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Exploring Rice Root Microbiome; The Variation, Specialization and Interaction of Bacteria and Fungi In Six Tropic Savanna Regions in Ghana

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Abstract: We investigated the root microbiomes of rice sampled from six major rice-producing regions in Ghana using Illumina MiSeq high-throughput amplicon sequencing analysis. The result showed that both bacterial and fungal community compositions were significantly varied across the regions. Bacterial communities were shaped predominantly by biotic factors, including root fungal diversity and abundance. In contrast, fungal communities were influenced by abiotic factors such as soil nitrate, total carbon and soil pH. A negative correlation between the diversity and abundance of root fungi with soil nitrate (NO_3^-) level was observed. It suggested that there were direct and indirect effects of NO_3^- on the root-associated bacterial and fungal community composition. The gradient of soil nitrate from North to South parts of Ghana may influence the composition of rice root microbiome. Bacterial community composition was shaped by fungal diversity and abundance; whereas fungal community composition was shaped by bacterial abundance. It suggested the mutualistic interaction of bacteria and fungi at the community level in the rice root microbiome. Specific bacterial and fungal taxa were detected abundantly in the ‘Northern’ regions of Ghana, which were very low or absent from the samples of other regions. The analysis of indicator species suggested that an ‘ecological specialization’ may have occurred which enabled specific microbial taxa to adapt to the local environment, such as the low-nitrate condition in the Northern regions.

Keywords: Ghana; rice; root microbiome; community analysis; regional variation; nitrogen gradient; ecological specialization; bacterial-fungal interaction

1. Introduction

The global human population is projected to reach eight to nine billion by 2050 [1]. With population growth, overexploitation of natural resources, including fossil fuels and nonrenewable raw materials has emerged as a great challenge for agriculture. Since the production of inorganic fertilizer relies heavily on nonrenewable resources [2–4], it is crucial to find renewable alternatives to chemical fertilizers, especially in developing countries [5]. Ghana, in West Africa, has attempted to promote rice production to feed its growing population [6], yet productivity has been low. The reported low rice yield across the nation is attributed to several factors. However, the major factors had been inadequate soil nutrient and inefficient fertilizer management due to low soil fertility, productivity remains low [7]. Therefore, it is urgent to find alternative, renewable ways to improve soil fertility and enhance plant's access to soil nutrients.

Root-associated microorganisms play a significant role in improving plant productivity [8]. Several root-associated bacteria and fungi are known to promote plant growth and increase plant tolerance to multiple environmental stresses [9,10]. Rhizobia are the best-known bacterial mutualistic symbionts, capable of fixing atmospheric nitrogen through nodulation in the roots of leguminous plants [11,12]. Arbuscular mycorrhizal fungi (AMF) are the most common mutualistic fungal symbionts, colonizing about 80% of land plants. They provide multiple benefits for host plants, including the supply of inorganic nutrients and enhanced disease resistance [13]. They promote the function and stability of ecosystems, including agricultural land [14]. Endophytic fungi (nonmycorrhizal fungal symbionts in roots) are capable of improving the environmental fitness of plant, including nutrient acquisition, enhancing disease resistance and drought tolerance [15–17]. In natural conditions, root-associated microorganisms coexist and interact as a root microbiome. Thus, it is vital to understand the whole microbiome to improve plant productivity and crop yield [18].

In general, the assembly of the host-associated microbiome, including the root-associated microbiome, is formed by colonization from regional microbial species, followed by host filtering [19]. In the terrestrial ecosystem, multiple environmental biotic and abiotic factors, are reported to affect the composition of the microbial species pool in soil [20]. Edwards et al. [21] conducted a wide-ranging investigation of the rice root-associated bacterial community in northern California, USA, and found regional variation. Further controlled experiments revealed the effect of environmental factors, including soil fertility [22] and soil salinity [23], on root-associated bacterial communities. Root fungal communities are found to be also determined by stochastic processes represented by geochemical differences, as well as by niche differences, including environmental factors [24]. However, no previous survey has focused on Ghanaian rice root microbiomes.

Evidence suggests that there are interactions between bacteria and fungi on many spatial scales, including that of plant roots, such as endosphere [25]. AMF and *Rhizobium* species partially share a symbiotic signaling pathway, the Common Symbiosis Pathway [26], which exerts a synergetic effect on the host plant, increasing nutrient acquisition [27]. Endophytic bacteria and fungi also interact on host rice roots to improve the host's physiological properties [28]. At the field-scale experiments, interactions between bacteria and fungi have been reported in several studies on leaf litter [29] and soil [30]. In the case of the root microbiome, Agler et al. [31] profiled the interactions of bacteria and fungi in the root community of *Arabidopsis thaliana* and found taxon-level interactions. However, field-scale understanding of the interactions between root-associated bacteria and fungi is still limited.

In Brazil, investigations of the functionality of soil microorganisms showed a substantial variation in the diversity and structure of bacterial communities. This was deemed as being due to geographic differences between tropical savanna climates [32]. Differences in function and environmental adaptability of root-associated microorganisms from different origins have also been reported [33,34]. From the findings, we infer that root-associated bacteria and fungi that are abundantly presenting in the specific region could have the potential for enhancing host adaptation and fitness to climatic variations.

Moreover, in a recent study [35] we reported that colonization intensity and taxonomic compositions of AMF in rice roots vary significantly among major rice-producing regions in Ghana,

with available phosphorus found as the essential factor driving AMF community structures in rice roots. We found that certain environmental factors such as C/N, NO_3^- and importantly available phosphorus influenced community structuring of AMF in rice in Ghana. Our hypothesis was that similar to AMF other microbial community composition in the roots may also be different among these regions and shaped by the environmental variables. If that was true, which environmental factors were important in forming the microbiome community composition among these regions. We did not come across any study that investigated the root microbiome of rice in those regions.

An understanding of the ecology of root-associated microorganisms is key to the development of sustainable rice production in Ghana. The hypotheses of our study were (1) rice root-associated microbiomes vary across the different regions, with local abiotic and biotic environmental factors involved in shaping the microbiome; (2) abundantly presenting bacterial and fungal in a different region could be key players in host rice productivity and (3) specific bacterial and fungal taxa may be correlated and interact in the rice root microbiome in Ghana. We used a high-throughput amplicon sequencing platform to reveal the structure and variation of bacterial and fungal communities. The goal of this study is to explore the possibility of applying root-associated microbiomes for sustainable rice production.

2. Materials and Methods

2.1. Study Site

Samples of soil and rice roots were collected from six Ghanaian rice-producing regions, including Upper West (UW), Upper East (U.), Northern (North), Ashanti (Ash), Brong-Ahafo (BA) and Volta (Vol) (Figure 1.). These selected regions are under three agroecological zones including; Guinea savannah, forest-savannah transitional and deciduous forest. The annual total precipitation means of 1063.7, 1161.3 and 1404.8 mm ranged within 0.0–257.4 mm, 4.4–1040 mm and 2.0–846.3 mm per month and with average annual temperatures of 28.3, 27.0 and 27 °C, ranged from 23.5–32.4 °C, 23.530.4 °C and 26.0 °C, respectively [36,37]. Overall, we survey a total of 20 fields across the six regions.

