Enhanced Nutrient Uptake in Side-Row Maize and Improved Microbial Community Diversity in Wide-Strip Intercropping of Maize and Peanut

Xinhua Zhao
Shenyang Agricultural University
Qiqi Dong
Shenyang Agricultural University
Yi Han
Shenyang Agricultural University
Kezhao Zhang
Shenyang Agricultural University
Xiaolong Shi
Shenyang Agricultural University
Xu Yang
Shenyang Agricultural University
Yang Yuan
Shenyang Agricultural University
Dongying Zhou
Shenyang Agricultural University
Kai Wang
Shenyang Agricultural University
Xiaoguang Wang
Shenyang Agricultural University
Chunji Jiang
Shenyang Agricultural University
Xibo Liu
Shenyang Agricultural University
He Zhang
Shenyang Agricultural University
Zhimeng Zhang
Shandong Peanut Research Institute
Haiqiu Yu (yuhaiqiu@syau.edu.cn)
Shenyang Agricultural University

Research Article

Keywords: maize, peanut, wide-strip intercropping, nitrogen content, 16S/ ITS, soil enzyme

Posted Date: June 24th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-643717/v1

Abstract

Background: Intercropping, a diversified planting pattern is currently the subject of major global research, but uncertainty remains about the rhizosphere interaction of intercropped maize and peanut, which increases nitrogen uptake. We explored the changes in soil physicochemical properties, nutrient uptake and use, and microbial community structure in wide-strip intercropped maize and peanut.

Results: The results from three treatments, sole maize (SM), sole peanut (SP) and intercropping of maize and peanut (IMP), showed that intercropping maize (IM) had a marginal advantage and that the nutrient content of roots, stems and grains in side-row maize was better than that of middle intercropping maize (MIM) and SM. And the yield of intercropped maize was higher than sole cropping. Compared with SM and SP, the soil nitrogen content (TN) in IM and intercropping peanut (IP) was lower and increased the soil enzyme activities of nitrate reeducates (NR) and peroxidase (POD), showing a significant negative correlation with soil TN. And decreased the soil enzymes activities of Pro and DHO, showing a positively correlation with soil TN. The diversity and richness of bacteria and fungi was decreased in IM rhizosphere soil, however, that richness of fungi was increased in IP rhizosphere soil. The *RB41*, *Candidatus-udaeobacter, Stropharia*, *Fusarium* and *Penicillium* were correlated with soil enzyme activity. In addition, intercropping enriched the functional diversity of bacterial community and reduced the pathogenic fungi.

Conclusion: IMP changed the rhizosphere soil bacterial and fungal community structure and composition, enriched nitrogen-fixing bacteria in the IP rhizosphere soil, promoted the nitrogen content of IM and provided a scientific basis for promoting IMP in northeastern China.

Background

Maize and peanut are major grain and oil crops and are important in ensuring food security in China. Sole cropping has been widely used in recent decades to facilitate planting, field management and mechanization. Sole cropping and improving yield by increasing fertilizer application are, however, not only detrimental to grain production in China [1] but also disturb the ecological stability of the soil microbial community and limit environmental sustainability [2]. Previous studies have found that wide-strip intercropping has the advantages of using marginal effects to increase yield, to optimize population structure, and to promote light energy utilization [3, 4]. This method is also suitable for mechanized seeding, fertilization and field management [5]. Maize and peanut strip intercropping can not only improve crop yield and water and fertilizer utilization efficiency, but also can reduce competition for major soil nutrients, increase beneficial soil microorganism numbers and diversity and reduce pathogenic and poisonous microorganisms, effectively improving the ecological environment of farmland [6, 7]. While helping to reduce carbon emissions and to increase the economic value of the ecosystem [8].

Previous studies have showed that plant nutrient uptake is affected by soil nutrient distribution, and the presence of neighboring plants greatly affects crop nutrient uptake in an intercropping system [9]. In the Loess Plateau of China, the intercropping of proso millet and mung bean have been reported to increased

the nitrogen absorption efficiency by 96% and 71.6%, respectively, due to complementarity of crops[10]. The maize grain nitrogen uptake was increased by 25.5% in the maize and soybean strip intercropping in southwest of China[11]. Intercropping with legumes, through the legumes' symbiotic relationship with nitrogen-fixing bacteria to obtain nitrogen from the air, allows neighboring plants to obtain more nitrogen from the soil [12]. Intercropping can improve the efficient utilization of soil nutrient resources through the complementary niche of crop root growth time and space.

Soil microbial activities are critical to nutrient mobilization and mineralization, directly affects the nutrient utilization efficiency of plants [13],and an important source of soil enzymes [14]. Previous studies have reported that intercropping changes the composition and function of microbial communities. The abundance of the nitrogen-fixing microbes *Rhizobium hainanense*, *R. leguminosarum* and *Frankia* spp. were promoted in the rhizosphere of peanut when intercropped with maize [15]. Intercropping of cassava and peanut induced ethylene release resulted in the increase of *Actinomycetes* abundance in the rhizosphere of peanut and promoted the absorption of soil available nutrients, thus increasing the yield of peanut [16]. Some studies have also shown that root exudates affect the structure and function of rhizosphere microbial community, promote soil organic matter mineralization, and thus affect the rhizosphere nitrogen cycle [17]. In the study of intercropping of maize and faba bean, maize root exudates increased nodule formation and biological nitrogen fixation in faba bean root, except that flavonoids in leguminous root exudates stimulated NOD gene expression in rhizobia [18]. The nitrate absorption pathway of mycorrhizal symbiosis has also been shown to promote the nitrogen absorption efficiency of gramineous crops [19]. The presence of maize increased the secretion of carboxylate in alfalfa root system, improved the availability of soil phosphorus, and then promoted the growth of maize in the aboveground and absorption of phosphorus [20]. Therefore, the relationship between microorganisms, plants and soil under intercropping of maize and peanut can not only reveal the adaptation of plants to the microecological environment but also improve resource utilization efficiency, reduce fertilizer application, protect the ecological environment and help to develop sustainable agriculture.

Northeastern China has a temperate monsoon climate, drought and less rain during the growing season, and there has been limited systematic research into the characteristics and mechanism of nitrogen uptake by crop and correlation between soil physicochemical properties and soil microorganisms at genus level. Through a field experiment in the study, the purpose of 1) the changes in structure and diversity of bacterial and fungal community at the genera level in intercropping, compared with sole cropping, which genera are beneficial, 2) Whether there is a correlation and the mechanism of soil enzyme activities and bacterial and fungal communities at the genus level, 3) We also clarified microecological changes in farmland ecosystems under maize and peanut strip intercropping in Northeast China maintain the balance of the soil ecosystem and increase productivity through sustainable agriculture.

Results

Effect of intercropping on the nitrogen content and yield of maize and peanut

Changes in the nitrogen content of maize and peanuts were similar between 2018 and 2019. The IM had higher nitrogen contents than those of SM, and the IP was lower than SP. The nitrogen content in the roots of IM was clearly higher than that of SM, The stems and leaves of IP significantly lower than those of SP (Fig. 1), indicating that intercropping increased the nitrogen content in maize. Compared with MIM, the IM was significantly higher than MIM. The IP was lower than MIP, indicating that intercropped maize has the marginal advantage. In addition, ear length and number of grains per spike significantly affected maize yield. Compared with SM, the yield of intercropped maize was significantly increased, by 30.34% (2018) and 24.8% (2019) (Table S1). The yield of peanut was significantly affected by 100 kernel weight. Intercropped peanut yield decreased by 33.49% (2018) and 2.4% (2019), compared with SP.

Changes in soil TN content and soil enzyme activity at IMP

The soil TN content of SM and SP were significantly higher than that of IM and IP, showed that intercropping increased soil nutrient consumption. And the soil TN content of IM and IP were lower than SIM and SIP, respectively (Table 1). It was speculated that the interspecific root interaction between IM and IP could promote soil nutrients uptake and utilization. The soil TN of II between IM and IP were no significantly difference. (Table S2).

Table 1
Soil total nitrogen (TN) content of different soil samples(mg/Kg)

Stage	**Trumpeting Stage**	**Heading Stage/**	**Anthesis and Silking stage/**	**Grain Filling Stage/**	**Mature stage**
Sample		**Seedling Sage**	**Flowering stage**	**Podding Stage**	
SM	235.43 ± 1.59a	224.44 ± 2.66a	186.01 ± 2.56a	157 ± 0.85a	125 ± 1.18a
MIM	4.34 ± 0.89c	6.34 ± 0.33b	5.19 ± 0.09b	6.59 ± 0.5b	2.91 ± 0.49b
IM	7.39 ± 0.15b	6.63 ± 0.37b	5.62 ± 0.19b	4.93 ± 0.54c	2.31 ± 0.7b
SP	258.16 ± 1.42a	217.66 ± 1.62a	179.37 ± 1.37a	133.91 ± 1.36a	85.93 ± 0.61a
MIP	5.87 ± 0.68b	7.73 ± 0.16b	6.5 ± 0.17b	11.47 ± 0.53b	2.31 ± 0.65b
IP	6.98 ± 0.5b	6.4 ± 0.31b	7.13 ± 0.22b	9.67 ± 0.29b	3.38 ± 0.05b

Compared with SM, the activities of nitrate reductase (NR) and peroxidase (POD) (Duncan test, $P < 0.05$) in IM soil increased, and the activity of protease (Pro) (Duncan test, $P < 0.05$) and dehydrogenase (DHO) decreased (Fig. 2). The POD in IP soil was increased and the activity of NR, Pro (Duncan test, $P < 0.05$) and DHO (Duncan test, $P < 0.05$) were decreased than SP (Fig. 2). Compared with monoculture, IMP significantly altered the activities of Pro, POD and DHO. Compared with the middle row of intercropping, the change of soil enzyme activities in the side row was similar to the above (Fig. 2). Furthermore, four soil enzyme activities were no significant difference in IM, IP and II (Table S3), which indicated soil enzyme activities tended to be stable under intercropping. Correlation analysis showed that POD activity was significantly negatively correlated with TN, while other enzyme activities were significantly positively correlated with TN (Figure S1). The results showed that IMP changes the soil physicochemical properties compared with sole cropping, and it affected the change of soil nutrient content.

