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## Metagenomic insights into the bacterial community structure and functional potentials in the rhizosphere soil of maize plants

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### ABSTRACT

Plant rhizosphere zones are hotspots for microbial diversity consisting of different communities when contrasted with surrounding bulk soils. Rhizosphere microorganisms play significant roles in plant development. We investigated the bacterial community and metabolic potentials of maize rhizosphere and bulk soils at two distant geographical locations in the North West Province of South Africa using shotgun metagenomics. We further characterized bacterial genes contributing to plant-beneficial functions present in the soils. Genes involved in plant-beneficial functions like nitrogen fixation and potassium transport were uncovered. Overall, 51 OTUs were identified in the soils. Shared OTUs between soils were 10.9% and 17.2% at Ventersdorp and Mafikeng, respectively. Significant differences in bacterial taxonomic composition and functional categories between soils ( $P < 0.05$ ) were revealed. *Acidobacteria* and *Firmicutes* dominated the rhizosphere soils while *Actinobacteria* and *Gemmatimonadetes* were predominant in bulk soils. Proper understanding of soil indigenous microbiome can help ascertain prospective targets for imminent crop breeding and management.

### ARTICLE HISTORY

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### KEYWORDS

Rhizobiome; soil metagenomics; bacteria; *Zea mays*; root exudates; bacterial genes

### Introduction

Maize (*Zea mays*), a principal staple crop in contemporary agriculture, is utilized for an assortment of food and industrial intents (Beirinckx et al. 2020; Fadiji et al. 2020; Walters et al. 2018). Owing to its emergent agricultural significance, maize has been implemented as a model organism (Hake and Ross-Ibarra 2015). Plants and their associated microbiota can handle biotic and abiotic pressures. Plant-soil microbiome interactions are intricate and are able to directly and indirectly enhance the health and development of plants (Berlanas et al. 2019; Igiehon and Babalola 2018).

Roots not only provide mechanical support, water and nutrients to plants; they are also involved in more explicit functions such as the exudation of various chemical substances. The rhizosphere is an interface between plant roots and soil microbes where rhizodeposits containing metabolites such as organic acid and carbohydrates are secreted by roots (Adedeji and Babalola 2020; Soussi et al. 2016). Root exudates contain signaling molecules that attract microorganisms to the rhizosphere. The rhizosphere is a hot-spot for microbial diversity containing more active, abundant and distinct microbial communities in contrast to proximate bulk soils (de Vries and Wallenstein 2017; Praeg et al. 2019). Bacteria are the most abundant microbes in the rhizosphere and are used as indicators of soil quality and fertility due to their rapid response to environmental alterations (Enagbonma et al. 2020). The actions of bacteria are vital because they expedite most biogeochemical progressions, thereby influencing mineral nutrient availability in soils (Amoo and Babalola 2019). Rhizospheric bacterial communities can resist disease-causing pathogens, and

promote tolerance to abiotic stressors, thereby promoting plant growth and development (Meena et al. 2017).


Although the maize root microbiome has been explored by various studies (Akinola et al. 2021; Aliche et al. 2021; Fadiji et al. 2021), the impact of plants on microbes is still understudied. It is therefore imperative to ascertain microbes that are effective in the rhizosphere and their associated functional genes (Li et al. 2014; Praeg et al. 2019). Herein, to discriminate the influences of plants, we studied bacterial communities in the rhizosphere of maize and the nearby bulk soils. We also investigated the differences in the functional composition of the soils. We hypothesized that the structure and metabolic potentials of maize rhizosphere bacterial communities would vary compared to the bulk soil. A good understanding of the metabolic abilities of rhizosphere microorganisms is important because functional diversity is a sensitive indicator of soil management and quality. It also facilitates the intensification of microbiome functions since an understanding of molecular and biochemical determinants in the rhizosphere zone regulates selective microbial enrichment.

### Material and methods

#### Site description and soil sampling

The maize fields being investigated in this study include Molelwane in Mafikeng and a private farm in Ventersdorp in the North West Province. In Ventersdorp, the rainfall levels are significantly higher than in Mafikeng. Both sites were intentionally selected based on the geographic location and the availability of maize plants. The geographical

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coordinates of North-West University Research Farm (Molelwane) are 25°48'S, 25°38'21"E, and those of the Ventersdorp private farm are 26°19'37.2"S, 26°53'19.1"E. The two sites have summer temperatures ranging from 17°C to 31°C and winter temperatures ranging 3°C to 21°C. The annual rainfall in both area ranges between 300 and 600 mm with more rain falling in the summer than in the winter.

The rhizosphere and bulk replicate soil samples were collected from the two commercial maize fields (8 replicate rhizosphere and bulk soils from both sites), at 8 cm diameter of maize plant and 15 cm depth. The soil samples were placed separately and were sieved and stored in plastic bags in the dark at 4°C. The samples were transported to the laboratory on ice and stored until further use.

