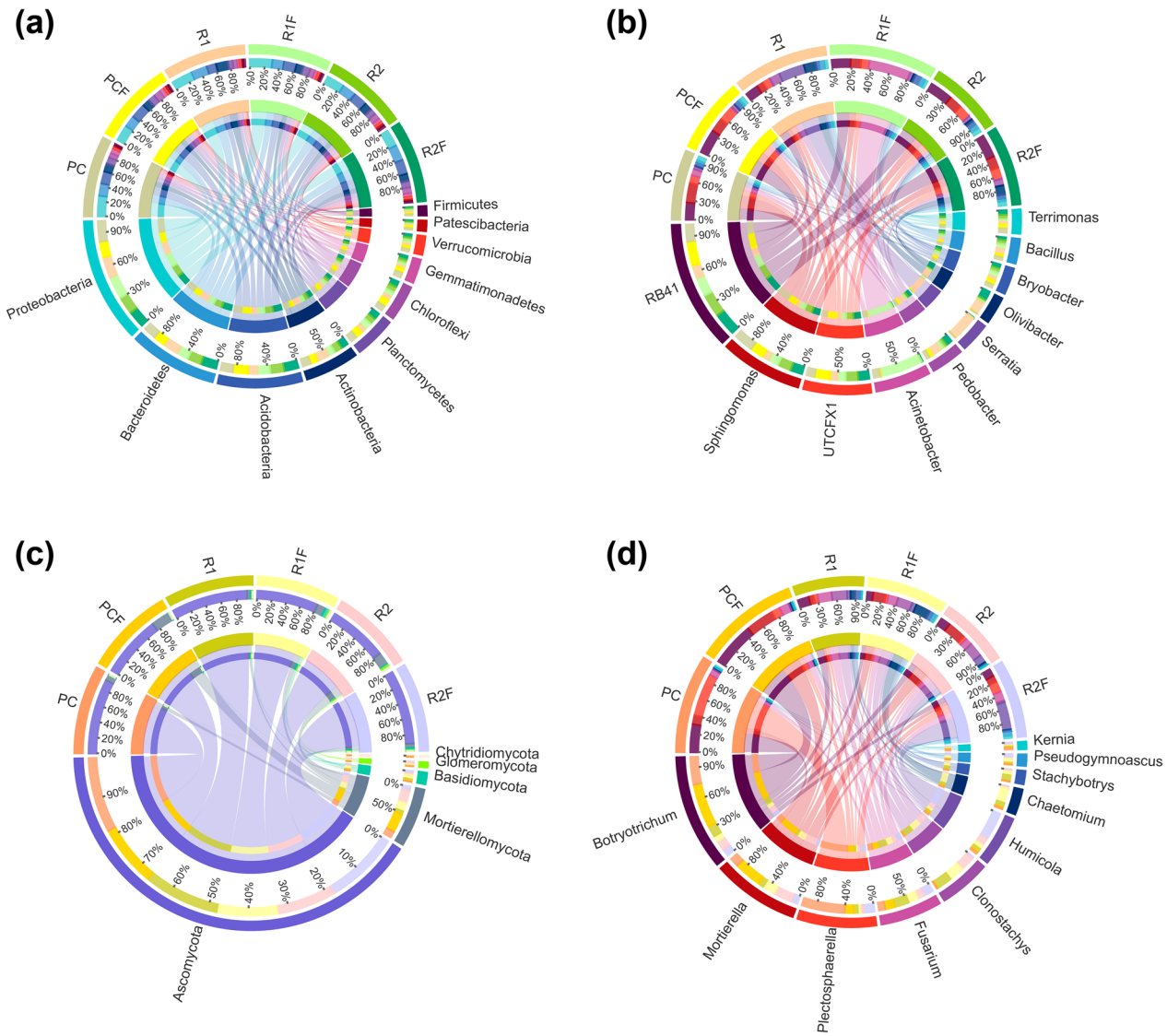


Fig. 5 Circos diagram of bacteria and fungi at different taxonomic levels under different film mulching and crop rotation treatments. **a**, **b** Relative abundance of bacteria in the top 10 at the phylum level and the top 10 at the genus level. **c**, **d** Relative abundance of fungi in the top 5 at the phylum level and the top 10 at the genus level. The upper side of the Circos diagram is microbial information, and the lower side is sample information. PC, potato continuous cropping without film mulching; PCF, potato continuous cropping with film mulching; R1, potato–broad bean rotation without film mulching; R1F, potato–broad bean rotation with film mulching; R2, potato–pea rotation without film mulching; R2F, potato–pea rotation with film mulching

