



Review Relationship between Plant Roots, Rhizosphere Microorganisms, and Nitrogen and Its Special Focus on Rice

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Abstract: Fertilization is an important practical measure in agricultural production. As an important nutrient element of plants, nitrogen (N) has a significant impact on the plant productivity and microbial function. Rhizosphere microorganisms affect plant growth and development, nitrogen uptake and utilization, and ecological adaptability. The interaction mechanism between plant and rhizosphere microorganisms is one of the hotspots in life science research and the key program of agricultural microorganism utilization. In this article, the relationship among plant root morphology and physiology, rhizosphere microorganisms, and nitrogen is reviewed, summarized, and prospected.

Keywords: nitrogen; root morphology; root physiology; soil enzyme; rhizosphere microorganisms

1. Introduction

Nitrogen (N) is one of the most important nutrient factors affecting crop growth and yield formation [1]. N application is still one of the most effective ways to improve rice yield [2-4]. To understand how plant root rhizosphere microorganisms affect the nitrogen absorption and transformation, it is necessary to understand what forms of nitrogen that plants can absorb in, the availability of these compounds in the soil, and how can plant characteristics affect the N nutrient absorption [5]. The inorganic forms of nitrogen absorbed by plants are usually nitrate and ammonium salts [6]. The root system is the main organ for plants to absorb nutrients and water, and the root growth condition directly affects the growth of plant shoots [7]. Plant roots can adapt to the changes of soil nutrient conditions through active physiological regulation to absorb more nutrients [8,9]. N uptake and utilization of roots plays an important role in yield formation [10]. The application of N fertilizer also affects the growth and development of rice roots. The rhizosphere is the center of the interaction among plant roots, soil, microorganisms, and environment [11,12]. N, P, K, and other nutrients get into the plants by rhizosphere so that these nutrients can join in the nutrient cycle [13]. The plant will establish beneficial connections with some microorganisms in the rhizosphere [14], and these microbes are listed as part of the plant microbiome [15–17], which have a positive impact on plant health and productivity [18,19]. Microbiomes include eukaryotes, prokaryotes, and viruses, which can interact with different plant tissues or organs, such as phyllosphere and the endosphere. There is a special thin layer of soil around the root system, and the microbes in the soil are called rhizosphere microorganisms [20]. The rhizosphere can affect plant growth and respond to root exudates in the root soil area by the "rhizosphere deposition" process [21]. Plants provide carbon sources for the microbial growth activities and the community composition through rhizosphere deposition [22,23]. Some important symbiotic microorganisms have been identified to reduce the incidence of plant diseases and promote nutrient utilization



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). rate in host plants [24–26]. In recent years, lots of newly identified microorganisms have been identified to participate in N cycling networks, such as *ammonia-oxidizing archaea* and *denitrifying eukaryotic foraminifera* [27]. Furthermore, the application of microorganisms and their biotechnology on plant breeding and variety improvement is an effective method to improve crop yield and quality [28,29], which would be widely applied to field production in the future. Therefore, it is necessary to understand the relationship among the plant root–microorganisms interaction system, N fertilizer, and the application to crop production.

2. Major Nitrogen Cycle Pathways

The nitrogen cycle in the soil mainly consists of nitrogen fixation, nitrification, denitrification, ammonization after nitrogen fixation, and ammonium reduction (Figure 1), which are mainly driven by nitrogen-fixing microorganisms, nitrifying functional bacteria, denitrifying functional bacteria, and nitrate dissimilatory reduction to ammonium process functional microorganisms [27,30]. Microbial nitrogen fixation refers to the process of transforming gaseous N_2 into NH_4^+ [31]. N-fixing microorganisms are the important microbial functional flora in plant soil, which usually exist in the plant rhizosphere ecosystem and play an important role in regulating N nutrition in plants [32]. The process of N conversion formed by microorganisms is usually described as a cycle of six ordered reaction processes [27]. This view holds that a nitrogen molecule (N_2) is first transformed into ammonia by nitrogen fixation, ammonia into biological organic nitrogen by assimilation and absorption, and organic nitrogen finally into ammonium salt by oxygen deficiency [31]. Ammonium salt is then oxidized to nitrate $(NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-)$ by nitrification and finally reduced to a nitrogen molecule $(NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2)$ by denitrification or to a nitrogen molecule by anaerobic ammonia oxidation (Figure 1). The N-fixing microorganisms discovered at present belong to the prokaryotic community and are divided into 59 genera: aerobic authigenic nitrogen-fixing bacteria, including Azotobacter, Azotomonas) etc., microaerobic authigenic nitrogen-fixing bacteria, including Dexia, Methylomonas, etc., facultative anaerobic authigenic nitrogen-fixing bacteria, including Bacillus, etc., specific anaerobic authigenic nitrogen-fixing bacteria, including Clostridium, Desulfotomaculum, etc., aerobic combined nitrogen-fixing bacteria, including Beijerinckia, etc., microaerobic combined nitrogen-fixing bacteria, including Alcaligenes, Arthrobacter, etc., facultative anaerobic combined nitrogen-fixing bacteria, including Enterobacter, Klebsiella, etc., and photoautotrophic nitrogen-fixing bacteria, including *Rhodobacter*, *Rhodospirillum*, etc. [33].



