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1 The impact of the rice production system (irrigated vs lowland) on root-

2 associated microbiome from farmer's fields in western Burkina Faso

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20 Abstract

21 As a consequence of its potential applications for food safety, there is a growing interest in 22 rice root-associated microbial communities, but some systems remain understudied. Here, we 23 compare the assemblage of root-associated microbiota in rice sampled in 19 small farmer's 24 fields from irrigated and rainfed lowlands in western Burkina Faso, using an amplicon 25 metabarcoding approach 16S (Prokaryotes, three plant sample per field) and ITS (fungi, one 26 sample per field). In addition to the expected structure according to the root compartment 27 (root vs. rhizosphere) and geographical zones, we show that the rice production system is a 28 major driver of microbiome structure, both for prokaryotes and fungi. In irrigated systems, we 29 found a higher diversity of prokaryotic communities from rhizosphere and more complex co-30 occurrence networks, compared to rainfed lowlands. Core taxa were different between the two 31 systems, and indicator species were identified: mostly within Bacillaceae and 32 Bradyrhizobiaceae families in rainfed lowlands, and within Burkholderiaceae and 33 *Moraxellaceae* in irrigated areas. Finally, phylotypes assigned to putative phytobeneficial and 34 pathogen species were found. Mycorrhizal fungi Glomeromycetes abundance was higher in 35 rainfed lowlands. Our results highlight deep microbiome differences induced by contrasted 36 rice production systems that should consequently be considered for potential microbial 37 engineering applications.

38 Key words

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

41 Introduction

42 Soil and rhizosphere host megadiverse and dynamic communities of microorganisms that are 43 crucial to the plants they associate with. Their role is particularly recognized for crops as the 44 below-ground microbiota supply plants with nutrients and provide protection against 45 pathogens (Singh et al. 2020; Chialva et al. 2021). Recent research suggests that root-46 associated microbes can improve plant tolerance to environmental stressors (Chialva et al. 47 2020), and modify phenology (Lu et al. 2018) and morphological traits (Senthil Kumar et al. 48 2018). Cultivated plants and their associated microbial communities are thus increasingly 49 studied jointly, as holobionts (a concept reviewed by Vandenkoornhuyse *et al.* in 2015), 50 because a deeper understanding of their interaction might help to develop microbial 51 engineering applications for modern sustainable agricultural systems (Chialva et al. 2021). 52 While much progress has been made, the mechanisms that control root-associated 53 microbiome assembly (i.e., structure, composition and dynamics) remain difficult to 54 disentangle (Brunel et al. 2020). Vertical transmission of the microorganisms (i.e., across 55 plant generation) exists as well as the horizontal transmission (i.e., recruitment from the soil 56 "seed bank"), with variable contributions of the seed and soil to the seedling microbiome 57 (Rochefort et al. 2021; Walsh et al. 2021). Beyond the environmental drivers known to shape 58 bulk soil communities (e.g., climate, soil properties, agricultural practices; see Vieira et al. 59 2020), the influence of the cultivated plant in structuring communities is more and more 60 documented and now referred as the extended root phenotype (see de la Fuente Cantó et al. 61 2020). Indeed, while the root-associated micro-habitats (i.e., rhizosphere, rhizoplane, 62 endosphere) modulate the intensity of assembly processes (Beattie, 2018), the plant identity 63 (e.g., species, genotype, age, etc.) plays a major role in recruiting specific microbial taxa 64 shared across multiple environmental conditions (Schweitzer et al. 2008).

65 Rice is the most important food crop in the world, grown in variable climatic conditions and 66 representing the staple food of more than half of the world's population, mostly in Asia, 67 Africa and Latin America (Pandey et al. 2010). The major species cultivated worldwide is 68 Oryza sativa L. (known as 'Asian rice'). This is also true in Africa, where a second rice 69 species was domesticated (O. glaberrima, referred to as 'African rice'), but is much less 70 grown because of lower yield (Linares, 2002). Given its importance for food security, and the 71 impact of microbiota on plant productivity, it is not surprising that many recent studies used 72 metabarcoding approaches to describe the microbiome of O. sativa (Kim & Lee, 2020),

73 particularly its root-associated microbial communities (reviewed by Ding *et al.* in 2019). Rice 74 has the particularity to be cultivated in flooded paddy soils over most growth stages, so that 75 the rhizosphere is located in an oxic-anoxic interface (Ding et al. 2019). Microbial 76 communities inhabiting rice roots are distinct from those found in other crops, with for 77 example an enrichement in Deltaproteobacteria, Euryarchaeota, Chytridiomycota (Ding et al. 78 2019). On the other hand, like for other crops, their structuring is driven both by the host plant 79 (in terms of root compartment / microhabitats, plant genotype) and its environment 80 (geographical zone, bioclimate, soil properties, agricultural practices; Ding et al. 2019). In 81 terms of microhabitats, differences between rhizophere and endosphere compartments are 82 clear (see e.g. Edwards et al. 2015; Guo et al. 2021) and recent evidence shows differences in 83 microbiota composition between root types and along root axes (Kawasaki et al. 2021). An 84 effect of host genotype (subspecies/cultivars) were evidenced in some cases (Edwards et al. 85 2015; Alonso et al. 2020), but it is generally weak compared to other factors, or even absent 86 (Edwards et al. 2018; Guo et al. 2021). Environmental factors, such as the geographical zone 87 and agricultural practices, are important drivers of microbiota structuring as well. For 88 example, Edwards et al. (2015) evidenced an effect of geographical location and cultivation 89 practices, namely organic vs conventional farming. Other environmental factors, such as 90 drought stress (Santos-Medellín et al. 2017), water management (Chialva et al. 2020), 91 phosphorus (Long & Yao, 2020), were also shown to affect rice root-associated microbiota. In 92 addition, bacterial and archaeal communities evolve during the vegetative phase of plant 93 growth, then shift and stabilize compositionally at the transition to reproductive growth at 94 flowering stage (Edwards et al. 2018). Vertical transmission through seeds seems quite weak 95 or even absent (Guo et al. 2021). However, the rice microbiome has been poorly explored in 96 African context in spite of (1) the importance to investigate a diversity of geographical areas 97 (as a consequence of the effect of geographical zone, see above) and document the diversity 98 of cultural practices, and (2) the growing importance of rice in Africa (recent surge in rice 99 consumption with 8% yearly increase from 2009 to 2019, Soullier et al. 2020). An exception 100 is the recent work by Kanasugi et al. (2020) that evidenced an effect of the region in 101 structuring of rice microbiome described in six tropic savanna regions in Ghana. More 102 generally, there is a lack of knowledge concerning crop-associated microbiota in the African 103 continent, that results in a biased view of the microbial world associated with crops due to 104 worldwide sampling repartition (Brunel et al. 2020; Hughes et al. 2021), and it is of particular 105 concern due to the potential of microbial engineering for future agriculture.

106 Rice is grown around the world in a diversity of rice-growing systems, three of them being the 107 most important (Rao et al. 2017). First, irrigated lowlands, with full water control, produce 108 75% of the global rice production. Second, rainfed lowlands (including flood prone), 109 represents around 19% of the world's rice production. Finally, rainfed upland rice, only 110 possible under high rainfall, results in 4% of the global total rice production. In Burkina Faso, 111 irrigated rice represents small areas (costly infrastructures representing less than 30% of 112 harvested areas; CountrySTAT, 2020), but produces more than half of the national rice 113 production (MAHRH, 2011). On the other hand, rainfed lowlands represent the majority of 114 rice growing surfaces (67% between 1984 and 2009), but only 42% of the production as a 115 consequence of lower yields compared to irrigated areas (MAHRH, 2011). Irrigated areas 116 (IR) and rainfed lowlands (RL) host different agricultural practices in West Africa (Nonvide 117 et al. 2018). In western Burkina Faso in particular, these contrasted practices have been 118 documented, showing that the possibility to grow rice twice a year was restricted to irrigated 119 areas, while direct sowing was performed only in rainfed lowlands, and that mineral 120 fertilization was more frequent in irrigated areas (Barro et al. 2021). In terms of the host plant 121 genetic diversity however, a study led in six locations from western Burkina Faso revealed 122 few differences between irrigated areas and rainfed lowlands in general, except in one of the 123 study sites, the rainfed lowland of Karfiguela, highly differentiated from the other five sites 124 (Barro et al. 2021).

125 This study aims at describing rice root-associated microbial communities in farmer's fields 126 from western Burkina Faso. More specifically, we investigate whether rice roots from two 127 contrasted rice growing systems (irrigated and rainfed lowland areas) host different microbial 128 communities. To this aim, we collected rice root and rhizosphere samples in farmer's fields 129 from three geographical zones, each consisting of an irrigated rice-growing area and 130 neighboring rainfed lowlands. A previous study performed in the same study sites 131 documented more intensive agricultural practices in irrigated areas, compared to rainfed 132 lowlands (Barro et al. 2021). Considering the effect of intensification on belowground 133 biodiversity and microbial network complexity (Banerjee et al. 2019; Tamburini et al. 2020), 134 we hypothesize an effect of the rice growing system on root-associated microbial 135 communities diversity and complexity. If true, this may have consequences on plant health 136 (Wei et al. 2015), particularly in this system where rice diseases were shown to circulate at 137 higher levels in irrigated areas compared to rainfed lowlands (Barro et al. 2021).

