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RESEARCH ARTICLE

Faba bean enhances soil multifunctionality through shaping rhizosphere microbial communities in legume-cereal crop rotations

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KEYWORDS

Crop rotation, faba bean, legume crops, microbial diversity, soil multifunctionality

HIGHLIGHTS

- Faba bean-based rotation was found to be beneficial for soil physicochemical properties.
- Legume species and regional difference significantly affected soil property variation.
- Desulfobacterota and Planctomycetota were enriched in the faba bean rotation.
- Co-occurrence analysis revealed different keystone- and legume-sensitive OTUs.

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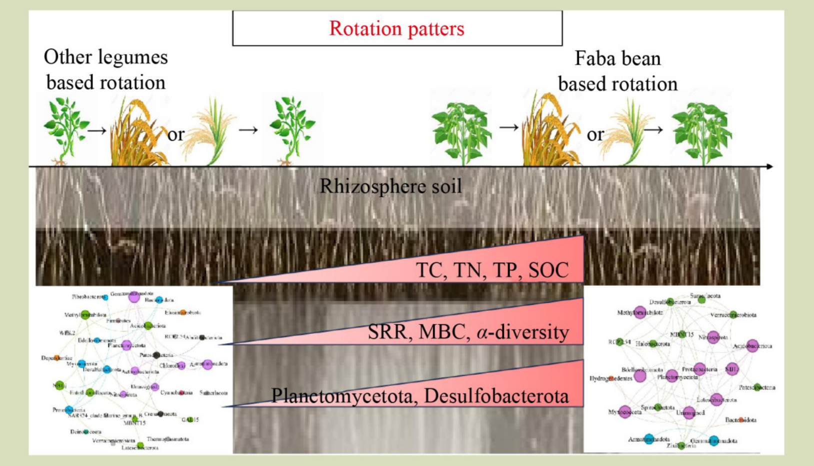
GRAPHICAL ABSTRACT



ABSTRACT

Crop rotation is a beneficial and sustainable agricultural practice that facilitates increased opportunities for smallholders. This study investigated the impact of eight commonly used crop rotations in China on soil properties and microbial communities. The faba bean (*Vicia faba*) rotation increased soil water content, total carbon, total nitrogen, total phosphorus and organic carbon content by 29.1%, 40.9%, 55.9%, 18.9%, and 61.6%, respectively, compared to other rotations. The faba bean rotation also exhibited increased soil microbial biomass and soil respiration rates. The effect sizes of the faba bean rotation on soil properties were larger than those of other rotations. The richness and diversity of the microbial community were significantly higher in the faba bean rotation than in other rotations. Desulfobacterota and Planctomycetota had a positive correlation with soil multifunctionality. The faba bean rotation was potentially beneficial to soil fertility and water-use efficiency, creating a favorable niche for microbial growth. With increased microbial activity and potential for nutrient mineralization, legume-microbe interactions had been improved through crop rotation. This resulted in enhancing nutrient cycling efficiency in the faba bean rotation, potentially improving soil properties.

1 Introduction

Soil is crucial for agriculture and ecosystems[1]. It encompasses sustainable ecosystem function and the provision of essential services, including supporting plant life, regulating nutrient cycling and providing habitats for many organisms[2–4]. Soil degradation, including soil erosion, compaction, nutrient depletion and organic matter loss, has occurred frequently in recent years. Given the growing concern over soil degradation from human activities and global climate change, the importance of global soil health has increasingly been acknowledged[5–7]. Intentional soil management practices, including crop rotation, nutrient management and monitoring have frequently been highlighted because of their ability to enhance soil quality and ecosystem services sustainably[8,9].

Crop rotation is an effective and widespread practice in sustainable agriculture worldwide[6,10,11]. This practice involves growing different crop species spatially or temporally in a particular area and includes crop rotation, intercropping and agroforestry[10,12]. Crop rotation enhances agricultural sustainability by increasing biodiversity, enhancing soil fertility and health, reducing pests and diseases, maintaining ecosystem services and enhancing agronomic productivity[13–15]. Crop rotation is one of the most common systematic processes used by smallholders. Soil microbial communities change with crop planting, which can disrupt pest and disease life cycles. Growing different crop species can support the growth of different microorganisms. Therefore, certain rotation combinations can increase soil fertility, health and quality. This, in turn, leads to a healthier soil microbiome, resulting in increased plant productivity and crop yield[16–18]. Legume-based crop rotation has been widely endorsed by researchers owing to its numerous benefits[19].

Also known as pulses, grain legume crops can fix atmospheric nitrogen through a symbiotic relationship with nitrogen-fixing bacteria in their root nodules[20]. In the field, the bacteria in root nodules can provide plants with up to 80% of their nitrogen needs[20,21]. This could decrease the requirement for N fertilizer input in legume cropping, which can provide the soil with a consistent N source[20,22]. The N-fixation process also has considerable benefits for agricultural systems, including improved soil quality and decreased environmental degradation caused by excess N and phosphorus runoff[23,24]. Altering legume crops in rotation could not only reduce the costs for farmers by reducing mineral fertilizer input but also decrease the negative environmental impacts of mineral fertilizers[23,25,26]. N-fixation by certain legume crops can increase soil organic content. This enhances the growth of beneficial soil organisms and leads to long-term soil improvements[27]. The symbiotic relationship between legume crops and nitrogen-fixing bacteria is complex and depends on environmental factors. Legume crops release compounds known as flavonoids that signal to bacteria that the plant is ready to form nodules. Bacteria provide some legume crops with mineralized nutrients for trading energy sources from legume crops, such as carbohydrates[20,28].

Legume crops differ in their interactions with soil microbes[29]. Consequently, microbial communities in the rhizospheres of different legume crops vary. Roots in soil typically interact with Proteobacteria, Actinobacteria and Bacteroidetes, which are associated with soil structure and nutrients[30]. Certain microbes, such as *Rhizobium* and arbuscular mycorrhizal fungi, form strict symbioses with specific legume crops[30]. These interactions can lead to significant variations in the influence of soil microbial communities on crop productivity among different legume crops[31,32]. Determining the role of microbes in nutrient cycling, soil organic matter decomposition, and soil health under different legume crop rotations can help farmers rotating between different plantings of grain and legume crops choose more effectively from different agricultural systems[33,34]. For example, soybean has been found to enrich microbial communities with *Bradyrhizobium*, *Mesorhizobium* or *Allorhizobium* as cores, and to recruit varying abundances of *Blastococcus*, *Nocandioides*, and *Nitrospira* for nitrogen uptake[35]. Similarly, both faba bean and field pea have typical rhizobia strains and *Rahnella aquatilis* in their nodules; faba bean also enrich *Serratia plymuthica* for nutrient uptake and salt stress modification[36]. Determining these correlations would be beneficial to the agronomic performance and elucidate the complex interactions between legumes and their associated microbial communities, as well as their impact on agricultural sustainability[37,38]. Conversely, harnessing the benefits of legumes and their associated microbial communities is essential for sustainable agricultural practices[39,40].

Productivity advantages, soil health and microbial communities of a rotation system with certain mono-legume crops has been a key research focus. However, more comprehensive elucidation is necessary to determine the effects of different legume crops on soil health. In this study, different soil samples were collected from legume-based rotations across China to study the effects of different legume-based rotations on soil. The aim of this study was to identify the shifts in soil properties, microbial communities and driving mechanisms under different legume-based rotations.