Figure 1. The nitrogen cycle and involved key functional genes.

The main process of nitrogen cycling is as follows: synthesis of organic nitrogen in organisms (organic nitrogen \rightarrow NH₃ \rightarrow NH₄⁺), microbial nitrogen fixation (N₂ \rightarrow NH₃ \rightarrow NH₄⁺), denitrification process (NH₃ \rightarrow NH₄⁺ \rightarrow NH₂OH \rightarrow NO₂⁻ \rightarrow NO \rightarrow N₂O), nitrification-coupled denitrification (NH₃ \rightarrow NH₂OH \rightarrow NO₂⁻ \rightarrow NO₃⁻ \rightarrow NO₂ \rightarrow →NO \rightarrow N₂O), and anaerobic ammonia oxidation pathway (NO₂⁻ \rightarrow NO \rightarrow N₂).

3. Nitrogen-Root-Rhizosphere Microorganisms Interaction Network

The photosynthesis above the ground and absorption of water and nutrients by the roots are unified during the systematic growth and development process in plants [10]. The results show that it is necessary to have a good above-ground "leaf light system" and a good "root-rhizosphere soil system" to achieve the goal of high yield and quality of rice [34,35]. A good rhizosphere soil system can transform variety potential and cultivation technology into the advantage of high yield [36], because the rhizosphere of plants is affected by root morphology and physiology, which has higher microbial activity than bulk soil and is also the microdomain for the direct exchange of nutrients and energy between soil microorganisms and crops [32,37]. The high-yield nitrogen fertilizer must first affect the plant rhizosphere soil system and then influence the growth of above-ground plants, so it is important to clarify the mechanism of nitrogen on plant roots and the rhizosphere soil system (Figure 2) [38,39]. These microorganisms can encode more genes than host plants and form a stable community structure through collaboration, competition, and other relationships [40–42], which are crucial for plant growth and development as well as nutrient uptake, etc.



Figure 2. The relationship among nitrogen and root system, rhizosphere microorganisms.

4. Effects of Nitrogen Fertilizer on Plant Root Morphology

Plant root morphological characteristics play an extremely important role in nutrient absorption and yield formation and are influenced by nitrogen fertilizer management [7]. The number and activity of rice roots are closely related to the production and distribution of assimilation products and rice senescence [43]. The material exchange and information exchange between the root system and the above-ground part directly affect the growth

and development of the above-ground part and yield formation [44]. Previous studies have shown that mild nitrogen deficiency can promote root growth and facilitate root deep ligation [45]. Appropriate nitrogen supply can promote the increase of root density, quality, and quantity, but excessive nitrogen supply inhibits root growth [46]. The decline of nitrogen utilization efficiency at a high nitrogen level cannot be attributed to the decrease of plant root biomass [47]. The increase of nitrogen fertilizer dosage is beneficial to the increase of root injury intensity and root oxidation ability, which can effectively delay root senescence [48]. The slow-release, controlled-release nitrogen fertilizers are beneficial to increase the root content of the deep soil of rice, expand the range of root nutrient absorption, and improve root vitality [46].

