The impact of the rice production system (irrigated vs lowland) on root-associated microbiome from farmer's fields in western Burkina Faso

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Abstract

Due to their potential applications for food safety, there is a growing interest in rice root-associated microbial communities, but some systems remain understudied. Here, we compare the assemblage of root-associated microbiota in rice sampled in 19 small farmer's fields from irrigated and rainfed lowlands in Burkina Faso, using an amplicon metabarcoding approach of the 16S rRNA gene (prokaryotes, three plant sample per field) and ITS (fungi, one sample per field). In addition to the expected structure by root compartments (root vs. rhizosphere) and geographical zones, we showed that the rice production system is a major driver of microbiome structure. In irrigated systems, we found a higher diversity of prokaryotic communities from the rhizosphere and more complex co-occurrence networks, compared to rainfed lowlands, while fungal communities exhibited an opposite pattern (higher richness in rainfed lowlands). Core taxa were different between the two systems, and indicator species were identified: mostly within *Bacillaceae* in rainfed lowlands, and within *Burkholderiaceae* and *Moraxellaceae* in irrigated areas. Finally, a higher abundance in rainfed lowlands was found for mycorrhizal fungi (both compartments) and rhizobia (rhizosphere only). Our results highlight deep microbiome differences induced by contrasted rice production systems that should consequently be considered for microbial engineering applications.

Key words

Irrigated rice; Metabarcoding; *Oryza sativa*; Rainfed lowlands; Rice production system; Root associated micro-organisms; West Africa.

Introduction

Soil and rhizosphere host megadiverse and dynamic communities of microorganisms that are crucial to the plants they associate with. Their role is particularly recognized for crops as the below-ground microbiota supply plants with nutrients and provide protection against pathogens (Singh *et al.* 2020; Chialva *et al.* 2021). Recent research suggests that root-associated microbes can improve plant tolerance to environmental stressors (Chialva *et al.* 2020), and modify phenology (Lu *et al.* 2018) and morphological traits (Senthil Kumar *et al.* 2018). Cultivated plants and their associated microbial communities are thus increasingly studied jointly, as holobionts (a concept reviewed by Vandenkoornhuyse *et al.* in 2015), because a deeper understanding of their interaction might help to develop microbial engineering applications for modern sustainable agricultural systems (Chialva *et al.* 2021). While much progress has been made, and despite the growing interest in crop microbiome, the mechanisms that control root-associated microbiome assembly (i.e., structure, composition and dynamics) remain difficult to disentangle (Brunel *et al.* 2020).

Rice is the most important food crop in the world, grown in variable climatic conditions and representing the staple food of more than half of the world's population, mostly in Asia, Africa and Latin America (Pandey *et al.* 2010). The major species cultivated worldwide is *Oryza sativa L.* (known as 'Asian rice'). This is also true in Africa, where a second rice species was domesticated (*O. glaberrima,* referred to as 'African rice'), but is much less grown because of lower yield (Linares, 2002). Given its importance for food security, and the impact of microbiota on plant productivity, metabarcoding approaches are more and more used to describe the microbiome of *O. sativa* (Kim & Lee, 2020), particularly its root-associated microbial communities (reviewed by Ding *et al*. 2019). Rice has the particularity to be cultivated in flooded paddy soils over most growth stages, so that the rhizosphere is located in an oxic-anoxic interface (Ding *et al.* 2019). Compared to other crops, rice root microbial communities are particularly enriched in *Deltaproteobacteria*, *Euryarchaeota*, *Chytridiomycota* (Ding *et al.* 2019). Communities' structuring is driven both by the host plant (in terms of root compartment / microhabitats, plant genotype) and its environment (geographical zone, bioclimate, soil properties, agricultural practices; Ding *et al.* 2019). The host genotype has been shown to structure rice associated microbial communities (Edwards *et al.* 2015; Alonso *et al.* 2020), but its influence is generally weak compared to environmental factors (Edwards *et al.* 2018; Guo *et al.* 2021). For instance, Edwards et al. (2015) evidenced

important effects of pedoclimate and agricultural practices. Other environmental factors, such as drought stress (Santos-Medellín *et al.* 2017), water management (Chialva *et al.* 2020), phosphorus (Long & Yao, 2020), were also shown to affect rice root-associated microbiota. However, the rice microbiome has been poorly explored in the African context in spite of **(1)** the importance to document the soil diversity as shaped by cultural practices, and **(2)** the growing importance of rice in Africa (Soullier *et al.* 2020). An exception is the recent work by Kanasugi *et al.* (2020) that evidenced an effect of the region in structuring of rice microbiome described in six tropic savanna regions in Ghana. More generally, there is a lack of knowledge concerning crop-associated microbiota in the African continent, that results in a biased view of the microbial world associated with crops due to an unbalanced worldwide sampling repartition (Brunel *et al.* 2020; Hughes *et al.* 2021).

Rice is grown around the world in three dominant growing systems (Rao *et al.* 2017). First, irrigated lowlands, with full water control, produce 75% of the global rice production. Second, rainfed lowlands (including flood prone), represents around 19% of the world's rice production. Finally, rainfed upland rice, only possible under high rainfall, results in 4% of the global total rice production. In Burkina Faso, irrigated rice represents small areas (costly infrastructures representing less than 30% of harvested areas; CountrySTAT, 2020), but produces more than half of the production, because of a relatively high productivity, up to 4 to 7 t/ha per year (MAHRH, 2011). Because of lower yields (for example, only 2 t/ha per year was estimated in Dano, Serpantié *et al.* 2019), rainfed lowlands that cover a wider area (ca. 70%), represent only 42% of the production (MAHRH, 2011). The full water control is the typical feature that distinguishes irrigated areas (IR) and rainfed lowlands (RL). In addition, different agricultural practices characterize each system in West Africa (see Nonvide *et al.* 2018 in Benin). In western Burkina Faso, these contrasted practices have been documented with three main differences: i) the possibility to grow rice twice a year is restricted to irrigated areas, ii) mineral fertilization is more frequent in irrigated areas, and iii) direct sowing is performed only in rainfed lowlands (Barro *et al.* 2021a). The effect of these two contrasted production systems on rice microbiota have never been investigated.

This study aims at describing rice root-associated microbial communities in farmer's fields from western Burkina Faso. More specifically, we investigate whether rice roots from two contrasted rice growing systems (irrigated and rainfed lowland areas) host different microbial communities. Our study focused on prokaryote communities, investigated through 16S rRNA

sequencing of three samples per analyzed field, and we also characterized fungal communities by ITS sequencing, with a lower sample size (one sample per field, see methods). We collected rice root and rhizosphere samples in farmer's fields from three geographical zones, each consisting of an irrigated rice-growing area and neighboring rainfed lowlands. A previous study performed in the same study fields documented more intensive agricultural practices in irrigated areas, compared to rainfed lowlands (e.g. mineral fertilization, two rice cropping per year; Barro *et al.* 2021a). Considering the effect of intensification on belowground biodiversity and microbial network complexity (Banerjee *et al.* 2019; Tamburini *et al.* 2020), we hypothesize an effect of the rice growing system on root-associated microbial communities. If true, this may have consequences on plant health (Wei *et al.* 2015), particularly in this system where rice diseases were shown to circulate at higher levels in irrigated areas compared to rainfed lowlands (Barro *et al.* 2021a).

Material and methods

Study sites in western Burkina Faso

The study sites are located in three geographic zones in western Burkina Faso, with maximum distance between each zone about 90 kilometers (Fig. 1a). Each zone comprises one irrigated area and the neighboring rainfed lowland, with maximum distance between the two rice growing systems of each zone being 7 kilometers (Fig. 1b). The climate consequently do not differ between rice growing systems within each zone, but average precipitation during the rice growing season (July to early December) differ between the three geographical zones (WorldClim 2 data; Fick & Hijmans, 2017; see Fig. S1). The three geographical zones were not studied for themselves, but instead considered as replicates to investigate how rice growing systems affect microbial communities in general. These six sites were studied from 2016 to 2019, with the characterization of agricultural practices and the follow-up of major rice diseases symptoms (Barro *et al.* 2021a; see further details on the methodology and raw data at: https://doi.org/10.23708/8FDWIE).

Rice root sampling

Within the six sites, we investigated a total of 19 fields, with three fields per site, except in Bama, the largest irrigated perimeter studied, where four fields were sampled (Fig. 1b). Rice

genotyping data are available for these 19 fields, with as much as 18 different rice genotypes (only two fields with the same rice genotype), illustrating the high rice genetic diversity in the area (see Barro *et al.* 2021b).

Each field studied corresponds to a square of approximately 25 meters on a side. Root and rhizosphere sampling was performed at rice maturation, between October, 16th and December 3rd 2018. We chose this developmental stage based on a previous study showing that the rice microbiome composition evolves during the growing season until a 'mature' microbiome at the flowering stage (Edwards *et al.* 2018). Within each field, we sampled three plants located on the square diagonal, with at least 5 meters distance. These three plants per field were also inspected visually and specific symptoms of the major rice diseases were reported (as in Barro et al 2021a). Sampling was performed with gloves and scissors (ethanol sanitized between two sampling) and involved nodal, bases and seminal roots (all sampled together). Roots were roughly shaken to remove non-adherent soil, and placed in 50 mL sterile tubes containing sterile Phosphate Buffered Saline (PBS) solution for a rapid (15s) rinse and then stored in another 50 mL sterile PBS-containing tube. We placed the tubes in a cooler and then at 4°C when back to the laboratory, on the same day.