138

139 Material and methods

140 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).

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.* 2021; see further details on the methodology and raw data at: https://doi.org/10.23708/8FDWIE). Rice genotyping data on samples collected in 2018 in these six sites are also available (see Barro *et al.* 2021).

153 Rice root sampling

154 Within the six sites, we investigated a total of 19 fields, with three fields per site, except in 155 Bama, the largest irrigated perimeter studied, where four fields were sampled (Fig. 1b). Each field studied corresponds to a square of approximately 25 meters on a side. Root and 156 rhizosphere sampling was performed at rice maturation, between October, 16th and December 157 3rd 2018. We chose this developmental stage based on a previous study showing that the rice 158 159 microbiome composition evolves during the growing season until a 'mature' microbiome at 160 the flowering stage (Edwards et al. 2018). Within each field, we sampled three plants located 161 on the square diagonal, with at least 5 meters distance. Sampling was performed with gloves 162 and scissors (ethanol sanitized between two sampling) and involved nodal, bases and seminal 163 roots (all sampled together). Roots were roughly shaken to remove non-adherent soil, and 164 placed in 50 mL sterile tubes containing sterile Phosphate Buffered Saline (PBS) solution for

165 a rapid (15s) rinse and then stored in another 50 mL sterile PBS-containing tube. We placed

166 the tubes in a cooler and then at 4° C when back to the laboratory, on the same day.

167 Soil physicochemical properties: data acquisition and analysis

168 The three geographical zones studied (Fig. 1a) are characterized by Lixisols (soils with 169 subsurface accumulation of low activity clays and high base saturation) according to the

170 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.

174 INERA/GRN service performed the analyses of soil samples according to a standardized 175 methodology. Briefly, soil physical properties were assessed by soil particle size distribution 176 following Bouyoucos (1962), pH was estimated according to AFNOR (1981), total organic 177 carbon with the Walkley & Black (1934)'s method, total concentrations of nitrogen with 178 Kjeldhal method Hillebrand et al. (1953), and finally phosphorus and potassium content as 179 described respectively in Novozansky et al. (1983) and Walinga et al. (1989). Then, cation 180 exchange capacity (CEC), a measure of fertility, nutrient retention capacity, and the capacity 181 to protect groundwater from cation contamination, was estimated, as well as sorptive 182 bioaccessibility extraction (SBE), that relates to the environmental mobility, partitioning and 183 toxicity of soil pollutants, following Metson (1956). Soil data are publicly available on the 184 IRD Dataverse : https://doi.org/10.23708/LZ8A5B.

185 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

193 rhizoplane. DNA extraction from the rhizosphere and root samples were performed on the 194 same day as the process of compartment separation.

195 For DNA extractions, 0.25g of root samples (crushed in liquid nitrogen beforehand) and 0.25g 196 of rhizosphere were extracted using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany), 197 following manufacturer's recommendations. DNA quality and quantity was verified using a 198 NanoDrop ND-1000 spectrophotometer. PCR amplification, library and MiSeq Illumina 199 sequencing were performed by Macrogen (Seoul, South Korea) using primers 341F (16S 200 V3F, 5'-CCTACGGGNGGCWGCAG-3') 785R (16S V4R. 5'and 201 GACTACHVGGGTATCTAATCC-3') to amplify the V3 -V4 regions of the 16S rRNA gene, 202 and using primers ITS1f: CTTGGTCATTTAGAGGAAGTAA and ITS2: 203 GCTGCGTTCTTCATCGATGC to amplify the internal transcribed spacer 1 (ITS1) region. 204 We specifically focused on the factors structuring prokaryotic communities (16S sequencing), 205 but were also interested to see whether similar tendencies hold for fungal communities (ITS 206 sequencing). We consequently chose the following approach: sequencing was performed for 207 each sampled plant (19 fields * 3 plants = 57 samples per compartment) for 16S sequencing 208 and for a composite sample (3 plant samples were pooled to result in one sample per field, so 209 that the total number of samples per compartment is 19) for the ITS marker. Negative controls 210 (three for 16S 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.

213 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* 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, and paired sequences were trimmed. Sequences were dereplicated, and the unique sequence pairs were denoised using the *dada* function. Primers and adapters were screened and removed using a custom script with *cutadapt* (Martin, 2011). Next, paired-end sequences were merged, and chimeras were

223 removed. Contaminants were identified and removed using negative controls and the 224 decontam package. Rarefaction curves were drawn for each sample, using the rarecurve 225 function of the *vegan* R package, and the rarefaction plateau was reached for all samples (Fig. 226 S2). To account for differences in sequencing depths, samples were rarefied to 4236 and 7828 227 for 16S and ITS, respectively. Taxonomy assignments were determined against the UNITE 228 2021 (Abarenkov et al. 2021) and the SILVA SSU r138 (Quast et al. 2013) taxonomic 229 databases for ITS and 16S, respectively using the *idtaxa* function from the *decipher* R 230 package. Mitochondria and chloroplast sequences were then removed. ASVs not seen more 231 than once in at least 2% of the samples were removed. We then obtained 2 116 969 (8260 232 ASVs) final sequences for 16S and 172 719 (566 ASVs) sequences for ITS. A figure showing 233 the phyla relative abundances was constructed and presented according to the rice-growing 234 system, site and 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 and ITS communities (including both rhizosphere and roots compartments) in each rice grown system using the prevalence threshold of 60%.

240 For 16S dataset only, we inferred co-occurrence networks using the *SpiecEasi* pipeline (Kurtz 241 et al. 2015), independently for each rice growing system: rainfed lowland and irrigated areas. 242 Networks were calculated for ASVs present in more than 15% of the samples using the 243 method 'mb' and setting the lambda.min.ratio to 1e-3 and nlambda to 50. We identified hub 244 taxa, i.e. the potential keystone of the microbial network belonging to the most connected 245 ASVs, based on their node parameters (method developed by Berry & Widder, 2014): a low 246 betweenness centrality (lower quantile, < 0.9), and a high closeness centrality (higher 247 quantile, > 0.75), transitivity (higher quantile, > 0.25) and degree (higher quantile, > 0.75). 248 The node and network parameters were determined using the R package *igraph* (Csardi & 249 Nepusz, 2006) and *qgraph* (Epskamp *et al.* 2012). Complete networks were further described 250 by calculating the number of nodes and hubs, the network mean degree, mean closeness and 251 betweenness centralities, the total number of edges, and the positive to negative edges ratio. 252 Core taxa (prevalent in more than 60% of the samples) were also identified for each system.

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 and ITS ASVs in order to search the genus or species names of a number of pathogen species listed a priori (Table S6), 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 and ITS ASV assigned to a species name's including "oryz", as this may correspond to a specific interaction (pathogen or beneficial) with rice.

261 Statistical analyses

262 We first analyzed soil physico-chemical parameters. To this purpose, PERMANOVA were 263 performed on soil physical properties (texture, i.e. relative amount of sand, silt, and clay) on 264 the one hand, and soil chemical properties (7 variables: pH, total carbon, total nitrogen, total 265 phosphorus, total potassium, SBE, CEC) on the other hand. For both models, we included as 266 explanatory factors the 'geographical zone' and 'rice-growing system', as well as their 267 interaction, using *adonis2* function from the *vegan* R package (Oksanen *et al.* 2007), with 999 268 permutations. Posthoc tests were done using the pairwiseAdonis function 269 (https://github.com/pmartinezarbizu/pairwiseAdonis). In addition, we performed Kruskal-270 Wallis non-parametric tests on each soil variable independently, testing for an effect of the 271 geographical zone or the rice growing system, using the *kruskal_test* function from the *rstatix* 272 package. Dunn tests (dunn_test function) were then performed in case of significant effect 273 evidenced to identify statistically differing groups.

274 For microbial communities, PERMANOVA were used to test for significant effects of root 275 compartments, geographical zones and rice growing systems on microbial β-diversity, based 276 on a Bray-Curtis dissimilarity matrix. Sites differences were further tested using the 277 pairwise.adonis function of the same vegan package. The graphical representation of β -278 diversity was based on Non-metric Multi-Dimensional Scaling (NMDS, *metaMDS* function). 279 The effect of edaphic variables (i.e., pH, total organic carbon, total phosphorus, total nitrogen, 280 total potassium, CEC and SBE) in structuring β -diversity was tested using the *envfit* function 281 (9999 permutations) in R package *vegan*. Structuring soil properties were thus fitted onto the 282 ordination space.