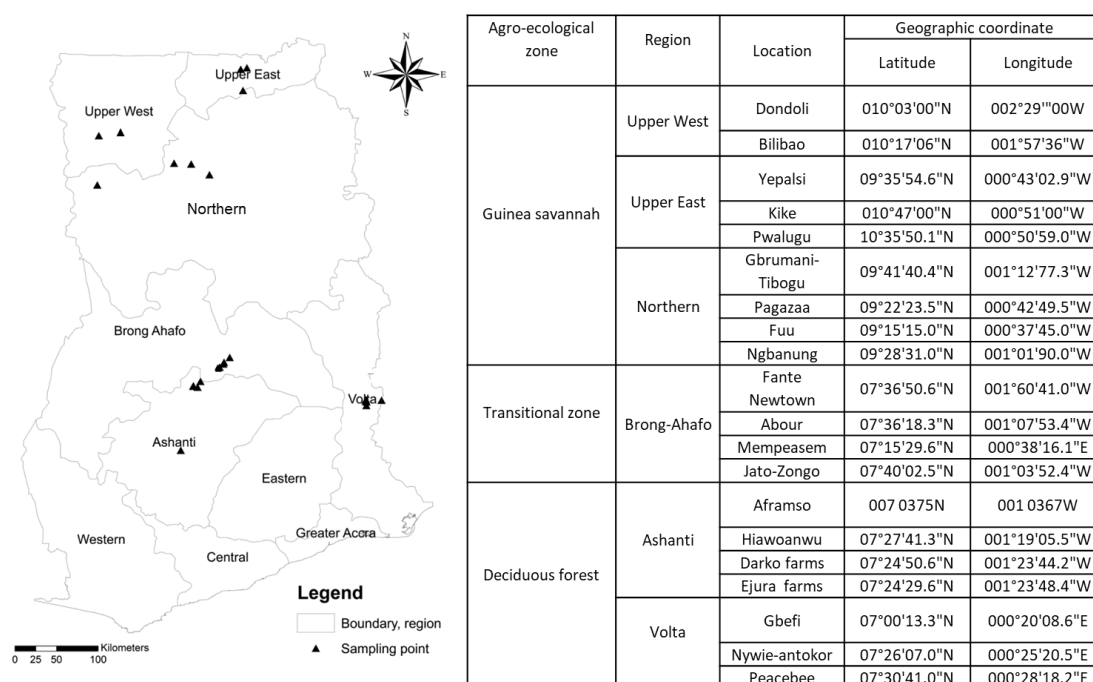


Figure 1. Sampling sites in rice cultivated fields of Ghana. The map shows 20 sampling areas marked with black triangles among six different regions under three agroecological zones of Ghana.

2.2. Root and Soil Sampling

Healthy rice plants (cv. Jasmine85, commonly cultivated in Ghana) in their vegetative stage (thus maximum tillering), at an average height of about 55 cm (BBCH code, identifying phenologically development stages of crop plants: 29, maximum tillering detected), were collected randomly from eight different spots within each cultivated field were sampled. Upon sampling, rice roots were detached and washed gently with tap water to remove soil debris. Cleaned roots from the same field were mixed and dried by pressing with absorbent tissue paper. Soil (0–15 cm depth) on the other hand, were collected randomly from eight different core spots within each sampling field and mixed as a sample. Sampled rice roots and soil were transferred to the laboratory in an icebox and stored at -80°C until further use. In the laboratory, soil samples were dried at room temperature (approximately 25°C) and sieved with 2 mm mesh to remove all other foreign materials. Samples were then stored at 5°C until analyzed.

2.3. Soil Property Analysis

As soil properties, pH, soil moisture, total carbon (TC), total nitrogen (TN), and the soil carbon–nitrogen ratio (C/N) were measured by the standard protocols for tropical soils [38]. The available phosphorus was determined by the Truog-soluble method extracted with sulfuric acid and ammonium sulfate $((\text{NH}_4)_2\text{SO}_4$, pH 3.0) solution. Ammonia (NH_4^+) and nitrate (NO_3^-) were extracted from 10 g soil with 2-M KCl solution [39], and concentrations were determined by the continuous flow injection analysis method and the indophenol-blue colorimetric procedure, respectively [40]. The cation exchange capacity (CEC) was measured by the Schollenberger and Simon method [41].

2.4. Root DNA Isolation and PCR

A total of 20 rice root samples were frozen-crushed with liquid nitrogen using a sterilized pestle and mortar. Total DNA was extracted from 100 mg of finely ground samples using the Dneasy Plant Mini kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations, and was stored at -20°C .

The bacterial 16S rRNA V4 region was amplified using the primer pair 515f (5'-GTGYCAGCMGCCGCGGTAA-3') and 806r (5'-GGACTACNVGGGTWTCTAAT-3') described by Walters et al. [42] with AmpliTaq Gold DNA Polymerase (Applied Biosystems). PCR was performed with a master mix in a 12.5- μL reaction mixture with 0.0625 μL polymerase, 5 pmol of each barcoded primer (a unique combination for each sample), 2 mM dNTP and 2.5 ng of template DNA. We ran an initial denaturation of 10 min at 95°C , then 25 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 1 min, followed by a 72°C for 9 min for a final extension. The fungal ITS2 region was amplified using the primer pair ITS7-f (5'-GTGAATCATCGAATCTTTG-3') described by Ihrmark et al. [43] and ITS4-r (5'-TCCTCCGCTTATTGATATGC-3') described by White et al. [44] with KOD DNA polymerase each) and a tenth of the products of the first PCR. We ran an initial denaturation of 10 min at 95°C , then 8 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 1 min, then at 72°C for 9 min for a final extension. The PCR products were purified with FastGene Gel/PCR Extraction kit (Nippon Genetics Co., Japan) and sequenced using Illumina MiSeq high-throughput sequencing.

2.5. Statistical Analysis

High-quality sequences were clustered into operational taxonomic units (out) with 97% similarity by QIIME software and taxa were assigned by BLAST search in GenBank. The resultant microbial OTUs were primarily analyzed with R version 3.6.1 [45]. As with the microbial α -diversity index, Shannon–Wiener and Simpson indices were calculated with the diversity() function in vegan. Chao richness was calculated with the chaorichness() function in the iNEXT software package [46]. Correlations between bacterial and fungal community composition and abiotic and biotic environmental

factors were predicted by the `envfit()` function in `vegan`. Pearson's correlation coefficients were calculated using the `ggscatter()` function in R package `ggpubr` [47]. Community analyses were performed with the package `vegan` [48]. The OTUs with <10 read counts were removed. Prior to community analyses, all samples were normalized to 6000- and 7500-read counts for bacteria and fungi, respectively, due to their minimum read counts among samples (7812 for bacteria and 7983 for fungi), by random subsampling without replacement using the function `rarefy()` in `vegan`. Microbiomes in six regions were visualized by nonmetric dimensional scaling (NMDS) based on the Bray–Curtis dissimilarity index using the `vegdist()` function in `vegan`. Root-associated microbial community composition differences were tested by Permutational analysis of variance (PerMANOVA) with the `adonis()` function and analysis of similarity (ANOSIM) with the `anosim()` function in `vegan`.

