

# Study of Diversity of the Anaerobic Micro-Organisms Commonly Found in the Paddy Fields

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**ABSTRACT:** Rice fields harbor a bewildering diversity of soil microbes and soil fauna—including nitrogen fixers, nitrifiers, methanogens, plant-growth regulators, methane oxidizers, phosphate-dissolving microbes, and sulfur oxidizers along with decomposers and nutrient recyclers. A variety of microbial species and taxa reside in the rhizosphere and the phyllosphere of plants and also have multiple roles as symbiotic endophytes while living within plant tissues and even cells. They alter the morphology of host plants, enhance their growth, health, and yield, and reduce their vulnerability to biotic and abiotic stresses. The findings of both agronomic and molecular analysis show ways in which microorganisms regulate the growth, physiological traits, and molecular signaling within rice plants. However, many significant scientific questions remain to be resolved. Advancements in high-throughput multi-omics technologies can be used to elucidate mechanisms involved in microbial–rice plant associations. Prospectively, the use of microbial inoculants and associated approaches offers some new, cost-effective, and more eco-friendly practices for increasing rice production.

**KEYWORDS**–rice, paddy, anaerobic, micro-organisms, diversity, fields, soil

## I.INTRODUCTION

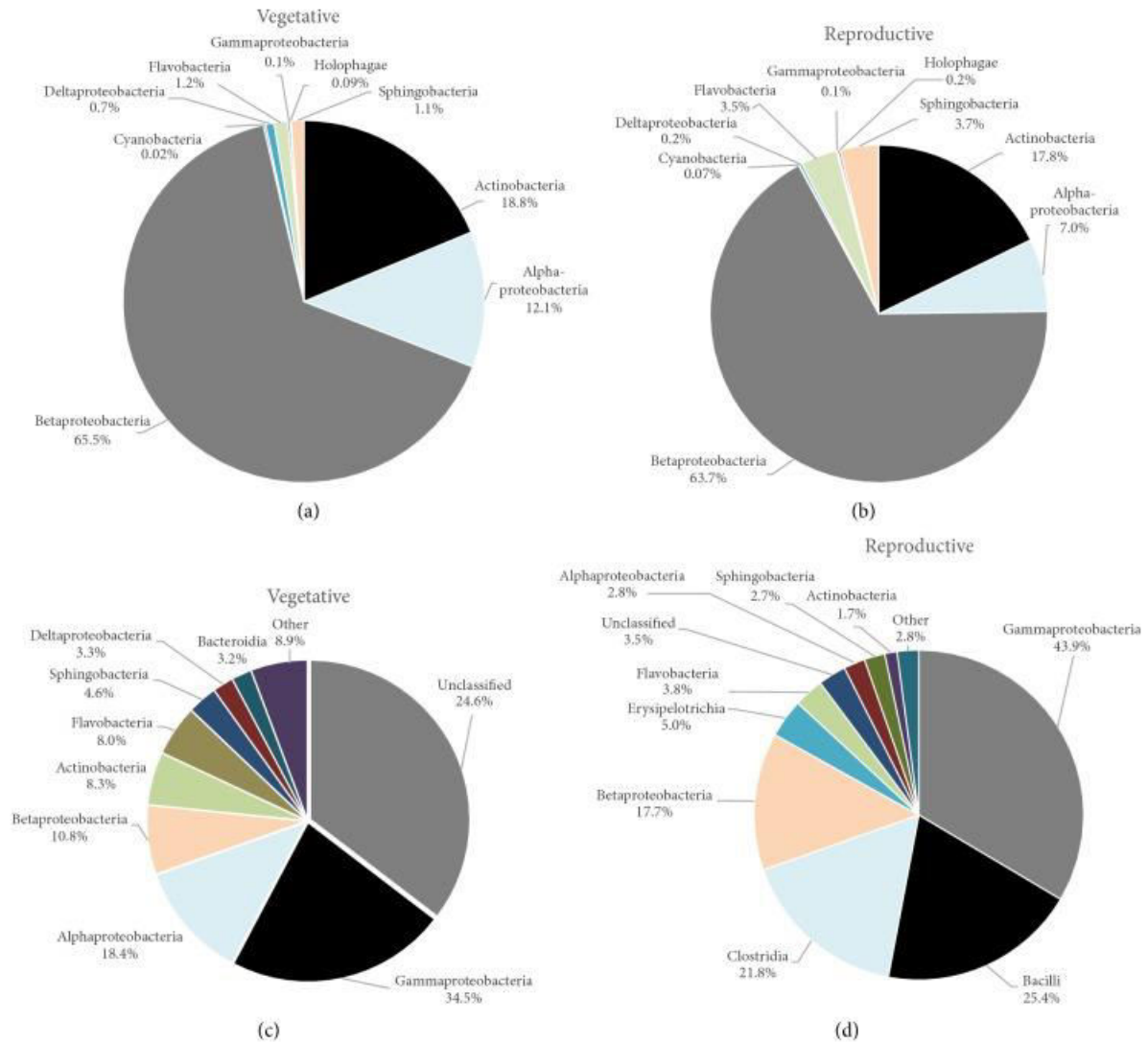
Microbes are ubiquitous and cosmopolitan, are associated with plants, animals and to different ecosystems. Microorganisms are the indirect nutrient source for plants, promoting plant growth and development. A number of microbial communities are associated with the plant root system in soil ecosystem [1]. The cropping systems are affected directly by the abundance of microbial entities in this ecosystem. The best example for this type of plant microbe interaction is PGPR (Plant growth promoting rhizobacteria) associated with the plant roots [1-3]. There are several mechanisms that are continuously running in this rhizospheric region that leads to the change in microbial dynamics by the time. This potent plant growth promoting rhizobacterial group has ability to provide several essential and soluble form of metabolites to the plant so that they can achieve a proper and healthy growth [4,5]. The interaction of these microbial communities is beneficial for both microbes and plant as well. Although, there are different cropping system, paddy field is an important crop to study various microbial interactions in its rhizospheric region along with the microbiome dynamics.

Paddy field is one of the major staple crops of many countries and its yield and production is a major concern as well. Moreover its production is also affected by the availability of water. In developing countries, farmers are still dependent on monsoon for water required for the cultivation of the rice plant. Researchers have elucidated several microbial interactions in the paddy fields. Cyanobacteria, methanogens and methylotrophs other than methanotrophs are interconnected microbial communities in the rice fields. The present editorial is to focus the role of microbial communities in sustaining the paddy ecosystem and involvement of microbial communities in the global carbon and nitrogen cycling by interacting with the plant ecosystem. The methanogenic, methanotrophic and cyanobacterial communities are described and elaborated that how they are interacting in the field.

