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ORIGINAL ARTICLE

Microbial community structure in the rhizosphere of the orphan legume Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Marechal & Baudet]

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Abstract
The presence of microbial communities in the rhizosphere of plants is an important determinant of plant health and soil organic matter composition. Plant species play significant roles in selecting the specific microbial communities that inhabit the root zone. However, till now, there is no solid information regarding the presence of specific plant-microbiome in the rhizosphere of many plants, especially under-exploited and under-researched species such as Kersting's groundnut. This study assessed the effect of five Kersting's groundnut landraces on the structure of microbial communities in rhizosphere of field-grown plants. The five tested Kersting's groundnut landraces (Belane Mottled, Boli, Funsi, Puffeun and Heng Red Mottled) were found to exert a marked selective influence on bacteria associated with their rhizospheres, measured using 16S rDNA MiSeq illumina sequencing. Community differences in microbial composition and relative abundance were both significant. Numerous phyla in the rhizosphere were affected by the test landraces. Except for Belane mottled whose rhizospheres were dominated by *Proteobacteria*, the rhizosphere soils of the other landraces were dominated by *Bacteroidetes*. With the exception of landrace Puffeun which showed only *Mesorhizobium* in its rhizosphere, all the other test landraces revealed the presence of *Bradyrhizobium* and *Rhizobium* species of alpha *Proteobacteria*. Furthermore, the rhizosphere of all landraces were abundant in species of the indole-3-acetic–acid producing *Sphingomonas* and cellulose-degrading *Fibrobacteres*. The results of this study suggest that Kersting's groundnut landraces can shape bacterial community composition in the rhizosphere via plant-related changes in the rhizosphere soil.

Keywords Phosphate solublizing bacteria · IAA producing bacteria · Cellulose degrading bacteria · Uncultivated bacteria · Diversity · Legumes · 16S rDNA · MiSeq illumina sequencing

Introduction

The Leguminosae represents a major family within the plant Kingdom, as it comprises more than 20,000 species [1]. This family is divided into the three subfamilies Mimosoideae, Caesalpinioideae and Faboideae [2]. The first two subfamilies consist of woody species with a more tropical distribution, while the Faboideae is the largest subfamily with both woody and herbaceous species and a cosmopolitan distribution.

Of the family leguminosae, grain legumes are considered a vital source of dietary protein in most developing countries. However, many traditional food legumes have remained neglected and underutilized despite their contribution to both food and nutritional security [3]. Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Marechal & Baudet] is a less cultivated grain legume believed to have originated from northern Togo or central Benin in West Africa [4]. There is apparently a large genetic distance between domesticated and wild accessions of Kersting's groundnut, suggesting that the wild progenitor of this legume may be a crop still unknown [4].

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Kersting's groundnut is an annual legume cultivated in the West African savanna, which stretches from Mali to Nigeria through to Burkina Faso and Benin [4]. Although there is no reliable statistical data on its production and trade, Kersting's groundnut is commonly found in village markets of West African countries such as Ghana, Togo, Benin and Nigeria [5, 6]. The legume is adapted to the marginal Guinea savanna soils, and grows well even under poor rainfall conditions [5]. The seeds of Kersting's groundnut are rich in protein (23.1%) and carbohydrate (61.53–73.3%), with a high proportion of essential amino acids (42%) [7, 8]. Unfortunately, the crop is facing extinction despite its good taste, high nutritional and medicinal value and N_2-fixing ability [8–11].

The gradual extinction of Kersting's groundnut is partly due to the fact that its cultivation is done by elderly people for superstitious reasons [12]. The nutritional prospects and the possible strategies to reverse the disappearance of Kersting's groundnut have been comprehensively reviewed [8]. Kersting's groundnut has potential for inclusion in cropping systems due to its ability to fix N_2 in root nodules. Few studies have revealed the crop's nitrate-tolerant symbiosis [13], variable response to inoculation in the field [11] and the ability to form effective root nodules with diverse species in the genus *Bradyrhizobium* leading to high N_2 fixation [14].

Exudation of phenolic compounds into the rhizosphere of legume plants can attract rhizobia towards root hairs during the process of nodule formation [15]. Additionally, some components of legume root exudates (e.g. flavonoids, amino acids, aromatic acids and other dicarboxylic acids) also serve to attract other microorganisms to the rhizosphere region [16]. The community of microbes in the rhizosphere usually includes plant growth promoters, pathogens, as well as bacteria that are antagonistic to other microbes within the rhizosphere [17]. Bacterial communities in the rhizosphere of soybean were found to exhibit marked changes in population dynamics at different plant growth stages (vegetative, flowering and maturative), with an increase in *Proteobacteria* and a decrease in abundance of *Acidobacteria* and *Firmcutes* in the rhizosphere, which ultimately resulted in the domination of the rhizosphere by potential plant growth-promoting bacteria such as species of *Bacillus*, *Bradyrhizobium* and *Rhizobium* [18]. Members of the *Rhizobiales* found in soybean rhizosphere are known for their N_2-fixing ability when in symbiosis with suitable host plants [19].

