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Review

Nitrogen Distribution and Soil Microbial Community Characteristics in A Legume–cereal Intercropping System: A Review

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Abstract: Intercropping systems can flexibly use resources such as sunlight, heat, water, and nutrients in time and space, improve crop yield and land utilization rates, effectively reduce continuous cropping obstacles and the occurrence of diseases and insect pests, and control the growth of weeds. Thus, intercropping is a safe and efficient ecological planting mode. The legume–cereal intercropping system is the most common planting combination. Legume crops fix nitrogen from the atmosphere through their symbiotic nitrogen fixation abilities, and the fixed nitrogen can be transferred to and utilized by cereal crops in various ways. The symbiotic nitrogen fixation efficiency of legume crops was improved by reducing the inhibition of soil nitrogen on nitrogenase activity through competitive absorption of soil nitrogen. However, the effects of nitrogen transformation and distribution in intercropping systems, and microbial community structure characteristics on nitrogen transfer need to be further explored. In this review, i) we present the transformation and distribution of nitrogen in the legume–cereal intercropping system; ii) we describe the soil microbial community characteristics in intercropping systems; and iii) we discuss the advantages of using modern biological molecular techniques to study soil microorganisms. We conclude that intercropping can increase the diversity of soil microorganisms, and the interaction between different plants has an important impact on the diversity and composition of the bacterial and fungal communities. The extensive application of modern biological molecular techniques in soil microbial research and the great contribution of intercropping systems to sustainable agriculture are particularly emphasized in this review.

Keywords: intercropping; nitrogen transfer; microbial community structure; microbial activity; DNA-SIP; high-throughput sequencing; metagenomes

1. Introduction

Nitrogen is the basic substance in the protoplasm composition of plant cells and the basis of plant life activities; it is the most important nutrient element for crop growth [1]. Nitrogen is generally distributed in the most active parts of plants (new leaves, meristem, reproductive organs, etc.). Therefore, nitrogen supply directly affects the growth and development of crops. Nitrogen demands are critical at certain stages of crop growth, such as the tillering stage and spike differentiation stage of cereal crops, flower and boll stage of cotton, rapid growth of cash crops and formation stages of economic products. Sufficient nitrogen nutrition at these stages can promote crop growth and increase yield.

After the application of chemical fertilizer, crop yield and nitrogen uptake capacity gradually increased, but yield per hectare and marginal benefit gradually decreased. It is evident that this nitrogen, which is not absorbed by crops, enters the environment through ammonia volatilization, nitrification, denitrification, leaching and runoff [2]. Therefore, the higher the nitrogen application rate is, the higher the nitrogen cycling intensity is. Accordingly, a high crop yield, low efficiency of nitrogen use and high loss will occur.

The legume–cereal intercropping pattern has a long planting history in China [3]. In legume–cereal intercropping systems, legumes can effectively fix nitrogen in the atmosphere, and this fixed nitrogen can be used by cereal crops [4]. The symbiotic nitrogen fixation efficiency of legume crops was improved by reducing the inhibition of soil nitrogen on nitrogenase activity through competitive absorption of soil nitrogen [5]. Therefore, intercropping systems can effectively reduce the use of chemical nitrogen fertilizer, which is of great significance in solving the environmental problems caused by irrational application of nitrogen fertilizer and has been widely studied.

In legume–cereal intercropping, microorganisms cross-migrate from the roots of one plant to those of another. In fact, intercropping with legumes can improve soil properties by enhancing the functional diversity of the soil microbial community, thus improving plant growth. Clearly, the soil microbial community is important to soil function because it plays a role in mineral nutrient cycling, organic turnover, nitrification, mineralization, and soil structure formation, which are essential for plant growth.

2. Transformation and distribution of nitrogen in the legume–cereal intercropping system

2.1. Fixation of nitrogen in legumes

Considerable amounts of fertilizer input have failed to produce good agricultural results and have had low nitrogen utilization rates, resulting in serious negative effects on soil, air, water and other ecological environments [6]. Biological nitrogen fixation has been implemented in solving agricultural production and environmental problems [7]. In 1886, Helligel found that the nodules on leguminous roots had the ability to assimilate N_2 [8]. A large number of studies later confirmed that rhizobia on legume crops can convert N_2 in the air into synthetic ammonia without demanding reaction conditions [9,10]. The nitrogen fixed by legumes is not only used for their own growth but also provides nitrogen-containing organic matter to the accompanying plants. Symbiotic nitrogen fixation converts atmospheric N_2 into ammonia under the action of nitrogenase [11,12], and this process is the result of complex interactions between host plants and rhizobia. There is a symbiotic relationship between rhizobia and legumes. A large part of the nitrogen and nutrients required for rhizobium growth and physiological metabolic activities come from legume plants, and the amount of cogrowth nitrogen fixation of mature legume plants accounts for 25%-66% of the total nitrogen. Symbiotic nitrogen fixation of legume crops can reach 75-150 kg per hectare per year and even 300 kg ha^{-1} when conditions are favorable [13]. Legume nodules act as a small nitrogen-processing plant, and taking advantage of this symbiotic relationship can reduce the dependence on chemical fertilizers and reduce environmental pollution caused by excessive fertilization.