Rice field is an excellent example of the methane sink [6] and a huge content of the methane gas is released because of the abundance of the methanogenic communities in the flooded rice field [7]. The subsequent chain of microbial transformations induces the release of methane in the paddy field. Subsequently, this enormous methane in the rice field is utilized by the methanotrophic bacterial communities present in the same ecosystem, converting methane into biomass. Therefore methane in the environment is diminished by the methanotrophs present in this flooded rice field. Interesting point is that, the methanotrophic activity is triggered by the presence of enzyme mmo (methane mono oxygenase) along with oxygen [8]. This oxygen requirement is fulfilled by the presence of the green algal communities to the surface of the flooded paddy field. Since these algal communities are very keen in the release of oxygen, it is trapped by the methanotrophic communities easily that is required for the enzymatic activity of mmo (methane mono oxygenase).

In agricultural systems, interactions between plants and microorganisms are important to maintaining production and profitability. In this study, bacterial communities in floodwaters of rice fields were monitored during the vegetative and reproductive stages of rice plant development using 16S amplicon sequencing. The study was conducted in the south of Brazil, during the crop years 2011/12 and 2012/13. Comparative analyses showed strong differences between the communities of floodwaters associated with the two developmental stages. During the vegetative stage, 1551 operational taxonomic units (OTUs) were detected, while less than half that number (603) were identified in the reproductive stage. The higher bacterial richness observed in floodwater collected during the vegetative stage may have been favored by the higher concentration of nutrients, such as potassium, due to rhizodeposition and fertilizer application. Eighteen bacterial phyla were identified in both samples. Both communities were dominated by Gammaproteobacteria. In the vegetative stage, Alphaproteobacteria and Betaproteobacteria were more abundant and, in contrast, Bacilli and Clostridia were the more dominant classes in the reproductive stage. The major bacterial taxa identified have been previously identified as important colonizers of rice fields. The richness and composition of bacterial communities over cultivation time may contribute to the sustainability of the crop.

The sequences were distributed among 18 different bacterial phyla. The most species-rich phyla in both the reproductive and vegetative stages were Proteobacteria, which contained 53.34% of the OTUs detected in both samples, Bacteroidetes (16.80% of the OTUs), Actinobacteria (7.84%), and Firmicutes (5.52%). In total, 83.50% of the total OTUs detected in these two communities was associated with these four phyla. Several phyla with lower richness estimates were also detected in these samples, defined as phyla containing less than 1% of the total OTUs detected in this analysis. Based on this criterion, a total of twelve rare phyla were detected including Chloroflexi, Deferribacteres, Deinococcus-Thermus, Fusobacterium, Gemmatimonadetes, OP10, Planctomycetes, SR1, Spirochaetes, TM7, Tenericutes, and Verrucomicrobia, which were detected in both stages of crop development. The phyla Acidobacteria (1.94% of the OTUs) and Cyanobacteria (1.25%) were also present, but at low frequencies ( $\leq 2\%$  of the OTUs).



Of the 169 genera identified in the two developmental rice stages, 22 (13%) occurred in both stages .Beijerinckia, Curvibacter, Pelomonas, and Rhodoferax occurred in high counts in both phenological stages. In general, the abundances of Beijerinckia, Pelomonas, and Rhodoferax were similar in flood waters from both communities; however, the relative abundance of Curvibacter was slightly higher in vegetative stage. The abundances of the genera Aeromonas, Bradyrhizobium, Emticicia, Massilia, Methylibium, and Spirosoma were lower and did not differ significantly between vegetative and reproductive stages . Polynucleobacter (29.5%), Curvibacter (23%), and Rhodoferax (10.3%) represented the most abundant genera in the vegetative stage, while Curvibacter (18.3%), Alcaligenes (11.8%), and Flavobacterium (11.3%) were more abundant in the reproductive stage .[1,2,3]

## II.DISCUSSION

A study was proposed to determine the changes in soil microflora of paddy fields in relation to the type of farming system. Soil samples were collected from Kumarakom region, South-West part of Kuttanadu, Kerala from two paddy fields each with single rice farming and fish-rice rotational farming. A total of 11 bacterial species were isolated from paddy field soil whereas 15 were obtained from fish-rice rotational farming soil. The highest frequency of occurrence among paddy field isolates was the Bacillus spp. followed by Klebsiella pneumonia, K. oxytoca and Pseudomonas spp. Similarly the fungal species in rice field with paddy alone showed 13 species and fish rotational field with paddy showed 19 fungal species. Aspergillus sp. recorded highest frequency of occurrence among the paddy field isolates. Soil heavy-metal pollution leads to excessive

heavy metals in rice and other food crops, which has caused serious impacts on the ecological environment and on human health. In recent years, environmental friendly treatment methods that reduce the bioavailability of heavy metals in soil by soil microorganisms improving the tolerance of heavy metals in rice and reducing the transfer of heavy metals from the roots to the above-ground parts of rice have attracted much attention. This paper reviews the role and mechanism of soil microorganisms in alleviating heavy-metal stress in rice at home and abroad in recent years. At present, microorganisms tolerant to heavy metals mainly include bacteria and fungi, and their mechanisms include the adsorption of heavy metals by microorganisms, the secretion of growth-promoting substances (growth hormone, ACC deaminase, IAA), changing the physical and chemical properties of the soil and the composition of the microbial community, changing the transport mode of heavy metals in soil, the improvement of the antioxidant capacity of rice, etc. Hence, soil microorganisms have good application value and prospects in rice and other crops. However, the vast majority of current research focuses on a single strain, the screening principles of strains are limited, the pathogenicities of the strains have not been evaluated, and there are still few field experiments under natural conditions. In the future, we should strengthen the action of soil microorganisms on rice in response to the above problems in heavy metals, to better promote the microbial remediation technology.

the rhizosphere is a small soil compartment that is around the rice roots and is affected by the rice roots. In flooded paddy soils, O<sub>2</sub> is secreted through the aerenchyma of rice roots leading to oxic zones around the roots (e.g. the rhizosphere) which are surrounded by the anoxic bulk soil. The rhizoplane is the surface of the rice roots (including root hairs). The endosphere is a compartment inside the rice roots.