The practice of inoculating legumes with desirable rhizobial strains in order to enhance nodulation and N_2-fixation is also known to modify rhizosphere microbial communities as a result of complex interactions [16]. For example, inoculating common bean with *Rhizobium etli* containing trifolitoxin encoding genes decreased the diversity of trifolitoxin-sensitive members of the α-*Proteobacteria* in the rhizosphere of field-grown plants [20]. Similarly, inoculating alfalfa with *Sinorhizobium meliloti* L33 resulted in a decrease in the rhizosphere population of γ-Proteobacteria and an increase of α-Proteobacteria [20]. Kawasaki et al. [21] also observed that pyrene contamination of the rhizosphere of legumes, including White clover, caused an increase in the populations of *Actinobacteria* and *Verrucomicrobia* in the rhizosphere, with a concomitant increase in the numbers of the genus *Denitratisoma*. The interaction of plant root exudates and microbes in the rhizosphere is globally important for biogeochemical cycling, plant health and productivity [22, 23]. The roots of plants play a major role in plant–microbe interaction as they are able to transport 20–50% of photosynthate into the soil environment, thus aiding microbial colonization by both plant pathogens and plant growth-promoting rhizobacteria (PGPR) [24]. The PGPRs aid the survival and growth of plants through nutrient provision and disease suppression in soils under unfavorable conditions [25], as well as decomposition of organic matter [26]. While culture-based techniques have allowed isolated microbes to be studied in detail, molecular techniques such as metagenomics have increasingly permitted the identification of microbes in situ.

Numerous factors can shape the microbial communities associated with the rhizosphere of plants. Although microbial inoculants have been used to boost legume and cereal production [27], resource partitioning in soils is influenced by the rhizosphere microbiome that regulates plant niche differentiation and plant diversity [28, 29]. More than 50,000 bacterial species, most of which are uncultured, are estimated to be present in a gram of soil [30, 31]. Variable soil conditions as well as plant root exudates are known to influence the diversity of rhizosphere bacteria.

There is however little information on the rhizosphere microbiology of Kersting's groundnut. Although there is no available information on the phytochemical composition of the crop's root exudates, the Kersting's groundnut landraces used in this study have been shown to attract diverse rhizobia leading to the formation of effective symbiotic relationships that confer variable adaptation to soil nutrient limitation, especially nitrogen [11, 14], although there is scanty information regarding the specific pathogens and diseases affecting Kersting's groundnut, black seeded landraces are reported to exhibit resistance the crop's key storage beetle, *Callosobruchus maculatus*, when compared to their white or brown seeded counterparts [32, 33].

Recently, 16S rDNA and throughput (large-part of genome) sequencing has been used to examine rhizosphere bacterial communities of various plant species [34, 35]. This study is an attempt to understand the extent to which Kersting's groundnut landraces can influence and shape the microbial community (composition and diversity) in their rhizospheres in order to know the plant–microbe

interactions for agricultural sustainability, using MiSeq illumina sequencing.

Materials and methods

Field planting of Kersting's groundnut

Seeds of five Kersting's groundnut landraces of variable seed coat pigmentation [Boli (white), Funsi (brown), Belane Mottled (brown mottled), Puffeun (black) and Heng Red Mottled (brown mottled)] were each planted on plots measuring 3 m × 2 m at Tamale (09°24′27″N 00°51′12″W) in the Northern Region of Ghana. Aside their phenotypic differences, the test landraces were recently shown to exhibit some genetic variation [11] Of the test landraces in this study, the seeds of Boli, Funsi and Puffeun were also shown to have similar anthocyanin profiles (i.e. Delphinidin-3-O-glucoside, Cyanidin-3-O-glucoside, Petunidin-3-O-glucoside), with the black seeded Puffeun recoding higher levels of these anthocyanin pigments in their seeds when compared to Boli or Funsi [36]. Planting was done at a spacing of 20 cm between plants and 50 cm between rows, resulting in ~ 45 plants per plot. Three replicate plots were established per each landrace and laid in a randomized complete block design. Plots were separated by 1 m spacing while a path of 1.5 m was left between blocks. The field used for the trial had no history of inoculant application.

Soil sampling and preparation

Before planting, soils were sampled (0–20 cm depth) from 20 points on the field and bulked together for analysis of chemical properties (Table 1) at the Institute for Plant Production, Elsenburg in the Western Cape, South Africa. At 50% flowering, rhizosphere soils were collected from each plot. Plants were carefully dug up using a spade and the soil attached to roots (rhizosphere soils) was collected by shaking it off into plastic zip-lock bags. The rhizosphere soils of each landrace from the three replicates plot were pooled together to obtain one composite sample per landrace. Soils were stored at − 20 °C suggested by Vestergaard et al. [37].