Regionally more abundantly presenting, root-associated microbial indicator OTUs were identified by the `mulipatt()` function in the R package `indicspecies` [49] and clustered into six groups, UW, UE, North, Ash, BA and Vol; and into two groups, North Region (UW, UE, North) and southern region (Ash, BA Vol), independently based on abundance. In the indicator analysis, we only focused on the OTUs with >500 reads as common OTU, and the OTUs with <500 reads were removed as rare OTUs [50]. From common OTUs, OTUs with relative abundance of <0.2% were also moved as low frequent taxa.

Cooccurrence network analysis was conducted using the CoNet method [51], using a multiple ensemble correlation (`row_minocc` filter = 20). Five similarity measures were calculated: Bray–Curtis and Kullback–Leibler nonparametric dissimilarity indices, mutual information (MI) similarities and Pearson and Spearman correlation coefficients. Distribution of all pairwise scores between OTUs was computed for all six regions to enrich the network with OTU nodes. Based on OTU distributions, initial thresholds were selected such that the initial network contained 1000 positive and 1000 negative edges, consistent across all five correlation measures. For each measure and each edge, 1000 renormalized permutation and bootstrap scores were generated [52,53]. Eventually, the cooccurrence network model was displayed by Cytoscape version 3.7.2.

3. Results

Soil properties of each sampling site are shown in Table 1. We found significant variations in those soil properties. Remarkably, we found the gradient of NO_3^- content from the Northern regions (UW, UE, North) to the South regions (BA, Ash, Vol) of Ghana.

After quality filtering and chimera removal, Illumina MiSeq high-throughput paired-end sequencing yielded of a total 4,400,432 sequences from the bacterial 16S V4 region and 3,401,411 from the fungal ITS region, from 20 samples each. After the removal of the nontarget taxa, we obtained 1,171,062 reads assigned as bacteria and 1,947,129 reads assigned as fungi. A total of 36,956 bacterial OTUs and 7101 fungal OTUs were obtained. As bacterial and fungal taxa, 54 and 12 phyla (including subphylum *Glomeromycotina*: former phylum *Glomeromycota*), 149 and 48 classes, 242 and 126 orders, 313 and 279 families, 531 and 562 genera, 24 and 639 species were observed in whole samples, respectively. After removal of low read count, OTUs with <10 read counts, rarefaction curves were predicted for both 16S and ITS (Figure S1).

Table 1. Variations of chemical and physical properties of soil samples from each sampling site in six different regions in Ghana.

Site	Region	pH	Moisture	NO ₃ ⁻	P	TC	TN	CN	C.E.C.
Don	UW	4.70 ± 0.20b	7.000 ± 0.655k	25.77 ± 1.28i	0.3603 ± 0.1107gh	5.216 ± 0.058k	0.2633 ± 0.0042k	10.39 ± 0.01l	5.466 ± 0.042n
Bib	UW	5.10 ± 0.24ab	13.14 ± 1.74i	44.20 ± 1.14h	0.2560 ± 0.0307gh	6.084 ± 0.039k	0.6703 ± 0.8110i	11.69 ± 0.01h	2.577 ± 0.291m
Yep	UE	5.00 ± 0.16ab	13.22 ± 1.15e	58.25 ± 0.35g	0.1520 ± 0.0272h	12.90 ± 0.01h	2.097 ± 0.011e	15.08 ± 0.01a	14.76 ± 0.05h
Kike	UE	5.10 ± 0.10ab	11.66 ± 0.65h	67.78 ± 1.07f	0.5370 ± 0.0450g	13.09 ± 0.03h	1.424 ± 0.014h	13.02 ± 0.01c	14.56 ± 0.11h
Pw	UE	4.80 ± 0.12b	14.60 ± 1.21d	13.27 ± 0.34j	1.221 ± 0.121d	12.08 ± 0.03h	2.500 ± 0.101d	12.37 ± 0.02f	10.87 ± 0.06k
GT	NO	4.70 ± 0.20b	18.62 ± 0.69jk	24.89 ± 0.57i	2.146 ± 0.054b	5.837 ± 0.029k	0.3593 ± 0.0149jk	9.840 ± 0.021n	9.917 ± 0.121l
PAGA	NO	5.10 ± 0.10ab	8.440 ± 0.287jk	24.22 ± 0.98i	4.602 ± 0.090a	8.833 ± 0.028j	0.3510 ± 0.0045jk	9.337 ± 0.026p	12.45 ± 0.09i
FUU	NO	4.70 ± 0.20b	15.40 ± 0.49gh	26.52 ± 1.29i	1.183 ± 0.033d	10.84 ± 0.059i	1.446 ± 0.003gh	10.61 ± 0.02k	11.73 ± 0.07j
Ngb2	NO	5.00 ± 0.10ab	9.280 ± 0.59lj	44.79 ± 0.63h	1.633 ± 0.036c	7.789 ± 0.055j	0.4733 ± 0.0244j	9.517 ± 0.017°	10.79 ± 0.08k
Aframso	Ash	5.80 ± 0.16a	15.58 ± 0.99h	106.6 ± 2.0d	1.017 ± 0.027de	28.36 ± 0.04d	1.374 ± 0.014h	12.89 ± 0.02d	22.43 ± 0.08d
Hia	Ash	5.00 ± 0.16ab	23.24 ± 1.89cd	126.6 ± 0.7b	0.2540 ± 0.146gh	29.64 ± 0.03c	2.638 ± 0.025cd	10.85 ± 0.03j	20.84 ± 0.09e
Dar	Ash	5.00 ± 0.16ab	10.82 ± 1.21f	96.50 ± 2.32e	0.7487 ± 0.0373ef	24.84 ± 0.04e	1.839 ± 0.025f	10.17 ± 0.03 m	22.94 ± 0.03d
Ejura	Ash	5.30 ± 0.3ab	9.840 ± 0.52g	148.8 ± 1.04a	0.8963 ± 0.0103de	25.87 ± 0.06e	1.608 ± 0.037g	11.81 ± 0.01h	22.96 ± 0.04d
FanteN	BA	5.10 ± 0.10ab	17.22 ± 1.02e	129.5 ± 0.27b	0.5313 ± 0.0218fg	20.23 ± 0.04f	2.191 ± 0.020e	12.19 ± 0.02g	15.54 ± 0.17g
Abour	BA	5.50 ± 0.16ab	6.784 ± 0.54c	126.7 ± 1.87b	0.5093 ± 0.0124fg	17.69 ± 1.01g	2.715 ± 0.043c	12.88 ± 0.004d	14.45 ± 0.03h
Mem	BA	5.60 ± 0.10ab	11.88 ± 0.91a	146.9 ± 0.7a	0.4500 ± 0.0271fgh	25.93 ± 0.03e	3.917 ± 0.010a	12.66 ± 0.004e	16.85 ± 0.03f
Jato	BA	5.00 ± 0.16ab	16.80 ± 2.71f	149.0 ± 0.4a	1.072 ± 0.042de	24.86 ± 0.07e	1.845 ± 0.029f	11.63 ± 0.02i	15.95 ± 0.03g
Gbe	Vol	5.60 ± 0.24ab	13.40 ± 1.13a	126.9 ± 2.0b	0.5037 ± 0.0088fg	39.92 ± 0.02a	3.918 ± 0.010a	12.88 ± 0.01d	24.94 ± 0.01c
Ny1	Vol	5.10 ± 0.10ab	18.76 ± 0.88b	108.3 ± 0.8d	0.4027 ± 0.0096gh	38.44 ± 0.02b	3.486 ± 0.028b	13.97 ± 0.01b	29.97 ± 0.09a
Pea2	Vol	5.60 ± 0.29ab	19.60 ± 0.32e	118.9 ± 0.8c	0.5177 ± 0.0110fg	38.31 ± 0.02b	2.229 ± 0.034e	12.47 ± 0.03f	27.55 ± 0.05b