In Arabidopsis thaliana, mild nitrogen deficiency increased the primary root length by 16% [49]. The typical response of maize root growth to mild low nitrogen stress is the decrease of crown root number and the increase of axial root elongation, including initial rooting and crown root [50]. Under severe low nitrogen stress, the elongation of the main root will also be enhanced [50]. Compared with full nitrogen application, low nitrogen application can increase the length of the axon by about 40%, and the elongation of the axon begins to increase slowly after low nitrogen treatment [50]. Similar results were found in rice and wheat studies, and low nitrogen stress also promoted root elongation in rice [51–53] and wheat [54–57]. In crops, nitrogen deficiency can promote or inhibit lateral root growth [50,51]. These two opposite results may be related to the degree of nitrogen deficiency or crop species [50,51,54]. In maize, nitrogen deficiency treatment for three days had little effect on lateral root length. However, nitrogen deficiency treatment promoted lateral root growth after four days [49]. With continuous nitrogen deficiency, the lateral root growth began to be inhibited after eight days [50]. From these data, we summarized and plotted the regulatory network map of root morphological changes at different nitrogen levels (Figure 3). When maize had just captured a signal of nitrogen deficit, the first reaction was to promote lateral root growth in an attempt to obtain more nitrogen from the soil [47]. But in the case of persistent nitrogen deficiency, plants could not obtain enough nitrogen to support stem growth [52,54]. Plants are adjusted to reduce C and N consumption by reducing lateral root growth [49]. In this situation, rapid elongation of plant axial roots is used to explore greater space around the soil, as found in *arabidopsis* [49]. Gao et al. found that when the nitrogen concentration in the stem decreased by more than 30% or the carbon–nitrogen ratio increased by more than 40%, the root axis elongation was significantly promoted [50]. These results indicate that the nitrogen concentration and carbon-nitrogen ratio in the stem can reflect the nitrogen state of the plant and regulate the root morphology. Root growth rates are regulated by two coordinated processes: cytokinin and auxin [58]. In wheat, the root elongation enhancement observed under low nitrogen conditions was associated with increased cell length, and a similar situation was observed in maize [55–57]. High concentrations of N also inhibit maize cell division [59]. However, it is unclear how nitrogen regulates cell division and elongation. Nevertheless, there is evidence that the phytohormone auxin (IAA), cytokinin (CTK), ethylene, abscisic acid (ABA), brassinosteroids, monopodolactone, and nitric oxide (NO) signaling pathways are involved in this process [50–62].



Figure 3. Regulation of nitrogen on changes of root morphology.

5. Effects of N Fertilizer on Plant Root Physiology and Biochemistry

As a component of plants, the root system is an important organ to synthesize many physiological active substances, which is a key point in the growth and development of plants [63]. At present, there is a popular direction to research the root and crown characteristics of varieties with high nitrogen uptake and utilization by comparing genotypes with obvious differences in nitrogen uptake and utilization [64,65]. General nitrogen-efficient rice varieties have strong root power, developed root system, large root biomass, root volume, root absorption surface area and active absorption area, high root oxidation capacity, and strong affinity of root system to NH₄⁺ [66,67]. The root characteristics of *indica* rice showed that with the increase of nitrogen application, the root activity, total absorption surface area, and active absorption surface area of rice increased gradually, the IAA concentration in bleeding sap amount of root increased first and then decreased, and the ABA concentration decreased gradually [68]. Studies on Japonica rice also showed that increasing nitrogen application could enlarge the root absorption area, increase bleeding sap amount of root, and improve the root oxidation power after heading [69]. Zhao et al. studied the effect of nitrogen application on reactive oxygen species metabolism differences between leaves and roots in winter wheat and the results showed that the response of root systems to stress environment was more sensitive than that of leaves [70]. Nitrogen application can increase root activity, reduce root-to-crown ratio, and increase leaf and root biomass. Excessive nitrogen application could inhibit some antioxidant enzyme activities in the enzymatic

system [70,71]. When the amount of nitrogen was applied in the range of medium nitrogen (150~240 kg/hm²), the enzyme activity indexes in winter wheat plants was relatively optimal, which was beneficial to the early growth and development of winter wheat, and was in line with the principles of reducing fertilization and increasing production efficiency [71]. Rape applied with 240 kg/hm² nitrogen can significantly improve soil enzyme activity, microbial abundance, root growth, and nutrient absorption ability [72]. Under low nitrogen stress in rape, the enzyme activity of root plasma membrane H⁺-adenosine triphosphate (H⁺-ATP) increased significantly. The enzyme activity of root plasma membrane H⁺-ATP in rape under three nitrogen levels has a significant difference [73]. Liang et al. found that both nitrogen and zinc significantly affected root dry weight, root length, root surface area, root nitrate reductase activity, root glutamine synthase activity, root indoleacetic acid oxidase activity, root ribonuclease activity, root nitrogen, and zinc content. The suitable combination of nitrogen and zinc has a positive synergistic effect of nitrogen and zinc, which is beneficial to the establishment of good root morphology, the regulation of root physiological activity, and the uptake and utilization of nitrogen and zinc nutrients, thus promoting the high and stable yield of wheat [74].