Table 1 Physico- chemical properties of the bulk soil sample

pH and minerals	Concentration
Carbon	0.26%
Iron	42.70 mg/kg
Potassium	48 mg/kg
Magnesium	0.35 cmol(+)/kg
Sodium	9 mg/kg
P (citric acid)	1 mg/kg
Calcium	2.58 cmol(+)/kg
NH_4 nitrogen	0.029%
pH	6.8

DNA extraction from rhizosphere soils

Genomic DNA was extracted from 0.5 g of rhizosphere soil for each landrace, using PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. The DNA samples were sent to Macrogen, South Korea, for sequencing and analysis. The DNA concentrations were determined using PicoGreen (Invitrogen, cat. # P7589) method and Victor 3 fluorometry. Fluorescence was measured for 3 cycles of 30 s at 25 °C in 96 well plates, and a standard curve generated by means of the fluorescence results used to determine DNA concentration, which was adjusted to a final concentration of 3.5 ng/µl.

Library preparation

Random fragments of the DNA samples were used to prepare the library, followed by 5′ and 3′ adapter ligation. PCR amplification was done using adapter-ligated primer pairs 5′TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTACGGGNGGCWGCAG3′ and 5′GTCTCGTGGGCT CGGAGATGTGTATAAGAGACAGGACTACHVGGG TATCTAATCC3′ targeting variable regions V3-V4 of the 16S rDNA gene [38] in 25 µl reaction volume containing 12.5 µl KAPA HiFi hotstart ready mix (2×), 5 µl each of forward and reverse primer (1 µM) and 2.5 µl sterilized double distilled water with the standard temperature profiles [30″ − 95 °C, 25 × (30″ − 95 °C, 30″ − 55 °C, 30″ − 72 °C), 5′ − 72 °C]. PCR amplified products were purified using AMPure XP beads. The library was loaded into a flow cell for cluster generation.

Sequencing and data assembling

Illumina SBS technology was utilized for paired end sequencing. During data analysis and alignment, the newly identified sequence reads were then aligned using FLASH to reference genome. The raw sequences were processed in CD-HIT-OTU for quality control (QC) assessment. All processed and QC passed cluster files were analysed using Quantitative Insights into Material Ecology (QIIME) pipe line [39]. The representative reads from non-chimeric clusters were grouped using a greedy algorithm into operational taxonomic units (OTUs) at a user-specified OTU cut-off (e.g. 97% ID at species level). The alpha diversity index (Chao1, Shanon, and Simpson) was calculated for each sample at both distances. The taxonomical abundance (%) of microbial communities

of each rhizosphere soil was calculated using read files as queries against removed and de-replicated set of sequences from the small subunit (SSU) UCLUST [40]. After normalization of sequences of each sample, rarefaction was analysed at species level for sampling adequacy by using alpha_rarefaction.py of QIIME.

Statistical analysis

To seek the order of the samples along the axes of principal coordinates to explain the variance in samples, principal coordinate analysis (PCoA) was carried out using QIIME with output data of beta diversity (pairwise sample dissimilarity). The differences in rhizosphere bacterial community structure among the landraces were assessed using Unifrac weighted distance metric. A one-way analysis of variance was used to compare the individual bacterial phyla identified in the rhizosphere of the test landraces. Duncan multiple range test (DMRT) was applied to separate the means at $p \leq 0.05$. The data were submitted to NCBI Sequence Read Archive under project Bioproject ID PRJNA335714.

Results and discussion

Plants can form close symbiotic relationships with soil microbes. Such plant-microbial communities play a crucial role in agricultural productivity and the resilience of the ecosystem to climatic stress. Understanding plant–microbe interaction can reveal novel ways of using these microorganisms to support plant health and productivity, without altering ecosystem functioning. Metagenomics can elucidate the diversity, abundance, and dynamics of microbial genes and pathways participating in biogeochemical transformations of mineral nutrients in a variety of ecosystems. In this study, the bacterial richness and community structure in the metagenome of the rhizosphere soil of five Kersting's groundnuts were analysed using MiSeq Illumina sequencing (Fig. 1).

Many uncultured bacterial populations were observed in this study, a finding consistent with the report by Aslam et al. [41] which found abundant uncultured microbes in their study site. Our results showed distinct bacterial communities in the rhizospheres of the five Kersting's groundnut landraces, which probably denotes differences in bacterial community function across these environments. Results indicated that Funsi had maximum sequence bases in its

Fig. 1 A field grown view of Kersting's groundnut plant, pod and seeds with different seed coat colours

Table 2 Information about the number of raw sequences generated, quality of sequences and chao1

Sample name	Total bases	Raw read count	GC (%)	Q20 (%)	Q30 (%)	Chao1	Good coverage
Belane mottled	59921540	132841	54.87	96.86	86.92	680.57	0.98
Boli	90362348	200133	54.16	97.1	87.71	840.71	0.99
Funsi	94982600	210705	54.37	97.11	87.69	981.82	0.99
Puffeun	56900504	123953	52.77	96.5	86.08	609.35	0.99
Heng red mottled	47007778	103559	53.18	96.22	85.25	574.3	0.98

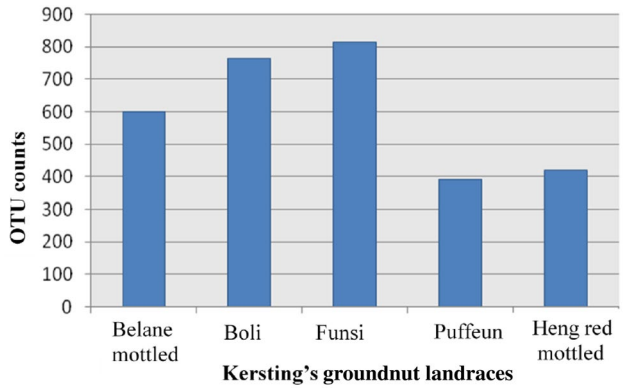

Fig. 2 Distribution and number of OTUs that found in the rhizosphere of test Kersiting's landraces