genus level and the environmental factors (Fig. 8). *Sphingomonas* was significantly positively correlated with soil pH and EC, but significantly negatively correlated with soil AN, AK, TC and TN. *Terrimonas* was significantly positively correlated with EC, but negatively correlated with AK and AP. AK was significantly positively correlated with *Olivibacter* and *Pedobacter* (Fig. 8a). *Botryotrichum* and *Plectosphaerella* were significantly positively correlated with pH and EC, but significantly negatively correlated with AN, AK, TC and TN. *Clonostachys*, *Stachybotrys*, *Chaetomium*, and *Pseudogymnoascus* were significantly negatively correlated with pH, but significantly positively correlated with AN and AK (Fig. 8b).

Discussion

Effects of film mulching and legume rotation on rhizosphere soil properties

The rotation of legumes fixes atmospheric N and increases soil organic matter, and plastic film mulching can play a role in maintaining the soil moisture content

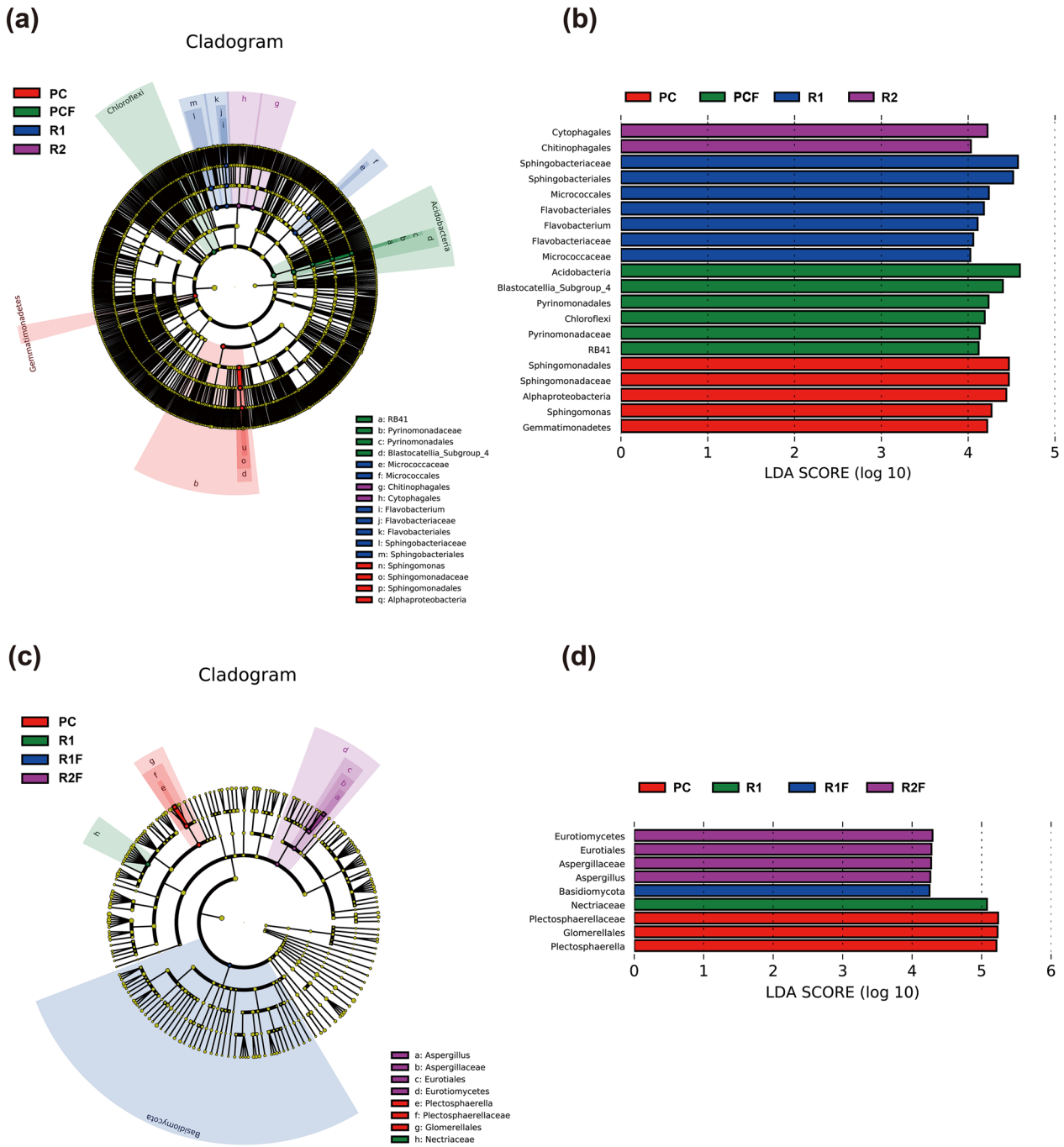


Fig. 6 Linear discriminant analysis effect size (Lefse) analysis of bacteria and fungi under different film mulching and crop rotation treatments. **a**, **c** Cladogram of bacteria and fungi included in the LEfSe analysis; phylum, class, order, family, and genus is represented from the center to the margin. The size of the small circle represents the relative abundance of the species at the taxonomic level. Species with no significant differences are marked with yellow, and species with significant differences are indicated by different colors. **b**, **d** LDA scores for bacteria and fungi with LDA score ≥ 4.0, $p < 0.05$. PC, potato continuous cropping without film mulching; PCF, potato continuous cropping with film mulching; R1, potato–broad bean rotation without film mulching; R1F, potato–broad bean rotation with film mulching; R2, potato–pea rotation without film mulching; R2F, potato–pea rotation with film mulching