We tested for an effect of the rice growing system on obtained indices of alpha diversity (Shannon diversity index and observed richness). To this purpose, we performed nonparametric statistical tests (*kruskal_test* function from the library *rstatix*) independently for

each kingdom (prokaryotes and fungi respectively) and each compartment (rhizosphere and
root associated). In addition, for 16S data only (because of insufficient sample size for ITS),
we also tested for an effect of the specific site, using Kruskal-Wallis test, and then performed
posthoc tests using *dunn_test* function.

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.

294 Finally, we analyzed the repartition of ASVs assigned to the class *Glomeromycetes* 295 (arbuscular mycorrhizal fungi, AMFs). The summed abundance of all ASVs which best blast 296 hit is within the *Glomeromycetes* class was modeled by generalized linear mixed models 297 (GLMMs), with Poisson distribution, with the package *lme4* (Bates et al. 2015), followed by 298 Type III ANOVA with the package *car* (Fox & Weisberg, 2019). The compartment and the 299 rice growing system were included as fixed effects and the geographical zone as a random 300 effect. In addition, differential repartition analysis was performed using DESeq2 (Love et al. 301 2014) to identify *Glomeromycetes* ASVs showing significant enrichment in one of the two 302 rice growing systems.

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

304

305 **Results**

306 Structure of rice soil properties and rice root associated microbial communities

307 PERMANOVA analysis performed on the Bray-Curtis distance matrix of soil characteristics 308 to describe the overall soil properties, highlighted no significant influence of the rice growing 309 system, but a differentiation according to the geographical zone, for both physical (F = 6.420, 310 r^2 = 0.448, *p* = 0.006, Fig. 1b) and chemical (F = 4.121, r²= 0.346, *p* = 0.026) soil parameters. 311 More precisely, we found no effect of the rice growing system but a significant effect of the 312 geographical zone on the clay and sand contents, as well as total Phosphorus, total Potassium,

313 SBE and CEC (Table S1). Posthoc tests revealed that the geographic zones that differed 314 statistically were the same for the six above-mentioned variables, namely Banzon and 315 Karfiguela zones (Fig. 1c).

316 To determine whether the geographical zones (Karfiguela, Bama or Banzon), the rice-growing 317 systems (irrigated vs. rainfed lowlands), or their interactions, structured root-associated or 318 rhizosphere microbial communities, we performed PERMANOVA analysis on the Bray-319 Curtis distance matrix of 16S and ITS ASVs, respectively (Table S2, Fig. S2). As different 320 structures were revealed between root and rhizosphere communities 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 321 322 both datasets to observe the relative influence of the rice-growing systems and the 323 geographical zones in shaping root and rhizosphere communities separately (Table 1, Fig. 2).

324 In the rhizosphere, prokaryotic communities were mainly structured by the rice growing system (F=5.096, $r^2 = 0.079$, p < 0.001, Table 1), the geographical zone (F=2.118, $r^2 = 0.069$, 325 p < 0.001) and the interaction between rice growing system and zone (F=1.877, r² = 0.058, p 326 327 < 0.001). Posthoc tests revealed that most of the pairs of sites (i.e., irrigated and lowland from 328 the same geographical zone) were significantly different, but interestingly revealed no 329 significant difference between communities originating from irrigated systems, whereas all 330 communities from rainfed lowland sites exhibited distinct structures (Table S3). Fungal 331 communities of the rhizosphere were also mostly structured by the rice growing system (F=2.452, $r^2 = 0.115$, p < 0.001, Table 1). The geographical zone (F=1.555, $r^2 = 0.146$, p = 0.146332 333 0.007) and the interaction between rice growing system and the geographical zone (F=1.335, $r^2 = 0.126$, p = 0.036) were also driving the rhizosphere fungal microbiome. The low number 334 of samples did not allow to detect, if any, statistically significant differences between sites in 335 336 communities' structures for ITS (Table S3).

As observed for rhizosphere communities, the root-associated prokaryotic communities were 337 mainly shaped by the rice growing system (F=5.155, $r^2 = 0.079$, p < 0.001, Table 1), the 338 interaction between rice growing system and the geographical zone (F=2.451, $r^2 = 0.075$, p =339 0.002) and the geographical zone (F=2.247, $r^2 = 0.069$, p < 0.001). Most of the pairs of sites 340 341 (from the same geographical zone) were significantly different. No significant difference was 342 detected between communities originating from irrigated systems, whereas all communities from rainfed lowland sites exhibited distinct structures (Table S3). Root-associated fungal 343 communities were also mostly influenced by the rice growing system (F=2.289, $r^2 = 0.111$, p 344

345 = 0.002, Table 1), and by the interaction between rice growing system and the geographical 346 zone (F=1.457, r^2 =0.141, p = 0.013). The effect of the geographical zone on root-associated 347 fungal communities was not evidenced (F=1.206, r^2 = 0.117, p =0.123). As for rhizosphere,

348 posthoc tests on root-associated fungal communities were all non-significant (Table S3).

349 The influence of soil chemical parameters on microbial community structure is reported in 350 Fig. 2 as arrows and in Table S4. We noticed that the prokaryotic communities of both 351 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 352 rhizosphere, and $r^2 = 0,204$, p = 0,003 for roots) and total phosphorus ($r^2 = 0,132$, p = 0,023 353 for rhizosphere, and $r^2 = 0.179$, p = 0.004 for roots). For fungi, although various parameters 354 355 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). 356

357 Composition of rice root microbiomes and comparison of alpha-diversity

While 16S 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. S4). 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 Basicomycota.

364 We tested the effect of the rice growing system on the diversity indices (alpha-diversity). No 365 effect could be evidenced on the fungal (Shannon: H = 0.026, p = 0.87 for the rhizosphere, and 366 H = 0.107, p =0.74 for root-associated communities), or root-associated prokaryote diversities 367 (Shannon: H = 1.05 p = 0.306). On the other hand, the rice growing system had a significant 368 effect on the prokaryote diversity of the rhizosphere (Shannon: H = 11.6, p <0.001), with a 369 higher Shannon diversity index in irrigated areas (5.03 ± 0.13) , compared to rainfed lowlands 370 (4.39 ± 0.12) and a higher observed richness (275.6 ± 26.4) in irrigated areas compared to 371 rainfed lowlands (143.7 \pm 17.4) (Fig. 3). We noticed however an opposite pattern for fungal communities of the rhizosphere with higher observed richness in rainfed lowlands, compared 372 373 to irrigated areas (Fig. 3).

374 This effect of the rice growing system on 16S rhizosphere data was also clearly observed 375 when plotting the diversity indices by site (Fig. S5). In addition, we found that the specific 376 site had an effect on the prokaryotic communities of the rhizosphere, and also, but to a lesser 377 extent, in roots (Fig. S5 and Table S5). In the rhizosphere, the highest diversity was found in 378 the irrigated perimeter of Karfiguela, and to a lesser extent in the irrigated area of Bama. A 379 particularly low diversity was found in the rainfed lowland of Karfiguela zone, and to a lesser 380 extent in the rainfed lowland of Banzon. Conversely, we noticed a slightly higher diversity in 381 fungal root associated communities in the rainfed lowland of Karfiguela (Fig. S5).

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

383 ASVs belonging to the core microbiome of lowland vs. irrigated rice were respectively 384 identified with a prevalence threshold set to 60%. For 16S, we identified 26 core ASVs 385 associated with the irrigated systems, and two core ASVs in lowlands (Fig. 4). Among the 386 core taxa in irrigated areas, the vast majority of phylotypes (25/26) belonged to the 387 Burkholderiaceae family, with 24 assigned to Ralstonia pickettii and one to Paraburkholderia 388 kururiensis. One of the core ASVs is common to both irrigated area and rainfed lowlands 389 systems. Its best blast hit corresponds to Bradyrhizobium tropiciagri (Bradyrhizobiaceae) 390 with a 99.5% sequence similarity. Another core phylotype in rainfed lowlands is assigned to 391 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 (Table 2). 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).

401 None of the identified hub taxa were also core in any of the two rice growing systems. Only
402 one ASVs was identified as a hub in both irrigated and rainfed lowland systems, assigned to
403 *Enterobacter mori* (Enterobacteriaceae). Hub taxa in irrigated areas (15 ASVs) were assigned

to 8 different species from 5 families, while hub taxa in rainfed lowland (20 ASVs) only

405 corresponded to 4 species from 2 families (Table 2).