### Physical and chemical parameters

The rhizosphere and bulk soils physical and chemical properties were evaluated using standard procedures. The soil pH was measured using a pH-meter in the ratio of 1:2.5 (soil: water) and the total carbon was assessed using the dry combustion method as described by (Santi et al. 2006). Soil nitrate and ammonium were determined by KCl extraction method. Organic carbon in the soil was determined by the Walkley Black method (Walker and Black 1934). Potassium was examined after extraction using 1 M ammonium acetate at pH 7.0. Extraction by 0.1 M HCl solution of sulphur analysis was conducted for rhizosphere and bulk soils (Yang et al. 2016). Phosphorus was extracted from the soil samples using Bray 1 method [0.03 M ammonium fluoride (NH<sub>4</sub>F) + 0.025 M hydrochloric acid (HCl)] (Gutierrez Boem et al. 2011). Soil organic matter was determined using mass loss on ignition method (LOI) (Roper et al. 2019).

### DNA extraction and metagenomic sequencing

The metagenomic DNA was extracted from each sample collected from maize rhizosphere and bulk soil using the DNeasy PowerSoil® DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. The extracted DNA was stored at -80°C while awaiting sequencing. Illumina Novaseq platform (San Diego, CA, USA) was used for sequencing the extracted DNA. All the datasets were created by the metagenome shotgun sequencing at Molecular Research LP (MR DNA, Shallowater, TX, USA). An amount of 50 ng of DNA from each sample was used to construct a library sequencing via the Nextera DNA Sample Preparation Kit (Illumina) following the manufacturer's user guide. These adapters are utilized during a limited-cycle PCR in which unique indices such as diluted Nextera read 1, 2 and index primers are added to the sample. The average library size was determined using the Agilent 2100 Bioanalyzer (Agilent Technologies). The library insert size ranged from 617 bp to 873 bp. The libraries were pooled, diluted (to 0.6nM) and sequenced paired end for 300 cycles.

### Metagenomic data analysis

The raw sequences of each metagenome were uploaded to the metagenomics rapid annotation online server (MG-RAST) at <https://www.mg-rast.org> (accessed on 8 July 2020) (Meyer et al. 2008). In the MG-RAST server, the

sequences were subjected to quality control. This included dereplication – that is, the removal of artificial sequences produced by sequencing artifacts, removing host-specific species sequences, ambiguous base filtering (removing sequences with >5 ambiguous base pairs with a 15 phred score cutoff) and length filtering (removing sequences with a length of >2 standard deviations from the mean). Following quality control (QC), the sequences were annotated using the BLAT (the BLAST-like alignment tool) algorithm (Kent 2002) against the M5NR database (Wilke et al. 2012), which provides a nonredundant integration of many databases. SEED subsystem was used for taxonomic profiling of bacterial communities and their functional categories assessments were performed using SEED subsystem level 1. Also, the genes that contribute to maize plant-beneficial functions were manually curated from the file generated from the SEED Level (function) databases. These were done under the following conditions: an e-value of 1e-5, a minimum identity of 60%, and a maximum alignment length of 15 base pairs. No further analyses were conducted on sequences that failed annotation. Our focus was on bacteria; hence, sequences obtained from viruses, archaea and eukaryotes were discarded. The normalized data option of MG-RAST was used to decrease the effect of experimental noise/error. The resulting bacterial table was agglomerated accordingly to each taxon, and unclassified reads were retained for statistical purposes. Next, the taxa abundances were transformed into percentages. The average values of the relative abundances of all 4 samples from each site (F4R, F4B, F3R, and F3B) were used for statistical analysis. The quality sequences for each site can be found on NCBI SRA dataset under the bio-projects PRJNA647806 and PRJNA647797 for Mafikeng and Ventersdorp rhizosphere and bulk soil samples respectively.

### Statistical analysis

The differences in soil properties between the maize rhizosphere soil samples and the bulk soil samples were assessed using a one-way analysis of variance (ANOVA) with Tukey's pairwise comparison test. The Evenness and Shannon diversity indices were assessed for each sample and these indices were compared between rhizosphere and bulk soil samples using a Kruskal-Wallis test. Statistical analysis was done using PAST version 3.20 (Hammer et al. 2001). The beta diversity was depicted using the principal coordinate analysis (PCoA) based on a Bray-Curtis distance matrix, and the one-way analysis of similarities (ANOSIM) via 999 permutations was used to test for differences in community composition between the groups of samples (Clarke and Green 1988). We conducted a canonical correspondence analysis (CCA) to identify the environmental variables that best described the bacterial composition and applied a forward selection of environmental variables; a significance test was conducted using the Monte Carlo permutation test of 999 random permutations. As explanatory variables, all the environmental variables mentioned in Table 1 were included in the CCA analysis. The PCoA and CCA were plotted via CANOCO 5 (Microcomputer Power, Ithaca, NY).