2 Materials and methods

2.1 Soil collection and preparation

The selected legume-cereal rotation systems were implemented using legumes, and rice (*Oryza sativa*) or wheat (*Triticum aestivum*) (Fig. S1). Soil samples were collected between June and October 2023 from legume-based agriculture fields, which spanned 21.66° to 48.02° N and 86.29° to 125.26° E across China. When legume crops were harvested, an ‘S’-shaped method was used to collect soil samples from a depth of 20 cm using a 3.5-cm soil auger. After ensuring uniformity, a single composite sample was collected from each site. During the sampling process, GPS instruments were used to record latitude and longitude. Additionally, a soil ring knife was employed to collect undisturbed soil for measuring soil bulk density. After drying and sieving (2-mm), the soil was divided into three subsamples for the analysis of various physical and chemical properties, as well as microbial structures. Of these samples, 229 were obtained from soil from legume-cereal rotations, including nine cowpea (*Vigna unguiculata*), 87 faba bean (*Vicia faba*), 21 kidney bean (*Phaseolus vulgaris*), 62 mung bean (*Vigna radiata*), 19 field pea (*Lathyrus oleraceus*), 21 adzuki bean (*Vigna angularis*) and 10 soybean (*Glycine max*) soil samples. Meanwhile, 32 samples were obtained from soil from less common legume rotations, including chickpea (*Cicer arietinum*), milk vetch (*Astragalus sinicus*), alfalfa (*Medicago sativa*) and scallion (*Allium fistulosum*). Climate factors, such as mean annual temperature (MAT) and mean annual precipitation (MAP) and agronomic management practices, including irrigation, mineral and organic fertilizer, pesticides, mulch, and legume rotations in supplementary materials.

2.2 Soil property measurement

Soil pH and electrical conductivity (EC) were measured using a pH meter (Mettler-Toledo, Shanghai, China) at a 1:2.5 and 1:5 weight ratios of soil and water, respectively. Soil water content (SWC) and bulk density (BD) were determined as described in a previous study[41]. Soil total carbon (TC) and total nitrogen (TN) were analyzed using a Vario Max element analyzer (Mettler-Toledo, Shanghai, China). Soil organic carbon (SOC) was determined using the dichromate oxidation method. Dissolved organic carbon was extracted using 0.5 mol·L^{-1} potassium sulfate and measured with a total organic carbon analyzer (Multi N/C 3100; Jena, Germany). The molybdenum blue method was used to measure the soil total phosphorus (TP) and available phosphorus (OlsenP) using a UV-Vis spectrophotometer. Nitrite nitrogen and ammonium nitrogen were extracted using 1 mol·L^{-1} KCl (1:10, m:v), and the resulting filtrate was assessed using automated segmented flow analysis (AA3; Seal Analytical GmbH, Norderstedt, Germany). Undried soil samples were used to determine the soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) content using the chloroform fumigation-extraction method. The soil respiration rate (SRR) was calculated by releasing the total amount of C in the glucose-unamended soil over one day of incubation using a ratio mass spectrometer (EA-IRMS; MAT 253, Thermo Finnigan, USA).

We measured the activities of β-1,4 glucosidase (BG), β-1,4-xylosidase (XYL) and cellobiohydrolase (CBH) to represent the activity of C-acquiring enzymes. Activity of β-N-acetyl glucosaminidase (NAG) as a soil N-acquiring enzyme was used to represent the activity of phosphatase (alkaline phosphatase, Phos) as a P-acquiring enzyme using the fluorogenic methylumbelliferone-based substrates method (Symergy H1, BioTek, Winooski, VT, USA). In addition, soil multifunctionality indexes were calculated using the aforementioned data through standardized scores and factor analysis.

2.3 DNA extraction, polymerase chain reaction (PCR) amplification and Illumina sequencing

DNA was extracted from the soil samples using a PowerSoil DNA isolation kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. The extracted DNA was sent to Biozeron Biological Technology Co., Ltd. (Shanghai, China) for PCR amplification and high-throughput sequencing. PCR amplification was performed in triplicate for each DNA sample using universal primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), ITS5-1737F (TCCGTAGGTGAACCTGCGG) and ITS2-2043R (GCTGCGTTCTTCATCGATGC) targeting the V3–V4 hypervariable region of the bacterial 16S rRNA gene and fungal ITS1 using a PCR instrument (GeneAmp 9700, ABI, USA). PCR amplicons were extracted from 2% agarose gels, purified using a QIA quick PCR Purification Kit (QIAGEN, CA, USA) and pooled in equimolar amounts. Purified amplicons were subjected to paired-end sequencing at 250 × 2 using an Illumina MiSeq PE250 platform (San Diego, CA, USA), following established protocols.

2.4 Data processing and analysis

To assess the variation in soil properties, enzyme activities and microbial traits across treatments, mixed effect model and one-way analysis of variance (ANOVA) were employed using SPSS 26.0 (SPSS, Inc., USA) and R software (version 4.4.0). Figures and charts were generated using Microsoft PowerPoint, Origin 2019b (OriginLab, USA) and R package “ggplot2”[42]. The raw sequence files (.fastq) of the samples were analyzed to generate operational taxonomic unit (OTU) tables using QIIME2. Measures of α-diversity, including richness and Shannon, Simpson and Pielou indices, were calculated to estimate species richness and evenness.

The effect size was used to study the response of the soil microbial community and soil properties under different legume rotations. Cohen’s d was used as the effect size to assess and visualize the variations and was calculated i using the R package “esc”[43], which use the formula:

$$\mathrm{D} = \frac{M_1 - M_2}{s} \tag{1}$$

$$s = \frac{\sqrt{(n_1-1)\,{s_1}^2 + (n_2-1)\,{s_2}^2}}{n_1 + n_2 - 2} \tag{2}$$

where, M_1 and M_2 are the means of the two groups compared, s_1 and s_2 are their standard deviations, and n_1 and n_2 are the sample sizes.

Three effect size levels were used to determine the magnitude of the difference detected, that is, small (0–0.2), medium (0.21–0.5) and large (0.51 and above). Greater differences and stronger influences between groups were identified as larger effect sizes[44].

Complementary approaches were used to identify the OTUs responsible for the observed effects, in accordance with Lavoie et al.[45] OTUs that were present in at least 50 samples were filtered, with each sample containing at least one instance of an OTU. We used correlation-based indicator species analysis and likelihood ratio tests with the R packages “indicspecies” and “edgeR”. OTUs, whose abundances were identified as differing between one or more of the different rotations at a false discovery rate corrected value of $P < 0.05$, were classified as responsive to the legume rotation. We defined OTUs confirmed by both indicator species analysis and likelihood ratio tests as legume-sensitive OTUs. Multiple correlation analysis was used to determine the relationship between environmental factors and microbial communities. Correlation analysis, the Mantel test, and principal coordinates analysis (PCoA) were performed using the *vegan* package in R software.

3 Results

3.1 Effects of different rotations on soil properties

The SWC and EC of the faba bean rotation were significantly increased by 29.1% and 75.82%, respectively, compared to other rotations. However, the BD of the faba bean rotation decreased by 5.1% compared to other rotations ($P < 0.001$) (Fig. 1). Soil carbon content exhibits significant differences among rotations via total effect (Fig. 1). The faba bean rotation maintained the highest levels of TC, SOC, and MBC, which increased by 40.9%, 61.6%, and 77.1%, respectively, compared with the other rotations ($P < 0.05$). This is mainly owed to differential and climate variations in regions. In addition, XYL activity in the faba bean rotation decreased compared to other rotations.