406 Indicator taxa of the two rice growing systems

407 For 16S data, we found 128 indicator taxa in irrigated areas, including ASVs from eight 408 bacterial families, most of them assigned to Acinetobacter, Ralstonia, Aeromonas, 409 Comamonas, Clostridium and Enterobacter (Table 3). On the other hand, only 63 were 410 identified in rainfed lowlands, most of them within the Bacillaceae family, including ASVs 411 assigned to Exiguobacterium and Priestia, and Bradyrhizobiaceae family, genus 412 Bradyrhizobium (Table 3). The ASV assigned to Paraburkholderia kururiensis 413 (Burkholderiaceae) revealed as indicator in irrigated areas (Table 3) was also a core taxa in 414 irrigated areas. Also, among the 24 indicator ASVs in irrigated areas assigned to Ralstonia 415 pickettii (Burkholderiaceae), 16 were also core in irrigated areas. In addition, four indicator 416 ASVs in irrigated areas were also hubs in this system: two assigned to Aeromonas hydrophilai 417 (Aeromonadaceae), another assigned to Enterobacter cloacae (Enterobacteriaceae), and 418 finally one corresponding to Acinetobacter soli (Moraxellaceae). Three ASVs assigned to Priestia flexa (Bacillaceae) were hubs in rainfed lowlands. 419

420 For ITS data, we found 16 indicator taxa in irrigated areas, and 27 in rainfed lowlands (Table 421 3). Indicator taxa in irrigated areas were assigned to seven classes: Agaricomycetes, 422 Chytridiomycetes, Dothideomycetes, Geoglossomycetes, Leotiomycetes, Sordariomycetes, and 423 Ustilaginomycetes. Indicator taxa in rainfed lowlands were assigned to nine classes: 424 Agaricomycetes, Chytridiomycetes, Dothideomycetes, Glomeromycetes, Saccharomycetes 425 Schizosaccharomycetes, Sordariomycetes, Tremellomycetes, Ustilaginomycetes. One ITS 426 ASV identified as indicator taxa in irrigated, with best hit Pulveroboletus sinensis 427 (Agaricomycetes), was also core in this rice growing system, and two indicator taxa in rainfed 428 lowlands were also core in this system: one assigned to *Pseudobaeospora wipapatiae* 429 (Agaricomycetes) and the other to *Paraphaeosphaeria michotii* (Dothideomycetes).

430 Putative pathogen or phytobeneficial taxa

First, responses for an '*oryz*' query within assignation and blast, found matching records only
in the 16S 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*.

437 Next, we made a subset of the ITS dataset for ASVs assigned to the *Glomeromycetes* class (total of 14 ASVs). AMF summed abundance was affected by the compartment ($\chi^2 = 101.22$, 438 p < 0.001) and by the rice growing system ($\chi^2 = 951.12$, p < 0.001), with higher abundances in 439 440 rhizosphere compartment and in rainfed lowlands (Fig. 5). In addition, differential abundance 441 testing between rice growing systems detected an ASV assigned to Racocetra crispa as 442 preferentially found in rainfed lowlands (12FC = 24.59; p<0.001). We also noticed that 443 another Glomeromycetes (Dentiscutata savannicola) was identified as indicator taxa in 444 rainfed lowland environments (Table 3).

445 We then screened the list of all assigned ASVs for a set of pathogen species defined a priori 446 (see the list in the Table S6). For Prokaryotes (16S data), a number of ASVs corresponded to 447 the genera of pathogens, but only Burkholderia glumae (two ASVs), Acidovorax avenae (four 448 ASVs) and Dickeya chrysanthemi (six ASVs) were identified at the species level. These 12 449 ASVs identified at the species level were however only found in one sample. The 450 Xanthomonas genus was found, but with no assignation to X. oryzae (instead, assignation to 451 X. theicola which is phylogenetically closed to the rice associated X. sontii (Bansal et al. 452 2020). A similar situation was observed for the genera Pseudomonas, Pantoea, and 453 Sphingomonas. The same analysis of putative pathogens for ITS revealed the presence of the 454 following ten genera: Alternaria, Bipolaris, Ceratobasidium, Curvularia, Fusarium, 455 Helminthosporium, Microdochium, Rhizoctonia, Sarocladium. We notice that one ASV 456 whose best blast hit was Curvularia chonburiensis was core in both irrigated and rainfed 457 lowlands (Fig. 4).

458 **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, and that the diversity of prokaryotic community from the rhizosphere was higher in irrigated areas compared to rainfed lowland. 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 databases, this study shed light on some drivers of assemblage of rice root associated microbial communities in a sparsely documented African system.

469 The structuring of microbial diversity is affected by the rice growing system

470 Although Edwards et al. (2018) showed that the root-associated microbiome of distant field 471 sites converge in similarity during the growing season, our study performed at the maturity 472 stage of rice still evidenced some drivers of rice root-associated microbial communities 473 structure. First, as for most rice microbiome studies, we found an effect of the compartment / 474 micro-habitat (Edwards et al. 2015; Santos-Medellín et al. 2017; Guo et al. 2021; Kawasaki et 475 al. 2021), and the geographical zone (Edwards et al. 2015; Kanasugi et al. 2020) on the beta-476 diversity of rice root-associated microbiome. In addition, our study shows that the contrasted 477 rice-growing systems, namely irrigated perimeters vs rainfed lowlands, harbor contrasted rice 478 root-associated microbial communities, both for prokaryotic and fungal communities, and for 479 rhizosphere and root compartment. We notice that the soil physicochemical properties weakly 480 differ between irrigated areas and rainfed lowland, the soil composition was instead mostly 481 affected by the geographical zones. Consequently, we evidence a structuring effect of the rice-482 growing system that was only slightly related to contrasted soil physicochemical properties. 483 Our results are in line with a previous study comparing microbiomes from two contrasted 484 water management conditions (upland vs lowland rice) in controlled settings (a field 485 experiment in northern Italy), which showed differentiation in microbial communities, 486 particularly for root microbiome, and to a lesser extent in soil samples (Chialva et al. 2020).

Only a few other studies compared variable water management agricultural systems. Cui *et al.* (2019) showed that irrigation water quality affected bacterial community alpha and beta diversity in maize, with pH and available phosphorus being the major factors shaping microbiome soil composition. Mavrodi *et al.* (2018) evidenced a slight effect of the three seasons of irrigation on the overall diversity within the rhizosphere microbiome in wheat, but significant differences in the relative abundances of specific taxa.

In our study, some of the soil physicochemical parameters affected rice root-associatedmicrobial communities. In particular, CEC and SBE, that reflect soil exchange capacity and

495 bioaccessibility, were the most important soil parameters for the structure of both rhizosphere 496 and root prokaryotic communities. These parameters are not commonly measured, nor 497 identified as important, in other studies of the root-associated microbiome. Our results argue 498 for including them in soil chemical characterization, to investigate whether their impact in 499 microbiome structure is general or not. In addition, phosphorus content significantly 500 structured the prokaryotic communities, both in rhizosphere and roots. Such an effect of 501 phosphorus is known for the rice root associated microbiome (Long & Yao, 2020). On the 502 other hand, the soil chemical parameter evidenced in this study to structure fungal rhizosphere 503 communities was the total nitrogen (N). This is in accordance with a study by Chen et al. 504 (2019) showing that nitrogen input drives changes in the microbial root-associated community 505 structure in wheat. Moreover, Wang & Huang (2021) showed the effect of optimized N 506 application on fungal community structure from paddy soils. Kanasugi et al. (2020) also 507 evidenced an effect of soil nitrate on rice root fungal communities in Ghana.

508 Effect of rice growing system on alpha-diversity and network topology

We found a higher taxonomic diversity in irrigated areas, compared to rainfed lowlands, for prokaryotic communities of the rhizosphere. Our findings differ from Chialva *et al.* (2020)'s results, where the 16S diversity was similar in lowland and upland rice. Chialva *et al.* (2020) also show significantly higher ITS diversity in lowland rice compared to upland, which may relate to the tendency (not significant, maybe as a consequence of the low sample size for ITS) observed here in the rhizosphere.

It is important to note that our sampling was performed in farmer's fields, while most results, including those of Chialva *et al.* (2020), were obtained in field trials, potentially explaining the differences. Indeed, in our study, various factors, such as rice cultivars, fertilization regime and rotation, exhibit large variability. However, rice genetic diversity was shown to be comparable in irrigated areas and rainfed lowlands (Barro *et al.* 2021), 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.

522 Considering the irrigated areas as systems with more intensive agricultural practices, 523 compared to rainfed lowland, we expected an opposite pattern of microbiome diversity. 524 Indeed, agricultural intensification was shown to reduce microbial network complexity and 525 the abundance of keystone taxa in roots (Banerjee *et al.* 2019). In addition, the fertilization

526 regime is known to have strong impact on root-associated microbiota (Ding et al. 2019; Xiong 527 et al. 2021). Various studies showed that organic fertilization enhances microbial diversity 528 (Liu et al. 2020). For example, recommended fertilization preserved belowground microbial 529 populations, compared to the fertilization mostly used ('conventional fertilization') that 530 depressed bacterial diversity, in experiments performed in China (Ullah et al. 2020). We 531 considered the irrigated areas as more intensified systems, compared to rainfed lowland, 532 particularly because only irrigated areas allow growing rice twice a year, and because only 533 rainfed lowland sites presented fields with no mineral fertilization at all (Barro et al. 2021). 534 We noticed however that organic fertilization remained rare, and its frequency was not 535 drastically affected by the rice growing system. Finally, transplantation was always performed 536 in irrigated areas, while direct sowing was the most common practice in rainfed lowlands.