The proportion of common and unique operational taxonomic units (OTUs) between rhizosphere and bulk soil

**Table 1.** Soil properties in rhizosphere and bulk soils.

Physical and chemical parameters	F3R	F3B	F4R	F4B
pH (H <sub>2</sub> O)	6.92 ± 0.18a	6.48 ± 0.47ab	5.17 ± 0.46b	5.37 ± 0.06b
Total C (%)	1.22 ± 0.04a	1.19 ± 0.18a	0.45 ± 0.06b	0.45 ± 0.04b
Organic Carbon (mg/kg)	1.06 ± 0.02a	0.94 ± 0.12a	0.26 ± 0.00b	0.25 ± 0.00b
Organic Matter (mg/kg)	4.09 ± 0.14a	4.02 ± 0.20a	1.46 ± 0.11b	1.54 ± 0.06b
NH <sub>4</sub> <sup>+</sup> (mg/kg)	3.27 ± 0.07a	5.55 ± 1.52ab	9.31 ± 1.83b	4.86 ± 1.43ab
NO <sub>3</sub> <sup>-</sup> (mg/kg)	16.49 ± 0.10a	14.28 ± 1.48a	1.44 ± 0.66b	1.95 ± 1.34b
K (mg/kg)	384 ± 65.05a	315 ± 53.74a	115.50 ± 7.78b	102.50 ± 3.54b
S (mg/kg)	8.10 ± 11.46a	2.97 ± 2.57a	0.34 ± 0.48a	0 ± 0a
P (mg/kg)	22.02 ± 3.21a	39.68 ± 22.14a	43.10 ± 19.49a	18.57 ± 4.02a

Notes: Mean ± standard deviation ( $n = 4$ ). Different letters (a, b) on the same row indicate values that are significantly different ( $P < 0.05$ ) based on Tukey's pairwise significant difference test. (F3R = Ventersdorp rhizosphere; F3B = Ventersdorp bulk; F4R = Mafikeng rhizosphere; and F4B = Mafikeng bulk).

samples was presented by the Venn diagram (Oliveros 2007–2015). Statistical Analysis of Metagenomic Profiles (STAMP) software was used to visualize functional profiles of bacteria in rhizosphere and bulk soil samples from Mafikeng and Ventersdorp (<http://kiwi.cs.dal.ca/Software/STAMP>). The Heatmap was drawn using the heatmapmer online tool ([www1.heatmapmer.ca/expression/](http://www1.heatmapmer.ca/expression/)) with  $z$ -score-transformed relative abundance of bacterial categories (Štajner et al. 2013). The Circos program (<http://circos.ca/>) was used to map a graph of the abundance of genes curated from SEED Level (function) databases for plant-beneficial functions.

## Results

### Physical and chemical characterization of maize rhizosphere and bulk soils

The soil physical and chemical analysis shows that the soil pH ( $F = 12.30$ ,  $P = 0.0173$ ), total C (%) ( $F = 38.81$ ,  $P = 0.0020$ ), organic carbon ( $F = 101.30$ ,  $P = 0.0003$ ), organic matter ( $F = 265.20$ ,  $P = 0.0001$ ), ammonium ( $F = 6.799$ ,  $P = 0.0476$ ), soil nitrate ( $F = 114.30$ ,  $P = 0.0002$ ), and potassium ( $F = 22.36$ ,  $P = 0.0058$ ) were significantly different across the maize farm samples. However, sulphur ( $F = 0.8106$ ,  $P = 0.5506$ ) and phosphate ( $F = 1.359$ ,  $P = .3750$ ) were not significantly different across the maize farm samples.