In the farmland system, bean intercropping with cereal has obvious advantages compared to outputs achieved with monoculture, mainly because during bean and cereal common growth, the roots of the two crops become interwoven with each other, and the competition for root nutrition is relatively intense in cereals. The competitiveness of cereal crops in obtaining soil nitrogen is further strengthened, and the obstacle of “nitrogen repression” of legume root nodules is reduced so that both legume and cereal crops show obvious yield advantages [14]. The formation of an intercropping advantage is closely related to the operation of nitrogen fertilizer in the intercropping system.

Nitrogen application amounts had a substantial impact on the proportion of nitrogen derived from the atmosphere (%Ndfa) and the amount of nitrogen fixed (Ndfa) by intercropped alfalfa [15]. With the addition of nitrogen fertilizer (N1, 200 mg N kg^{-1} soil), the %Ndfa and Ndfa of intercropped alfalfa were lowered by 41% and 46%, respectively. Cereals compete more aggressively for nutrient absorption than legumes in the legume–

cereal intercropping system [16] because of their faster root growth rate and wider root distribution, which reduces the nitrogen content in rhizosphere and improves the nitrogen fixation capacity of legumes. Pasture-legume intercropping improved the efficiency of biological nitrogen fixation (BNF), and the amount of fixed nitrogen increased by 18% compared with that of legume crops alone [17,18]. The quantity and biomass of soybean root nodules were significantly affected by the nitrogen delivery rate in a soybean-sugarcane intercropping system [19]. In comparison to conventional nitrogen intake, the nodule number increased by 2.1-4.1 times and nodule biomass increased by 1.8-2.0 times when nitrogen input was lowered. The same conclusion showed that the nitrogen fixation ratio of pea under the N1 (225 kg N ha^{-1}) nitrogen level was significantly lower than that under the N0 level because fertilization increased soil mineral nitrogen and then inhibited symbiotic N_2 fixation [20]. Hei et al. [21] designed a field experiment of rice-water mimosa intercropping with zero nitrogen (ZN, 0 kg N ha^{-1}), reduced nitrogen (RN, 140 kg N ha^{-1}) and conventional N (CN, 180 kg N ha^{-1}). The study found that mimosa had the highest biological nitrogen fixation efficiency under the RN treatment. In conclusion, the nitrogen fixation capacity, nitrogenase activity, nitrogen transformation and distribution in intercropping systems of different regions and different crop species were also inconsistent. Therefore, in the process of improving rational nitrogen utilization through legume–cereal intercropping, it is worth studying how to stimulate legume biological nitrogen fixation to the maximum extent and accurately quantify nitrogen distribution in legume–cereal intercropping systems.

Legumes have a stronger proton releasing capacity than cereals and can significantly acidify the rhizosphere, thus facilitating the activation and absorption of soil insoluble phosphorus [22], meeting the supply of adenosine triphosphate (ATP) required for nitrogen fixation in legumes, and maintaining a stable nitrogen and phosphorus stoichiometric relationship in intercropped legumes (Figure 1). Amarildo et al. [23] found that legumes could replace nitrogen application in the case of low phosphate capacity and no nitrogen application. Seeding and incorporation of phosphorus fertilizer in guinea grass cultivation not only improves pasture yield but also improves nitrogen use efficiency regardless of phosphorus solubility. Intercropping broad bean with maize significantly boosted grain output and aboveground biomass compared to wheat–maize in another field trial at low P rates (75 kg P_2O_5 ha^{-1}) but not at high P rates (>112.5 kg P_2O_5 ha^{-1}). Broad beans overproduce because they have a different growing season and rooting depth than maize, so intercropping on low-phosphorus soils can greatly increase yields [24].

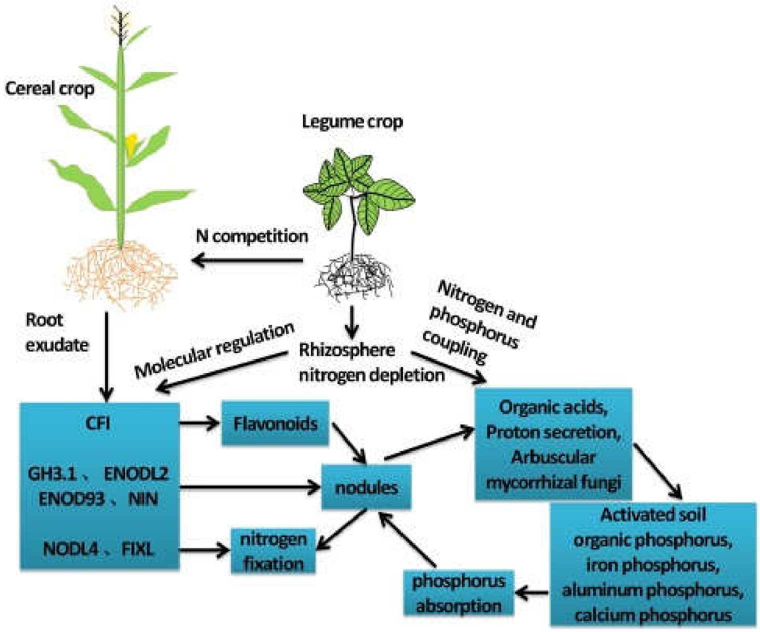

Figure 1. The mechanism by which legume–cereal intercropping improves nodule formation and symbiotic nitrogen fixation of legumes.

