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Research article

Rhizospheric, seed, and root endophytic-associated bacteria of drought-tolerant legumes grown in arid soils of Namibia

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ABSTRACT

Plant growth-promoting bacteria (PGPB) are of increased interest as they offer sustainable alternatives to the more common chemical fertilisers. Research, however, has increased into the use of PGPB as bioinoculants to improve yields. Legumes are known to interact with diazotroph PGPB which increase nutrient uptake, prevent pathogenic infections, and actively fix nitrogen. This study aimed to comprehensively describe PGPB associated with legumes grown in Namibia through analysis of the site-specific bacterial microbiomes. In the present study, we used the 16S rRNA sequencing approach to determine the structure of rhizosphere, root, and seed endosphere microbiomes of five drought-tolerant legume species: *Macrotyloma uniflorum*, Vigna radiata, *Vigna aconitifolia*, Vigna unguiculata and *Lablab purpureus*. Several important phyla were identified including Actinobacteriota, Bacteroidota, Firmicutes, Proteobacteria and Verrucomicrobiota. Overall, Proteobacteria was the most abundant phylum followed by Actinobacteria. The most important genera identified were *Bacillus, Mesorhizobium*, *Pseudomonas, Bradyrhizobium* and the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* group. The relative abundance of these genera varied across sample types and legume species. This study identified important diazotrophs across all the legume species. *Bacillus*, an important PGPB, was found to be the most abundant genus among all the niches analysed and legume species, while *Rhizobium* spp. was particularly enriched in roots. This study ultimately provides previously undescribed information on legume-associated bacterial communities in Namibia.

1. Introduction

Agriculture in Namibia is largely restricted and limited because of the arid climate and nutritionally poor soils [1,2]. As such, the cultivation of legumes (and other crops) in Namibia is limited. This is due to several reasons including recurring droughts [3]. Furthermore, there is limited arable land (approximately 1 %) and limited resources, which often result in farmers reporting low yields [4,5].

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Legumes, mostly grown in the northern regions by smallholder subsistence farmers, offer nutritious crop alternatives. They are an important class of vegetables across the globe. They are highly nutritious and offer significant levels of proteins, fatty acids and other functional compounds [6]. They are also important food crops and are grown on almost 96 million hectares around the world. They are often rainfed and require minimal fertiliser inputs [7]. The legumes in this study, however, are of great importance to Namibia because they are known to tolerate extended periods of drought, and, therefore, thrive in arid regions [8,9]. This characteristic is crucial for the arid climate of Namibia.

In addition, legumes, like other plants, are known to have plant growth-promoting symbiotic assemblages with both bacteria and fungi [10,11]. These are part of the general soil health and are found in the roots and seeds as endophytes [12] and the rhizosphere as rhizobacteria [13]. Seeds, through vertical transmission [14], also contribute to the plant growth-promoting (PGP) microbiome thanks to the seed endophytes. As such, these legumes can be used in crop rotations to reduce nitrogen runoff [15] and essentially reduce the need for environmentally harmful chemical fertilisers [16]. Furthermore, some drought-tolerant legumes form key interactions with PGPB that are crucial for biofertilizer development targeted for arid climates [17]. The microbiomes in these cases will then offer support to the plants. This is achieved by the production of phytohormones, phyto-stimulators and the formation of biofilms [18].

Research has found an increased interest in these plant growth-promoting bacteria (PGPB) in recent years. This is due to their low-cost production, low environmental impact and increased performance in agricultural production [19,20]. Nitrogen-fixing rhizobia, such as *Bradyrhizobium*, and *Rhizobium,* promote plant growth and improve soil quality in the long run. This is in addition to other PGP properties like 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production [21], antifungal activity [22], indole acetic acid (IAA) production [23] and siderophore production [24,25] that may be key factors that contribute to improved plant growth and yield in an arid environment [19]. However, there remain gaps within Namibia, (and Africa), on the description of microbiomes particularly in association with plants [26]. Therefore, analysis of the microbial communities associated with these legumes could provide more information useful for the development of bioinoculants relevant to these legumes in Namibia.

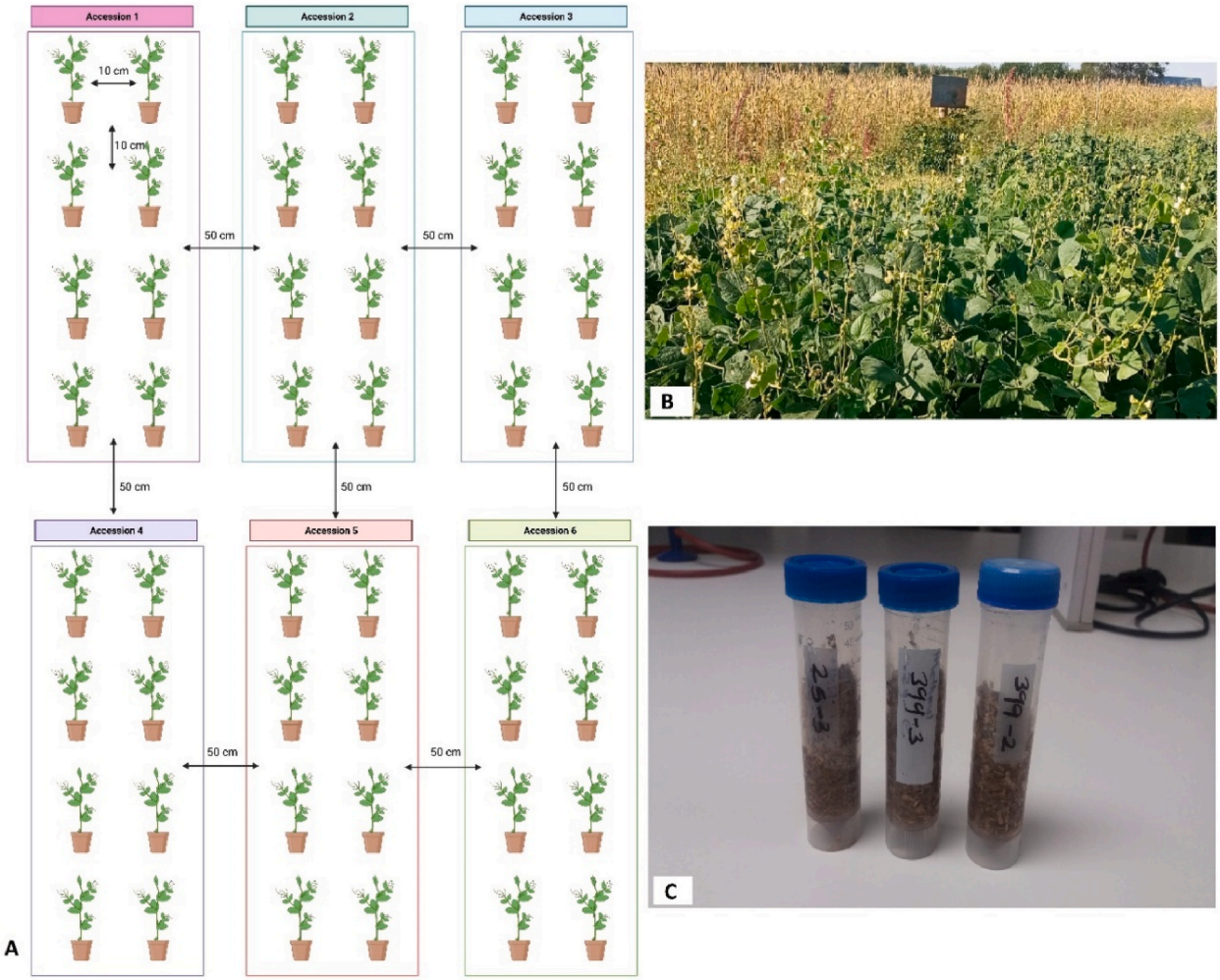

Fig. 1. **A**) Potting strategy used for the 6 accessions. **B**) Bagani Research Station study field in Bagani in the Kavango East region in the north-eastern parts of Namibia. **C**) Falcon tubes in which surface sterilised seeds were germinated in sterile sawdust.