The root system, as the organ to connect and exchange surface between plant and soil, releases various compounds (organic acids, malic acids, succinic acids, amino acids, polyamines, etc.) around the root through root secretion while absorbing water and nutrients from the soil, which produces the rhizosphere effect, and then regulates or affects the growth and development of the plant [37]. These compounds secreted by plant roots into the external environment during life activities are called root exudates [75–77]. Root exudates improve the bioavailability of nutrients in rhizosphere soil and promote crop growth by changing the physical, chemical, and supporting specific microbial activities [78]. On the other hand, as an important part of root physiology, root exudates play an important role in regulating plant growth and development [79]. To obtain the benefits of the microbial community, plant hormones are important. It can be divided into two aspects: (1) enhanced ecological plasticity and adaptability based on improved nutrient and water supply and protection against biotic and abiotic stresses, and (2) optimized plant/root growth and development due to microbial support for plant regeneration, growth, and lateral root and root hair formation. It depends on hormone concentration, hormone homeostasis, plant species, plant development stage, environmental stimulation, and so on [80].

6. Effects of Nitrogen Fertilizer on Soil Enzyme Activity in Plants

Soil enzymes are proteins that catalyze soil biochemical reactions, which are mainly derived from soil microorganisms and plant roots, as well as from soil animals and animal and plant residues entering the soil [81]. The effect of plant roots on soil enzyme activity is that plant roots can secrete extracellular enzymes, and they may stimulate the activity of soil microorganisms [82,83]. Soil enzyme plays an important role in driving almost all biochemical reactions in soil, promoting the circulation and transformation of various main elements in the soil ecosystem, and maintaining the normal operation of the ecosystem [84]. Soil enzyme activity is widely applied to the study of soil ecological environment as a quick index to measure the microecological environment of rhizosphere [85]. Therefore, it is of great significance to study soil enzyme activity to evaluate and improve soil fertility and promote crop high yield. As one of the most important cultivation measures, nitrogen fertilizer has been reported for its effect on soil enzyme activity. Previous studies showed that the application of urea increased the enzyme activity of rhizosphere soil in the middle and late filling stage, which was beneficial to the improvement of grain yield and nitrogen utilization efficiency [59]. Su et al. took the desertification grassland as the research object and discovered that nitrogen application significantly inhibited the activities of soil urease, sucrase, catalase, and dehydrogenase, and the different enzyme activities were different in nitrogen level, soil depth, and interannual [86]. A study on maize found that the addition of nitrogen fertilizer significantly reduced soil pH and available phosphorus levels but increased the activity of soil phosphatase [87]. The addition of biochar to soil significantly

increased soil microbial biomass carbon and the activities of urease, alkaline phosphatase, dehydrogenase, and N-acquisition-related enzymes [88]. Moreover, the increase of activity varies greatly with soil properties, biochar-related properties, application amount, and experimental type [88]. Among them, the combined application of biochar and nitrogen fertilizer can improve soil quality, form a more stable microbial community structure, and make soil viability more active [89]. In the maize pot experiment, it was found that adding oxytetracycline to the soil can inhibit the activity of soil enzymes [90]. However, adding different fertilizers to the soil, especially microbial inoculants, can reduce the impact of oxytetracycline on the soil. It also shows that microorganisms can improve soil quality by adjusting soil enzyme activity [91].

Different nitrogen fertilizer operation methods also have an important effect on rice rhizosphere microorganisms [86]. Some studies have shown that the proper backward shift of nitrogen fertilizer is beneficial to promote the growth of bacteria, actinomycetes, ammoniated bacteria, nitric acid bacteria, and aerobic authigenic nitrogen-fixing bacteria in the middle and late rhizosphere soil of rice, and indirectly improve the activity of urease and acid phosphatase in rhizosphere soil, which creates a good rhizosphere nutrient environment and material basis for the rapid growth of rice plants [92].