537 On the other hand, paddy soils studied in western Burkina Faso (all over the six sites) are 538 particularly poor if compared for example to a study of more than 8 000 soils in Hunan 539 Province (Duan et al. 2020), where average organic carbon was 1.972%, compared to 0.922% 540 in our study, total nitrogen was 0.191%, higher than 0.072%, and total phosphorus was 0.71g.kg⁻¹, compared to 0.24g.kg⁻¹. The studies previously cited evidencing fertilization 541 542 effects, were performed in soils with higher carbon and nitrogen contents (see for example 543 Ullah et al. 2020, where minimum average organic carbon was 2% and total nitrogen 0.1%). 544 The effect of fertilization on microbial diversity may actually depend on various aspects, 545 including the soil type. Notably, a positive relationship was found between rice fertilization 546 and soil bacterial richness and diversity in a 19-years inorganic fertilization assay in a reddish 547 paddy soil in southern China (Huang et al. 2019); while Wang & Huang (2021) showed an 548 effect of the fertilization on paddy soils microbial community composition but no effect on 549 the diversity. In poor soil systems such as in this study, fertilization input may actually 550 increase microbial diversity.

Finally, a complementary hypothesis could be the higher fragmentation of rainfed lowlands compared to irrigated areas. Indeed, irrigated sites correspond to larger areas cultivated in rice, possibly with two rice seasons per year, so that rice fields are likely to be more connected to each other than in rainfed lowlands. Higher connectivity generally leads to higher biodiversity (Fletcher *et al.* 2016). The principles of metacommunity theory could also be applied to micro-organisms, with reduction in host habitats and fragmentation potentially

557 increasing extinction rates (Mony *et al.* 2020), but as our study misses an explicit 558 characterization of the rice landscape structure, this hypothesis could not be formally tested.

559 Distant rainfed lowlands differ more than distant irrigated perimeters

560 Our results showed that the prokaryotic communities in the rice rhizosphere and roots from 561 the three irrigated sites do not differ significantly from each other. On the other hand, the 562 same analysis revealed significant differentiation between the three rainfed lowland study 563 sites (in all three cases for rhizophere and two out of three comparisons in roots). Also, we 564 found very few core phylotypes in rainfed lowland, with only two core ASVs for 16S, what 565 reinforces the above-mentioned observation. These results are likely driven by a higher 566 heterogeneity between rainfed sites, in terms of water control, agricultural practices or rice 567 genotypes.

568 Indeed, in irrigated rice, the farmer has the potential to control irrigation water during the 569 whole growing season. On the other hand, irrigation in rainfed lowland is dependent on 570 precipitations that differ between the three geographical zone sampled within the rice growing 571 season. In addition, we showed a high heterogeneity of agricultural practices in rainfed 572 lowlands: for example, legume rotation was common in the rainfed lowland of Bama zone, 573 but rare or absent in the two other rainfed lowland sites, and organic fertilization was more 574 frequent in the rainfed lowland of Karfiguela zone, than in the other sites (Barro et al. 2021). 575 Finally, in terms of rice genetics, a high rice genetic differentiation was found between the 576 rainfed lowland site of Karfiguela zone and the five sites: a distinct genetic group O. sativa 577 Aus, and other distinct landraces were found in this peculiar site, compared to the five others 578 where only O. sativa indica was grown (Barro et al. 2021). These specificities of the rainfed 579 lowland from Karfiguela zone, in terms of rice grown and agricultural practices, may also 580 drive its specific patterns of alpha diversity, with a particularly low prokaryote diversity (in 581 rhizosphere and also, in a lesser extent, in roots), and a tendency for higher fungal diversity in 582 roots.

583 Our sampling size was much lower for ITS and this likely explains the absence of such a 584 pattern, with no significant differences obtained between pairs of sites. Alternatively, the 585 pattern may be different for fungal diversity, as suggested by the higher number of core taxa 586 in rainfed lowlands than in irrigated areas.

587

588 Identification of core microbiota and hub phylotypes

589 The prevalent taxa, indicator taxa and hubs may be considered as having an important 590 ecological role in microbiome assembly and ecosystem functions (Banerjee et al. 2018). In 591 this study, we identified the core prokaryote and fungal microbiota in both irrigated and 592 rainfed lowland environments. While four fungal taxa were found to be cores in both systems, 593 only one bacterial core taxa was shared between the two rice growing systems: assigned to 594 Bradyrhizobium tropiciagri, a nitrogen-fixing symbiont isolated from tropical forage legumes 595 (Delamuta *et al.* 2015), known as rice root endophytes capable of fixing N_2 (Chaintreuil *et al.* 596 2000; Ding et al. 2019). In addition, the core taxa in irrigated areas likely includes 597 Paraburkholderia kururiensis, a bacterium with potential phytobeneficial properties 598 (bioremediation, biofertilization and biocontrol of pathogens; Dias et al. 2019). Various ASVs 599 identified as core in irrigated areas were assigned to Ralstonia pickettii, an ubiquitous 600 Betaproteobacteria found in water and soil, and capable to thrive in low nutrient 601 (oligotrophic) conditions (Ryan et al. 2007). Isolated from the plant rhizosphere, R. pickettii 602 injected in tomato stem could reduce bacterial wilt disease caused by its congeneric pathogen 603 *R. solanacearum*, and could consequently be considered as potential biocontrol agent (Wei *et* 604 al. 2013). Noteworthy, this bacterium is also described as human emerging pathogen, causing 605 nosocomial infections (Ryan et al. 2006).

One ASVs assigned to *Enterobacter mori* (Enterobacteriaceae) was identified as hub of the 16S-based co-occurrence networks both in irrigated areas and rainfed lowland site. On the other hand, most of the bacterial taxa identified as hubs differed between irrigated areas and rainfed lowlands reflecting a highly contrasted structuring of bacterial communities in the two rice growing systems.

611 Characterization of indicator taxa in each rice growing system and contrasted612 repartition of AMFs

We identified indicator taxa for each rice growing system, most of them being in irrigated for procaryotes (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

617 Paraburkholderia kururiensis and Ralstonia pickettii as well as Aeromonas hydrophila,
618 Enterobacter cloacae and Acinetobacter soli. In rainfed lowlands, it was the case for Priestia
619 flexa. In particular, we notice that Acinetobacter soli was identified as potent phosphorus
620 solubilizer in rice and consequently promising for plant growth promotion (Rasul et al. 2019).

621 For fungi, we identified as both indicator and core taxa: Pulveroboletus sinensis in irrigated 622 areas, as well as both Pseudobaeospora wipapatiae and Paraphaeosphaeria michotii in 623 rainfed lowlands. Eight ASVs assigned to Chytridiomycetes were identified as indicator 624 species in rainfed lowland systems. Members of this fungal division of aquatic fungi (Barr, 625 2001) were found in the rhizosphere compartment in our study. They are known as 626 particularly abundant in microbial communities associated with rice roots, compared to other 627 crops (Ding et al. 2019) and were preferentially associated to lowland conditions compared to 628 upland (Chialva et al. 2020).

629 We identified a few potentially beneficial taxa that could be investigated further. In particular, 630 AMFs of the class *Glomeromycetes* were found preferentially in rainfed lowlands, with one 631 ASV, Racocetra crispa, enriched in rainfed lowland system compared to irrigated areas, and 632 one ASV, Dentiscutata savannicola, identified as indicator in rainfed system. This was 633 expected considering the lower frequency of mineral fertilization in rainfed lowlands 634 compared to irrigated areas. Indeed, AMF colonization was shown to be affected by farming 635 regimes: the rice roots cultivated in the conventional agrosystem (N and P fertilization and 636 pesticides) or under permanent flooding showed no AMF colonization, while the rice plants 637 grown with organic conditions showed typical mycorrhization patterns (Lumini et al. 2011).

638 Towards the identification of putative pathogens and the study of interactions

639 between microbiome and diseases

640 Various pathogen species are suspected from the sequence variants identified in this study. In 641 particular, the 16S dataset contained ASVs assigned to Burkholderia glumae, Acidovorax 642 avenae and Dickeya chrysanthemi. All three remained rare, with only one sample containing 643 each of these sequences. The presence of B. glumae was described in Burkina Faso (but with 644 no molecular data; Ouedraogo et al. 2004), and targeted detection performed in two sites 645 failed to detect B. glumae and A. avenae (Bangratz et al. 2020). For fungal pathogens, we 646 found ASVs assigned to ten genera comprising rice pathogens, including *Bipolaris*, 647 Curvularia, Fusarium and Rhizoctonia. It has to be noted that the major rice pathogens

648 causing foliar diseases, namely Pyricularia oryzae and Xanthomonas oryzae, known from 649 symptom observations to be present in the study sites (Barro et al. 2021), were not detected in 650 this root-associated metabarcoding data. Some of these putative fungal pathogens are 651 frequent, particularly one, whose best blast hit is Curvularia chonburiensis, identified as core 652 taxa in both irrigated perimeters and rainfed lowlands. Various *Curvularia* species are known 653 to be pathogenic in rice, potentially causing contrasted symptoms (Gao et al. 2012; Majeed et 654 al. 2015), and their widespread repartition evidenced here argues for more work in plant 655 pathology to better understand the interactions between *Curvularia* and rice.