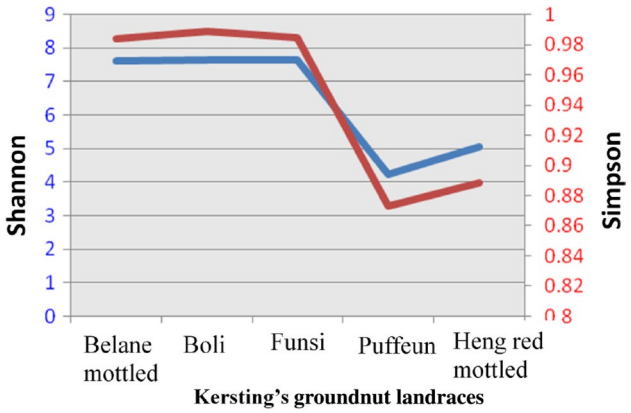

Fig. 3 Microbial community in Shannon (Blue line) and Simpson (Red line) diversity index of microbial communities in the rhizosphere soils of the test Kersting's groundnut. (Color figure online)

rhizosphere soils (> 90 million), while the lowest sequences were observed in the rhizosphere soil of Heng Red Mottled (< 5 million) (Table 2). Variations were observed in read output across the samples, with > 100,000 reads being obtained for each sample. A total of 0.1 to 0.21 million reads were generated. Boli and Funsi landraces had > 200,000 reads (Table 2). The highest OTU (816) was observed in the metagenome of Funsi samples, with Puffeun showing the least (Fig. 2). The Shannon diversity index showed similar bacterial diversity (7.6) in the rhizoshere soils of landraces Belane Mottled, Boli and Funsi, which was higher than the diversity observed in the rhizosphere soils of Puffeun (4.2) and Heng Red Mottled (5.1) (Fig. 3). Furthermore, the results of OTU richeness reached a plateau at 14,000 sequences per sample. The alpha rarefaction graph (Fig. 4) was not reaching the plateau, indicating that more reads were needed to capture all the diversity. Based on rarefaction analysis, the prokaryotic diversity at species level was highest in the rhizosphere soil of Funsi, and lowest in the rhizosphere of the Puffeun landrace (Fig. 4). The profiles of microbial communities in the rhizosphere soils of Boli and Heng Red mottled were almost similar at the phylum, class, order and family levels, despite some little differences in the relative abundance of some bacteria (Fig. 5).

This atlas of bacterial communities associated with the rhizosphere of Kersting's groundnut could aid in our understanding of how bacterial communities function with each landrace as a host plant. Although reads were also assigned as far as the species level, the taxonomic differences between the various rhizosphere soils were indicated by their PCoA (Fig. 6). The PCoA results indicated that there were differences in microbial community composition and showed that the first axis of PC1 versus PC2 could explain 73.25% of the variance in the data, indicating that the relative abundance of most OTUs were diverse between the rhizosphere soils of all test landraces (Fig. 6). The number of sequences attributed to each taxon were compared between the rhizospheres of the five Kersting's groundnut landraces based on distance matrix estimated with UPGMA algorithm presented by the phylogenetic tree in Fig. 7. The results yielded two main clusters (Cluster I & II), which clearly showed the presence of diverse microbial community structures in the test rhizosphere soils.

Sequences were classified and summarized according to bacterial phyla, and these showed differences between and among the rhizosphere soils of the five Kersting's groundnut landraces, as well as revealed the overall composition of the community at a high phylogenetic resolution. The variation in OTU richness, both within and among phyla, decreased non-linearly with landraces, which suggests that selection of rhizosphere microsymbionts was highly plant-specific [28, 42]. The results also showed that microbial communities in the rhizosphere of the black seeded Puffeun landrace were slightly different (i.e. less cover of bacterial phyla) in