Soil physicochemical properties: data acquisition and analysis

The three geographical zones studied (Fig. 1a) are characterized by Lixisols (soils with subsurface accumulation of low activity clays and high base saturation) according to the Harmonized Word Soil Database (HWSD) map (FAO/IIASA/ISRIC/ISSCAS/JRC, 2012).

Soil sampling was performed on the same day as rice root sampling, and in three locations nearby sampled plants, using a 10 cm depth auger. Back from the field, sampled soil was dried in the shade at room temperature and stored until analysis.

INERA/GRN service performed the analyses of soil samples according to a standardized methodology. Briefly, soil physical properties were assessed by soil particle size distribution following Bouyoucos (1962), pH was estimated according to AFNOR (1981), total organic carbon with the Walkley & Black (1934)'s method, total concentrations of nitrogen with Kjeldhal method Hillebrand *et al.* (1953), and finally phosphorus and potassium content as described respectively in Novozansky *et al.* (1983) and Walinga *et al.* (1989). Then, cation exchange capacity (CEC), a measure of fertility, nutrient retention capacity, and the capacity

to protect groundwater from cation contamination, was estimated, as well as sorptive bioaccessibility extraction (SBE), that relates to the environmental mobility, partitioning and toxicity of soil pollutants, following Metson (1956). Soil data are publicly available on the IRD Dataverse: https://doi.org/10.23708/LZ8A5B.

Rice root conditioning, DNA extraction and sequencing

Less than 24h after sampling, root samples (rice roots including rhizosphere) stored at 4°C were processed. In order to separate the different root compartments, the tubes were vortexed vigorously one minute and then, roots were removed from the PBS solution using sterile forceps. The remaining PBS solution was considered as the 'rhizosphere' compartment. Roots were then surface-sterilized with 70% alcohol (30s), 1% bleach (30s) and finally rinsed three times in sterile water. We considered these surface-sterilized roots as the 'root' compartment, which comprises both endosphere microorganisms as well as persistent DNA from the rhizoplane. DNA extraction from the rhizosphere and root samples were performed on the same day as the process of compartment separation.

For DNA extractions, 0.25g of root samples (crushed in liquid nitrogen beforehand) and 0.25g of rhizosphere were extracted using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany), following manufacturer's recommendations. DNA quality and quantity was verified using a NanoDrop ND-1000 spectrophotometer. PCR amplification, library and MiSeq Illumina sequencing were performed by Macrogen (Seoul, South Korea) using primers 341F (16S V3F, 5'-CCTACGGGNGGCWGCAG-3') and 785R (16S V4R, 5'-GACTACHVGGGTATCTAATCC-3') to amplify the V3 -V4 regions of the 16S rRNA gene (Thijs et al., 2017), and using primers ITS1f: CTTGGTCATTTAGAGGAAGTAA and ITS2: GCTGCGTTCTTCATCGATGC to amplify the internal transcribed spacer 1 (ITS1) region (Op De Beeck et al., 2014). We specifically focused on the factors structuring prokaryotic communities (16S rRNA gene sequencing), but were also interested to see whether similar tendencies hold for fungal communities (ITS sequencing). We consequently chose the following approach: sequencing was performed for each sampled plant (19 fields * 3 plants = 57 samples per compartment) for 16S rRNA gene sequencing and for a composite sample (3 plant samples were pooled to result in one sample per field, so that the total number of samples per compartment is 19) for the ITS marker. Negative controls (three for 16S rRNA gene and one for ITS) were sequenced to remove potential contaminants.

Sequence data are retrievable from NCBI (National Center for Biotechnology Information) under the Bioproject ID: PRJNA763095.

Bioinformatic analyses of obtained sequences

All bioinformatics and statistical analyses were performed in R software v 3.6.3 (R core Team, 2018) and the package *ggplot2* (Wickham, 2016) was used for the visualization.

Raw sequences were processed using a custom script from the *dada2* pipeline, which is designed to resolve exact biological sequences (ASVs for Amplicon Sequence Variants) from Illumina sequence data without sequence clustering (Callahan *et al.* 2016). Raw sequences were first demultiplexed by comparing index reads with a key. Primers and adapters were screened and removed using a custom script with *cutadapt* (Martin, 2011). Paired sequences were trimmed. Sequences were dereplicated, and the unique sequence pairs were denoised using the *dada* function. Next, paired-end sequences were merged, and chimeras were removed. Taxonomy assignments were determined against the SILVA SSU r138 (Quast *et al.* 2013) and the UNITE 2021 (Abarenkov *et al.* 2021) taxonomic databases for 16S rRNA gene and ITS, respectively using the *idtaxa* function from the *decipher* R package (Wrigth, 2016). Mitochondria and chloroplast sequences were then removed, and contaminants were identified and removed using negative controls and the *decontam* package (Davis et al., 2018). Rarefaction curves were drawn for each sample, using the *rarecurve* function of the *vegan* R package (Oksanen et al., 2007), and the rarefaction plateau was reached for all samples (Fig. S2). Two filtering steps were performed, first, to account for differences in sequencing depths, samples were rarefied to 4236 and 7828, for 16S rRNA gene and ITS, respectively removing 1092 prokaryote and 350 fungal ASVs. Then, ASVs not seen more than once in at least 2% of the samples were removed. We then obtained 2 116 969 (8260 ASVs) final sequences for 16S rRNA gene and 172 719 (566 ASVs) sequences for ITS. A figure showing the phyla relative abundances was constructed and presented according to the rice-growing system, the geographic zone and the root compartment.

Indices of α-diversity (observed richness and Shannon diversity index) were calculated using the *estimate_richness* function from the *phyloseq* package (McMurdie & Holmes, 2013). Members of the core microbiota were identified for 16S rRNA gene and ITS communities (including both rhizosphere and roots compartments) in each rice grown system using the prevalence threshold of 60% of samples. This threshold allows identifying the ecological core

microbiome in the soil heterogeneous matrix considering the degree of accuracy generated by the processing into exact sequences (ASV) while ensuring not to miss lower abundance taxa that have fallen below the threshold of detection in one or more samples, but are of functional and ecological importance (Neu et al, 2021).

For 16S rRNA gene dataset only, we inferred co-occurrence networks using the *SpiecEasi* pipeline (Kurtz *et al.* 2015), independently for each rice growing system: rainfed lowland and irrigated areas. Networks were computed for ASVs present in more than 15% of the samples to meet the data requirement to draw correlations. They were calculated using the method 'mb' and setting the lambda.min.ratio to 1e-3 and nlambda to 50. We identified hub taxa, i.e. the potential keystone of the microbial network belonging to the most connected ASVs, based on their node parameters (method adapted from Berry & Widder, 2014): a low betweenness centrality (lower quantile, < 0.9), and a high closeness centrality (higher quantile, > 0.75), transitivity (higher quantile, > 0.25) and degree (higher quantile, > 0.75). The node and network parameters were determined using the R package *igraph* (Csardi & Nepusz, 2006) and *qgraph* (Epskamp *et al.* 2012). Complete networks were further described by calculating the number of nodes and hubs, the network mean degree, mean closeness and betweenness centralities, the total number of edges, and the positive to negative edges ratio. The taxonomic affiliation of all ASVs was refined using nucleotide basic local alignment search tool (BLASTn) analyses on NCBI nr database. We screened the table of blast best hits of all 16S rRNA gene and ITS ASVs (present in the rarefied but unfiltered dataset) in order to search the genus or species names of a number of pathogen species listed a priori (Table S1), based on the reference book *Compendium of Rice Diseases* (Cartwright *et al.* 2018). We also searched within blast best hits for ITS ASVs assigned to the *Glomeromycetes* class, as well as 16S rRNA gene and ITS ASV assigned to a species name's including "rhizob" to identify rhizobia, and including "oryz", as this may correspond to a specific interaction (pathogen or beneficial) with rice.

Statistical analyses

We first analyzed soil physico-chemical parameters. To this purpose, PERMANOVA were performed on soil physical properties (texture, i.e. relative amount of sand, silt, and clay) on the one hand, and soil chemical properties (7 variables: pH, total carbon, total nitrogen, total phosphorus, total potassium, SBE, CEC) on the other hand. For both models, we included as

explanatory factors the 'geographical zone' and 'rice-growing system', as well as their interaction, using *adonis2* function from the *vegan* R package, with 999 permutations. Posthoc tests were done using the *pairwiseAdonis* function (https://github.com/pmartinezarbizu/pairwiseAdonis). In addition, we performed linear models (*lm* function) on each soil variable independently, after having tested for normality (function *shapiro.test*) function, and transformed the variable (using the package *bestNormalize*, Peterson, 2021) if required. The linear models included as explanatory variables: the rice growing system, the geographical zone, and their interaction (the last one being removed from the final model if revealed non-significant). Correction for multiple testing (10 soil parameters) was performed using the FDR (false discovery rate) method, using the function *p.adjust* (option method "BH") from the package *stats* (R core Team, 2018).