Current research into biofertilizers in Africa has identified several bacterial strains with plant growth-promoting traits. Studies currently aim to characterise the plant microbiome in Africa while also characterising species of interest for use within African climatic conditions [26]. A study previously done in Namibia observed growth improvement by *Bradyrhizobium* in the lab. Subsequent field studies that were carried out in Namibia supported this evidence [27]. These field trials feed into biofertilizer production processes as described by Raimi et al. This is described to begin with the screening phase in the laboratory, greenhouse trials, product formulation and field trials before mass production for sale [28]. Biofertilizer production for crop improvement, therefore, follows the proven abilities of PGPB in improving crop health, and abiotic and biotic stress tolerance [29].

In this study, microbiomes associated with six accessions of five drought-tolerant legumes; horse gram (*Macrotyloma uniflorum* Var. Madhu), mung bean [Vigna radiata (L.) R. Wilczek var. radiata], moth bean [*Vigna aconitifolia* (Jacq.) Marechal], cow pea (Vigna unguiculata L. Walp) and dolichos [*Lablab purpureus* (L.) Sweet var. Lignosus Prain] were comparatively analysed. The microbiomes were assessed (using culture-independent techniques) to be indicative of PGPB associated with these drought-tolerant legumes. This study aimed to describe culture-independent bacteria associated with drought-tolerant legumes grown in Namibia. It also sought to compare the different site-specific microbiomes associated with these legumes. Therefore, this study presents the first report of microbiomes associated with horse gram, mung bean, moth bean, cowpea, and dolichos grown in Namibia.

2. Materials and methods

2.1. Study area

Soil from the Bagani Research Station study field in Bagani, Kavango East (−18° 5′ 43.6914″,21° 33′ 41.796″) was collected in clean marked bags and transported to the laboratory in Windhoek in June 2020. Once in the laboratory, the soil was sieved to remove large debris and non-organic material. Thereafter, the soil was placed in clean pots in preparation for planting following the strategy shown in Fig. 1A. The soil at the Bagani Research Station is low nutrient with organic carbon typically around 0.4 % while nitrogen ranges between 0.03 % and 0.16 %. The pH ranges between 5.5 and 7.5 with the lower end of the spectrum observed more often [30].

2.2. Potting strategy

Seeds obtained from the Indian Plant Genetic Resources Centre in July 2019 were used in this study. Seeds of six accessions from the five species were used. The accessions were Himala (*M. uniflorum*), IC39399 (*V. radiata*), Gujarat (*V. unguiculata*), IC0623025 (*L. purpureus*) and 2 accessions from *V. aconitifolia* which were IPCMO-880 and RMB-25. The seeds were surface sterilised as previously described by Chimwamurombe, Grönemeyer, & Reinhold-Hurek, [31]. Seeds were washed twice with sterile distilled water before being incubated in 70 % ethanol for 20 s. The seeds were washed again with sterile distilled water. Thereafter, seeds were incubated in 5 % NaOCl for 30 s before being washed with excess sterile distilled water. Surface sterilisation was verified by inoculating 100 μL of the final wash onto sterile LB agar plates for both roots and seeds. Samples with growth were excluded from further analysis.

To determine seed endophytes, seeds were placed in falcon tubes (two seeds per tube) with sterile saw dust (Fig. 1C) and germinated in sterile conditions at 30 °C. After 7 days, germinated roots and shoots were surface sterilised as above. The assessment of rhizospheric and root endophytic microbiomes was done from potted plants with soil obtained from Bagani, Kavango East (Fig. 1B). Samples were grown in a growth chamber maintained at 25 % humidity, 30 °C with 12 h light cycles at the University of Namibia. Seeds (two) from each accession were planted into eight pots to have two plants growing in each pot. Individual pots were kept 10 cm apart while pots of different accessions were kept 50 cm apart (Fig. 1A). Pots were watered twice a week receiving a uniform amount of water (200 mL). After six weeks, four plants that showed the least necrosis and greatest plant growth were selected for microbiome analysis. Bulk soil was analysed as a reference (control) of the overall microbial profile of the Bagani soil.

2.3. DNA extraction from seeds, roots and rhizospheric soil samples

Roots were carefully uprooted and prepared for DNA extraction following the method described by Grönemeyer, Burbano, Hurek, & Reinhold-Hurek [32]. Roots were aseptically cut off from the rest of the plant and placed in 14 mL sterile falcon tubes. To these tubes, 10 mL of sterile phosphate buffer (per 1 L- KH_2PO_4 6.75 g; K_2HPO_4 8.75 g) was added. Samples were vortexed for 5 min to remove the rhizospheric soil around the root before being centrifuged for 10 min at 10,000×*g*. Root samples were carefully removed, and surface sterilised as above. (The remaining soil in the transport buffer was reserved for DNA isolation from the rhizosphere). Thereafter, DNA was extracted using QIAGEN® DNeasy® Plant Mini Kit (Qiagen, USA, Valencia, CA) following the manufacturer's instructions.

Tubes containing rhizospheric soil and transport buffer from the previous step were centrifuged for 5 min at 10,000×*g*. The supernatant was carefully removed avoiding the pellet. DNA was extracted from rhizospheric soils using Zymo Research™ Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine, CA) following the manufacturer's instructions. DNA from all samples was quantified using the ThermoScientific NanoDrop (NanoDrop One UV–Vis Spectrophotometer, Thermo Scientific, USA). Samples were sequenced individually and grouped according to sample type, accession, and legume species.

2.4. 16S rRNA metabarcoding and Illumina sequencing

Sequencing library preparation was done following the Illumina MiSeq System manual. DNA samples were amplified using 16S amplicon PCR primers: forward = 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG 3′ and 16S Reverse

Primer = 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTAC HVGGGTATCTAATCC 3' [33]. The following PCR parameters were used; 95 °C for 5 min, 25 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s before a final extension of 72 °C for 5 min and held at 4 °C.

2.5. Amplicon data processing

The sequenced amplicon profiling data were processed with workflow based on DADA2 (v1.12.1, https://github.com/Guan06/DADA2_pipeline) [34]. Forward and reverse reads were demultiplexed. Raw sequencing reads were subsequently truncated to 260 bp (forward) or 240 bp (reverse) and filtered with the command maxN = 0, maxEE = c [2,2], truncQ = 2, rm.phix = TRUE. After learning the error rates, ASVs were generated by merging the corrected forward and reverse reads, and chimeras were removed.

2.6. Community diversity analysis at the phylum level

Merged reads were aligned to the SILVA database implemented in the QIIME2 package as described by Bolyen et al. [35]. Taxonomic annotation at different taxonomic levels ranging from phylum to genus was performed based on ASV composition and relative abundance. Community richness, diversity indices and rarefaction curves were determined using the QIIME diversity core-metrics-phylogenetic command for alpha and beta diversity analysis in the QIIME2 package. We estimated the Shannon diversity (H′) OTU richness indices using the package Phyloseq in R [36]. Statistical analysis for alpha diversity was done with the function Kruskal.test or pairwise.Wilcox.test in the R base. For beta-diversity analyses, OTU tables were normalized by the variance stabilizing transformation (VST) method using the package DESeq2 in R. Bray-Curtis distance was calculated from the normalized OTU tables using the function ordinate of the R package Vegan [37]. Principal Coordinate Analysis (PCoA) and Canonical Analysis of Principal coordinates, (CAP) analysis using the unweighted Unifrac distance was calculated using the plot_ordination function from the R package Phyloseq and Vegan. Permutational multivariate analysis of variance (PERMANOVA) was determined with the function adonis in the R package Vegan and a maximum of 999 permutations. Sequences were submitted to NCBI and were assigned the reference accession PRJNA834937, and specific accession numbers are shown below in Table 1.