Differences of each soil property were compared by ANOVA. Different alphabets stand for statistical differences in Turkey's test. UW—Upper West region, including Don, Bib; UE—Upper East region, including Yep, Kike, Pw; NO—Northern region, including GT, PAGA, FUU; Ngb; Ash—Ashanti region, including AframsoU, Hia, Dar, Ejura; BA—Brong-Ahafo, including FanteN, Abour, Mem, Jato; Vol—Volta region; including Gbe; Ny, Pea.

Ordination analysis was performed, to investigate patterns of microbiome assemblages of rice roots, across the six locations. Two-dimensional nonmetric multidimensional scaling (NMDS), based on Bray–Curtis dissimilarity, did not show any noticeable pattern of variation of bacterial communities across the regions (Figure 2a). For fungal communities, we found that there were significant differences between the northern regions of Ghana (UW, UE, NO) and those from the southern regions (BA, Ash, Vol), as displayed by NMDS. plot (Figure 2b). In contrast, PerMANOVA detected a significant difference in both root-associated bacterial and fungal communities, between the six regions (P : <0.001 and 0.0092, respectively), albeit with a low pseudo- F value (Table 2).

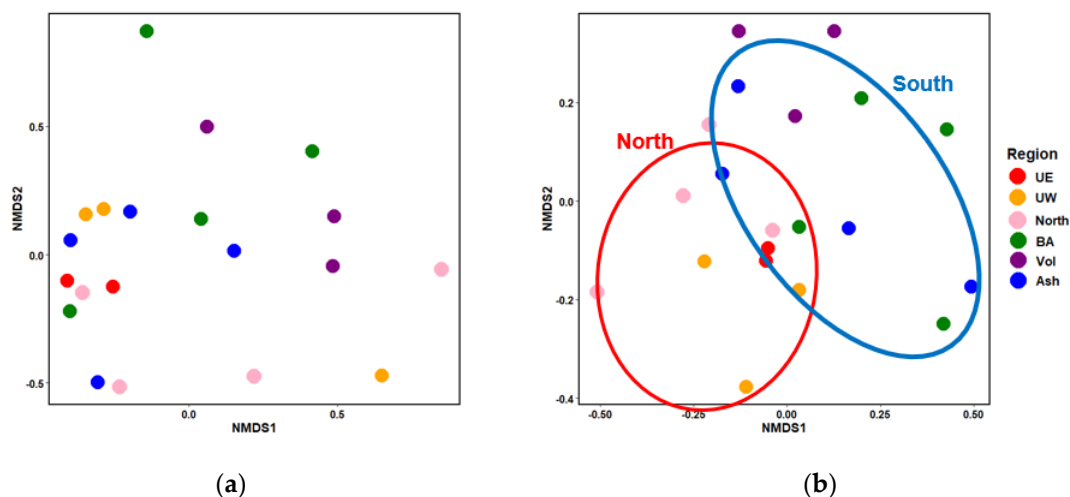


Figure 2. Nonmetric multidimensional scaling (NMDS) of Bray–Curtis pairwise dissimilarity of (a) bacterial and (b) fungal community. Regions coded by color. UW—Upper East region; UE—Upper East region; NO—Northern region; Ash—Ashanti region, including AframsoU, Hia, Dar and Ejura; BA—Brong-Ahafo, including FanteN, Abour and Mem, Jato; Vol—Volta region; including Gbe, Ny and Pea; Upper—North regions (UW, UE and NO); South-South regions (BA, Ash and Vol).

Table 2. Difference between rice root-associated bacterial and fungal community composition among six different regions in Ghana.

Kingdom	PerMANOVA		ANOSIM	
	Pseudo- F	P	R	P
Bacteria	2.436	<0.001	0.2114	0.0338
Fungi	1.589	0.0092	−0.0228	0.5786

Bacterial and fungal community differences were tested by PerMANOVA and ANOSIM after rarefying each OTU table to 6000 and 7500 reads, respectively.

We considered several biotic and abiotic factors to explain the variation in the microbiome composition of rice roots, across the locations studied (Table 3, Tables S4 and S5). Vector analysis revealed that assemblies of bacterial community composition were significantly correlated with one abiotic factor: soil moisture ($P = 0.0405$). The fungal diversity was also correlated with bacterial composition, evidenced by Shannon index ($P = 0.0292$) and Simpson index ($P = 0.0404$). Assemblies of fungal community composition were significantly correlated with four soil abiotic factors ($P < 0.05$): pH ($P = 0.0104$), NO_3^- concentration ($P < 0.001$), total carbon (TC, $P < 0.001$) and cation exchange capacity (CEC, $P = 0.0226$). Bacterial read number ($P = 0.0251$) was correlated with assemblies of fungal community composition.