OTUs and diversity of the rhizosphere soil microbial community

Through OTUs analysis, combined with the species represented by OTUs, common microorganisms in different environments can be found. Compared with SM, the OTUs of bacteria and fungi in IM were decreased markedly by 9.7% and 10.4%, respectively. However, compared with SP, the variation of OTUs in IP was different. The number of OTUs was lower (by 3.9%) in the bacterial community, while the number of fungal OTUs was higher (by 7.9%) (Figure S2). IMP changed the number of OTUs bacteria and fungi in rhizosphere soil, and the change was different among crop types. Bacterial OTUs analysis indicated that 4 OTUs were common to all samples. The number of OTUs shared by IM and SM was 1, IP and SP was 4, IP and II was 1 (Fig. 3-A). In the fungal community, of the number of OTUs shared by IM and SIM was 1, IM and II was 1,IP and SP was 7, IP and SIP was 2, IP and II was 3, respectively (Fig. 3-B).

The diversity and richness of bacteria and fungi in IM were lower than that of SM and SIM, and richness of fungi in IP were higher than that of SP and SIP. The bacterial diversity and richness of were higher than IP and IM, respectively. While the fungal diversity and richness of were lower than IM and IP (Fig. 4).

Composition and function of the microbial community in rhizosphere soil

Although reduced the diversity and richness of microorganisms in intercropping. However, compared with sole cropping and middle row of intercropping, the abundance of some species increased. Such as *RB41*, *Haliangium*, *Ramlibacter* and *Candidatus-udaeobacter* were higher in IM and IP (Fig. 5-A). The abundance of *Ellin6067*, *MND1*, *RB41* and in II were higher than in those in IP and IM, whereas *Ramlibacter* and *Candidatus-udaeobacter* were lower (Fig. 5-A). Specificaly, the abundance of *Sphingomonas* was increased in IP (Fig. 5-A). To identify the representative microbial of the samples, soil microbial from the different treatment groups were compared using LEfSe analysis. In the genus level, *Ramlibacter* and

MND1 were significantly enriched in IP and II, respectively (Fig. 6-A). Analysis for KEGG pathway using LEfSe, the functions of the first level of the genus bacteria were mainly metabolism (Figure S3). The functions of the second level included amino acids metabolism, carbohydrates metabolism and other amino acids metabolism accounted for high relatively abundance (Fig. 7-A).

Compared with SM and SIM, the abundance of *Fusarium*, *Neocosmospora*, *Chaetomium*, *Cladosporium*, *Staphylotrichum* and *Penicillium* were higher in IM (Fig. 5-B). The abundance of *Mortierella*, *Fusarium*, *Chaetomium*, *Cladosporium* and *Penicillium* in IP were higher than those in SP (Fig. 5-B). In case of II, the abundance of *Mortierella*, *Staphylotrichum*, *Saccharomyces* and *Tausonia* were higher than those in IM and IP, whereas the abundance of *Fusarium*, *Chaetomium*, *Cladosporium* were lower (Fig. 5-B). The abundance of *Chaetomium* and Stropharia were significantly enriched IM, and the abundance of *Penicillium* and *Fusarium* were significantly enriched IP (Fig. 6-B). According to the function analysis of the fungal community, it was found that most fungal species in the rhizosphere soil were saprophyrotrophic fungi, while pathotroph fungi had the least species (Fig. 7-B). Therefore, IMP reduced the varities of pathogenic bacteria in the rhizosphere soil.

Correlation between bacterial and fungal communities and soil physicochemical properties

RB41, Candidatus-udaeobacter were significantly positively correlated with POD activity, extremely significantly negatively correlated with TN, Pro and DHO, and significantly negatively correlated with Pro. *Vicinamibacter, Gemmatimonas* and *Ellin6067* were significantly positively correlated with TN, Pro and DHO (Fig. 8-A). In the fungal community, *Mortierella* was significantly positively correlated with TN. *Chaetomium, Fusarium* and *Penicillium* were extremely significantly and significantly positively correlated with POD activity, and extremely significantly or negatively correlated with DHO, Pro and TN. *Stropharia*, *Staphylotrichum* and *Cladosporium* were extremely significantly and significantly negatively correlated with TN and DHO, respectively (Fig. 8-B).

Discussion

Nitrogen is an essential element for plant growth and development and is the element most closely related to yield. In a gramineous and leguminous intercropping system, nitrogen content was clearly promoted nitrogen content and yield in the gramineous crop, and improved land productivity [21]. In the current study, maize had marginal advantage under IMP. The nitrogen content in the IM roots, stems and leaves was significantly higher than SM and MIM, respectively (Fig. 1), which was consistent with the findings of another [4]. Due to the adjustment of root length density and root distribution, the nitrogen uptake per unit root length was increased, compared with that of monoculture [22]. Combined with other research reports, maize competes strongly for nitrogen and absorbs more nitrogen than peanut, so significantly increasing the nitrogen content in IM and promoting the yield of maize [23]. In the middle and late growth period, the shading of IM was intensified, and photosynthesis was weakened on the IP, which further affected the absorption of nutrients by the peanut (Fig. 1). In the intercropping of maize and

soybean, light transmittance was increased after defoliation of the top two leaves of the maize, and the nitrogen absorption of soybean was increased by 5% (grain), 10%(stem) and 14% (root), respectively [24]. The shading of maize inhibited the growth of peanut and the yield of intercropped peanut decreased (Table S1). Ear length, number of grains per spike and fruit weight per hundred were the main yield components (Table S1), which were the same as the findings of previous studies [4].

In this study, it was found that interspecific root interactions of IMP promoted soil nutrient uptake and utilization, compared with monoculture (Table 1). The results were consistent with the decrease of soil TN in intercropping Chinease milk vetch and rape[25]. It is reported, the root density distribution was different under different soil depths, which affected the absorption and utilization of soil nutrients [26], and IMP maintained the basic stability of soil chemical properties and the diversity effect of soil nitrogen accumulation (Table S2-3) [14, 27].

It is needed to explore the interactions of rhizosphere microbial community in intercropping, and the inner mechanism of IMP was elucidated through analyzing the physicochemical properties of and rhizosphere soil microbial community [26]. Soil microorganisms are one of the main sources of soil enzymes, and there is a correlation between soil enzyme activity and microorganisms [11, 28], it's consistent with this study show that *RB41*, *Candidatus-udaeobacter* and *Chaetomium* were significantly positively correlated with soil POD, and negatively correlated with Pro and DHO (Fig. 8). Besides, *Fusarium, Stropharia* and *Penicillium* also were negatively correlated with Pro and DHO (Fig. 8-B). *Candidatus-udaeobacter* within the Verrucomicrobia phylum is pervasive in soils around the world, sacrificing metabolic versatility for efficiency to become dominant in the soil environment. The *Chaetomium*, which is a beneficial fungi with biocontrol effect and antagonistic to soil pathogenic bacteria [29].In our study, the soil POD was negatively correlated with soil TN content across all soil (Figure S1), due to it is involved in the degradation of hydrocarbons and their intermediates [30]. Soil Pro and DHO activities were positively correlated with TN (Figure S1), which was consistent with previous studies [31]. Soil Pro are involved in the conversion of amino acids and other nitrogen-containing organic compounds present in soil, and their hydrolysates are one of the nitrogen sources for higher plants [32]. Soil DHO participates in soil carbon cycle and promotes dehydrogenation of carbohydrates and organic acids [31].

Soil microorganisms play a key role in soil nutrient cycling and crop nutrient uptake [13]. Through the analysis of the dominant bacteria in the soil components, it was found that the intercropping varieties had increased the relative abundance on the dominant bacteria species (Fig. 5-A). The *Ramlibacter* and *MND1* were significantly enriched in IP and II, respectively (Fig. 6-A). The functions of *MND1* need to be investigated more deeply. The *Ramlibacter* within the Proteobacteria, comprising an enormous range of metabolic diversity [26]. In addition, *Sphingomonas* also increased in IP than SP (Fig. 5-B), which is the characteristics of promoting nitrogen fixation and dehydrogenation [33] and the uptake of nutrients in the rhizosphere, improving the soil environment in the rhizosphere of IP, and maintaining the soil nitrogen balance. This result could be related to the reduced abundance of denitrifying bacteria *Haliangium*, which reduces nitrates in the soil to nitrogen and releases them into the air [34]. So that, intercropping improved the bacterial community structure and increased the abundance of beneficial bacteria. IMP changed the

abundance of bacteria in soil, affected soil enzyme activities and soil nutrients, and ultimately affected crop nutrient uptake (Fig. 9). Other bacterial genera had no significant correlation with soil enzyme activities (Fig. 8). The reason is that there are many kinds of bacteria in the soil, and the correlation between soil enzyme activity and bacteria is not specific and unique. A single bacterium can affect a variety of enzyme activities, so it is necessary to further explore or study the functional properties of each bacterium and the mechanism of soil enzyme activities themselves [35]. It was found that the transport and metabolism of amino acids, carbohydrate transport and metabolism, and metabolism of other amino acids in the secondary functional areas were relatively high in abundance, which was consistent with previous findings [32, 36]. Furthermore, it was speculated that the relative abundance of *RB41* and *Candidatus-udaeobacter* increased. Soil microorganisms to transport amino acid metabolism, carbohydrate metabolism to produce a series of material, when they were plants as signal perception, can cause related enzyme activity in plant or related gene expression changes, ensure the survival of the bacteria, and then adjust the plant physiological metabolism and nutrient accumulation levels, which promote plant growth and development [37, 38].

We found that, compared with SP, the change in OTUs and richness of IP was higher than those of SP (Figure S2 and 4). These results were consistent with the promotion of fungal community growth in the IMP, intercropping promotes fungal community growth [39]. On the one hand, many biotic and abiotic factors can alter the fungal community, such as soil chemical properties, plant functional diversities and management practices. On the other hand, the variety and quantity of root exudates affect the abundance of the rhizosphere fungal community owing to the presence of different crop species [40, 41].

Soil fungi decompose organic matter in crop residues and fertilizers, which is beneficial to soil nutrient supply and crop growth, but also affects soil enzyme activity [35]. *Mortierella* decomposes organic matter and promotes mineral uptake by plant roots. It also has the potential to secrete antimicrobials that inhibit pathogenic bacteria such as *Fusarium* [42–44]. However, many fungi are plant pathogens and cause fungal diseases [35]. The relative abundance of soil pathogens such as *Fusarium* increased (Fig. 5-B). IMP can reduce the damage of pathogenic fungi in soil to plants. The unique of *Saccharomyces* promoted crop yield increases under IMP. One possible reason is the secretion of hormones such as gibberellin (GA), cytokinin (CTK) and auxin (IAA), which promote metabolic activity and stimulate crop growth and development. Another reason may be that the large concentrations of bacteria around the rhizosphere are advantageous to *Saccharomyces*, effectively reducing pathogenic bacterial infection and improving crop resistance. The reason for the increased abundance of *Saccharomyces* in II is, however, unclear. Overall, the fungal structure in IMP, SM and SP was relatively stable but still had some differences in distribution. This is because different crops release chemical substances to the surrounding environment through allelopathy produced by secondary substances in intercropping. Another reason is that the physicochemical properties of soil are related. Saprotrophic fungi are concentrated in rhizosphere soil as a dominant functional group and can obtain nutrients by degrading dead host cells (Fig. 7-C). They are closely involved in the cycle of decomposition of organic matter and nutrients and can also produce a series of hydrolases and oxidases, which contribute to the decomposition of carbohydrates and increase the nutrients in soil organic matter [45]. Compared with

that of sole cropping, the soil IMP micro ecological environment is complex and microorganisms and plants are interdependent. This characteristic provides a theoretical basis for further understanding the mechanism of plant nutrient absorption. Our results indicated that the staggered superposition of roots and secretion of secondary metabolites among root systems in IMP promoted the reproduction of rhizosphere fungi and improved microbial diversity [12]. In this regard, managing rhizosphere microbes and maintaining the balance of the soil microbial community assist plant growth and nutrient uptake.