Table 1
Sequence accession numbers from NCBI submission for each sample set.

Accession	Sample Name	SPUID
SAMN28085006	*V. unguiculata* seed	CS1
SAMN28085007	*V. unguiculata* rhizosphere	Crhi
SAMN28085008	*V. unguiculata* root	CowRT1
SAMN28085009	*V. unguiculata* root	CRo2
SAMN28085010	*V. unguiculata* root	CRTS3
SAMN28085011	Bulk soil	CBu1
SAMN28085012	Bulk soil	CB So2
SAMN28085013	*V. radiata* seed	MuBS1
SAMN28085014	*V. radiata* seed	MBSS2
SAMN28085015	*V. radiata* rhizosphere	MBRhi1
SAMN28085016	*V. radiata* rhizosphere	MnBRh
SAMN28085017	*V. radiata* root	MBRoot1
SAMN28085018	*V. radiata* root	MBRs2
SAMN28085019	Bulk soil	MBBulksoil1
SAMN28085020	*M. uniflorum* seed	HGS1
SAMN28085021	*M. uniflorum* seed	HG2
SAMN28085022	*M. uniflorum* rhizosphere	HRhi1
SAMN28085023	*M. uniflorum* root	HRoot1
SAMN28085024	*M. uniflorum* root	HRs2
SAMN28085025	*L. purpureus* seed	DS1
SAMN28085026	*L. purpureus* seed	DBSeed2
SAMN28085027	*L. purpureus* rhizosphere	DolRhi1
SAMN28085028	*L. purpureus* root	DolichosR1
SAMN28085029	*L. purpureus* root	DRs2
SAMN28085030	Bulk soil	DBeanBS1
SAMN28085031	*V. aconitifolia* seed	IPC880S1
SAMN28085032	*V. aconitifolia* seed	IPCSS2
SAMN28085033	*V. aconitifolia* rhizosphere	IPCRhi1
SAMN28085034	*V. aconitifolia* rhizosphere	IP8C8R0h2
SAMN28085035	*V. aconitifolia* root	IPCR1
SAMN28085036	*V. aconitifolia* root	880IPCRt2
SAMN28085037	*V. aconitifolia* seed	RMothBS1
SAMN28085038	*V. aconitifolia* seed	RMBean 25SS2
SAMN28085039	*V. aconitifolia* rhizosphere	RMBRhizosphere1
SAMN28085040	*V. aconitifolia* rhizosphere	RMBRh2
SAMN28085041	*V. aconitifolia* root	RMBRoots1

3. Results

3.1. Sequencing data

A total of 4670 taxa were identified from the soil, rhizosphere, root, and seed samples sequenced. Low abundance taxa with less than 50 reads among all the samples (3387) were removed from further analysis to avoid unwanted technical variations. These taxa represented 220 genera. Reads annotated as chloroplast made up 70 % of the sequences and were excluded from the data set. The total number of reads was 246230, ranging from 2 to 16517. No operational taxonomic units (OTUs) were identified as Archaea. The highest number of reads, as shown in Table 2, were from bulk soil samples. Average reads were found to be lowest in seed samples as shown in Table 2 below. The lowest number of reads were from *V. aconitifolia* (IPCMO-880) seeds with 2 reads. The highest average number of reads was found in *M. uniflorum* seeds approximating 5981.

The diversity of microbial communities within samples was compared and shown by the alpha diversity plot. Species richness was highest in bulk soil and rhizosphere samples. A trend was observed with a decrease in diversity with bulk soil being the most diverse. This was followed by the rhizosphere, roots and finally seeds with the least diversity. The lowest diversity was found in seed samples. The diversity within these seeds was particularly low for *V. aconitifolia*, *V. radiata* and *V. unguiculata* in which the diversity was approximately zero as shown by the Alpha-Diversity (Shannon) plot Fig. 2.

The diversity between samples by principal component analysis (shown in Fig. 3) was found to be influenced largely by seed endophytes with the least number of reads. Distance measurements showed an absence of significant differences among bulk soil, rhizosphere, and root samples. However, a significant difference was observed between seeds and the rest of the sample types. The seeds, mostly showing values greater than 0.25 on axis 1, had the least influence on variation. The PCoA based on the unweighted UniFrac distance measure showed that seeds samples formed a distinct cluster to bulk soil, rhizosphere, and root samples. To infer significant differences among seeds and the rest of the root samples, we performed a PERMANOVA test on the unweighted UniFrac distances comparing different groups (with 999 permutations in all tests). Significant differences were detected for seeds samples compared to rhizosphere ones (pseudo-$F = 7.9$, $p = 0.001$), seeds samples compared to bulk soil ones (pseudo-$F = 6.9$, $p = 0.003$) and seeds samples compared to root samples (pseudo-$F = 5.7$, $p = 0.002$) shown in Supplementary Fig. S1.

3.2. Structure of bacterial communities

Several different phyla were identified from the samples analysed. The most abundant phyla as seen in Fig. 4 were Proteobacteria (14–52 %), Firmicutes (5–24 %), and Actinobacteriota (5–24 %). On the other hand, Elusimicrobiota, RCP2-54, FCPU426 and WPS-2 were the least abundant taxa. Proteobacteria was the most abundant phylum across all samples. It was identified in all samples but the seeds of *V. aconitifolia*, *V. radiata* and *V. unguiculata*. The phylum Proteobacteria was found most abundant in the rhizosphere and roots of *V. aconitifolia* accessions. Fig. 5 shows the abundance of the top 13 phyla in the different samples and species. The phyla observed less than 1 % were glommed together. Seed samples of *V. aconitifolia*, *V. radiata* and *V. unguiculata* had the lowest relative abundance and diversity of the different phyla.

The microbiomes at the genus level were dominated by *Acidibacter*, *Ammoniphius*, *Bradyrhizobium*, *Bacillus*, *Flavobacterium*, *Mesorhizobium*, *Pseudomonas* and *Streptomyces* (Figs. 6 and 7). Uncultured groups, WD2101 soil group, 67-14 and RB41 are non-specific isolates that were also identified within the sequences. The most abundant genera identified in the samples include *Bacillus, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* genus group, uncultured group and *Niastella*. The heatmap (Fig. 7) further details the differences in the abundance of the 50 most abundant taxa.

Table 2
Average number of sequences counts subset by species.

Species	Common name	Accession	Sample	Average sequence counts	Average number of reads
Vigna unguiculata	Cowpea	Gujarat 5	Roots	60554	3151
			Rhizosphere	62236	12381
			Seeds	67704	18
Vigna radiata	Mungbean	IC39399	Roots	58101	7954
			Rhizosphere	57657	13165
			Seeds	61987	8
Macrotyloma uniflorum	Horsegram	Himala	Roots	64509	11628
			Rhizosphere	70659	13569
			Seeds	59136	5981
Lablab purpureus	Dolichos	IC0623025	Roots	56299	6902
			Rhizosphere	39893	7417
			Seeds	61631	2512
Vigna aconitifolia	Mothbean	IPCMO-880	Roots	54052	4659
			Rhizosphere	70433	12497
			Seeds	57302	2
		RMB 25	Roots	32144	2469
			Rhizosphere	39101	7313
			Seeds	54861	5
			Bulk soil	60788	13625

