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Combined metagenomics and metabolomic analysis of microbial community structure and metabolic function in continuous soybean cropping soils of Songnen Plain, China

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Abstract

Continuous cropping has a negative effect on soybean yield. In this study, a positioning experiment was conducted starting in 2015, with three treatments: maize–soybean rotation (SMR), 2-year maize, 2-year soybean rotation cropping (SC2), and 8-year soybean continuous cropping (SC8). We determined soybean yields (2015–2022) and analyzed soil microbial communities, functions, and metabolites composition in the 0–20 cm tillage layer using metagenomics technology and GC–MS technology during soybean flowering in 2022. Results indicated that continuous cropping (SC8) significantly reduced soybean yield compared to crop rotation (SMR) during the experimental period, while SC8 showed higher yield than SC2 in 2022. Compared to SMR, SC8 significantly increased soil N content and significantly decreased pH and TP, AP, and AK content. However, the pH and AK contents of SC8 were significantly higher than those of SC2. LeFSe analysis showed that *Friedmanniella*, *Microlunatus*, *Nitrososphaera*, *Rubrobacter*, *Geodermatophilus*, *Nitriliruptor* were enriched in SC8. *Gaiella*, *Sphaerobacter*, *Methyloceanibacter* were enriched in SC2. *Sphingomonas*, *Cryobacterium*, *Marmoricola*, *Haliangium*, *Arthrobacter*, *Ramlibacter*, *Rhizobacter*, *Pseudolabrys*, *Methylibium*, *Variovorax* were enriched in SMR. And the relative abundance of *Cryobacterium*, *Marmoricola*, *Haliangium*, *Arthrobacter*, *Ramlibacter*, *Rhizobacter*, *Methylibium*, *Variovorax* was significantly positively correlated with yield, while the relative abundance of *Gaiella* and *Sphaerobacter* was significantly negatively correlated with yield. SC8 significantly increased the abundance of genes in nitrogen metabolism and significantly decreased the abundance of genes related to phosphorus and potassium metabolism compared with SMR. However, the abundance of genes in potassium metabolism was significantly higher in SC8 than in SC2. Metabolomic analysis showed that compared to SMR, SC8 decreased the abundance of carbohydrates, ketones, and lipid. However, the abundance of carbohydrates, ketones, and lipid was significantly higher in SC8 than in SC2. Mantel test showed that soil pH and AK significantly affected soil microbial community, function, and metabolite composition. Correlation analysis showed significant correlation between soil metabolites and microorganisms, metabolic functions.

Keywords Soybean continuous cropping, Soybean yield, Soil microorganism, Soil metabolites

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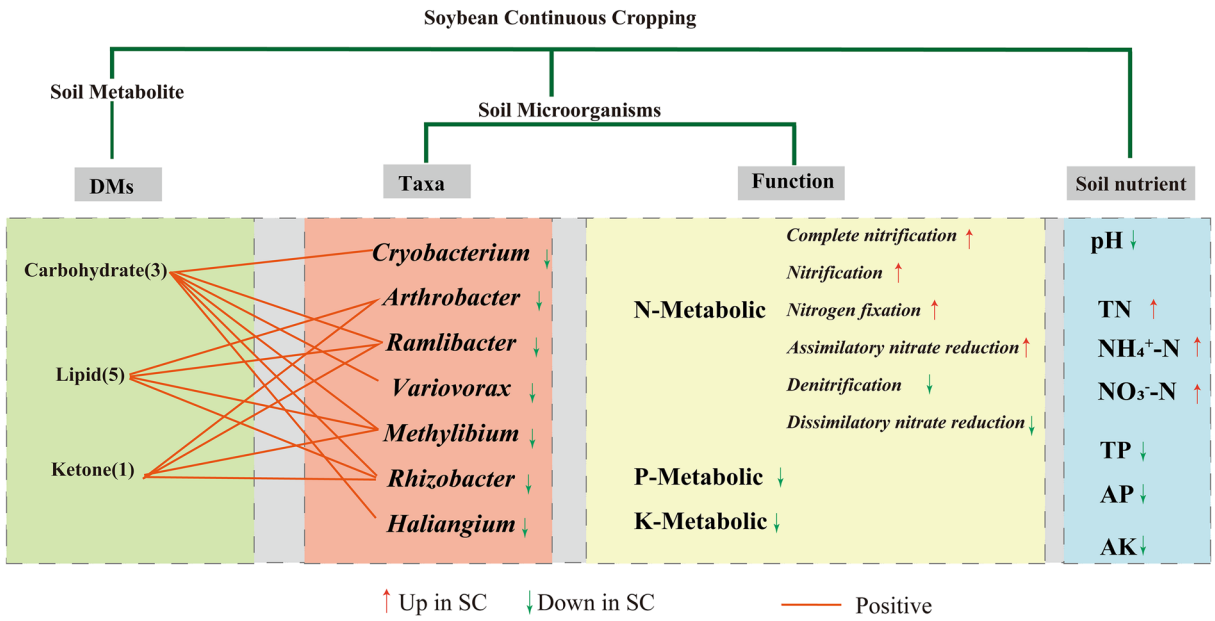
Graphical Abstract



Introduction

The Songnen Plain, located in Northeast China, is the main production area of soybean in China. Due to the limitation of arable land area and climatic factors, the phenomenon of soybean planting in consecutive years occurs commonly. Soybean is a sensitive crop for continuous cropping, continuous cropping will lead to deterioration of soil physicochemical properties [1], reduction of soil enzyme activities [2], and accumulation of harmful metabolites [3], imbalance of microbial community structure [4, 5], which in turn negatively affects yield [6].

Soil microorganisms participate in nutrient cycling and soil organic matter degradation, and play an important role in improving soil ecological functions and maintaining plant health [7]. Continuous cropping profoundly affects soil microbial community structure [6, 8]. Continuous cropping of soybean will increase the relative abundance of soil phytopathogens, aggravate soybean soil-borne diseases, and reduce beneficial microorganisms [6, 9]. However, other studies have found that soybean continuous cropping for 5–36 years partially restores beneficial soil microorganisms, suppresses soil-borne diseases, and contributes to restoring the health of the soil microbial environment [10–12]. Continuous cropping and continuous cropping years also changed the function of soil microbial communities. Previous studies have shown that soybean continuous cropping reduced soil nitrogen cycle and phosphorus cycle gene abundance [13, 14]. Chen et al. found that microbial metabolic functions associated with soil fatty acid metabolism and amino acid biosynthesis declined significantly with prolonged cucumber continuous cropping time [15]. Compared with 5 years continuous cropping, long-term continuous cropping of barley (10 years, 20 years) increased the abundance of functional genes related to soil C degradation and P cycling [16]. The effect of soybean continuous cropping on the structure and function of soil microbial community in Songnen Plain needs further study.

Soil metabolites are sensitive factors for assessing soil health, and their composition and concentrations are closely related to microbial community structure [17] and expression of functional genes [18]. Continuous cropping altered the composition of soil metabolites. Zhao et al. found that long-term continuous cropping (10 and 20 years) of barley up-regulated nucleosides, nucleotides, and analogues, and down-regulated alkaloids and derivatives compared with 5 years continuous cropping [16]. Wang et al. found that the abundance of phenolic acid metabolites in alfalfa root secretions increased with the increasing years of continuous cropping, which promoted the growth of some disease-causing fungi and led to the development of alfalfa root rot [19]. In addition, soil metabolites are closely related to microorganisms, with the greatest correlation between soil carbohydrates and bacteria [20]. Soil metabolites are closely related to

bacterial Shannon diversity and community composition [21]. Comprehensive analysis of soil metabolites and microbial community changes can better explore the mechanisms of soybean continuous cropping barriers.

This study was initiated in 2015 to investigate the effects of different crop rotation systems on soybean cultivation. Three treatments were set up: maize–soybean rotation (SMR), 2-year maize, 2-year soybean rotation cropping (SC2), and 8-year soybean continuous cropping (SC8). Soybean yields were measured from 2015 to 2022, and soil metagenome and metabolomes were measured at the soybean flowering stage in 2022, assess the changes of soybean yields, soil nutrient count and explore the impact of continuous cropping on the structure and potential functions of soil microbial communities and soil metabolites. These findings will provide valuable insights into the obstacle mechanism of soybean continuous cropping in the Songnen Plain.