2.2. Nitrogen uptake by cereals in intercropping systems

Legume plants can transfer a certain amount of nitrogen to cereal crops in intercropping systems [25]. The absorption of legume-fixed nitrogen by cereal crops mainly occurs through the decomposition of legume litter and the deposition of root and nodule tissue [26] (Figure 2). Previous studies have shown that the transfer rate of nitrogen from legumes to cereal in intercropping systems ranges from 0 to 73%, depending on the species and planting method of legumes and cereals [27]. Maize + legume - wheat intercropping was more beneficial than monoculture and could save 25% of the nitrogen for subsequent wheat [28]. All intercropping treatments left enough residual fertility to significantly increase the yield of the wheat crop without fertilization. From a cropping system perspective, legume intercropping provides significant nitrogen savings, resulting in lower tillage costs and improved soil health indicators.

Most of the earliest studies on nitrogen transfer and distribution in intercropping systems focused on mixed pasture systems [29]. Legume–cereal intercropping systems transfer nitrogen from legumes to adjacent cereal crops through root and mycorrhizal networks, which improve nitrogen uptake of cereals [30]. Cereal crops can rapidly consume nitrogen in the soil, and their root exudates can induce nodulation in the root system of broad bean, thus increasing the symbiotic nitrogen fixation rate of legumes and ultimately increasing the soil nitrogen pool [31]. Researchers established a basic nitrogen cycling network between crops long ago, but the exact proportions of nitrogen distribution were unclear. The nitrogen distribution in legume–cereal intercropping systems could be analyzed by using ^{15}N isotope-labeled nitrogen and leaf labeling techniques. Yong et al. [32] used ^{15}N isotope dilution labeling and direct ^{15}N leaf feeding measurement to conduct a pot experiment with a wheat–maize–soybean rotation and discovered that there was bidirectional nitrogen transfer in the symbiotic stage of crops, and the amount of ^{15}N transfer (N transfer) and percentage (%NT) decreased as the soil fertilization level increased. Maize-to-wheat nitrogen transfer was 16.1%-163.0% higher than wheat-to-maize nitrogen transfer. The nitrogen transfer from soybean to maize was 1.7-6.0 times that of maize to soybean, and the %NT from soybean to maize was 6.7-22.2 times that of maize to soybean. The researchers concluded that bidirectional nitrogen transfer is the main factor in improving crop nitrogen use efficiency. Ledgard et al. [33] studied nitrogen transfer in a clover-ryegrass intercropping system by using the leaf feeding method of ^{15}N, and the results showed that 2.2% of nitrogen in clover was directly transferred to ryegrass. In a two-year indoor pot experiment, Shao et al. [34] measured the influence of root contact on nitrogen transfer in a maize-alfalfa intercropping system. The majority of nitrogen transfer occurred in one direction, from alfalfa to maize. Nitrogen transfer from alfalfa to maize was 204.56 mg pot^{-1} (without a root barrier) and 165.13 mg pot^{-1} (with a nylon net barrier) in the N0 treatment, accounting for 4.72 and 4.48 percent of the total nitrogen accumulation in maize, respectively. Nitrogen transfer from alfalfa to maize was 197.70 mg pot^{-1} (without root barrier) and 139.04 mg pot^{-1} (with nylon net barrier) under the N1 (10.2 g N pot^{-1}) treatment, accounting for 3.64% and 2.36% of the total nitrogen accumulation in maize, respectively. Furthermore, regardless of the supplemented nitrogen dosage, the amount of nitrogen transfer without a root barrier was 1.24 to 1.42 times that with a nylon net barrier. The findings emphasize the significance of root interactions in improving nitrogen transfer in maize-alfalfa intercropping systems.

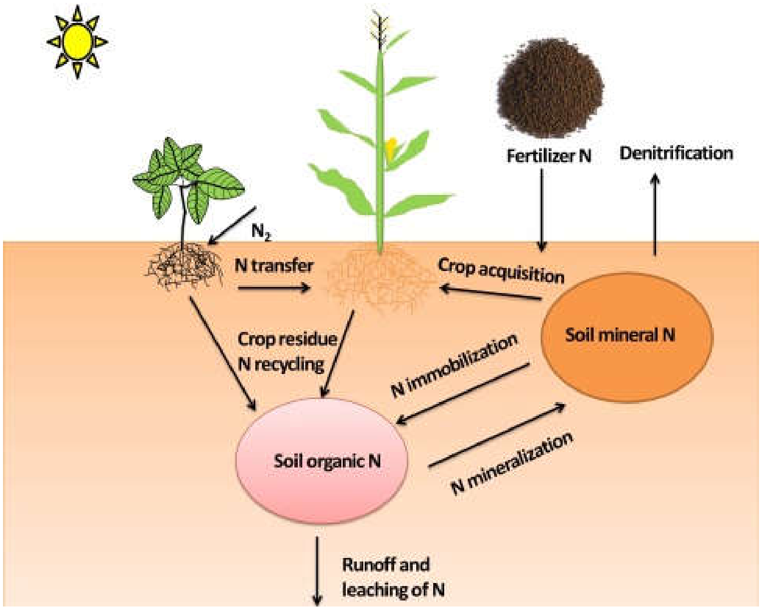

Figure 2. Nitrogen transfer and distribution in the legume–cereal intercropping system.