The abundance, diversity and composition of microbial communities, including bacterial, archaeal and fungal communities residing in the rice rhizosphere, have been widely investigated. Bacterial populations have been found to dominate the rice rhizosphere, and the abundances of rhizosphere bacterial, archaeal and fungal populations were at least twice those of the bulk soil, respectively, irrespective of rice growth stages and soil depth (Hussain et al. 2012; Lee et al. 2015; Breidenbach, Pump and Dumont 2016). This phenomenon is commonly termed the 'rhizosphere effect', which is ascribed to the rhizodeposition of plant-derived carbohydrates stimulating microbial growth and multiplication (Smalla et al. 2001). Unlike microbial abundance, both alpha diversities of bacterial and archaeal communities in the rice rhizosphere were close to, or even significantly less than in the bulk soil (Edwards et al. 2015; Breidenbach, Pump and Dumont 2016).[4,5,6]

The structures of microbial communities, especially bacterial communities, in the rice rhizosphere are, however, diverse and dynamic. They might be primarily shaped by soil- and plant-related conditions such as geographic location, soil type and rice genotype (Edwards et al. 2015; Santos-Medellin et al. 2017). Indeed, alterations in these conditions could change soil environments for microbial growth and proliferation (e.g. soil pH and root exudation patterns), and ultimately lead to variations in the rhizosphere microbial community structure. In addition, agricultural management (e.g. cultivation practice and drainage) and growth stages also exerted substantial influence on the rice rhizosphere microbiome (Breidenbach and Conrad 2015; Edwards et al. 2015; Breidenbach, Pump and Dumont 2016).

Here we focused on a number of high-throughput sequencing-based studies (detailed information regarding literature), collected taxonomic information of bacterial, archaeal and fungal communities at the genus level in the rice rhizosphere and made a comparison at the phylum level between rice and other crops/plants including maize (*Zea mays*), soybean (*Glycine max L.*), potato (*Solanum tuberosum*), *Populus* and *Arabidopsis*. These independent studies have indicated that the bacterial community in the rice rhizosphere is largely occupied by Proteobacteria (mainly Alpha-, Beta- and Deltaproteobacteria classes), Acidobacteria, Actinobacteria and Chloroflexi phyla. Not surprisingly, the bacterial community composition in the rice rhizosphere is distinct from that in the rhizosphere of other crops/plants (i.e. maize, soybean, potato, *Populus* and *Arabidopsis*) based on the selected studies. Notably, the Deltaproteobacteria class in the rice rhizosphere is markedly enriched when compared with that in the rhizosphere of other crops/plants. This could be partly because the oxic-anoxic characteristic of the rice root environment might favor the growth and functioning of multiple anaerobic microbes belonging to the Deltaproteobacteria class, for instance, *Geobacter* and *Desulfococcus* genera, which participate in ferric iron [Fe(III)] and/or sulfate reduction (Ding et al. 2015; Sun et al. 2015). Additionally, Alpha- and Betaproteobacteria classes are also abundant in the rice rhizosphere. They are essential for ecosystem functioning, including regulating cycles of carbon (C), nitrogen (N), Fe and sulfur (S) (Lee et al. 2006; Hernández et al. 2015; Sun et al. 2015), producing phytohormones that benefit plant growth (Lee et al. 2006)

and synthesizing antibiotics in response to bacterial and fungal pathogens (Raaijmakers and Mazzola 2012). Given the potential importance of Alpha-, Beta- and Deltaproteobacteria in nutrient availability, growth promotion and disease inhibition, their colonization probably makes the rhizosphere tend to be a strictly limited compartment of beneficial interactions between symbiotic microbes and rice plants. However, it should be noted that the comparison of the rhizosphere microbial community composition (including bacterial, archaeal and fungal community) between rice and the selected non-rice crops/plants might be affected by targeted regions of the 16S rRNA gene, different sequencing approaches and sequencing depths across different studies. Moreover, we are aware of the fact that the results of the microbial diversity (including bacterial, archaeal and fungal diversity) in the rhizosphere of rice and those non-rice crops/plants are limited to the selected studies. This was also the case with the microbial diversity in the other compartments of rice roots (i.e. the rhizoplane and endosphere) as discussed below. Although the selected studies might be representative, a larger fraction of relevant studies should be included in future work to obtain a comprehensive understanding of the microbial diversity of rice root-associated compartments.[6,7,8]

### III.RESULTS

Archaeal communities in the rice rhizosphere are commonly composed of Crenarchaeota, Thaumarchaeota and Euryarchaeota phyla based on the selected studies. These are markedly different from those in the rhizosphere of other crops/plants (e.g. maize, soybean, potato and Populus), which are usually dominated by the Thaumarchaeota phylum. In particular, the great enrichment of the Euryarchaeota phylum in the rice rhizosphere could be due to the fact that methanogens (e.g. Methanosarcina and Methanosaeta genera) which participate in CH<sub>4</sub> production in paddy soils are affiliated with this phyla (Conrad 2007; Lee et al. 2014). This special microbial functional group will be discussed in the next section in detail. Unlike bacterial communities, the diversity of archaeal communities in the rice rhizosphere remains relatively constant and is tolerant to environmental variations, for instance, growth season (Breidenbach, Pump and Dumont 2016), drainage (Breidenbach and Conrad 2015) and soil depth (Lee et al. 2015). Nevertheless, the activity of the rhizosphere archaeal communities might fluctuate although their diversity was relatively stable (Lee et al. 2014).

As with the bacterial and archaeal communities, fungal community composition in the rice rhizosphere greatly differs from that in the rhizosphere of soybean and Populus, particularly with the enrichment of Chytridiomycota phylum based on the selected studies. Most members affiliated with this phylum have been considered as aquatic fungi, and are ubiquitous in aquatic environments (e.g. lakes) and habitats with high moisture (e.g. ditches and pond banks) (Barr 2001), and hence would be favored by paddy soils. Several members within this phyla, for instance, Rhizophlyctis and Cladochytrium genera which have been detected in the rice rhizosphere, are effective decomposers of carbohydrates like cellulose and thus possibly contribute to the C biogeochemical cycle in paddy soils (Gleason et al. 2011; Eichorst and Kuske 2012). It has been realized that fungi play significant roles in ecosystem functioning such as phosphorus (P) solubilization and synthesis of indole acetic acid (IAA) for plant growth promotion (Mendes, Paolina and Jos 2013). In the rice rhizosphere, fungi-like members of Aspergillus genus (within Ascomycota phylum) can greatly promote P solubilization, and arbuscular mycorrhizal fungi (AMF) (e.g. Rhizophagus spp.) can facilitate P transport and uptake (Mendes, Paolina and Jos 2013; Mwajita et al. 2013). Notably, the arbuscular mycorrhizal (AM) symbiosis can contribute up to 80% to P uptake by the rice roots (Yang et al. 2012), but a high level of soil-soluble P has a negative impact on this symbiosis (Mendes, Paolina and Jos 2013). The effectiveness of AMF colonization of roots probably relies on rice genotypes and is affected by crop management practices (e.g. fertilization regime) (Diedhiou et al. 2016; Mbodj et al. 2018). Moreover, soil types and rice genotypes could impose significant effects on the diversity of overall fungal community in the rice rhizosphere; despite that a considerable fraction of fungal operational taxonomic units (OTUs) have yet to be classified using high-throughput sequencing (Santos-Medellin et al. 2017).