Materials and methods

Study site description

The experiment was conducted at the Xiangyang Experimental Practice Base of Northeast Agricultural University in Xiangfang District, Harbin City, Heilongjiang Province, China (126° 22′–126° 50′ E; 45° 34′–45° 46′ N). It is located in the central part of the Songnen Plain in China, with a cold-temperate continental climate, with rain and heat in the same season. The annual precipitation ranges from 500 to 550 mm. The duration of the frost-free period spans approximately 140 days, while the accumulated temperature surpasses 2700 °C above 10 °C. The temperature and monthly average rainfall data are shown in Additional file 1: Fig. S1 (https://data.cma.cn/).

The experiment began in 2015 on a test site with black soil clay loam (Mollisol). The cropping system was implemented once a year. Initial measurements of soil fertility were recorded in Additional file 1: Table S1. The experiment consisted of three treatments (Fig. 1): maize–soybean rotation (SMR), 2-year maize, 2-year soybean rotation cropping (SC2), and 8-year soybean continuous cropping (SC8). Each treatment was repeated three times. The experimental area consisted of 40 ridges (ridge with a length of 60 m and a width of 0.65 m) per plot and a total area of 1,5,60 m^2. Sowing around May 1 every spring, soybean variety was Kenfeng 16, planting density was about 280,000 plants/ha. The fertilization rates for soybean included diammonium hydrogen phosphate (P_2O_5: 46%; N: 18%) at 150 kg/ha, potassium sulfate (K_2O:

Fig. 1 Schematic diagram of planting crops in different years. *SMR* maize–soybean rotation, *SC8* 8-year soybean continuous cropping, *SC2* 2-year maize, 2-year soybean rotation cropping

50%) at 75 kg/ha. The maize variety was Guoyu 49, with a planting density of about 60,000 plants/ha. The fertilization rates for maize included diammonium hydrogen phosphate (P_2O_5: 46%; N: 18%) at 150 kg/ha, potassium sulfate (K_2O: 50%) at 75 kg/ha, and urea (N: 46%) at 300 kg/ha. The rest of the field management practices (e.g., seed coating, herbicide application) were comparable to high-yielding fields.

Soil sampling and determination

On July 9, 2022, during the soybean flowering period, ten sampling points were randomly selected between the soybean plants in each plot using auger with a diameter of 3 cm. These samples were mixed to form a repetition. The soil layer sampled had a depth of 0–20 cm. Three replicates were processed, resulting in a total of nine samples. Any impurities such as plant residues and stones were removed from the samples, which were then passed through a 2 mm sieve, mixed thoroughly, and stored in a − 80 °C. These samples will be further analyzed for soil metagenome and metabolome. Additionally, the soybean yield was measured during the maturity stage from 2015 to 2022. For each plot, five areas of 19.5 m^2 (with a ridge length of 1.5 m and two ridges) were selected where soybean growth was uniform, and the yield was measured. Soil nutrient determinations are described in Additional file 1: Text S2 of the Supplementary Information.

Metagenomic assembly and gene annotation

Soil samples collected were used for soil DNA extraction using the Tengen magnetic bead kit (TianGen, China, Beijing). Sequencing libraries were generated using the NEBNext®Ultra™DNA Library Preparation Kit for Illumina (NEB, USA). After clustering, library preparations were sequenced on the Illumina NovaSeq platform and paired-end reads were generated. Raw Data obtained from Illumina HiSeq sequencing platform were first preprocessed, and Readfq (V8, https://github.com/cjfields/readfq) was used to remove reads in the raw data that were more than 40 bp in length and had a quality score of less than 38, and reads with an N base of more than 10 bp. And removing reads with overlap of more than 15 bp with Adapter, an average of about 6.46G clean bases were obtained per sample. Assembly was performed using MEGAHIT software (V1.0.4-beta).

We utilized the MetaGeneMark software to predict open reading frames (ORFs) for every sample. ORFs that were 100 bp or longer were then translated into amino acid sequences. To eliminate redundancy, we employed the CD-HIT software (V4.5.8, http://www.bioinformatics.org/cd-hit/) on the ORF prediction results, resulting in a non-redundant initial gene catalogue. For comparison, the clean data from each sample were aligned with the initial gene catalogue using Bowtie2 (Bowtie2.2.4). By applying Bowtie2 (Bowtie2.2.4), we compared the clean data of each sample with the initial gene catalogue, determining the gene read count for each sample. Genes with a read count equal to or less than 2 were filtered out from each sample, resulting in the final gene catalogue (Unigenes) for subsequent analysis. To classify and functionally annotate each sample, we further employed the BLASTP software (V0.9.9.110, https://github.com/bbuchfink/diamond/) to compare the non-redundant gene catalogues with both the NR database (e-value ≤ 1e−5) and KEGG database. Metagenome sequencing raw data had been uploaded to NCBI under accession number PRJNA1082034.

Analyses of soil metabolome

The soil samples were first freeze-dried using vacuum conditions and then ground to a powdered form using a grinder (30 Hz, 30 s). Next, 0.5 g of the sample was weighed, and 1 mL of a solution containing methanol, isopropanol, and water in a ratio of 3:3:2 (V/V/V) was added. The resulting mixture was shaken for 3 min at room temperature and then sonicated for 20 min in an ice-water bath. Afterward, the samples underwent centrifugation at 12,000 r/min for 3 min at 4 °C. Carefully transfer the supernatant into a sample vial and add 0.020 mL internal standard (10 μg/mL) to evaporate under nitrogen flow. The evaporated samples were transferred to the lyophilizer for freeze-drying. The residue was used for the further derivatization. The GC–MS analysis of the extracting solution was conducted using an Agilent 8890 gas chromatograph coupled to a 5977B mass spectrometer. For the analysis, a DB-5MS (30 m length × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, USA) was employed. The operating parameters and data processing were described in Additional file 1: Text S2.

Data analysis

All analyses were performed in R software (version 4.2.2). The significance of differences in soybean yield, relative abundance of soil microbial taxa, and relative abundance of microbial functions, abundance of metabolites was analyzed first by the normality test, and after the test of variance alignment, one-way ANOVA (Duncan's test) was used. If the data do not conform to a normal distribution, a non-parametric test (Kruskal–Wallis) is performed. Based on the Bray–Curtis distance, Principal co-ordinates analysis (PCoA) was performed to identify the distribution patterns of microbial community composition across treatments. A permutation multivariate analysis of variance (Adonis) and an analysis of similarity (ANOSIM) were performed using the "vegan" package,

respectively, to test the degree of significance of the differences in microbial communities between treatments, and the number of permutations was 999. The mantel test and procrustes analysis were used to examine the correlation between soil metagenome and metabolome. Microbial taxa and functions, soil differential metabolites, and soybean yield were analyzed using the R package "corplot" for Spearman's correlation analysis, and relative abundance of microbial functions was visualized in the R environment (version 4.2.2) using "pheatmap" for heatmap visualization to reveal the differences among soil samples. Correlation network graph visualization in Gephi. LeFSe analysis was performed in the R package "microeco".

Result

Effect of continuous cropping on soybean yield and soil nutrient

Additional file 1: Table S2 presents the soybean yield for different years. In comparison to the SMR, the soybean yield of the SC8 exhibited a decrease of 11.04%, 10.21%, 13.12%, and 13% in 2016, 2018, 2020, and 2022, respectively. Similarly, compared with the SMR, the SC2 experienced a reduction in soybean yield of 6.98% and 16% in 2018 and 2022, respectively. Furthermore, compared with SC2 treatment, the yield of SC8 treatment decreased by 9.7% and 13.9% in 2017 and 2021, respectively. Notably, in 2022, the yield reduction of the SC8 treatment (13%) was smaller than that of the SC2 treatment (16%).

As shown in Additional file 1: Table S3, compared to SMR, SC2 and SC8 significantly decreased pH, AP and AK contents. SC8 also significantly increased soil N content (TN, NH_4^+–N, NO_3^-–N) and significantly decreased soil TP content compared to SMR. Additionally, compared to SC2, SC8 significantly increased soil pH and soil TN, nitrate nitrogen, and AK content, and significantly decreased soil TP content.

Effects of continuous cropping on the structure and function of soil microbial communities

Metagenetic sequencing revealed that, following quality control, a total of 845,961 ORFs were obtained, with an average length of 491.12 bp for each ORF (Additional file 1: Table S3). Comparison of the species in the NR database indicated that the majority of microorganisms in the soil samples were bacteria, accounting for over 93% of the relative abundance. Additionally, a small number of archaea, eukaryotes, and viruses were also detected (Additional file 1: Table S4).