Soil nutrient content of the faba bean rotation also remained high compared to other rotations ($P < 0.05$). Faba bean rotation significantly increased TN and TP by 55.9% and 18.9%, respectively. Soil multifunctionality index calculated by standardized scores and factor analysis (Multi1 and Multi2) had a significantly high score in the faba bean rotation (Fig. 2(b) and Table S1). Relationships between faba bean and soil multifunctionality were assessed using linear regression analysis (Fig. 2(c)). Faba bean-based rotation was significantly positively correlated with soil multifunctionality index.

3.2 Changes in soil properties and effect sizes under different rotations

Generally, faba bean-based rotations had positive effects, with medium or greater effect sizes, on both bacterial and fungal α-diversity, compared to all other legume rotations (Fig. 2(a)). However, bacterial richness index in legume-based rotations had negative effects, with greater effect sizes observed ($F = 6.64$, $P < 0.05$, $\eta^2 > 0.14$) (Fig. 2(a), Table S2). Among the legume-

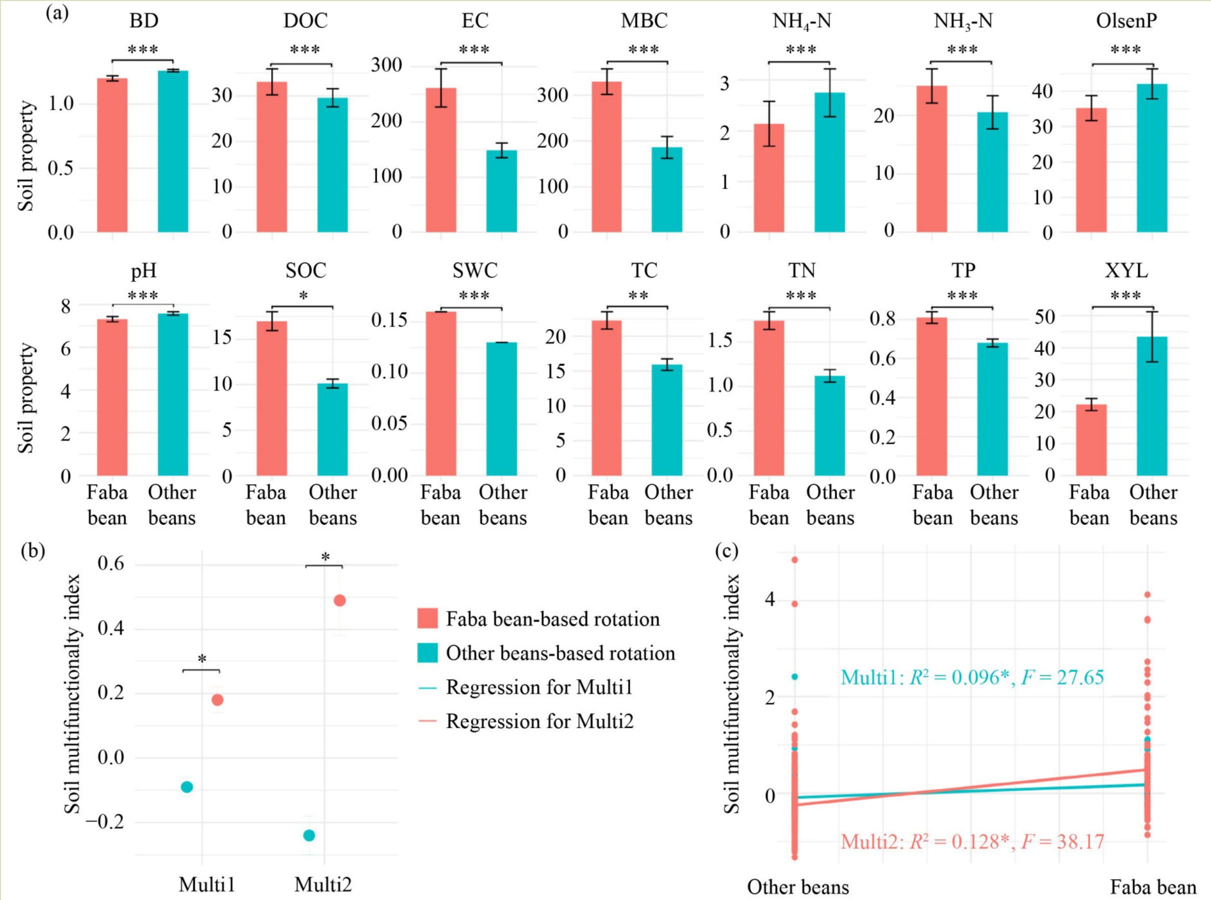


Fig. 1 Soil properties and multifunctionality index under faba bean-based and other rotation systems. (a) Soil physiochemical properties under different rotation systems. (b) Soil multifunctionality index under different rotation systems. (c) Relationships between faba bean-based rotation and soil multifunctionality index. Significance is indicated by * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. BD, Bulk density; DOC, dissolved organic carbon; EC, electrical conductivity; MBC, microbial biomass carbon; NH_4-N, soil ammonia nitrogen content; NO_3-N, soil nitrate nitrogen content; SOC, soil organic carbon; SWC, soil water content; TC, soil total carbon content; TN, soil total nitrogen content; TP, soil total phosphorus content; XYL, β-1,4-xylosidase; Multi1, soil multifunctionality index by standardized scores; and Multi2, soil multifunctionality index by factor analysis.

based rotations, the faba bean rotation had greater soil multifunctionality indices ($\eta^2 > 0.14$) (Fig. 2(a)). With respect to soil nutrient content and microbial activity, faba bean exhibited considerable effect size on SOC, TN, TP, MBC and SRR ($\eta^2 > 0.1$). The effects of different legume-based rotations on other enzymes were only accompanied by small-to-medium effect sizes (Table S2).

The random forest analysis confirmed that region and legume species was correlated with changes in soil properties, with similar importance levels (Fig. 2(b)). We then examined the effects of different legume species on soil properties. Legume species had significant differences in SWC, MBC, SOC and other properties. The faba bean rotation exhibited significantly positive relationships with most selected soil properties. TN, TP, SOC and MBC were also predicted to be significantly important through the random forest analysis (Fig. 2(c)).

3.3 Effects of different rotations on soil microbial community

The data of the diversity index conform to a normal distribution; therefore, ANOVA was performed. Fungal α-diversity was similar across the rotation systems (Fig. 3(a)), with no significant variation observed in the richness, Shannon, Simpson and Pielou indices ($P > 0.05$). However, significant variation was noted in the bacterial richness, Shannon and Pielou indices ($P < 0.05$). The Shannon, Simpson