Assembly of the rhizosphere microbiome is, to some extent, operated by the root exudates (Backer et al. 2018; Zhalnina et al. 2018), which contain diverse organic compounds such as organic acids, amino acids, polysaccharides and other primary and/or secondary metabolites (Bais et al. 2006). The root exudates are genetically regulated and can selectively attract the rhizosphere microbes, making the rhizosphere microbiome unique and representative in different plant genotypes and species (Paterson et al. 2007; Backer et al. 2018). For allelopathic rice varieties, allelochemicals as a kind of root exudate could significantly affect the rhizosphere microbial community structure, for example, decreasing the populations of culturable microbes (including

bacteria and fungi) and total phosphor lipid fatty acid (PLFA) at an early stage of rice growth (Kong et al. 2008). Recently, Zhalnina et al. (2018) have demonstrated that rhizosphere bacteria of *Avena barbata* had substrate preferences for the root exudates which could be predicted from genome sequences, and suggested that the establishment of the rhizosphere microbial community might be determined by both chemical composition of root exudates and microbial substrate preferences. We can also use advanced meta-omics techniques (e.g. metagenomics, metatranscriptomics and metabolomics) to explore the mechanisms of how root exudates mediate interactions among rice-soil-microbiome in the future.

## MICROBIAL FUNCTIONAL GROUPS RESPONSIBLE FOR BIOGEOCHEMICAL CYCLES IN THE RICE RHIZOSPHERE

As described above, the rice rhizosphere is characterized by the oxic-anoxic interface and the presence of abundant rhizodeposition carbon, and could potentially offer a unique niche for various microbial functional groups regulating biogeochemical processes, for example, C, N and P cycling and arsenic (As) transformation. [8,9,10]

## METHANE CYCLING

Rice cultivation worldwide makes a major contribution to global greenhouse gas emissions, mainly CH<sub>4</sub>. CH<sub>4</sub> fluxes from paddy soils are an integrated result of CH<sub>4</sub> formation, oxidation and release from soil to atmosphere through the rice vascular system, which involves complex consortia comprising hydrolytic and fermentative bacteria, methanogens and methanotrophs (Conrad 2009). The release of CH<sub>4</sub> is mostly quantified via the net balance between the activities of methanogens and methanotrophs.

In paddy soils, CH<sub>4</sub> is formed by the methanogens mainly through acetotrophic or hydrogenotrophic routes, in which either methyl group of acetate or carbon dioxide (CO<sub>2</sub>) is reduced (Conrad 2007). The methanogenic archaea which belong to Euryarchaeota phylum function under anaerobic conditions are commonly thought to reside in the bulk soil of submerged paddy where it is poor in O<sub>2</sub>. Unexpectedly, extensive studies have detected their presence in the rice rhizosphere and even on the rice roots which are relatively rich in O<sub>2</sub>. One probable explanation for this surprising phenomenon could be that several methanogenic families such as Methanosarcinaceae and Methanocellaceae (formerly Rice cluster I) are possibly tolerant to O<sub>2</sub> toxicity, as they harbor diverse genes encoding enzymes involved in O<sub>2</sub> detoxification (Angel, Matthies and Conrad 2011). Another probable explanation could be that the methanogens with strict anaerobic metabolism employ a spatial strategy, dwelling where O<sub>2</sub> is absent, for instance in old root segments or anoxic sites within the rhizosphere (Lu et al. 2005; Lee et al. 2015). Intriguingly, several studies have even found that the rice rhizosphere supported higher CH<sub>4</sub> production and abundances of methanogenic archaea than the bulk soil (Lee et al. 2015; Mao, Yin and Deng 2015). This might be a result of relatively less supply of energy or organic substrates in the bulk soil without rice root exudates. It has been reported that hydrogenotrophic Methanocellales and aceticlastic Methanosarcinaceae played crucial roles in CH<sub>4</sub> formation in the rice rhizosphere, and Methanomicrobiaceae and Methanosarcinaceae were the active rhizosphere methanogens as well (Lu and Conrad 2005; Zhu et al. 2014a). The rhizosphere methanogenic community composition for rice plants has been suggested to be relatively stable under field conditions, while their abundance and activities might vary during the rice growth period (Lee et al. 2014, 2015).

CH<sub>4</sub> can be metabolized via either aerobic or anaerobic pathways in paddy soils (Reim et al. 2012; Shen et al. 2014). Considering the extremely low rate of anaerobic CH<sub>4</sub> oxidation, CH<sub>4</sub> is proposed to be oxidized mostly by the aerobic methanotrophic bacteria which are affiliated with Proteobacteria (e.g. type I and type II methanotrophs) and Verrucomicrobia phyla in paddy soils (Conrad 2007; Lee et al. 2014; Shen et al. 2014). It is estimated that a substantial proportion (~10–30%) of the CH<sub>4</sub> synthesized by the methanogenic archaea in paddy soils is oxidized by the methanotrophic bacteria related to rice roots, hence alleviating CH<sub>4</sub> emissions (Shrestha et al. 2010).

Aerobic methanotrophs are prone to inhabit the oxic-anoxic interfaces in paddy soils, e.g. the rhizosphere and soil-floodwater interface where both CH<sub>4</sub> and O<sub>2</sub> are present (Conrad 2007). In the rice rhizosphere, type I methanotrophs (e.g. *Methylobacterium*, *Methylobacteria*, *Methylosarcina* and *Methylobacterium*) possibly contribute more to aerobic CH<sub>4</sub> oxidation than type II methanotrophs (e.g. *Methylocystis*) under in

situ conditions (Qiu et al. 2008). Moreover, the composition, abundance and activities of aerobic methanotrophs in the rice rhizosphere are dynamic and expected to change depending on various environmental factors, in particular those related to O<sub>2</sub> availability (e.g. distance to the roots and depth of the rhizosphere soil) as well as rice growth stage (Eller and Frenzel 2001; Reim et al. 2012; Lee et al. 2014, 2015).