Effect of continuous cropping on soil microbial diversity

Through the comparison of α-diversity index (Table 1), it was determined that there existed no notable distinctions within the α-diversity indexes among the treatments. This result suggests that the α-diversity of microbial communities remained unaffected by continuous cropping. PCoA analysis at the genus level and species level (Fig. 2a, b) showed, the microbial communities (genus level and species level) of SC2, SC8, and SMR could be well separated, which indicated that continuous cropping changed the microbial community (genus level and species level). ANOSIM and Adonis analyses (Fig. 2a, b) also showed that there were significant differences in microbial community structure (genus level and species level) among treatments.

Table 1 The α-diversity index of microbial community

	Shannon	Simpson	Chao1	Ace
SC2	4 ± 0a	1 ± 0a	7091 ± 44a	7103 ± 44a
SC8	4 ± 0a	1 ± 0a	7122 ± 14a	7132 ± 15a
SMR	4 ± 0a	1 ± 0a	6983 ± 100a	6988 ± 100a

Results are means ± sd, different letters indicate 0.05 level differences

SMR maize–soybean rotation, *SC8* 8-year soybean continuous cropping, *SC2* 2-year maize, 2-year soybean rotation cropping

The effect of continuous cropping on the composition of soil microbial communities

The dominant microbial phyla in each treatment were Actinobacteria, Proteobacteria, Acidobacteria (Fig. 3a), the dominant microbial genera in each treatment were *Bradyrhizobium*, *Nocardioides*, *Sphingomonas* (Fig. 3b), and the dominant microbial species in each treatment were *Sphingomonas* sp. *URHD0057*, *Actinobacteria bacterium 13_1_20CM_4_69_9*, *Bradyrhizobium japonicum* (Additional file 1: Fig. S2).

In the phyla (relative abundance > 1%), compared to SMR, the SC2 and SC8 treatments significantly increased the relative abundance of Chloroflexi and Verrucomicrobia (Additional file 1: Fig. S3e, f). Furthermore, compared to SMR, SC2 also exhibited a significant decrease in the relative abundance of Proteobacteria (Additional file 1: Fig. S3b) and a significant increase in the relative abundance of Gemmatimonadetes (Additional file 1: Fig. S3d).

At the microbial genus level (Additional file 1: Fig. S4a), compared to the SMR treatment, the SC2 and SC8 treatments significantly reduced the relative abundance of *Sphingomonas*, *Marmoricola*, *Phycicoccus*, *Arthrobacter*, and *Rhodoplanes*. In addition, compared to SMR, SC2 significantly decreased the relative abundance of *Blastococcus* and significantly increased the relative abundance of *Gaiella*. SC8 significantly decreased the relative abundance of *Nocardioides* and significantly increased the relative abundance of *Nitrospira*, *Friedmanniella*. Compared to SC2, SC8 significantly increased the relative abundance of *Cryobacterium*, *Nitrospira*, *Friedmanniella*,

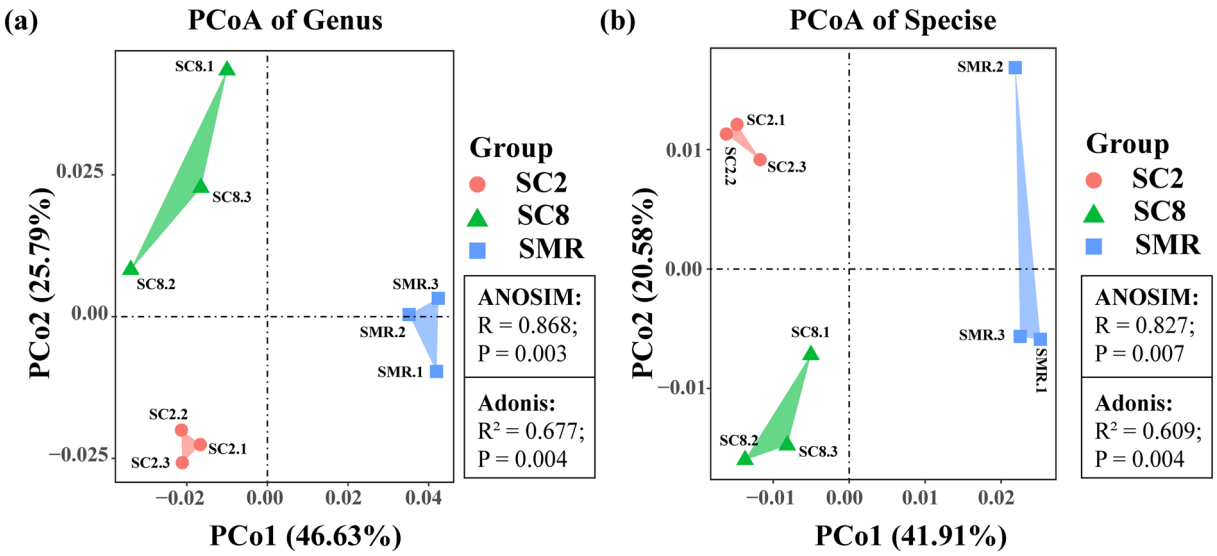


Fig. 2 Microbial PCoA analysis and ANOSIM, Adonis test at the genus level (**a**) and species level (**b**). *SMR* maize–soybean rotation, *SC8*: 8-year soybean continuous cropping; *SC2* 2-year maize, 2-year soybean rotation cropping

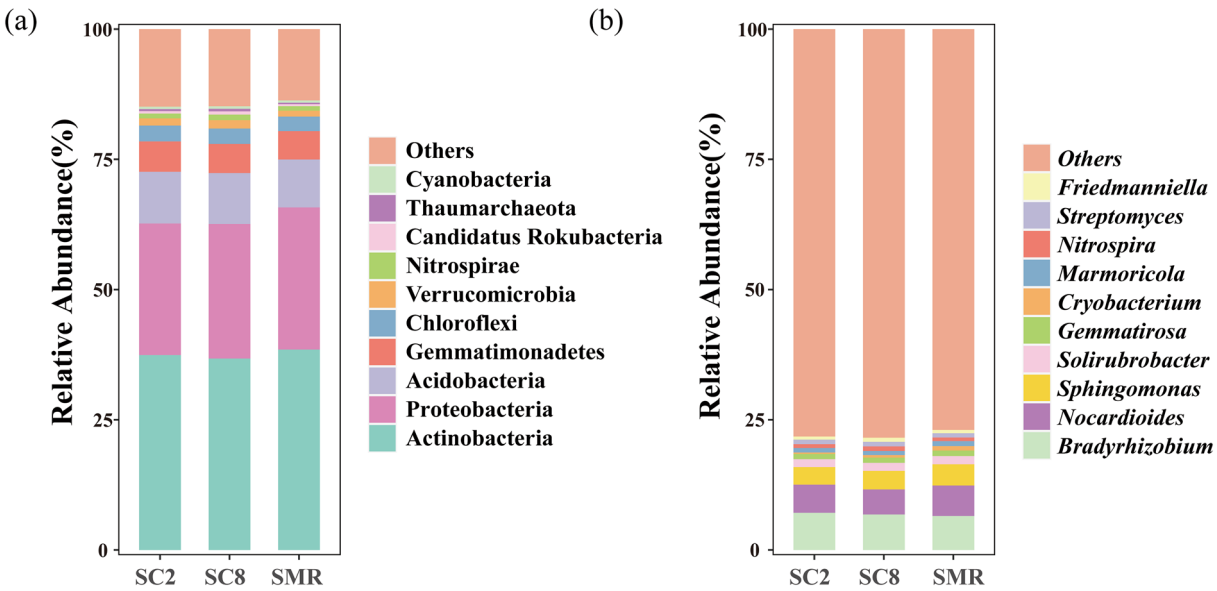


Fig. 3 Relative abundance of microbial taxa stacked histogram at the phylum level (**a**), genus level (**b**). *SMR* maize–soybean rotation, *SC8* 8-year soybean continuous cropping, *SC2* 2-year maize, 2-year soybean rotation cropping

Mycobacterium, and *Blastococcus*, and significantly decreased the relative abundance of *Nocardioides*, *Marmoricola*, and *Gaiella*.