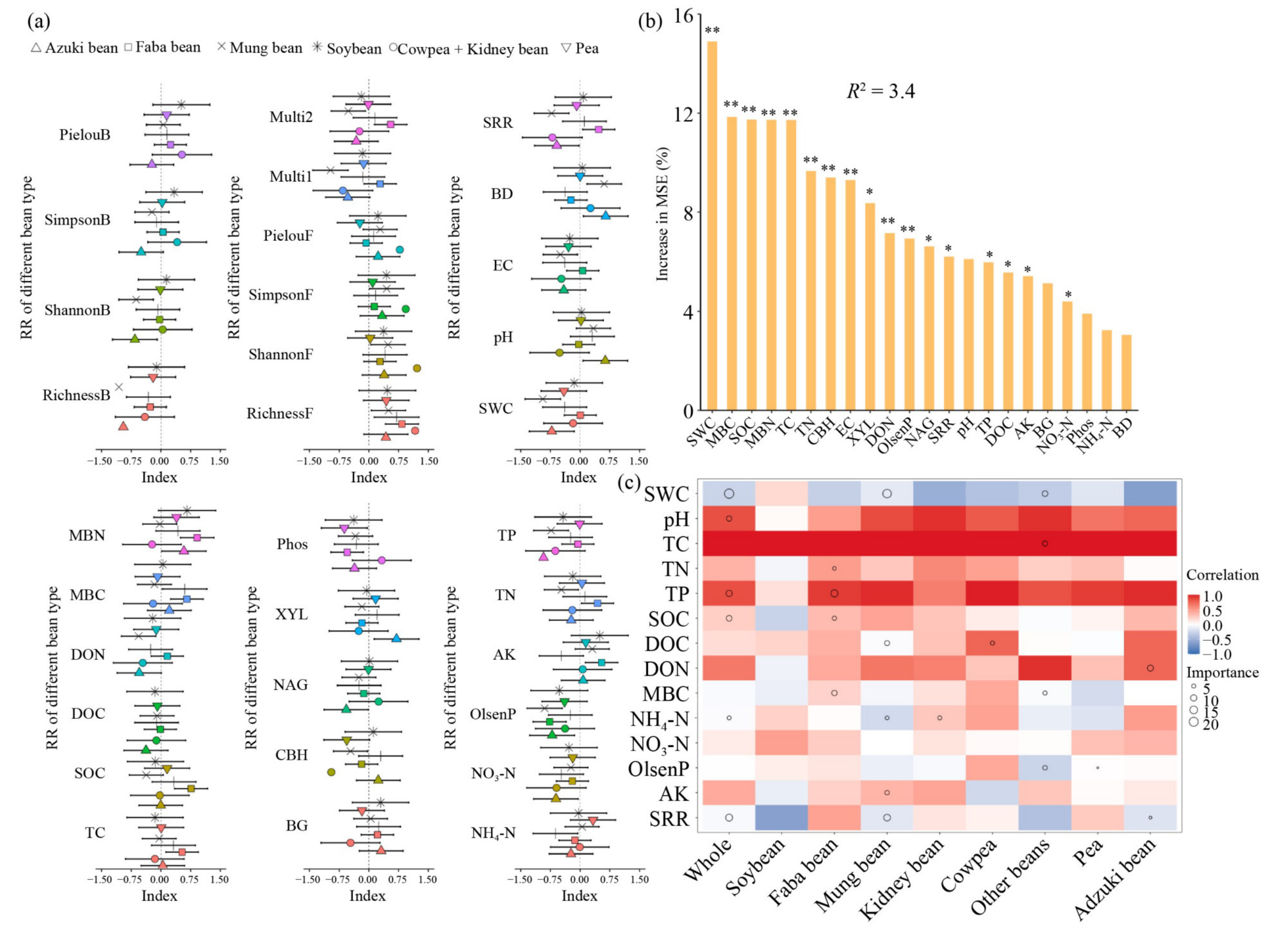


Fig. 2 (a) Effect size of soil properties on different legume rotation compared to other legumes. The error bars are the 95% confidence intervals. The sample sizes of cowpea, faba bean, kidney bean, mung bean, field pea, adzuki bean and soybean for each variable were 9, 87, 21, 62, 19, 21 and 10, respectively. (b) Random forest mean predictor importance of different legume rotation for soil properties. Elevations in the mean squared error (MSE) of variables were used to estimate the importance of these predictors, more important predictors maintained relatively high MSE% values (variation explained was 20.6%, $P < 0.01$). (c) Pearson correlation heatmap between selected soil properties and different rotations with mean predictor importance. AK, available potassium; BD, bulk density; BG, *β*-glucosidase; CBH, cellobiohydrolase; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, soil microbial biomass carbon; MBN, soil microbial biomass nitrogen; Multi1, soil multifunctionality index by standardized scores; Multi2, soil multifunctionality index by factor analysis; NAG, N-acetyl-*β*-glucosaminidase; NH_4-N, soil ammonia nitrogen content; NO_3-N, soil nitrate nitrogen content; Phos, phosphatase, SOC, soil organic carbon; SRR, soil respiration rate; SWC, soil water content; TC, soil total carbon content; TN, soil total nitrogen content; TP, soil total phosphorus content; and XYL, *β*-1,4-xylosidase . * and ** indicate $P < 0.05$ and $P < 0.01$, respectively.

and Pielou indices of the adzuki bean rotation were the lowest whereas the richness index of other rotations (2650 ± 110), the Shannon index of the soybean rotation (10.2 ± 0.13), and the Simpson index of the cowpea rotation (1.0 ± 0.00) were the highest. The *β*-diversity of bacterial and fungal communities under different legume-based rotation were assessed using PCoA (Fig. 3(b,c)). Bacterial community composition was divided by legume species, and PERMANOVA confirmed that legume species significantly altered bacterial community composition. Although, fungal statistics were non-significant. Bacterial PCoA1 and PCoA2, fungal PCoA1 and PCoA2 explained 43.0% and 23.5%, 57.8% and 24.1% of the variance, respectively, with a cumulative explanatory power of 66.5% and 81.9%.

The soil samples analyzed in this study were dominated by Actinobacteria (33.8%), Proteobacteria (21.0%), Chloroflexi (15.4%), Acidobacteria (10.1%), Planctomycetota (5.9%), and other bacterial phyla, as shown in Fig. 4. The combined abundance of Actinobacteria was greater in legume-based