Collectively, it is evident that both methanogenic and methanotrophic communities in the rice rhizosphere determine the CH<sub>4</sub> emissions in paddy soils. Manipulating these microbial populations by means of managing the rhizosphere environments towards favoring the CH<sub>4</sub> oxidation and inhibiting the CH<sub>4</sub> production could be potentially feasible approaches for mitigation of the CH<sub>4</sub> release. Water management like midseason drainage is an efficient strategy that not only reduces CH<sub>4</sub> release but also provides the economic benefits of rice cultivation, for instance, improved yields and water saving (Li et al. 2002; Pandey et al. 2014). The penetration of O<sub>2</sub> into paddy soils resulting from drainage enables various oxidation processes, e.g. Fe(II) to Fe(III) and sulfide to sulfate. Fe(III) and sulfate benefit the growth of iron- and sulfate-reducing microbes, respectively. These microbes could outcompete the methanogens for common electron donors such as acetate and hydrogen (H<sub>2</sub>), the two most key methanogenic substrates, and thus inhibit the CH<sub>4</sub> production (Achtlich, Bak and Conrad 1995). The same effect is achieved by direct addition of Fe(III) (e.g. ferrihydrite) or sulfate (e.g. ammonium sulfate fertilizers) in paddy soils (Conrad 2002; Jäkel, Russo and Schnell 2005). As was the case with Fe(III) or sulfate, nitrate (NO<sub>3</sub><sup>-</sup>) could also suppress the CH<sub>4</sub> production (Chidthaisong and Conrad 2000). In rice agriculture, N fertilizers are usually applied in the form of ammonium (NH<sub>3</sub>) or urea. They can be rapidly converted into NO<sub>3</sub><sup>-</sup> in the rice rhizosphere (Arth, Frenzel and Conrad 1998), and, consequently, deep incorporation of N fertilizers could also result in the inhibition of CH<sub>4</sub> production in rooted paddy soils (Bodelier et al. 2000).[10,11,12]

## NITROGEN LOSS

The amount of N fertilizers applied to paddy ecosystems has been increasing over recent decades, particularly in China (Ding et al. 2014). Nonetheless, the uptake efficiency of N fertilizers by rice roots is rather poor (usually <40%) due to various processes involved in N loss, in particular denitrification (Cassman et al. 1996). The rice rhizosphere with the oxic-anoxic interface could offer a favorable microhabitat for coupled nitrification-denitrification (Arth, Frenzel and Conrad 1998; Wei et al. 2017), and thus is likely to be a hotspot of N loss from paddy soils. Within the rice rhizosphere, O<sub>2</sub> released from the roots supports the oxidation of NH<sub>3</sub> to NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> via nitrification regulated by aerobic nitrifiers. The produced NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> can diffuse to the surrounding anoxic sites and then is stepwise reduced to nitric oxide (NO), nitrous oxide (N<sub>2</sub>O) and dinitrogen gas (N<sub>2</sub>) via denitrification regulated by heterotrophic denitrifiers. The occurrence of nitrification and denitrification has been detected at a distance of <2 and 1.5–5.0 mm from the root surface of rice, respectively (Arth and Frenzel 2000).

The aerobic oxidation of NH<sub>3</sub> to NO<sub>2</sub><sup>-</sup> is a key step in the nitrification which is mediated by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) (Chen et al. 2010a). These ammonia-oxidizing prokaryotes targeted by amoA gene (encoding ammonia monooxygenase) have been identified as affiliated with Thaumarchaeota phylum for AOA, and primarily with Nitrosospora and Nitrosomonas of Betaproteobacteria for AOB in the rice rhizosphere (Chen et al. 2008b; Wang et al. 2009; Hussain et al. 2011; Li et al. 2018). The community of AOB in the rice rhizosphere has been shown to be highly responsive to rice genotypes and environmental variations such as soil depth, rice growth stages and N fertilization regime, while that of AOA remained relatively stable (Briones et al. 2002; Wang et al. 2009; Hussain et al. 2011). Moreover, it has been reported that soil pH was significantly positively correlated with the abundance of AOB, but not with that of AOA in various soils (Chen et al. 2011; Yao et al. 2011). Considering the root-mediated pH changes in the rice rhizosphere (Begg et al. 1994), soil pH is also a potentially important factor influencing the community size of rhizosphere AOB. Additionally, a few studies have explored the relative prevalence of AOA and AOB in the rice rhizosphere, and suggested that AOA contributed more to the aerobic NH<sub>3</sub> oxidation than AOB because of greater archaeal amoA abundances (Chen et al. 2008b; Hussain et al. 2011), whereas the community size of AOA/AOB represented by their abundances may not be directly linked with either functional activity of AOA/AOB or nitrification activity under field conditions. Using <sup>13</sup>C<sub>2</sub>O<sub>2</sub>-based stable isotope probing (SIP) of DNA/RNA in combination with functional gene analysis, AOA have been proven to drive the autotrophic nitrification in acid paddy soils, while AOB are the sole players in alkaline paddy soils (Jiang et al. 2015).

The reduction of  $\text{NO}_2^-$  to  $\text{NO}$  is a crucial step in the denitrification and is mediated by denitrifying bacteria harboring  $\text{NO}_2^-$  reductase genes *nirK* and *nirS* (Chen et al. 2010b). Unlike ammonia-oxidizing prokaryotes which have been only assigned to several genera, the *nirK*- and *nirS*-harboring denitrifying community is mainly consisted of taxonomically and phylogenetically diverse bacterial populations (Ishii et al. 2011). Denitrifiers containing *NirK* related to those of known Rhizobiales, and *NirS* related to those of known Burkholderiales and Rhodocyclales, have been identified from paddy soils (Yoshida et al. 2009; 2010). Intriguingly, *nirK*-harboring denitrifying community composition in the rice rhizosphere was largely dominated by unclassified taxa from the soil source (Hussain et al. 2011). Furthermore, it has been reported that the *nirK*-containing denitrifying community prevailed over the ammonia oxidizers (based on *amoA* gene) irrespective of rice growth stage, implying that denitrifiers were more adaptive to rhizosphere (Hussain et al. 2011). As with AOB, the rhizosphere *nirK*-harboring denitrifying community structure has been found to vary during rice-growing stages.  $\text{O}_2$  availability and carbohydrates derived from roots are the keys to shaping the rhizosphere ammonia-oxidizing and denitrifying guilds for rice plants, and thus influence the potential of nitrification and denitrification processes (Hussain et al. 2011; Chen et al. 2014). However, how these two distinct microbial functional groups interact with each other is not clear yet, and warrants more investigation.

