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Effects of pepper–maize intercropping on the physicochemical properties, microbial communities, and metabolites of rhizosphere and bulk soils

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Abstract

Background Intercropping increases land use efficiency and farmland ecological diversity. However, little is understood about whether and how soil biota, metabolites, and nutrients change under interspecific competition among plants. Thus, this study aimed to explore the changes in the physicochemical properties, microbial communities, and metabolites of rhizosphere and bulk soils of pepper monocropping and pepper–maize intercropping systems.

Results Intercropping significantly increased the contents of available phosphorus (AP) and available potassium (AK), and decreased the pH value, whereas it had little effect on the total nitrogen (TN) and organic matter (OM) in the rhizosphere and bulk soils, compared with those in monocropping pepper. Moreover, the OM content was higher in rhizosphere soil than in bulk soil. The microbial community structures and metabolite profiles also differed between the two systems. The diversity of bacteria and fungi increased in intercropped pepper. The relative abundances of *Actinobacteria*, *Chloroflexi*, *Cyanobacteria*, and *Ascomycota* were higher while those of *Proteobacteria*, *Planctomycetes*, *Mucoromycota*, and *Basidiomycota* were significantly lower in the rhizosphere and bulk soils from the intercropping system than in those from the monocropping system. Linear discriminant analysis revealed that the predominant bacteria and fungi in the rhizosphere soil from the intercropping system belonged to the order *Sphingomonadales* and genera *Nitrospira*, *Phycicoccus* and *Auricularia*, whereas those in the bulk soil from the intercropping system belonged to the phylum *Acidobacteria* and genera *Calocera*, *Pseudogymnoascus*, and *Trichosporon*. Intercropping promoted the secretion of flavonoids, alkaloids, and nucleotides and their derivatives in the rhizosphere soil and significantly increased the contents of organoheterocyclic compounds in the bulk soil. Furthermore, the AP and AK contents, and pH value had strong positive correlations with bacteria. In addition, co-occurrence network analysis also showed that asebogenin, trachelanthamidine, 5-methyldeoxycytidine, and soil pH were the key factors mediating root-soil-microbe interactions.

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Conclusion Intercropping can alter microbial community structures and soil metabolite composition in rhizosphere and bulk soils, enhancing soil nutrient contents, enriching soil beneficial microbes and secondary metabolites (flavonoids and alkaloids) of intercropped pepper, and provided a scientific basis for sustainable development in the pepper-maize intercropping system.

Keywords Pepper, Intercropping, Rhizosphere and bulk soil, Soil nutrient, Bacterial and fungal community structures, Soil metabolite profiles

Background

Intercropping, also known as mixed cropping, involves simultaneously growing more than one species on a field. It has great potential for enhancing water and nutrient use efficiency and improving plant productivity, yield stability, and resilience to biotic and abiotic stresses, including those triggered by climate change [1]. The yield advantage of cereal and legume intercropping systems is evident in various intercropping patterns [2–5]. Intercropping pepper (*Capsicum annuum* L.) with other crops is an effective and economic measure to restrict the spread of *Phytophthora capsici* across rows in the soil [6, 7]. Understanding the critical roles of underground interspecific interactions is important to regulate the nitrogen (N) cycling of plant–soil systems and mediate the mineralisation of soil organic matter (OM) [8–10].

The rhizosphere is a key area for roots to obtain water and nutrients, and it interacts closely with soil physical, chemical, and biological components [11]. However, continuous monocropping and unreasonable fertilisation disrupt the balance of soil–microbe–plant relationships and alter soil physicochemical and biological characteristics [12, 13]. Plant roots release exudates, which are the main nutrient source driving bacterial communities and activities [14]. Thus, root–soil interactions are critical for soil health, sustainable food security, and resource use efficiency [15].

Intercropping improves soil nutrient cycling and land productivity by regulating microbial community activities [16]. In maize (*Zea mays*) -peanut (*Arachis hypogaea*) intercropping system, the diversity and richness of bacteria and fungi decrease in maize rhizosphere soil, whereas the richness of fungi increases in peanut rhizosphere soil [8]. The abundance of beneficial microbes (*RB41* and *Chaetomium*) in the rhizosphere and the nitrogen content of maize are also increased when intercropped with peanut [8]. Apple (*Malus pumila*)-marigold (*Tagetes erecta*) intercropping significantly increases the relative abundance (RA) of plant growth-promoting bacteria, such as *Rhizobiales*, *Pseudomonadales*, and *Bacillales*, in rhizosphere soils. Moreover, the amount of carbohydrates is higher in intercropping systems than in monocropping systems [17]. Many studies reported that intercropping improves yield and resistance against soilborne Phytophthora disease in pepper [6, 7]. However, few studies have clearly demonstrated the interactions between the microbial community structure and the soil metabolite profiles during intercropping. Specifically, the effects of maize–pepper intercropping on the nutrients, microbial communities, and metabolites in the soil remain unclear.

Considering that various intercropping patterns differentially affect soil physicochemical properties and microbial characteristics, we aimed to examine the changes in the bacterial and fungal communities in the pepper–maize intercropping system. We hypothesised that intercropping would greatly affect soil physicochemical properties, increase microbial diversities, and alter microbial community structures. Thus, the objectives of this study were to (1) explore the effects of pepper–maize intercropping on soil physicochemical properties and metabolite profiles; (2) compare the responses of bacterial and fungal diversities and community composition to intercropping with monoculture plantations of pepper; and (3) determine the relationships between soil microbial communities and soil physicochemical properties and metabolite profiles.

Materials and methods

Experimental site and soil sample collection

The experimental site was in Dali City, Yunnan Province, China (26°12'79" N, 99°96'91"E). This region has a subtropical climate with an annual precipitation of 719.2 mm and an average annual temperature of 14.2 °C. The soil at the test site was brown and loamy soil. Six core soil samples were collected from each cropping ridge using a soil sampler and mixed as one replication sample per group (Additional file 1: Fig. S1). Thus, 12 samples were collected and divided into four groups with three replications each: rhizosphere soil of the pepper–maize intercropping system (IPr1, IPr2, and IPr3), bulk soil of the pepper–maize intercropping system (IB1, IB2, and IB3), rhizosphere soil of the pepper monocropping system (MPr1, MPr2, and MPr3), and bulk soil of the pepper monocropping system (MB1, MB2, and MB3) (Additional file 1: Fig. S1). The effects of planting pattern on the physicochemical properties, microbiome, and metabolites of the soil samples were then evaluated. All samples were placed in an ice box and brought to the laboratory. Each soil sample was passed through a 2 mm sieve and then divided into two subsamples: one portion was used for the determination of soil physicochemical properties,

whereas the remaining portion was stored at −80 °C for subsequent microbiome and metabolome analyses.

Determination of soil properties

The physicochemical properties of the soil samples were analysed as previously described [18, 19]. Soil pH was determined using pH meter (FE28, METTLER-TOLEDO, USA) with a soil-to-water ratio of 1:5 (wt/vol). Available phosphorus (AP) was assessed using the ascorbic acid reductant method (SpectraMax 190 Microplate Reader, Molecular Devices, USA), and available potassium (AK) was determined through atomic absorption (Atomic absorption spectrometer zeenit 700Q, Jena, Gemany). Samples were combusted by high-temperature reactor (SPH120 Digestion System, Jnan Alva Instrument Co., LTD, China), after used to detect total nitrogen (TN) concentration by Kjeldahl method (KN-520 Automatic Kjeldahl Nitrogen Analyzer, Jnan Alva Instrument Co., LTD, China). The dichromate oxidation (Titrette® 50 mL Digital Bottletop Burette, Brand, Germany) and external heating method (HH-S Thermostatic Lab Digital Oil Bath, Changzhou LangYue Instrument Co., LTD, China) was applied to explore the OM content of the soil.