At the microbial species level (Additional file 1: Fig. S4b), compared to the SMR, the SC2 and SC8 treatments significantly reduced the relative abundance of *Solirubrobacterales bacterium 70-9*, *Marmoricola* sp. *URHB0036*, *Sphingomonas* sp. *URHD0057*, and significantly increased the relative abundance of *Chloroflexi bacterium RBG_16_70_13*. Compared to the SMR, SC2 also significantly increased the relative abundance of *Actinobacteria bacterium 13_1_20CM_4_69_9*, *Actinobacteria bacterium 13_2_20CM_68_14*, *Actinobacteria bacterium RBG_16_68_12*, *Gaiella* sp. *SCGC AG-212-M14*, and SC8 significantly increased the relative abundance of *Friedmanniellaluteola*. Compared to

SC2, SC8 significantly reduced the relative abundance of *Bradyrhizobium erythrophlei*, *Actinobacteria bacterium 13_2_20CM_68_14*, *Gaiella* sp. *SCGC AG-212-M14*, *Actinobacteria bacterium RBG_16_68_12*, *Actinobacteria bacterium 13_1_20CM_4_69_9*, *Marmoricola* sp. *URHB0036*, but significantly increased the relative abundance of *Friedmanniella luteola*.

LeFSe analysis

To find the differential microorganisms among the three treatments, further LeFSe analysis was carried out (Fig. 4). The results showed that at the genus level, SC8 was enriched for 6 genera, namely *Friedmanniella*, *Microlunatus*, *Nitrososphaera*, *Rubrobacter*, *Geodermatophilus*, *Nitriliruptor*. SC2 enriched 3 genera, i.e., *Gaiella*, *Sphaerobacter*, *Methyloceanibacter*, SMR enriched 10 genera, i.e., *Sphingomonas*, *Cryobacterium*, *Marmoricola*, *Haliangium*, *Arthrobacter*, *Ramlibacter*, *Rhizobacter*, *Pseudolabrys*, *Methylibium*, *Variovorax*.

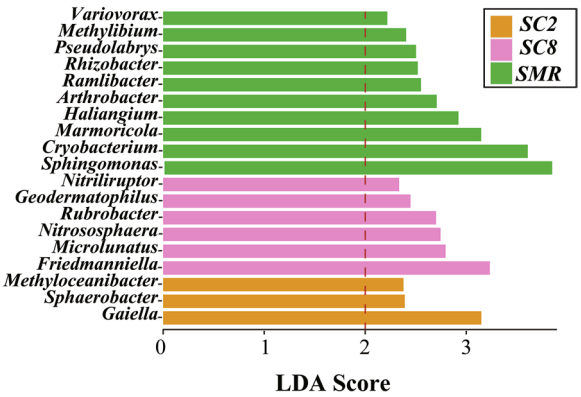


Fig. 4 LeFSe analysis of genus. *SMR* maize–soybean rotation, *SC8* 8-year soybean continuous cropping, *SC2* 2-year maize, 2-year soybean rotation cropping

Effect of continuous cropping on soil microbial functions

Further analysis of microbial function, after functional comparison in the KEGG database, the pathway with the highest number of genes annotated to KEGG pathway level 1 was metabolism (Fig. 5a). All samples annotated to KEGG pathways level 2 were mainly carbohydrate metabolism, amino acid metabolism, energy metabolism, nucleotide metabolism (Fig. 5b). PCoA analysis showed significant differences in microbial function between treatments (Additional file 1: Fig. S5).

Among the top 20 relative abundance of KEGG pathways level 2 (Fig. 5a; Additional file 1: Table S6), compared to SMR, the SC2 and SC8 treatments significantly reduced the relative abundance of carbohydrate metabolism, amino acid metabolism, energy metabolism, nucleotide metabolism, lipid metabolism, signal transduction. Furthermore, the relative abundance of energy metabolism in SC8 was significantly higher than that in SC2.

Screening of the genes annotated to be involved in the nitrogen metabolism process (map00910) in the KEGG database, a total of 49 relevant genes were found to be annotated; 22 of them were found to be directly involved in seven nitrogen metabolism modules (Fig. 6). Compared to SMR, both SC2 and SC8 significantly increased the abundance of nirfK and norC genes, significantly decreased the abundance of narG, narZ, nxrA, napA, and nirB genes, and SC2 significantly decreased nasB. The continuous treatment

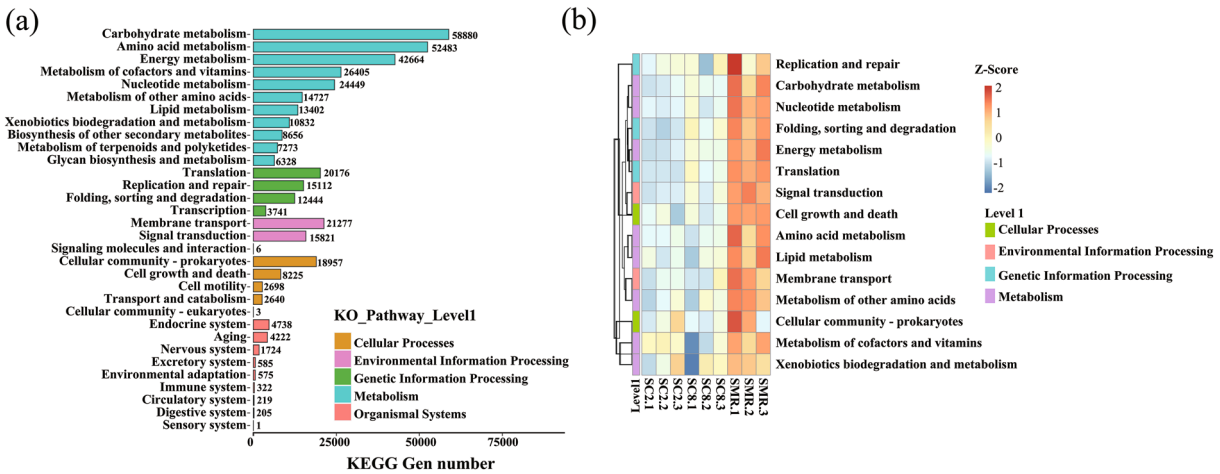


Fig. 5 The statistics of gene annotation of the KEGG pathway analysis (**a**). Heatmap of microbial relative abundance of the top 15 KEGG pathways level 2 (**b**). The *Z*-values obtained after normalizing the functional relative abundance for each row are presented as the values corresponding to the middle heatmap. *SMR* maize–soybean rotation, *SC8* 8-year soybean continuous cropping, *SC2* 2-year maize, 2-year soybean rotation cropping