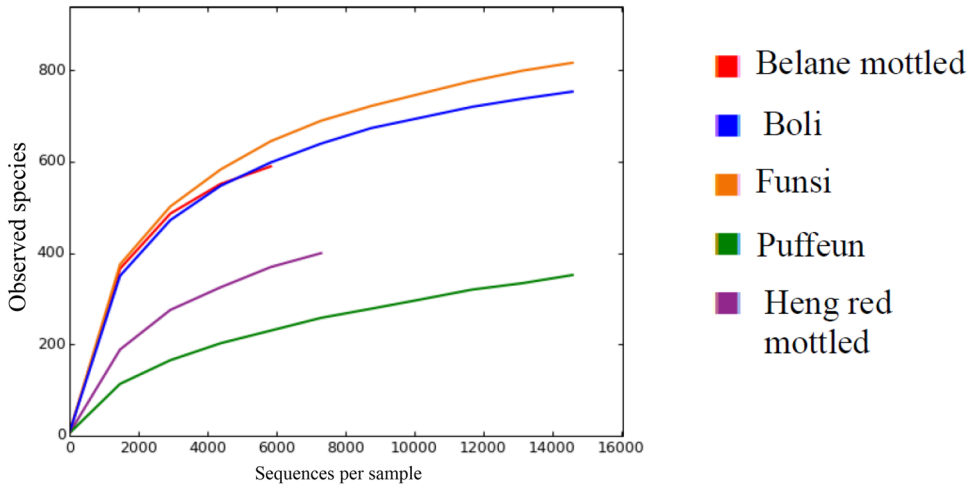

Fig. 4 Rarefaction analysis of microbial communities at the species levels to determine whether sampling depth was sufficient to accurately characterize

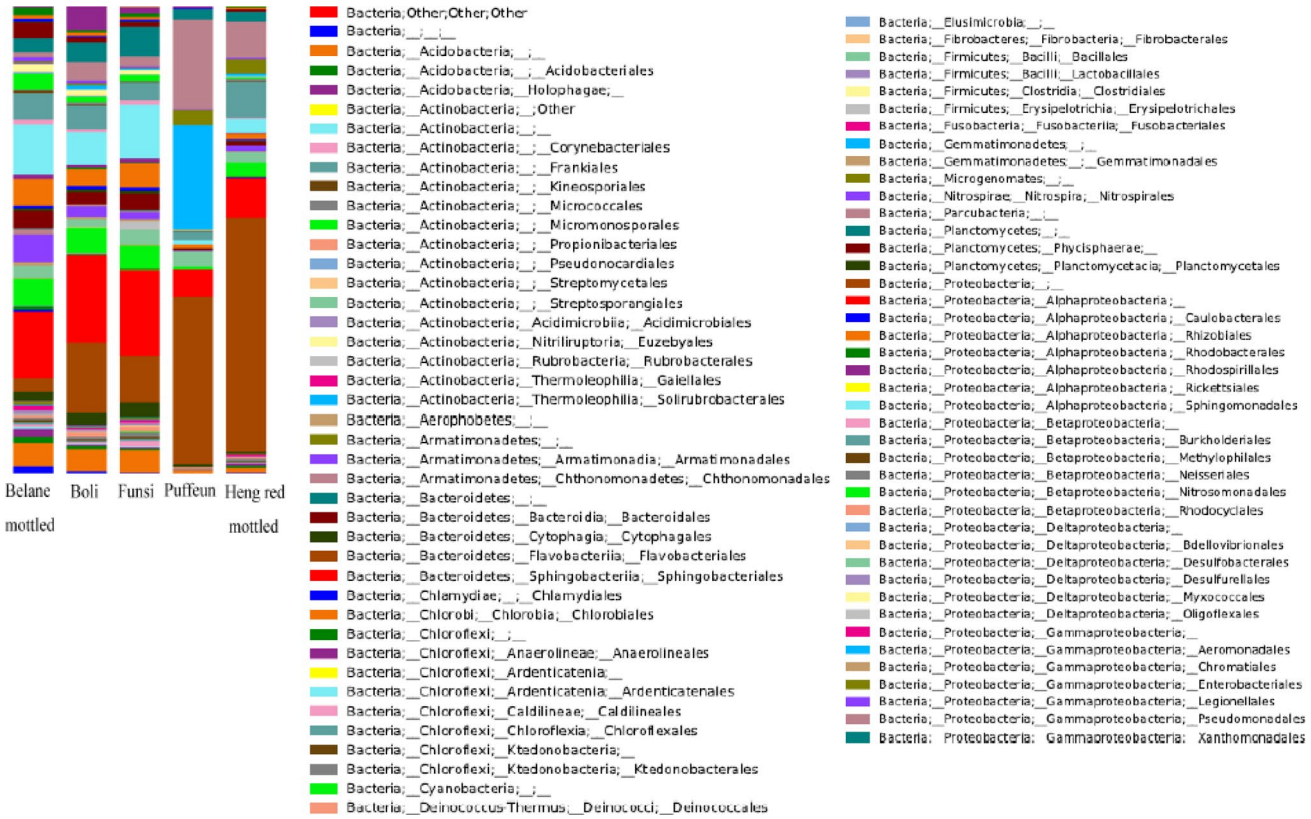

Fig. 5 Taxonomic distribution based on 16S rDNA sequences at the bacterial phylum to order levels in rhizosphere soils of Kersting's landraces. (x axis = sample name; y axis = OTU proportions)

comparison to the rhizosphere soils of the other landraces. In contrast, the rhizosphere of Belane Mottled harboured many bacterial genera, which suggests that bacterial community composition and its development in the rhizosphere is regulated by plant-induced changes in the soil [28].

Despite the presence of diverse bacterial species in the rhizosphere soil of the five Kersting's groundnut landraces, most of these bacteria were unclassified. For example, the rhizosphere soils of Belane Mottled, Boli, Funsi, Puffeun and Heng Red Mottled, respectively, contained 39.91, 36.86, 37.73, 30.35 and 34.25% unclassified bacteria (Table 3). Except Puffeun, other landraces contain beta proteobacteria *Methylophilaceae* in their rhizosphere.