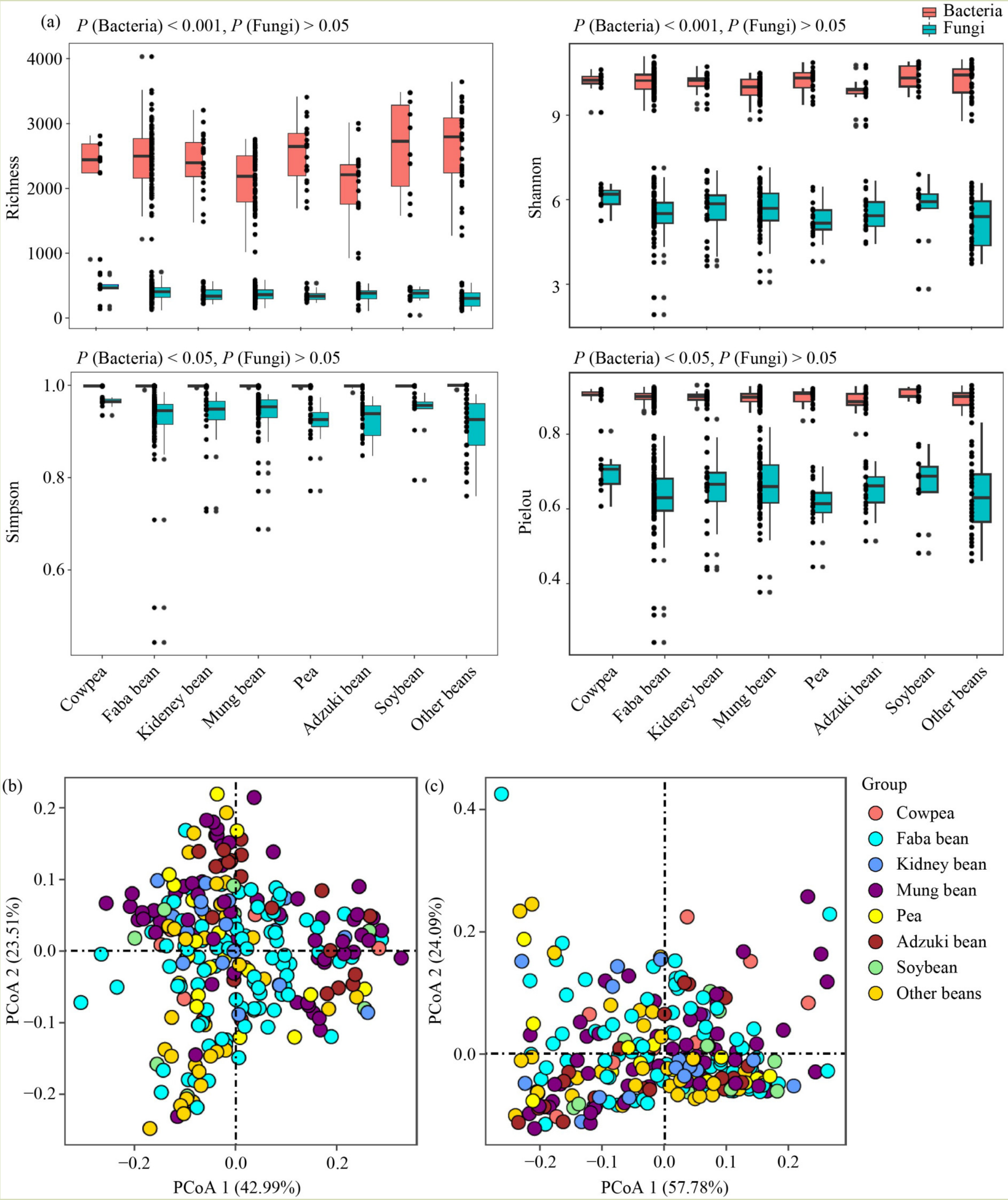


Fig. 3 Bacterial and fungal α-diversity of soil under different legume rotations (a), principal coordinate analysis of bacterial (b) and fungal (c) community composition based on Bray-Curtis distance different rotation systems.

rotations than in the other legume rotations. On average, Actinobacteria abundance in legume-based rotations increased by 18.3% compared with other legume rotations ($P < 0.05$). The abundance of Firmicutes decreased by an average of 40.3% under legume-based rotations compared with the other legume rotations ($P < 0.05$). The soybean rotation had the highest abundance of Planctomycetota (6.92%), whereas the lowest abundance was observed in the adzuki bean rotation (4.18%)

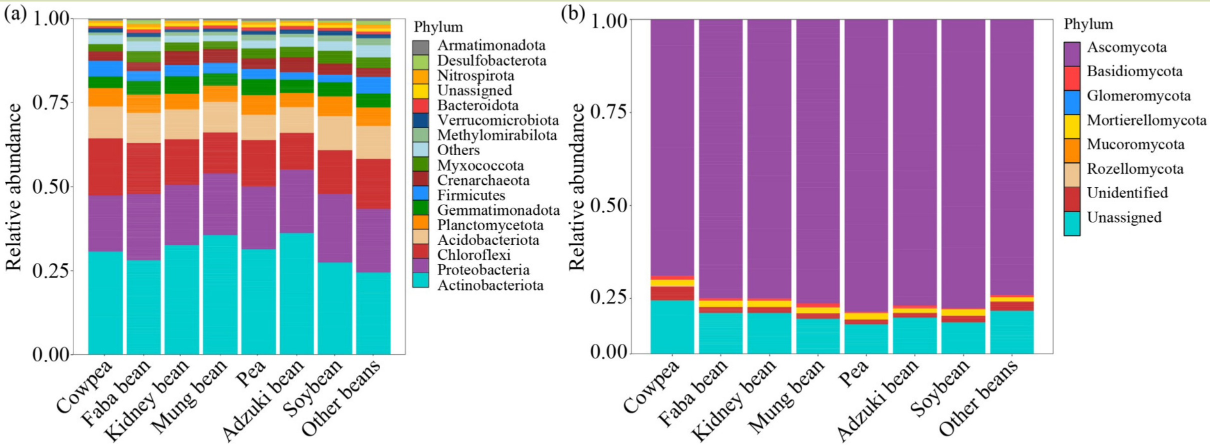


Fig. 4 Bacterial (a) and fungal (b) phylum level composition under various legume rotations.

(Fig. 4). Nine fungal phyla were identified which were Ascomycota (> 80%), an unassigned phylum (> 10%), Mortierellomycota (> 1%), Sordariomycetes (> 1%), an unidentified phylum (> 1%), Basidiomycota (~0.9%), Glomeromycota (~0.1%), Rozellomycota (~0.05%) and Mucoromycota (~0.03%).

To investigate microbial association, a bacterial and fungal co-occurrence network was built based on Spearman's correlation among phyla (Fig. 5(a)). The network was composed of 34 phyla (29 bacterial phyla and 5 fungal phyla) and 90 edges (78 bacterial-bacterial connections, 6 bacterial-fungal connections, and 6 fungal-fungal connections). The nodes revealed the dominant phyla like Desulfobacterota, Latescibacterota, NB1.j, Zixibacteria, and MBNT15.

Rotation changed the soil co-occurrence network, and the modularity class and community members varied under different legume crop treatments (Fig. S2, Table S3). The soybean and other beans rotations tended to have larger networks with more nodes than the other rotations. The number of communities in the legume-based rotation in the modularity calculation was higher than that of other rotations, which had only two (Fig. S2).

We identified phyla with a high level of degree, eigenvector centrality, closeness centrality and betweenness centrality in the network as the keystone phyla. Gemmatimonadota, NB1.j, and Proteobacteria were the key phyla in the cowpea, field pea, and soybean rotations, respectively (Fig. 5(a), Table S4). Desulfobacterota is regarded as the keystone phylum of faba bean, mung bean and other beans rotations. In addition, these rotations had different bridge phyla, such as Latescibacterota, Planctomycetota, and Hydrogenedentes, which had the highest betweenness centralities. The kidney bean and adzuki bean were found to contain Proteobacteria and Armatimonadota as keystone phyla and Methylomirabilota and Planctomycetota as bridge phyla (Table S4).

Legume species was correlated with fungal and bacterial diversity, and bacterial structure. In addition, legume species exhibited a significant spearman correlation with TC, SOC, TN, AK, MBC, MNB and SRR. Fungal diversity and structure were strongly correlated with four environmental factors. Meanwhile, bacterial diversity and structure were correlated with six factors, indicating that bacteria responded relatively strongly to different legume rotations (Fig. 5(b)). Compared with fungal diversity, fungal structure, and bacterial diversity and structure are more sensitive, so could be used as traceability indicators.

The contents of SOC, SWC, TN and TP significantly influence bacterial community differences, with relevant bacteria listed (Fig. 5(c)). In addition to the aforementioned factors, fungi are influenced by available nitrogen and potassium, but only three phyla had significant correlations with soil properties (Fig. 5(d)).