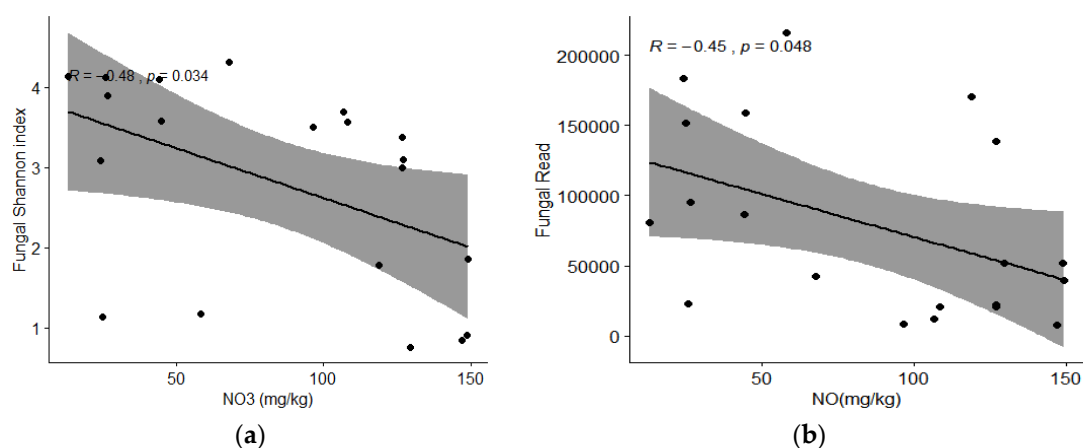
Table 3. Abiotic and biotic environmental variables involved in the assembly of rice root-associated bacterial and fungal community composition in six different regions in Ghana.

Kingdom	Factor	Variable	NMDS1	NMDS2	r ²	P
Bacteria	abiotic	Moisture	0.9773	0.2118	0.3112	0.0405
		TN	0.2311	0.9729	0.2428	0.0915
	biotic	ReadFun	0.7245	−0.6893	0.4304	0.0078
		ObsFun	0.15572	−0.9878	0.2625	0.0740
		ShnFun	−0.8061	−0.5918	0.3374	0.0292
		SimFun	−0.7407	−0.6718	0.3150	0.0404
Fungi	abiotic	pH	0.45549	0.8903	0.4114	0.0104
		NO ₃ [−]	0.7563	0.6543	0.5797	<0.001
		TC	0.3870	0.9221	0.5601	<0.001
		TN	0.6247	0.7809	0.2796	0.0629
		CEC	0.38942	0.92106	0.3546	0.0226
	biotic	ReadBac	−0.08821	−0.9961	0.3546	0.0251

Abiotic and biotic environmental factors involved in the assembly of bacterial and fungal community composition revealed by vector analysis. Bac—bacteria; Fun—fungi; Read—read counts; Shn—Shannon index; Sim—Simpson index; Read—read number; Obs—observed OTU number; TN—total nitrogen; TC—total carbon; CEC—cation exchange capacity. NMDS1 and NMDS2 stand for the axes in NMDS. (Nonmetric multidimensional scaling) plotting.

Further analysis of Pearson's correlation between abiotic and biotic environmental factors indicated that the concentration of soil NO₃[−] significantly influenced fungal α -diversity indices, including observed OTU number, fungal Chao richness and Shannon index, while total soil carbon influenced bacterial read number (Figure 3, Table S5).

Analysis of indicator species detected more abundantly presenting bacterial and fungal OTUs in the root-associated microbiome in 6 regions. Overall, the northern regions (UW, UE, NO) tended to harbor such bacterial and fungal OTUs in rice root-associated microbiome, to a greater extent than the southern regions (Ash, BA, Vol). For example, one bacterial OTU (*Sporomusa* sp. OTU_B4896) was abundantly presenting in three northern regions of Ghana; UW, UE and NO (Table S6). Among fungal communities, we found that one fungal OTU was abundantly present in the UW, UE and Northern regions and six OTUs were common to three regions (UW, UE, NO), but were absent from the southern regions (Table S7).

**Figure 3.** Cont.

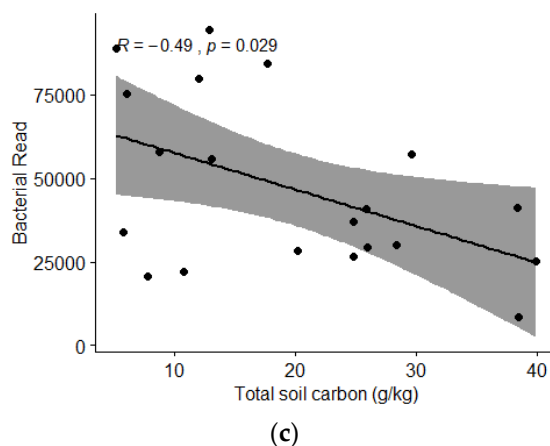


Figure 3. Correlation between abiotic and biotic environmental factors within six different regions in Ghana. Pearson's correlation coefficient was obtained between (a) soil NO_3^- concentration and fungal Shannon index, (b) soil NO_3^- concentration and fungal read number and (c) soil total carbon (TC) and bacterial read number (ReadBac).

Further analysis of indicator species detected root-associated bacterial and fungal OTUs abundantly presenting to the northern regions (UW, UE, NO) of Ghana (Tables 4 and 5). Among bacterial communities, we found seven OTUs abundantly present in the northern regions, five of which were taxa previously reported as endophytes, and one of them was a taxon reported as pathogenic (Table 4). Among fungal communities, we found 16 fungal OTUs abundantly present in the northern regions; six taxa were reported as endophytic, three taxa as pathogenic, and six taxa were reported as being both endophytes and pathogens (Table 5). We found no such bacterial and fungal OTUs in the southern regions of Ghana.

Cooccurrence network analysis disclosed potential interactions between root-associated bacterial and fungal species (Figure 4). We found a complex bacterial network, consisting of 64 OTUs, but only two fungal OTUs were associated with bacterial OTUs (Figure 4). Bacterial and fungal taxa correlated with each other were *Kaistobacter* sp. (OTU_B22284) and *Mycosphaerella tassiana* (OTU_F7631), *Acinetobacter rhizosphaerae* (OTU_B38216) and an unidentified *Ascomycota* (OTU_F3422), respectively. Fungal OTUs correlated each other were *Mycosphaerella tassiana* OTU_F7631 and an unidentified fungus OTU_F5419 and *Cercospora jatrophiophila* OTU_F9290 and Glomeraceae OTU_F7924.

Table 4. Bacterial indicator OTUs detected in northern regions of Ghana and their characteristics on host plant.