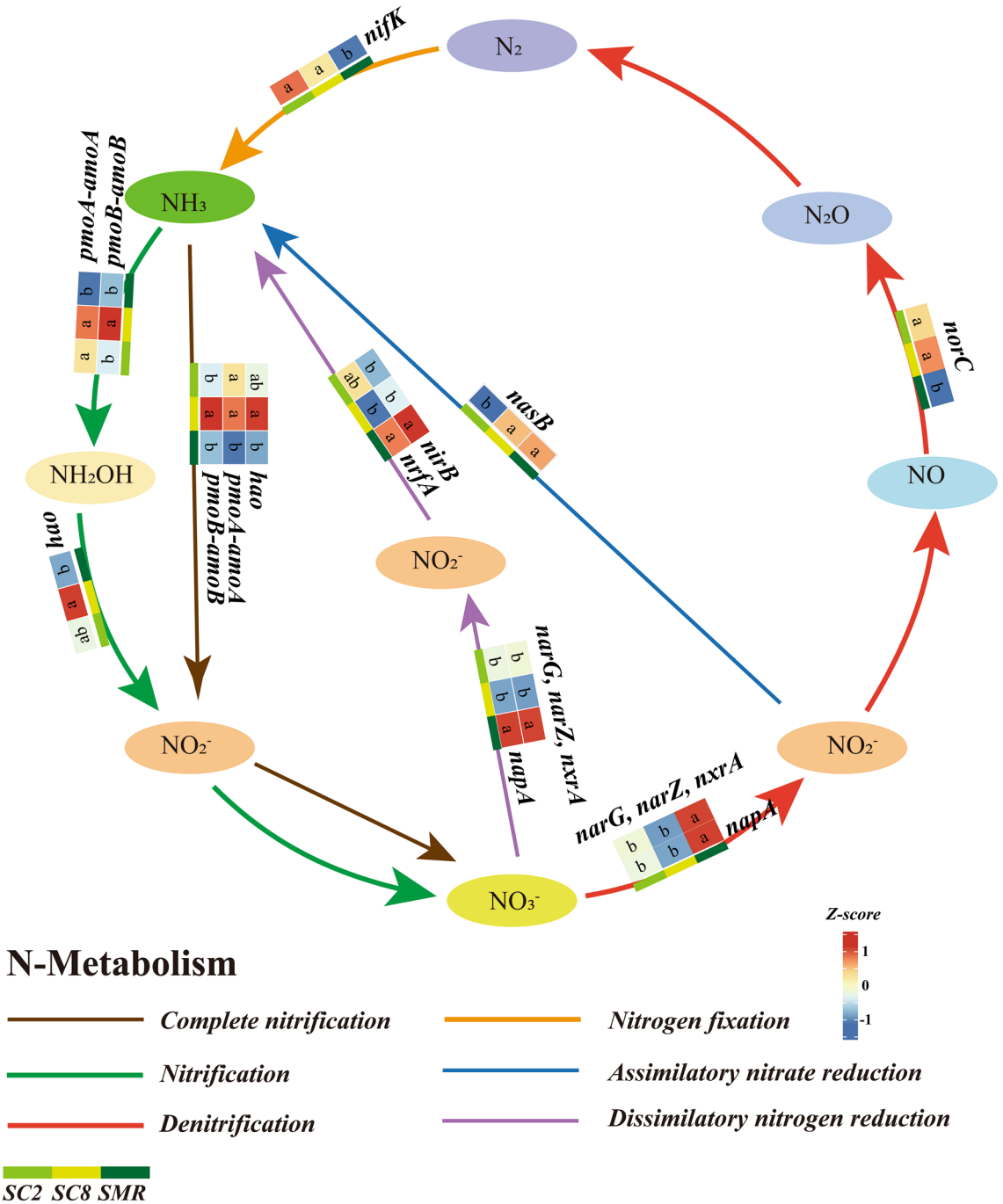


Fig. 6 Heatmap of abundance of genes related to nitrogen metabolism. The *Z*-values obtained after normalizing the functional relative abundance for each row are presented as the values corresponding to the middle heatmap. *SMR* maize–soybean rotation, *SC8* 8-year soybean continuous cropping, *SC2* 2-year maize, 2-year soybean rotation cropping

increased the abundance of genes for anammox, complete nitrification, nitrification, and nitrogen fixation modules, decreased the abundance of genes for denitrification and dissimilatory nitrogen reduction.

The abundance of genes with significant differences was shown after comparison of genes related to phosphorus metabolism and potassium metabolism by KEGG database (Additional file 1: Fig. S6). In

Glycerophospholipid metabolism, SC8 significantly increased the abundance of K01622, E2.5.1.41. Both SC2 and SC8 treatments significantly reduced the abundance of mmsA, iolA, ALDH6A1 genes in inositol phosphates. SC8 also significantly reduced the abundance of most genes involved in the oxidative phosphorylation, phosphotransferase system, and pentose phosphate pathway. Additionally, in putative flavoprotein involved in K^+ transport metabolic pathway, K07222 gene abundance was significantly higher in SC8 than in SC2.

Effect of continuous cropping on soil metabolites

A total of 111 metabolites were detected using GC–MS. The soil metabolites exhibited different responses in rotation and continuous cropping soils (Fig. 7a). Compared to SMR, 45 metabolites were down-regulated and 4 metabolites were up-regulated in the SC2 treatment (Fig. 7b). Compared to SMR, 22 metabolites were down-regulated and 5 metabolites were up-regulated in the SC8 treatment (Fig. 7c). Similarly, compared to SC2, 4 metabolites were down-regulated and 32 metabolites were up-regulated in the SC8 treatment (Fig. 7d). These

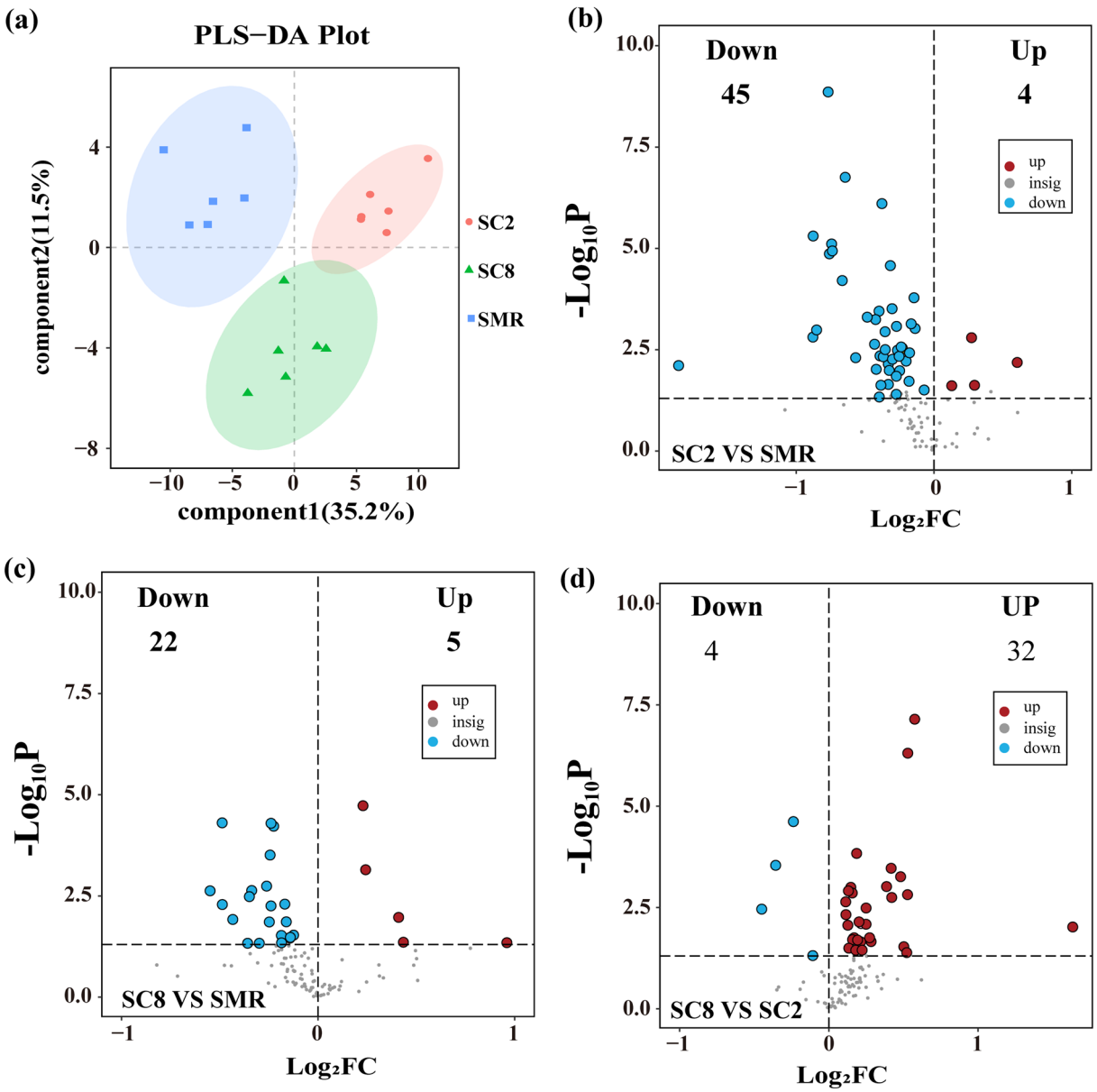


Fig. 7 Metabolite PLS-DA analysis (**a**) and differential metabolite volcano plots of SC2 vs. SMR (**b**), SC8 vs. SMR (**c**), SC8 vs. SC2 (**d**). *SMR* maize–soybean rotation, *SC8* 8-year soybean continuous cropping, *SC2* 2-year maize, 2-year soybean rotation cropping

differential metabolites belong to lipids, alcohols, aromatics, aldehydes, acids, carbohydrate, etc. (Additional file 1: Fig. S7a–c).

A total of 21 differential metabolites were selected in continuous cropping (SC8) and crop rotation (SC2, SMR) (Additional file 1: Fig. S7d), these metabolites belong to acid (2), alcohol (4), aldehyde (1), aromatics (1), carbohydrate (3), ketone (1), lipid (5), nitrogen compounds (1), others (3). Compared with SMR, the abundance of glycerin (alcohol) and 1,8-diaminoanthra-9,10-quinone (aromatics) significantly increased in SC2 and SC8 treatment, while the abundance of other differential metabolites decreased significantly. Compared to SC2, SC8 significantly increased 3,7-dimethyl-undecane, glycerophosphoric acid, 1-methoxy-1,3-propanediol, phthalic acid, cyclobutyl tridecyl ester, palmitic acid, 3-hexyl-7,8,9,10-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo(b,d) pyran-1-ol, muco-Inositol, 3-trifluoromethylbenzylamine, *N*,*N*-dinonyl, chrysophanol, 4-(2-methylbutanoyl)sucrose, 1,8-diaminoanthra-9,10-quinone, stigmasterol 2.