We then examined the distribution patterns of legume-sensitive OTUs in the meta-co-occurrence patterns of bacteria in legume-soil communities (Fig. 6(a)). The effect of different legumes on soil communities was apparent with five discrete modules (Modules 1, 3 and 5–7) in the soil network containing legume-sensitive OTUs specific to different legume crops. We observed that the five modules were separated from each other and each module had its own legume-sensitive OTUs. The

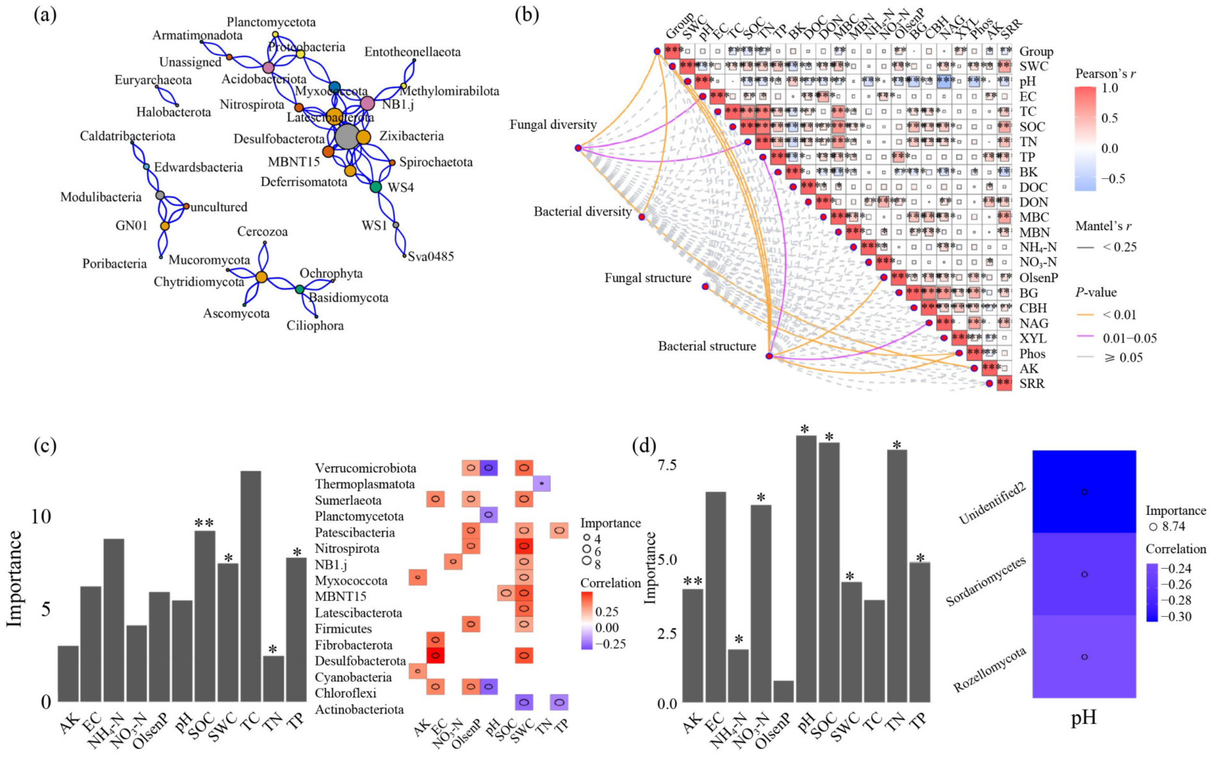


Fig. 5 (a) Co-occurrence network of microbial communities. Each edge denotes a robust and significant correlation, and the size of each node is proportional to its degree (FDR-adjusted $P < 0.01$). Blue edges refers Spearman's $\rho > 0.6$ and red edges < −0.6. The colors of the nodes indicate the different dominant microbial phyla. (b) Spearman correlations and Mantel test between environmental factors and microbial communities. The lines connecting from environmental factors to microbial communities indicate their correlation. The solid squares show the correlation between environmental variables. (c) Random forest mean predictor importance and Pearson correlation heatmap with mean predictor are important to soil properties and bacterial communities. (d) Random forest mean predictor importance and Pearson correlation heatmap with mean predictor importance between soil properties and fungal communities. AK, available potassium; BG, *β*-glucosidase; BD, bulk density; CBH, cellobiohydrolase; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; EC, electrical conductivity; MBC, soil microbial biomass carbon; MBN, soil microbial biomass nitrogen; NAG, N-acetyl-*β*-glucosaminidase; NH_4-N, soil ammonia nitrogen content; NO_3-N, soil nitrate nitrogen content; Phos, phosphatase, SOC, soil organic carbon; SRR, soil respiration rate; SWC, soil water content; TC, soil TC content; TN, soil total nitrogen content; TP, soil total phosphorus content; and XYL, *β*-1,4-xylosidase. *, **, and *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

legume species was the main driver in soil communities, and numerous legume-sensitive OTUs were mainly located in Module 3. Module 1 contained partial cowpea-specific OTUs, whereas Modules 5–7 primarily maintained favorable bean-specific OTUs (Fig. 6(b)). Legume-sensitive OTUs are identified at phylum level (Fig. 6(c)). Bacterial community composition of legume-sensitive OTUs were obviously separated into different dimensions (Fig. 6(d)). PCoAs 1 and 2 explained 64.6% and 21.9% of the variance with a cumulative explanatory power of 86.6%. And most phyla are correlated with indicators of soil nutrient cycling processes (Fig. 6(e)).

In summary, the faba bean rotation enhanced both soil properties and relative bacterial communities relative to other beans rotations investigated. The rotation also exhibited significantly increased soil TC, TN, TP and SOC, and in the meantime exhibited relatively high SRR, MBC and *α*-diversity. In addition, Desulfobacterota and Planctomycetota were enriched in faba bean rhizosphere (Fig. 7(a)). The structural equation models accounted for 90% of the variance in soil multifunctionality. Legume species had indirect effects on soil multifunctionality through their negative correlations with soil properties and nutrient content. Soil properties and nutrient content positively directly influenced soil multifunctionality, whereas indirectly influence microbial community affected soil multifunctionality. (Fig. 7(b)).