For microbial root and rhizosphere communities respectively, PERMANOVA based on a Bray–Curtis dissimilarity matrix were used to test for significant effects geographical zones, rice growing systems and their interaction on β-diversity. Of significant, differences were further tested using the *pairwise.adonis* function of the *pairwiseAdonis* package (version 0.4). The graphical representation of β-diversity was based on Non-metric Multi-Dimensional Scaling (NMDS, *metaMDS* function). The effect of edaphic variables (i.e., pH, total organic carbon, total phosphorus, total nitrogen, total potassium, CEC and SBE) in structuring β-diversity was tested using the *envfit* function (9999 permutations) in R package *vegan*. Structuring soil properties were thus fitted onto the ordination space.

We tested for an effect of the rice growing system, the geographical zone and their interaction on obtained indices of alpha diversity, using linear models (*lm* function) for Shannon diversity indices, and generalized linear models (*glm* function, with a Poisson distribution) for observed richness). Models used for ITS dataset did not include random effect (function lm for Shannon index and glm for observed richness). On the other hand, as three samples of different plants but same rice fields were included in the 16S dataset, we built generalized linear mixed models (GLMMs) for prokaryote kingdom to include the particular field as random factor. The package *lme4* (Bates et al. 2015) was used for this purpose (function *lmer* for Shannon index and *glmer* for observed richness. These models were run independently for each compartment (rhizosphere on the one hand, and root associated on the other) and were analyzed by Type III ANOVA with the package *car* (Fox & Weisberg, 2019).

Then, we identified particular soil taxa that were associated with lowlands or irrigated systems using indicator analyses with the function *multipatt* implemented in the *indicspecies* package (De Cáceres *et al.* 2010). The algorithm determines both fidelity and consistency to a system.

Finally, we analyzed the repartition of ASVs belonging to rhizobia, and assigned to the class *Glomeromycetes* (arbuscular mycorrhizal fungi, AMFs). The summed abundance of all ASVs which best blast hit is within the two groups were modeled with Poisson distribution, and analyzed with Type III ANOVA with the package *car*. Explanatory variables included the rice growing system, the geographical zone, and their interaction. For ITS data (Glomeromycetes), GLMs were used with no random factor. For 16S rRNA gene data (rhizobia), GLMMs were built, including the particular field as a random factor.

R code used to perform the analyses and generate the figures are available upon request.

Results

Structure of rice soil properties and rice root associated microbial communities

PERMANOVA analysis performed on the Bray-Curtis distance matrix of soil characteristics to describe the overall soil properties, highlighted no significant influence of the rice growing system, but a differentiation according to the geographical zone, for both physical ($F = 6.420$, $r^2 = 0.448$, $p = 0.006$, Fig. 1b) and chemical ($F = 4.121$, $r^2 = 0.346$, $p = 0.026$) soil parameters. More precisely, we found no effect of the rice growing system but a significant effect of the geographical zone on the clay and sand contents, as well as total Phosphorus, total Potassium, SBE and CEC (Table S2). Posthoc tests revealed that the geographic zones that differed statistically were the same for the six above-mentioned variables, namely Banzon and Karfiguela zones (Fig. 1c).

To determine whether the geographical zones (Karfiguela, Bama or Banzon), the rice-growing systems (irrigated vs. rainfed lowlands), or their interactions, structured root-associated or rhizosphere microbial communities, we performed PERMANOVA analysis on the Bray-

Curtis distance matrix of 16S rRNA gene and ITS ASVs, respectively. As different communities' structures were revealed between root and rhizosphere of both prokaryotes (F= 6.863, $r^2 = 0.052$, $p < 0.001$) and fungi (F= 3.753, $r^2 = 0.080$, $p < 0.001$), we further subsetted 16S and ITS datasets to better observe the relative influence of the rice-growing systems and the geographical zones in shaping root and rhizosphere communities separately (Table 1, Fig. 2).

In the rhizosphere, prokaryotic communities were structured by the rice growing system, the geographical zone and the interaction between rice growing system and zone (Table 1). Posthoc tests revealed that all pairs of sites (i.e., irrigated and lowland from the same geographical zone) were significantly different, but interestingly revealed no significant difference between communities originating from irrigated systems, whereas all communities from rainfed lowland sites exhibited distinct structures (Table S3). Fungal communities of the rhizosphere were mostly structured by the rice growing system (Table 1). The geographical zone and the interaction between rice growing system and the geographical zone were also driving the rhizosphere fungal microbiome. The lower number of samples did not allow to detect, if any, statistically significant differences between sites in communities' structures for ITS (Table S3).

As observed for rhizosphere communities, the root-associated prokaryotic communities were mainly shaped by the rice growing system, the interaction between rice growing system and the geographical zone and the geographical zone (Table 1). For all pairs of sites (within the same geographical zone), irrigated areas and rainfed lowlands were significantly different. No significant difference was detected between communities originating from irrigated systems, whereas in two out of three comparisons, communities from rainfed lowland sites exhibited distinct structures (Table S3). Root-associated fungal communities were also mostly influenced by the rice growing system and by the interaction between rice growing system and the geographical zone (Table 1). The effect of the geographical zone (three zones) on root-associated fungal communities was not evidenced. As for rhizosphere, posthoc tests on root-associated fungal communities were all non-significant (Table S3).

The influence of soil chemical parameters on microbial community structure is reported in Fig. 2 as arrows and in Table S4. We noticed that the prokaryotic communities of both rhizosphere and roots were affected by the same three parameters: SBE (r^2 =0,482, p < 0,001 for rhizosphere, and r^2 = 0,175, p = 0,006 for roots), CEC (r^2 =0,314, p < 0,001 for

rhizosphere, and r^2 = 0,204, p = 0,003 for roots) and total phosphorus (r^2 = 0,132, p = 0,023 for rhizosphere, and r^2 = 0,179, p = 0,004 for roots). For fungi, although various parameters were marginally significant in each compartment (Table S4), we only detected a significant effect of total nitrogen on rhizosphere communities (r^2 =0,320, p = 0,043).

Composition of rice root microbiomes and comparison of alpha-diversity

While 16S rRNA gene data were assigned at the genus level for 64% of ASVs, only 34% of ITS ASVs could be assigned (see assignations at the phyla level in Fig. S4). Assignations at the phylum level were obtained for all (100%) 16S ASVs, but only for 62% for ITS ASVs (see Fig. S3). Assigned prokaryotic taxa represent 17 phyla, most abundant ones being Proteobateria, Firmicutes, Mixoccoccota and Acidobacteriota. For ITS, seven phyla were found, with the most abundant ones being Ascomycota followed by Basidiocomycota.

We tested the effect of the rice growing system on the diversity indices (alpha-diversity). No effect of the rice growing system could be evidenced on the root-associated compartment, although the interaction between rice growing system and geographical zone significantly affected observed richness for both prokaryotes and fungi (Table 2). In addition, the rice growing system had a significant effect on the prokaryote diversity of the rhizosphere (Table 2), with a higher Shannon diversity index in irrigated areas (5.03 ± 0.13), compared to rainfed lowlands (4.39 ± 0.12) and a higher observed richness (275.6 ± 26.4) in irrigated areas compared to rainfed lowlands (143.7 ± 17.4) (Fig. 3). An opposite pattern was found for fungal communities of the rhizosphere with higher observed richness in rainfed lowlands (75.78 ± 4.34), compared to irrigated areas (63.80 ± 4.51) (Table 2 and Fig. 3).

Core microbiome and co-occurrence networks in the two rice-growing systems

ASVs belonging to the core microbiome of lowland vs. irrigated rice were respectively identified with a prevalence threshold set to 60%. For the 16S rRNA gene, we identified 26 core ASVs associated with the irrigated systems, and two core ASVs in lowlands (Fig. 4). Among the core taxa in irrigated areas, the vast majority of phylotypes (25/26) belonged to the *Burkholderiaceae* family, with 24 assigned to *Ralstonia pickettii* and one to *Paraburkholderia kururiensis*. One of the core ASVs is common to both irrigated area and rainfed lowlands systems. Its best blast hit corresponds to *Bradyrhizobium tropiciagri*

(Bradyrhizobiaceae) with a 99.5% sequence similarity. Another core phylotype in rainfed lowlands is assigned to the same species with 99.3% sequence similarity.

For ITS, we identified 5 core ASVs in the irrigated systems, compared to 11 core ASVs associated with the lowlands, 4 of them being common to both rice growing systems (Fig. 4).

Then, we compared the prokaryotic co-occurrence networks in each rice growing system respectively (Fig. 5). We identified 15 hub ASVs in the irrigated systems and 20 in rainfed lowlands. We found a higher edge number in irrigated compared to rainfed lowlands: 1720 positive and 269 negative resulting in 2029 total edges in irrigated areas, while only 1163 positive and 85 negative resulting in 1248 total edges were found in rainfed lowlands. Finally, the network computed from irrigated areas had higher connectivity compared to the one from rainfed lowlands (9.8 vs 7.9 node mean degrees, respectively). None of the identified hub taxa were also core in any of the two rice growing systems. Only one ASVs was identified as a hub in both irrigated and rainfed lowland systems, assigned to *Anaeromyxobacter dehalogenans* (Anaeromyxobacteraceae). Hub taxa in irrigated areas (15 ASVs) were assigned to 15 different species from 12 families, while hub taxa in rainfed lowland (20 ASVs) only corresponded to 17 species from 11 families (Fig. 5).