3. Soil microbial community characteristics in the legume–cereal intercropping system

Soil microorganisms are the link and bridge between aboveground and underground ecosystems and play an important role in regulating soil ecological functions, the transformation of organic matter in soil, and carbon and nitrogen cycles [35]. The intercropping system not only affected the physical and chemical properties of the soil but also the microorganisms in the soil. Due to the different root distributions of different crops, nutrients in different spaces can be fully utilized to change the physiological characteristics of crops and root exudates, thus indirectly affecting the composition of the soil microbial community and soil enzyme activities and improving soil fertility. Plant roots are the best space for microbial communities to gather [36]. Phenotypic traits such as root structure may affect microbial growth and reproduction, and plant roots can secrete primary metabolites such as organic acids or amino acids [37], which play an important role in shaping the rhizosphere environment by changing the chemical composition of soil near plant roots and serving as substrates for the growth of some soil microorganisms [38]. The composition of plant root exudates changes qualitatively and quantitatively according to the plant's nutritional status, growth stage, and even the root position in time and space [39]. These secretions produce strong selection pressures in the rhizosphere, prompting plants to drive the selection of specific rhizosphere microbial communities [40].

3.1. Soil microbial composition and diversity in the legume–cereal intercropping system

Intercropping systems have a greater diversity of roots and residues, which improves the energy supply of soil microbial biomass by releasing exudates such as amino acids and organic acids [41]. As a result, the effects of these leftovers on soil microbial biomass in intercropping were more noticeable than those in monocropping. The diversity of the rhizosphere soil microbial community structure in the intercropping system was generally higher than that in the monocropping system [42]. Common networks dominated by Acidobacteria, Alphaproteobacteria and Bacteroidetes were found in wheat-pea intercropping systems, and these findings suggest a more complex rhizosphere bacterial network in intercropping [43]. When the cooccurrence network between populations becomes more complex, the functional expression of the subsurface microbiome increases [44]. Bean–oat intercropping significantly increased the diversity of nitrogen-fixing microbes and changed their community composition [45]. Legume–cereal intercropping significantly altered the composition and structure of functional microbial communities, particularly those microorganisms involved in nitrogen transformation in the rhizosphere soil, such as nitrogen fixation, ammonia oxidation, denitrification, nitrous oxide reduction and organic nitrogen decomposition [46]. Strip intercropping methods have also been demonstrated to increase soil nitrogen sequestration by improving biological nitrogen fixation and lowering greenhouse gas emissions in recent research [47]. Yu et al. [35] evaluated changes in the microbial composition, structure and abundance of nitrogen cycling genes in sweet maize intercropped with soybean in tropical and subtropical regions under specific conditions for the first time. It was found that maize–soybean intercropping could promote the soil microbial community. The composition of the arbuscular mycorrhizal fungal community was affected by the planting method and nitrogen application rate [48]. Previous studies showed that intercropping had positive effects on root colonization of arbuscular mycorrhizal fungi (AMF) [41]. The expression of *amoA*, *nirK*, *nirS*, and *nosZ* was found to be closely connected to the structure of the bacterial community, suggesting that bacterial community structure may have a substantial impact on soil nitrification, denitrification, and nitrogen fixation. AMF of adjacent maize crops could reduce N_2O emissions from soybean stubble mainly by changing the abundance and community structure of denitrifying bacteria [49]. The abundance of the *nifH* gene was significantly positively correlated with planting methods and nitrogen fixation rate. Sugarcane-peanut intercropping significantly increased the Shannon diversity of nitrogen-fixing bacteria in sugarcane crops, and the rhizosphere bacterial richness in sugarcane-peanut intercropping increased by 9.28% compared with monoculture sugarcane [50]. The intercropping roots of the two plants changed the soil physical and chemical properties and microbial community structure and increased the content of soil available nutrients [51]. In conclusion, legume–cereal intercropping, as a composite population formed by the combination of different crop species, has a great impact on the number, biomass, population structure and diversity of rhizosphere microorganisms (Table 1 and Table 2).