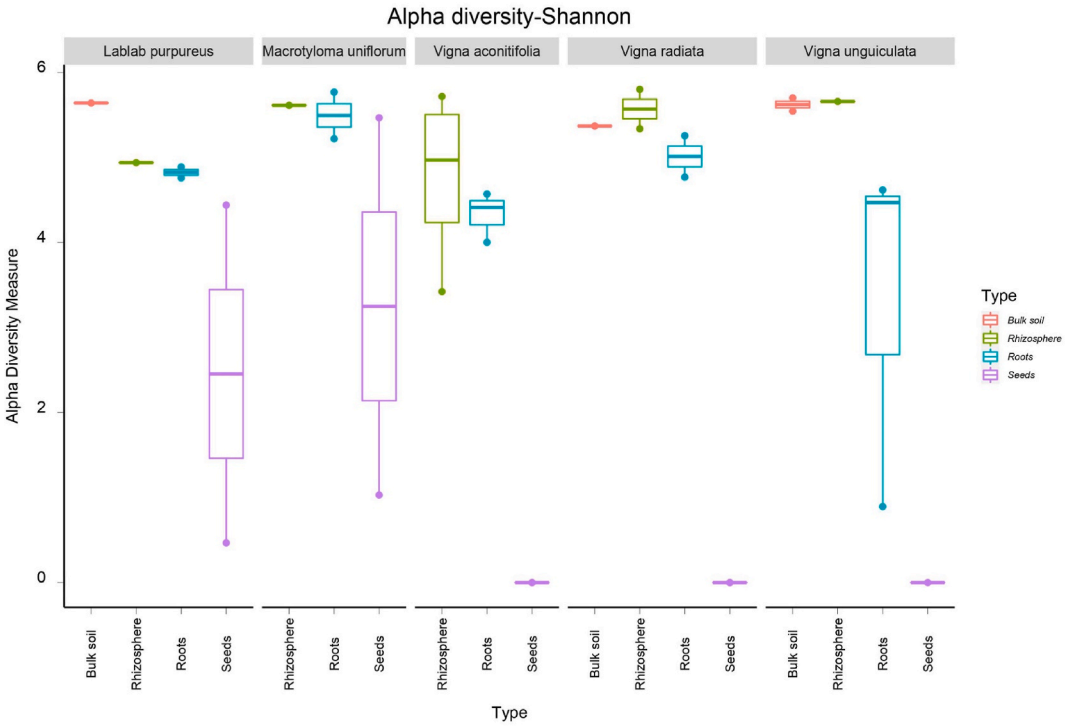

Fig. 2. Shannon index of the microbiome in the rhizosphere soil, seed, bulk soil, and roots of legume. The larger the Shannon index, the better the sample uniformity. In the case of the same species richness, the greater the uniformity of each species in the community, the greater the diversity of the community.

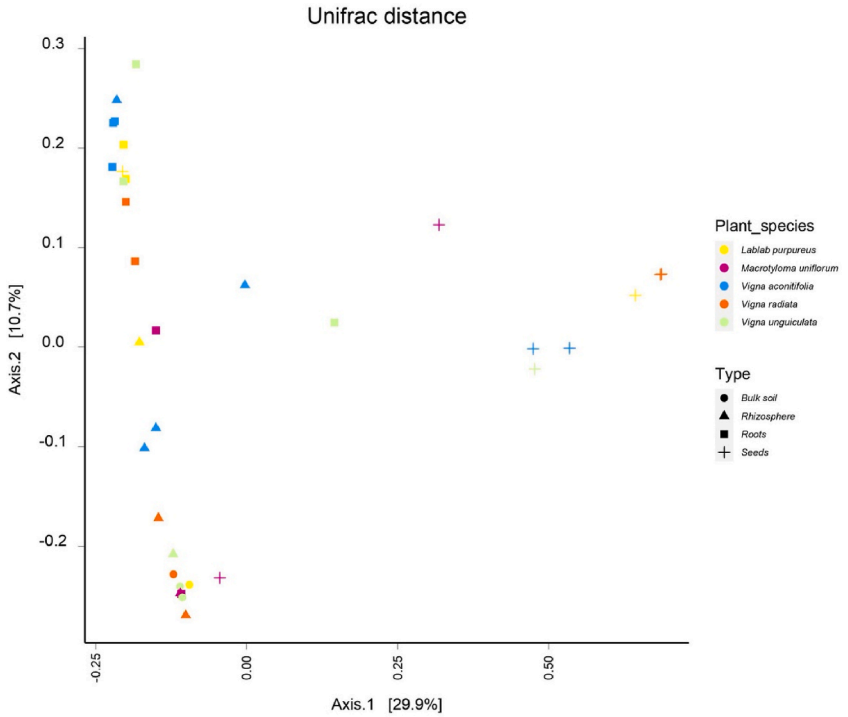

Fig. 3. Principal component analysis (PCA) based on unweighted unifrac distance calculated on rhizosphere, roots, seeds, and bulk soil samples. Statistical significance has been inferred using PERMANOVA (see Supplementary Fig. S1).

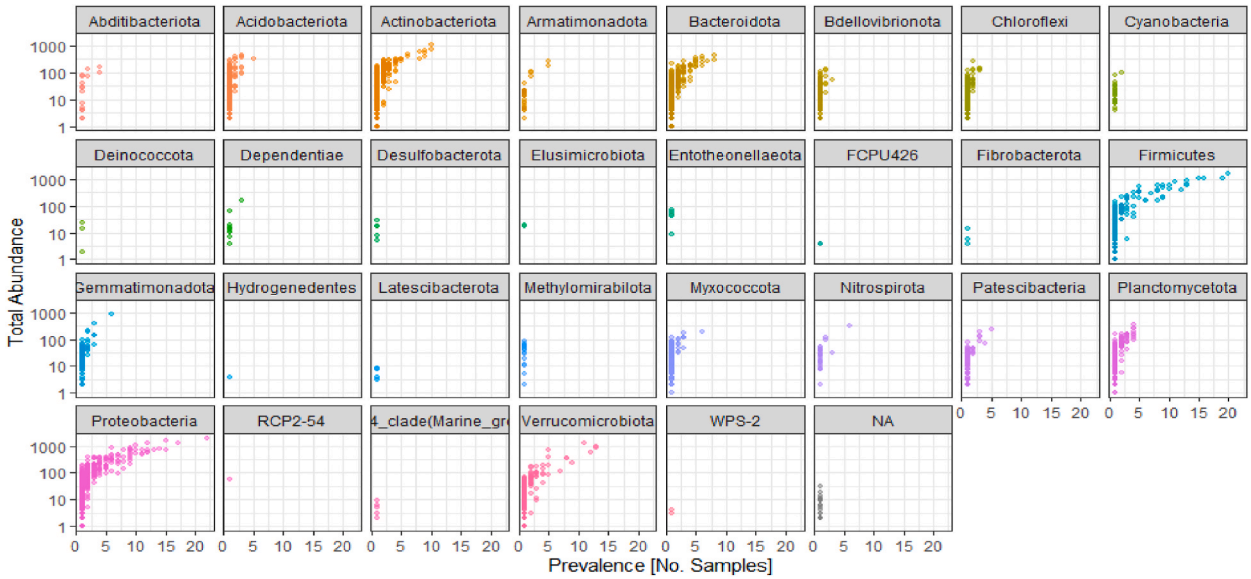

Fig. 4. Overall abundance of phyla identified.

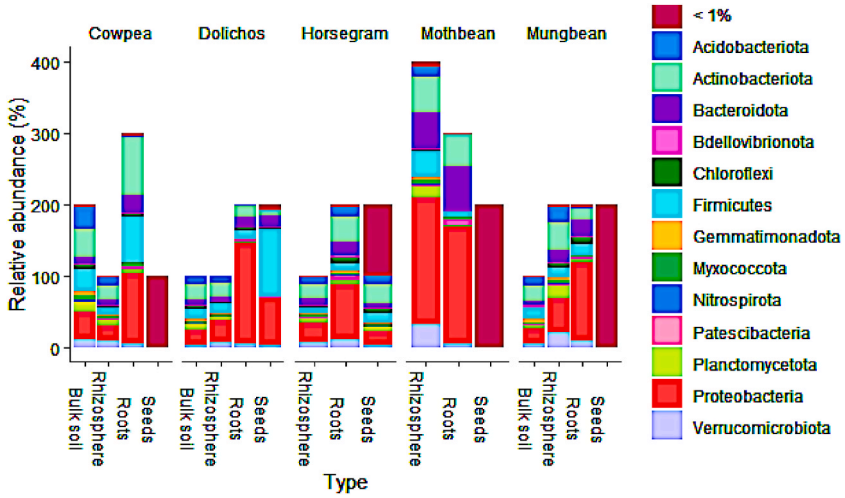

Fig. 5. Phylum abundance according to sample type of the top 13 phyla.