Indicator taxa of the two rice growing systems

For 16S rRNA gene data, we found 128 indicator taxa in irrigated areas, including ASVs from 24 bacterial families, most of them assigned to *Acinetobacter, Ralstonia, Comamonas* and *Clostridium* (Table 3). On the other hand, only 63 were identified in rainfed lowlands, most of them within the Bacillaceae family, including ASVs assigned to *Exiguobacterium* and *Priestia* (Table 3). The ASV assigned to *Paraburkholderia kururiensis* (Burkholderiaceae) revealed as indicator in irrigated areas (Table 3) was also a core taxa in irrigated areas. Also, among the 21 indicator ASVs in irrigated areas assigned to *Ralstonia pickettii* (Burkholderiaceae), 16 were also core in irrigated areas. In addition, four indicator ASVs in irrigated areas were also hubs in this system: one assigned to *Pseudogulbenkiania subflava* (Chromobacteriaceae), one to *Clostridium intestinale* (Clostridiaceae), another to *Enterobacter cloacae* (Enterobacteriaceae), and finally one corresponding to *Pseudomonas flavescens* (Pseudomonadaceae). One ASVs assigned to *Priestia flexa* (Bacillaceae) and one assigned to *Methylosinus trichosporium* (Methylocystaceae) were hubs in rainfed lowlands.

For ITS data, we found 16 indicator taxa in irrigated areas, and 27 in rainfed lowlands (Table 3). Indicator taxa in irrigated areas were assigned to four classes: *Agaricomycetes*, *Microbotryomycetes* and *Sordariomycetes*,. Indicator taxa in rainfed lowlands were assigned to five classes: *Chytridiomycetes*, *Dothideomycetes*, *Saccharomycetes, Sordariomycetes* and *Ustilaginomycetes*. One ITS ASV identified as indicator taxa in irrigated, with best hit *Pulveroboletus sinensis* (Agaricomycetes), was also core in this rice growing system, and two indicator taxa in rainfed lowlands were also core in this system: one assigned to *Paraphaeosphaeria michotii* (Dothideomycetes) and the other to *Coniochaeta rosae* (Sordariomycetes).

Putative pathogen or phytobeneficial taxa

First, responses for an '*oryz*' query within assignment and blast, found matching records only in the 16S rRNA gene dataset. A total of 200 ASVs included '*oryz'* in their names, from 21 different genera, none of these species corresponded to pathogens from Table S6. Among them, putative beneficial taxa were found, particularly the following: *Azospirillum oryzae, Novosphingobium oryzae, Paenibacillus oryzae,* and *Rhizobium oryzae, R. rhizoryzae and R. straminoryzae.*

Next, we made a subset of the 16S rRNA gene dataset for ASVs assigned to rhizobia. This corresponds to a total of 96 ASV among which one was indicator species for rainfed lowlands (Table 3), two are core in rainfed lowlands while one is core in irrigated (Fig. 4) and finally, one is hub in irrigated (Fig. 5). Summed abundances of rhizobia of the rhizosphere are affected by the rice growing system (Table 4), with higher abundances in rainfed lowlands (Fig. 6). In roots, summed abundances of rhizobia were affected by the geographic zone, as well as the interaction between rice growing system and geographic zone (Table 4, Fig. 6).

The same kind of analysis was then performed for the *Glomeromycetes* class (total of 14 ASVs) in ITS dataset. AMF summed abundance was affected by the rice growing system, the geographical zone and their interaction, both for rhizosphere and root compartments (Table 4). Higher abundances of *Glomeromycetes* are observed in rainfed lowlands, compared to irrigated areas (Fig. 6). .

We then screened the list of all assigned ASVs for a set of pathogen species defined *a priori* (see the list in the Table S1). For prokaryotes (16S rRNA gene data), a number of ASVs

corresponded to the genera of pathogens, but only *Burkholderia glumae* (two ASVs), *Acidovorax avenae* (four ASVs) and *Dickeya chrysanthemi* (six ASVs) were identified at the species level. These 12 ASVs identified at the species level were however only found in one sample. The *Xanthomonas* genus was found, but with no assignment to *X. oryzae* (instead, assigned to *X. theicola* which is phylogenetically closed to the rice associated *X. sontii*, Bansal *et al.* 2020), although two sampled plants had specific symptoms of bacterial leaf streak (caused by *X. oryzae* pathovar *oryzicola*). A similar situation (assignment at the genus level) was observed for the genera *Pseudomonas, Pantoea*, and *Sphingomonas*. The same analysis of putative pathogens for ITS revealed the presence of the following ten genera: *Alternaria*, *Bipolaris*, *Ceratobasidium*, *Curvularia*, *Fusarium*, *Helminthosporium*, *Microdochium*, *Rhizoctonia*, *Sarocladium*. *Bipolaris* spp. causes brown spot symptoms, which were frequently observed (33 out of 57 sampled plants, i.e. 57.9%). On the other hand, one sampled plants presented rice leaf blast symptoms, but no *Pyricularia oryzae* was found in ITS dataset.

Discussion

This study aimed at describing the rice root-associated microbiome by comparing contrasted rice growing systems in farmer's fields in Burkina Faso. We found that the rice growing system was a structuring factor for rice root-associated microbiomes, with an effect on alpha diversity in the rhizosphere, higher in irrigated areas for bacteria, while rainfed lowlands harbor higher fungal richness. In addition, we identified a number of phylotypes with potential key roles (hub, core, indicators) in the two contrasted systems, as well as putative phytobeneficial and pathogen species. Although the results on fungi (ITS region) must be taken with caution due to a smaller sample size and the poor representation of obtained sequences in available taxonomic databases, this study shed light on some drivers of assemblage of rice root associated microbial communities in a sparsely documented African system.

Structure and diversity of the root-associated microbiomes is affected by the rice growing system

Although Edwards *et al.* (2018) showed that the root-associated microbiome of distant field sites converge in similarity during the growing season, our study performed at the maturity stage of rice still evidenced some drivers of rice root-associated microbial communities

structure. First, as for most rice microbiome studies, we found an effect of the compartment / micro-habitat (Edwards *et al.* 2015; Santos-Medellín *et al.* 2017; Guo *et al.* 2021; Kawasaki *et al.* 2021), and the geographical zone (Edwards *et al.* 2015; Kanasugi *et al.* 2020) on the beta-diversity of rice root-associated microbiome. In addition, our study shows that the contrasted rice-growing systems, namely irrigated perimeters *vs* rainfed lowlands, harbor contrasted rice root-associated microbial communities, both for prokaryotic and fungal communities, and for rhizosphere and root compartment. We notice that the soil physicochemical properties weakly differ between irrigated areas and rainfed lowland, the soil composition was instead mostly affected by the geographical zones. Consequently, we evidence a structuring effect of the rice-growing system that was only slightly related to contrasted soil physicochemical properties. Our results are in line with a previous study comparing microbiomes from two contrasted water management conditions (upland *vs* lowland rice) in controlled settings (a field experiment in northern Italy), which showed differentiation in microbial communities, particularly for root microbiome, and to a lesser extent in soil samples (Chialva *et al.* 2020).

In our study, some of the soil physicochemical parameters affected rice root-associated microbial communities. In particular, CEC and SBE, that reflect soil exchange capacity and bioaccessibility, were the most important soil parameters for the structure of both rhizosphere and root prokaryotic communities. These parameters are not commonly measured in other studies of the root-associated microbiome, and our results argue for including them in soil chemical characterization, to investigate whether their impact in microbiome structure is general or not. In addition, phosphorus content significantly structured the prokaryotic communities, both in rhizosphere and roots. Such an effect of phosphorus is known for the rice root associated microbiome (Long & Yao, 2020). On the other hand, the soil chemical parameter evidenced in this study to structure fungal rhizosphere communities was the total nitrogen (N). This is in accordance with a study by Chen *et al.* (2019) showing that nitrogen input drives changes in the microbial root-associated community structure in wheat. Moreover, Wang & Huang (2021) showed the effect of optimized N application on fungal community structure from paddy soils. Kanasugi *et al.* (2020) also evidenced an effect of soil nitrate on rice root fungal communities in Ghana.

We found a higher taxonomic diversity in irrigated areas, compared to rainfed lowlands, for prokaryotic communities of the rhizosphere, while the opposite (higher diversity in rainfed lowlands compared to irrigated areas) was found for fungal communities. Chialva *et al.*

(2020)'s found that the 16S rRNA gene diversity was similar in lowland and upland rice, but they found a significantly higher ITS diversity in lowland rice compared to upland. It is important to note that our sampling was performed directly in farmer's fields, with no control of management practices, while most results, including those of Chialva *et al.* (2020), were obtained in field trials. Indeed, in our study, various factors, such as rice cultivars, fertilization regime and rotation, exhibit large variability. However, levels of rice genetic diversity were shown to be comparable within each site, both in irrigated areas and rainfed lowlands (Barro *et al.* 2021b), therefore the effect of rice growing system on alpha-diversity of microbiomes could not be attributed to result from difference in terms of genetic diversity of the host plant.