### Analysis of sequencing data of the rhizosphere and bulk soil samples

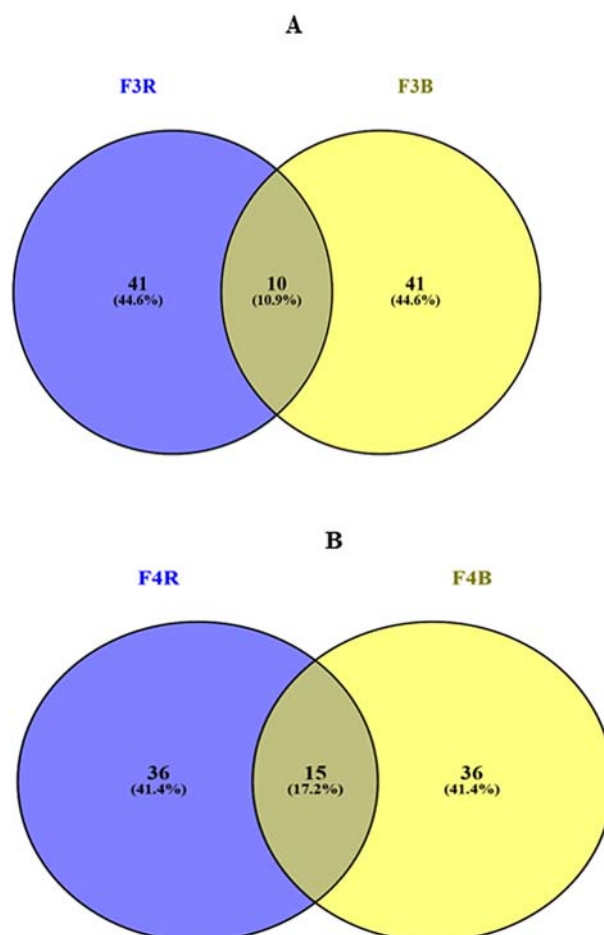
An estimated number of uploaded sequences were 15,742,603 (F4R) and 16,926,039 (F3R) sequence reads for the maize rhizosphere soils and 15,049,855 (F4B) and 13,417,316 (F3B) sequence reads for the bulk soil samples. Following quality assurance (QC) in MG-RAST (Meyer et al. 2008), the estimation of sequences were 14,469,753 (F4R), with an average G + C content of 65.9% and 15,470,556 (F3R), with a median G + C content of 65.4% for the maize rhizosphere soil samples, while the sequences of the maize bulk soil samples were 13,771,970 (F4B), with a median G + C content of 66.85% and 12,383,932 (F3B), with a median G + C content of 66.9%, respectively. In addition, 7,758,156 (F4R) and 8,800,127 (F3R) sequence reads contained predicted proteins with unknown functions from maize rhizosphere soil samples, while 7,374,147 (F4B) and 6,964,324 (F3B) sequence reads contained predicted proteins with unknown functions from maize bulk soil samples.

Sequences were clustered by OTUs at 97% similarity, and the abundance of different OTUs in all samples was obtained. The Venn diagram (Figure 1) demonstrated that the

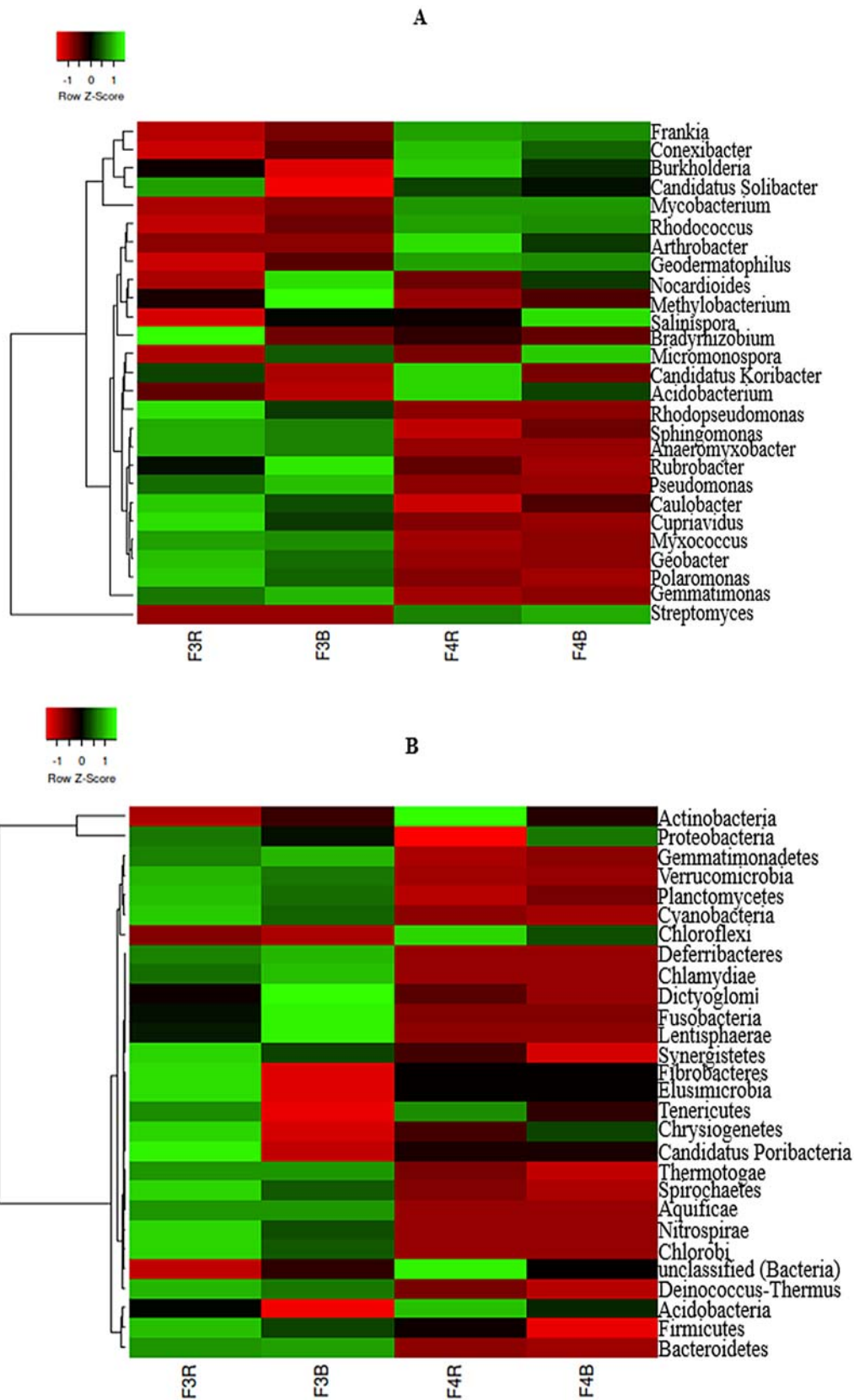
Ventersdorp rhizosphere (F3R) and bulk (F3B) soil samples shared 10.9% of OTUs, while the of Mafikeng rhizosphere (F4R) and bulk (F4B) soils shared 17.2% of OTUs (Supplementary Table 3).