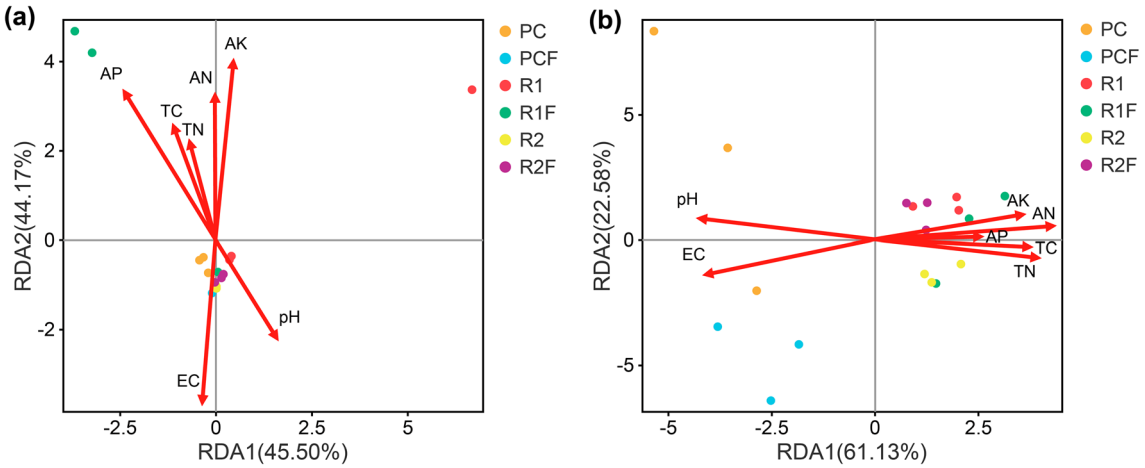


Fig. 7 Redundancy analysis of bacteria (**a**) and fungi (**b**) with environmental factors at the genus level under different film mulching and crop rotation treatments. Alkaline nitrogen (AN), available phosphorus (AP), available potassium (AK), total carbon (TC), and total nitrogen (TN). PC, potato continuous cropping without film mulching; PCF, potato continuous cropping with film mulching; R1, potato–broad bean rotation without film mulching; R1F, potato–broad bean rotation with film mulching; R2, potato–pea rotation without film mulching; R2F, potato–pea rotation with film mulching

and improving soil microenvironment of the crop rhizosphere [28, 29]. The present study found that both legume rotation and legume rotation with film mulching lowered soil pH 7.0–7.5, which enhance the availability of nutrients to crops [30]. Meanwhile, legume rotation and legume rotation with film mulching decreased soil EC. This could be attributed to the fact that leguminous plants have a positive impact on increasing soil organic matter content and cation exchange capacity, while also improving soil buffer performance against both acid and alkali [23, 31]. The nitrogen fixation by leguminous rhizomes, the return of their residues to the soil, and also possibly the planting density of them, resulted in the increase of mineral nutrient contents in the soil, in addition to the fact that plastic film mulching facilitates soil nutrient retention [6, 32]. In our study, the contents of soil AN, AK, TC and TN in the legume rotation, as well as AN, AP, AK, TC and TN in the legume rotation with film mulching were significantly higher than those in the continuous cropping treatments, suggesting that rotation with legumes improved the availability of soil nutrients. The roots and branch residues from the legume rotation enter the soil to provide a stable source of nutrients [4, 11].