DNA extraction, library construction, and metagenomic sequencing

Soil DNA was extracted from 0.5 g of soil using the EZNA® Soil DNA Kit (Omega Biotek, Inc., Norcross, GA, USA) in accordance with the manufacturer's instructions. The concentration of DNA was measured using the NanoDrop 2000-UV spectrophotometer (Thermo Scientific, Waltham, MA, USA), and the quality of DNA was monitored on 1% agarose gels. For the library construction, 1 μg of DNA per sample was used. Sequencing libraries were generated using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations. Briefly, the DNA samples were fragmented by sonication to a size of 350 bp. DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing with further PCR amplification. Finally, the PCR products were purified (AMPure XP system, Beckman Coulter, Brea, CA, USA), and libraries were analysed for size distribution on an Agilent2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and quantified using real-time PCR. After cluster generation of the index-coded samples, the libraries were sequenced on an Illumina NovaSeq platform (Illumina PE150), and paired-end reads were generated.

Metagenome assembly, gene prediction, and functional annotation

To obtain high-quality clean data for subsequent analyses, we trimmed the raw data from the Illumina PE150 platform by using Readfq (Version 8.0, https://github.com/cjfields/readfq). Briefly, sequences with low-quality bases (default quality threshold value ≤ 20), N bases reaching 10 bp long, and final lengths < 50 bp were removed. The obtained clean data were assembled and analysed using MEGAHIT software (Version 1.0.4) [20]. Then, the assembled Scaftigs were interrupted from the N connection, and the Scaftigs without N were retained. Scaftigs longer than 500 bp were used to predict the open reading frame. The metagenomic DNA sequences were assigned taxonomic labels using the Kraken 2 program [21], and then the abundance of microbes in each sample at different phylogenetic levels (phylum, class, order, family, genus, and species) was estimated using Bracken (Bayesian Reestimation of Abundance after Classification with KrakEN) [22]. The clean reads were BLAST searched against the Uniref90 database using Humann2 software (based on Diamond) [23]. The annotation information and RA table from each functional database were obtained according to the corresponding relationship between Uniref90 ID and each database [24, 25].

Metabolite profiling and analysis

The soil samples were homogenised for 1.5 min at 30 Hz in a mixer mill (MM 400, Retsch, Hann, Germany) containing zirconium beads. A 100 mg sample from each replicate was weighed and extracted overnight at 4 °C with 1.2 mL of aqueous methanol (70%). After centrifugation at 12,000 rpm for 10 min, the extracts were filtered and subjected to ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

The identified metabolites were annotated through the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and then mapped to the KEGG pathway database. Metabolite enrichment analysis was conducted using MetaboAnalyst (Version 6.0), employing the hypergeometric test. Variable significance in projection, VIP > 1, and absolute Log2fold change > 1 were used to assess whether the metabolites were significantly regulated across the groups. VIP values were extracted from the orthogonal partial least squares discriminant analysis (OPLS-DA) results using the MetaboAnalyst (Version 6.0).

Statistical analysis

Principal coordinate analysis (PCoA) based on a Bray–Curtis distance matrix was conducted to visualize the distribution of microbial community in soils with different planting patterns. Principal Component Analysis (PCA) was performed using the data, which was scaled and logarithmically transformed by the SIMCA software package (Version 16.0.2), to investigate the changes in metabolites in soils with different planting patterns. Differential metabolites were screened using OPLS-DA.

Linear discriminant analysis with effect size (LEfSe) was conducted, and heat maps were drawn using the OECloud tools (https://cloud.oebiotech.com/#/bio/tools?cat_id&tag_id&search). Spearman's correlation analysis of metabolites and dominant microbes, and co-occurrence network analysis were performed using the OmicShare tools (http://www.omicshare.com/tools). Kruskal–Wallis tests ($p<0.05$) (http://cloudtutu.com.cn/) were used to compare the abundances of dominant microbes in the samples at the phylum and genus levels. Mantel test correlation analysis of soil physicochemical properties and soil microorganisms was completed using the Wekemo Bioincloud (https://www.bioincloud.tech) [26]. Statistical analyses also were performed using SPSSAU (Version 24.0) (https://www.spssau.com.) and GraphPad Prism 8.0.

Results

Intercropping affects the physicochemical properties of rhizosphere and bulk soils

The variations in edaphic factors in the rhizosphere and bulk soils under the two cultivation systems are shown in Table 1. The nutrient contents in the rhizosphere and bulk soils were significantly higher ($p<0.01$) in the intercropping system than in the monocropping system. Specifically, the contents of AP in the rhizosphere and bulk soils increased by fourfold and sixfold, respectively, and those of AK enhanced by twofold and onefold, respectively. The pH values in the rhizosphere and bulk soils were significantly lower in the intercropping system than in the monocropping system. No significant differences in OM and TN contents were found between the two cultivation systems. However, soil OM content was higher in the rhizosphere soil than in the bulk soil. These results suggested that intercropping altered the physicochemical properties and enhanced the total nutrient contents in the soils.

Intercropping changes the microbial composition and structure in rhizosphere and bulk soils

The correlation coefficients of the abundance of unigenes in both rhizosphere and bulk soils between different planting patterns were close to 1, indicating that the higher the similarity of gene abundance between samples, the more reliable the experiment and the more reasonable the sample selection (Additional file 1: Fig. S2).

A moderate divergence in the numbers of genes was found between groups. Bacteria were the most abundant group in the rhizosphere and bulk soils from the monocropping and intercropping systems. As shown in Additional file 1: Fig. S3, the number of bacterial and fungal genes ranged from 1,092,690 (MPr) to 1,153,157 (MB) and 299 (MPr) to 315 (IPr), respectively. PCoA results showed obvious differences in the bacterial and fungal communities of both the rhizosphere soil and bulk soil between the different planting patterns (Fig. 1). The bacterial and fungal communities could be separated along the second and first coordinate axes, respectively.

The interaction between crops increased the diversity of soil microbial communities and the abundance of some bacteria and fungi. The results of microbial diversity analyses revealed that the Shannon and Simpson index values of bacteria and fungi in the rhizosphere and bulk soils were higher under the intercropping system than under the monocropping system (Fig. 2). The RAs of the top 10 most abundant phyla and genera showed evident variations between the different planting patterns (Fig. 3A, B, Additional file 1: Fig. S4A, B). At the phylum level, *Proteobacteria* (34.21–38.02%), *Actinobacteria* (22.62–28.80%), and *Acidobacteria* (17.61–18.36%) were the most dominant soil bacteria identified in both cropping systems, followed by *Chloroflexi* (6.78–7.42%), *Gemmatimonadetes* (2.84–3.68%), *Candidatus Rokubacteria* (2.64–3.22%), *Nitrospirae* (0.84–0.99%), *Planctomycetes* (0.80–0.96%), *Cyanobacteria* (0.54–0.60%), and *Verrucomicrobia* (0.54–0.60%) (Fig. 3A). However, the RAs of *Actinobacteria*, *Chloroflexi*, and *Cyanobacteria* were higher in IPr and IB than in MPr and MB. Meanwhile, the RA of *Planctomycetes* was significantly lower ($p<0.01$) in IPr and IB than in MPr and MB (Fig. 3B). Additionally, the RA of *Verrucomicrobia* was significantly lower ($p<0.05$) in the bulk soil from the intercropping system than in that from the monocropping system. Among the top 10 bacterial genera, eight had higher RAs in IPr and IB than in MPr and MB (Additional file 1: Fig. S4A, B). Noticeably, *Sphingomonas* had a higher RA in IPr than in MPr. In the fungal community (Fig. 3C, D, Additional file 1: Fig. S4C, D), the RAs of the predominant phyla varied amongst the different planting patterns. The RA of *Ascomycota* was considerably higher in IPr and IB; those of *Basidiomycota*, *Chytridiomycota*, and *Mucoromycota* were higher in MPr and MB; and that of *Zoopagomycota*