### Structural composition analysis of the bacterial community

At genus level (Figure 2(A), Supplementary Table 4), *Candidatus koribacter*, *Acidobacterium*, *Arthrobacter*, *Geodermatophilus*, *Burkholderia*, *Frankia* and *Conexibacter* were more predominant in the Mafikeng rhizosphere soil (F4R), while *Streptomyces*, *Micromonospora*, and *Salinispora* were more abundant in the Mafikeng bulk soil (F4B). *Bradyrhizobium*, *Rhodopseudomonas*, *Anaeromyxobacter*, *Sphingomonas*, *Caulobacter*, *Cupriavidus*, *Geobacter*, and *Polaromonas* were more



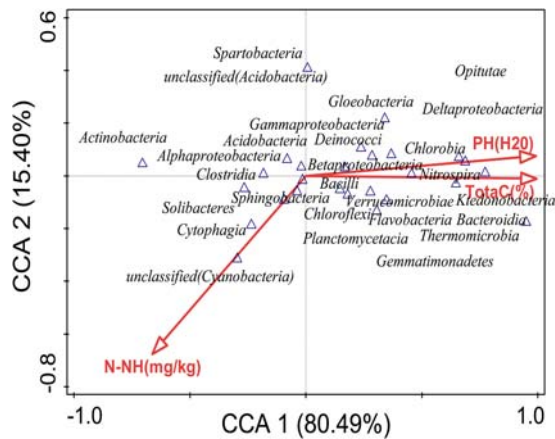
**Figure 1** Venn diagram of shared OTUs between the rhizosphere and bulk soils at class level. (F3R = Ventersdorp rhizosphere, F3B = Ventersdorp bulk soil, F4R = Mafikeng rhizosphere, F4B = Mafikeng bulk).



**Figure 2.** Relative abundance of the bacterial communities at the (A) genus level and (B) phylum level across the sites. The scale bar shows the color saturation gradient based on the relative abundances, with a z-score-transformed relative abundance of the bacterial communities.

predominant at the Ventersdorp rhizosphere soil (F3R), while *Rubrobacter*, *Nocardioideis*, *Methylobacterium*, *Pseudomonas*, and *Gemmatimonas* were more predominant in the Ventersdorp bulk soil (F3B). At the phylum level (Figure 2(B), Supplementary Table 5), *Actinobacteria*, *Chloroflexi*, *Acidobacteria* and *Tenericutes* were more abundant in the Mafikeng rhizosphere soil (F4R). *Planctomycetes*,

*Verrucomicrobia*, *Nitrospirae*, *Aquificae*, *Spirochaetes*, *Chlorobi*, *Fibrobacteres*, *Elusimicrobia*, *Chrysiogenetes*, *Candidatus Poribacteria* and *Cyanobacteria* were more predominant in the Ventersdorp rhizosphere soil (F3R), while *Gemmatimonadetes*, *Lentisphaerae*, *Deferribacteres*, *Chlamydiae*, *Dictyoglomi*, *Fusobacteria* were more abundant in the Ventersdorp bulk soil (F3B).



**Figure 3.** Canonical correspondence analysis (CCA) of the bacterial community distribution and soil physicochemical properties of both rhizosphere and bulk soil samples.

### Influence of environmental factors on the bacterial community

Canonical Correspondence analysis (CCA) (Figure 3) was used to determine the effects of physical and chemical parameters (Table 1) on the bacterial community distribution. All the soil physical and chemical properties (Table 1) were used for the CCA plot (Figure 3). The CCA plot indicated that the composition of the bacterial communities was affected by the soil properties. The vector length of total carbon (%) (on axis 1) positively correlated with *Ktednobacteria*, *Gemmatimonadetes*, *Nitrospira*, *Verrucomicrobiae*, *Bacteroidia*, *Chloroflexi*, *Bacilli*, *Flavobacteria* and *Thermomicrobia*. On axis 2, the vector length of pH and total carbon positively correlated with *Opitutae*, *Chlorobia*, *Deinococci*, *Deltaproteobacteria*, but negatively correlated with *Solibacteres*, *Cytophagia* and *Clostridia*.