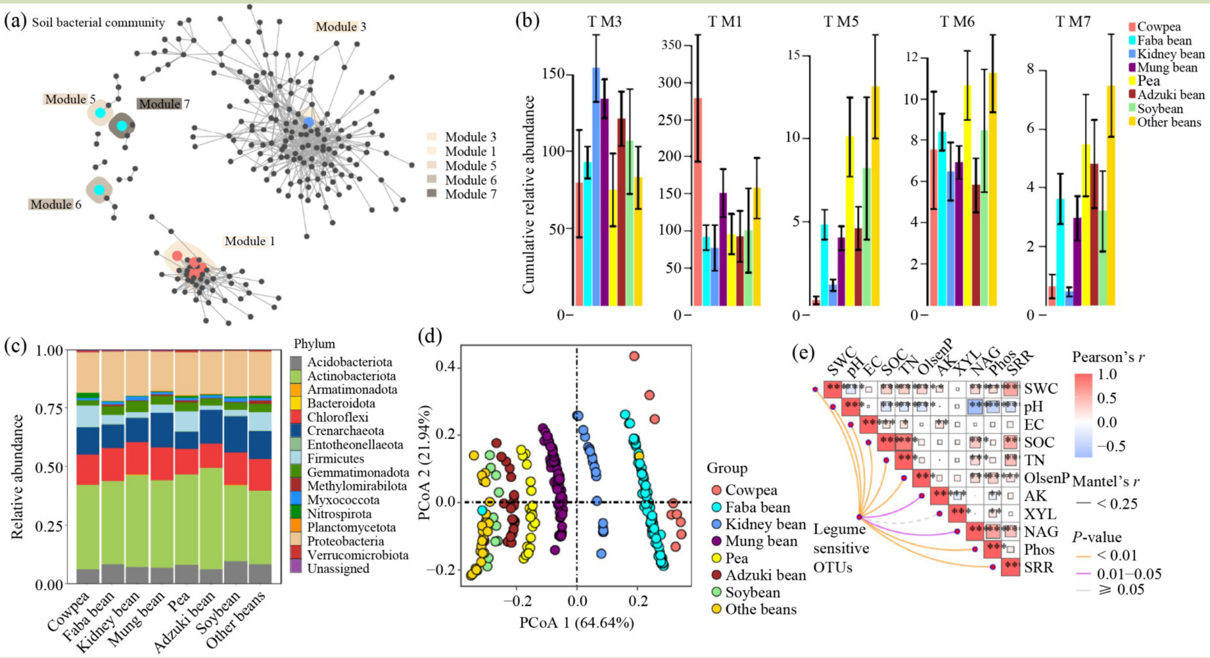


Fig. 6 Co-occurrence patterns of legume-sensitive OTUs. (a) Co-occurrence networks visualizing significant correlations ($P > 0.7$, $P < 0.001$; indicated with gray lines) between bacteria OTUs in different rotation communities. (b) Cumulative relative abundance (as counts per million, CPM; *y*-axis in × 1000) of all bacteria of the rotation-sensitive modules in different rotation networks. (c) Qualitative taxonomic composition of legumes sensitive OTUs is reported as proportional OTUs numbers per phylum rotation by rotations. OTU, operational taxonomic unit. (d) Principal coordinate analysis of legume sensitive OTUs based on Bray-Curtis distance different rotation systems. (e) Mantel test between bacterial legumes sensitive OTUs and environmental factors. AK, available potassium; EC, electrical conductivity; NAG, N-acetyl-*β*-glucosaminidase; Phos, phosphatase, SOC, soil organic carbon; SRR, soil respiration rate; SWC, soil water content; TN, soil total nitrogen content; and XYL, *β*-1,4-xylosidase. *, **, and *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

4 Discussion

4.1 Soil properties and soil multifunctionality improved better in faba bean-based rotation than other legumes-based rotation

In this study, we investigated soil properties and soil multifunctionality under varying rotation systems. Our results revealed significant variation in the soil physicochemical properties and soil multifunctionality across the different rotation systems. The faba bean rotation resulted in superior soil properties and soil multifunctionality. Owing to the extensive sampling area, we used the mixed-effects model and random forest analysis to determine the effect of legume species, climate and regional differences (Fig. S3 and Table S5). Legume species and region were both contributed to soil variation, while climate was correlated TC, MBC, MBN, BG, CBH and TP (Fig. 2 and Table S5). In addition, soil multifunctionality are significantly higher in faba bean rotation than all other rotations due to the legume species effect (Fig. 1 and Table S5). Lavoie et al. found that soil respiration varied with soil temperature and nitrogen limitation, with MAT exhibiting optimal predictability for soil respiration in winter. This result indicated an association between MAT and SOC. MAP was positively correlated with TP, TC, precipitation-related soil erosion, and soil fertility in Inner Mongolia. The linear model indicated that MAP and MAT were not suitable for higher values, indicating that MAP and MAT in the faba bean rotation were important factors influencing soil properties[45–47].

The positive impacts of the rotation or faba bean on soil multifunctionality and maintaining SOC, nitrogen cycling, phosphorus cycling and water savings under diversified cropping have been widely reported[46–49]. Similarly, in our study, faba bean significantly increased the soil multifunctionality, soil water content, total carbon, SOC, total nitrogen and total phosphorus, potentially benefiting soil fertility and water-use efficiency (Fig. 1). Soil pH is a key factor in shaping microbial communities, activities and controlling C turnover[50]. However, the influence of pH in the present study

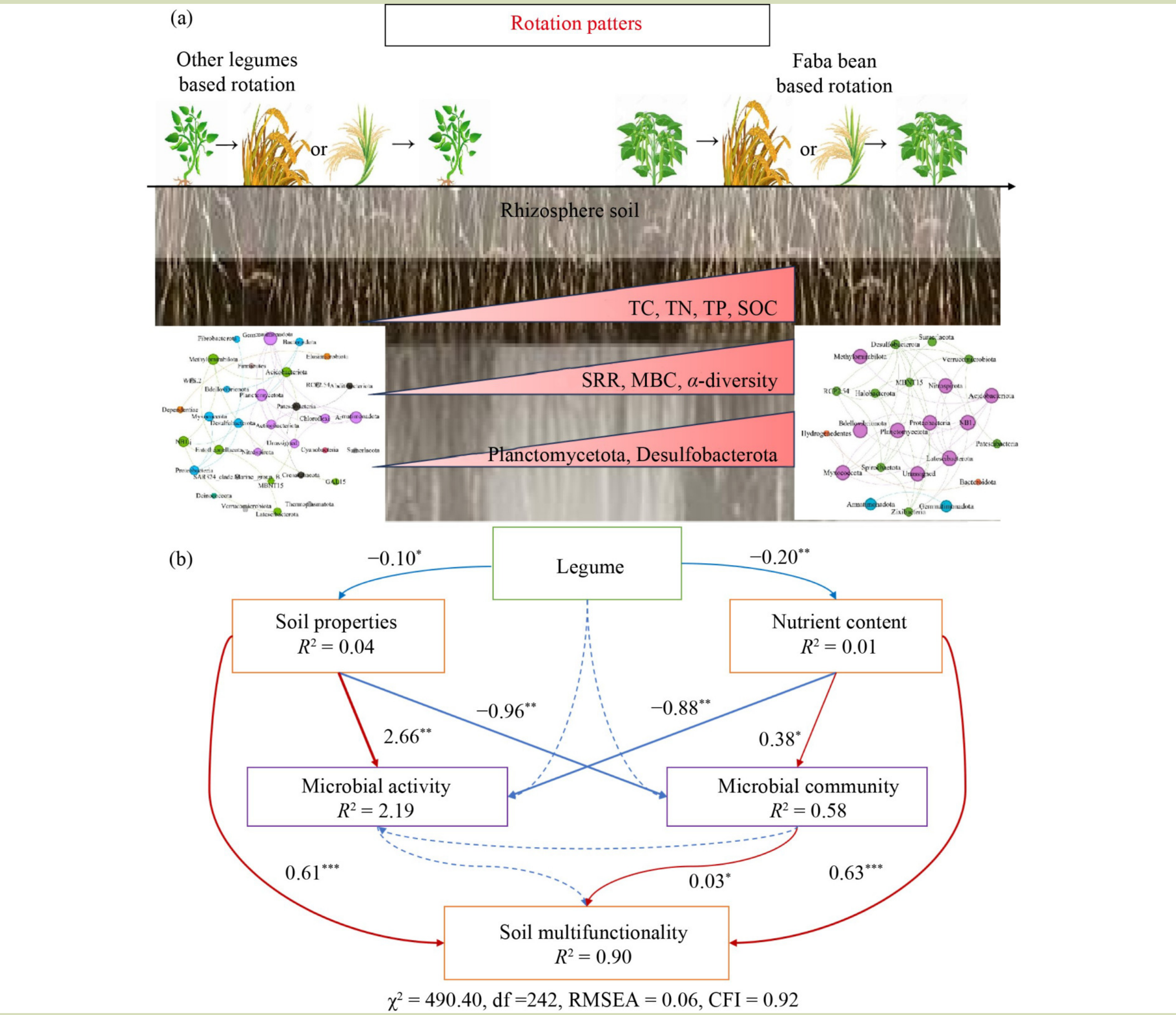


Fig. 7 Schematic diagram of legume crops on soil properties and microbial communities. (a) Effects of faba bean-based rotations is better than all other legumes on soil properties and relative communities. (b) Structural equation models reveal the direct and indirect effects of different legume species, soil physicochemical properties (soil properties and nutrient content), microbial attributes (microbial activity and microbial community) on soil multifunctionality index. Numbers adjacent to arrows are the path coefficients and are indicative of the effect size of the relationship. Continuous and dashed arrows represent significant and non-significant effects, respectively. R^2 explained the proportion of variance. Significance is indicated by $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. MBC, soil microbial biomass carbon; SOC, soil organic carbon; SRR, soil respiration rate; TC, soil total carbon content; TN, soil total nitrogen content; and TP, soil total phosphorus content.

was less important than that of region and legume differences. The high microbial biomass and specific respiration rate in the faba bean systems likely explain the increased nutrient content in these soils (Fig. 1). This indicates a relatively high level of microbial activity and greater potential for organic nitrogen and phosphorus mineralization. Legumes such as faba bean can enhance the formation and stabilization of soil aggregates, thereby protecting SOC from mineralization[51]. In contrast, the barley significantly reduced SOC content because of the dominant role of barley in SOC mineralization. Different leguminous plants within a crop rotation have diverse effects on soil properties, particularly by modifying soil microbial structures[51].