The most abundant prokaryotes in the rhizosphere of the landrace Belane Mottled was the *Proteobacteria*. In contrast, Boli, Funsi, Puffeun and Heng Red Mottled harboured

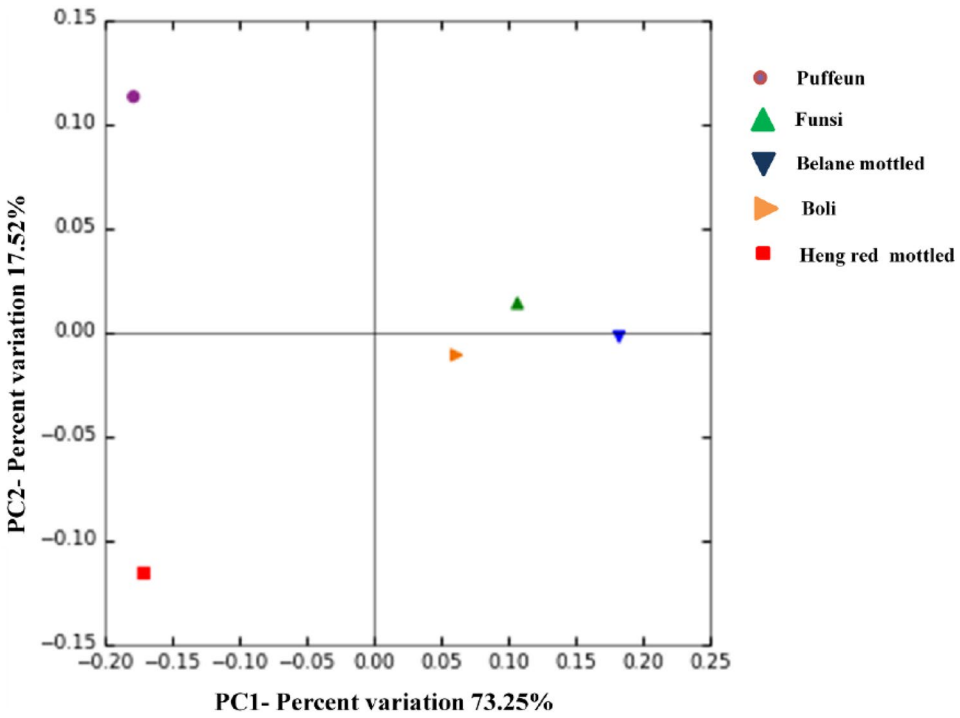

Fig. 6 PCoA plot based on weighted unifrac distances showing the differences in the composition of microbial communities in the rhizospheric soil samples of Kersting's groundnut landraces

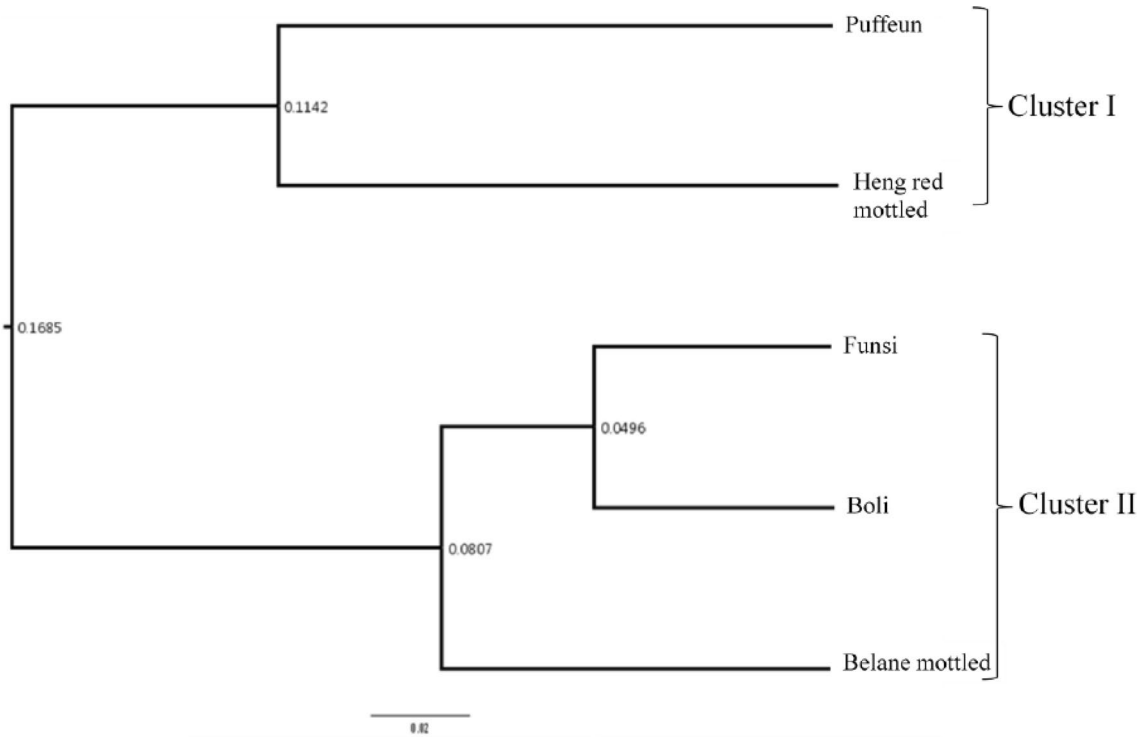

Fig. 7 Hierarchical cluster analysis based on distance matrix with the UPGMA algorithm for five metagenomes of rhizosphere of Kersting's groundnut