Table 3 Mantel test correlation of bacterial and fungal community structure (at the genus level) with soil properties under different film mulching and crop rotation treatments

Microbial community	Environmental factor	*R*	*P*
Bacteria	pH	0.142	0.149
	EC	0.469	0.001*
	AN	0.207	0.026*
	AP	0.239	0.078
	AK	0.471	0.001*
	TC	0.167	0.126
	TN	0.14	0.164
Fungi	pH	0.462	0.001*
	EC	0.526	0.001*
	AN	0.561	0.001*
	AP	0.207	0.042*
	AK	0.331	0.003*
	TC	0.443	0.001*
	TN	0.564	0.001*

AN alkaline nitrogen, *AP* available phosphorus, *AK* available potassium, *TC* total carbon, *TN* total nitrogen

* Indicates significant correlation between items ($p < 0.05$)

Effects of film mulching and legume rotation on rhizosphere soil microbial community diversity

Soil microbial diversity is closely related to soil ecosystem stability and nutrient transformation [33]. In the study, number of soil fungal OTUs was higher in the broad bean rotation and broad bean rotation with mulching than in the potato continuous crop. Legume rotation causes changes in soil nutrients, while the difference in utilization of soil nutrients leads to the difference in OUT diversity of bacteria and fungi [34]. Essel et al. [11] showed that crop diversification significantly alters the abundance and diversity of soil microorganisms. In this study, there was no significant change in the bacterial alpha diversity in the treatment, indicating that soil bacterial diversity was not affected by the

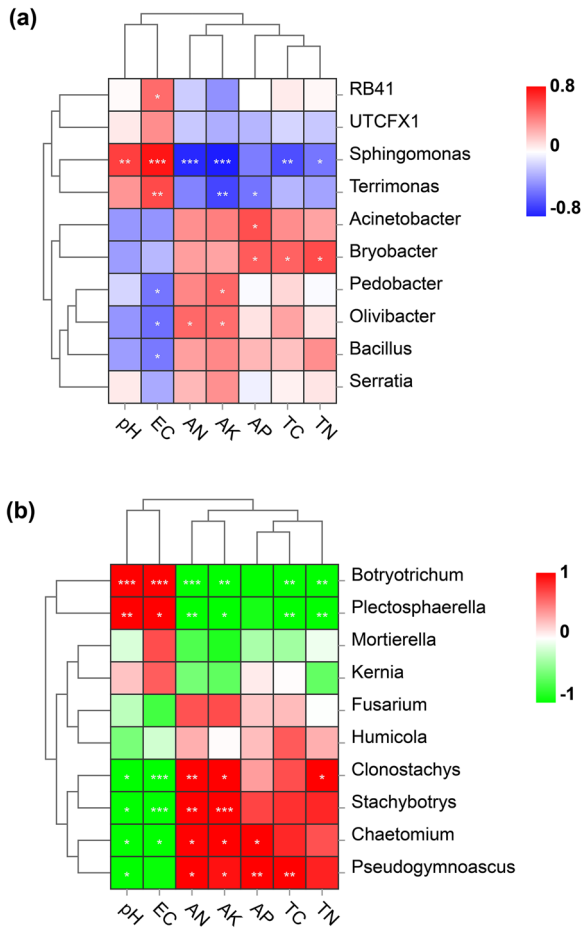


Fig. 8 Pearson correlation analysis of environmental factors with the main bacterial and fungal communities at the genus level. **a** Bacteria; blue indicates a negative correlation and red indicates a positive correlation. **b** Fungi; green indicates a negative correlation and red indicates a positive correlation. The shade of color in (**a**) and (**b**) reflects the strength of the correlation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. *AN* alkaline nitrogen, *AP* available phosphorus, *AK* available potassium, *TC* total carbon, and *TN* total nitrogen

legume rotation and film mulching in the semi-arid region of the Loess Plateau. R1F and R2 significantly altered the alpha diversity of the fungi. This result is basically consistent with previous research done in Zeng et al. [26].