Conclusion

Compared with sole maize and peanut, IMP improved the microecological environment of rhizosphere soil, and the bacterial and fungal diversity of maize rhizosphere soil decreased (Fig. 9). However, the relative abundance of beneficial bacteria in the genus *RB41* and *Candidatus-udaeobacter* were increased in IM. On the contrary, the diversity and richness of fungi in IP was increased. IMP can alleviate the imbalance of rhizosphere soil fungal community structure caused by perennial continuous cropping and benefit the abundance of fungi *Chaetomium*. In addition, IMP changed soil enzyme activities, affected the distribution of soil bacteria and fungi, activated the transport and metabolism of bacterial amino acids and carbohydrates, and reduced the species of pathogenic fungi. Overall, the IMP system can stimulate soil microbial communities, that are beneficial to plant growth, and its use is appropriate in agricultural practice. Our study clearly illustrated that the microbial community composition in rhizosphere soil has an important relationship with nutrient uptake and thus provides a theoretical basis for the use of IMP and nutrients in northeastern China.

Methods

Methods

Field sites and experimental design

Field experiments were conducted in 2018 and 2019 in long-term plots at Shenyang Agricultural University, Shenyang city, China (41°82' N, 123°56' E). We used a single-factor randomized block design with three treatments comprising sole maize (SM), sole peanut (SP) and intercropping of maize and peanut (IMP) and included three replicate plots. The maize was hybrid Liang-yu 99 (*Zea mays* L.), a semicompact plant with high nutrient efficiency (Dandong Denghai Seed Industry Co. Ltd., China). The peanut was Nong-hua 9 (*Arachis hypogaea* L.), which is upright and sparsely branched, has strong shade resistance and good comprehensive resistance (Peanut Research Institute of Shenyang Agricultural University, China). The sowing and harvest dates of maize and peanuts are shown in Supplementary Table S4. The field site was previously used for sole peanut, and the soil was a brown loam (Table S5). We used a planting pattern of 8:8 wide belts to intercrop maize and peanut, and the crop rows were oriented north–south (Fig. 9). Intercropping maize was grown at a row distance of 60 cm, with plant distance in the row of 25 cm, resulting in a density of 66670 plants/hm^2. Intercropping peanut was a

small ridge and double rows with a row distance of 60 cm, with a plant distance in the row of 12.3 cm, resulting in a density of 135508 plants/hm^2. Apply base fertilizer before sowing, intercropped maize with conventional compound fertilizer 750 kg/hm^2 (N-P_2O_5-K_2O = 27-13-15) and peanut with potassium phosphate compound fertilizer 750 kg/hm^2 (N-P_2O_5-K_2O = 14-16-15) (Table S6). Sole maize and peanut plots comprised 24 rows, and plant density and fertilizer were the same as in the intercropping pattern. Other cultivation management measures were consistent with conventional field production.

Sample collection

Plant samples

Maize (trumpeting stage, heading stage, anthesis and silking stage, grain filling stage and mature stage) and peanut (seedling stage, flowering stage, podding stage, and mature stage) were sampled from the main growth stages. We selected three plants of uniform size in each intercropped plot from the side row (IM for maize, IP for peanut), middle row (MIM for maize, MIP for peanut) and sole cropping middle row (SM for maize, SP for peanut).

Soil samples

We sampled maize and peanut soil at the same time. Samples were taken from in the side row (IM, IP) and middle row of the intercropped ridge (MIM, MIP), the shared soil of intercropping maize and peanut (II), and the middle of sole maize (SM) and sole peanut (SP). Soil samples and three replicates were taken at 0–20 cm from each point. The nitrogen content (TN) of the soil was determined after air drying and sieving. In 2019, the activities of soil enzymes were measured in soil samples at maize of anthesis and silking stage /peanut of flowering stage.

Rhizosphere soil was collected during the flowering stages of peanut in 2019. And selected from the side row of the intercropped ridge (IM, IP), the shared soil of intercropping maize and peanut (II) and the side (SM, SP) and middle of (SIM, SIP) in sole cropping. The roots were carefully uprooted from the soil and shaken gently to remove loosely attached soil. A sterile brush was used to collect soil from depths of 5–15 cm that had adhered firmly to the roots, and then the rhizosphere soil was sieved through 0.9-mm mesh [46]. Soil samples were separated into two parts: one part was stored at − 80°C for soil DNA extraction, and the other was stored at 4°C for analysis of soil physicochemical properties.

Measurement of nutrients and soil enzyme

The organs of maize (roots, stems, leaves and grains) and peanut (roots, stems, leaves and pods) were heated to 105°C for 30 min and dried at 80°C to constant weight. The total nitrogen (TN) content of the sample was determined by the Kjeldahl method (Kjeltec 8400, Foss, Denmark). Soil samples from different depths in each treatment were sieved and air-dried to determine soil TN content by the same method.

The nitrate reductase (NR), peroxidase (POD), protease (Pro) and dehydrogenase (DHO) of soil enzymes activities were measured using an ELISA kit (MLBIO, Shanghai, China). The kit assay Soil NR, Pro, Pod and DHO level in the sample, use Purified Soil NR antibody to coat micro titer plate wells, make solid-phase antibody, then add NR to the wells, Combined antibody which With HRP labeled, become antibody-antigen-enzyme-antibody complex, after washing Completely, Add TMB substrate solution, TMB substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometric ally at a wavelength of 450 nm. The concentration of NR in the samples is then determined by comparing the OD of the samples to the standard curve.

DNA extraction, PCR amplification and high-throughput sequencing

Soil genomic DNA was isolated using the PowerSoil® DNA Isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's protocol.

The 16S rRNA was amplified for each sample using primer sets of 27F (5'-AGRGTTTGATYNTGGCTCAG-3') and 1492R (5'-TASGGHTACCTTGTTASGACTT-3') with adapter sequences and barcode sequences. The ITS was amplified for each sample using primer sets of ITS9 munngs (F—CTTGGTCATTTAGAGGAAGTAA) and ITS4 ngsUni (R—TCCTCCGCTTATTGATATGC) with adapter sequences and barcode sequences. PCR was performed as follows: an initial denaturation at 95°C for 5 min, followed by 95°C for 30 s, 50°C for 30 s and 72°C for 1 min/1 kb and 72°C for 7 min, for 25–30 cycles. After the electrophoretic results were obtained, all PCR products were quantified by ImageJ software (version 1.4.3.67). After quantification, the samples were mixed according to the required output data amount and fragment size of each sample, and then recovered and purified with 0.8x magnetic beads to form a sequencing library (SMRT Bell), and the library was subjected to quality inspection [47, 48].

The qualified libraries were sequenced with PacBio sequencing platform, and SMRT Cell method was used to sequence marker genes in Biomarker Technologies Co.,Ltd, Beijing, China. To obtain the raw tags, paired-end reads were merged by FLASH (version 1.2.11 http://ccb.jhu.edu/software/FLASH/) [49]. Tags with an average quality score < 20 in a 50 bp sliding window were truncated using Trimmomatic (version 0.33) [50], and tags shorter than 350 bp were removed. We identified possible chimeras by employing UCHIME (version 4.2) [51], high-quality Tags sequences were obtained.

Statistical and bioinformatics analysis

The plant nutrient content, yield and soil physiochemical properties and diversity indices were tested for differences among wetlands restoration with the one-way Analysis of Variance (ANOVA) using SPSS 23.0 for Windows (IBM SPSS Inc., USA). Significance differences were defined at $p < 0.05$. Using OriginPro version 9.0 (OriginLab Corporation, Northampton, MA, United States) and R software (version 4.0.3) for drawing.

The high-quality sequences were clustered using USEARCH (version 10.0), and tags with similarity ≥ 97% were regarded as OTUs.

The high-quality sequences were clustered using USEARCH (version 10.0) [52] and tags with similarity ≥ 97% were regarded as a OTU. Taxonomy was assigned to all OTUs by searching against the Silva databases (Release128, http://www.arb-silva.de) [53], and the UNITE database (Release 8.1, http://unite.ut.ee/index.php) [54], and then were identified down to phylum, class, order, family and genus level using Ribosomal Database Project (RDP, version 2.2, http://sourceforge.net/projects/rdpclassifier/), the confidence threshold was 0.8.

Alpha diversity indices referring to community richness (Chao1) and community diversity (Shannon) were calculated by Mothur (version v.1.30, http://www.mothur.org/).

PICRUSt software (http://kiwi.cs.dal.ca/Software/STAMP) was used to predict the functional gene composition of the samples by comparing the species composition information obtained from 16S sequencing data, so as to analyze the functional differences between different samples or groups. Pair T-test was performed between different groups and the p-value was 0.05 [55]. Fungi Functional Guild (FUN Guild) was used to speculate the differential functional gene composition in bacterial and fungal samples to analyze the functional differences between different samples or groups.

Abbreviations

SM	Sole maize
SP	Sole peanut
SIM	The middle row of sole maize
SIP	The middle row of sole peanut
IM	Intercropping maize
IP	Intercropping peanut
II	The shared of intercropping maize and peanut
MIM	The middle intercropping maize
MIP	The middle intercropping peanut
NR	Nitrate reductase
Pro	Protease
POD	Peroxidase
DHO	Dehydrogenase
GA	Gibberellin
CTK	Cytokinin
IAA	Auxin
TN	Total nitrogen
OTUs	Operational Taxonomic Units
KEGG	Kyoto Encyclopedia of Genes and Genomes

Declarations

Acknowledgments: The research was supported by the China Agricultural Research System (CARS-13). We thank Lijun Zhang for guidance in the paper. We thank Pei Guo for drawing the simulated diagram in the paper.