Table 1 Basic physicochemical parameters in the rhizosphere and bulk soils under pepper intercropping and monocropping modes

Samples	pH	OM (g/kg)	TN (g/kg)	AP (mg/kg)	AK (mg/kg)
IPr	7.63 ± 0.06 ^{B}	76.88 ± 3.91 ^{AB}	4.68 ± 0.08 ^{A}	229.93 ± 9.11 ^{A}	553.51 ± 4.07 ^{A}
MPr	8.10 ± 0.02 ^{A}	79.81 ± 0.03 ^{A}	4.60 ± 0.09 ^{A}	46.77 ± 8.72 ^{B}	178.93 ± 28.77 ^{C}
IB	7.67 ± 0.01 ^{B}	72.05 ± 1.77 ^{B}	4.41 ± 0.04 ^{A}	221.44 ± 11.01 ^{A}	375.15 ± 15.25 ^{B}
MB	8.04 ± 0.01 ^{A}	74.19 ± 2.90 ^{AB}	4.13 ± 0.64 ^{A}	31.54 ± 6.97 ^{B}	186.44 ± 0.34 ^{C}

Data (means ± SD, $n=3$) followed by different letters indicate significant differences at $p<0.01$

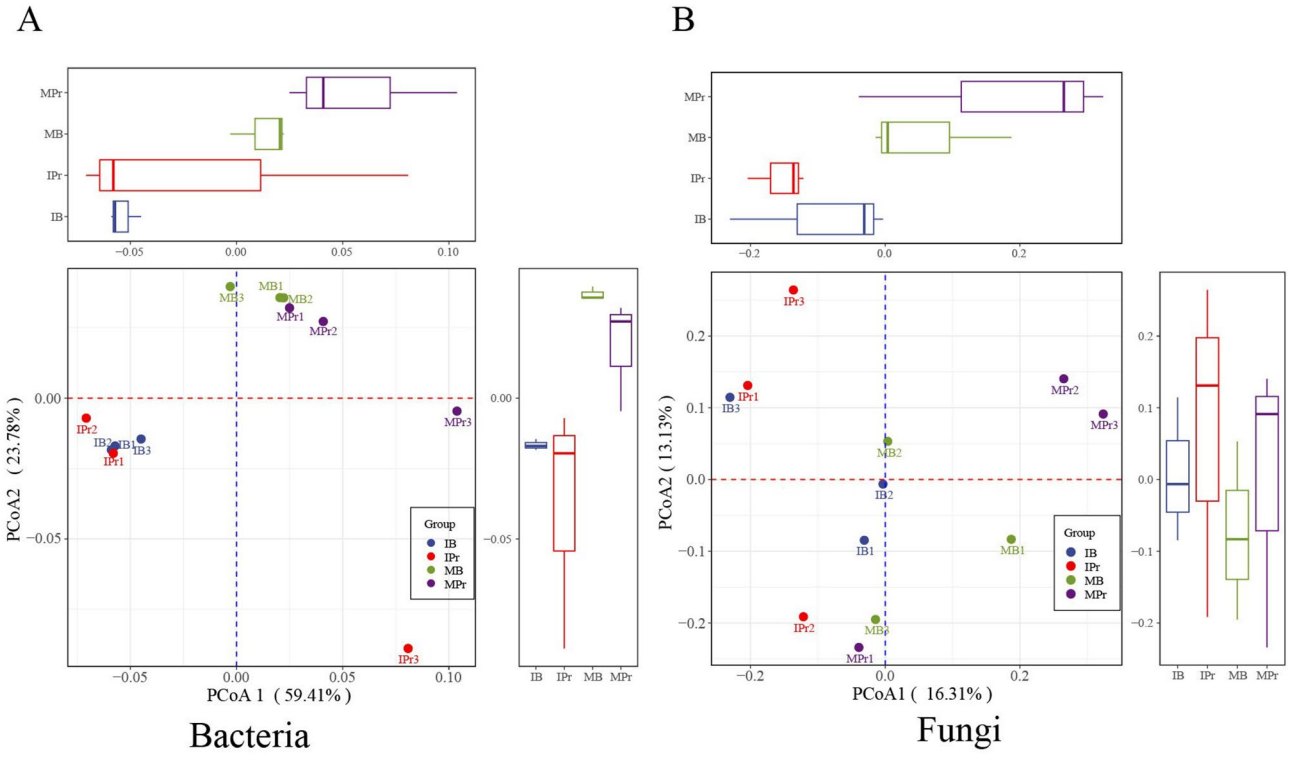

Fig. 1 Bacterial (**A**) and fungal (**B**) community composition by PCoA, based on 97% sequence similarity. IPr: intercropped pepper rhizosphere soil, IB: bulk soil of pepper-maize intercropping system, MPr: monocropped pepper rhizosphere soil, and MB: bulk soil of pepper monocropping system

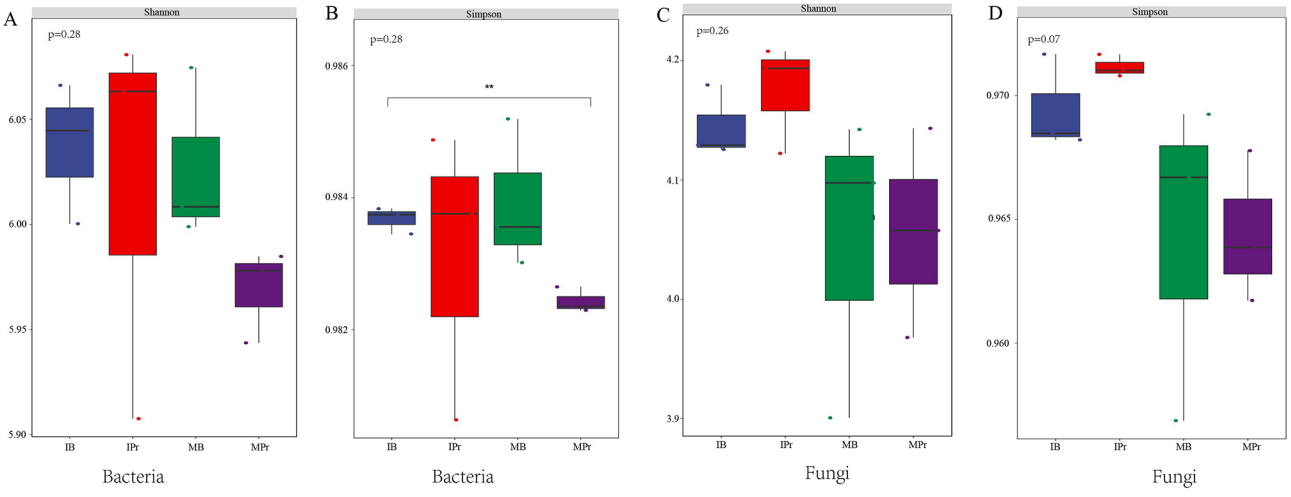

Fig. 2 Diversity of the bacterial (**A, B**) and fungal (**C, D**) communities in IPr, IB, MPr, and MB.** means significantly at $p < 0.01$ level

was higher in MPr and IB. The fungal genera *Coniosporium* and *Pyrenophora* had higher RAs in the intercropping system than in the monocropping system. Moreover, the RA of *Penicilliopsis* increased in IB (Additional file 1: Fig. S4C, D).