Considering the irrigated areas as systems with more intensive agricultural practices, compared to rainfed lowland, we expected to observe higher diversity of microorganism in rainfed systems, as it was the case for fungal communities, but not the opposite one obtained for bacterial communities. Indeed, agricultural intensification was shown to reduce microbial network complexity and the abundance of keystone taxa in roots (Banerjee *et al.* 2019). In addition, the fertilization regime is known to have strong impact on root-associated microbiota (Ding *et al.* 2019; Xiong *et al.* 2021). Various studies showed that organic fertilization enhances microbial diversity (Liu *et al.* 2020). For example, recommended fertilization preserved belowground microbial populations, compared to the fertilization mostly used ('conventional fertilization') that depressed bacterial diversity, in experiments performed in China (Ullah *et al.* 2020). We considered the irrigated areas as more intensified systems, compared to rainfed lowland, particularly because only irrigated areas allow growing rice twice a year, and because only rainfed lowland sites presented fields with no mineral fertilization at all (Barro *et al.* 2021a). We noticed however that organic fertilization remained rare, and its frequency was not drastically affected by the rice growing system. Finally, transplantation was always performed in irrigated areas, while direct sowing was the most common practice in rainfed lowlands.

On the other hand, paddy soils studied in western Burkina Faso (all over the six sites) are particularly poor if compared for example to a study of more than 8 000 soils in Hunan Province (Duan *et al.* 2020). The studies previously cited evidencing fertilization effects, were performed in soils with higher carbon and nitrogen contents (see for example Ullah *et al.* 2020). The effect of fertilization on microbial diversity may actually depend on various aspects, including the soil type. Notably, a positive relationship was found between rice

fertilization and soil bacterial richness and diversity in a 19-years inorganic fertilization assay in a reddish paddy soil in southern China (Huang *et al*. 2019); while Wang & Huang (2021) showed an effect of the fertilization on paddy soils microbial community composition but no effect on the diversity. The poverty of soils in the sampled area may be involved in the complexity of fertilization-diversity relationships observed in this study.

Our results showed that the prokaryotic communities in the rice rhizosphere and roots from the three irrigated sites do not differ significantly from each other. On the other hand, the same analysis revealed significant differentiation between the three rainfed lowland study sites (in all three cases for rhizophere and two out of three comparisons in roots). Also, we found very few core phylotypes in rainfed lowland, with only two core ASVs for 16S rRNA gene, which reinforces the above-mentioned observation. These results are likely driven by a higher heterogeneity between rainfed sites, in terms of water control, agricultural practices or rice genotypes. Indeed, in irrigated rice, the farmer has the potential to control irrigation water during the whole growing season. On the other hand, irrigation in rainfed lowland is dependent on precipitation that differs between the three geographical zones sampled within the rice growing season. In addition, we showed a high heterogeneity of agricultural practices in rainfed lowlands: for example, legume rotation was common only in the rainfed lowland of Bama zone, and organic fertilization was especially frequent in the rainfed lowland of Karfiguela zone (Barro *et al.* 2021a). Finally, in terms of rice genetics, a high rice genetic differentiation was found between the rainfed lowland site of Karfiguela zone and the five other sites: a distinct genetic group *O. sativa Aus*, and other distinct landraces were found in this peculiar site, compared to the five others where only *O. sativa indica* was grown (Barro *et al.* 2021b). Lower sampling size for ITS may explain the absence of significant differences obtained between pairs of sites. Alternatively, the pattern may be different for fungal diversity, as suggested by the higher number of core taxa in rainfed lowlands than in irrigated areas.

Key taxa reveal different ecological functioning between irrigated systems and lowlands

The core microbiome is hypothesized to represent the most ecologically and functionally important microbial associates of that environment under the conditions sampled (Neu *et al* 2021), so that describing the cores and determining whether it is shared between irrigated

systems and rainfed lowlands is of major interest. While four fungal taxa were found to be cores in both systems, only one bacterial core taxa was shared between the two rice growing systems: assigned to *Bradyrhizobium tropiciagri*, a nitrogen-fixing symbiont isolated from tropical forage legumes (Delamuta *et al.* 2015). Some legumes (crops in rotation, or wild plants such as *Aeschynomene* or *Stylosanthes*) may share the lowland soils with rice, and bacteria could interact with different plant host. Indeed, several strains of *Bradyrhizobium spp.* isolated from rice roots were described to be capable of fixing N_2 (Chaintreuil *et al.* 2000; Ding *et al.* 2019). In addition, the core taxa in irrigated areas likely includes *Paraburkholderia kururiensis*, a bacterium with potential phytobeneficial properties (bioremediation, biofertilization and biocontrol of pathogens; Dias *et al.* 2019). Various ASVs identified as core in irrigated areas were assigned to *Ralstonia pickettii*, an ubiquitous Betaproteobacteria found in water and soil, capable to thrive in low nutrient (oligotrophic) conditions (Ryan *et al.* 2007), considered as potential biocontrol agent against its congeneric pathogen *R. solanacearum* (Wei *et al.* 2013), and also, noteworthy, described as human emerging pathogen causing nosocomial infections (Ryan *et al.* 2006).

Hub taxa are potential keystones, i.e., taxa that drive community composition and function irrespective of their abundance (Banerjee *et al.* 2018). Only one ASVs was identified as hub of the prokaryote co-occurrence networks both in irrigated areas and rainfed lowland site. It is assigned to *Anaeromyxobacter dehalogenans* (Anaeromyxobacteraceae), a versatile soil bacterium capable of denitrification in the presence of iron (Onley *et al.* 2018). On the other hand, although various ASVs indicator in either irrigated areas or rainfed lowlands were assigned to the same species, we also found many bacterial taxa identified as hubs that differ between irrigated areas and rainfed lowlands (even at the family level), reflecting a highly contrasted structuring and functioning of bacterial communities in the two rice growing systems.

To identify specific ASVs that characterized microbiome variations between rice growing systems, we further analyzed our data for the presence of candidate indicator taxa. Most indicator taxa were found in irrigated systems for prokaryotes (128, *vs* only 63 in rainfed lowlands) while the opposite was found for fungi (27 in rainfed lowlands *vs* only 16 in irrigated areas). For prokaryotes, five taxa identified as indicator species in irrigated areas were also core or hub: the previously mentioned *Paraburkholderia kururiensis* and *Ralstonia pickettii* as well as *Pseudogulbenkiania subflava*, *Clostridium intestinale Acinetobacter soli*

and *Pseudomonas flavescens*. In rainfed lowlands, it was the case for *Priestia flexa* and *Methylosinus trichosporium*. In particular, we notice that *Acinetobacter soli* was identified as a potent phosphorus solubilizer in rice and consequently promising for plant growth promotion (Rasul *et al.* 2019). For fungi, we identified as both indicator and core taxa: *Pulveroboletus sinensis* in irrigated areas, as well as both *Coniochaeta rosae* and *Paraphaeosphaeria michotii* in rainfed lowlands. Two ASVs assigned to Chytridiomycetes were identified as indicator species in rainfed lowland systems. These aquatic fungi (Barr, 2001), found in the rhizosphere compartment in our study, are known as particularly abundant in microbial communities associated with rice roots, compared to other crops (Ding et al. 2019) and were preferentially associated to lowland conditions compared to upland (Chialva et al. 2020).

We identified a few potentially beneficial taxa that could be investigated further. In particular, rhizobia abundance in the rhizosphere was higher in rainfed lowlands compared to irrigated areas. This was expected considering that rainfed lowlands corresponds to lower fertilizer input, and, in some cases legume rotations between rice cycles, frequent at least in some rainfed lowland site (rainfed lowland of Bama zone, see above). Rhizobia populations are released from nodules when legume plant dies, and rhizobia are rice-colonizing bacteria (Chi et al., 2005). We also showed that AMF abundance is affected by the rice growing system (in both rhizosphere and roots). This is congruent with other studies reporting AMF colonization depending on farming regimes. For example, the rice roots cultivated in the conventional agrosystem (N and P fertilization and pesticides) or under permanent flooding showed no AMF colonization, while the rice plants grown with organic conditions showed typical mycorrhization patterns (Lumini *et al.* 2011).

Various pathogen species were suspected from the sequence variants identified in this study. In particular, the 16S rRNA gene dataset contained ASVs assigned to *Burkholderia glumae*, *Acidovorax avenae* and *Dickeya chrysanthemi*. All three remained rare, each of these sequences only found in one sample. The presence of *B. glumae* was described in Burkina Faso (but with no molecular data; Ouedraogo *et al.* 2004), and targeted detection performed in two sites failed to detect *B. glumae* and *A. avenae* (Bangratz *et al.* 2020). For fungal pathogens, we found ASVs assigned to ten genera comprising rice pathogens, including *Bipolaris*, *Curvularia*, *Fusarium* and *Rhizoctonia*. Some of these putative fungal pathogens are frequent, particularly one, whose best blast hit is *Curvularia chonburiensis*, identified as

core taxa in both irrigated perimeters and rainfed lowlands. Various *Curvularia* species are known to be pathogenic in rice (Gao *et al.* 2012; Majeed *et al.* 2015), and their widespread repartition evidenced here argues for more plant pathology work on the interactions between *Curvularia* and rice.