Increased crop diversity through diverse crop rotations increases soil chemical diversity, supporting the propagation and growth of diverse microbial taxa [35, 36]. The increased effect of crop diversification on soil fungal diversity is attributed to root secretions and plant residues from diverse plants. In addition, the impact of mulching on microbial diversity is evident [25]. Based on the PCoA analysis, soil microbial beta diversity significantly changed with the change in planting sequence, which is consistent with previous findings [4]. Broad bean rotation led to the bacterial beta diversity, and legume rotation and legume rotation with film mulching resulted in higher fungal beta diversity. This difference is a result of soil microorganisms being influenced by the nitrogen fixation capacity of legumes and the alteration of soil nutrients [13].

Effects of film mulching and legume rotation on rhizosphere soil microbial community structure

Soil microbial communities in agroecosystems are extensive [37] and are susceptible to factors such as crop types, cultivation patterns, and tillage practices [38, 39]. In this study, the dominant populations in all soil samples were Proteobacteria, Bacteroidetes, Acidobacteria, and Actinobacteria at the bacterial phylum level (Fig. 5a). Similarly, Acidobacteria and Proteobacteria are the most common bacterial taxa in the soils of the North China Plain, the Northeast Blackland, and the Loess Plateau [4, 11, 40]. However, Nacke et al. [41] reported that the most dominant bacterial taxa in the rhizosphere soil of European beech and Norway spruce were Acidobacteria. Fierer et al. [42] observed that Acidobacteria were the most abundant bacterial taxa in soils in temperate forests. These results indicate that different dominant species exist in diverse ecosystems. In addition, we observed that the relative abundance of Proteobacteria was higher under rotation with broad bean crops, and that Proteobacteria were diverse and ecologically functional, with many being nitrogen-fixing and highly adaptable to the soil environment [40]. Rotation with broad bean and mulching promoted increase in the soil OM content, provided sufficient metabolic substrate for Proteobacteria and promoted their growth and reproduction [6, 25, 43].

At the genus level, RB41, *Sphingomonas*, UTCFX1, and Acinetobacter were the dominant bacterial genera common to all treatments, agree well with previous studies [4]. The higher relative abundance of *Olivibacter* in the crop rotation was attributed to the facts that *Olivibacter* belongs to the Bacteroidetes, which are important for decomposition of OM. Rotation with legumes increased soil OM content and soil nutrient, thus promoting the propagation of *Olivibacter* [44, 45]. However, the relative abundance of *Sphingomonas* was reduced in the crop rotation, which may be related to crop type and ecological environment.

Ascomycota, Mortierellomycota, Basidiomycota, Glomeromycota, Chytridiomycota and Mortierellomycota were the dominant soil fungal phyla in the study. The dominance of Ascomycota is consistent with previous findings that Ascomycota is the dominant soil fungal phylum and is an important driving force for soil nutrient cycling [25]. Meanwhile, their relative abundance in

different habitats varies owing to the influence of crops types [46, 47].

Basidiomycota and Glomeromycota abundance was significantly higher in the crop rotation than the continuous cropping treatments. Because annual deep plowing disturbs the soil after the legume rotation, providing a suitable soil environment for Basidiomycota and *Glomus* to better futilize degradable plant residues, and promoting the rapid growth and reproduction of the fungal flora [3, 4].

The principal genera of fungi recorded were *Botryotrichum*, *Mortierella*, *Plectosphaerella*, and *Fusarium*. *Botryotrichum* is a common invasive pathogen of fruits and vegetables during postharvest storage, widely distributed parthenogenic parasite [48]. Rotation of legume reduced the abundance of *Botryotrichum*. Therefore, reduction in the number of harmful fungi may be one mechanism by which crop rotation changes soil quality. In the present study, R1F and R2 reduced the relative abundance of *Mortierella*. As a group of fungi with a strong cellulolytic capacity, *Mortierella* is more effective for enhancing soil nutrient effectiveness and improving soil structure, hence the genus shows high abundance in cultivated land [49]. However, some species of *Mortierella* are phytopathogenic fungi that cause plant seedling death [50], thus high *Mortierella* abundance in soils may increase the risk of fungal diseases.