many more *Bacteroidetes*, followed by *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Firmicutes*, *Nitrospirae*, *Planctomycetes* and *Verrucomicrobia* in their rhizospheres. Furthermore, the *Cynobacteria* were in high proportion in all test rhizosphere soils. These major groups were earlier identified in a PCR-based and metatranscriptomic study of

Table 3 The Phylum showing significant differences among the samples

Phylum	Belane mottled	Boli	Funsi	Puffeun	Heng Red mottled	F statistics
Acidobacteria	7.44 ± 0.15a	3.83 ± 0.36b	1.76 ± 0.54c	0.32 ± 0.17c	6.25 ± 0.58a	54.9***
Actinobacteria	6.29 ± 0.76b	6.08 ± 0.36b	9.07 ± 0.04a	3.47 ± 0.20c	4.89 ± 0.38bc	24.1**
Arthropoda	0.38 ± 0.04a	0.22 ± 0.00bc	0.28 ± 0.02ab	–	0.25 ± 0.08ab	7.4*
Ascomycota	0.35 ± 0.05bc	0.47 ± 0.06b	0.74 ± 0.30a	0.22 ± 0.03 cd	0.13 ± 0.05d	32.7***
Bacillariophyta	0.88 ± 0.01ab	0.89 ± 0.11ab	0.68 ± 0.06b	0.16 ± 0.01c	0.99 ± 0.12a	18.5**
Bacteroidetes	8.38 ± 0.49d	16.11 ± 0.66c	14.75 ± 0.11c	34.61 ± 1.48a	23.76 ± 1.29b	111.8***
Basidiomycota	0.08 ± 0.03a	0.07 ± 0.03a	0.13 ± 0.02a	–	–	2.6 ns
Chlamydiae	0.35 ± 0.09a	–	0.12 ± 0.0a	–	0.29 ± 0.12a	4.1 ns
Chloroflexi	1.22 ± 0.39a	1.17 ± 0.24a	1.45 ± 0.46a	0.40 ± 0.08a	0.57 ± 0.21a	2.1 ns
Chlorophyta	0.09 ± 0.01bc	0.31 ± 0.05a	0.14 ± 0.02b	0.001 ± 0.00c	0.08 ± 0.00bc	14.8**
Cyanobacteria	2.51 ± 0.02c	3.86 ± 0.55ab	4.42 ± 0.37a	1.01 ± 0.11d	3.12 ± 0.03bc	19.5**
Deinococcus-Thermus	0.13 ± 0.10a	–	–	–	0.10 ± 0.00a	1.0 ns
Firmicutes	5.96 ± 1.72a	5.88 ± 0.05a	6.44 ± 0.79a	4.77 ± 0.31a	6.24 ± 1.07a	0.43 ns
Gemmatimonadetes	0.63 ± 0.01a	0.37 ± 0.01b	0.57 ± 0.08ab	–	0.41 ± 0.08b	16.7**
Nitrospirae	1.60 ± 0.18a	0.98 ± 0.16b	0.73 ± 0.11b	0.11 ± 0.01c	0.60 ± 0.15bc	15.7**
Planctomycetes	1.40 ± 0.75a	1.80 ± 0.57a	1.55 ± 0.54a	0.59 ± 0.23a	1.06 ± 0.62a	0.7 ns
Proteobacteria	12.60 ± 2.81a	11.53 ± 1.31a	11.21 ± 0.97a	20.97 ± 5.69a	10.32 ± 0.61a	2.17 ns
Spirochaetes	0.54 ± 0.19a	0.25 ± 0.09a	0.25 ± 0.12a	–	0.27 ± 0.16a	1.8 ns
Streptophyta	1.67 ± 0.13a	1.25 ± 0.14b	1.11 ± 0.12bc	0.60 ± 0.10d	0.77 ± 0.00 cd	14.4**
Synergistetes	0.10 ± 0.02	–	–	–	–	–
unclassified (derived from Bacteria)	39.91 ± 0.13a	36.86 ± 0.95a	37.73 ± 1.58a	30.35 ± 6.07a	34.25 ± 2.57a	1.4 ns
unclassified (derived from Eukaryota)	0.59 ± 0.02a	0.45 ± 0.03b	0.65 ± 0.05a	0.19 ± 0.01c	0.42 ± 0.01b	46.1***
Verrucomicrobia	4.79 ± 0.93a	5.19 ± 0.99a	4.04 ± 1.36a	1.33 ± 0.46a	3.20 ± 0.56a	2.8 ns

Values (Mean ± SE) with dissimilar letters in a row are significantly different at $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***)

both bulk and rhizosphere soils [42–45]. Although in this study the *Actinobacteria* and *Bacteriodetes* contributed more to their respective phyla, it was the *Proteobacteria* that showed greater diversity. In fact, this study shows that the major microbial communities associated with Kersting's groundnut rhizosphere were *Flavobacteriales*, *Sphingobacteriales*, *Rhizobiales*, *Sphingomonadales*, *Burkholderiales* and *Xanthomonadales,* which are well known for their interaction with plants in the rhizosphere [46]. From this study, changes in rhizosphere microbial communities can be attributed to differences in the genetic make-up of Kersting's groundnut landraces [28] as well as variations in the relative phytochemical compositions of their seed and root exudates [36].