The literature shows that higher microbiome diversity may be associated with a lower infection rate (see for example Rutten *et al.* 2021). For rice in western Burkina Faso, when compared to irrigated perimeters, we found that rainfed lowlands harbor higher rhizosphere fungal richness and lower prevalence of major rice diseases, particularly bacterial leaf streak and the fungal rice blast disease, based on the observation of foliar symptoms (Barro *et al.* 2021a). On the other hand, for prokaryote communities, the pattern is actually opposite, with a higher diversity in irrigated areas compared to rainfed lowlands. The potential relationship between the diversity of root-associated microbiome and diseases may actually be complex, requiring other studies, including under controlled conditions. More generally, scientific interest in the relationship between root-associated microbiota and plant diseases is growing (Vannier *et al.* 2019; Trivedi *et al.* 2020). These aspects constitute a promising avenue of research in this system as well, especially considering reciprocal effects evidenced in rice from experimental studies (Spence *et al.* 2014; Tian *et al.* 2021).

Perspectives

We are only at the beginning of understanding the complexity of rice root microbial communities, especially for rice cultivation in Africa. The originality, but also a limitation of our study, lies in the fact that the samples were collected in farmer fields, and it globally compares the two contrasting rice production systems that differ in various management practices, so that it could not tease apart the specific effect of each individual factor (water management, cultivar, fertilization, etc). More investigations are now required to decipher each structuring factor at a smaller scale: in particular between fields within each site, where the rice cultivar and specific agricultural practices are likely to play a significant role (Delitte *et al.* 2021).

Describing rice microbiota through metabarcoding is a first mandatory step that needs to be combined with culturomics for a greater accuracy and a deeper description, in particular in such systems where some taxa are poorly described in taxonomic databases. Experimental work in an integrative approach is also required to move on towards microbiota management

methodologies. Such microbiota-based strategies could contribute to improving rice health and productivity (Sessitsch & Mitter, 2015), while preserving human health. They are consequently an important component of the toolbox of science-based strategies to achieve zero-hunger in Africa.

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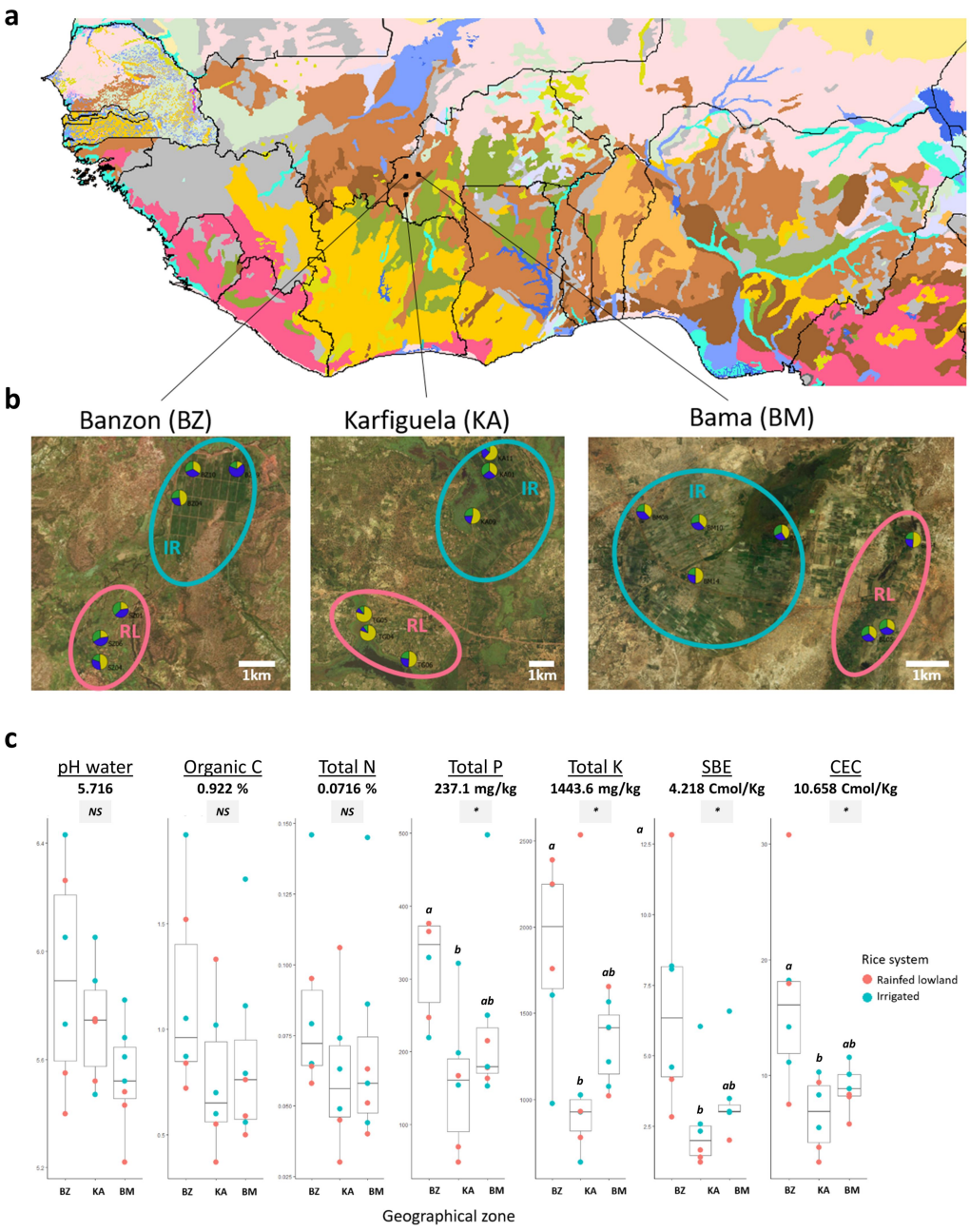


Figure 1

Location of the study sites and soil physico-chemical properties

Location of the study sites in western Burkina Faso on the soil Harmonized Word Soil Database (HWSD) map of West Africa (https://webarchive.iiasa.ac.at/Research/LUC/External-World-soil-database/HTML/). The three geographical zones studied are in Lixisols (LX: soils with subsurface accumulation of low activity clays and high base saturation)

Location of the field studied within each geographical zones. Soil texture, are indicated for each of the 19 studied fields, with pie charts representing relative proportions of sand (in yellow), silt (in green), and clay (in blue). Soil chemical properties estimated in each geographical zones, with colors representing the rice growing system (irrigated areas in blue and rainfed lowlands in red). Each point corresponds to one field studied. Averages over the 19 studied fields are indicated for each parameter, as well as the results of statistical tests for the geographical zone effect.

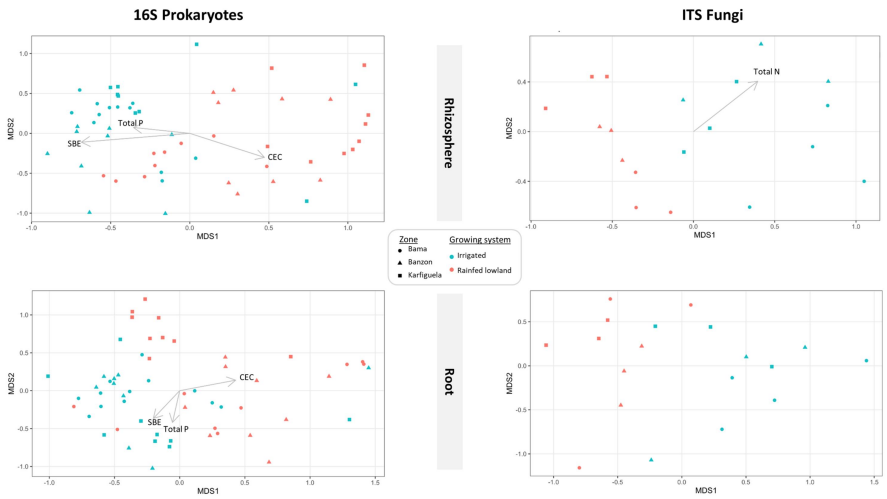


Figure 2

NMDS ordination showing the three factors identified as drivers of the structuration of rice root microbial communities: the color of points represent the rice growing system (irrigated vs rainfed lowland), while the shape shows the geographical zone (Banzon, Karfiguela and Bama).

On the left side are presented the analyses based on 16S rRNA gene reflecting Prokaryote communities, where one point corresponds to one plant. On the right side are shown the analyses based on ITS reflecting fungal communities, where one point corresponds to one field. The root compartment is presented on the upper side of the figure while the rhizosphere data appear on the bootom side. Only the soil physicochemical parameter that revealed as having a significant effect (see Supplementary Table S4) are represented with arrows: cation exchange capacity (CEC), Sorptive Bioaccessibility Extraction (SBE), total concentrations of phosphorus (Total P), total concentrations of nitrogen (Total N), total organic carbon (Organic C).