7. Effects of Nitrogen Fertilizer on Microorganisms in Rhizosphere Soil of Plants

Soil microorganism is the most active factor in the soil system, which participates in material transformation and nutrient cycle in soil and is an important factor of soil fertility [93,94]. Microbes in different groups of soil can perceive the change of soil properties caused by nitrogen fertilizer and make different responses [90,95], which will affect soil fertility. Liu et al. have found that long-term application of chemical and organic fertilizers in both dry and paddy soils can not only increase soil urease and catalase activity but also increase the number of bacteria and actinomycetes [47,96]. However, it has also been pointed out that excessive application of chemical fertilizer, especially nitrogen fertilizer, is considered to be the main reason for the decrease of soil quality and soil enzyme activity in the agricultural ecosystem [97]. Besides, nitrogen addition usually inhibits microbial biomass, but the effects of different ecosystems may be different [98]. The addition of nitrogen can alleviate the limitation of nitrogen on some microorganisms [99], especially nitrifiers and denitrifiers that use inorganic nitrogen as their energy or an electron acceptor. On the other hand, the number and activity of nitrogen-fixing emblem organisms may decrease with the increase of nitrogen, and some microorganisms with weak tolerance to high permeability potential may be killed, resulting in a decrease in microbial diversity [100]. In the study of wheat, it was found that both nitrogen fertilizer application and crop growth stage could affect wheat root-related microorganisms, especially bacteria. The application of nitrogen fertilizer significantly increased the abundance of plant growth-promoting bacteria (PGPR), and its abundance was significantly related to the content of organic acids, indicating that crops may recruit beneficial microorganisms by secreting organic acids [38].

Soil microbes play an important role in maintaining the material circulation, energy flow, and information transmission in the ecosystem [27]. They are the repositories of important elements such as carbon and nitrogen in soil and one of the important indexes to evaluate soil quality [101]. The availability of nitrogen (N) and phosphorus (P) strongly influence the cycling and fixation of carbon (C) in the landfall ecosystem [102]. Soil microbial diversity refers to all microbial species in the soil ecosystem, the genes they have, and the diversity of the interactions between these microorganisms and plant roots, microorganisms can be divided into rhizosphere microorganisms and non-rhizosphere microorganisms [105]. The rhizosphere is the soil around the root system affected by the growth of the root system [106]. It is the interface of soil–plant–microbial interaction and a special habitat for microbial diversity, there was no significant difference in the total bacterial community number between rhizosphere and non-rhizosphere soil [108],

but the rhizosphere and non-rhizosphere soil had different physicochemical conditions. Rhizosphere could utilize carbon secreted by crop roots [109,110], so rhizosphere bacterial community diversity was higher than that of non-rhizosphere soil, but the abundance of different types of bacteria was different in rhizosphere and non-rhizosphere.

8. Relationship between Rhizosphere Microorganisms and Crop Yield

Rhizosphere microorganisms are closely related to crop growth [107,111]. Nitrogen, as an important nutrient element of plants, has a significant impact on the control of plant productivity and microbial function [38,112,113]. At present, there are many studies on the relationship between rhizosphere microorganisms and crop yield [114,115]. Abdelaziz et al. found that the addition of root endophytic fungus Piriformospora indica to the soil could promote the growth of tomato and increase the yield of crops, and also induce the resistance of tomato to salt stress. This may also be applied to future soil by adding fungi to cope with soil salinization [116,117]. In the study of Angelica Sinensis, with the increase of continuous cropping years, the total number of culturable microorganisms, bacteria, and actinomycetes in soil decreased, while the number of fungi increased significantly. The microbial diversity index decreased significantly, and the total yield of Angelica Sinensis decreased significantly [118]. Studies on wheat, corn, and other crops showed that rhizosphere bacteria, fungi, and actinomycetes were positively correlated with crop yield [119,120]. Studies in maize and peas have found that direct inoculation of isolated rhizosphere microorganisms into the rhizosphere of crops also showed that rhizosphere microorganisms can be promoted to increase crop yield [121,122]. Among them, plant growth promoting rhizobacteria (PGPR) inoculation only changed the number of maize microaerophilic nitrogen-fixing microorganisms in the crop reproduction stage, while fertilization only influenced the number of cellulose decomposition, nitrification, and microaerophilic nitrogen-fixing microorganisms in the nutrition stage [122].

Besides, inoculation of arbuscular mycorrhizal fungi and selenium application are environmental protection strategies to improve the overall yield, quality, and selenium content of scallion bulbs [123]. Rice rhizosphere microorganisms are also closely related to yield, and the number of bacteria, fungi, and actinomycetes in the rhizosphere of rice with different yield fields is inconsistent with the growth period [124]. During the same growth period, there were also significant differences between rhizosphere bacteria, fungi, and actinomycetes in different rice yield fields, among which the rice field with a large number of rhizosphere microorganisms also had a high yield [124]. The comprehensive analysis based on three diversity indexes of Shannon, Simpson, and McIntosh showed that the rhizosphere microorganism of high-yield paddy soil was more abundant, that the metabolism ability was stronger, that the microbial community distribution was more uniform, that the diversification degree was higher, and that the carbon source species could be used more abundantly [125]. Among them, the number and variety of rhizosphere microorganisms, strong metabolic ability, and metabolic types are the keys to the vigorous growth and high yield formation of high-yielding rice, and the total number of cultured microorganisms in the rhizosphere of high-yielding rice is close to two times that of the control. From the above studies, the conclusion can be drawn that the variety and quantity of microorganisms in rhizosphere soil are beneficial to the increase of rice yield [125].