Author Contributions

XZ and HY designed this study. QD and XZ conducted the data analysis and wrote the manuscript. YH, ZZ, YZ, YY, XY and KW carried out the field experiments. LS, and HZ helped data analysis. GW, JJ, BL and MZ revised the manuscript. All authors have read and approved the final manuscript.

Funding: This study was made possible by the China Agricultural Research System (CARS-13).

Availability of data and materials

All raw sequences have been deposited into a NCBI Sequence Read Archive with the accession number PRJNA728390 (Bacteria) and PRJNA728391 (Fungi).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1] Peanut Research Institute, Shenyang Agricultural University, Shenyang 110161, China.

[2] Shandong Peanut Research Institute, Qingdao 266100, Shandong, China.

References

1. Zhang Q, Chu Y, Xue Y, Ying H, Chen X, Zhao Y, Ma W, Ma L, Zhang J, Yin Y *et al*: **Outlook of China's agriculture transforming from smallholder operation to sustainable production**. *Global Food Security* 2020, **26**.
2. Misra P, Maji D, Awasthi A, Pandey SS, Yadav A, Pandey A, Saikia D, Babu CSV, Kalra A: **Vulnerability of Soil Microbiome to Monocropping of Medicinal and Aromatic Plants and Its Restoration Through Intercropping and Organic Amendments**. *Front Microbiol* 2019, **10**:2604.
3. Yang F, Liao D, Wu X, Gao R, Fan Y, Raza MA, Wang X, Yong T, Liu W, Liu J *et al*: **Effect of aboveground and belowground interactions on the intercrop yields in maize-soybean relay intercropping systems**. *Field Crops Res* 2017, **203**:16–23.
4. Wang R, Sun Z, Zhang L, Yang N, Feng L, Bai W, Zhang D, Wang Q, Evers JB, Liu Y *et al*: **Border-row proportion determines strength of interspecific interactions and crop yields in maize/peanut strip intercropping**. *Field Crops Res* 2020, **253**.
5. Du J-b, Han T-f, Gai J-y, Yong T-w, Sun X, Wang X-c, Yang F, Liu J, Shu K, Liu W-g *et al*: **Maize-soybean strip intercropping: Achieved a balance between high productivity and sustainability**. *Journal of Integrative Agriculture* 2018, **17**(4):747–754.

6. Du Q, Zhou L, Chen P, Liu X, Song C, Yang F, Wang X, Liu W, Sun X, Du J *et al*: **Relay-intercropping soybean with maize maintains soil fertility and increases nitrogen recovery efficiency by reducing nitrogen input**. *The Crop Journal* 2020, **8**(1):140–152.
7. He Y, Ding N, Shi J, Wu M, Liao H, Xu J: **Profiling of microbial PLFAs: Implications for interspecific interactions due to intercropping which increase phosphorus uptake in phosphorus limited acidic soils**. *Soil Biol Biochem* 2013, **57**:625–634.
8. Sun T, Zhao C, Feng X, Yin W, Gou Z, Lal R, Deng A, Chai Q, Song Z, Zhang W: **Maize-based intercropping systems achieve higher productivity and profitability with lesser environmental footprint in a water-scarce region of northwest China**. *Food and Energy Security* 2020.
9. Zhang D, Lyu Y, Li H, Tang X, Hu R, Rengel Z, Zhang F, Whalley WR, Davies WJ, Cahill JF, Jr. *et al*: **Neighbouring plants modify maize root foraging for phosphorus: coupling nutrients and neighbours for improved nutrient-use efficiency**. *New Phytol* 2019.
10. Gong X, Dang K, Liu L, Zhao G, Lv S, Tian L, Jin F, Feng Y, Zhao Y, Feng B: **Intercropping combined with nitrogen input promotes proso millet (Panicum miliaceum L.) growth and resource use efficiency to increase grain yield on the Loess plateau of China**. *Agric Water Manage* 2021, **243**.
11. Fu Zd, Zhou L, Chen P, Du Q, Pang T, Song C, Wang Xc, Liu Wg, Yang Wy, Yong Tw: **Effects of maize-soybean relay intercropping on crop nutrient uptake and soil bacterial community**. *Journal of Integrative Agriculture* 2019, **18**(9):2006–2018.
12. Mommer L, Kirkegaard J, van Ruijven J: **Root-Root Interactions: Towards A Rhizosphere Framework**. *Trends Plant Sci* 2016, **21**(3):209–217.
13. Guo Z, Wan S, Hua K, Yin Y, Chu H, Wang D, Guo X: **Fertilization regime has a greater effect on soil microbial community structure than crop rotation and growth stage in an agroecosystem**. *Applied Soil Ecology* 2020, **149**.
14. Wang Z-g, Bao X-g, Li X-f, Jin X, Zhao J-h, Sun J-h, Christie P, Li L: **Intercropping maintains soil fertility in terms of chemical properties and enzyme activities on a timescale of one decade**. *Plant Soil* 2015, **391**(1–2):265–282.
15. Chen J, Arafat Y, Wu L, Xiao Z, Li Q, Khan MA, Khan MU, Lin S, Lin W: **Shifts in soil microbial community, soil enzymes and crop yield under peanut/maize intercropping with reduced nitrogen levels**. *Applied Soil Ecology* 2018, **124**:327–334.
16. Chen Y, Bonkowski M, Shen Y, Griffiths BS, Jiang Y, Wang X, Sun B: **Root ethylene mediates rhizosphere microbial community reconstruction when chemically detecting cyanide produced by neighbouring plants**. *Microbiome* 2020, **8**(1):4.
17. Keiluweit M, Bougoure JJ, Nico PS, Pett-Ridge J, Weber PK, Kleber M: **Mineral protection of soil carbon counteracted by root exudates**. *Nature Climate Change* 2015, **5**(6):588–595.
18. Coskun D, Britto DT, Shi W, Kronzucker HJ: **How Plant Root Exudates Shape the Nitrogen Cycle**. *Trends Plant Sci* 2017, **22**(8):661–673.
19. Wang S, Chen A, Xie K, Yang X, Luo Z, Chen J, Zeng D, Ren Y, Yang C, Wang L *et al*: **Functional analysis of the OsNPF4.5 nitrate transporter reveals a conserved mycorrhizal pathway of nitrogen**

acquisition in plants. *Proc Natl Acad Sci U S A* 2020.

20. Wang L, Hou B, Zhang D, Lyu Y, Zhang K, Li H, Rengel Z, Shen J: **The niche complementarity driven by rhizosphere interactions enhances phosphorus-use efficiency in maize/alfalfa mixture**. *Food and Energy Security* 2020, **9**(4).
21. Jensen ES, Carlsson G, Hauggaard-Nielsen H: **Intercropping of grain legumes and cereals improves the use of soil N resources and reduces the requirement for synthetic fertilizer N: A global-scale analysis**. *Agronomy for Sustainable Development* 2020, **40**(1).
22. Liu Y-X, Sun J-H, Zhang F-F, Li L: **The plasticity of root distribution and nitrogen uptake contributes to recovery of maize growth at late growth stages in wheat/maize intercropping**. *Plant Soil* 2019, **447**(1–2):39–53.
23. Fan Y, Wang Z, Liao D, Raza MA, Wang B, Zhang J, Chen J, Feng L, Wu X, Liu C *et al*: **Uptake and utilization of nitrogen, phosphorus and potassium as related to yield advantage in maize-soybean intercropping under different row configurations**. *Sci Rep* 2020, **10**(1):9504.
24. Raza MA, Bin Khalid MH, Zhang X, Feng LY, Khan I, Hassan MJ, Ahmed M, Ansar M, Chen YK, Fan YF *et al*: **Effect of planting patterns on yield, nutrient accumulation and distribution in maize and soybean under relay intercropping systems**. *Sci Rep* 2019, **9**(1):4947.
25. Zhou Q, Chen J, Xing Y, Xie X, Wang L: **Influence of intercropping Chinese milk vetch on the soil microbial community in rhizosphere of rape**. *Plant Soil* 2019, **440**(1–2):85–96.
26. Tang X, Zhong R, Jiang J, He L, Huang Z, Shi G, Wu H, Liu J, Xiong F, Han Z *et al*: **Cassava/peanut intercropping improves soil quality via rhizospheric microbes increased available nitrogen contents**. *BMC Biotechnol* 2020, **20**(1):13.
27. Xue Y, Xia H, Christie P, Zhang Z, Li L, Tang C: **Crop acquisition of phosphorus, iron and zinc from soil in cereal/legume intercropping systems: a critical review**. *Ann Bot* 2016, **117**(3):363–377.
28. Diamantidis G, Effosse A, Potier P, Bally R: **Purification and characterization of the first bacterial laccase in the rhizospheric bacterium** *Azospirillum lipoferum*. *Soil Biology & Biochemistry* 2000, **32**:919–927.
29. Gao Z, Han M, Hu Y, Li Z, Liu C, Wang X, Tian Q, Jiao W, Hu J, Liu L *et al*: **Effects of Continuous Cropping of Sweet Potato on the Fungal Community Structure in Rhizospheric Soil**. *Front Microbiol* 2019, **10**:2269.
30. Liu Y, Yang F, Yang W, Wu F, Xu Z, Liu Y, Zhang L, Yue K, Ni X, Lan L *et al*: **Effects of naphthalene on soil fauna abundance and enzyme activity in the subalpine forest of western Sichuan, China**. *Sci Rep* 2019, **9**(1):2849.
31. Chae Y, Cui R, Woong Kim S, An G, Jeong SW, An YJ: **Exoenzyme activity in contaminated soils before and after soil washing: ss-glucosidase activity as a biological indicator of soil health**. *Ecotoxicol Environ Saf* 2017, **135**:368–374.
32. Tang X, Zhang Y, Jiang J, Meng X, Huang Z, Wu H, He L, Xiong F, Liu J, Zhong R *et al*: **Sugarcane/peanut intercropping system improves physicochemical properties by changing N and P cycling and organic matter turnover in root zone soil**. *PeerJ* 2021, **9**:e10880.