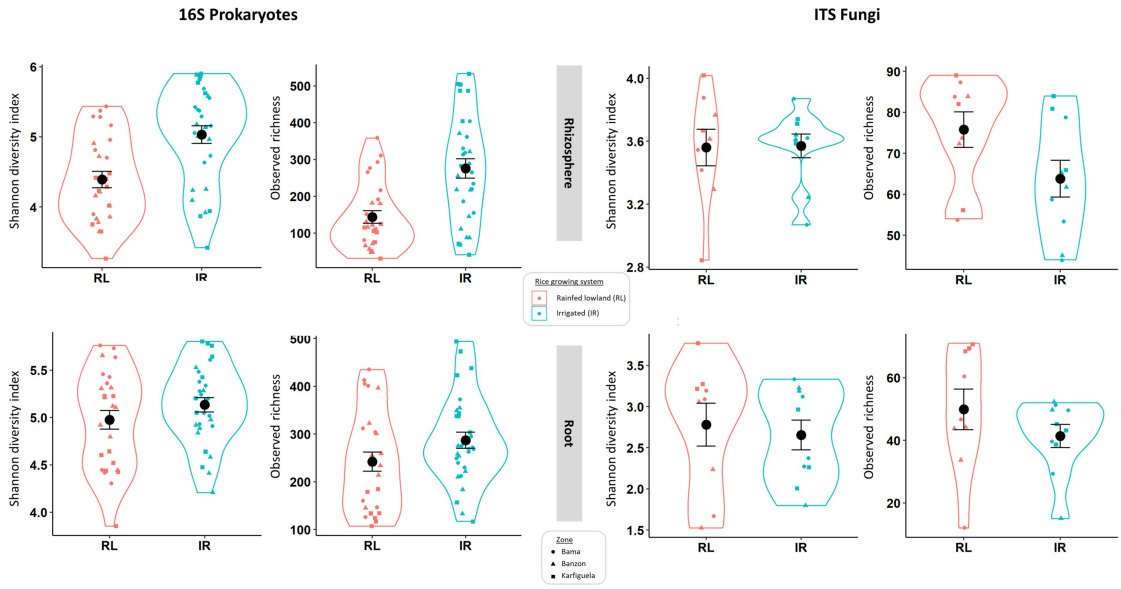


Figure 3

Comparison of root-associated microbiota α-diversity in contrasted rice-growing systems: irrigated (in blue) vs rainfed lowland (in red).

Observed richness and Shannon indices are reported for each sample (i.e. one plant for 16S and one field for ITS), as violin diagram for each rice growing system. The left side of the figure presents the results obtained for 16S microbiome, while the right side shows the results obtained for ITS analysis. On top are shown the results of the rhizosphere compartment and on the bottom are the results obtained for the root associated compartment. The shape of the points shows the geographical zone (Banzon, Karfiguela and Bama)

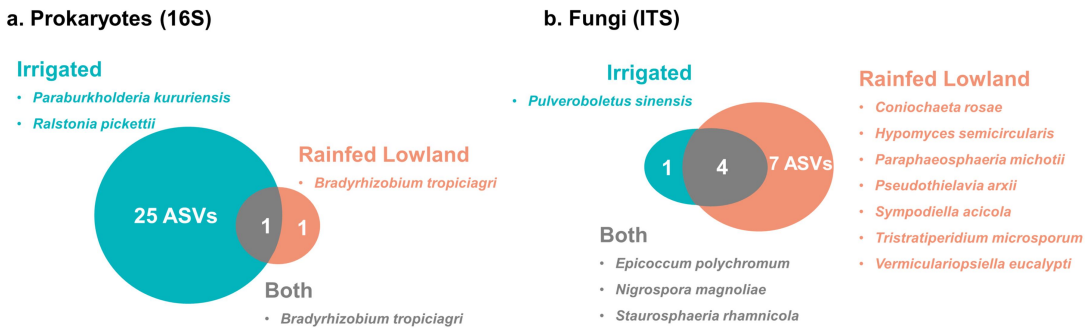
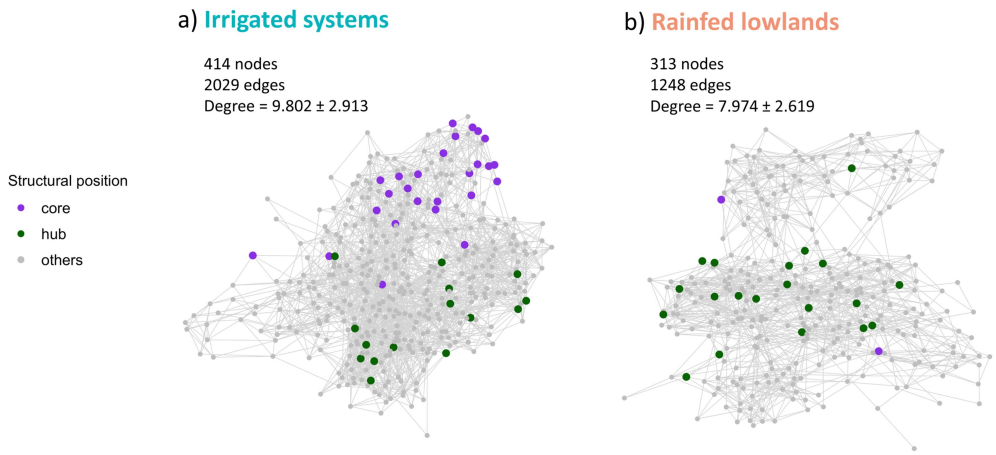


Figure 4

Venn diagram representing the core sequence variants for each rice growing system : irrigated vs rainfed lowlands. A. For prokaryotes B. For fungi



Number of hubs	15	20
Hub's families	Hub species (Number of ASV)	
Acidobacteriaceae	*Acidipila rosea (1)* *Occallatibacter savannae (1)*	*Acidipila rosea (1)*
Anaeromyxobacteraceae	*Anaeromyxobacter dehalogenans (1)*	
Bacillaceae	*Bacillus zanthoxyli (1)*	*Bacillus zanthoxyli (1)* *Neobacillus cucumis (4)* *Priestia flexa (2)*
Bryobacteraceae	*Paludibaculum fermentans (1)*	
Burkholderiaceae		*Rubrivivax gelatinosus (1)*
Chromobacteriaceae	*Pseudogulbenkiania subflava (1)*	
Clostridiaceae	*Clostridium intestinale (1)*	*Clostridium huakuii (1)*
Comamonadaceae		*Albitalea terrae (1)*
Enterobacteriaceae	*Enterobacter mori (1)*	*Enterobacter hormaechei (1)* *Enterobacter mori (1)*
Methylocystaceae	*Methylosinus trichosporium (1)*	*Methylosinus trichosporium (1)*
Moraxellaceae	*Acinetobacter brisouii (1)* *Acinetobacter soli (1)*	*Acinetobacter modestus (1)* *Acinetobacter seifertii (1)* *Acinetobacter soli (1)*
Pseudomonadaceae	*Pseudomonas flavescens (1)* *Pseudomonas weihenstephanensis (1)*	*Pseudomonas paralactis (1)*
Sphingomonadaceae	*Sphingomonas pituitosa (1)*	
Sterolibacteriaceae	*Methyloversatilis universalis (1)*	
Thermosporotrichaceae		*Thermosporothrix hazakensis (1)*

Figure 5

Network properties for each of the co-occurrence networks obtained from 16S rRNA data: a) Samples from irrigated perimeters, and b) samples from rainfed lowlands. Graphical

representation is shown, as well as a table showing the assignation of hub ASV in each rice growing system.

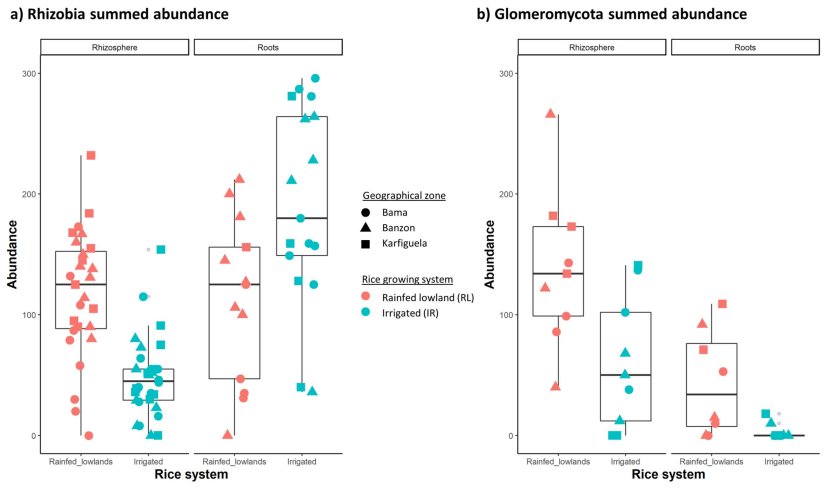


Figure 6

Spatial repartition of summed abundance of potentially phytobeneficial families in the two rice root microbiome compartments (rhizosphere and roots), and in each rice growing system (irrigated perimeters vs rainfed lowlands). a) Summed abundance for 14 ITS ASVs assigned to the class *Glomeromycetes*; b) Summed abundance for 96 ITS ASVs assigned to rhizobia. The shape of the point corresponds to each geographic zone.