9. Relationship between Root Microorganisms and N Utilization Efficiency

In soil, plant roots interact with microorganisms. Plant roots, in addition to fixing plants and serving as organs for absorbing water and nutrients, are also places where microorganisms gather and inhabit and multiply [126]. Rhizosphere microorganisms can be promoted for plants to obtain nutrients from the soil. For example, mycorrhizal fungi dissolve phosphorus and rhizobia fix nitrogen [127,128]. In contrast, rhizosphere microorganisms can also compete with host plants for nutrients in the soil or attack plants as pathogenic bacteria [18]. Asian cultivated rice (*Oryza sativa* L.) is mainly divided into *indica* rice and *japonica* rice. Compared with *japonica* rice, *indica* rice usually shows higher nitrogen

utilization efficiency [129]. Studies have shown that the natural variation of some genes such as NRT1.1B in indica rice plays a very important role in improving the utilization efficiency of *indica* rice nitrogen fertilizer [129]. However, it is still unclear whether the microbial composition of the root system affects the nitrogen fertilizer utilization efficiency. By comparing 68 indica rice and 27 japonica rice varieties grown in the field, Bai Yang Research Group and Chu Chengcai Research Group of the Institute of Genetics and Development Biology of the Chinese Academy of Sciences found that *indica* rice and *japonica* rice formed significantly different root microbiomes [16]. The diversity of root-enriched microbiomes in *indica* rice was significantly higher than that in *japonica* rice, and the characteristics of root-enriched microbiomes could be used as biomarkers to distinguish rice from rice. The specifically enriched microbiomes of *indica* rice roots were distributed in *Deltaproteobacteria*, Actinobacteria, Acidobacteria, and Bacteroidetes, etc., while the microorganisms enriched by japonica roots were mainly concentrated in Alphaproteobacteria. The microbial function of the *indica* rice root system was predicted and analyzed [16]. It was found that the pathways involved in nitrogen metabolism were enriched, including the nitrate ammonification and nitrite ammonification pathway and the nitrate respiration, nitrite respiration, and nitrogen respiration pathway. Indica rice roots are enriched with more microbial groups related to the nitrogen cycle than *japonica* rice, thus having a more active nitrogen conversion environment, which may be one of the important reasons for the higher nitrogen utilization efficiency of *indica* rice than *japonica* rice. Genetic experiments showed that the absence of NRT1.1B and the natural variation between *indica* rice and *japonica* rice significantly affected the root microbiomes of rice, most of which had the function related to the nitrogen cycle [16]. As a result, rice changes the rhizosphere microenvironment by NRT1.1B regulating the microorganisms with nitrogen conversion ability of the root system and then affects the nitrogen fertilizer utilization efficiency in rice fields. Through the improved high-throughput microbial isolation culture and identification system, 70% of the bacterial species of the rice root system were successfully isolated and cultured, and the first systematic resource bank of rice root bacteria was established [16]. The specific enriched flora of *indica* rice and *japonica* rice was artificially reconstituted by rice root bacterial resource bank. It was found that enrichment flora of *indica* rice could promote the growth of rice under organic nitrogen better than that of *japonica* rice. It was further confirmed that the difference in nitrogen utilization efficiency between *indica* rice and *japonica* rice was related to root microbiomes [16]. As a result, the study not only revealed the relationship between root microbiomes and nitrogen fertilizer utilization efficiency but also proved the key role of NRT1.1B in regulating rice root microbiomes. More importantly, the first rice root culturable bacterial resource bank was established, which laid an important foundation for studying the interaction and function between root microbiome and rice, and also laid a foundation for applying beneficial microorganisms and reducing nitrogen fertilizer application. In wheat, the use of bacteria that promote plant growth may be another way to improve nitrogen use efficiency, because these bacteria can improve root development and increase access to nutrients, including nitrogen fertilizer [130]. Microbial mineralization of organic nitrogen in the rhizosphere improves nitrogen utilization and nitrogen availability in roots [131]. The transformation from N_2 to NH_3 is accomplished by non-nodding nitrogen-fixing bacteria, which naturally exist in soil, including the rhizosphere of wheat [132,133], and inoculation of nitrogen-fixing bacteria can increase the yield of wheat [134–136].