Correlations among soybean yield, soil nutrients, soil metabolites, soil microbial communities and functioning

Mantel's test showed that pH, NH_4^+–N, TP, AP, and AK significantly affected soil microbial community composition and KEGG functional composition, and pH, NH_4^+–N, and AK significantly affected soil metabolite composition (Fig. 8a). Differential microorganisms were significantly correlated with yield (Fig. 8b). Relative abundance of *Cryobacterium*, *Haliangium*, *Arthrobacter*, *Rhizobacter*, *Variovorax* showed highly significant positive correlation with yield, and *Marmoricola*, *Ramlibacter* showed significant positive correlation with yield. Most of these microorganisms enriched in SMR were significantly positively correlated with pH, AP, AK and significantly negatively correlated with NH_4^+–N. *Gaiella*, *Sphaerobacter* were significantly negatively correlated with yield, AP, AK and pH in soil nutrients. Soil metabolites were also significantly correlated with yield. Carbohydrate, ketone, lipid, nitrogen compounds were significantly and positively correlated with yield (Additional file 1: Fig. S10).

Metabolites were significantly correlated with differential microorganisms (Fig. 9a). Carbohydrate, ketone, lipid, aromatics, aldehyde had more significant positive correlation with microorganisms. Acid, alcohol had more significant negative correlation with differential microorganisms. For example, carbohydrate was significantly positively correlated with *Cryobacterium*, *Marmoricola*, *Pseudolabrys*, *Sphingomonas*. *Ramlibacter*, *Variovorax*, *Rhizobacter*, *Haliangium*, Ketone was significantly positively correlated with *Cryobacterium*, *Pseudonocardia*.

Figure 9b shows that differential metabolites have more significant positive correlations with differential genes (N and P). Carbohydrate among the metabolites had the most correlation with differential genes (N and P). Among the genes related to nitrogen metabolism, carbohydrate was significantly positively correlated with the abundance of nasB, narH, narY, nxrB, nirB, and significantly negatively correlated with the abundance of nifD, NifK. Among the P metabolism-related genes, carbohydrate was significantly correlated with the abundance of ATPF1A, atpA, ATPF1B, atpD, ccoN, CYTB, petB, ND1, ND5, nuoB, nuoD, nuoF, nuoH, nuoI, PTS-EI. PTSI, ptsI, PTS-Fru-EIIA, fruB, sdhA, frdA. sdhA, frdA, sdhB, frdB and significantly negatively correlated with

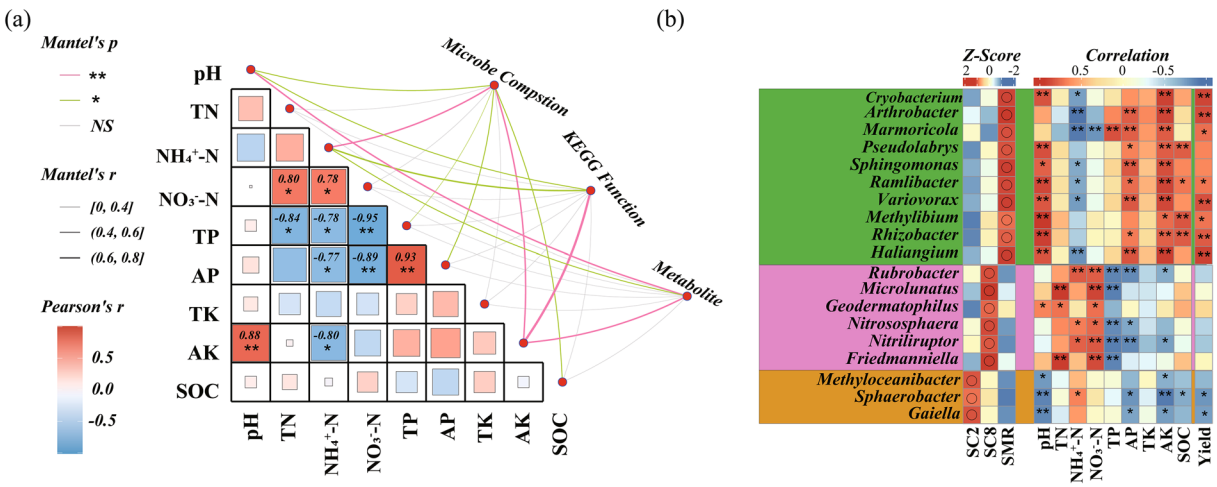


Fig. 8 Correlation analysis. **a** Mantel test of soil nutrients with microbial community, KEGG function, and metabolite composition. **b** Correlation analysis of yield, soil nutrient and differential microbial

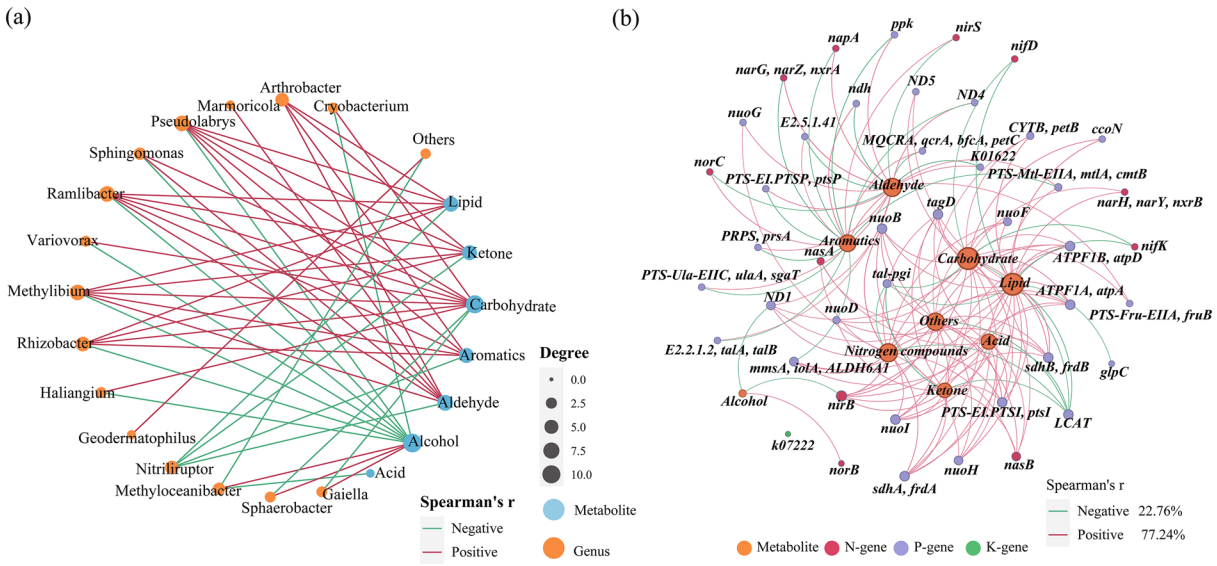


Fig. 9 Correlation analysis of microbial and metabolite. **a** Correlation analysis of different microbial and the categories of the significantly different metabolite. **b** Correlation analysis of functional genes (N, P, K) and the categories of the significantly different metabolite

the abundance of tal-pgi, LCAT. In addition, metabolites such as Lipid, Ketone, and Nitrogen compound also showed extensive significant correlations with N and P metabolism genes. The above results indicated that the metabolites were significantly correlated with soil N and P cycling genes. The above results indicated that the metabolites were significantly correlated with soil nitrogen and phosphorus cycling genes. In addition, the yield was significantly positively correlated with carbohydrate, lipid, ketone, nitrogen compounds of differential metabolites (Additional file 1: Fig. S10a).

Discussions

Continuous cropping changes the structure of soil microbial communities

Microbial community structure is very sensitive to changes in cropping practice [9, 22]. In the present study, continuous cropping had a significant effect on microbial community structure (Fig. 2). Actinobacteria, Proteobacteria, and Acidobacteria are common dominant phylum of the soil [23, 24], their responses to cropping systems are different. This study revealed that SC2 significantly reduced the relative abundance of Proteobacteria compared to SMR, while the relative abundance of Actinobacteria and Acidobacteria did not show significant variation across the three treatments (Additional file 1: Fig. S3). Li et al. found that the relative abundance of Proteobacteria was significantly reduced in the 10-year continuous cropping treatment of soybean [6]. While Liu et al. found a significant increase in the relative abundance of Deltaproteobacteria after 13 years of continuous cropping compared to short-term cropping (3, 5 years) [8]. The significant decrease in the relative abundance of Proteobacteria in SC2 treatment in this study may be due to the fact that continuous cropping was unfavorable to soil nutrient accumulation [10, 25], the relative abundance of Proteobacteria was further reduced [23, 26]. The relative abundance of Proteobacteria in SC8 treatment was not significantly different from that of SMR (Additional file 1: Fig. S3), which may be attributed to the improvement of the soil microbial environment and alleviation of soil nutrient deterioration after long-term continuous cropping [8, 10], and the restoration of the relative abundance of Proteobacteria. In addition, previous studies found that continuous cropping significantly reduced the relative abundance of Chloroflexi, Verrucomicrobia [6, 8, 24], but the results of our study were opposite (Additional file 1: Fig. S2), which might be due to the competition from other dominant phyla [27], which resulted in a decrease in the relative abundance of Chloroflexi, Verrucomicrobia in the SMR treatment. The different findings may be due to the differences in sampling time, sampling site, sequencing method and years of continuous cropping [28].