OTU.ID.	Probability		Indicator Value Index	P	Characteristic	Reference	Host
	A	B					
<i>Sporomusa</i> _sp. OTU_B4896	0.9971	1.0000	0.999	0.001	Free living or unknown	[54]	
Aeromonadaceae OTU_B18812	0.9760	1.0000	0.988	0.020	Endophyte	[55]	rice
<i>Clostridium</i> sp. OTU_B44691	0.9752	1.0000	0.988	0.011	Endophyte	[56]	maiden silver grass
Micromonosporaceae OTU_B6546	0.9312	1.0000	0.965	0.015	Endophyte	[57]	Leguminous and actinorhizal plants
<i>Pleomorphomonas</i> sp. OTU_B24241	0.9698	0.8889	0.928	0.012	Endophyte	[58]	rice
<i>Achromobacter</i> sp. OTU_B36942	0.9506	0.8889	0.919	0.046	Endophyte	[59]	wheat
Xanthomonadaceae OTU_B14074	0.7998	1.0000	0.894	0.032	Endophyte and Pathogen	[60]	rice and other plants

Abundantly presenting bacterial indicator OTUs in North regions (UW, UE, NO) were revealed by indicator species analysis after removing low frequent OTUs (<500 reads, 0.2% sequence) from bacterial OTU tables.

Table 5. Fungal indicator OTUs detected in northern regions of Ghana and their characteristics on host plant.

OTU.ID.	Probability		Indicator Value Index	P	Characteristic	Reference	Host
	A	B					
<i>Pleosporales</i> OTU_4099	0.9971	1.0000	0.999	0.001	Endophyte and Pathogen	[61]	rice and other plants
<i>Curvularia_prasadii</i> OTU_6231	0.9963	1.0000	0.998	0.001	Pathogen	[62]	<i>Achyranthes aspera</i>
<i>Pleosporales</i> OTU_3272	0.9651	1.0000	0.982	0.003	Both endophyte and Pathogen	[61]	rice and other plants
<i>Sebacinales</i> OTU_1840	0.9631	1.0000	0.981	0.002	Endophyte	[63]	barley
<i>Stachybotryaceae</i> OTU_3669	0.9448	1.0000	0.972	0.031	Endophyte	[64,65]	black cottonwood, wild rice
<i>Myrothecium_verrucaria</i> OTU_7577	0.9996	0.8889	0.943	0.002	Pathogen	[66]	rice and other plants
<i>Bambusicolaceae</i> OTU_3283	0.9925	0.8889	0.939	0.012	Endophyte and Pathogen	[67]	bamboo
<i>Macrophomina_phaseolina</i> OTU_4358	0.8767	1.0000	0.936	0.003	Pathogen	[68]	rice and other plants
<i>Xylariales</i> OTU_8174	0.9755	0.8889	0.931	0.032	Endophyte	[65,69]	wild rice and other plants
<i>Thielavia_terrestris</i> OTU_8307	1.0000	0.7778	0.882	0.002	Free living or unknown	[70]	
<i>Sordariomycetes</i> OTU_2298	1.0000	0.7778	0.882	0.002	Endophyte and Pathogen	[71]	rice and other plants
<i>Chaetomium</i> OTU_10228	0.8635	0.7778	0.819	0.014	Endophyte	[72]	rice
<i>Dothideomycetes</i> OTU_7194	1.0000	0.6667	0.816	0.006	Endophyte and Pathogen	[73,74]	<i>Lycopodium annotinum</i> , wheat
<i>Pleosporales</i> OTU_11327	1.0000	0.5556	0.745	0.004	Endophyte and Pathogen	[61]	rice and other plants
<i>Chaetomium</i> OTU_4724	0.9999	0.5556	0.745	0.030	Endophyte	[72]	rice
<i>Pezizaceae</i> OTU_11633	1.0000	0.4444	0.667	0.032	Endophyte	[75]	<i>Quercus</i> , <i>Pinus</i> , <i>Populus</i>

Abundantly presenting fungal indicator OTUs in north regions (UW, UE and NO) were revealed by indicator species analysis after removing low frequent OTUs (<500 reads, 0.2% sequence) from fungal OTU tables.

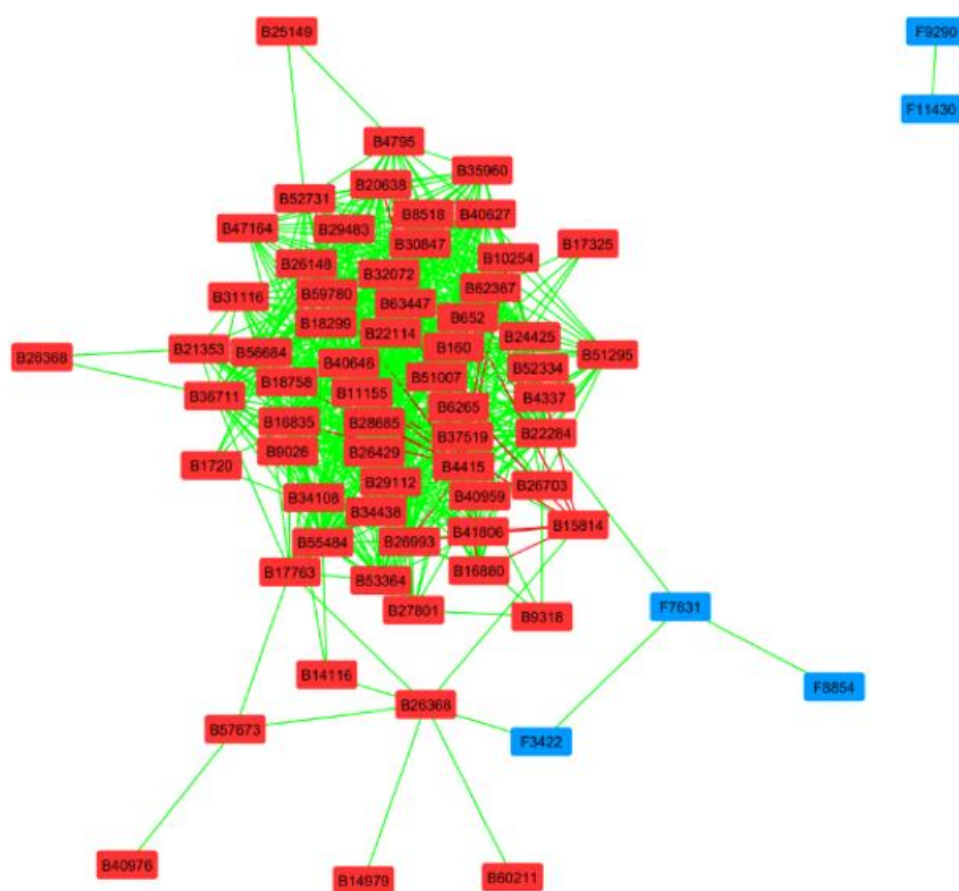


Figure 4. Cooccurrence network of bacterial and fungal OTUs within the rice root microbiome among six regions in Ghana. OTUs with low frequency (<500 reads, 0.2% sequence) were removed from both bacterial and fungal OTU data. Each kingdom was coded by colors; bacteria with the red box and fungi with the blue box. Green lines show a positive correlation between OTUs, while the red lines show a negative correlation between OTUs.