The LEfSe analysis revealed differences in the dominant bacteria among the treatments. The critical bacteria in the PC were *Sphingomonas* and Gemmatimonadetes. In previous studies, the higher abundance of *Sphingomonas* was associated with soil nutrient absorption [4]. In the PCF, the crucial bacteria were RB41 and Chloroflexi. RB41 belongs to Acidobacteria, which are bacteria of nutrient-poor soils that are highly sensitive to soil pH, and a soil environment of low nutrient status is more conducive to their survival [51]. Under the legume rotation, the critical bacteria in the R1 were Micrococcales (Actinobacteria), Flavobacteriales (Bacteroidetes), and Sphingobacteriales (Bacteroidetes), and the crucial bacteria in the R2 were Cytophagales and Chitinophagales of the phylum Bacteroidetes. Actinobacteria produce enzymes associated with OM degradation, and thus accelerate the decomposition of OM and increase their relative abundance [52]. As the main mineralizer of organic C, Bacteroidetes increase soil organic C content and provide energy for microbial growth and soil enzyme activity. Crop rotation can change soil organic C content by affecting the input of litter and root exudates, and providing favorable conditions for the growth and reproduction of Bacteroidetes and Actinobacteria [53]. In terms of fungi, *Plectosphaerella* (Ascomycota) was significantly enriched in the PC. *Plectosphaerella* causes fungal diseases of plants, such as root rot of melon (*Cucumis melo*) [54]. The crucial fungi in the R1 were Nectriaceae of the phylum Ascomycota. These pathogenic bacteria cause root rot in the rhizosphere [55]. Whether the increased relative abundance of Nectriaceae in the R1 leads to root rot requires further verification. In the R1F, Basidiomycota, which are an important component of the mycorrhizal fungal florai, can antagonize pathogenic bacteria in plant roots and provide a healthy soil microenvironment for plant growth [56]. Thecrucial fungus in the R2F was *Aspergillus*, which can degrade cellulose and lignocellulose, promote nutrient uptake by plant roots, and ensure the supply of soil nutrients to the plant rhizosphere [57]. The high risk of fungal diseases in potato with continuous cropping requires additional attention. In the current study, legume rotations increased the relative abundance of potentially beneficial fungi, and film mulching had an ameliorating effect on microbial populations, suggesting that legume rotation and film mulching are effective means of reducing the incidence of potato fungal diseases, which supports our findings [2, 6].

Prediction of microbial metabolic pathways in rhizosphere soils under film mulching and legume rotation

The dominant factor for changes in bacterial and fungal functions was crop rotation, and legume rotation increased the diversity of functional groups of bacteria, enhancing the ability of bacteria to absorb nutrients and enhancing their active metabolism [13, 58]. In the study, we found that three types of functional genes (Metabolism, Environmental Information Processing and Genetic Information Processing) in the soil, promotes the uptake of nutrients and small molecules by the soil bacterial community to accelerate its growth, resulting in vigorous metabolism of soil bacteria and increased bacterial functional community diversity [58]. Pathotroph-Saprotroph-Symbiotroph and Saprotroph were the most dominant predicted fungal functional types in the present study, which may be associated with the dominance of the fungal phylum Ascomycota. Most Ascomycota are saprophytic fungi that decompose refractory OM in the soil and play an important role in nutrient cycling [47].

At the same time, the increased diversity of functional groups of different nutritional types of fungi strengthened their ability to participate in carbon source metabolism. Fungi not only decompose the carbon sources used by bacteria but also indirectly contribute to releasing carbon sources from the root system to the soil [59]. The abundance of the functional groups Pathogens-Saprophytes, Pathogens-Symbionts, Saprophytes, Saprophytes-Symbionts, and Pathogens-Saprophytes was higher under the crop rotation treatments than those under the continuous cropping treatments to varying degrees, possibly

because the legume rotation treatments significantly changed the structure of the fungal community. Besides, the number of fungi increased, resulting in the increase in functional groups with corresponding functions, and it may also be associated with the increased abundance of Unassigned fungi in the legume rotation treatments, which requires further investigation.

Relationship between microbial community composition and soil properties

Bacteria and fungi are the main soil microbial taxa and are extremely sensitive to the microenvironment, such as soil nutrient status and hydrothermal conditions, which directly affect the species diversity and abundance of soil microorganisms [16, 60, 61]. The present RDA analysis showed that EC, AN and AK were the main environmental factors that dominated the changes in soil bacterial community structure. Wang et al. [4] observed that pH and AN were the main drivers of changes in bacterial community structure, which differed from the present study, possibly because of differences in the regional environment, crop species and sampling time. Simultaneously, pH, EC, AN, AP, AK, TC and TN were the main environmental factors that affected soil fungal community structure. Previous studies have shown that legume rotation can influence the decomposition of crop residues, which promotes the accumulation of soil organic C and causes changes in the structural composition of soil fungal communities [27]. Different conclusions have been drawn on the effects of environmental factors on soil microbial communities [15, 25, 43, 62], which may be associated with regional differences and crop type.