10. Effects of Cultivated Habitats on Rhizosphere Microorganisms

Soil microbes showed characteristic community structure differences in different soil types and different cultivation methods, so as to adapt to the changes of the living environment [137]. The direct seeding and mechanical transplanting significantly increased the richness and abundance of soil microorganisms in paddy fields and changed the community structure of microorganisms, but the effect of seedling throwing on soil microbial community structure and diversity was not significant compared with the traditional

cultivation methods of transplanting [138]. Different environmental conditions and rice ecotypes can lead to the recombination of root microbiome [29]. Upland and lowland environment had a significant effect on the composition of root microbial community of two ecological rice varieties, and the environmental effect was greater than that of ecological variety [139]. The overall response of root microorganisms to upland environment is driven by *enterobacteriaceae*, *serratia*, and *ascomycota* [139]. The process of lowland rice ecotype from paddy field to upland can be specifically enriched by some Gram-positive microbes, such as thermoleophilia and actinobacteria [139]. Upon further isolation and screening of root system functional microorganisms in upland rice, it was found that root microbes in upland rice could promote plant growth under drought and irrigation conditions, and may be an effective microbial resource to promote crop growth and drought tolerance [140]. The formation of paddy-upland rotation alleviates the internal environment and soil fertility accumulation, which makes the paddy field more resistant to external environmental disturbance than the dry farming ecosystem [141]. The rice field is the main source of artificial methane and is affected by nitrogen fertilizer [142]. Methane is produced mainly by methanogenic bacteria using carbohydrates in soil metabolism [143]. Decreased CH₄ emissions under deep nitrogen application are attributed to a decrease in the methanogen/methanotroph ratio [144]. The addition of nitrate will affect the transcriptional and functionally active methanogens and may reduce methane production associated with straw improvement in paddy soil culture by competing for common substrates between nitrate-using organisms and methanogenic bacteria [145].

11. Summary and Outlook

Whether from the perspective of microbial diversity or the perspective of root exudates, soil enzymes, plant growth, and development have a certain role in promoting rhizosphere microorganisms in the study of the relationship among plant roots, rhizosphere microorganisms, and nitrogen. Different nitrogen management also has a certain effect on plant rhizosphere microorganisms. There is an interaction between soil microorganisms and nitrogen loss in the rice field. For example, nitrogen source, carbon source, water, and pH value changes have an impact on nitrogen transformation, but these property changes only indirectly affect the function of related microorganisms by changing the living environment of microorganisms, and the essence of nitrogen transformation is the biochemical process in which soil microorganisms participate. Therefore, the mechanism of microbial action on nitrogen transformation is key to nitrogen loss research. It is not clear how many of the microorganisms are uncultured. Our understanding of these microorganisms is still limited, because the mainstream research focuses on rhizobia, arbuscular mycorrhizal, and important pathogens, which are only a small part of the plant microbiome, and forgets the steering role of the physico-chemical habitat conditions. Moreover, due to the limitation of research techniques, traditional methods rely on the interaction between plants and single microorganisms under laboratory conditions, and rarely study the molecular mechanism of microbiome coexistence with host plants in the natural state. Plant microbiology researches take all microbes associated with plants as a whole at the community level to explore how it affects the physiological phenotypes of crops. At the same time, more attention is paid to microbes and the interaction between microbial groups and host plants [146]. The method of isolation and culture cannot elucidate the interaction between different species [147]. Besides, the isolated and cultured microorganisms will adapt to the laboratory environment and cannot fully reflect their true situation in nature [148]. Microbial communities play an important role in human health, environmental protection, and industrial development. The method of accurate gene editing in microbial communities will greatly promote our understanding of microbes. PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) technology has some shortcomings. For example, due to the inability to accurately understand the phylogenetic relationship and community diversity of a large number of bacteria in the sample, its sensitivity is relatively low. Sampling and sample processing may cause errors in DNA extraction [149].