656 The literature shows that higher microbiome diversity may be associated with a lower 657 infection rate (see for example Rutten et al. 2021). Our results for rice from western Burkina 658 Faso are somehow opposite as irrigated perimeters harbor more diverse prokaryotic 659 communities of rhizosphere compared to rainfed lowlands (this study), but also higher 660 prevalence of major rice diseases, particularly bacterial leaf streak and the fungal rice blast 661 disease, based on the observation of foliar symptoms (Barro et al. 2021). On the other hand, 662 for fungal communities, the pattern may actually be opposite; a tendency for higher diversity 663 in rainfed lowlands is observed but low sample size prevents from obtaining significant 664 results. Also, some diseases, such as the viral yellow mottle disease (Barro et al. 2021), are 665 not affected by the rice growing system but by the specific site. The relationship between the 666 diversity of root-associated microbiome and diseases is complex and remains to be studied in 667 more details, including under controlled conditions.

668 More generally, scientific interest in the relationship between root-associated microbiota and 669 plant diseases is growing (Vannier et al. 2019; Trivedi et al. 2020). In rice, various studies 670 evidenced an inhibition of disease development by root-associated micro-organisms (see 671 Yasmin et al. 2016 for bacterial diseases; and Spence et al. 2014; Law et al. 2017 for rice 672 blast). On the other hand, disease was shown to affect root-associated microbiomes, with for 673 example the effect of *Magnaporthe grisea* inoculation on microbial endosphere diversity 674 (Tian et al. 2021). A promising avenue of research is consequently to investigate the 675 relationship between root-associated microbiota and rice diseases in the particular rice-676 growing systems of Burkina Faso.

677

678 Perspectives

679 We are only at the beginning of understanding the complexity of rice root microbial 680 communities, especially for rice cultivation in Africa. The originality, but also a limitation of 681 our study, lies in the fact that the samples were collected in farmer fields, and it globally 682 compares the two contrasting rice production systems that differ in various management 683 practices, so that it could not tease apart the specific effect of each individual factor (water 684 management, variety, fertilization, pesticides, etc). More investigations are now required to 685 decipher each structuring factor at a smaller scale: in particular between fields within each 686 site, where the rice cultivar and specific agricultural practices are likely to play a significant 687 role (Delitte et al. 2021).

688 Describing rice microbiota through metabarcoding is a first mandatory step that needs to be 689 combined with culturomics for a greater accuracy and a deeper description, in particular in 690 such systems where some taxa are poorly described in taxonomic databases. Experimental 691 work in an integrative approach is also required to move on towards microbiota management 692 methodologies. Such microbiota-based strategies could contribute to improving rice health 693 and productivity (Sessitsch & Mitter, 2015), while preserving human health. They are 694 consequently an important component of the toolbox of science-based strategies to achieve 695 zero-hunger in Africa.

696

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709

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- 960 Growth Promotion and Suppression of Bacterial Leaf Blight in Rice by Inoculated Bacteria.
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962

963 **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/ExternalWorld-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 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.



Figure 4 Venn diagram representing the core sequence variants for each rice growing system : irrigated vs rainfed lowlands. A. For prokaryotes B. For fungi



Figure 5 Spatial repartition of summed abundance of 14 ITS ASVs assigned to the class *Glomeromycetes* in the two rice root microbiome compartments (rhizosphere and roots), and in each rice growing system (irrigated perimeters vs rainfed lowlands).

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.

			Prol	caryotes 10	6S	Fungi ITS					
,		Df	SumOfSqs	R2	F	Pr(>F)	Df	SumOfSqs	R2	F	Pr(>F)
	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
Rhizosphere	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

	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
Root	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

Properties of co-occurrence networks of Prokaryote taxa in rhizosphere and root-associated samples from irrigated areas in the one hand, and rainfed lowlands in the other hand.

	Irrigated areas	Rainfed lowlands
Number of nodes	414	313
D	0.002 . 0.012	
Degree	9.802 ± 2.913	7.974 ± 2.619
Closeness	0.00076 ± 0.00006	0.00093 ± 0.00009
Betweenness	450.271 ± 336.298	389.978 ± 370.222
Total edges	2029	1248
(Positive + Negative edges)	(1760 + 269)	(1163 + 85)
Number of hubs	15	20
Hub's families	Hub species (Number of ASV)	
Aeromonadaceae	Aeromonas hydrophila (2)	
Bacillaceae		Bacillus zanthoxyli (6)
		Neobacillus cucumis (5)
		Priestia flexa (4)
Bradyrhizobiaceae	Bradyrhizobium oligotrophicum (1)	
	Enterobacter cloacae (2)	
Enterobacteriaceae	Enterobacter hormaechei (2)	
	Enterobacter mori (5)	Enterobacter mori (5)
Moraxellaceae	Acinetobacter modestus (1)	
	Acinetobacter soli (1)	
Weeksellaceae	Elizabethkingia anophelis(1)	

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
	Acidobacteriaceae		Occallatibacter savannae (2)
	Aeromonadaceae	Aeromonas hydrophila (7)	
	Bacillaceae		Bacillus zanthoxyli (1)
			Priestia flexa (10)
			Exiguobacterium acetylicum (25)
			Exiguobacterium indicum (19)
	Bradyrhizobiaceae		Bradyrhizobium tropiciagri (6)
	Burkholderiaceae	Paraburkholderia kururiensis (1)	
		Ralstonia pickettii (24)	
Prokaryotes	Clostridiaceae	Clostridium beijerinckii (10)	
		Clostridium huakuii (9)	
	Comamonadaceae	Comamonas testosteroni (19)	
	Enterobacteriaceae	Enterobacter cloacae (7)	
		Enterobacter mori (1)	
	Moraxellaceae	Acinetobacter modestus (1)	
		Acinetobacter soli (46)	
	Pseudomonadaceae	Pseudomonas glareae (2)	
	Weeksellaceae	Elizabethkingia anophelis (1)	
	Number of ASVs	128	63
	rumber of rib vs	120	00
		Corneriella bambusarum (1)	Cortinarius violaceomaculatus (1)
		Corneriella bambusarum (1) Pseudosperma notodryinum (1)	Cortinarius violaceomaculatus (1)
	Agaricomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1)
	Agaricomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1)
	Agaricomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1)
	Agaricomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6)
	Agaricomycetes Chytridiomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6) Lobulomyces poculatus (1)
	Agaricomycetes Chytridiomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6) Lobulomyces poculatus (1) Operculomyces laminatus (1)
Fungi	Agaricomycetes Chytridiomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1) Aureoconidiella foliicola (2)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6) Lobulomyces poculatus (1) Operculomyces laminatus (1) Bambusicola splendida (1)
Fungi	Agaricomycetes Chytridiomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1) Aureoconidiella foliicola (2) Bambusicola didymospora (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6) Lobulomyces poculatus (1) Operculomyces laminatus (1) Bambusicola splendida (1) Muyocopron laterale (1)
Fungi	Agaricomycetes Chytridiomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1) Aureoconidiella foliicola (2) Bambusicola didymospora (1) Gordonomyces nucovaginatus	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6) Lobulomyces poculatus (1) Operculomyces laminatus (1) Bambusicola splendida (1) Muyocopron laterale (1)
Fungi	Agaricomycetes Chytridiomycetes Dothideomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1) Aureoconidiella foliicola (2) Bambusicola didymospora (1) Gordonomyces mucovaginatus (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6) Lobulomyces poculatus (1) Operculomyces laminatus (1) Bambusicola splendida (1) Muyocopron laterale (1) Neocamarosporium salsolae (1) Barnah coort houring with otii (1)
Fungi	Agaricomycetes Chytridiomycetes Dothideomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1) Aureoconidiella foliicola (2) Bambusicola didymospora (1) Gordonomyces mucovaginatus (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6) Lobulomyces poculatus (1) Operculomyces laminatus (1) Bambusicola splendida (1) Muyocopron laterale (1) Neocamarosporium salsolae (1) Paraphaeosphaeria michotii (1)
Fungi	Agaricomycetes Chytridiomycetes Dothideomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1) Aureoconidiella foliicola (2) Bambusicola didymospora (1) Gordonomyces mucovaginatus (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6) Lobulomyces poculatus (1) Operculomyces laminatus (1) Bambusicola splendida (1) Muyocopron laterale (1) Neocamarosporium salsolae (1) Paraphaeosphaeria michotii (1) Poaceascoma filiforme (1) Stamphylium katricesum (2)
Fungi	Agaricomycetes Chytridiomycetes Dothideomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1) Aureoconidiella foliicola (2) Bambusicola didymospora (1) Gordonomyces mucovaginatus (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6) Lobulomyces poculatus (1) Operculomyces laminatus (1) Bambusicola splendida (1) Muyocopron laterale (1) Neocamarosporium salsolae (1) Paraphaeosphaeria michotii (1) Poaceascoma filiforme (1) Stemphylium botryosum (2) Valagaia milija (1)
Fungi	Agaricomycetes Chytridiomycetes Dothideomycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1) Aureoconidiella foliicola (2) Bambusicola didymospora (1) Gordonomyces mucovaginatus (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6) Lobulomyces poculatus (1) Operculomyces laminatus (1) Bambusicola splendida (1) Muyocopron laterale (1) Neocamarosporium salsolae (1) Paraphaeosphaeria michotii (1) Poaceascoma filiforme (1) Stemphylium botryosum (2) Valsaria rudis (1)
Fungi	Agaricomycetes Chytridiomycetes Dothideomycetes Geoglossomycetes Glomeromycetes	Corneriella bambusarum (1) Pseudosperma notodryinum (1) Pulveroboletus sinensis (1) Lobulomyces poculatus (1) Aureoconidiella foliicola (2) Bambusicola didymospora (1) Gordonomyces mucovaginatus (1) Hemileucoglossum pusillum (1)	Cortinarius violaceomaculatus (1) Pseudobaeospora wipapatiae (1) Clydaea vesicula (6) Lobulomyces poculatus (1) Operculomyces laminatus (1) Bambusicola splendida (1) Muyocopron laterale (1) Neocamarosporium salsolae (1) Paraphaeosphaeria michotii (1) Poaceascoma filiforme (1) Stemphylium botryosum (2) Valsaria rudis (1)