In L. *purpureus* samples, the genus *Bacillus* was the most predominant particularly in seeds. In *M. uniflorum* samples, the most abundant genera were *Bacillus, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* genus group and an uncultured group. These observations were in line with those in *V. aconitifolia* samples in addition to *Luteolibacter* which was abundant in rhizosphere samples. In *V. radiata*, however, the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* genus group was most abundant in root samples while the rhizosphere had more of the uncultured genus group and *Candidatus Udaeobacter*. *V. unguiculata* roots had the highest abundance of *Bacillus* and *Streptomyces* compared to other species. Like *V. radiata* roots, *V. unguiculata* roots had a high amount of the uncultured genus group and *Candidatus Udaeobacter*.

The abundance of the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* genus group was found to range from 0.2 to 1.8 % (Fig. 8). The *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* genus group was found to be least abundant in *V. unguiculata* and *M. uniflorum* while the highest amounts were observed in *V. radiata* and *L. purpureus*. *Bradyrhizobium,* though present in both bulk soil and the rhizosphere, was found in relatively low amounts ranging from 0.25 to 1 relative to the heatmap.

3.3. Core microbiome

To examine the existence of an identifiable common core microbiome [38], we defined a core as the group of members shared among the microbial community and represented the core by overlapping areas in the circles in a Venn diagram at 97 % identity

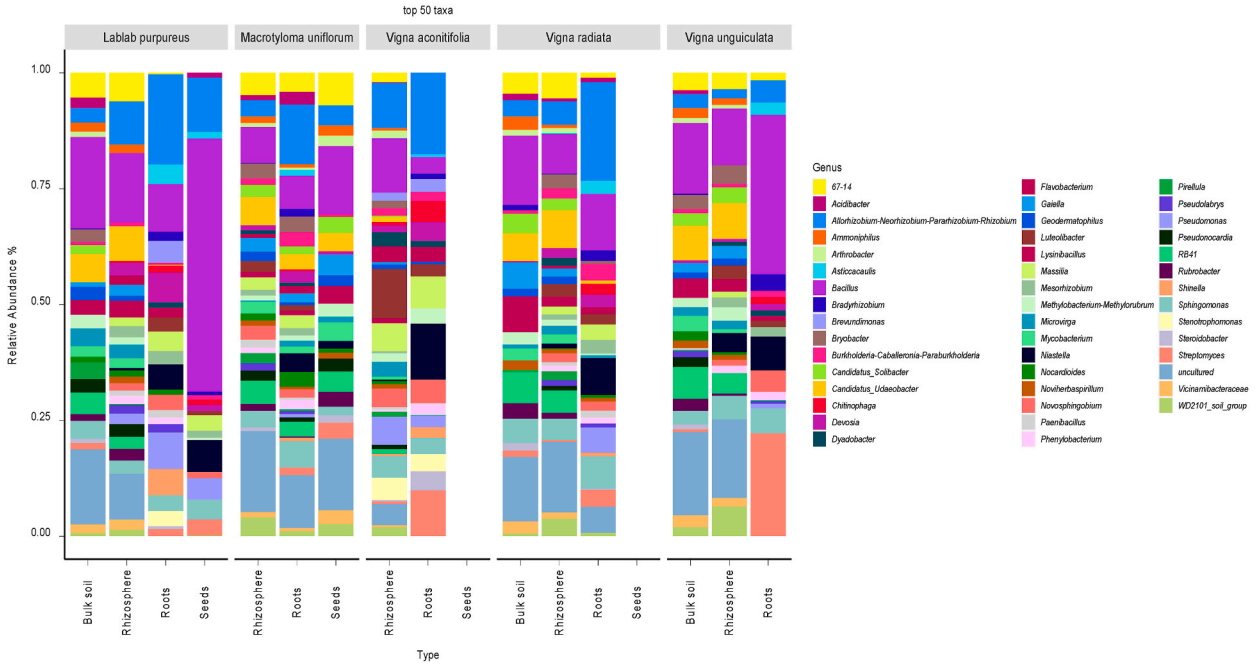

Fig. 6. Genera abundance according to sample type of the top 50 genera.

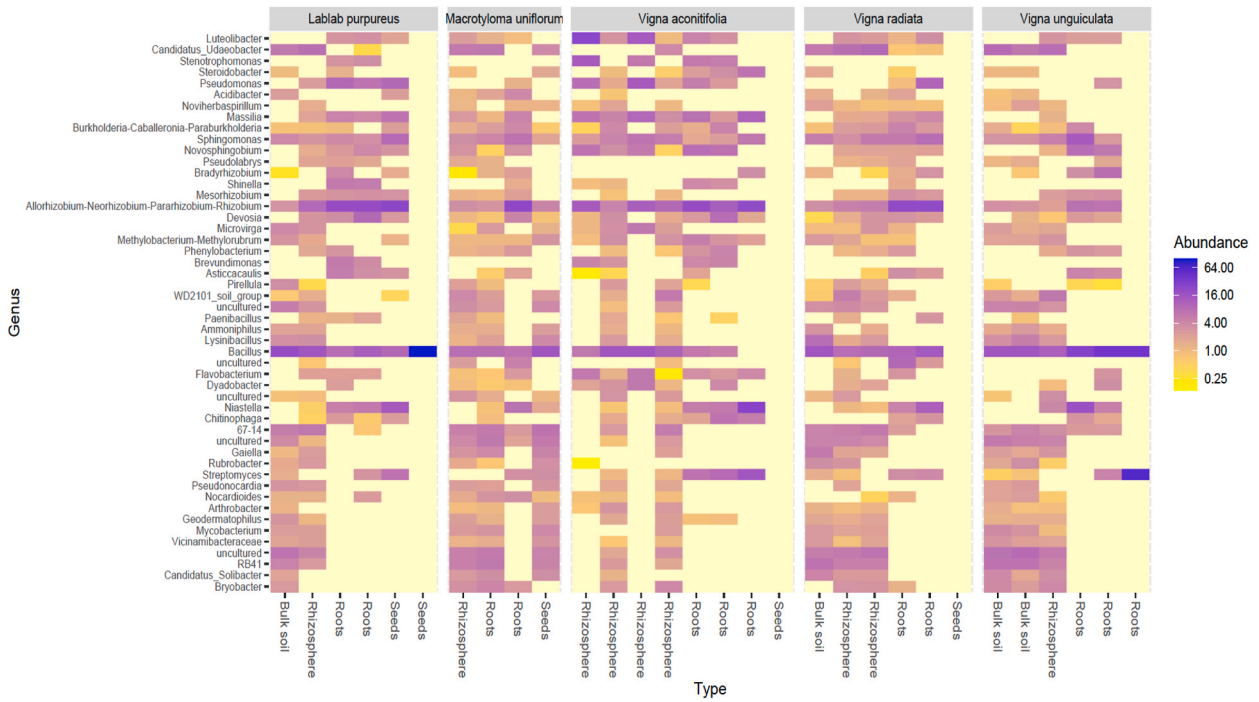

Fig. 7. Heat map with the relative abundances of the bacterial genera in the five different legume crops and rhizosphere soil, and the endophytes of the roots and seeds.

(Fig. 9). We identified 1034, 1633, 1144, and 310 OTUs in the bulk soil, rhizosphere, roots, and seeds respectively. As shown in Figs. 8 and 633 OTUs were shared among the four groups, occupying 13.6 % of all OTUs. These shared taxonomic members can be regarded as the core microbiome of roots, seeds, and soil as well as the rhizosphere.