Sphingomonas is involved in soil C, N, and P cycling and can produce gibberellins to promote potato growth [29, 30]. *Phycicoccus* and *Rhodoplanes* are involved in soil nitrogen cycling [31, 32], which is helpful to improve nitrogen availability. *Marmoricola* and *Arthrobacter* are highly interconnected with the carbon and nitrogen cycling processes in soil, play a significant role in suppressing soil-borne pathogens [33, 34]. *Cryobacterium*

may play a role in nitrogen and phosphorus cycling in cold environments [35]. In this study, we found that the relative abundance of *Sphingomonas*, *Phycicoccus*, *Rhodoplanes*, *Marmoricola*, *Arthrobacter*, and *Cryobacterium* were significantly reduced in the SC2 and SC8 treatments (Additional file 1: Fig. S4a), indicated that continuous cropping may have reduced soil nutrient cycling and inhibition of pathogenic bacteria, which adversely affected soil health. Meanwhile, *Cryobacterium*, *Marmoricola*, and *Arthrobacter* were significantly positive correlated with soybean yield (Fig. 8b), indicated that the reduction of beneficial microorganisms might be one of the important factors for yield reduction in continuous cropping. In peach tree bark, *Friedmanniellas* is beneficial microorganisms antagonistic to peach gummosis disease pathogens [36]. *Nitrospira* is involved in soil nitrification and plays an important role in the nitrogen cycle [37, 38]. *Blastococcus* is beneficial to the accumulation of soil organic matter and soil nutrients [39, 40]. In this study, SC8 significantly increased the relative abundance of *Friedmanniellas*, *Nitrospira*, *Blastococcus*, and *Cryobacterium* compared to SC2 (Additional file 1: Fig. S4a), indicated SC8 treatment was associated with an increase in the relative abundance of microorganisms for soil nutrient cycling and inhibition of pathogens, which is favorable for soybean growth.

In species level, correlation analysis (Additional file 1: Fig. S9b) showed that *Gaiella sp. SCGC AG-212-M14*, *Chloroflexi bacterium RBG_16_70_13* were significantly negatively correlated with yield, and *Solirubrobacterales bacterium 70-9*, *Marmoricola* sp. *URHB0036* were significantly and positively correlated with yield, the role of these microorganisms in the soil is not yet clear and needs further study.

Continuous cropping changed soil microbial function and soil nutrient

Soil microorganisms actively contribute to the process of nutrient cycling and the degradation of soil organic matter, thereby fulfilling a crucial function in enhancing soil ecological functions and preserving the health of plants. Liu et al. found that soybean continuous cropping significantly reduced the copy number of genes related to soil N and P transformations [13]. Extending the continuous cropping years of chili peppers resulted in a reduction of genes for soil energy metabolism, carbohydrate metabolism and amino acid metabolism [41]. In this study, the relative abundance of soil energy metabolism, amino acid metabolism and carbohydrate metabolism were significantly reduced in the SC2 and SC8 treatments (Fig. 5a), and the reduction of these functions may be one of the reasons for the creation of continuous cropping obstacle. Previous studies have shown that continuous cropping of soybean reduced the copy number of genes related to soil nitrogen cycling [13, 14], but in this study, we found that continuous cropping (SC8) increased the abundance of genes for complete ammonia oxidation (hao, pmoB-amoB, pmoA-amoA), nitrification (hao, pmoB-amoB, pmoA-amoA), and nitrogen fixation (nifK) in nitrogen metabolism (Fig. 6). The differences in the study may be due to the different period of continuous cropping [28]. After long-term continuous cropping of soybean, some beneficial microorganisms recovered [10], which in turn favored the accumulation of soil N metabolism-related genes. Continuous cropping reduced the abundance of genes related to phosphorus and potassium metabolism, consistent with the P and K content. This suggests that although long-term continuous cropping of soybean favors nitrogen accumulation, it is not conducive to phosphorus metabolism, which in turn leads to nutrient imbalance.

Soil chemical properties are one of the important soil health indicators [3]. Numerous studies have shown that crop succession can lead to soil nutrient imbalance [6, 8]. In this study, soil pH, TP and available nutrients (AP and AK) were significantly higher in SMR than in SC8 (Additional file 1: Table S3), which is consistent with previously reported results [8]. However, the pH and AK contents of SC8 were significantly higher than those of SC2 (Additional file 1: Table S3), probably because the SC2 treatment caused a certain degree of continuous cropping obstacle after 2 years of continuous soybean planting, which has been shown by a previous study to cause nutrient imbalance after 2 years of continuous planting [42]. The differences in SOC content between treatments in this study were not significant, which may be due to the low sensitivity of carbon content to different cropping practices [8]. In addition, we found that SC8 significantly increased soil TN and nitrate N content and significantly decreased soil TP and AP content compared to rotational cropping (SMR, SC2), which was related to the increased abundance of N metabolism genes (Fig. 6) and the abundance of P metabolism genes (Additional file 1: Fig. S6).

Continuous cropping changed soil metabolite composition

Carbohydrates are an important component of soil organic matter [43]. D-Mannose, D-galactose can be used as a carbon and nitrogen source directly utilized by microorganisms [44]. D-Galactose was found to induce colonization of beneficial bacteria by Liu et al. [43]. In this study, compared with SMR, continuous cropping treatments (SC2, SC8) significantly reduced the abundance of D-galactose and D-mannose in carbohydrates (Additional file 1: Fig. S7), reduced the microbial carbon source supply. Lipids in soil are important nutrient sources for microorganisms and play a role in chemical signaling in

plant–microbe interactions [45, 46]. The abundance of lipids in the differential metabolites of the present study was significantly reduced in the SC2 and SC8 treatments (Additional file 1: Fig S7), and the plant–microbe signaling aspects were adversely affected. Heneicosane, a volatile organic compound, is beneficial in suppressing phytopathogenic bacteria in soil [47, 48]. Palmitic acid, a saturated long-chain fatty acid compound, is not only utilized as a carbon source by microorganisms [49], but also inhibits the growth of plant soilborne pathogens [42, 50]. Stigmasterol is a phytosterol that enhances the nitrogen uptake and has the potential to promote the growth of duckweed plants [51]. In this study, we found that the abundance of heneicosane, palmitic acid, and stigmasterol was significantly reduced in the continuous cropping treatments (SC8) (Additional file 1: Fig. S7), indicated that continuous cropping reduces the supply of soil carbon and the potential to inhibit the growth of pathogenic fungi, and at the same time, may reduce the growth potential of the crop. In addition, compared to SC2, SC8 significantly increased the abundance of metabolites such as palmitic acid and stigmasterol (Additional file 1: Fig. S7), and soil function was partially restored, which may be one of the reasons why the yield of SC8 treatment was higher than that of SC2 treatment.

Correlation of yield with others indicators

Soil metabolites composition is significantly correlated with soil microbial communities [21, 52]. A well-structured microbial community maintains healthy soil microenvironments and allows crops to utilize soil nutrients more efficiently, thus achieving higher production potential. The significant effect of pH and available nutrients on microbial communities is widely reported [15, 21–23]. In this study, mantel's test showed that pH, NH_4^+–N, and AK significantly affected soil microbial community composition, KEGG functional composition, and soil metabolite composition (Fig. 8a). Based on the correlation of yield, differential metabolites, and differential microorganisms, we constructed a schematic diagram of the correlation among the three (Fig. 10), the results demonstrated that continuous cropping had a negative impact on the relative abundance of *Cryobacterium*, *Arthrobacter*, *Ramlibacter*, *Variovorax*, *Methylibium*, *Rhizobacter*, *Haliangium*. It also decreased the relative abundance of microbial carbohydrate metabolism, amino acid metabolism, and energy metabolism. Additionally, there was an unfavorable accumulation of carbohydrate, ketone, lipid, and nitrogen compounds. These metabolites, beneficial genera, and functions were found to be significantly and positively correlated with yield. The decrease in energy substances available for microorganisms, the weakening of metabolic functions, and the reduction in the abundance of beneficial microorganisms in continuous cropping soils are likely the main reasons for the decline in yield in continuous cropping soybeans.