Number of ASVs	16	27
Ustilaginomycetes		Farysia acheniorum (1)
	Violaceomyces palustris (1)	Cintractiella scirpodendri (1)
1 remetioniyeeles		Papiliotrema zeae (1)
Tremellomycetes		Hannaella surugaensis (1)
	Ophiostoma pityokteinis (1)	
	Idriellomyces eucalypti (1)	
Sordariomycetes	Acremonium persicinum (1)	Tristratiperidium microsporum (1
	Achroiostachys aurantispora (1)	Diatrypella vulgaris (1)
Schizosaccharomycetes		Schizosaccharomyces cryophilus (
Saccharomycetes		[Candida] boidinii (1)
Leotiomycetes	Curviclavula anemophila (2)	

Figure S1: Monthly precipitation (on the left) and average temperature (on the right) over 1970-2000 period, for each of the six study sites in Western Burkina Faso during the rice growing season (July to November).

WorldClim 2 data (Fick & Hijmans, 2017): https://worldclim.org/data/worldclim21.html



Figure S2: Rarefaction curves (plot of the number of ASVs obtained against the number of analysed reads) for each analyzed samples. On the left, are shown 16S metabarcoding data, representing prokaryotic communities. On the right are presented the ITS metabarcoding data, representing fungal communities.



Figure S3: 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 compartments (rhizosphere vs roots) and the geographical zone (Banzon, Karfiguela and Bama).

a. Analysis based on 16S rRNA gene reflecting Prokaryote communities. One point corresponds to one plant.

b. Analysis based on ITS reflecting fungal communities. One point corresponds to one field.



a 16S Prokaryotes



Figure S4: Prokaryote (16S) and fungi (ITS) taxonomic diversity obtained for each study site and each compartment

Figure S5: Comparison of root-associated microbiota α -diversity in the six study sites: for Prokaryotes (16S data) on the left and for fungi (ITS data) on the right.

Data obtained for the rhizosphere compartment are presented on top and roots data are on the bottom of the figure. The study sites from irrigated areas are represented in blue, while the ones from rainfed lowlands appears in red.



Non-parametric tests (Wilcoxon tests) on the soil physico-chemical parameters

		Rice growing sys	stem	Geographical zor	ne
			P-		P-
		W	value	W	value
Dhuaiaal	Clay %	43,000	0,902	9,765	0,008
Physical	Silt %	41,000	0,775	2,831	0,243
parameters	Sand %	49,000	0,775	8,382	0,015
	pH water	23,500	0,086	3,456	0,178
	Organic C	27,000	0,156	3,574	0,168
Chambrel	Total N	29,000	0,205	2,952	0,229
Cnemical	Total P	31,500	0,288	7,577	0,023
parameters	Total K	60,000	0,243	6,063	0,048
	SBE	24,000	0,094	8,282	0,016
	CEC	27,000	0,153	7,577	0,023

		Pr	okaryotes 16S	5		Fungi ITS						
	Df	SumOfSqs	R2	F	Pr(>F)	Df	SumOfSqs	R2	F	Pr(>F)		
Root compartment	1	2.418	0.052	6.863	0.001	1	1,187	0,080	3,753	0.001		
Rice growing system	1	2.256	0.048	6.402	0.001	1	1,178	0,080	3,725	0.001		
Geographical zone	2	2.258	0.048	3.205	0.001	2	1,278	0,087	2,020	0.001		
Rice growing system * Geographical zone	2	2.155	0.046	3.059	0.001	2	1,306	0,089	2,065	0.001		
Residual	107	37.700	0.806	NA	NA	31	9,804	0,665	NA	NA		
Total	113	46.788	1.000	NA	NA	37	14,754	1.000	NA	NA		

Results of permanova analysis on 16S and ITS microbiome data (all dataset for each of the two markers)

Results of Posthoc tests realized after permanova analysis on 16S and ITS microbiome data, and each compartment independently.

Tomas			16S	Prokary	otes				Ι	TS Fung	ji		
Type of	Pair of sites	Rhiz	osphere			Roots			Rhizosphere			Roots	
comparison		F	R2	p adj	F	R2	p adj	F	R2	p adj	F	R2	p adj
	BZ-IR vs												
Within-zone	BZ-RL	2,265	0,124	0,015	3,667	0,186	0,045	1,608	0,287	1,000	1,575	0,283	1,000
between rice	KA-IR vs												
systems	KA-RL	3,225	0,168	0,015	2,982	0,157	0,015	1,799	0,310	1,000	2,108	0,345	1,000
systems	BM-IR vs												
	BM-RL	3,342	0,150	0,045	3,343	0,150	0,015	1,730	0,257	0,960	1,605	0,243	0,855
	BM-IR vs	1 5 1 5	0.000	0.100	1 = 40	0.004	0 600		0.101	1 000	1 0 0 0	0.4.67	1 0 0 0
Between	BZ-IR	1,717	0,083	0,120	1,749	0,084	0,600	1,177	0,191	1,000	1,003	0,167	1,000
zone, within	BM-IR vs	1 (02	0.000	0.260	1 650	0.000	0.005	1 (70	0.051	0 425	1 2 6 4	0.014	1 000
irrigated sites	KA-IK	1,693	0,082	0,360	1,658	0,080	0,285	1,679	0,251	0,435	1,364	0,214	1,000
	BZ-IK VS	1 759	0.000	0.220	2 075	0 1 1 5	0 105	1 220	0.244	1 000	1 5 1 0	0.274	1 000
	RA-IK BM DL vo	1,730	0,099	0,330	2,075	0,115	0,195	1,209	0,244	1,000	1,310	0,274	1,000
Between	BWI-KL VS	2 299	0.126	0.015	1 688	0.095	1 000	1 3 2 7	0.249	1 000	1 004	0 201	1 000
zone within	BM-RI vs	2,299	0,120	0,015	1,000	0,075	1,000	1,327	0,247	1,000	1,004	0,201	1,000
rainfed	KA-RL	3.075	0.161	0.015	3.768	0.191	0.015	1.952	0.328	1.000	1.915	0.324	1.000
lowland sites	BZ-RL vs	0,070	0,101	0,010	0,700	0,171	0,010	1,201	0,020	1,000	1,7 10	0,02	1,000
	KA-RL	1,728	0,097	0,015	3,022	0,159	0,015	1,293	0,244	1,000	1,355	0,253	1,000
	BM-RL vs		,	,	,	,		,	,	,	,	,	,
	BZ-IR	2,536	0,137	0,030	3,480	0,179	0,045	2,209	0,356	1,000	1,736	0,303	1,000
Othors	BM-RL vs						ŗ						
Others	KA-IR	3,891	0,196	0,015	2,623	0,141	0,105	2,092	0,343	1,000	1,825	0,313	1,000
	BM-IR vs												
	BZ-RL	3,091	0,140	0,015	3,245	0,146	0,015	1,767	0,261	0,375	1,254	0,200	1,000

BM-IR vs KA-RL	3,866	0,169	0,015	3,934	0,172	0,015	1,743	0,259	0,360	1,769	0,261	0,420
BZ-IR vs KA-RL	2,811	0,149	0,015	4,113	0,205	0,015	1,457	0,267	1,000	2,031	0,337	1,000
BZ-RL	2,704	0,145	0,030	2,458	0,133	0,015	1,804	0,311	1,000	1,219	0,234	1,000

Results of the statistical analyses testing for the effect of soil chemical parameters on microbiome communities (each compartment analyzed separately).