33. Leys NM, Ryngaert A, Bastiaens L, Verstraete W, Top EM, Springael D: **Occurrence and phylogenetic diversity of Sphingomonas strains in soils contaminated with polycyclic aromatic hydrocarbons**. *Appl Environ Microbiol* 2004, **70**(4):1944–1955.
34. Zhou X, Wang Z, Jia H, Li L, Wu F: **Continuously Monocropped Jerusalem Artichoke Changed Soil Bacterial Community Composition and Ammonia-Oxidizing and Denitrifying Bacteria Abundances**. *Front Microbiol* 2018, **9**:705.
35. Wang X, Song D, Liang G, Zhang Q, Ai C, Zhou W: **Maize biochar addition rate influences soil enzyme activity and microbial community composition in a fluvo-aquic soil**. *Applied Soil Ecology* 2015, **96**:265–272.
36. Zeng J, Liu J, Lu C, Ou X, Luo K, Li C, He M, Zhang H, Yan H: **Intercropping With Turmeric or Ginger Reduce the Continuous Cropping Obstacles That Affect Pogostemon cablin (Patchouli)**. *Frontiers in Microbiology* 2020, **11**.
37. Sekar J, Raju K, Duraisamy P, Ramalingam Vaiyapuri P: **Potential of Finger Millet Indigenous Rhizobacterium Pseudomonas sp. MSSRFD41 in Blast Disease Management-Growth Promotion and Compatibility With the Resident Rhizomicrobiome**. *Front Microbiol* 2018, **9**:1029.
38. Choudoir M, Rossabi S, Gebert M, Helmig D, Fierer N: **A Phylogenetic and Functional Perspective on Volatile Organic Compound Production by Actinobacteria**. *mSystems* 2019, **4**(2):e00295-00218.
39. Liu H, Pan F-j, Han X-z, Song F-b, Zhang Z-m, Yan J, Xu Y-l: **A comprehensive analysis of the response of the fungal community structure to long-term continuous cropping in three typical upland crops**. *Journal of Integrative Agriculture* 2020, **19**(3):866–880.
40. Li W-h, Liu Q-z: **Changes in fungal community and diversity in strawberry rhizosphere soil after 12 years in the greenhouse**. *Journal of Integrative Agriculture* 2019, **18**(3):677–687.
41. Sun Z-c, Li G-t, Zhang C-l, Wang Z-m, Lin Q-m, Zhao X-r: **Contrasting resilience of soil microbial biomass, microbial diversity and ammonification enzymes under three applied soil fumigants**. *Journal of Integrative Agriculture* 2020, **19**(10):2561–2570.
42. Miao CP, Mi QL, Qiao XG, Zheng YK, Chen YW, Xu LH, Guan HL, Zhao LX: **Rhizospheric fungi of Panax notoginseng: diversity and antagonism to host phytopathogens**. *J Ginseng Res* 2016, **40**(2):127–134.
43. Li R, Shen Z, Sun L, Zhang R, Fu L, Deng X, Shen Q: **Novel soil fumigation method for suppressing cucumber Fusarium wilt disease associated with soil microflora alterations**. *Applied Soil Ecology* 2016, **101**:28–36.
44. Lin W-p, Jiang N-h, Peng L, Fan X-y, Gao Y, Wang G-p, Cai K-z: **Silicon impacts on soil microflora under Ralstonia Solanacearum inoculation**. *Journal of Integrative Agriculture* 2020, **19**(1):251–264.
45. Phillips LA, Ward V, Jones MD: **Ectomycorrhizal fungi contribute to soil organic matter cycling in sub-boreal forests**. *ISME J* 2014, **8**(3):699–713.
46. Li Q, Chen J, Wu L, Luo X, Li N, Arafat Y, Lin S, Lin W: **Belowground Interactions Impact the Soil Bacterial Community, Soil Fertility, and Crop Yield in Maize/Peanut Intercropping Systems**. *Int J Mol Sci* 2018, **19**(2).

47. Zhu F, Ju Y, Wang W, Wang Q, Guo R, Ma Q, Sun Q, Fan Y, Xie Y, Yang Z *et al*: **Metagenome-wide association of gut microbiome features for schizophrenia**. *Nat Commun* 2020, **11**(1):1612.
48. Yang F, Sun J, Luo H, Ren H, Zhou H, Lin Y, Han M, Chen B, Liao H, Brix S *et al*: **Assessment of fecal DNA extraction protocols for metagenomic studies**. *Gigascience* 2020, **9**(7).
49. Magoc T, Salzberg SL: **FLASH: fast length adjustment of short reads to improve genome assemblies**. *Bioinformatics* 2011, **27**(21):2957–2963.
50. Bolger AM, Lohse M, Usadel B: **Trimmomatic: a flexible trimmer for Illumina sequence data**. *Bioinformatics* 2014, **30**(15):2114–2120.
51. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R: **UCHIME improves sensitivity and speed of chimera detection**. *Bioinformatics* 2011, **27**(16):2194–2200.
52. Edgar RC: **UPARSE: highly accurate OTU sequences from microbial amplicon reads**. *Nat Methods* 2013, **10**(10):996–998.
53. Wang Q, Garrity GM, Tiedje JM, Cole JR: **Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy**. *Appl Environ Microbiol* 2007, **73**(16):5261–5267.
54. Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R: **PyNAST: a flexible tool for aligning sequences to a template alignment**. *Bioinformatics* 2010, **26**(2):266–267.
55. Parks DH, Tyson GW, Hugenholtz P, Beiko RG: **STAMP: statistical analysis of taxonomic and functional profiles**. *Bioinformatics* 2014, **30**(21):3123–3124.

Figures

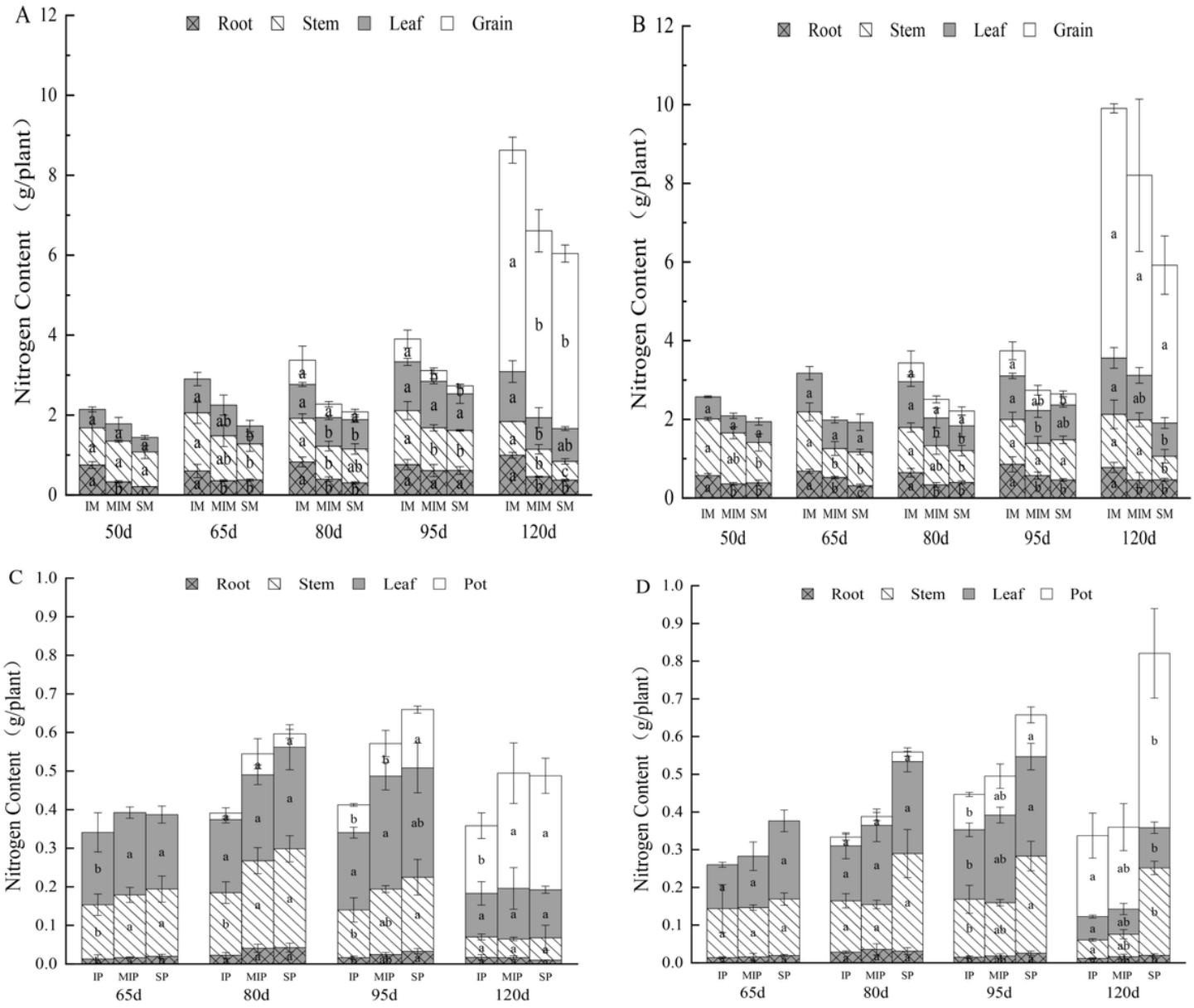

Figure 1

The nitrogen content of maize and peanut under IMP. (A) The nitrogen content in various organs of maize in 2018; (B) The nitrogen content in various organs of maize in 2019; (C) The nitrogen content in various organs of peanut in 2018; (D) The nitrogen content in various organs of peanut in 2019. 50d, trumpeting stage; 65d, heading stage/ seedling stage; 80d, anthesis and silking stage/ flowering stage; 95d, grain filling stage/ podding stage; 120d, mature stage.