Environmental factors have inhibitory, facilitative, or no noticeable effect on soil microbes and are important influences on soil microbial community composition [15, 62]. Correlation analysis in the present study showed that the higher relative abundance of bacterial populations, such as *Sphingomonas* of the Proteobacteria, was significantly correlated with soil pH, EC, AN, AK, TC and TN. Higher soil nutrient contents alter the relative abundance of bacterial communities in the soil owing to the synergistic interaction of soil fertility, soil environment, and soil bacterial communities [4]. The present study showed that soil EC and AK were the main environmental factors affecting the soil bacterial communities, which may reflect the unique habitat in the semi-arid region of the western Loess Plateau. In the study, soil pH was negatively correlated with *Clonostachys*, *Stachybotrys*, *Chaetomium* and *Pseudogymnoascus*.An excessively high soil pH limits the survival of fungi, and thus acidic conditions were more suitable for the growth of soil fungi [63]. The study found that soil fungal community was significantly correlated with soil pH, EC, AN and AK. Zhao, et al. [64] showed that fungal abundance in paddy soils was mainly regulated by soil pH. Furthermore, a previous study of soil diversity under continuous cropping of potato indicated that soil fungal community composition was positively influenced by soil characteristics, particularly AN, AP and AK [4].

Conclusion

This study reveals the changes in soil physicochemical properties and microbial responses to legume rotation and film mulching. Legume rotation with mulching had remarkable impacts on soil properties and microorganisms, favored an increase in soil nutrients of potato continuous cropping filed. Broad bean rotation with film mulching altered fungal α-diversity. Legume rotation favored an increase in the relative abundance of fungi, including the phylum Tameromycota and Glomeromycota, while broad bean rotation promoted the growth of the beneficial fungus Aspergillus. In addition, broad bean rotation with film mulching increased bacterial signal transduction and energy metabolism functions. Legume rotations increased the relative abundance of metabolic fungi, and altered the key differential microbial species and interrelationships among microbial taxa. In summary, legume rotation has been shown to improve soil quality through various mechanisms, including increasing soil fertility, altering soil microbial diversity and community structure, thus alleviating potato continuous cropping barrier. This study provides a basis for selecting suitable legume crops for rotation with potatoes and a theoretical support for the potato sustainable production in semi-arid agroecosystems.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00411-w.

Additional file 1: Table S1 The basic properties of soil in the experimental area. **Figure S1.** Number of tags and operational taxonomic units (OTUs) of (a) bacteria and (b) fungi under different film mulching and crop rotation treatments. **Figure S2.** Upset diagram of operational taxonomic units (OTUs) for (a) bacteria and (b) fungi under different film mulching and crop rotation treatments. PC, potato continuous cropping without film mulching; PCF, potato continuous cropping with film mulching; R1. potato–broad bean rotation without film mulching; R1F, potato–broad bean rotation with film mulching; R2, potato–pea rotation without film mulching; R2F, potato–pea rotation with film mulching. **Figure S3.** Relative abundance of potential functional pathways of the top 10 bacteria at level 2 under different film mulching and crop rotation treatments. Different lowercase letters indicate a significant difference between treatments ($p < 0.05$; Tukey HSD). **Figure S4.** Differences in soil fungal function based on FUNGuild predictions under different film mulching and crop rotation treatments. PC, potato continuous cropping without film mulching; PCF, potato continuous cropping with film mulching; R1, potato–broad bean rotation without film mulching; R1F, potato–broad bean rotation with film

mulching; R2, potato–pea rotation without film mulching; R2F, potato–pea rotation with film mulching.

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Author contributions
Conceptualization, methodology, and writing—original draft, MS; data curation and writing—review and editing, WZ and SQ; software, YK, XY, YL, andAQ4 AG; methodology, XY and AG; investigation, YW; formal analysis, RZ; funding acquisition, YK and SQ. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated and analysed during the current study are available in the NCBI repository, https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA869187, the accession number is PRJNA869187. The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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