### Functional analysis associated with the bacterial community in the maize rhizosphere and bulk soil samples

There are 28 major functional categories that were found at SEED Subsystem level 1 hierarchical gene annotation. The functions are related to bacterial communities in maize rhizosphere and bulk soils. Photosynthesis ( $P < 0.001$ ), potassium metabolism ( $P < 0.001$ ) and secondary metabolism ( $P < 0.001$ ) were significantly higher in Ventersdorp rhizosphere (F3R) soil as shown in Figure 4(A) (Supplementary Table 1) while dormancy and sporulation ( $P < 0.001$ ) was significantly higher in the bulk soil (F3B). Dormancy and sporulation ( $P < 0.001$ ) was however, significantly higher in Mafikeng rhizosphere soil (F4R) while photosynthesis ( $P < 0.001$ ), potassium metabolism ( $P < 0.001$ ), and secondary metabolism were significantly higher in the bulk soil (F4B) (Figure 4(B)).

### Alpha and beta diversity estimations of bacterial communities in maize rhizosphere and bulks soils

The Simpson, Shannon, and Evenness diversity indices were used to portray the alpha diversity (that is, diversity within the samples) of the bacterial communities. These indices demonstrated that there were no significant differences in

the alpha diversity of the bacterial taxonomic composition (Kruskal–Wallis,  $F3p$ -value = 0.51;  $F4p$ -value = 0.83) and their functional categories (Kruskal–Wallis,  $F3p$ -value = .51;  $F4p$ -value = 0.51) (Table 3). However, there was a significant difference in the beta diversity (that is diversity between samples) of the bacterial taxonomic composition (ANOSIM,  $p$ -values = 0.01;  $R = 0.58$ ), and the functional categories (ANOSIM,  $p$ -values = 0.01;  $R = 0.58$ ); this was shown using the PCoA (Figure 5).

### Bacterial community genes contributing to maize plant-beneficial functions identified in Mafikeng and Ventersdorp rhizosphere and bulk soils samples

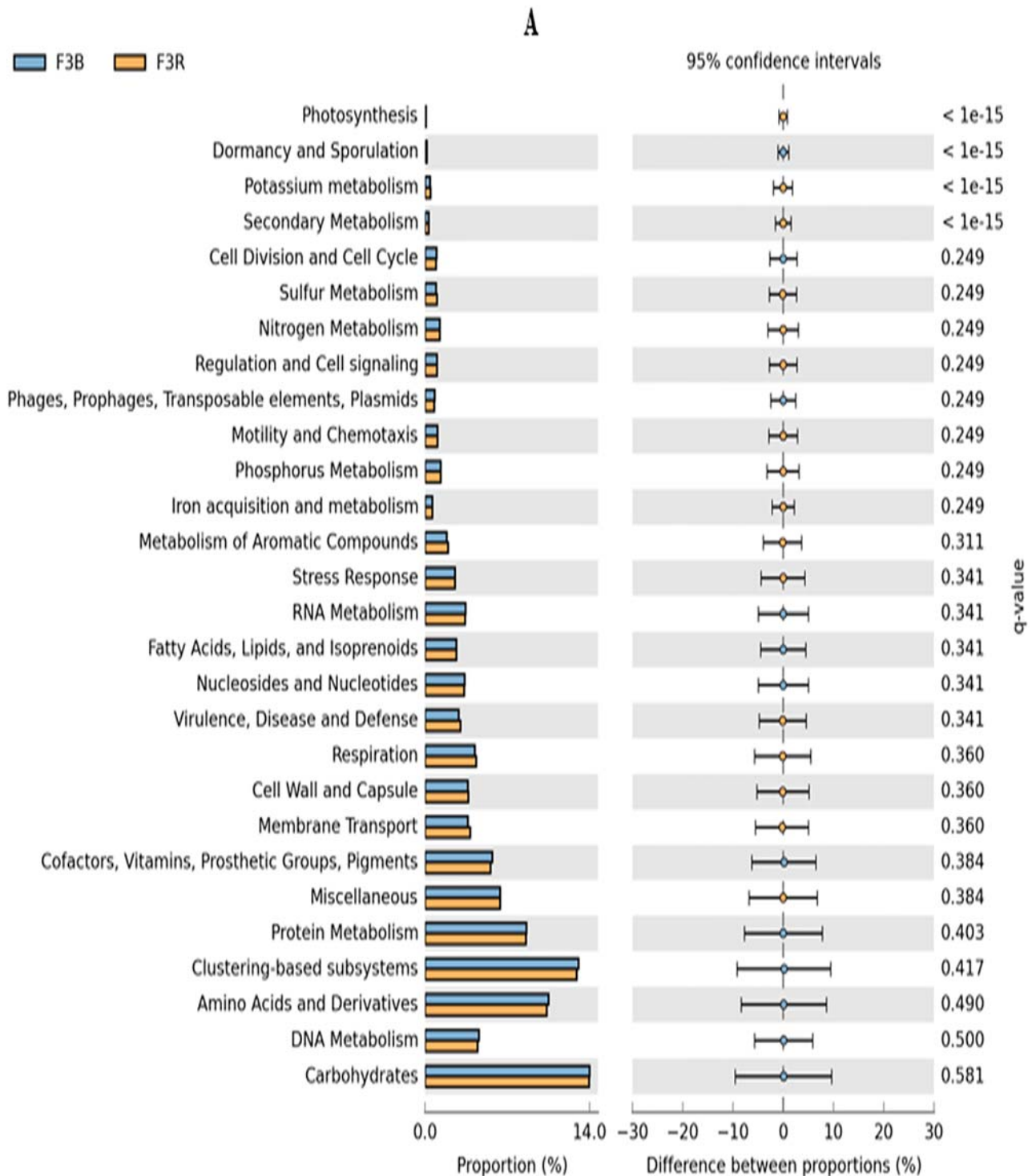
The Circos diagram (Figure 6, (Supplementary Table 2)) demonstrates more *treS*, *trkH*, *sodC*, *rimM*, *pvdIJ*, *phzF*, *groE*, *dnaJK*, *cysCIN*, *cspABCDEF*, *pqqBDEF*, and *pstABC* genes in the maize rhizosphere soils than the bulk soils from Mafikeng and Ventersdorp. However, the *nifDHK*, *opuD*, *mbtH*, *cspBF*, *sodC*, *budAB*, *pqqADF*, *krABD*, and *FhuABCD* genes were poorly represented in the maize rhizosphere and bulk soils of Mafikeng and Ventersdorp. The abundance of these bacterial community genes contributing to plant-beneficial functions ( $F3p$ -value = .51;  $F4p$ -value = .83) did not differ significantly within the maize rhizosphere and bulk soils at Mafikeng and Ventersdorp as shown with the arms of the Circos plot.