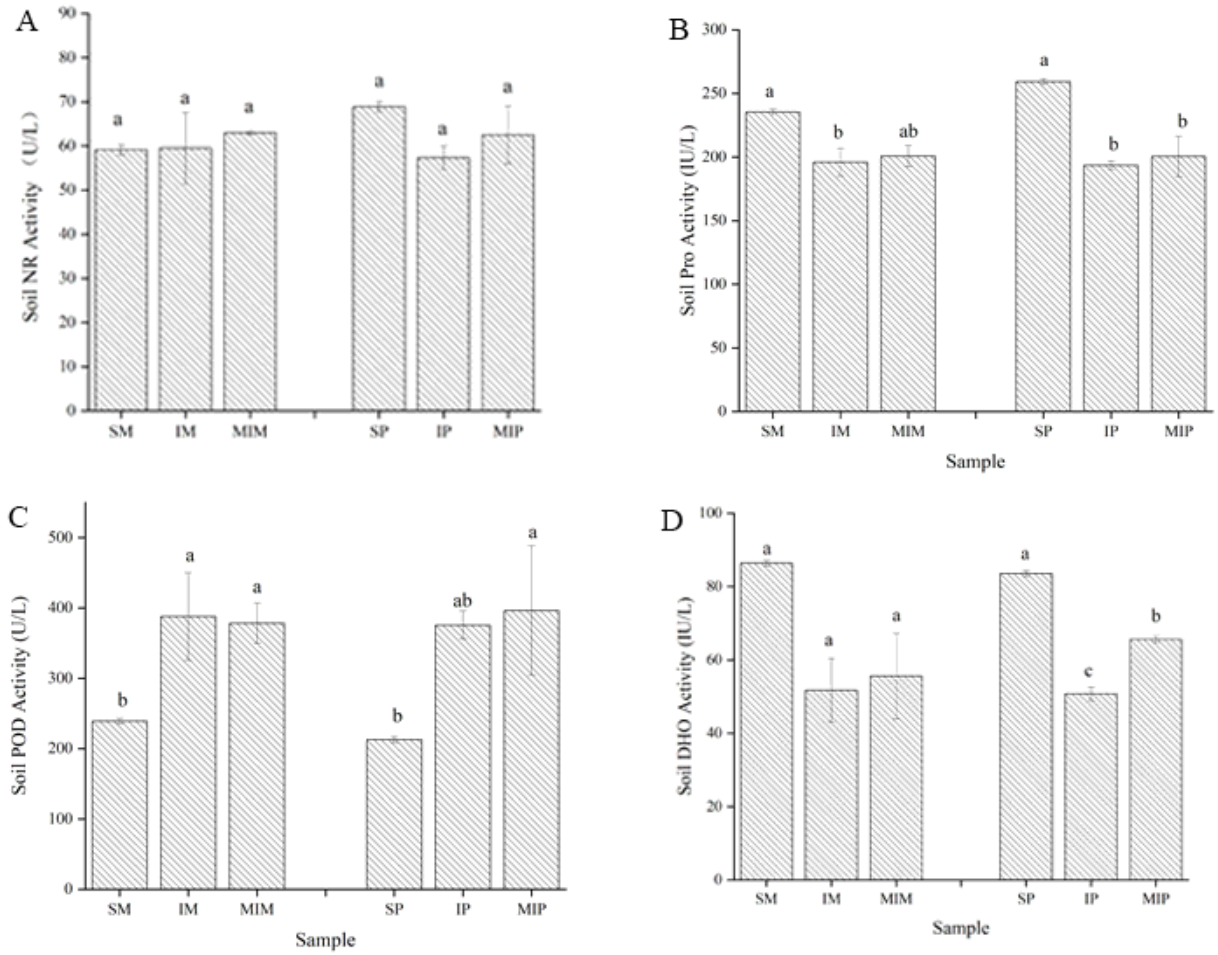

Figure 2

Changes in soil enzyme activities under IMP.

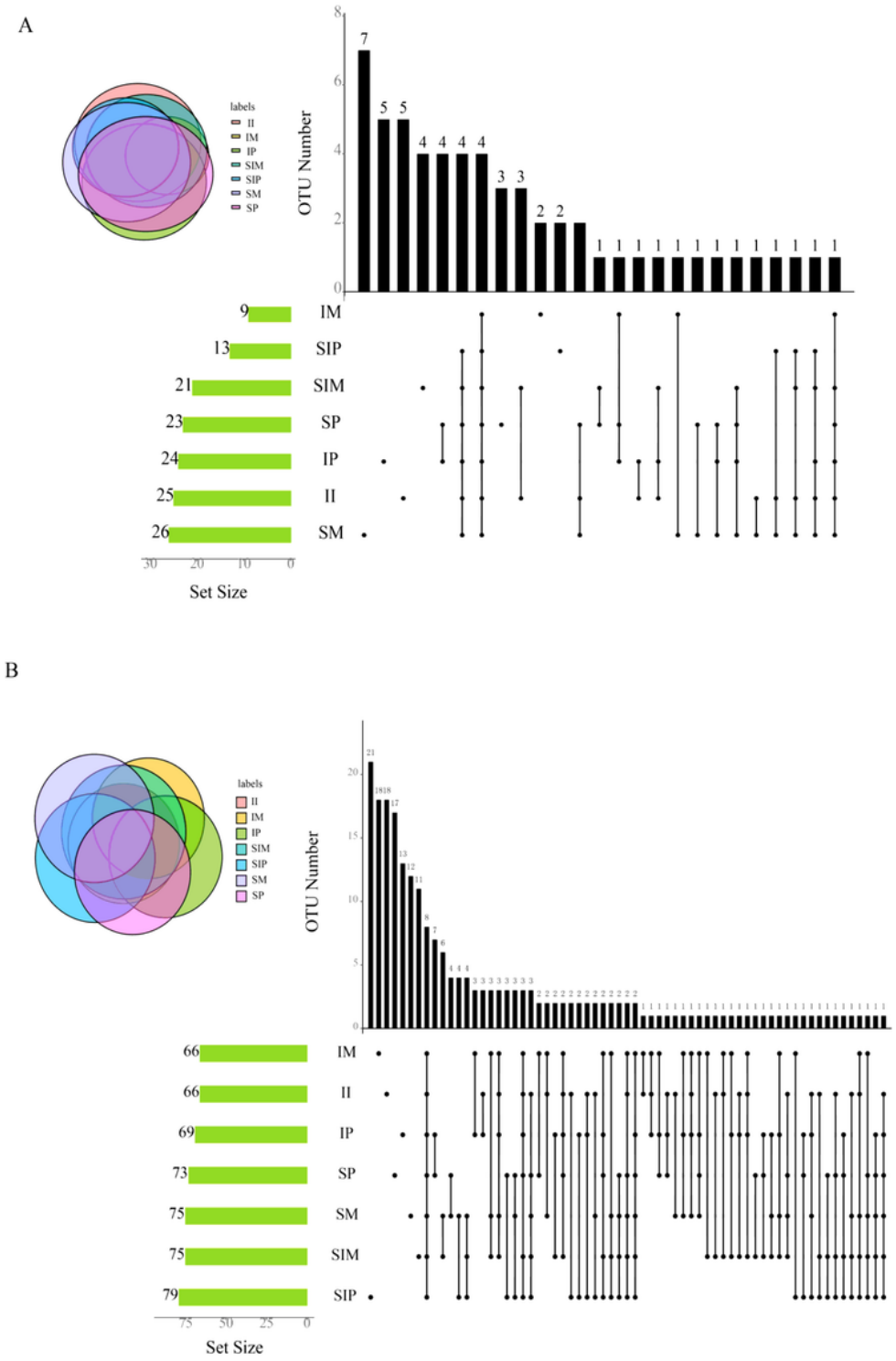

Figure 3

The Upset of the number distribution of OTUs in bacterial community (A), and fungal community (B).

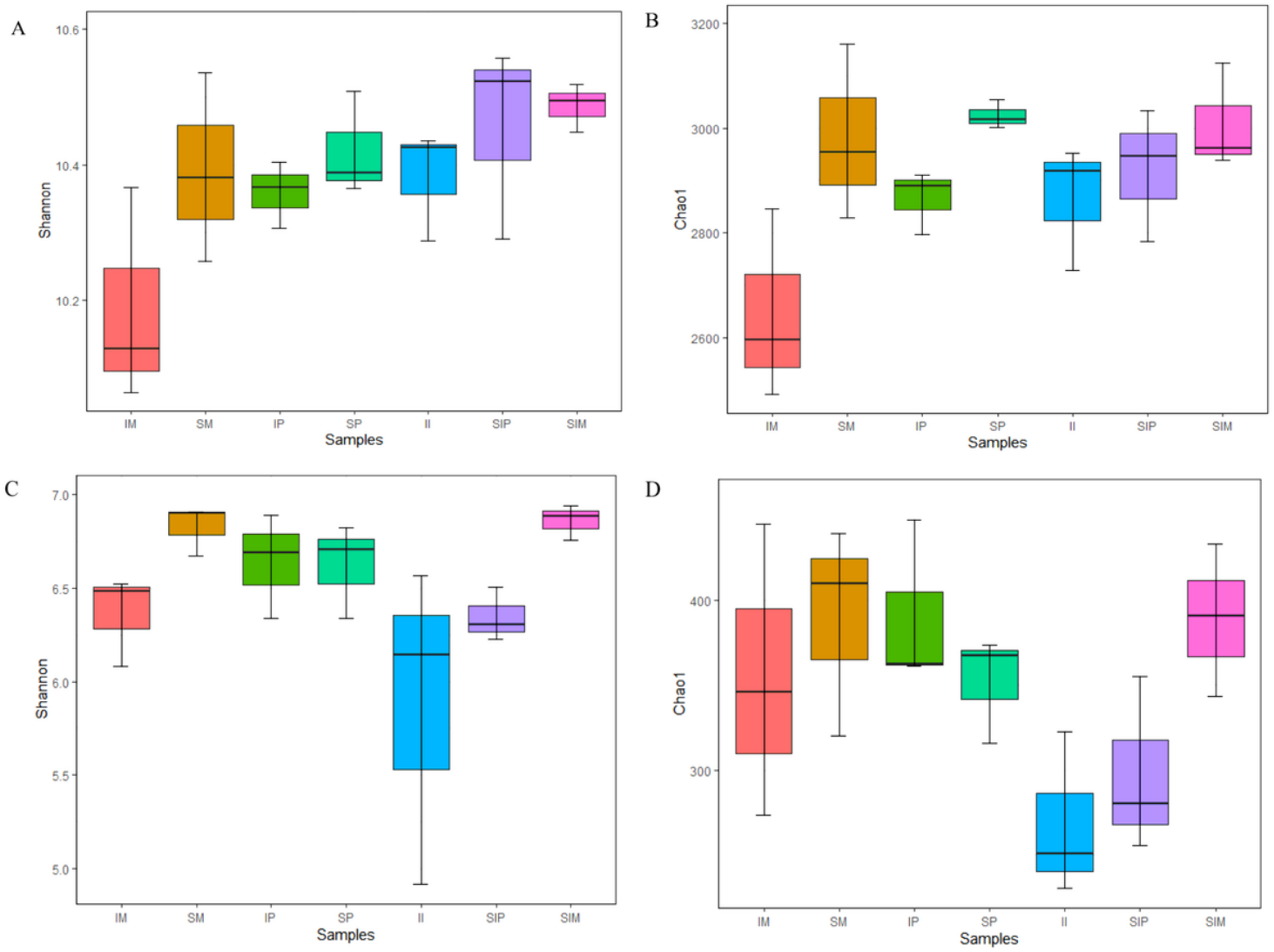

Figure 4

The diversity and richness of the bacterial community (A), and fungal community (B).