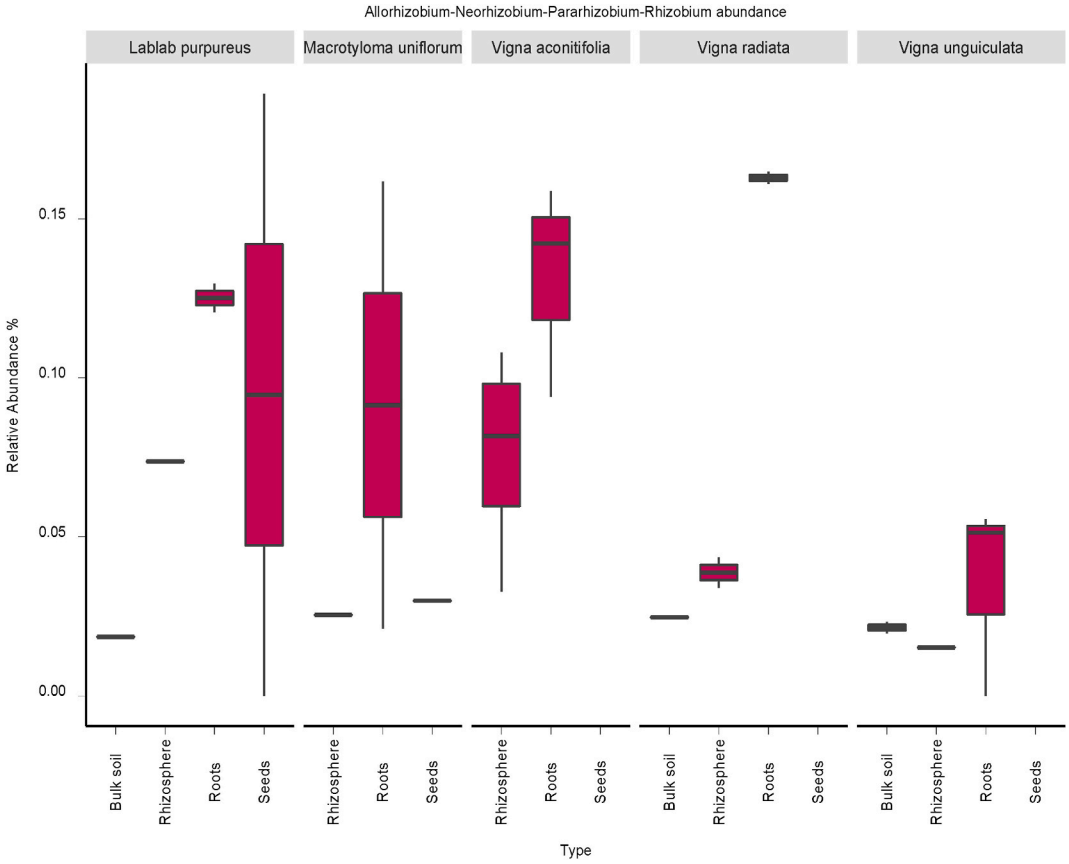

Fig. 8. Relative abundance of Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium group.

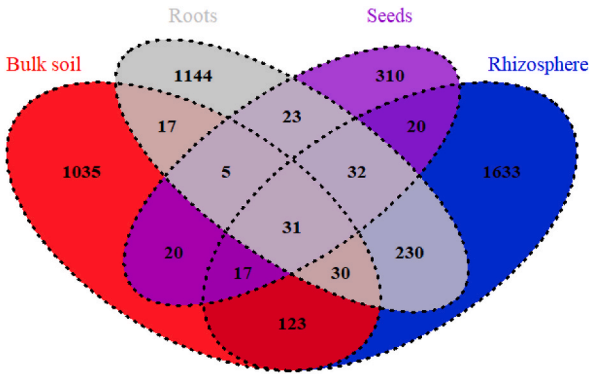

Fig. 9. Venn diagram of shared and unique genera between all the microbiomes observed in this study.

4. Discussion

Microbiome analysis offers a path to analyse a complete microbiome via culture-independent methods, providing a full picture of the total number of members of a microbial community [39]. In this study, the microbiomes of 6 accessions from 5 legume species were analysed to extrapolate the plant-microbial interactions of both culture-dependent and independent bacteria. However, one major limitation was identified. This study used 16S sequencing which limited the identification of *nifH* genes specific for nitrogen fixation. Therefore, strains with specific genes associated with plant growth-promoting traits could not be defined [40]. In addition, the culture-independent methodology used in this study limited the identification of active species or genera as it is DNA-dependent. Culture-dependent analyses would offer insight into active species for specific PGPB activity [41].

Significant differences in diversity were observed between sample types. The greatest biodiversity was observed in bulk soil

samples as expected [42]. The degree of diversity decreased significantly between the bulk soil and the rhizosphere reflecting the specific selectivity of roots and root exudates [12]. PGPB, therefore, may differ across different plant species, varieties and different plant niches within the same host. The diversity further decreases from the rhizosphere to root endophytes. This is largely due to the selective ability of very specialized bacteria to colonize the root systems [43].

Analysis of the core microbiome allowed for the assessment of core species related to different sampled sites. This analysis offered inference towards shared taxa in relation to different sites [18]. The core microbiome also points towards the stable components within the microbiomes [44]. In this study, it presented the core microbiome associated with drought-tolerant legumes. PCA on the other hand measured total variance [45]. A clear distinction was observed between the seeds (with foreign microbiome) and roots, rhizosphere and bulk soil all influenced by the Bagani soil.

PGPB are found in several different phyla with different characteristics. Actinobacteria, Bacteroidota and Verrucomicrobiota are phyla that make up the most common soil bacteria. These are often found in great abundance in the soil and rhizosphere of legumes [46]. Studies have also identified Proteobacteria and Actinobacteria constituting up to 54.90 % and 32.00 % respectively [47,48]. These observations were similar to the results obtained in this study. Proteobacteria, as shown in Fig. 4, was the most abundant phylum in all samples.

Actinobacteria and Firmicutes are both gram-positive phyla with a high G-C and low G-C content respectively. PGPB found under Actinobacteria include strains from *Streptomyces*, *Arthrobacter* and *Nocardia* genera. *Bacillus* and *Paenibacillus*, on the other hand, are important Firmicutes diazotrophs [49]. The genus *Bacillus* is of particular importance as it was strongly represented in *L. purpureus* seeds and *V. unguiculata* roots with a high abundance reflected on the heatmap. This study observed *Bacillus* being the most abundant genus across all *V. radiata* samples. Previous studies have also found *Bacillus* spp. along with *Arthrobacter* to be dominant in the rhizosphere of *V. radiata* [50]. *Streptomyces* strains were poorly represented in most samples except for in *V. unguiculata* roots. This genus was least represented in *V. aconitifolia* samples.

The analysis in this study found Proteobacteria to be the most abundant phylum dominating the root endosphere. By comparison, the abundance of Proteobacteria was relatively less in the bulk soil and rhizosphere in most samples. Root exudates are known to influence both the rhizosphere and root endosphere [51]. These vary distinctly among different plant species, resulting in the selective influence of the rhizosphere microbiome [52]. As a result, the soil microbiome composition often differs from the rhizosphere and root endosphere. This supports a study that found this phylum most abundant in the root endosphere [48].

This phylum contains several diazotrophic genera identified by the presence of *nifH* genes [53]. These include *Rhizobium*, *Sphingomonas* [51], Bradyrhizobium, *Burkholderia* [13] and *Pseudomonas* [54]. In bulk soil, *Bradyrhizobium* was observed to be in low abundance compared to other genera. However, higher levels were observed in roots and rhizosphere samples. *Pseudomonas* species were found in greater abundance in the rhizospheres compared to other sample types. These genera are often found in root nodules pointing to their nitrogen-fixing properties. *Rhizobium* and *Bradyrhizobium* are symbionts of *V. radiata* with increased abundance in root nodules [55].