At the species level (Additional file 1: Fig. S5), *Betaproteobacteria bacterium* RIFCSPLOWO2_12_FULL_65_14 and *Gemmatimonadetes bacterium* SCN 70−22 were significantly enriched in IPr. *Chloroflexi bacterium* RBG_16_69_14, *Chloroflexi bacterium* RBG_16_70_13, and *Chloroflexi bacterium* CSP1-4 were enriched in MPr. In the bulk soil, most *Actinobacteria* bacteria were the predominant species in IB, and the RAs of *Actinobacteria bacterium* 13_1_20CM_3_68_9 and *Solirubrobacterales bacterium* 70−9 were higher in MB than in IB. Among the fungal species (Additional file 1: Fig. S5B), several *Ascomycota* fungi and *Linnemannia elongata* were abundant in IPr, and the RAs of *Mucor ambiguus*, *Serendipita*

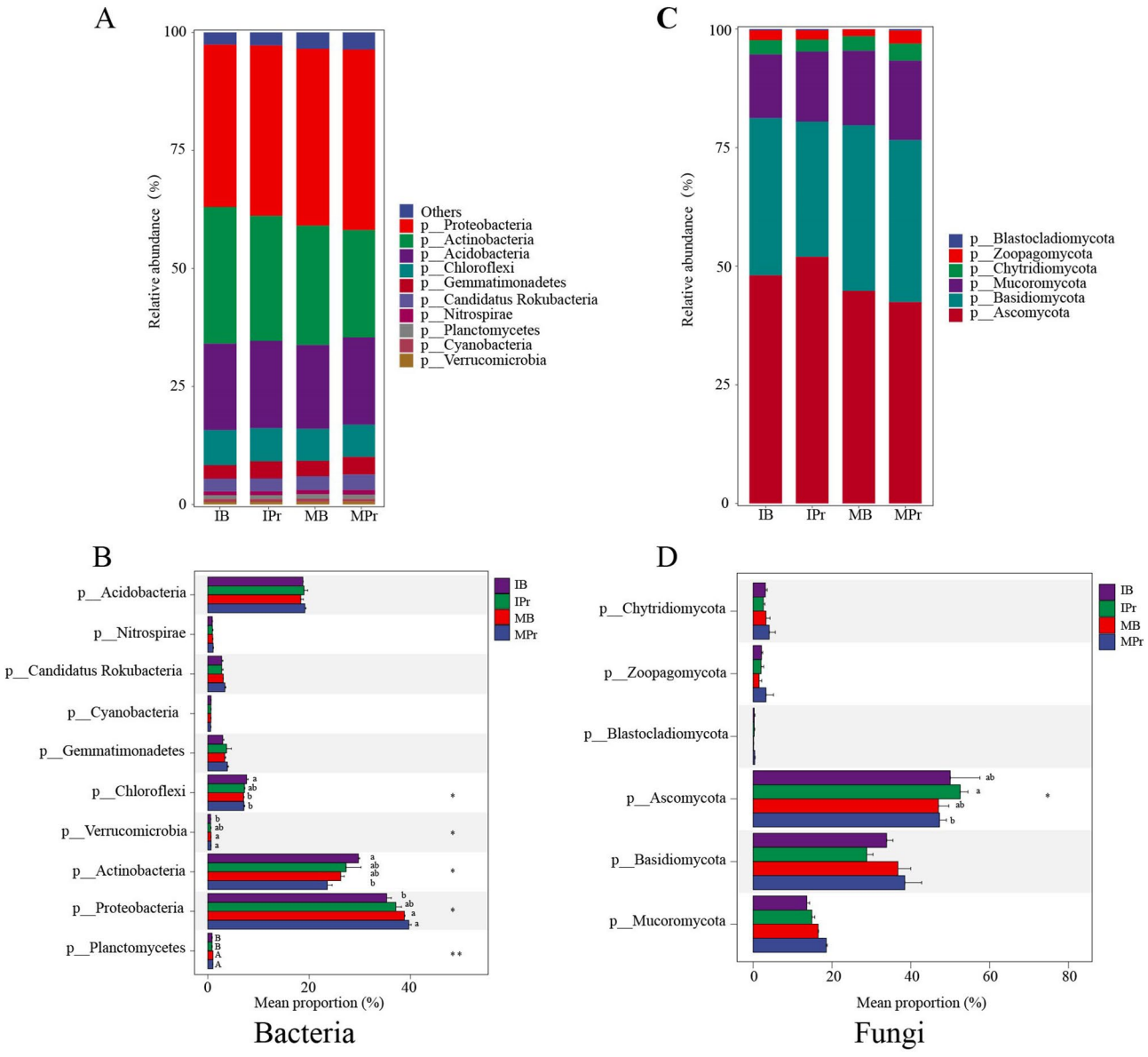

Fig. 3 RAs of the bacteria (**A, B**) and fungi (**C, D**) phyla in IPr, IB, MPr, and MB. *, and ** means significantly at $p < 0.05$ and $p < 0.01$ level, respectively. Different lower and uppercase letters means significantly at $p < 0.05$ and $p < 0.01$ level, respectively

vermifera, and *Choanephora cucurbitarum* were higher in MPr than in IPr. In the bulk soil, the predominant species were *Spizellomyces punctatus* and *Fonsecaea monophora* in IB, and *Kockovaella imperatae* and *Penicillium subrubescens* in MB.

These results revealed that pepper intercropping significantly changed the microbial community composition and structure in the rhizosphere and bulk soils.

Biomarker and functional analyses of soil microbial communities in rhizosphere and bulk soils under different planting patterns

LEfSe was employed to compare the bacterial and fungal communities between the monocropping and intercropping systems (Fig. 4). At the bacterial genus level, *Nitrospira* and *Phycicoccus* were enriched in IPr (Fig. 4A, B). Meanwhile, the relative abundances of *Pseudorhodoplanes*, *Pseudolabrys*, and *Woeseia* increased in MPr. In addition, *Methyloceanibacter* was significantly enriched in MB (Fig. 4A, B). However, the phylum *Candidatus Eisenbacteria* and class *Gammaproteobacteria* were significantly enriched in MPr. Compared with MPr and MB, the relative abundances of the order *Sphingomonadales* and the phylum *Acidobacteria* were higher in IPr and IB, respectively (Fig. 4A, B). In the fungal community (Fig. 4C, D), the abundances of *Auricularia*, *Grosmannia* and *Exophiala* were significantly enriched in IPr, MPr, and MB, respectively, whereas

Fig. 4 LEfSe for bacterial (**A, C**) and fungal (**B, D**) taxa in IPr, IB, MPr, and MB. Significant differences are defined at $p < 0.05$, LDA score > 2.0 in bacterial and fungal taxa

Calocera, *Pseudogymnoascus*, and *Trichosporon* were more abundant in IB. Overall, LEfSe analysis identified 49 differential microbes in rhizosphere soils between the monocropping and intercropping systems, and they were used for further analyses.

Analysis of bacterial/fungal community functional prediction

Analysis of the KEGG pathways revealed that the functions of the two levels of microbial community was mainly metabolism, and 24 major level-2 sub-systems were identified in the metagenome samples (Fig. 5). In the bacterial community, cell growth and death, carbohydrate metabolism, and amino acid metabolism were the top three pathways in level-2 sub-systems in all samples (Fig. 5A). However, in the fungal community, carbohydrate metabolism, amino acid metabolism, metabolism of cofactors and vitamins, and energy metabolism were enriched in all samples (Fig. 5B).