Changes in soil microbial composition and quantity affect crop growth, crop yield, crop nutrient absorption, and pest control. Compared with monoculture, the number of soil fungi, bacteria and actinomycetes in soybean-sugarcane intercropping increased by 115.5%, 43.6% and 57.3%, respectively [52]. A field experiment was conducted with a split-pot design to study the changes in rhizosphere microbial diversity driven by interspecific interactions in a maize–soybean intercropping system through Illumina MiSeq sequencing [53]. Intercropping had no significant effect on the α diversity of maize rhizosphere soil and root AMF but significantly increased the α diversity of soybean rhizosphere soil AMF. At the genus level, *Glomus_f_Glomeraceae* was the dominant strain of AMF in maize rhizosphere soil and roots, and its relative abundance was the highest. In contrast to the α diversity of root AMF, the relative abundance of *Glomus_f_Glomeraceae* in maize roots increased significantly with the increase in the nitrogen application rate. Intercropping significantly decreased the α diversity of ammonia-oxidizing archaea (AOA) and

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ammonia-oxidizing bacteria (AOB) in maize rhizosphere soil and significantly increased the α diversity of AOA in soybean rhizosphere soil.

A large number of studies have demonstrated that plant diversity must affect soil biological activity, particularly through the input of more diverse substrates in the soil, possibly affecting decomposer activity in plant growth [54]. Intercropping occurs when two or more crops are grown simultaneously in the same space for a substantial portion of their life cycle [55]. The simultaneous planting of different crops in the same soil space will inevitably lead to the diversity of root and mycorrhizal networks and affect the composition and quantity of microorganisms. Agroecosystem functioning encompasses not only a wide range of spatial interactions between plants and soil but also interactions that occur throughout time as a function of the plants' and microorganisms' developmental stages, as well as their association dynamics [56].

Table 1. Effects of legume–cereal intercropping on rhizosphere microorganisms of legumes.

Types of intercropping crops	Soil type	Increased microorganism	Reduced microorganism	Microorganisms that do not change significantly	reference
Soybean–maize	Brown soil	Actinomycetes, Firmicutes, *Corynebacter*, *Corynebacter*, *Cysts*, *Halophile bacillus*, *Phagocytes*	Proteobacteria, Bacteroidetes, *Burkholderia*, *Desulphurobacter*, *Neisseria*		[57]
Soybean-sugarcane	Red soil	*Streptomyces*, *Bacillus*, *Pantomyces*, *Enterobacter*, *Arthrobacter*, Symbiotic bacteria rhizobia, Chinese rhizobia			[58]
Peanut-sugarcane	Red soil	*Streptomyces*, *Bacillus*, *Pantomyces*, *Enterobacter*, *Arthrobacter*, Symbiotic bacteria rhizobia, Chinese rhizobia			[58,59]
Pea-wheat	Loamy soil	Acidobacteria, Proteobacteria, Bacteroidetes, Chlorobacteria			[43]
Soybean-sugarcane	Red soil	Proteobacteria, Acidobacteria, Chlorobacteria, Actinomycetes, Bacteroidetes			[60]
Peanut-maize	Hydragric anthrosol	*Actinobacteria*, *Acidobacteria*	*Gammaproteobacteria*, *Bacteroidetes*, *Firmicutes*	*Alphaproteobacteria*	[61]
Fata beans-maize	Clay loam	Firmicutes, Bacteroidetes, *Bacillus*, *Clostridium*,	*Methylobacter*, *Sphingomonas*	Acidobacteria, Floating mold, Blastomonas phylum	[62]

		Sporobacteria, Desulphuria, Alicyclobacter			
Mung bean-proso millet	Loess-like	Proteobacteria, Chlorobacteria, Blastomonas, Acidobacteria, Helicobacter nitrifying, *Firmicutes*	Actinomycetes	Phylum fungi, Ascomycetes, Morpita, basidiomycetes	[63]
Wild soybean-sorghum	Salined fluvoaquic soil	*Proteobacteria, Bacteroidetes*	*Firmicutes, Gemmatimonadetes*		[64]

Table 2. Effects of legume–cereal intercropping on rhizosphere microorganisms of cereals.

Types of intercropping crops	**Soil type**	**Increased microorganism**	**Reduced microorganism**	**Microorganisms that do not change significantly**	**reference**
Soybean–maize	Brown soil	Actinomycetes, Firmicutes, *Desulphurobacter, Erythrococcus, Kinetospora, Bacillus, Bacillus, phagocyte*	Proteobacteria, *Achromatobacteria, Burkholderia, Pseudomonas, Aikenella*	Bacteroidetes	[57]
Soybean-sugarcane	Red soil	*Streptomyces, Bacillus, Pantomyces, Enterobacter, Arthrobacter*, Symbiotic bacteria rhizobia, Chinese rhizobia	data		[58]
Peanut-sugarcane	Red soil	*Streptomyces, Bacillus, Pantomyces, Enterobacter, Arthrobacter*, Symbiotic bacteria rhizobia, Chinese rhizobia,Acidobacteria,Chloroflexi	Proteobacteria,Actinobacteria		[58,59]
Pea-wheat	Loamy Soil	Acidobacteria, Proteobacteria, Bacteroidetes, Chlorobacteria			[43]
Soybean-sugarcane	Red soil	Proteobacteria, Acidobacteria, Chlorobacteria, Actinomycetes, Bacteroidetes			[60]
Peanut-maize	Hydragric anthrosol	*Deltaproteobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes*	*Actinobacteria,*	*Alphaproteobacteria*	[61]