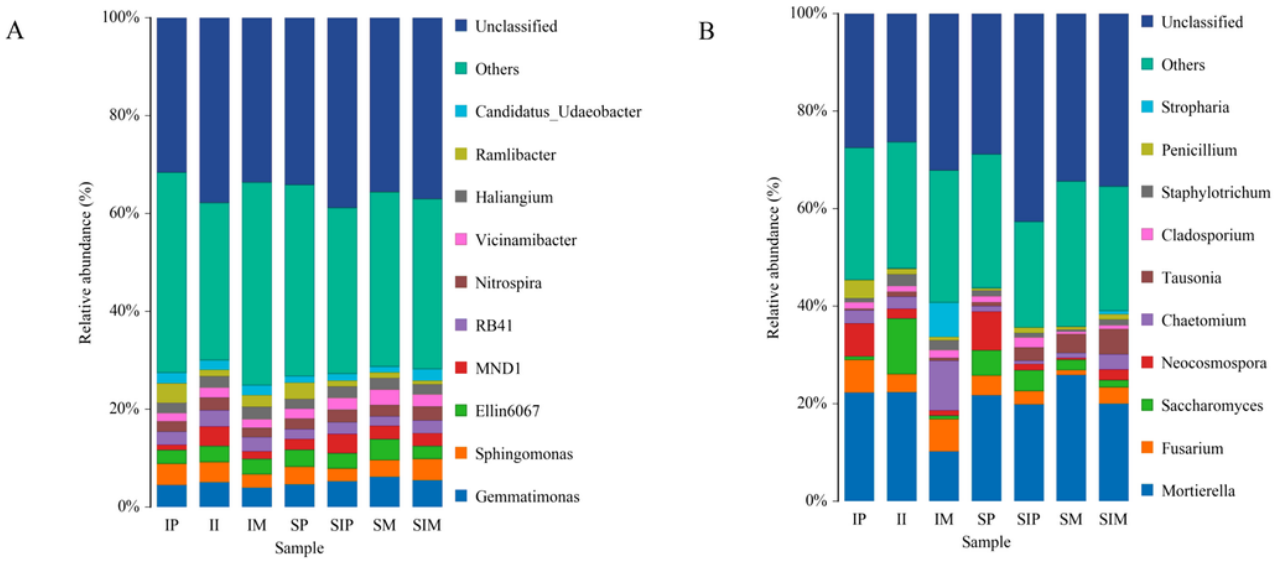

Figure 5

Composition of the bacterial community (A), and fungal community (B) at the genus level in the rhizosphere soil. The histogram of distribution figures shows the relative abundance of the top 10 rhizosphere soil bacteria and fungi at the genus level. A color represents a species, and the length of the color block represents the relative abundance ratio of species at the genus level. The heatmap of the relative abundance of the figure shows the genus-level RA of the 20% bacterial and fungal community of rhizosphere soil.

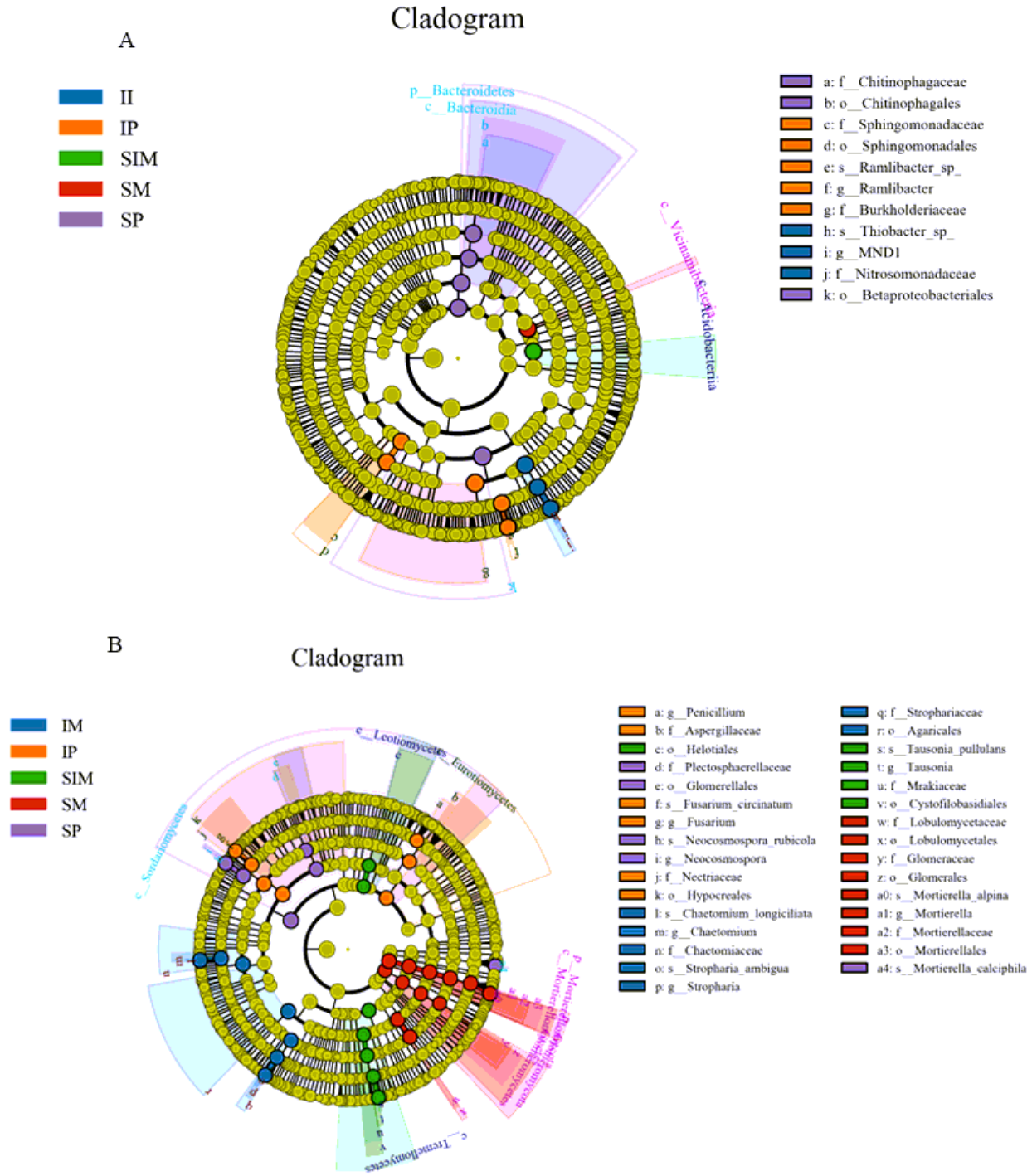

Figure 6

LEfse of difference analysis of bacterial community (A), and fungal community (B).

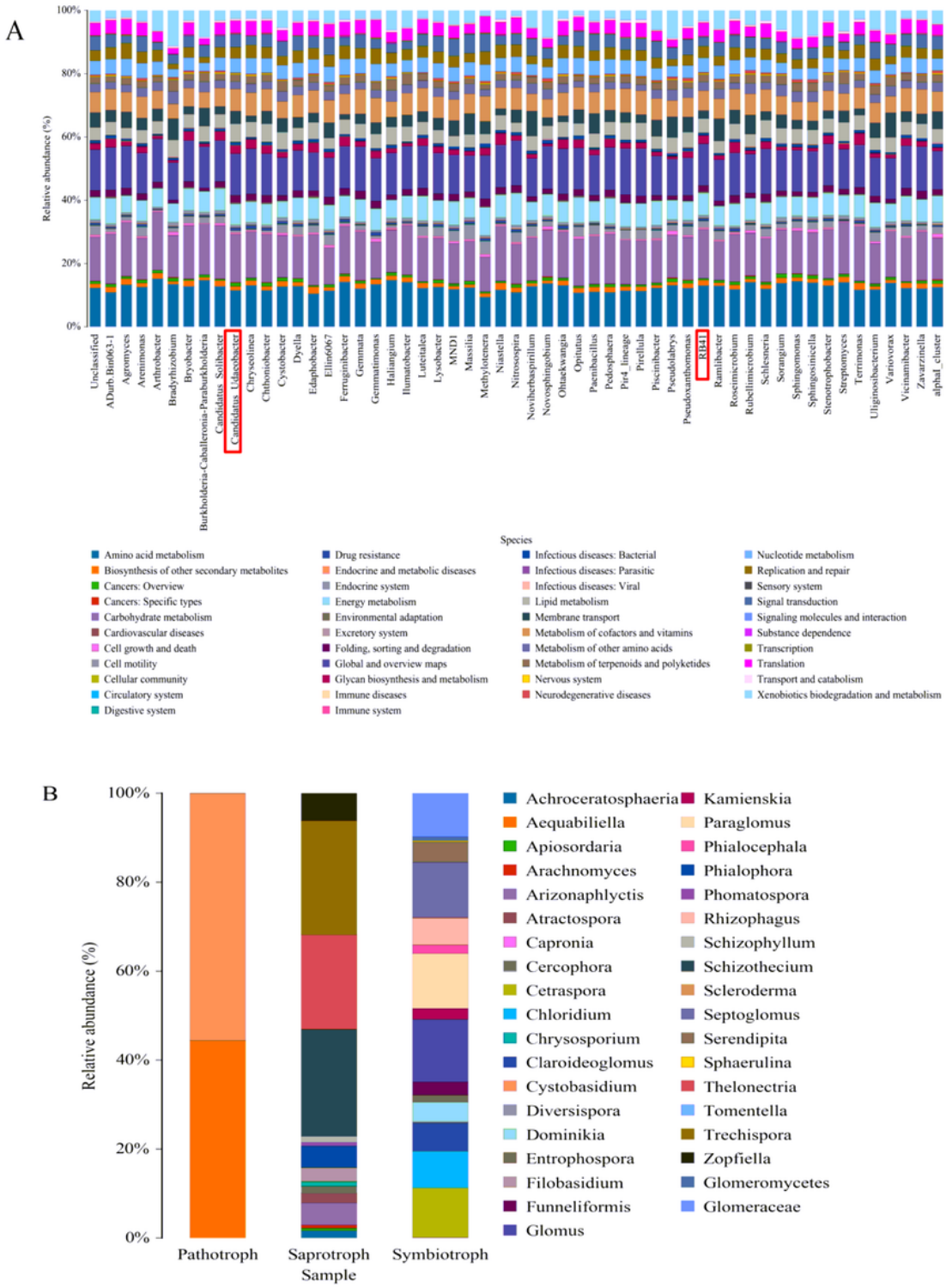

Figure 7

Cluster heatmap of correlation between the soil physiochemical properties and bacterial community (A) fungal community (B).