### Discussion

Rhizosphere microbes have been reported to significantly impact the growth and development of plants (Kumar and Dubey 2020). In the present study, employing a high-throughput-based evaluation, the metabolic potentials and structure of bacterial communities in bulk soils and the rhizobiome of maize were examined.

### Effects of soil parameters on the bacterial community structure and functional diversity

Our physical and chemical analysis showed that soil pH was significantly higher ( $P < 0.05$ ) in the maize rhizosphere soil relative to the bulk soil at Ventersdorp (Table 1). In contrast, the pH was significantly higher ( $P < 0.05$ ) in the maize bulk soil relative to the rhizosphere at Mafikeng. Researchers have reported that soil pH is a major factor determining bacterial diversity in different soils, including maize agricultural fields (Amoo and Babalola 2017; Cordero et al. 2020). pH affects essential nutrients and thus influences the physiology and growth of soil bacterial communities. Our study revealed that the relative abundances of predominant phyla differed along the pH gradient. In acidic soils, most micronutrients are more available to plants than in neutral-alkaline soils, and plant growth is much more favored to bacterial growth (Gentili et al. 2018). Under alkaline soils, the abundance of most macronutrients is improved (Gentili et al. 2018). In addition, Mafikeng rhizosphere (F4R) and Ventersdorp rhizosphere (F3R) had prevalent soil microorganisms relative to their bulk soils due to the increased levels of organic exudates in plant roots (Figure 2(B)) (Vieira et al. 2020). In general, our study is in agreement with previous studies that suggests that the potential of plants to influence microbial



**Figure 4.** Extended error bars plot identifying significant differences between proportions of microbial functions between maize rhizosphere and bulk soil samples from (A) Ventersdorp and (B) Mafikeng. *p*-values are shown at right.

diversity and selection in the rhizosphere may be linked to their ability to establish an environment high – in carbohydrate, aromatic compounds and amino acids.

Total carbon was higher in the rhizosphere soils although not significantly different from the bulk soil samples. The canonical correspondence analysis (CCA) plot (Figure 3) showed that total carbon alone explained 88.6% of the entire variation in the bacterial communities while  $N-NH_4$  explained 6.5% and the pH explained 4.8% (Table 2). Considering the vector length of  $N-NH_4^-$  and the total carbon in the CCA plot, it demonstrated that not only pH determines the shaping of bacterial communities. According to

Jacoby et al. (2017), sulphur, phosphorus and potassium present in rhizosphere soils also contribute to the composition of soil microbial communities for mineralization processes crucial for plant nutrition in natural ecosystems. The composition of root exudates is also a major factor which modulates the composition of rhizosphere microbial communities (Babalola et al. 2021a, 2021b; Korenblum et al. 2020).