In addition to coupled nitrification-denitrification, anaerobic ammonia oxidation (termed anammox), which is regulated by bacteria belonging to the order Brocadiales within the phylum Planctomycetes (Jetten et al. 2010), is also related to the N loss from diverse environments with  $\text{N}_2$  as the end product (Wang and Gu 2013; Nie et al. 2015; Zhou, Zhang and Wen 2017). Rhizosphere anammox bacteria in various ecosystems have been found to be affected by root exudates (Li et al. 2016), plant species (Chu et al. 2015; Zhou, Zhang and Wen 2017), phenological progression (Zheng et al. 2016), exogenous N (Pandey et al. 2018) and ecological niches (Wang and Gu 2013). The abundance, composition and diversity of anammox bacterial community in the rhizosphere strongly vary across different ecosystems. This may be attributed to the differences of specific environment established by different plant species (e.g.  $\text{O}_2$  diffusion, rhizodeposition and nutrient uptake) (Lee 2003; Richardson et al. 2009).

The occurrence of the anammox process in the rice rhizosphere has been demonstrated recently using the  $^{15}\text{N}$ -based isotope tracer technique combined with fluorescence microscopy (Nie et al. 2015). Not surprisingly, the rice rhizosphere harbored significantly higher anammox activities and anammox bacterial abundances than the bulk soil (Nie et al. 2015; Li et al. 2016). This positive effect could be explained by the transiently stimulated  $\text{NO}_2^-$  produced by nitrification as well as denitrification. As anammox is supported by the co-occurrence of oxidized ( $\text{NH}_4^+$ ) and reduced N ( $\text{NO}_2^-$ ) compounds, the rice rhizosphere, which can provide the oxic-anoxic interface, may supply an adequate habitat for anammox bacteria. It is noteworthy that although the anammox bacteria (e.g. *Candidatus Brocadia* genus) commonly grow under anoxic conditions (Strous et al. 1997), their presence and the anammox activity have been detected in the oxic regions of paddy soils like the rice rhizosphere (Nie et al. 2015) and surface soil layer (Bai et al. 2015). These imply that the anammox bacteria might be tolerant to  $\text{O}_2$  as with the methanogens discussed above (Kalvelage et al. 2011; Oshiki et al. 2011). The anammox bacterial community has been enriched from the upper layer of a paddy soil (Hu et al. 2013), while not yet from the rice rhizosphere. Still, more specific research on the microbial diversity of anammox process in the rice rhizosphere is required for a thorough understanding of N loss in terrestrial ecosystems. [11,12,13]

## PHOSPHORUS DISSOLUTION

Since alternating redox cycles have pronounced impacts on the weathering and transformation of mineral P in paddy soils, the role of microorganisms in soil P availability has been underestimated. However, the presence of phosphate-solubilizing microorganisms (PSMs) and the phosphatase activity in the rice rhizosphere indicate a potential role of PSMs for rice yields (Raghu and MacRae 1966; Ge et al. 2017b). The increase of available P in the rice rhizosphere has been found to be correlated with the decrease of soil pH (Jianguo and Shuman 1991), which might be, to some extent, induced by the production of organic (gluconic, oxalic and citric) acids and protons via the PSMs' metabolism (Uroz et al. 2009; Khan, Zaidi and Ahmad 2014). Specially, diverse phosphate-solubilizing bacteria (e.g. *Bacillus*, *Micromonospora*, *Streptomyces* and *Rhizobium* spp.) and fungi (e.g. *Aspergillus* and *Penicillium* spp.), which can synthesize various organic acids (Panhwar et al. 2012; Khan, Zaidi and Ahmad 2014), have been isolated from the rice rhizosphere (Sharma et al. 2013).



It has been reported that the release of P by PSMs in the rice rhizosphere varied from 22 to 826  $\mu\text{g P mL}^{-1}$  based on tricalcium phosphate solubilizing analysis (Prasanna et al. 2011). In comparison with PSMs isolated from other ecosystems, strains isolated from the rice rhizosphere have been shown to have a higher ferric phosphate solubilizing ability (Chung et al. 2005). However, the activity of PSMs in the rice rhizosphere was not only affected by rice growth season and air temperature (Ge et al. 2017b) but also soil P availability (Wei et al. 2017; Long et al. 2018). To date, the P-solubilizing genes encoding pyrroloquinoline quinone (pqqC), alkaline phosphatase (phoD), glucose dehydrogenase (gcd) and acid phosphatase (appA) have been widely applied to identify PSMs in different ecosystems (Golovan et al. 2000; Huang et al. 2009; Sashidhar and Podile 2010; Meyer et al. 2011).

#### ARSENIC TRANSFORMATION

Arsenic (As) is toxic and broadly distributed in various environments (Nordstrom 2002). Rice is efficient in taking up As from soil, and transporting As into rice grains (Williams et al. 2007, Duan et al. 2013, 2016; Zhu et al. 2014b), and rice consumption has been a major route of dietary As intake for people who live on rice (Li et al. 2011). Therefore, it is important to understand how As is transported and transformed in the soil-rice system. Diverse microbes in the rice rhizosphere can mediate a variety of As biotransformation processes, such as reduction, oxidation, methylation and de-methylation of As in the soil-rice system, and As accumulation and speciation in rice grains (Yoshinaga and Rosen 2014; Zhu et al. 2014b; Zhang et al. 2015). For instance, iron- and sulphate-reducing bacteria have been found to participate in arsenate release from soil minerals and arsenate reduction to arsenite, which can enhance As mobility in paddy soils (Wang et al. 2017; Qiao, Li and Li 2018; Yang et al. 2018). Furthermore, As methylation plays significant roles in regulating As accumulation and speciation in rice grains. Since rice plants do not have the ability to methylate As, dimethylarsinous acid (DMA) in rice grains is most likely produced by microbes inhabiting the bulk soil and rice rhizosphere, and then being absorbed by the rice roots (Jia et al. 2013). Arsenite methyltransferase (ArsM), methylating arsenite to organic As species, such as monomethylarsonous acid (MMA), DMA or volatile trimethylarsine (TMA), is broadly distributed in paddy soils (Ye et al. 2012). ArsM has been identified and characterized from several microorganisms, such as *Methanosarcina* spp. and a bacterial strain capable of sulfate reduction (Wang, Sun and Zhu 2014; Wang, Bao and Sun 2015). Huang et al. (2016) have also isolated SM-1 (belonging to Cytophagaceae family) from paddy soils, which showed high As methylation activity.