Metabolite changes in rhizosphere and bulk soils under different planting patterns

A total of 1294 peaks were detected in the chromatogram. Based on the agreement of the mass spectrum fingerprint and the retention index, 429 metabolites in the rhizosphere and bulk soils were identified and grouped into 15 classes. Of these compounds, flavonoids had the highest concentration, accounting for 16.55% of the total, followed by alkaloids (14.22%), organoheterocyclic compounds (9.32%), benzenoids (8.86%), and terpenoids (8.62%) (Additional file 1: Fig. S6A).

OPLS-DA results revealed a clear separation between the intercropping and monocropping systems (Fig. 6A), indicating that planting patterns significantly affected the soil metabolites. The first and second principal components accounted for 11.7% and 11.5% of the total variation, respectively. Score plots showed that the metabolites were statistically separated in IPr, IB, MPr, and MB. The OPLS-DA results also indicated that the model could better explain the differences between planting patterns ($R^2Y=1$, $Q2>0.5$; Fig. 6B, C), proving that the results of differential soil metabolites caused by intercropping were stable and reliable. The metabolites in MPr vs. IPr and MB vs. IB were clearly separated along the first principal component (Additional file 1: Fig. S6B, C).

A heatmap was used to visualise the differential expressed metabolites between the intercropping and monocropping systems (Fig. 7). In total, 28 (10 upregulated and 18 downregulated) and 29 (7 upregulated and 22 downregulated) were significantly enriched in MPr vs. IPr and MB vs. IB, respectively. In rhizosphere soil, IPr mainly drives the accumulation of flavonoids (such as morusin, chrysoeriol 7-apiosylglucoside, and asebogenin), alkaloids (tabernanthine, cheilanthifoline, and trachelanthamidine), nucleotide and its derivates (5′-S-methyl-5′-thioadenosine and 5-methyldeoxycytidine), and lipids (traumatic acid) (Fig. 7A). In bulk soil, organoheterocyclic compounds (such as cis-Zeatin and furan-3-carboxylic acid) and terpenoids (parthenolide) were greatly increased in IB (Fig. 7B). Overall, the results demonstrated that intercropping can significantly alter the metabolites in the rhizosphere and bulk soils.

Pathway enrichment analysis on these differential metabolites was used to illuminate the specific changes in soil metabolic pathways. Purine metabolism was the most significantly altered process in the soils. Phenylalanine, tyrosine, and tryptophan biosyntheses were significantly impacted in the rhizosphere soil, whereas tryptophan metabolism was significantly affected in the bulk soil (Additional file 1: Fig. S7).

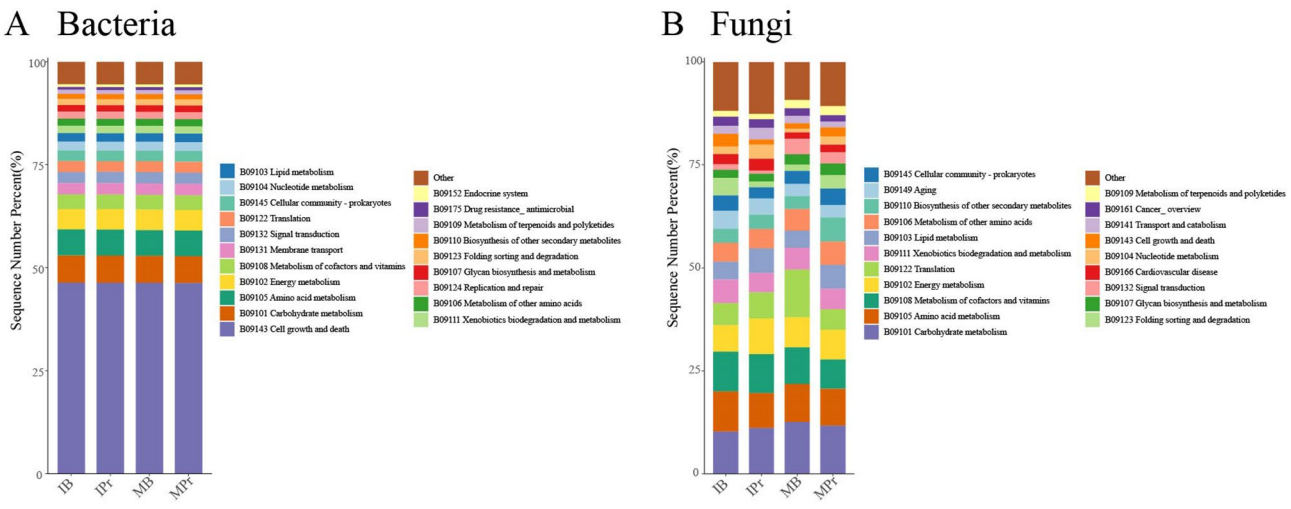

Fig. 5 The Predictions of function in the bacterial (**A**) and fungal (**B**) communities