Fata beans-maize	Clay loam	Firmicutes, Bacteroidetes, *Bacillus, Clostridium, Sporobacteria, Desulphuria, Alicyclobacter*	*Methylobacter, Sphingomonas*	Acidobacteria, Floating mold, Blastomonas phylum	[62]
Mung bean-proso millet	Loess-like	Proteobacteria, Chlorobacteria, Blastomonas, Acidobacteria, Helicobacter nitrifying, Firmicutes	Actinomycetes	Phylum fungi, Ascomycetes, Morpita, basidiomycetes	[63]
Wild soybean-sorghum	Salined fluvoaquic soil	*Proteobacteria, Bacteroidetes*	*Firmicutes, Gemmatimonadetes*		[64]

3.2. Soil microbial activity in the legume–cereal intercropping system

Soil microbial and enzyme activities are more sensitive than soil physical and chemical properties under changes in the soil environment and can effectively indicate changes in ecosystem function, which is an important indicator of soil fertility [65]. Soil enzymes and microorganisms participate in the transformation of organic matter, the biological cycle of various elements, the formation of humus and organic–inorganic colloids and have an important influence on the evolution of soil fertility [66]. Soil enzymes are proteins with microbial activity in soil, which is a key factor in soil metabolism and an important indicator of soil productivity and quality. Soil enzyme activity is closely related to soil physical and chemical properties and soil types.

There is a certain correlation between soil microbial activity and soil enzyme activity. Studies have found a significant positive correlation between bacteria and soil enzymes. Soil enzymes power the metabolism of organic matter in soil, and their activity directly reflects the activity of microorganisms, the ability of nutrient transformation and operation and the strength of soil biochemical reactions. It is one of the important indexes used to evaluate soil organic matter content, quality and health status [67]. Intercropping has strong interspecific root interactions, which can significantly improve soil microbial activity and promote the decomposition of humus and the transformation of organic matter and nutrients. The effects of intercropping on soil microbial activity were evaluated by total organic carbon, carbon management index, microbial biomass carbon, soil basal respiration and metabolic quotient. The results showed that cowpea-forage intercropping significantly increased microbial activity compared with monoculture forage [68]. Amit Kumar et al. [69] conducted monoculture and mixed cultivation experiments of broad bean, lupine and corn on medium fertility soil. Biomass and the kinetic parameters (maximum velocity (Vmax) and Miltonian constant (km)) of leucine aminopeptidase (LAP), β-1,4-n-acetylglucosaminopyrase (NAG) and phosphomonoesterase (PHO) in rhizosphere soil were determined. Maize and lupine are expected to have higher biomass under mixed cultivation than under monoculture. Second, LAP and NAG dynamic parameters were less responsive to monoculture and mixed culture. The Vmax and km of PHO in the faba bean rhizosphere were increased compared with those of maize and lupine. Under the condition of mixed culture, the absorption of phosphorus and nitrogen by *Vicia faba* seedlings was positively correlated with each other and showed net facilitative interactions. Dehydrogenase, nitrogen enzyme, rhizosphere and diazotrophic bacteria were significantly affected by the different cropping rotations [58]. The intercropping system significantly increased the number of soil microbes due to the abundance of microbes and the increase in soil enzyme activity. The data results of higher rhizosphere-specific OTUs and higher diversity indexes indicated that the intercropping system had positive effects on soil microbial activity. Peanut intercropping with atractylodes significantly increased the activities of urease and sucrose [70]. Continuous monoculture was harmful to soil enzyme activity. Soil catalase, urease and sucrase activities were significantly reduced under continuous monoculture [71]. Intercropping changes soil microbial activity through niche complementation. At the same time, the diversity of planting patterns affects the activities of soil microorganisms and enzymes.

3.3. Mechanism of plant–soil–microorganism interactions in the legume–cereal intercropping system

Agricultural intensification has long-term negative effects, mainly due to the overuse of fertilizers and the simplification of farming systems (e.g., continuous monoculture). However, increasing attention has been given to enhancing root-soil-microbial interactions by selecting crop varieties/genotypes, optimizing crop spatiotemporal combinations, improving nutrient inputs, developing smart fertilizers, and applying soil or microbiome inoculations. Enhancing root-soil-microbial interactions can increase yields and reduce pest and disease stress in farming systems, and it can also reduce greenhouse gas emissions and enhance soil carbon sequestration [72]. Biodiversity can enhance ecosystem services. The mechanism is usually explained as a complementary effect and selection effect [73,74]. Complementary effects refer to the separation of spatial and temporal ecological niches or the promotion of interspecific effects to improve the efficiency of resource utilization and productivity. The selection effect means that the productivity of a community is dominated by more productive species in a community with higher diversity. The selection effect and complementary effect are important mechanisms for increasing yield in intercropping. Previous studies have also proven that there is a positive correlation between the diversity effect and efficient resource utilization in intercropping systems [24].