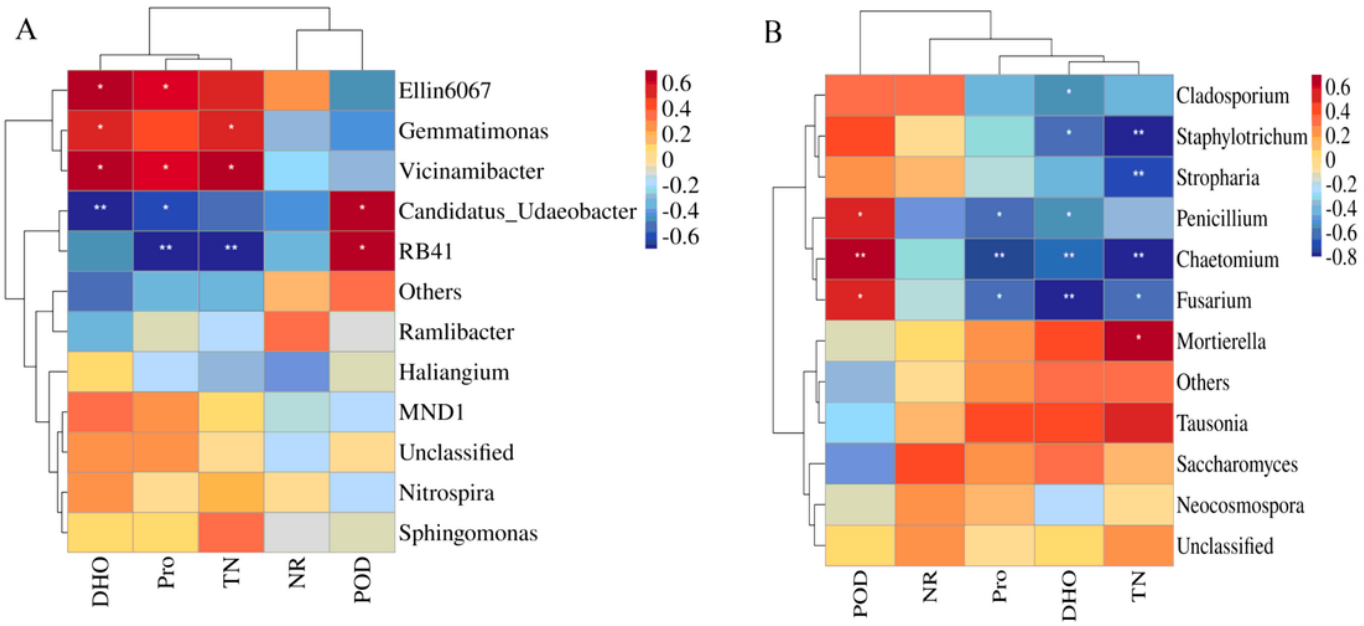

Figure 8

Predictions of functional genes in bacterial community (A), and fungal community (B).

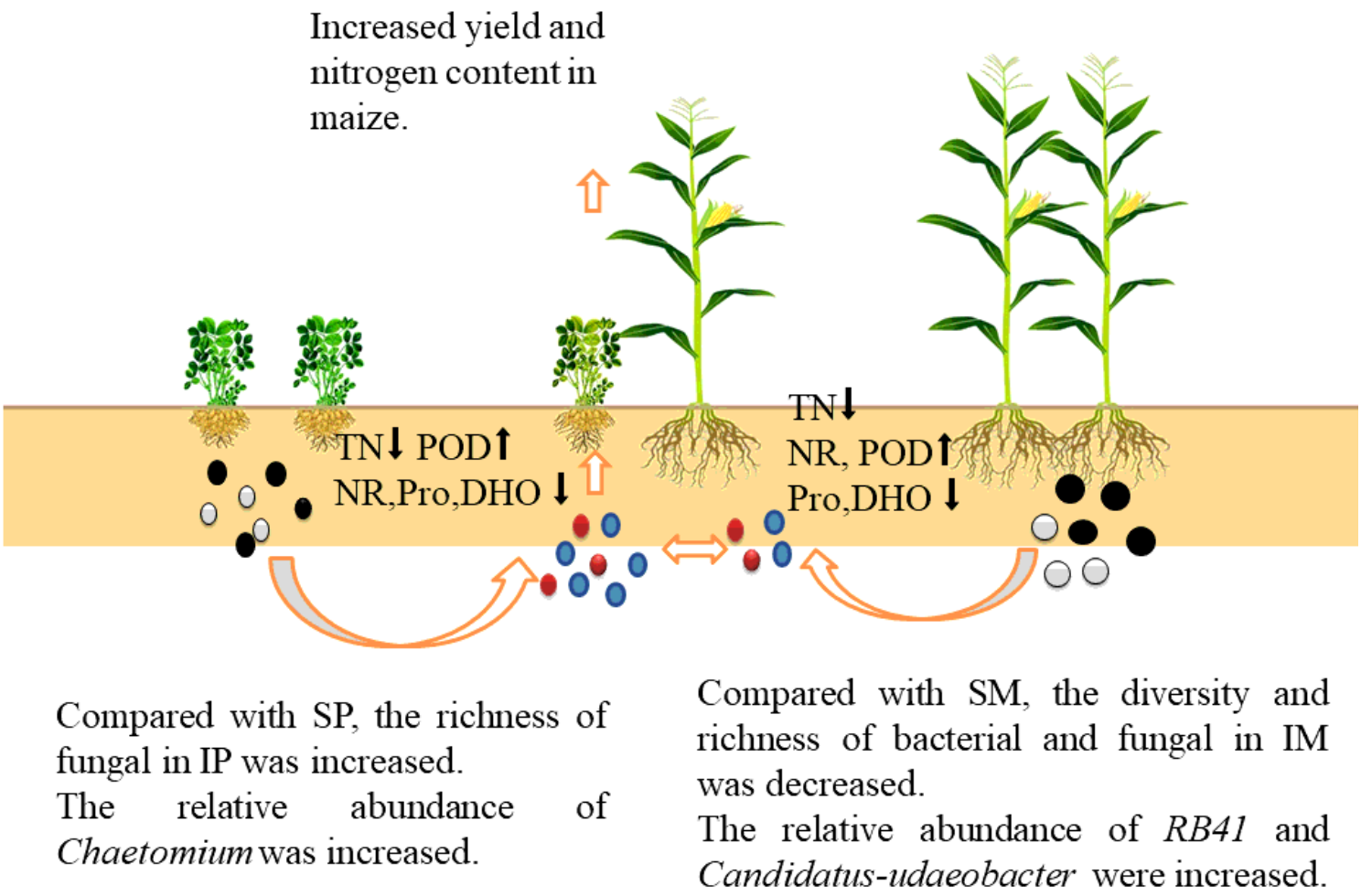

Figure 9

Overview of promoting nutrient uptake by root interaction IMP.

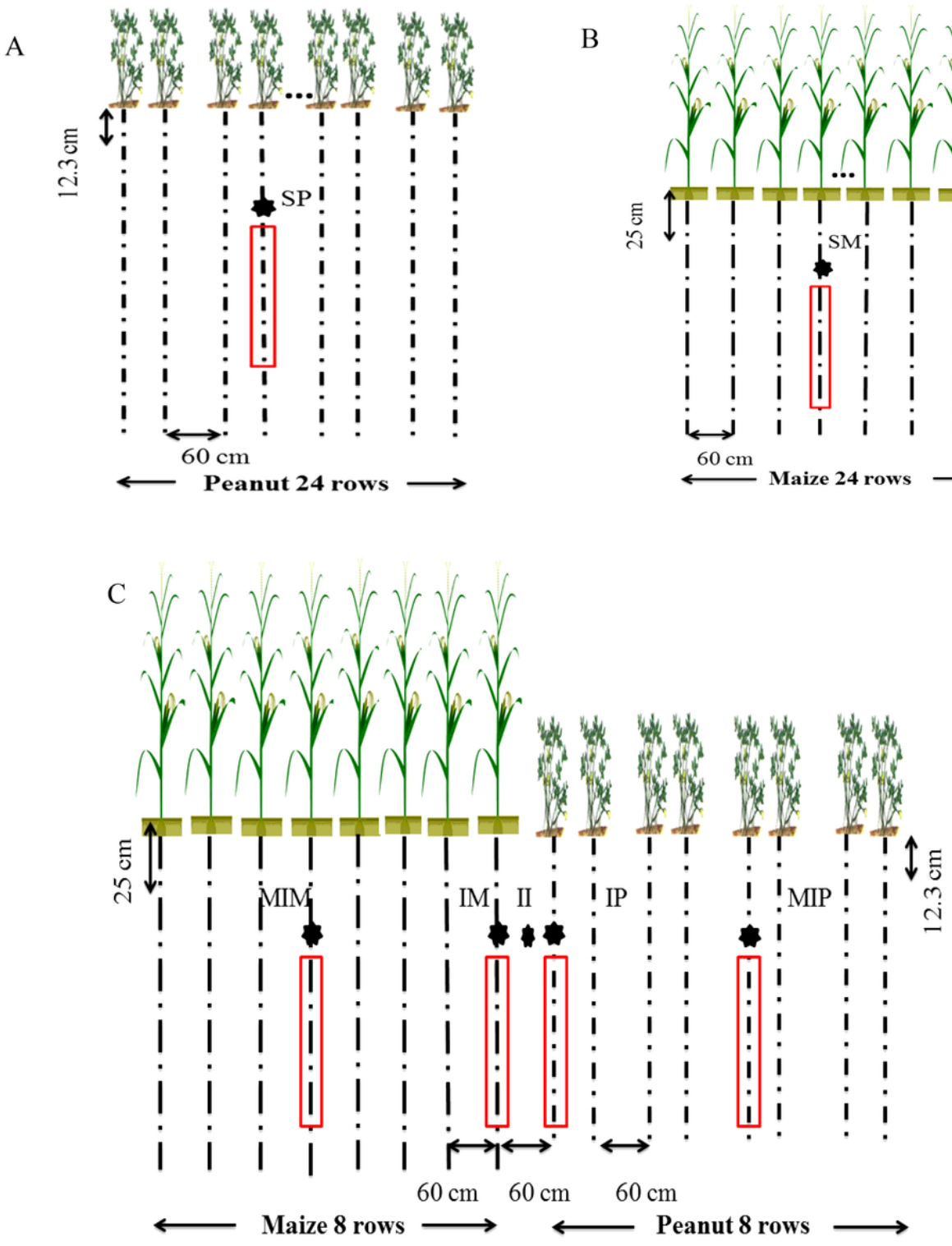

Figure 10

Please see the Manuscript file for the complete figure caption.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- BMCMICROBIOLGYSupplementary.docx