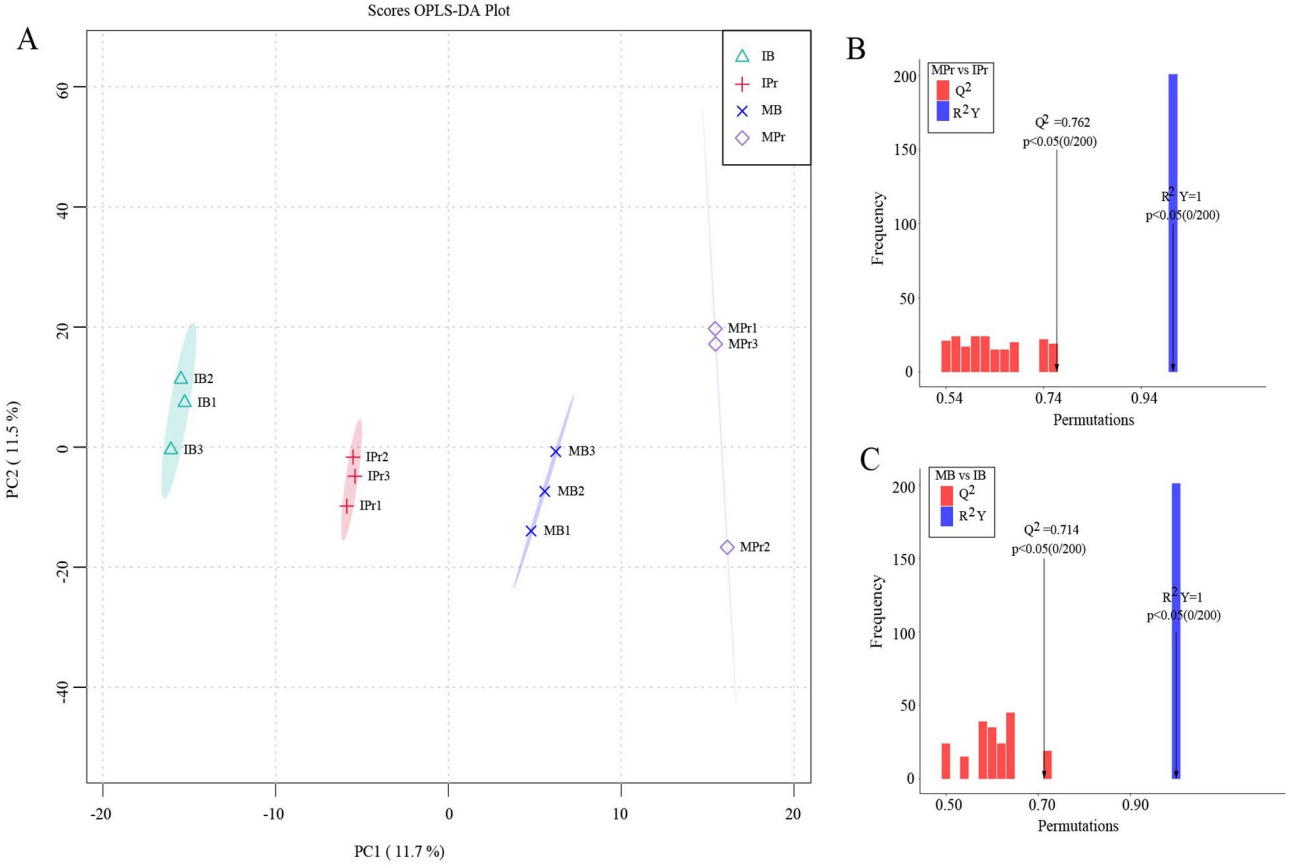

Fig. 6 OPLS-DA score plots (**A**) and models (**B, C**) of metabolites in rhizosphere and bulk soils

Correlation between the microbial community composition and soil physicochemical properties

The microbial community composition was highly associated with intrinsic edaphic factors. The association between the essential edaphic factors and microbial community composition was discerned using mantel test and co-occurrence network analysis (Fig. 8A, B). The results demonstrated that the taxonomic composition of bacteria had a significantly positive correlation ($p<0.01$) with soil pH, AP, and AK (Fig. 8A). Soil pH also had a positive correlation ($p<0.01$) with fungal community. The co-occurrence network revealed that soil pH was a key edaphic factor regulating the bacterial community composition (Fig. 8B). *Planctomycetes*, *Proteobacteria*, *Verrucomicrobia*, *Gemmatimonadetes*, and *Candidatus Rokubacteria* showed a significantly positive correlation with soil pH, whereas *Actinobacteria*, *Chloroflexi*, and *Cyanobacteria* revealed the opposite. Soil AP and AK had a significantly positive association with *Planctomycetes*. The fungal phyla *Ascomycota*, *Chytridiomycota*, and *Mucoromycota* were significantly correlated with soil pH, TN, and AK, respectively.

Correlations between the metabolism and microbial communities in pepper rhizosphere soil

Interactive networks were constructed to elucidate the relationship between the differential metabolites and microorganisms in rhizosphere soils from the monocropping and intercropping systems (MPr vs. IPr) (Additional file 1: Fig. S8, Fig. 8C). For upregulated metabolites, a significantly positive ($p<0.01$) correlation was observed between the trachelanthamidine and bacteria related to the family *Nitrospiraceae*, class *Gammaproteobacteria*, phylum *Nitrospirae*, and two *Acidobacteria bacterium* species. Three upregulated metabolites (5-methyldeoxycytidine, cheilanthifoline, and chrysoeriol 7-apiosylglucoside) showed a significantly positive ($p<0.01$) correlation with *Sorangium cellulosum* and *Planctomycetes bacterium* for MPr vs. IPr. Similarly, asebogenin also exhibited a significantly positive correlation with *S. cellulosum* for MPr vs. IPr. Three downregulated metabolites (ethyl 3,4,5-trimethoxybenzoate, styrene-cis-2,3-dihydrodiol, and galactinol) showed the most remarkable correlation with the rhizosphere soil bacterial and fungal taxa, being especially positively correlated with *Nitrobacter vulgaris* and *Phycicoccus duodecadis*. *Sphingomonadales* showed a significantly positive correlation with four

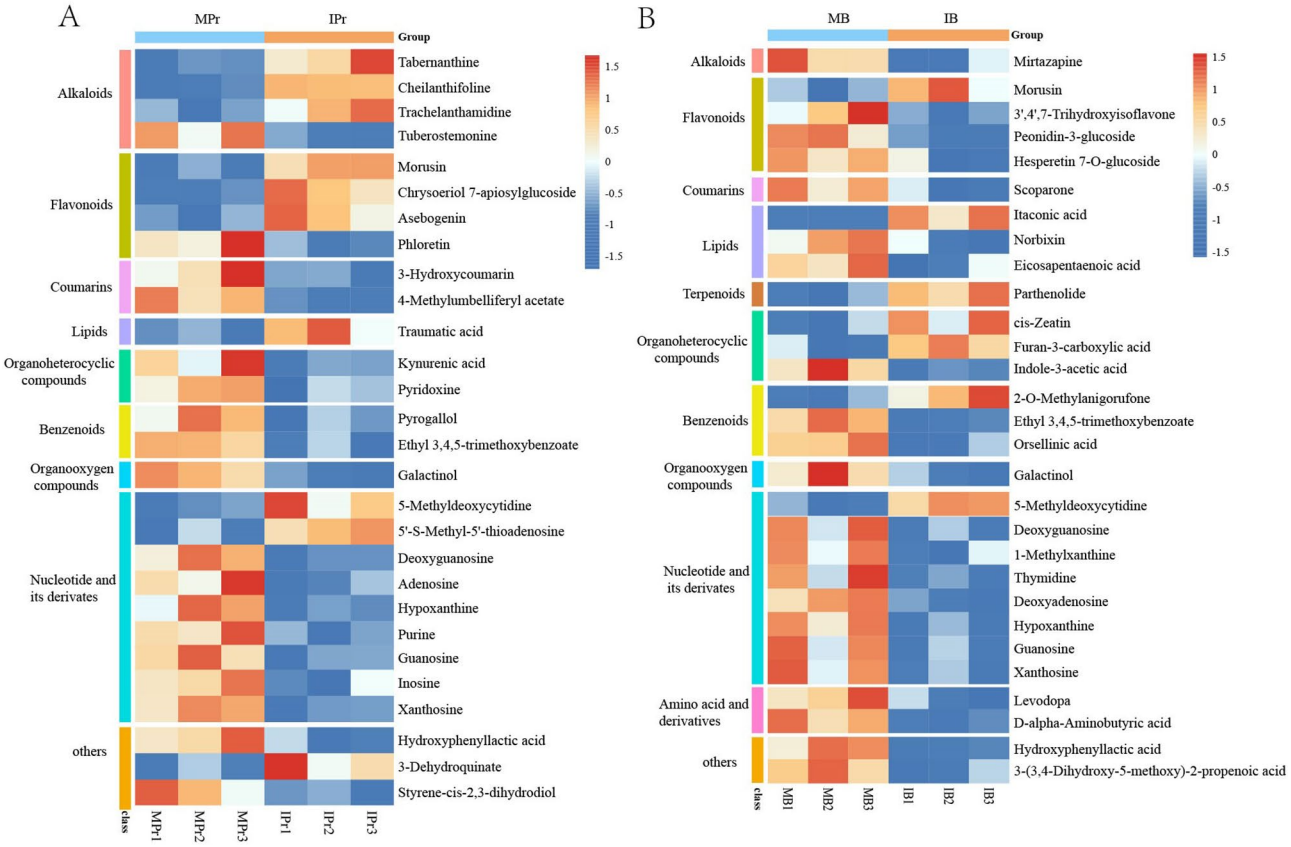

Fig. 7 Heatmap analysis of the differential expressed metabolites in MPr vs. IPr (**A**) and MB vs. IB (**B**)

downregulated metabolites, namely, inosine, adenosine, pyridoxine, and xanthosine. However, two fungal species (*Auricularia subglabra* and *Grosmannia clavigera*) displayed a significantly positive or negative correlation ($p<0.01$) with 11 differential metabolites (2 upregulated and 9 downregulated).