The genus *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* was identified in bulk soil, rhizospheres, and root endospheres with a lesser presence in seeds. This genus is of particular importance as is a diazotrophic genus known to have non-cyanobacteria species. It is often found within soils, but associated species are often found in roots contributing to nitrogen fixation [56]. As PGPB, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* species have been observed to improve sugarcane weight and sucrose content in the plants [47]. In addition to the PGP properties of *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, species within this genus have been found to positively contribute to soil bioremediation. A study found some species actively reducing the amount of di(2-ethylhexyl) phthalate (DEHP), an environmental contaminant used in plastic manufacture [57].

5. Conclusion

Several important plant growth-promoting bacteria phyla were identified from all the samples. These include Actinobacteria, Bacteroidota, Firmicutes and Proteobacteria. Within these groups, diazotrophic genera were identified. These legumes, grown in poor sandy soils of Bagani, were found to actively recruit plant growth-promoting bacteria. Recruitment was found to be selective for bacteria known to promote plant growth. These include *Rhizobium,* Bradyrhizobium, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium, Pseudomonas* and *Bacillus*. Significant differences were not observed between the rhizosphere and roots. The low reads in seeds resulted in a significant difference in biodiversity.

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Data availability statement

Sequences were submitted to NCBI and were assigned the reference accession PRJNA834937.

CRediT authorship contribution statement

Paidamoyo N. Mataranyika: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Cristina Bez:** Writing – review & editing, Formal analysis, Data curation. **Vittorio Venturi:** Writing – review & editing, Supervision, Resources.

Percy M. Chimwamurombe: Writing – review & editing, Supervision, Resources, Project administration. **Jean D. Uzabakiriho:** Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] B.J. Strohbach, Vegetation of the okavango river valley in Kavango west, Namibia, Biodiversity and Ecology 5 (2013) 321–339.
[2] B.J. Strohbach, J.T. Kutuahuripa, Vegetation of the eastern communal conservancies in Namibia : II . Environmental drivers, Koedoe 56 (1) (2014) 1–12.
[3] L.N. Horn, H.M. Ghebrehiwot, H.A. Shimelis, Selection of novel cowpea genotypes derived through gamma irradiation, Front. Plant Sci. 7 (2016) 1–13.
[4] J.L. Grönemeyer, T. Hurek, B. Reinhold-Hurek, Bradyrhizobium kavangense sp. nov., a symbiotic nitrogen-fixing bacterium from root nodules of traditional Namibian pulses, Int. J. Syst. Evol. Microbiol. 65 (12) (2015) 4886–4894.
[5] Food and Agriculture Organization, Arable land (% of land area) - Namibia. https://data.worldbank.org/indicator/AG.LND.ARBL.ZS?locations=NA, 2022.
[6] J. Miedzianka, M. Styczyńska, K. Łoźna, M. Aniołowska, J. Biernat, Nutritional value of raw legumes, Journal of Elementology 22 (2) (2017) 643–652.
[7] K. Swarnalakshmi, V. Yadav, D. Tyagi, D.W. Dhar, A. Kannepalli, S. Kumar, Significance of plant growth promoting rhizobacteria in grain legumes: growth promotion and crop production, Plants 9 (11) (2020) 1–25.
[8] M.W. Lestari, N. Arfarita, A. Sharma, B. Purkait, Tolerance mechanisms of Indonesian plant varieties of yardlong beans (Vigna unguiculata sub sp. sesquipedalis) against drought stress, Indian J. Agric. Res. 53 (2) (2019) 223–227.
[9] B. Tiwari, S. Kalim, N. Tyagi, R. Kumari, P. Bangar, P. Barman, et al., Identification of genes associated with stress tolerance in moth bean [Vigna aconitifolia (Jacq.) Marechal], a stress hardy crop, Physiol. Mol. Biol. Plants 24 (4) (2018) 551–561.
[10] H. Liu, L.C. Carvalhais, M. Crawford, E. Singh, P.G. Dennis, C.M.J. Pieterse, et al., Inner plant values: diversity, colonization and benefits from endophytic bacteria, Front. Microbiol. 8 (2552) (2017) 1–17.
[11] Y. Goudjal, O. Toumatia, N. Sabaou, M. Barakate, F. Mathieu, A. Zitouni, Endophytic actinomycetes from spontaneous plants of Algerian Sahara: indole-3-acetic acid production and tomato plants growth promoting activity, World J. Microbiol. Biotechnol. 29 (10) (2013) 1821–1829.
[12] E. Deyett, P.E. Rolshausen, Endophytic microbial assemblage in grapevine, FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 96 (5) (2020) 1–11.
[13] X. Xiao, M. Fan, E. Wang, W. Chen, G. Wei, Interactions of plant growth-promoting rhizobacteria and soil factors in two leguminous plants, Appl. Microbiol. Biotechnol. 101 (23–24) (2017) 8485–8497.
[14] S. Truyens, N. Weyens, A. Cuypers, J. Vangronsveld, Bacterial seed endophytes: genera, vertical transmission and interaction with plants, Environmental Microbiology Reports 7 (1) (2015) 40–50.
[15] Y. Yu, L. Xue, L. Yang, Winter legumes in rice crop rotations reduces nitrogen loss, and improves rice yield and soil nitrogen supply, Agron. Sustain. Dev. 34 (3) (2014) 633–640.
[16] E. Mayer, P.D. de Quadros, R. Fulthorpe, Plantibacter flavus, Curtobacterium herbarum, Paenibacillus taichungensis, and Rhizobium selenitireducens endopghytes provide host-specific growth promotion of Arabidopsis thaliana, basil, lettuce, and bok choy plants, Appl. Environ. Microbiol. 85 (19) (2019).
[17] G.T. Pambuka, T.R. Kinge, S. Ghosh, E.D. Cason, M.M. Nyaga, M. Gryzenhout, Plant and soil core mycobiomes in a two-year sorghum-legume intercropping system of underutilized crops in South Africa. https://doi.org/10.3390/microorganisms10102079, 2022.
[18] I.S. Petrushin, I.A. Vasilev, Y.A. Markova, Drought tolerance of legumes: physiology and the role of the microbiome. https://doi.org/10.3390/cimb45080398, 2023.
[19] B.S. Adeleke, O.O. Babalola, B.R. Glick, Plant growth-promoting root-colonizing bacterial endophytes, Rhizosphere 20 (2021) 1–12.
[20] R.N. Khandare, R. Chandra, N. Pareek, K.P. Raverkar, Carrier-based and liquid bioinoculants of Azotobacter and PSB saved chemical fertilizers in wheat (Triticum aestivum L.) and enhanced soil biological properties in Mollisols, J. Plant Nutr. 43 (1) (2020) 36–50.
[21] S. Pandey, A. Verma, D. Chakraborty, Potential use of rhizobacteria as biofertilizer and its role in increasing tolerance to drought stress, Recent trends in Biofertilizers. (1) (2015) 116–140.
[22] S.K. Verma, K. Kingsley, I. Irizarry, M. Bergen, R.N. Kharwar, J.F. White, Seed-vectored endophytic bacteria modulate development of rice seedlings, J. Appl. Microbiol. 122 (6) (2017) 1680–1691.
[23] N. Bhutani, R. Maheshwari, P. Suneja, Isolation and characterization of plant growth promoting endophytic bacteria isolated from Vigna radiata, Indian J. Anim. Res. 52 (6) (2018) 596–603.
[24] P.N. Bhattacharyya, D.K. Jha, Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture, World J. Microbiol. Biotechnol. 28 (4) (2012) 1327–1350.
[25] D.A. Gamit, S.K. Tank, Effect of siderophore producing microorganism on plant growth of Cajanus cajan (Pigeon pea), International Journal of Research in Pure and Applied Microbiology 4 (1) (2014) 20–27.
[26] T.P. Makhalanyane, O.K.I. Bezuidt, R.E. Pierneef, E. Mizrachi, A. Zeze, R.K. Fossou, et al., African microbiomes matter, Nat. Rev. Microbiol. 21 (8) (2023) 479–481.
[27] C.C. Luchen, J.D. Uzabikiriho, P.M. Chimwamurombe, B. Reinhold-Hurek, Evaluating the yield response to bio-noculants of Vigna unguiculata in the Kavango Region in Namibia, J. Plant Pathol. Microbiol. 9 (10) (2018).
[28] A. Raimi, A. Roopnarain, R. Adeleke, Biofertilizer production in Africa: current status, factors impeding adoption and strategies for success, Sci Afr [Internet] 11 (2021) e00694, https://doi.org/10.1016/j.sciaf.2021.e00694.
[29] A. Sessitsch, N. Pfaffenbichler, B. Mitter, Microbiome applications from lab to field: facing complexity, Trends Plant Sci. 24 (3) (2019 Mar 1) 194–198.