Based on species level PCoA, there was a plant effect on the total community structure in the rhizosphere. The dissimilarity in microbial community structure between rhizospheres of test landraces could be attributed to selection or depletion by plant root exudates. For example, we observed the selection of taxa with phosphate-solubilizing ability in the rhizosphere of Kersting's groundnut landraces. The rhizosphere of the landrace Puffeun was strongly enriched with species of *Flavobacterium* (previously discovered in soybean rhizosphere) and *Aeromonas,* a phosphate-solublizing bacterium [47, 48]. Both *Chryseobacterium* and *Flavobacterium* were abundant in the rhizosphere of Heng Red Mottled. Interestingly, species of *Chryseobacterium* were earlier isolated from bean roots and reported to have phosphate-solubilizing ability and a role as plant growth-promoting rhizobacteria [49–51]. The rhizospheres of Belane Mottled, Boli and Funsi landraces showed an abundance of the indole-3-acetic acid-producing bacterium *Sphingomonas*, reported earlier in the rhizosphere of soybean [18]. Some other Gram-negative bacteria such as *Massilia, Flavobacterium, Chitinophagaceae, Nitrospiraceae,* and *Bacillus* were also detected in the rhizosphere of Kersting's groundnut landraces. The absence of *Sphingomonas* in the rhizosphere of Puffeun and Heng Red Mottled could possibly be attributed to their production of antimicrobial compounds, or the inability of these bacteria to use plant-derived carbon sources which can lead to depletion of the taxa in the rhizosphere. Some cellulose-degrading bacteria such as *Fibrobacteres* [52] were found in the rhizospheres of Belane Mottled and Funsi, an observation that probably suggests the role of plant cell wall substances in shaping rhizosphere microbiome [53]. The nitrate-reducing *Methylophilaceae* of the beta-*Proteobacteria* was also present in the rhizosphere of all landraces, except Puffeun. Apparently,

members of the *Methylophilaceae* can reduce nitrate in the presence of methanol produced from pectin of plant cell walls origin, and this helps in bacterial colonization through the activation of C1 metabolism in the rhizosphere [42, 54].

Some bacterial taxa were thus common in all rhizosphere soils and this could suggest their general presence in plant rhizospheres. The microbial community in the rhizosphere of the black-seeded Puffeun was totally different in having a less diverse type of microbiome when compared to the rhizospheres of other landraces (Table 3). These results probably suggest that the black-seeded Puffeun releases antimicrobial compounds that select for a specific type of microbiome a further indication that the presence of a microbial community in the rhizosphere is plant-regulated. The impact of plant accessions on microbial community in the rhizosphere has been well documented for wheat [55], maize [56] and *Arabidopsis* [28]. In this study, Kersting's groundnut landraces have been shown to influence rhizosphere microbial communities with strong evidence of landraces of same plant species differently shaping the rhizomicrobial populations. As legumes, the rhizosphere soils collected from Kersting's groundnut landraces would be expected to contain N_2-fixing rhizobia. In that regard, Puffeun had a very low population of Rhizobiales (*Mesorhizobium*) in contrast to the other landraces which harboured *Bradyrhizobium, Rhizobium* and *Mesorhizobium* in their rhizospheres. This variation in rhizobial population could be due to exudates released by plant roots. The components of root exudates include organic acids, sugars, amino acids, fatty acids, vitamins, growth factors, hormones and antimicrobial compounds [17] which can play a major role in structuring the rhizosphere microbiome [57–62]. However, root exudate production and composition can vary with plant taxa, even among closely-related plant species, and/or within different accessions of the same plant species [28, 63, 64].

In this study, Miseq Illumina-based metagenomic analysis provided well-defined taxonomic groups in all the test rhizosphere soils and helped to contribute to our understanding of microbial community structure. Our results showed that the rhizosphere microbiomes were largely plant-based. The rhizosphere soil of Puffeun landrace had a very selective microbiome, while the other landraces were enriched with diverse microbiomes. In this study, phosphate-solublising bacterial (PSB) genus were richly present in the rhizospheres of Puffeun and Heng Red Mottled landraces while with Belane Mottled, Boli and Funsi, the rhizosphere harboured mostly the IAA-producing bacteria *Sphingomonas*. Cellulose-degrading and nitrate-reducing bacteria were also present in the rhizosphere soils of all Kersting's groundnut landraces except for Puffeun.

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Compliance with ethical standards

Conflict of interests The authors declare that they have no competing interests.

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