	Prokaryotes 16S				Fungi ITS							
Soil	Rhizo	osphere	Ro	oots	Rhizo	osphere	Roots					
chemical parameter _	\mathbf{r}^2	p-value	r ²	p-value	r ²	p-value	r ²	p-value				
pH water	0,001	0,985	0,024	0,519	0,272	0,081	0,201	0,166				
Organic C	0,089	0,079	0,076	0,122	0,293	0,061	0,266	0,085				
Total N	0,086	0,089	0,059	0,198	0,320	0,043	0,273	0,079				
Total P	0,132	0,023	0,179	0,004	0,181	0,205	0,303	0,055				
Total K	0,037	0,372	0,066	0,159	0,017	0,866	0,029	0,792				
SBE	0,482	0,000	0,175	0,006	0,106	0,416	0,265	0,083				
CEC	0,314	0,000	0,204	0,003	0,028	0,814	0,161	0,244				

<u>**Table S5**</u> Results of Posthoc tests on the effect of the particular site on alpha diversity indices (Shannon diversity index and observed richness) for 16S microbiome data only and for each compartment independently.

		Rhizosphere							Roots					
Type of	Dain of sites							Shanno	on diver	sity				
comparison	rail of sites	Shannon	diversity ir	ndex	Observ	ed richr	ness	i	ndex		Observ	ed richn	iess	
		statistic	р	p.adj	statistic	р	p.adj	statistic	р	p.adj	statistic	р	p.adj	
Within-	BZ-IR vs BZ-RL	-1,803	0,071	1,000	-1,725	0,084	1,000	1,164	0,244	1,000	0,263	0,793	1,000	
zone,	KA-IR vs KA-RL	-3,110	0,002	0,028	-3,352	0,001	0,012	-2,826	0,005	0,071	-3,742	0,000	0,003	
between rice	BM-IR vs BM-													
systems	KL	0,721	0,471	1,000	0,786	0,432	1,000	-0,053	0,958	1,000	-0,478	0,632	1,000	
Between	BM-IR vs BZ-IR	-1,556	0,120	1,000	-1,416	0,157	1,000	-1,844	0,065	0,977	-0,660	0,509	1,000	
zone, within	BM-IR vs KA-IR	-0,144	0,885	1,000	-0,201	0,841	1,000	0,615	0,539	1,000	1,199	0,230	1,000	
irrigated	BZ-IR vs KA-IR													
sites		1,321	0,187	1,000	1,136	0,256	1,000	2,300	0,021	0,321	1,740	0,082	1,000	
Between	BM-RL vs BZ-													
zone,	RL	-2,584	0,010	0,146	-2,315	0,021	0,309	-0,611	0,541	1,000	-0,802	0,422	1,000	
within	BM-RL vs KA-		0.010		• • • • •		o o - -	• • • • •						
rainfed	RL	-2,570	0,010	0,152	-2,805	0,005	0,076	-2,300	0,021	0,321	-3,068	0,002	0,032	
sites	BZ-RL vs KA-RL	0,014	0,989	1,000	-0,490	0,624	1,000	-1,690	0,091	1,000	-2,265	0,024	0,353	
	BM-RL vs BZ-IR	-0,781	0,435	1,000	-0,589	0,556	1,000	-1,775	0,076	1,000	-1,065	0,287	1,000	
	BM-RL vs KA-IR	0,540	0,589	1,000	0,547	0,585	1,000	0,525	0,599	1,000	0,675	0,500	1,000	
Others	BM-IR vs BZ-RL	-3,484	0,000	0,007	-3,260	0,001	0,017	-0,600	0,549	1,000	-0,380	0,704	1,000	
Oulers	BM-IR vs KA-RL	-3,469	0,001	0,008	-3,784	0,000	0,002	-2,406	0,016	0,242	-2,801	0,005	0,076	
	BZ-IR vs KA-RL	-1,789	0,074	1,000	-2,215	0,027	0,401	-0,525	0,599	1,000	-2,002	0,045	0,679	
	KA-IR vs BZ-RL	-3,124	0,002	0,027	-2,862	0,004	0,063	-1,136	0,256	1,000	-1,477	0,140	1,000	

1 Table S6

Kingdom	Pathogen species	Known rice diseases				
	Vanthomonas oruzas	Bacterial Leaf Blight (BLB) and				
		Bacterial Leaf Streak (BLS)				
	Pseudomonas fuscovaginae	Sheath brown rot, Manchado de				
		grano				
		Bacterial brown stripe, Sheat rot,				
	Pseudomonas syringae	Glume blotch, Grain rot, Halo				
		blight				
	Acidovorax avenae subsp. avenae	Bacterial brown stripe				
Bacteria	Burkholderia glumae	Bacterial panicle blight / Grain rot / Seedling rot				
	Burkholderia gladioli	Bacterial panicle blight / Grain rot				
	Burkholderia plantarii	Seedling blight				
	Erwinia spp	Brown stripe				
	Erwinia herbicola	Black rot				
	Dickeya chrysanthemi	Culm and root disease				
	Pantoea ananatis, P. stewartii, P.					
	agglomerans	Bacterial Leaf Blight ?				
	Sphingomonas					
	Pyricularia oryzae (syn. Magnaporthe	Diss blast				
	oryzae)	Rice blast				
	Bipolaris oryzae (syn Helminthosporium					
	oryzae, syn Cochliobolus miyabeanus, Syn					
	Drechslera oryzae)	Brown spot				
	Bipolaris spp					
	Exserohilum rostratum					
	Curvularia spp	Black kernel				
	Microdochium albescens (syn					
	Rhynchosporium oryzae, Gerlachia oryzae,					
	Grophosphaerella albescens,	Leaf Scald				
	Metasphaeria albescens, Micronectriella					
Fungi	pavgu, Monographella albescens)					
	Cercospora janseana (syn C. oryzae,	NI				
	Sphaerulina oryzina, Napiciaalum	Narrow brown leaf spot				
	Janseanum, Passalora Janseana)					
	fusikuroi)	Bakanae				
	Fusarium graminearum (syn Giberella					
	<i>rene</i>)	Scab				
	Fusarium spp					
	Achlya spp	Water mold				
	Pythium spp					
	Rhizoctonia solani (syn Thanatenhorus					
	cucumeris)	Sheath blight				

2 List of the pathogen species searched for within the microbiome data

Sarocladium oryzae (syn Acrocylindrium oryzae)	Sheath rot
Ustilaginoidea virens	False smut
Epicoccum sorghinum (syn Phoma sorghina, Phyllosticta oryzina)	Glume blight
Epicoccum spp.	Red blotch of Grains
Trichoniella padwickii (syn Alternaria padwickii, Trichoconis padwickii)	Stackburn
Nakataea oryzae (syn Leptosphaeria salvinii, Vakrabeeja sigmoidea	Stem rot
Athelia rolfsii (syn Sclerotium rolfsii)	Seedling blight
Eballistra oryzae (syn Entyloma oryzae)	Leaf smut
Drechslera gigantea	Eyespot
Sclerophthora macrospora (syn Sclerospora macrospora)	Downy mildew
Ramularia oryzae (syn Mycovellosiella oryzae)	White leaf streak
Ascochyta oryzae (syn Phomopsis oryzae- sativa)	Collar rot
Waitea circinata (syn Rhizoctonia oryzae)	Sheath Spot
Ceratobasidium setariae (syn Rhizoctonia oryzae-sativa)	Aggregate Sheat Spot
Calonectria morganii (syn Cylindrocladium scoparium)	Sheath Net Blotch
Gaeumannomyces graminis	Crown Sheat Rot
Myrothecium verrucaria	Myrothecium blotch
Pyrenochaeta acicula (syn P. oryzae)	Sheat Blotch
Globisporangium spinosum (syn Pythium spinosum)	Root rot
Tilletia spp.	Kernel smut
Balansia oryzae (syn Ephelis oryzae)	Udbatta
Nicrospora spp.	Minute Leaf and grain spot

3





С

Geographical zone

16S Prokaryotes



ITS Fungi

16S Prokaryotes





ITS Fungi

a. Prokaryotes (16S)

Irrigated

•

• Paraburkholderia kururiensis



b. Fungi (ITS)

Irrigated

Pulveroboletus sinensis



- Curvularia chonburiensis
- Nigrospora magnoliae
- Quixadomyces hongheensis
- Staurosphaeria rhamnicola

Rainfed Lowland

- Cladosporium chasmanthicola
- Operculomyces laminatus
- Paraphaeosphaeria michotii
- Pseudobaeospora wipapatiae
- Pseudothielavia arxii
- Sympodiella acicola