Intercropping between legumes and cereals has close physical roots, and root-soil-microbial interactions can lead to mixing of microbial communities between the two plant species during intercropping. In the rhizosphere, there is close exchange between plants and rhizosphere bacteria. In fact, plants can stimulate rhizosphere bacteria by secreting root exudates and phytochemical metabolites, thus causing the rhizosphere bacterial community to react with the plant [75]. Intercropping broad bean and wheat increased nodule biomass compared to monocropping wheat because of the interaction between the rhizosphere, and wheat promoted the secretion of root exudates containing flavonols, isoflavones, chalcone and hesperidin [76]. Flavonoids produced by legume roots influence the behavior of related bacteria [77]. It also acts as a chemical attractant to concentrate compatible rhizobia on the root surface [78]. In addition, it stimulates the production of lipid-chitosaccharides in rhizosphere-related rhizobia. The latter induces a signal cascade that regulates the expression of symbiotic genes to transmit rhizobia-mediated signals and promote nodule development [79]. The resulting nodules can then positively affect N levels in the rhizosphere, a fraction of which is absorbed and utilized by the grain. Under soil nutrient deficiency and salt and drought stress, rhizosphere microorganisms dissolve P, immobilize N, and produce hydrogen cyanide, ACC-deaminase, indole-3-acetic acid and siderophores through rhizosphere interactions to help plants resist stress [80,81]. Currently known salt-tolerant rhizosphere bacteria are *Zhinguel-Liuella*, *Agrobacterium Tumefaciens*, *Brachybacterium Saurashtrense*, *Vibrio*, *Brevibacterium Casei*, *Bacillus amyloliquefaciens*, *Haererohalobacter*, etc. [82,83].

Complex plant–soil–microbial interactions maintain the balance between the different members of the microbial community. Nitrogen-fixing bacteria can fix nitrogen (N_2) in the atmosphere and actively transport ammonium (NH_4^+) and nitrate (NO_3^-) into the host cell. Ammoniating bacteria convert organic N_2 in soil to NH_4^+, which is further converted to NO_3^- by nitrifying bacteria. Legumes and nitrogen-fixing bacteria form nodule symbiosis. Mycorrhizal fungi convert arginine (Arg) to urea, which in turn converts to NH_4^+. Microbial communities convert minerals into plant usable nutrients such as phosphate (Pi), nitrogen (NH_4^+) and potassium (K^+) by oxidizing, dissolving or chelating and release essential elements by producing organic acids and side spores. In addition, mycorrhizal fungi can transport elements directly into the host cytoplasm through specialized structures of mycelium and sporangium over long distances, thus increasing nutrient availability. Microorganisms stimulate plant growth by metabolizing tryptophan and other small molecules in plant secretions and producing plant hormones, including coenzymes, gibberellins, cytokinins and plant hormone analogs [84,85].

4. Application and development of modern biological detection technology in soil microorganisms

Modern molecular biology has confirmed that culturable microorganisms account for only 0.1%~1% of the total number of microorganisms [86]. Since most functional soil microorganisms cannot be cultivated in laboratory conditions, 99% of the species and functions of soil microorganisms are still in the "black box" [87]. Uncultured microorganisms are of inestimable value to human production and play an important role in plant productivity. They are a group with great potential and are a new field for scientists to study because of their huge resources. In recent years, with the development of the independent cultivation of proteomics and bioinformatics, molecular techniques such as DNA stable isotope probing (DNA-SIP), RNA stable isotope probing (RNA-SIP), high-throughput sequencing of functional genes, metagenomics and carbon and nitrogen functional gene chips have been widely used in the study of rhizosphere microbial community structure, which has allowed more microbes to be found and enhanced the understanding of the diversity of uncultivated microorganisms [88-90].

4.1. DNA-SIP

Stable isotope probing (SIP) technology can be used to track the flow of elements in different substances and organisms in the environment, thus helping scientists infer various chemical reactions or biological processes in nature [91-93]. In recent years, nucleic acid (DNA/RNA) detection has been widely combined with SIP technology to link microbial metabolic function with phylogenetic information in environmental samples such as sediment, soil and water [94,95]. The advantage of DNA-SIP technology is that it avoids the deficiency of traditional culture methods and facilitates the identification of target microorganisms by tracking labeled substrates [96]. ^{13}C-DNA-SIP is the most widely used method by scientists. For example, $^{13}CO_2$ continuous labeling was used to study the effect of transgenic rice on microbial community structure [97], which revealed that planting Bt transgenic rice had little effect on the soil bacterial community structure and rhizospheric microbiome. The application of ^{13}C-DNA-SIP revealed that the abundance of AOB in nitrogen-amended soil was significantly higher than that in soil without nitrogen application. Compared with AOB, the nitrogen application level had less influence on the abundance and transcriptional activity of AOA [95], and the ^{13}C-DNA-SIP technique was used to elucidate the direct translocation of ^{13}C-labeled photoassimilates from maize plants to phosphate solubilizing bacteria associated with extracellular free radical mycelium. Mediated mineralization and turnover of atmospheric soil organophosphorus [98]. However, there are few studies on the application of DNA-SIP technology in legume–cereal intercropping systems. To date, only Gou et al. [99] used DNA-SIP technology to screen and identify functional genes indicating nitrogen cycling at the enrichment position of soil ^{15}N-DNA in the sugarcane/soybean intercropping system. Ma et al. [100] only used ^{13}C, ^{15}N, ^{14}C, and ^{35}S labeling technology to verify whether corn and soybean could use amino acids containing nitrogen and sulfur when competing with soil microorganisms under monoculture and intercropping, without combining DNA with stable isotope tracer technology. Therefore, it will be a great breakthrough and innovation to apply DNA-SIP technology to the study of legume–cereal intercropping. However, the marker cost involved in DNA-SIP is high, and the operation procedure is cumbersome [101]. If the economy and conditions allow, carefully designed experiments and careful operation are required to avoid repeated work and obtain reliable results.