Much progress has been made in the study of soil biodiversity, but the understanding of soil biodiversity is still very limited, and basic questions such as how many species of organisms exist in the soil are still unclear. In the future, to better protect and utilize soil biodiversity, it is necessary to establish an in-situ monitoring platform to monitor the main ecosystem types and soil biological groups of important habitats in China, understand the resources, evolution rules, and threat factors of biodiversity, and provide scientific and technological support for the protection of soil biodiversity and the development of biological resources in China. At the same time, the big data-sharing platform of microbial resources and the new technology of microbial isolation and culture can provide a fully scientific basis for the full development and utilization of known soil biological resources and bring the function of soil biological into practice application. Modern soil biology should be based on soil biodiversity, take the unity of soil-biology-environment as the research object, take the ecological process and service function of soil biology as the ultimate goal, and construct a multi-dimensional theoretical system that integrates above-ground and underground, structure and function, and ecology and evolution, including various soil biological groups. Ultimately, based on scientific theories, there is a need to fully tap into the potential of soil biodiversity to ensure soil fertility, environmental and ecological health, promote plant health and agricultural product health, and ultimately serve human health and the health of the planet on which it depends. In the future microbial culture, experiments should be fully integrated with modern sequencing and bioinformatics techniques. Microbial culture isolation can be used to study detailed interaction mechanisms for screening specific traits and determining the genetic mechanisms behind phenotypes [21].

In the 20th century, genomics, transcriptomics, proteomics, and other disciplines have been established and formed a system [150], making the research of species more in-depth. The emergence and continuous updating of new sequencing methods such as 16 S amplicon sequencing, metagenome sequencing, meta-transcriptome sequencing, meta-proteome sequencing, and so on, open up new ideas for the study of microorganisms and provide technical support for the study of the interaction mechanism among plant roots, microorganisms, and nitrogen. Future researchers may introduce genome editing techniques to site-specific clustered regularly interspaced short palindromic repeats (CRISPR) genome editing in situ for specific species in any complex microbial community without prior isolation and culture. Before genome editing of microbial communities, it is necessary to know which species can effectively ingest and integrate exogenous nucleic acids. Experimenters developed new a method, Environmental Transformation Sequencing (ET-Seq), to identify from microbial communities which bacteria can be effectively genome-edited [151]. Through the ET-Seq method, the researchers also found the most suitable DNA delivery method for the next step of CRISPR genome editing. Notably, the authors also identified ten species that failed to isolate and culture in the laboratory. ET-Seq results suggest that they can also be edited by the genome [152]. Next, the authors used the CRISPR-associated protein, cas (CRISPR-Cas) Tn17 transposase system for genome editing of microbial communities [153–155]. The traditional CRISPR-Cas transposase system requires two or more plasmids, and in this study, the experimenter optimized the system and developed the DNA-editing All-in-one RNA-guided CRISPR-Cas Transposase (DART) system. As validation, the experimenter successfully performed genome editing on two specific bacteria in the microbial community [152]. Isolation, culture, transformation, and gene editing of environmental microorganisms usually takes a year and can easily fail. The in-situ microbial genetics strategy proposed here takes only a few weeks and does not require isolation and culture. In the future, we may need more effective tools to target genes that can be widely used and last for a long time. Traditional approaches to antibiotic resistance screening may not be suitable for genome editing in microbial communities because natural resistance may exist in complex communities. To improve genome editing efficiency, we also need more efficient nucleic acid delivery systems, and further optimized forward and reverse screening strategies. Cas-16S-seq is a method that uses CRISPR/Cas9 to target and cut plant 16S rRNA gene sequences to enrich bacterial sequences in amplified

products, and the proposed method can be easily integrated into the existing 16S-seq processes and can efficiently eliminate co-amplified high-abundance plant sequences [151].

The correlation between soil rhizosphere microorganisms and plant phenotype, genotype, soil enzyme activity, and the use of microorganisms to improve plant growth and nutrient absorption will be an important direction of plant research, but also open up a new way to realize new green agriculture. Therefore, the future research should focus on quantification of the relationship between plant and microbial traits and the crops' long-term experiments under field conditions. Besides, the complex feedback mechanism between nitrogen-plant-microorganisms should be studied in combination with botany, plant physiology, crop cultivation, soil ecology and soil physics, microbiology, and molecular biology, which is essential for crop production and nutrient absorption under global climate change. Previous microbial studies have been included only in the final stage of crop improvement programs or in the fertilization stage of field experiments, and it has been proven that optimizing the pairing of microorganisms and plant genotypes during plant breeding has great potential to maximize plant performance. Therefore, the study of plant microbes needs to be more involved in the process of plant breeding, combined with traditional plant breeding, precise fertilization, and water-efficient utilization, to effectively improve the growth, development, and output of plants under a series of stresses, and provide a transformative example for the sustainable development of plant nutrition and health, for low chemical-input agriculture.

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