In addition to direct function in As biotransformation, microbes in the rice rhizosphere can also indirectly influence As mobility in soil and As accumulation in rice grains through multiple processes related to cycles of C, N and Fe (Duan et al. 2013; Zhu et al. 2017a). For example, during microbial Fe(III) reduction, As absorbed by Fe(III) minerals can be released, enhancing As mobility in soil (Wang et al. 2017; Qiao, Li and Li 2018; Yang et al. 2018). On the contrary, during the denitrification process mediated by denitrifying bacteria, arsenite is oxidized to arsenate, immobilizing As in soil (Chen et al. 2008a). Hence, simultaneous application of Fe(II) and  $\text{NO}_3^-$  to contaminated paddy soils could favor the growth of nitrate-reducing Fe(II) oxidizers, and eventually reduce As accumulation in rice plants (Wang et al. 2018). Therefore, it is clear that microbes in the rice rhizosphere play pivotal roles in controlling As's fate in the soil-rice system, and harnessing microbiota in the rice rhizosphere could be a potential bioremediation strategy for mitigating health risks resulted from As contamination of paddy soils.[13,14,15]

#### RICE RHIZOPLANE MICROBIOME

The rhizoplane is the root surface where the host plants are in direct contact with the rhizosphere soils (Sasse, Martinoia and Northen 2018). The separation distance between the rhizoplane and rhizosphere is extremely close and thus both zones are commonly regarded as a continuum (Johri, Sharma and Viridi 2003). At the rhizoplane level, roots actively interact with the rhizosphere soils through releasing substrates and competing for nutrients, and hence, the rhizoplane is anticipated to be a compartment that drives nutrient exchange and transformation in the soil-plant systems. Moreover, the rhizoplane might act as a selective barrier in the establishment of plant root microbiome, as only a proportion of microbes in the rhizosphere could attach to the rhizoplane, and then a subset of them could invade and thrive in the endosphere (Edwards et al. 2015). It is evident that this model of root microbiome establishment is far more complicated than had been thought when taking the rhizoplane microbiome into account.

The knowledge of microbial diversity on the rhizoplane could shed light on the interactions within the soil-rice system. Generally, bacteria are the major population colonizing the rice rhizoplane, and can exert positive, negative or neutral influences on plant growth (Kimura, Murakami and Wada 1989; Giongo et al. 2010). *Bacillus* (e.g. *Bacillus velezensis*) and *Pseudomonas* genera (e.g. *Pseudomonas fluorescens*) have been reported to be typical rhizoplane inhabitants for rice plants on the basis of culture-dependent studies (Asanuma, Tanaka and Yatazawa 1979; Vlassak et al. 1992; Hwangbo et al. 2016). *B. velezensis* is capable of phosphate solubilization as it possesses genes encoding phosphorus-solubilizing proteins (Hwangbo et al. 2016). *P. fluorescens* has high antifungal activity due to the production of cyanide or siderophore, and is a putative antagonistic bacterium for the biocontrol of fungal pathogens of roots (Vlassak et al. 1992). Therefore, colonization of these bacterial populations on the rice rhizoplane could favor the growth of rice plants through improving P availability and antagonism of soil-borne phytopathogens. Additionally, microbes involved in the  $N_2$  fixation (e.g. *Azospirillum* spp.) and nitrification (e.g. *Nitrobacter* and *Nitrosospira* spp.) have also been isolated from the rice rhizoplane (Tomiya et al. 2001; Banik, Mukhopadhyaya and Dangar 2016), further indicating the potentially positive effects of rhizoplane microbes on the rice growth. It has been suggested that the rhizoplane microbes are promising for application as bio-fertilizers in paddy ecosystems (Etesami and Alikhani 2016).

Because of the limitation in isolating microorganisms through conventional cultivation methods, the application of metaproteomic and metagenomic techniques with higher resolution and broader coverage to investigate the rice rhizoplane microbiome has recently become of great interest. We have chosen several studies employing high-throughput sequencing and collected taxonomic information of bacterial and archaeal communities at the genus level on the rice rhizoplane. Proteins of Alpha-, Beta- and Deltaproteobacteria phyla have been found to be predominant groups on the rice rhizoplane using metaproteomic analyses (Knief et al. 2012). These proteins were assigned to multiple genera responsible for the biogeochemical cycles, like *Azospirillum* and *Bradyrhizobium* for  $N_2$  fixation, *Methylosinus* for  $CH_4$  oxidation, and *Anaeromyxobacter* and *Geobacter* for Fe(III) reduction (Knief et al. 2012). These findings are partly consistent with those culture-dependent studies described above. Furthermore, several bacterial taxa within Fibrobacteres and Spirochaetes phyla which are responsible for cellulose degradation have also been observed on the rice rhizoplane (Edwards et al. 2015). Plant species and agricultural practice (e.g. crop rotation and water management) have been shown to contribute to variations in the diversity, abundance and composition of rice rhizoplane microbiome (Breidenbach et al. 2017; Zecchin et al. 2017). However, the decisive factor of shaping the rice rhizoplane microbiome is still unclear and requires further investigation.[15,16,17]

## ENDOPHYTIC MICROBIOME OF RICE ROOTS

Endophytic microbiome is referred to as those microorganisms found inside surface-sterilized plant tissue or retrieved from plant interior (Hallmann et al. 1998; Mano and Morisaki 2008). The endophytes normally have no visibly detrimental effect on their host plants, and some of them are beneficial to their hosts. It has been well demonstrated that microbes can internally colonize plant roots to become the endophytes via either vertical or horizontal transmission routes. On the one hand, microbes, which colonized the seeds of the mother-generation previously, may vertically transmit as the endophytes when seeds are germinating (Kaga et al. 2009; Hardoim et al. 2012); on the other hand, the horizontal transmission can take place in the rhizosphere (Frank, Saldierna and Shay 2017). The rhizosphere microbes have the ability to colonize the roots via producing cell wall-degrading enzymes like cellulase and pectinase; or they are likely to accidentally enter through the root wounds or the emerging lateral roots (Hardoim et al. 2015).