4. Discussion

4.1. Variations in Rice Root Microbiome Composition and the Influence of Environmental Factors.

We tested hypothesis 1) that rice root-associated microbiomes vary across the different regions, with local abiotic and biotic environmental factors involved in shaping the microbiome. We did find a variation in root-associated microbiomes across the six regions. Both bacterial and fungal communities showed significant differences among regions, in PerMANOVA. However, we also found low pseudo-F values: 2.436 and 1.586, respectively (Table 2), implying a low effect-size of regional differences on microbial community differences.

We detected abiotic and biotic environmental factors that influenced the assembly of rice root microbiomes (Table 3). Based on these results, a conceptual model was constructed to show the factors which shape the assembly of the rice root-associated microbiome in six regions of Ghana (Figure 5). Several abiotic factors: NO_3^- , pH, CEC, and TC were shown to shape the fungal community. Two biotic factors, root fungal diversity and root fungal read count as abundance seemed to shape the bacterial community. Moreover, soil NO_3^- concentration was significantly correlated with root-associated fungal diversity, which indicates that this factor directly affects the assembly of the fungal community and indirectly affects the root-associated bacterial community by altering root fungal α -diversity.

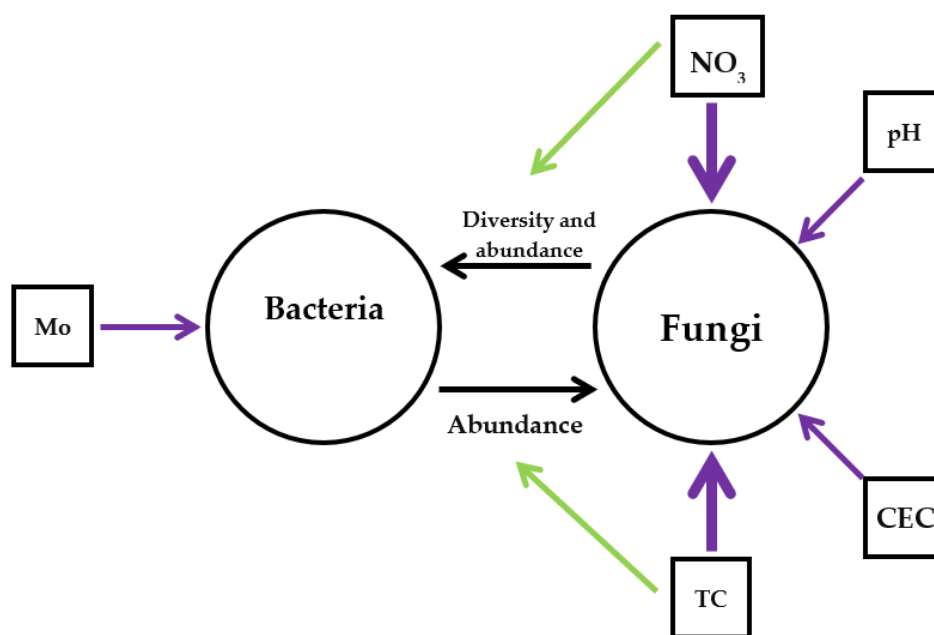


Figure 5. Conceptual model of factors that shape the assembly of rice root microbiome in six regions in Ghana. Model of bacterial and fungal community assembly was predicted based on the result of vector analysis (Tables S7 and S8; $P < 0.05$). Thickness of arrow stands for statistical significance: $P < 0.05$, $P < 0.01$, $P < 0.001$. Mo—moisture, CEC—cation exchange capacity; TC—total carbon.

Nitrogen is the primary limiting nutrient in most terrestrial ecosystems, with NO_3^- as the form of nitrogen available to both plants and soil microorganisms [54]. In soil property analysis, we found a NO_3^- gradient from the northern to southern regions of Ghana (Table 1). Fierer et al. [55] found that microbial communities varied along a soil nitrogen gradient in field-based long-term nitrogen fertilization experiments in the US. Chen et al. [56] also conducted a field-based long-term nitrogen enrichment experiment in semiarid Mongolia and found a significant decrease in soil fungal and bacterial biomass along the nitrogen gradient. Furthermore, root-associated fungi, such as arbuscular mycorrhizal fungi and ectomycorrhizal fungi showed an alteration in community structures, with a decline in diversity and richness along the soil nitrogen gradient [57,58]. Our results support their findings that nitrogen enrichment significantly affects the structure and diversity of root-associated microbiomes. We assume that the NO_3^- gradient that we observed may be due to farmer's fertilizer management. The Southern regions which were considered in this study belong to the Transition and Deciduous Forest agroecological zones, which have two rainy seasons, with annual precipitation of 1161.3 and 1404.8 mm, respectively. The studied regions in the north belong to the Savannah agroecological zone, which has only one rainy season, with an average rainfall of 1063.7 mm per year and a relatively low soil NO_3^- concentration [39]. Many farmers in the Southern regions, with better financial circumstances than those in the Northern regions, can also afford to apply chemical fertilizers to achieve higher yields [59].

The potential effect of soil NO_3^- enrichment on the assembly of root microbiome is due to the decrease in soil pH [60]. However, our soil property analysis showed a tendency toward lower soil pH in northern regions than in southern regions (Table 1). Therefore, we presume that other mechanisms may be involved in this phenomenon; influence of NO_3^- on rice root microbiome. In evolutionary history, plants have taken advantage of symbiotic relationships with microorganisms to enhance adaptability to multiple abiotic and biotic environmental stresses [61]. In the case of nitrogen deficiency, plants tend to change the composition and quantity of root exudates, potentially recruit beneficial microorganisms into their roots and discourage colonization by nonbeneficial microbes [62]. We hypothesize that such selection by the host may have influenced the assembly of rice root-associated microbes to adapt themselves to local NO_3^- conditions in six regions via unknown mechanisms. We also envisage that the

model could be more complex in our field-based observational survey than in other studies mentioned above which had tightly controlled experimental designs. Further investigation is needed to reveal the intricate relationships between microbiome networks, the host, and environmental factors.

4.2. Abundantly Presenting Bacterial and Fungal Taxa and Their Potential Functions in the Root-Associated Microbiome in the Six Regions

We tested hypothesis 2, that abundantly presenting bacterial and fungal in a different region could be key players in host rice productivity. We found that some bacterial and fungal OTUs were specific to the northern regions (UW, U., NO) harbor certain OTUs that are absent in the southern regions (BA, Ash, Vol). As mentioned above, the northern regions of Ghana belong to an agroecological zone called Guinea Savannah, and they are characterized by lower pH, NO_3^- , TC, TN and CEC (Table 1). The Guinea Savannah itself, including the northern regions, is characterized by higher temperatures than other zones (mean annual average, 28.3 °C [36]). The bacterial and fungal indicator OTUs in these regions may have adapted to the environment and could be the candidate of Grinnell's ecological "specialist" [63]. Evidence of such specialization of microorganisms has also been reported elsewhere (e.g., [64]). They found evidence of the ecological specialization of heterotrophic bacteria in different nitrogen enrichment in stream sediment. However, previous studies did not report the specialization of root-associated microorganisms at the regional scale, so ours may be the first study to do so.