Discussion

In this study, we characterised the effects of intercropping pepper with maize on soil microbial communities and metabolite profiles by using high-throughput sequencing and soil metabolomics. Previous studies revealed that interactions between crops affect microbial diversity [27]. Moreover, intercropping systems can promote soil microbial diversity and community composition, and biochemical property in the rhizosphere [28, 29] and non-rhizosphere soils [30]. In the present study, the diversity of the bacterial and fungal communities increased in the rhizosphere and bulk soils from the pepper intercropping system (Fig. 2). The nutrient contents (TN, AK, and AP) were also higher in the rhizosphere and bulk soils from the intercropping system than in those from the monocropping system (Table 1). However, soil OM content was higher in the rhizosphere soil than in the bulk soil.

Furthermore, intercropping systems notably affect bacterial community composition [31, 32]. In the current study, bacteria were the most abundant group in the rhizosphere soil of intercropped pepper (Additional file 1: Fig. S1). The RAs of *Actinobacteria*, *Chloroflexi*, and *Cyanobacteria* increased in the rhizosphere and bulk soils from the pepper intercropping system (Fig. 3B). This finding is consistent with the results of a previous study [27]. Specifically, IB had a significantly higher abundance of *Actinobacteria*. *Actinobacteria* have a ubiquitous distribution in the biosphere being a dominant taxon in soil microbial communities [33]. *Actinobacteria* contribute significantly to nitrogen fixation [34], decomposition of OM such as cellulose and lignin [33, 35], plant growth [36], organic acid production [37], and phosphate solubilisation [38] in soil. In addition, *Sphingomonas* had a higher RA in IPr than in MPr (Additional file 1: Fig. S4A, B). *Sphingomonas* promotes nitrogen fixation and dehydrogenation [39], which enhancing the uptake of nutrients in the rhizosphere, improving the rhizosphere soil environment of intercropped pepper, and maintaining the soil nitrogen balance.

Fungi are an important part of soil microorganisms and play a crucial role in soil ecosystems.

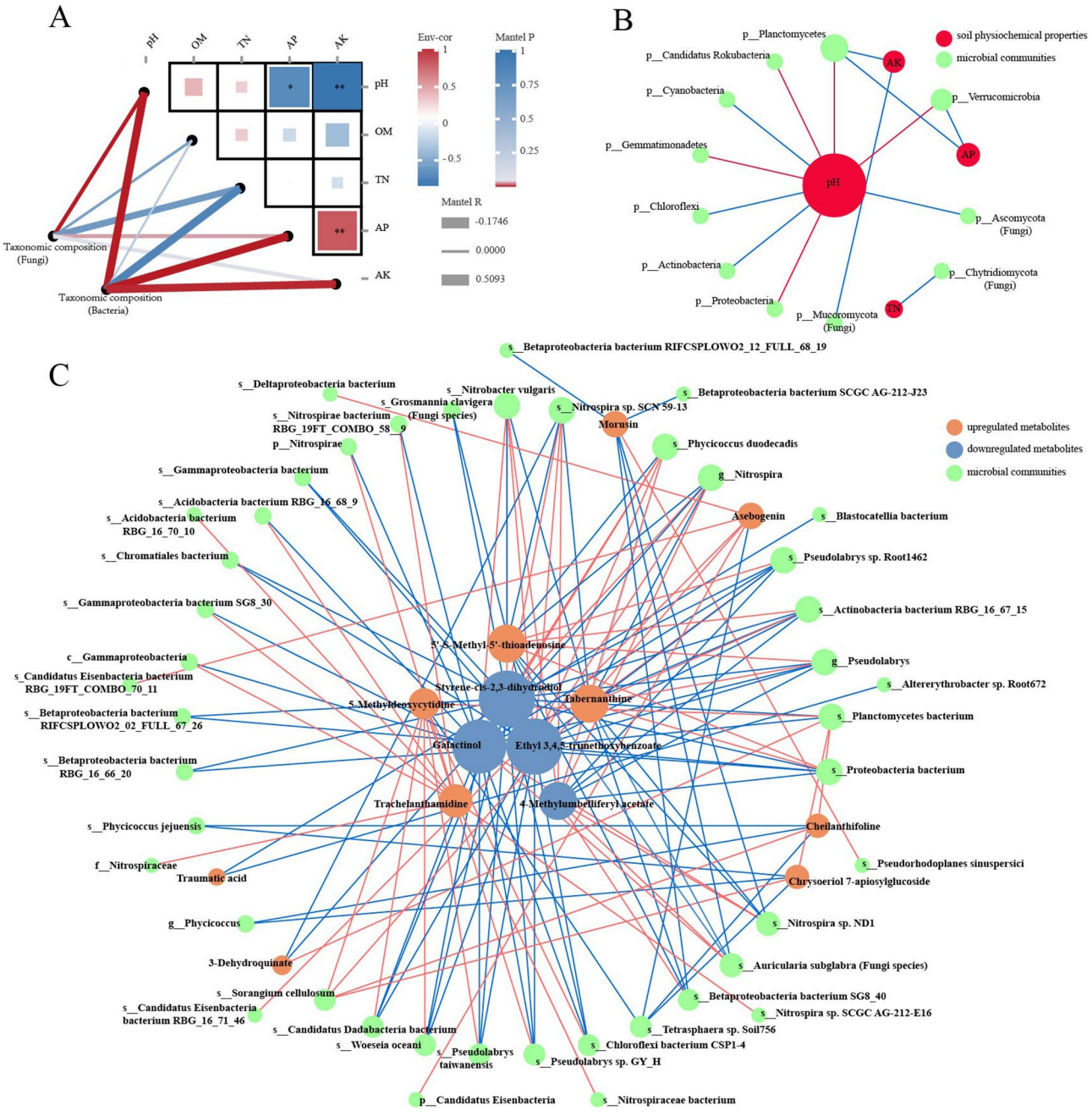

Fig. 8 The Mantel tests (**A**) and co-occurrence network analysis (**B, C**) of microbial communities with soil physiochemical properties and differential metabolites. Red and blue lines indicate positive and negative correlations, respectively

Compared with bacteria, fungi play critical roles as decomposers, symbionts, and pathogens in the soil [40]. Saprophytic fungi help decompose soil substrates, contributing to the soil nutrient cycle, while ectomycorrhizal fungi, such as symbiotrophs, improve the nutritional status of plants [41, 42]. In the present study, the diversity and gene number of fungal community increased in the pepper intercropping system (Fig. 2; Additional file 1: Fig. S3), consistent with the promotion of fungal community growth under intercropping [8]. The RAs of the phylum *Ascomycota* and genus *Pyrenophora* were higher in IPr than in MPr. However, the RA of the genus *Rhizoctonia* significantly decreased in the rhizosphere soil of intercropped pepper. The fungal community can be influenced by various biotic and abiotic factors. For instance, the diversity and amount of root exudates produced by different crops impact the abundance of the fungal community [8, 43].