4.2. High-throughput sequencing of *nifH* genes

In recent years, high-throughput sequencing technology has been widely used to study soil microbial species diversity, structural diversity, functional diversity and genetic diversity. This method overcomes some shortcomings of culture technology and obtains soil microbial information that cannot be isolated and cultured [102,103]. Currently, the major platforms for high-throughput sequencing include Roche's Roch GS FLX Sequencer, Illumina's Solexa Genome Analyzer, ABI's SOLiD Sequencer and Life Technologies' Ion Torrent PGM (Personal Genome Machine) and Ionproton. Illumina sequencing is most widely used in the study of soil microorganisms.

Biological nitrogen fixation is an important source of fixed nitrogen in the biosphere. *nifH* gene sequencing provides a large and rapidly growing database of sequences from different terrestrial and aquatic environments. However, it remains to be explored whether endogenetic and symbiotic nitrogen-fixing bacteria exist in different soil regions, such as the rhizosphere, under leguminous/grass intercropping systems. In view of this, high-throughput sequencing of the *nifH* gene showed that the intercropping system enriched soil organic carbon and was conducive to the propagation of nitrogen-fixing bacteria at the tillering stage [104]. Sugarcane-peanut intercropping could increase the abundance and diversity of soil microorganisms. *Devosia, Rhizobiales, Myxococcales, Allorrhizobium-Neorhizobium-Pararhizobium-Rhizobium, Bradyrhizobium, and Sphingomonas* proliferated profoundly [50]. Legume–cereal intercropping could accumulate more nitrogen-fixing bacteria, which was more closely related to the soil; moreover, the diversity of the rhizosphere bacterial community of different sugarcane varieties intercropping with soybean was significantly different. Using the *nifH* gene sequencing method will contribute to the design of intercropping systems, selection of optimal varieties, enrichment of bacterial community structure and creation of an environment conducive to crop growth, thus maximizing crop yield [89].

4.3. Metagenomics

Metagenomics, the study of the entire genome of soil biota, is currently a powerful tool for assessing the diversity of complex microbial communities, providing access to many new species, genes or molecules relevant to biotechnology and agricultural applications. The rhizosphere contains a powerful microbiome that plays an important role in improving soil fertility and maintaining plant health, productivity and nutrient cycling. The complexity of these microbiomes varies by plant species, soil type, host genotype and land cultivation system. They are the most complex ecosystems on earth and have studied the rhizosphere microbiome of a variety of crops and their biocontrol properties [105,106]. These studies and evidence reflect the importance of metagenomics applications as a tool for understanding the knowledge of biota affected by abiotic factors in sustainable agriculture.

To improve crop productivity, soil health and sustainable intensification to better understand the interactions between cropping systems and soil microbial diversity, metagenomics can be used to analyze the diversity, abundance and richness of rhizosphere soil microorganisms [107]. Through metagenomics research on traditional banana planting systems in the change of the microbial community, the results showed that planting systems were very important to support dynamic microbial diversity, and they were particularly useful to bacteria that help small farmers in sub-Saharan Africa (SSA) under the condition of scarce resources to promote collaborative interaction with soil and the total productivity of a plant [108].

The goal of metagenomics is not just to provide a collection of billions of biological projects but also to provide a unique opportunity to explore how microbial communities interact with soils and crops and ultimately harness the power of soil microbial communities to produce healthier, more robust crops or novel biomolecules.

5. Conclusions and future Prospects

The legume–cereal intercropping system can reduce the application of chemical fertilizer, improve the uptake of nitrogen by cereal plants, promote the nitrogen fixation of legume root nodules, expand the soil nitrogen pool, and reduce the loss of available nitrogen in the soil through leaching, nitrification and denitrification. Future research may focus on cropping systems rather than single crops. Therefore, the legume–cereal intercropping system has strong prospects for development in future agricultural systems. However, soil is a "black box", and the specific nitrogen allocation and utilization in legume–cereal intercropping systems is still unclear. The effects of different legume and cereal intercropping systems on microbial community structure, composition and activity need to be further studied. Using $^{15}N_2$ isotope and molecular biology technology clearly showed the specific distribution of nitrogen in the legume–cereal intercropping system, and ^{13}C-DNA-SIP, high-throughput sequencing and metagenomics molecular biology technology were used to explore rhizosphere microbial community characteristics, which helped us understand the mechanism of the underground part. Thus, rhizosphere regulation gives full play to the biological potential of crops and improves nutrient resource use efficiency and crop productivity [109], which is an important way to realize environmentally friendly and sustainable agricultural development.

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