Some of the microbial OTUs abundantly present to the northern regions are known to be endophytes (e.g. *Aeromonadaceae* sp., *Clostridium* sp. *Pleomorphomonas* sp.) or pathogens (e.g. *Xanthomonadaceae*. OTU_B14074). The family *Aeromonadaceae* includes well-known pathogens of mammals, including humans [65], and others are also reported to colonize rice roots as endophytes [66], but their function in the rice host is not known. The genus *Clostridium* includes species that are capable of nitrogen fixation in the root of *Miscanthus sinensis* (Chinese silver grass) [67]. The genus *Pleomorphomonas* includes the species *P. oryzae*, capable of nitrogen fixation in rice roots [68]. The family *Xanthomonadaceae* includes two plant-pathogenic genera, *Xanthomonas* and *Xylella* [69], with *Xanthomonas oryzae* specific to rice [70].

Among the abundantly presenting fungal OTUs, one taxon has been reported as a rice endophyte, *Chaetomium* (OTU_F10228 and OTU_F4724). Another is both an endophyte and a pathogen in rice, *Pleosporales* (OTU_F4099, F3272 and F11327). Three have been identified as rice pathogens, *Sordariomycetes*. (OTU_F2298), *Myrothecium verrucaria*. (OTU_F7577) and *Macrophomina phaseolina* (OTU_F4358) (Table 5.). The genus *Chaetomium* includes the species *C. globosum*, capable of colonizing rice root and leaves and producing antifungal metabolites [71,72]. The genus *Cochliobolus*, which belongs to the order *Pleosporales*, is a well-studied pathogen of monocots with a wide range of hosts, including rice [73]. The class *Sordariomycetes* includes the species *Magnaporthe grisea*, which is a known cause of rice blast disease [74]. *Myrothecium verrucaria* and *Macrophomina phaseolina* are plant pathogens with a wide range of hosts, including rice [75,76].

Endophytes from these abundantly presenting microbial taxa could be key players for improving rice production in the northern regions of Ghana. The genus *Pleomorphomonas*, in particular, is expected to improve nitrogen availability for rice by nitrogen fixation. On the other hand, we also detected abundantly presenting bacterial and fungal pathogenic taxa that could be the cause of rice disease needing to be controlled in the northern regions. We expect the abundantly presenting fungal endophyte *Chaetomium* (OTU_F10228 and OTU_F4724) could antagonize these abundantly presenting pathogens in the northern regions by producing pharmacologically active metabolites [71]. Further functional investigation by isolation with pure culturing and in vitro scale experiments is necessary to assess the roles of those abundantly presenting taxa for host and adaptability to local environments.

4.3. Potential Interactions Between Bacteria and Fungi in the Root Microbiome in Six Regions

We tested hypothesis 3, certain bacterial and fungal taxa may be correlated and interact in the rice root microbiome in Ghana. By vector analysis, we revealed the abiotic and biotic environmental factors

involved in the assembly of the root-associated microbiome, with evidence for interactions between root-associated bacteria and fungi. Cooccurrence network analysis revealed potential interactions among bacterial and fungal OTUs in the root microbiome as a cooccurrence pattern. We found few cooccurrence patterns between bacterial and fungal OTUs. On the other hand, we found significant correlations between the assembly of bacterial and fungal community composition and their diversity indices (Table 3), hence, we assume that there are, potentially, interactions between bacteria and fungi at the community level within rice root-associated microbiomes in Ghana.

In general, bacteria and fungi share microhabitats in a variety of environments in which they interact with each other [77]. Such interaction has been widely studied, especially by in vitro scale studies [78,79], although our understanding of bacterial and fungal interaction is still limited at the field scale [78]. In the case of the root-associated microbiome, they share the plant root endosphere as a habitat and interact at both molecular and physiological levels, playing an important role in host plant productivity [80]. Few studies have focused on these potential interactions at the field scale.

It is thought that root-associated bacteria–fungus interaction is mediated by signal exchange with the host plant and competition for resources, including infection sites [81]. Our results suggest the potential interaction of root-associated bacteria and fungi at a community level, rather than at the level of OTUs. As mentioned previously, bacterial abundance affects fungal community assembly. Conversely, fungal diversity affects bacterial community assembly (Table 3). It has been reported that fungal and bacterial communities shape each other [82,83]. Singh et al. [82] revealed the potential effects of an arbuscular mycorrhizal fungal community on the root-associated bacterial community, although the inverse effect was not detected. Our study is consistent with their findings on the effect of root-associated fungi on bacteria at the community level. Furthermore, our results indicate that the bacterial community does affect the fungal community, suggestive of a mutualistic interaction in the root microbiome, at the community level. On the other hand, the more complex cooccurrence pattern of bacterial OTUs suggested that OTU level interaction occurs only within bacterial communities in the rice microbiome. This may be the first study to show evidence of mutualistic interaction of bacteria and fungi in a root microbiome at the community level. However, the nature and mechanisms of the interaction of rice root-associated bacteria and fungi are unclear.

5. Conclusions

In this study, we found that the structure of the rice root-associated bacterial and fungal community varied significantly between regions and that local environmental factors influence the assembly of these community compositions. The bacterial communities tended to be shaped by fungal α -diversity and abundance, whereas the fungal communities tended to be shaped by abiotic factors, such as soil NO_3^- . The soil NO_3^- gradient from the North to South regions seems mostly responsible for the variation in the rice root microbiome directly and indirectly by affecting microbial diversity and abundance. We detected bacterial and fungal taxa abundantly present in the Northern regions, not in the Southern regions in Ghana reported as endophytic symbionts such as N-fixing bacteria. We suggest that those microbial traits may be candidates of ecological specialists, having adapted to a harsh, local environment and could be key players to improve host productivity. Finally, we uncovered potential interactions between rice root-associated bacteria and fungi at a community level; shaping each other at the community level, but not at OTU. However, the mechanisms shaping assembly of the rice root microbiome, the functions and role of the candidates of specialist microbial traits on the host plant, and their interactions are still uncertain. Further identification of functions, adaptabilities and interactions of those microbial traits by culturing method should be necessary. Overall, our study contributes to the further understanding of the ecology of the rice root microbiome and begins to explore its potential for the improvement of rice production in Ghana.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2071-1050/12/14/5835/s1>, Figure S1: Rarefaction curve, Table S1: Sample ID and sampling location, Table S2: α -diversity index, Table S3: Correlation between bacterial community and environmental factors, Table S4: Correlation between fungal community and environmental factors, Table S5: Correlation between abiotic and biotic factors, Table S6: indicator bacterial OTUs in six regions and their characteristics on host plant., Table S7: indicator Fungal OTUs in six regions and their characteristics on host plant.

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