Soil toxic substances are important factors affecting crop yield and soil microbial community structure.

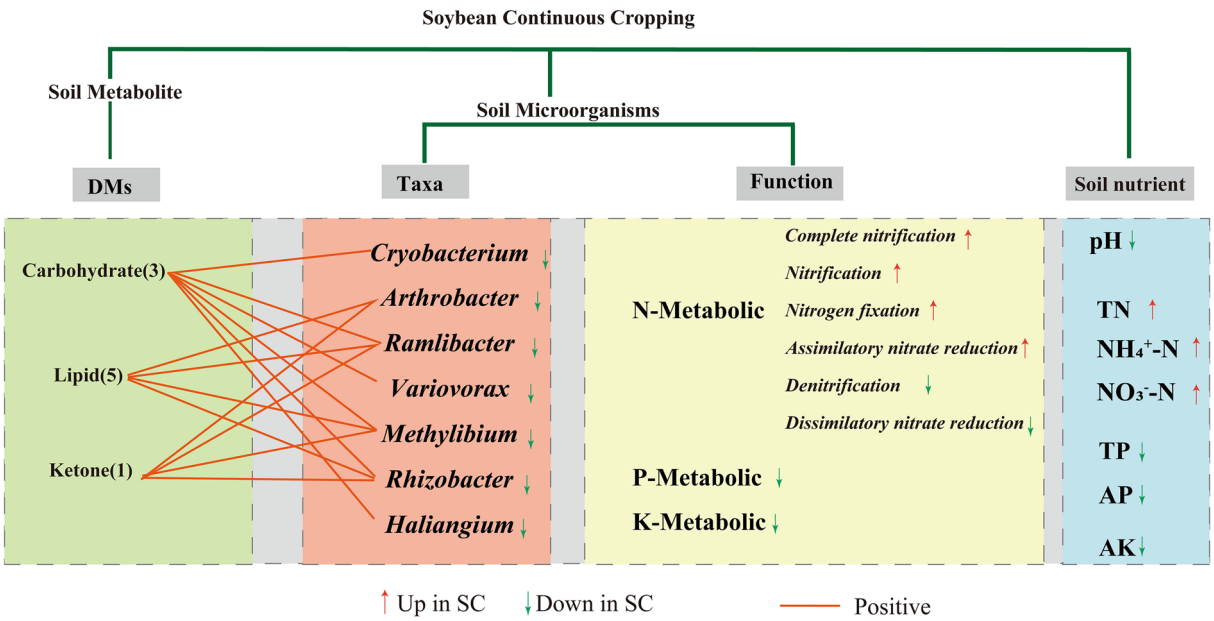


Fig. 10 Effect of continuous cropping on soil microorganisms, metabolites, and soil nutrients. Numbers in parentheses for metabolites represent the amounts of different metabolites. Red lines between metabolites and microbial taxa represent correlations between the two

However, this study did not measure soil toxic substance concentrations and, therefore, does not have a comprehensive understanding of soil health in continuous soybean. In the future, we will investigate the relationship between toxic substances and soil microorganisms in continuous soybean soils.

Conclusion

Compared to rotation, continuous cropping reduced soybean yield. The yield of soybean continuous cropping for 8 years was higher than that of continuous cropping for 2 years. Continuous cropping significantly reduced the abundance of beneficial bacteria involved in soil nutrient cycling and inhibition of pathogenic bacteria. And continuous cropping also significantly increased the abundance of genes in nitrogen metabolism and significantly decreased the abundance of genes related to phosphorus and potassium metabolism. Additionally, the abundance of metabolites such as carbohydrate, ketone, lipid, and nitrogen compounds were also reduced due to continuous cropping. These beneficial bacteria, metabolic functions, and metabolites showed a significant positive correlation with soybean yield. Compared to 2 years of soybean cropping, the relative abundance of beneficial bacteria, metabolic function and differential metabolites positively related to soybean yield increased significantly after 8 years of soybean continuous cropping. Declines in soybean yields can be attributed to soil nutrient imbalances, declines in the relative abundance of beneficial microorganisms and metabolic functions, as well as the decrease in energy substances.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00569-x.

Additional file 1: Text S1. Analyses of soil metabolome. **Text S2.** Analyses of soil nutrients. **Table S1.** Basal fertility of the experimental site. **Table S2.** Year of soybean planting and production. **Table S3.** Soil nutrients at soybean flowering in 2022. **Table S4.** Gene catalogue basic information catalogue. **Table S5.** Table of average relative abundance of species by treatment. SMR: maize–soybean rotation; SC8: 8-year soybean continuous cropping; SC2: 2-year maize, 2-year soybean rotation cropping. **Table S6.** ANOVA of the top 20 KEGG Level2 functional pathways in relative abundance (%). Results are means ± sd, different letters indicate 0.05 level differences. SMR: maize–soybean rotation; SC8: 8-year soybean continuous cropping; SC2: 2-year maize, 2-year soybean rotation cropping. **Table S7.** ANOVA of the top 20 KEGG Level3 functional pathways in relative abundance (%). Results are means ± sd, different letters indicate 0.05 level differences. SMR: maize–soybean rotation; SC8: 8-year soybean continuous cropping; SC2: 2-year maize, 2-year soybean rotation cropping. **Table S8.** ID of the differential metabolites. **Table S9.** ID of the microbial species level. **Figure S1.** Monthly average temperature and monthly average rainfall in the experiment site. **Figure S2.** Relative abundance of microbial taxa stacked histogram at the species level. **Figure S3.** Box line plot of microbial phyla with relative abundance greater than 1% between treatments. Different letters indicate significant differences at 0.05 level. SMR: maize–soybean rotation; SC8: 8-year soybean continuous cropping; SC2: 2-year maize, 2-year soybean rotation cropping. **Figure S4.** One-way ANOVA for the top 20 taxa in terms of relative abundance of microorganisms at the genus level (a) and species level (b). Different letters indicate significant differences at 0.05 level. SMR: maize–soybean rotation; SC8: 8-year soybean continuous cropping; SC2: 2-year maize, 2-year soybean rotation cropping. **Fig. S5.** KEGG pathway PCoA analysis and ANOSIM, Adonis test at the level2 level (a) and level3 level (b). SMR: maize–soybean rotation; SC8: 8-year soybean continuous cropping; SC2: 2-year maize, 2-year soybean rotation cropping. **Figure S6.** Heatmap of abundance of genes related to P and K metabolism. The Z-values obtained after normalizing the functional relative abundance for each row are presented as the values corresponding to the middle heatmap. SMR: maize–soybean rotation; SC8: 8-year soybean continuous cropping; SC2: 2-year maize, 2-year soybean rotation cropping. **Figure S7.** Abundance of categories of the significantly different metabolite in SC2 vs SMR (a), SC8 vs SMR (b) and SC2 vs SC8 (c). Heatmap analysis of changes in abundance of soil metabolites that were significantly different between SMR and continuous cropping (SC2, SC8). *Represents 0.05 level differences. SMR: maize–soybean rotation; SC8: 8-year soybean continuous cropping; SC2: 2-year maize, 2-year soybean rotation cropping. **Figure S8.** Network of Spearman's correlation between soil nutrients and abundance of N metabolite genes (a) P metabolite genes (b). **Figure S9.** Heatmap of Spearman's correlation between yield and abundance of the categories of the significantly different metabolite (a) and different metabolites (b). The different metabolites corresponding to the metabolites numbers in Fig. S4 are detailed in Table S6. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. SMR: maize–soybean rotation; SC8: 8-year soybean continuous cropping; SC2: 2-year maize, 2-year soybean rotation cropping.

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Author contributions
Letian Xu and Chunmei Ma designed the experiment. Letian Xu, Shun Jin and Yue Su completed the experiment. Letian Xu carried out data analysis and graphic production. Letian Xu participated in the writing of manuscript. Letian Xu, Chao Yan and Chunmei Ma performed the final editing of the manuscript. We thank Xiaochen Lyu and Shuangshuang Yan, Liang Cao for overseeing and enhancing the writing process. Our final manuscript was approved by all the authors. All authors have read and agreed to the published version of the manuscript.

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Data availability
Data will be made available on request.

Declarations

Competing interests
We declare that there are no competing interests.

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