[30] J.L. Grönemeyer, C.S. Burbano, T. Hurek, B. Reinhold-Hurek, Isolation and characterization of root-associated bacteria from agricultural crops in the Kavango region of Namibia, Plant Soil 356 (1–2) (2012) 67–82.
[31] P.M. Chimwamurombe, J.L. Grönemeyer, B. Reinhold-Hurek, Isolation and characterization of culturable seed-associated bacterial endophytes from gnotobiotically grown Marama bean seedlings, FEMS Microbiol. Ecol. 92 (6) (2016) 1–11.
[32] J.L. Grönemeyer, C.S. Burbano, T. Hurek, B. Reinhold-Hurek, Isolation and characterization of root-associated bacteria from agricultural crops in the Kavango region of Namibia, Plant Soil 356 (1–2) (2012) 67–82.
[33] A. Klindworth, E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn, et al., Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies, Nucleic Acids Res. 41 (1) (2013) 1–11.
[34] B.J. Callahan, P.J. Mcmurdie, M.J. Rosen, A.W. Han, A.J. Johnson, S.P. Holmes, DADA2: high resolution sample inference from Illumina amplicon data, Nat. Methods 13 (7) (2016) 581–583.
[35] E. Bolyen, J.R. Rideout, M.R. Dillon, N.A. Bokulich, C.C. Abnet, G.A. Al-Ghalith, et al., Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2, Nat. Biotechnol. 37 (8) (2019) 852–857.
[36] P.J. McMurdie, S. Holmes, Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data, PLoS One 8 (4) (2013).
[37] J. Oksanen, F.G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. Mcglinn, et al., Vegan: community ecology package, Community ecology package 2 (5–6) (2019) 1–296.
[38] C.A. Lozupone, M. Hamady, S.T. Kelley, R. Knight, Quantitative and qualitative diversity measures lead to different insights into factors that structure microbial communities, Appl. Environ. Microbiol. 73 (5) (2007) 1576–1585.
[39] K. Gururani, S. Sood, A. Kumar, D.C. Joshi, D. Pandey, A.R. Sharma, Mainstreaming Barahnaja cultivation for food and nutritional security in the Himalayan region, Biodivers. Conserv. 30 (3) (2021) 551–574.
[40] T.R. Turner, E.K. James, P.S. Poole, The plant microbiome, Genome Biol. 14 (209) (2013) 1–10.
[41] V. Riva, F. Mapelli, A. Bagnasco, A. Mengoni, S. Borin, A meta-analysis approach to defining the culturable core of plant endophytic bacterial communities, Available from: http://www.interactivenn.net/, 2022.
[42] E. Essel, J. Xie, C. Deng, Z. Peng, J. Wang, J. Shen, et al., Bacterial and fungal diversity in rhizosphere and bulk soil under different long-term tillage and cereal/legume rotation, Soil Tillage Res. 194 (1) (2019).
[43] J. Vacheron, G. Desbrosses, M.L. Bouffaud, B. Touraine, Y. Moënne-Loccoz, D. Muller, et al., Plant growth-promoting rhizobacteria and root system functioning, Front. Plant Sci. 4 (2013) 1–19.
[44] S. Jiao, W. Chen, G. Wei, Core microbiota drive functional stability of soil microbiome in reforestation ecosystems, Glob Chang Biol 28 (3) (2022 Feb 11) 1038–1047.
[45] Greenacre M, Groenen PJF, Hastie T, Iodice D'enza A, Markos A, Tuzhilina E. Principal component analysis. Available from: https://doi.org/10.1038/s43586-022-00184-w.
[46] M. Saleem, A.D. Law, M.R. Sahib, Z.H. Pervaiz, Rhizosphere impact of root system architecture on rhizosphere and root microbiome, Rhizosphere 6 (2018) 47–51.
[47] Z. Pang, N. Fallah, P. Weng, Y. Zhou, X. Tang, M. Tayyab, et al., Sugarcane–peanut intercropping system enhances bacteria abundance, diversity, and sugarcane parameters in rhizospheric and bulk soils, Front. Microbiol. 12 (2022).
[48] E.K. Mitter, JR De Freitas, J.J. Germida, Bacterial root microbiome of plants growing in oil sands reclamation covers, Front. Microbiol. 8 (2017) 1–14.
[49] I. Francis, M. Holsters, D. Vereecke, The Gram-positive side of plant-microbe interactions: minireview, Environ. Microbiol. 12 (1) (2010) 1–12.
[50] A.M.M. de los Reyes, E.T.M. Ocampo, Manuel MaCC, B.C. Mendoza, Analysis of the bacterial and fungal community profiles in bulk soil and rhizospheres of three mungbean [Vigna radiata (L.) R. Wilczek] genotypes through PCR-DGGE, Int. Lett. Nat. Sci. 77 (2020) 1–26.
[51] A.J. Fernández-González, P.J. Villadas, C. Gómez-Lama Cabanás, A. Valverde-Corredor, A. Belaj, J. Mercado-Blanco, et al., Defining the root endosphere and rhizosphere microbiomes from the world olive germplasm collection, Sci. Rep. 9 (1) (2019) 1–13.
[52] J. Wang, L. Liao, G. Wang, H. Liu, Y. Wu, G. Liu, et al., N-induced root exudates mediate the rhizosphere fungal assembly and affect species coexistence, Sci. Total Environ. 804 (2021) 150148, https://doi.org/10.1016/j.scitotenv.2021.150148.
[53] T. Hurek, T. Egener, B. Reinhold-Hurek, Divergence in nitrogenases of Azoarcus spp., Proteobacteria of the β subclass, J. Bacteriol. 179 (13) (1997) 4172–4178.
[54] B. Beckers, M.O. De Beeck, N. Weyens, W. Boerjan, J. Vangronsveld, Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees, Microbiome 5 (1) (2017) 1–17.
[55] S. Hakim, A. Imran, M.S. Mirza, Phylogenetic diversity analysis reveals Bradyrhizobium yuanmingense and Ensifer aridi as major symbionts of mung bean (Vigna radiata L.) in Pakistan, Braz. J. Microbiol. 52 (1) (2021) 311–324.
[56] Y. You, K. Aho, K.A. Lohse, S.G. Schwabedissen, R.N. Ledbetter, T.S. Magnuson, Biological soil crust bacterial communities vary along climatic and shrub cover gradients within a Sagebrush Steppe ecosystem, Front. Microbiol. 12 (2021).
[57] N. Bai, S. Li, J. Zhang, H. Zhang, H. Zhang, X. Zheng, et al., Efficient biodegradation of DEHP by CM9 consortium and shifts in the bacterial community structure during bioremediation of contaminated soil, Environ. Pollut. 266 (2020) 115112.