Numerous studies have uncovered a rather high diversity of rice endophytic microbiome, and the taxonomic information of the bacterial, archaeal and fungal communities at the genus level in the rice endosphere has been listed in For bacteria, Gammaproteobacteria, Firmicutes, Actinobacteria and Bacteroidetes phyla are common inhabitants in the rice endosphere (Reinhold-Hurek and Hurek 2011; Sessitsch et al. 2012), especially Enterobacteriaceae and Pseudomonadaceae families within the Gammaproteobacteria phylum, and *Bacillus* genus within the Firmicutes phylum (Hallmann et al. 1997; Hardoim, van Overbeek and van Elsas 2008). Additionally, a few taxa that typically reside in soil, such as Fusobacteria, Planctomycetes and Verrucomicrobia phyla, unexpectedly have been found inside the rice roots using metagenomic analysis (Sessitsch et al. 2012). Edwards et al. (2015) have explored three different, but overlapping microbiome in the rice rhizosphere, rhizoplane and endosphere, and uncovered that although the

alpha-diversity sharply reduced from the rhizosphere to endosphere, the latter still harbored a considerable diverse microbial community. This phenomenon has also been observed in other plants like Lotus japonicus and poplar trees (Zgadzaj et al. 2016; Beckers et al. 2017). Besides bacteria, fungi and archaea are also widely distributed inside the rice roots. Ascomycota phylum is the most prominent fungal population in the rice endosphere, particularly with Aspergillus, Fusarium, Penicillium and Trichoderma genera (Potshangbam et al. 2017; Santos-Medellin et al. 2017). Methanogenic archaea like Methanospirillum and Methanobacterium genera have also been frequently detected inside the rice roots (Sun et al. 2008; Edwards et al. 2015). However, the size of fungal and archaeal communities is much smaller than that of bacterial community in the rice endosphere (Santos-Medellin et al. 2017). The rice endophytic community structure might be indirectly affected by some abiotic factors like precipitation and soil type (Hallmann et al. 1997) although the endophytes have been previously thought to inhabit a relatively stable and protected environment in internal plant tissues (Krause et al. 2006). For example, precipitation might indirectly influence the endosphere microbial diversity in upland rice ecosystems via directly influencing root growth and the rhizosphere microbiome (Crusciol et al. 2013), as most endophytes are thought to be a sub-population of the rhizosphere microbiome (Compant, Clément and Sessitsch 2010).

Besides diverse microbial populations, the rice endophytic microbiome also harbors many transcriptional regulators like LysR-, AraC-, Crp- and IclR families (Sessitsch et al. 2012). These transcriptional regulators are related with diverse physiological responses, such as glycometabolism and the metabolism of amino acids, virulence and quorum sensing (Hardoim et al. 2015), and could respond to environmental changes and improve endophytic cellular homeostasis (Balleza et al. 2009; Sessitsch et al. 2012). For instance, the endophytes would encounter O<sub>2</sub> limitation inside the rice roots, and they could employ various metabolic pathways to adapt to different redox niches, such as overexpression of OxyR (a member of the LysR family of transcriptional regulators) in response to a hypoxia environment (Sarkar et al. 2017). Also, the endophytes are able to utilize plant-derived secondary metabolites to adapt and survive. Some rice endophytes can produce storage compounds like polyhydroxybutyrate (PHB), which may be able to remove the growth inhibitory metabolites (e.g. plant secondary metabolites) (Aneja et al. 2004). Other endophytes possess genes responsible for the degradation of multiple aromatic compounds synthesized from plants' secondary metabolism (Sessitsch et al. 2012). These metabolic adaptations of rice endophytes may have advantageous effects on their hosts in return (Mano and Morisaki 2008).

Diverse root endophytes have been revealed to promote rice growth, primarily through the synthesis of phytohormones (e.g. IAA and cytokinin) and siderophores (Sessitsch et al. 2012). Moreover, some root endophytes can indirectly benefit rice plants through pathogenic resistance. For example, it has been found that the members of Pseudomonas and Bacillus genera could stimulate induced systemic resistance (ISR), thus increasing the resistance of rice plants to pathogens (Hardoim 2015). The rice endophytes may also protect their hosts against N starvation. Sessitsch et al. (2012) have found that the microbial N cycle was almost entirely represented in the endophytes colonizing the rice roots by metagenomic analysis. They further suggested that the rice root endophytes have important impacts on N use efficiency and plant growth, although the rhizobacteria have been regarded as a major contributor to nitrification and aerobic ammonia oxidation so far (Li, Fan and Shen 2008). Furthermore, several rice root endophytes are capable of fixing N<sub>2</sub> (e.g. Azospirillum, Herbaspirillum, Bradyrhizobium and Sphingomonas spp.) with the presence of nifH gene encoding nitrogenase (Mano and Morisaki 2008; Hardoim 2015).[16,17,18]

#### IV. CONCLUSION

Overall, the microbiomes inhabiting rice root-related compartments, especially rhizosphere, are taxonomically and phylogenetically diverse, and shaped primarily by soil- (e.g. soil type) and plant-related conditions (e.g. rice genotype). Furthermore, the uniqueness of rice rhizosphere with the oxic-anoxic interface makes the rhizosphere microbial community structure distinct from that of the selected non-rice crops/plants. Although the rice root-related microbiomes are diverse, they all share similar beneficial functions to rice plants, such as mediating nutrient availability, producing phytohormones for plant growth promotion, and synthesizing antibiotics for phytopathogen inhibition, and ultimately determine the functioning of paddy ecosystems and improve yields. This reveals microbial redundant functions with possible distinct players in each root compartment. We are only at the beginning of understanding the complexity of rice root microbiome. With the advent of molecular and genomic tools, it is now possible to explore the diversity of rice root microbiome, but how to link microbial

diversity to functions remains a major challenge. We recommend that in the future attention should be paid to the following issues:

- Taking a holistic approach to investigating the diversity of microbes in the entire root microbiome (rhizosphere, rhizoplane and endosphere), thus to better understand the connectivity and interactions of the microbiomes within the system;
- Linking the microbial diversity to the functions through multi-‘omics’ approaches, such as metabolomics and proteomics as well as in situ measurement of biogeochemical fluxes;
- Using single-cell techniques to elucidate the complexity and novel functions of root microbiome at micro-scale, and also to explore the largely uncultivable microorganisms from the root system;
- Unraveling the spatial distribution at micro-scale and the evolution of root microbiomes and identifying the key driving factors, with the aim of harnessing the root microbiome for better productivity and environmental quality.[19]

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