Among the marker bacteria associated with pepper intercropping, *Nitrospira* and *Phycicoccus* were mostly

involved in soil N and carbon (C) cycling [44–47]. In addition, diverse metabolic capabilities of *Nitrospira* include utilising different organic compounds, nitrite, carbon dioxide, cyanate, or urea [48]. Ammonia-oxidizing microorganisms, including ammonia-oxidizing bacteria and ammonia-oxidizing archaea, which is important regulators of the nitrification process. Specifically, they oxidize ammonia to nitrite, which is subsequently oxidized to nitrate by nitrite-oxidizing bacteria [49]. Complete ammonia oxidizers, capable of oxidizing ammonia to nitrate, were discovered in nitrite-oxidizing bacteria of the genus *Nitrospira* [49]. Recently, a study reported that the relative abundance of *Phycicoccus* increased with N addition [50]. Atmospheric N deposition leads to N enrichment in soil, inducing changes in plant growth and soil biological activity, thereby affecting global C and N cycling [51]. In this study, the abundance of *Nitrospira* and *Phycicoccus*, and the content of TN and OM increased in IPr, suggesting that intercropping may promote nitrification, ammonification and carbon dioxide assimilation pathway, which may further enhance the carbon-nitrogen cycling in pepper rhizosphere.

Soil metabolites originate from plant root exudates, microbial metabolites, and soil OM decomposition by plants, microbes, and microorganisms [52]. Differential metabolites sensitive to pepper intercropping were firstly investigated. PCA showed that the metabolites in MPr vs. IPr and MB vs. IB were significantly separated, indicating that the root interaction significantly affected the distribution of soil metabolites (Additional file 1: Fig. S6). In this research, the main differential compounds were flavonoids, alkaloids, and nucleotide and its derivates in the rhizosphere. Flavonoids, secondary metabolites secreted by plant roots, have various biological activities, such as antioxidant and antimicrobial activities [53, 54]. Flavonoids play critical roles in the nodule fixation of nitrogen and regulation of interplant and plant–microbe interactions [2]. Previous studies indicated that interspecific interaction changes the flavonoid content and proportion in the rhizosphere soil of wheat, maize, and peanut [2, 55]. In the present study, four flavonoids were identified in MPr vs. IPr, and the levels of morusin, chrysoeriol 7-apiosylglucoside, and asebogenin were upregulated in the rhizosphere of intercropped pepper. Alkaloids exert inhibitory effects on pathogenic microorganisms, specifically, the five alkaloid compounds isolated from *Picrasma quassioides* exhibited highly significant preventive on apple valsa canker (AVC) [56]. In vivo, two carboline alkaloids, at the concentration of 1000 μg/mL, displayed good inhibitory activity (78% and 80%) on Pytophthora blight of pepper [57]. The levels of alkaloids, including tabernanthine, cheilanthifoline, and trachelanthamidine, were upregulated in the rhizosphere of intercropped pepper. Suggesting that intercropping could enhance the resistance of pepper to *P. capsici* by increasing the content of alkaloids in the pepper rhizosphere. These result indicated that interspecific interactions regulated the types and contents of secondary metabolites in pepper rhizosphere.

The soil bacterial community composition is responsive to soil environmental parameters [58]. In the present study, the bacterial community composition exhibited a significantly positive correlation ($p<0.01$) with soil pH, AP, and AK (Fig. 8A). Soil pH showed a significantly negative correlation with *Actinobacteria*, *Chloroflexi*, and *Cyanobacteria* (Fig. 8B), which enriched in the rhizosphere soil from the intercropping system. *Planctomycetes*, enriched in the rhizosphere soil from the monocropping system, demonstrated a significant and negtive association with soil AP and AK. This result was similar to previous reports in Sugarcane (*Saccharum officinarum*)-peanut and maize-peanut intercropping systems [31, 59], wherein environmental properties, such as pH, AP and AK were the principal determinant impacting bacteria dissimilarities in pepper rhizosphere soil under intercropping and monocropping systems. Thus, intercropping may regulate the composition and abundance of bacteria in the pepper rhizosphere soil by changing soil physicochemical properties. Some soil microorganisms are capable of solubilizing and mineralizing insoluble soil phosphorus (P) and potassium (K), then enhancing the content of available P and K and the growth of plants [60, 61].The contents of AP and AK were increased in the IPr and IB, indicated that intercropping increase the abundance of phosphate and potassium solubilizing microorganisms (PSMs and KSMs) in the pepper rhizosphere and buik soils, thus may promote the transformation of insoluble nutrients in soil. Three *Aspergillus* (PSMs and KSMs) species (*A. japonicus*, *A. lentulus* and *A. sclerotioniger*) were enriched in IPr. Whereas, *Streptomyces* (KSM) was enriched in the IB.

The significant relationship between soil metabolites and microbial communities can guide soil fertility conditions. The correlation analysis revealed that 28 different metabolites in MPr vs. IPr showed frequent significant correlations with bacterial and fungal communities, indicating that the microbiota can interact with metabolites and change to adapt to environmental stress. Flavonoids are associated with the regulation of symbiosis between plants and microbes and serve as quorum sensing inducers for communications among microbes [62]. In the present study, asebogenin was positively correlated with *Candidatus Eisenbacteria*, *Deltaproteobacteria bacterium* and *Sorangium cellulosum*. Morusin and chrysoeriol 7-apiosylglucoside were positively correlated with *Pseudorhodoplanes sinuspersici* and *Planctomycetes bacterium*, respectively. These results agree with those of a previous study [63]. However, in the intercropping

system, three metabolites (styrene-cis-2,3-dihydrodiol, ethyl 3,4,5-trimethoxybenzoate, and galactinol) negatively regulated pepper rhizosphere microecology, and these compounds were negatively correlated with most bacteria and one fungus.

Conclusion

In this study, pepper-maize intercropping significantly increased the contents of nutrients (TN, AP, and AK) and the diversity of bacteria and fungi, as well as altered the metabolite profiles, in the rhizosphere and bulk soils. Moreover, LEfSe results showed that the RAs of beneficial microorganisms, such as *Sphingomonadales, Phycicoccus* and *Nitrospira*, were significantly enriched in the rhizosphere soil of intercropped pepper. The differential metabolites of soils were enriched in flavonoids, alkaloids, organoheterocyclic compounds, and benzenoids. The levels of flavonoids (such as morusin, chrysoeriol 7-apiosylglucoside, and asebogenin), alkaloids (tabernanthine, cheilanthifoline and trachelanthamidine), and organoheterocyclic compounds (such as cis-zeatin and furan-3-carboxylic acid) were upregulated in the intercropping system. Correlation analysis showed that asebogenin and trachelanthamidine had a significantly positive correlation with *Candidatus Eisenbacteria* and *Nitrospirae*. The results of this study demonstrate that intercropping directly or indirectly effected community structure of pepper rhizosphere microbes by enhancing soil nutrient contents and changing soil metabolites. The results provided a theoretical basis for the effects of intercropping on microbial communities and metabolites in pepper rhizosphere soil.

Abbreviations

AP	Available P
AK	Available K
pH	Potential of hydrogen
TN	Total nitrogen
OM	Organic matter
IPr	Intercropped pepper rhizosphere soil
IB	Bulk soil of pepper–maize intercropping system
MPr	Monocropped pepper rhizosphere soil
MB	Bulk soil of pepper monocropping system
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
NCBI	National center for biotechnology information
KEGG	Kyoto Encyclopedia of Genes and Genomes
VIP	Variable importance in the projection
OPLS-DA	Orthogonal partial least squares discriminant analysis
PCoA	Principal coordinate analysis
PCA	Principal component analysis
LEfSe	Linear discriminant analysis with effect size
RA	Relative abundance

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Author contributions

CZ and MY conceived the study and wrote the initial draft of the paper. CZ, WZ and CL collected the soil samples, and analyzed the data. ZP, LZ, YX, SJ and DY performed the experiment. All authors read, revised, and approved the manuscript.

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Data availability

The original contributions presented in this study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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