4.2 Legumes-based rotation differently alter soil properties and soil multifunctionality through microbial diversity, community composition and co-occurrence network

Legumes-based rotations are climate-smart agricultural practices and positively affect microbial activity and diversity[52,53]. Microbial activity and diversity has been used to identify soil multifunctionality in different biomes[54]. Similar to the faba bean-based rotation, the kidney bean-based rotation also maintained high levels of SRR and MBC as the soil nutrient content increased (Table S1 and Table S2). The α-diversity of the faba and kidney bean-based rotation was significantly high compared to that of the other legume rotations ($P < 0.05$). This result indicated that faba and kidney bean-based rotation improved soil physical and chemical properties create a favorable niche for microbial growth[55]. Faba and kidney bean-based rotation rotations enhance soil quality and positively affect microbial communities[11]. However, the positive effect of the kidney bean-based rotation on soil properties and soil multifunctionality was substantially smaller than that of the faba bean-based rotation. The differences in soil nutrient content between the two rotations were not statistically significant (Table S1). We investigated two microbial community structure and found that both dominant phyla were Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria and Planctomycetota (Fig. S4). There is no significance on phyla and species level among legume-based rotation.

Co-occurrence networks can help expand analytical data for more useful information[56]. Keystone phyla are crucial for maintaining the stability and diversity of an ecosystem[57]. Keystone phyla do not have the highest relative abundance or biomass in an ecosystem. However, they make an irreplaceable contribution to the functioning of an entire ecosystem. At the phylum level, it was found that the legume-based rotation has the important nodes like Desulfobacterota, Latescibacterota, NB1.j, Zixibacteria and MBNT15. This might have resulted from soil-microbe, plant-microbe or microbe-microbe interactions[58].

In contrast to the soybean-rotation soil, the networks in other sampled soils remained relatively simple, and the MBN content in the soybean soil was extremely high. Nitrospirota, MBNT15, and Planctomycetota were significantly enriched in soybean soil, which illustrates the patterns reoccurring for soybean ($P < 0.05$). N-N-related cycling bacteria in soil were recruited, increasing network connectivity and complexity, and the available N content. Similar increases in soybean have been observed in other experiments[59,60]. MBNT15 abundance was linearly related to SRR and NAG, which could explain the higher N-related microbial activity in the soybean-based rotation.

Of the different legume meta-networks, we identified modules containing high proportions of OTUs that responded to legume crops. We found that legume-sensitive OTUs occupying five modules reflected the effects of different legume species on soil microbial communities (Fig. 6(a,b)). These also partially explained the changes in soil properties and microbial communities in the faba bean-based rotation. The 1118 legume-sensitive OTUs were identified as 224 legume-sensitive species, which were usually distributed in the top 16 phyla (Fig. 6(c)). This indicated that the legume species affected the highly co-occurring soil microorganisms. This also fits the situation of changing keystone and bridge phyla. Further experiments are required to identify how keystone- or legume-sensitive species directly and indirectly influence the microbiome, thereby affecting soil health.

4.3 Keystone and sensitive OTUs in co-occurrence networks under different legumes

Based on bacterial and fungal phylum level composition, we performed a random forest analysis, which indicated that Actinobacteriota, Fimicutes, MBNT15 and Verrucomicrobiota were correlated with relatively more important soil nutrient content (Fig. 5). The linear model results revealed a linear correlation between Desulfobacterota and OlsenP and Phos activity. Planctomycetota was linearly correlated with the available nitrogen content and NAG activity ($P < 0.05$, Fig. S2). MBNT15 was correlated with TC, SOC, SRR, MBC and NAG activities ($P < 0.05$, Fig. S2 and Table S5). Combining the results of co-occurrence networks, we conclude that Desulfobacterota and Planctomycetota make multifunctional contributions to nutrient mineralization, nitrogen fixation and plant growth promotion. The phylum Desulfobacterota and an unassigned phylum that were significantly enriched in the faba bean-based rotation were not in the top list for the kidney bean-based rotation ($P < 0.05$, Fig. S5). Desulfobacterota had a positive correlation with TN, OlsenP, SOC, Phos and SRR ($P < 0.05$, Fig. S2). This might explain the relatively high microbial activity observed in the faba bean-based rotation. Fang et al. also reported a higher abundance of Desulfobacterota, which was positively correlated with soil nutrients under crop rotation[61]. Desulfobacteria are pivotal for soil biogeochemistry[62] and in the degradation of organic matter in the soil, thereby releasing nutrients such as carbon, nitrogen and phosphorus required for plant growth[63,64].

Planctomycetota, another phylum that was significantly correlated with soil nutrient levels, decreased in the adzuki bean-based rotation. Adzuki bean-based rotation had significantly lower SOC, TN, TP and OlsenP contents with a lower relative abundance of Planctomycetota (Table S2). This might also indicate that Planctomycetota is an important contributor to bean nutrient cycling. A 19-year study revealed that Planctomycetota was enriched in the rotation rotation and might be related to ammoxidation, and carbohydrate and polysaccharide metabolism[59,65].

The faba bean-based rotation enhanced soil properties and relative bacterial communities to a greater extent than the other rotations investigated. The effect size indicated that the variation was species- and management-independent. Correlation analysis and random forest demonstrated that soil properties and relative bacterial communities that interacted through mutual influence and mutual promotion (Fig. 5(c,d) and Fig. S4). However, the mechanisms of the nutrient improvement in faba bean-based rotation and the variable impacts of different legume-based rotations on soil properties are still unknown. More detail and experiments are needed to explore these questions for sustainable and health soil and ecosystems.

5 Conclusions

This study has contributed to further understanding of the complex interactions between legumes and associated microbial communities and the potential impacts of faba bean-based crop rotations on soil multifunctionality. The faba bean-based rotation increased physical properties and nutrient content, but this was dependent on region and climate. Also, the faba bean-based rotation increased the abundance of Desulfobacterota and Planctomycetota, and microbial activity which might mediate the positive effects of faba bean-based rotation on soil multifunctionality. These findings enhance knowledge about the interactions of microbiome and soil multifunctionality under faba bean-based rotation, and indicates that legume rotation, particularly faba bean rotation, can promote sustainable agricultural practice and aid in the development of crop rotation strategies to enhance soil quality and ecosystem services.

Supplementary materials

The online version of this article at https://doi.org/10.15302/J-FASE-2025604 contains supplementary materials (Figs. S1–S5; Tables S1–S5).

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Compliance with ethics guidelines

Yixuan Chen, Zhijie Dong, Qiong Liu, Kailu Zhang, Ruohan Yin, Tida Ge, and Zhenke Zhu declare that they have no conflict of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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