Table 1

Results of PERMANOVA analysis performed independently for rhizosphere compartment and root compartment, for 16S and ITS microbiome data.

		Prokaryotes 16S					**Fungi ITS**				
		Df	SumOfSqs	R2	F	Pr(>F)	Df	SumOfSqs	R2	F	Pr(>F)
Rhizosphere	Rice growing system	1	1.890	0.079	5.096	0.001	1	0,687	0,115	2,452	0,001
	Geographical zone	2	1.646	0.069	2.218	0.001	2	0,872	0,146	1,555	0,007
	Rice growing system * Geographical zone	2	1.393	0.058	1.877	0.001	2	0,748	0,126	1,335	0,036
	Residual	51	18.919	0.793	NA	NA	13	3,644	0,612	NA	NA
	Total	56	23.848	1.000	NA	NA	18	5,951	1,000	NA	NA
Root	Rice growing system	1	1.614	0.079	5.155	0.001	1	0,846	0,111	2,289	0,002
	Geographical zone	2	1.407	0.069	2.247	0.001	2	0,891	0,117	1,206	0,123
	Rice growing system * Geographical zone	2	1.535	0.075	2.451	0.002	2	1,076	0,141	1,457	0,013
	Residual	51	15.966	0.778	NA	NA	13	4,803	0,631	NA	NA
	Total	56	20.522	1.000	NA	NA	18	7,616	1,000	NA	NA

Table 2

Results of the statistical tests performed on 16S and ITS alpha diversity indices (Shannon diversity index and observed richness) for rhizosphere and root compartments.

		Prokaryotes 16S						**Fungi ITS**					
		Shannon diversity index			Observed richness			Shannon diversity index			Observed richness		
		Df	Chisq	p-value	Df	Chisq	p	Df	Sum Sq	F value	p-value	Df	Chisq	p-value
Rhizosphere	Rice system	1	5,776	**0,016**	1	6,979	**0,008**	1	0,000	0,004	0,949	1	6,826	**0,009**
	Zone	2	5,087	0,078	2	5,510	0,064	2	0,014	0,070	0,932	2	0,056	0,973
	Rice system * Zone		ns			ns				ns		2	6,088	**0,048**
Root	Rice system	1	0,554	0,456	1	0,003	0,958	1	0,077	0,158	0,461	1	0,334	0,563
	Zone	2	1,084	0,586	2	7,422	**0,024**	2	0,505	0,522	0,604	2	32,476	**0.001**
	Rice system * Zone		ns		2	7,769	**0,021**			ns		2	13,440	**0,001**

The interaction between rice growing system and geographical zone was first included in the model, but then removed if non significant (indicated as 'ns' in the table). Mixed models (using the functions: lmer for Shannon index, and glmer with poisson family for observed richness) were used for 16S data, analyzed at the plant level, in order to include the field sampled as random effect, while no random effect were used for ITS data (functions: lm for Shannon index, and glm with poisson family for observed richness).

Table 3

List of species assignation and number of sequence variants (ASVs) identified as indicator taxa in irrigated and rainfed lowland environment.
The species in bold were also found as potential hub or core taxa.

Kingdom	Family (Prokaryotes) / Class (Fungi)	Irrigated	Lowlands
Prokaryotes	Acidobacteriaceae		*Occallatibacter savannae (2)*
	Anaerolineaceae	*Ornatilinea apprima (2)*	*Ornatilinea apprima (1)*
	Anaeromyxobacteraceae	*Anaeromyxobacter dehalogenans (2)*	
	Azospirillaceae	*Azospirillum soli (1)*	
	Bacillaceae	*Bacillus zanthoxyli (3)* *Neobacillus cucumis (2)* *Priestia flexa (1)* *Exiguobacterium acetylicum (4)*	*Bacillus zanthoxyli (2)* *Neobacillus cucumis (1)* ***Priestia flexa** (4)* *Exiguobacterium acetylicum (25)* *Exiguobacterium indicum (19)*
	Bradyrhizobiaceae	*Bradyrhizobium oligotrophicum (4)*	
	Bryobacteraceae	*Paludibaculum fermentans (2)*	
	Burkholderiaceae	*Burkholderia vietnamiensis (1)* ***Paraburkholderia kururiensis** (1)* ***Ralstonia pickettii** (21)*	*Burkholderia vietnamiensis (1)*
	Caulobacteraceae	*Caulobacter hibisci (1)* *Phenylobacterium panacis (1)*	
	Chitinophagaceae		*Flavisolibacter ginsenosidimutans (1)*
	Chromobacteriaceae	***Pseudogulbenkiania subflava** (1)*	
	Clostridiaceae	*Clostridium beijerinckii (8)* *Clostridium huakuii (5)* *Clostridium intestinale (1)*	
	Comamonadaceae	*Albitalea terrae (1)* *Comamonas testosteroni (9)*	
	Enterobacteriaceae	*Enterobacter cloacae (1)* *Enterobacter mori (1)* *Klebsiella variicola (1)*	
	Erythrobacteraceae	*Altericroceibacterium xinjiangense (1)*	
	Gallionellaceae		*Sideroxydans lithotrophicus (1)*
	Geobacteraceae	*Geobacter luticola (1)*	
	Kineosporiaceae	*Angustibacter speluncae (4)* *Kineococcus glutinatus (1)*	
	Methylobacteriaceae	*Methylobacterium radiotolerans (1)*	

	Methylocystaceae	*Methylocystis parvus (2)* *Methylosinus trichosporium (1)*	*Methylocystis parvus (1)* ***Methylosinus trichosporium (1)***
	Moraxellaceae	*Acinetobacter brisouii (1)* *Acinetobacter modestus (2)* *Acinetobacter pittii (1)* *Acinetobacter seifertii (1)* ***Acinetobacter soli** (20)*	*Acinetobacter soli (1)*
	Pseudomonadaceae	*Pseudomonas citronellolis (2)* ***Pseudomonas flavescens** (1)* *Pseudomonas glareae (1)* *Pseudomonas paralactis (2)* *Pseudomonas weihenstephanensis (2)*	*Pseudomonas paralactis (2)*
	Rhizobiaceae	*Rhizobium etli (1)*	
	Rhodanobacteraceae	*Dyella marensis (2)*	
	Shewanellaceae	*Shewanella xiamenensis (5)*	
	Sphingomonadaceae	*Sphingomonas limnosediminicola (1)*	
	Sterolibacteriaceae	*Sulfurisoma sediminicola (1)*	
	Thermosporotrichaceae		*Thermosporothrix hazakensis (1)*
	Number of ASVs	**128**	**63**
Fungi	Agaricomycetes	*Crepidotus albolanatus (1)* *Marasmiellus celebanticus (1)* *Pseudosperma notodryinum (1)* ***Pulveroboletus sinensis (1)***	
	Chytridiomycetes		*Clydaea vesicula (1)* *Protrudomyces lateralis (1)*
	Dothideomycetes	*Epicoccum polychromum (1)* *Muyocopron laterale (1)* *Poaceascoma filiforme (1)*	*Camarosporidiella melnikii (1)* *Cladosporium chasmanthicola (1)* *Dendryphion europaeum (1)* *Epicoccum polychromum (1)* *Gordonomyces mucovaginatus (1)* *Helminthosporium erythrinicola (1)* ***Paraphaeosphaeria michotii (1)*** *Stemphylium botryosum (1)*
	Microbotryomycetes	*Rhodosporidiobolus colostri (1)*	
	Saccharomycetes		*[Candida] boidinii (1)* *Nakaseomyces delphensis (2)*
	Sordariomycetes	*Diatrypella vulgaris (1)* *Neurospora lineolata (1)* *Ophiostoma pityokteinis (2)*	***Coniochaeta rosae (1)*** *Cordana bisbyi (1)* *Cordana ellipsoidea (1)*

	Vermiculariopsiella eucalypti (3)	*Diatrypella atlantica (1)* *Neurospora lineolata (5)* *Ophiostoma pityokteinis (1)* *Tristratiperidium microsporum (1)* *Vermiculariopsiella eucalypti (1)* *Xylotumulus gibbisporus (1)*
Ustilaginomycetes		*Violaceomyces palustris (1)*
NA	*Asteromyces cruciatus (1)*	
Number of ASVs	**16**	**27**

Table 4

Results of the statistical tests performed on summed abundance of Rhizobia on the one hand, and Glomeromycota on the other hand, for both rhizosphere and root compartment.

		Rhizobia			Glomeromycota		
		Df	Chisq	p	Df	Chisq	p
Rhizosphere	Rice system	1	20,043	**0,000**	1	26,159	**0,000**
	Zone	2	1,684	0,431	2	32,543	**0,000**
	Rice system * Zone		ns		2	282,909	**0,000**
Root	Rice system	1	1,090	0,297	1	106,760	**0,000**
	Zone	2	13,001	**0,002**	2	1999,660	**0,000**
	Rice system * Zone	2	6,069	**0,048**	2	20,710	**0,000**

The interaction between rice growing system and geographical zone was first included in the model, but then removed if non significant (indicated as 'ns' in the table). Mixed models (using the functions: glmer with poisson family) were used for 16S data, analyzed at the plant level, in order to include the field sampled as random effect, while no random effect were used for ITS data (functions: glm with poisson family).