The alpha diversity (diversity within the samples) showed that bacterial diversity and functions were not significantly different (*p*-value > 0.05) between the maize rhizosphere and bulk soil samples from Ventersdorp and Mafikeng (Table 3). This has shown that the maize

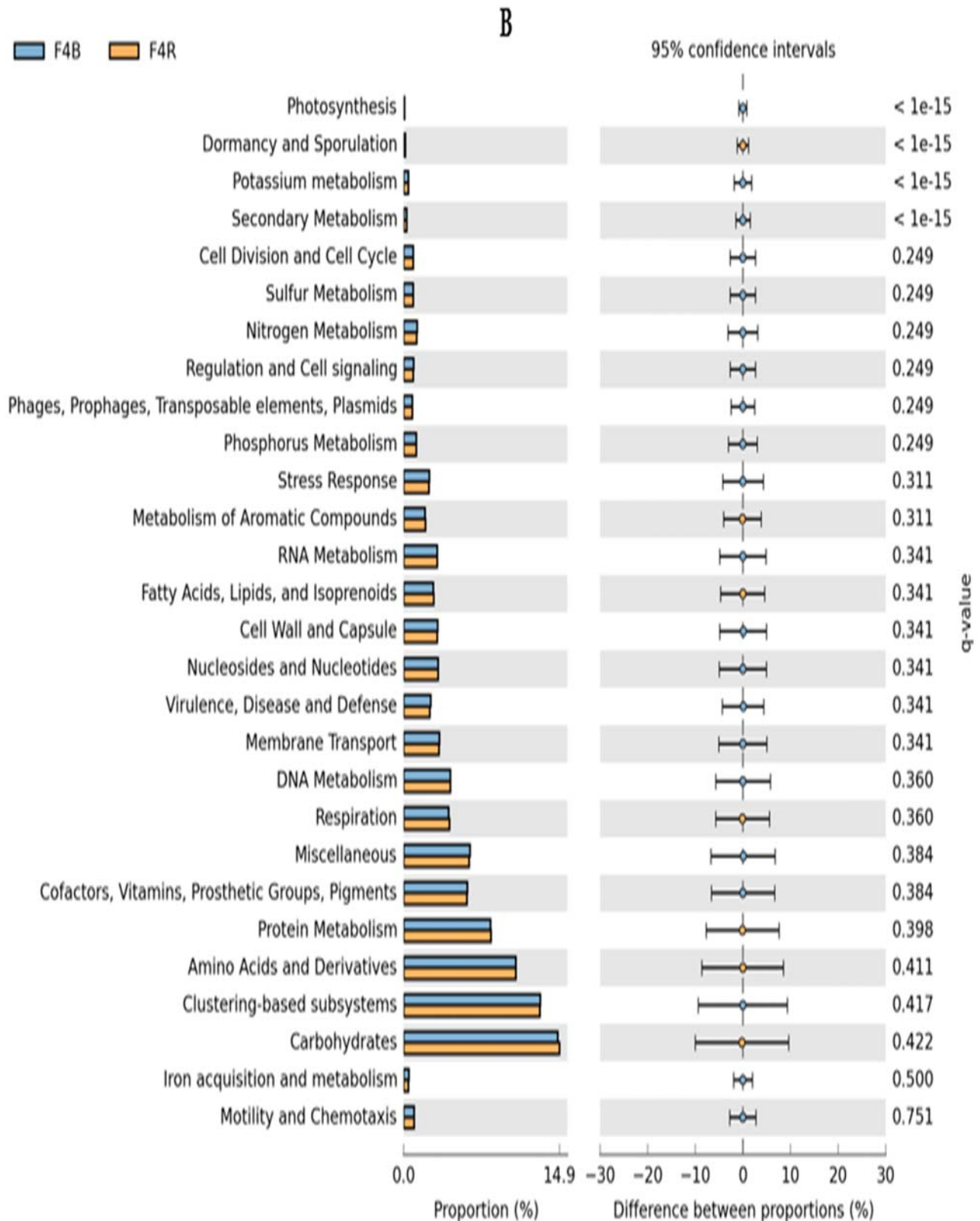


Figure 4 Continued

rhizosphere effect is the driving force of alpha diversity in this research. After secreting a wide range of nutrients and bioactive compounds into the rhizosphere, maize root exudates can influence bacterial diversity and functions in the rhizosphere and bulk soils (Praeg et al. 2019). The alpha diversity (Shannon diversity indices) also indicated that only the functional diversity represented by the bacterial metagenomes of the rhizosphere and bulks soil passed its hypothetical limit of 2.81 (Dinsdale et al. 2008), signifying

that bacterial metagenomes were most characterized in both maize rhizosphere and bulk soils from Mafikeng and Ventersdorp. The Evenness indices for the metagenomes across all samples were low (<1, Table 3), signifying that there are a few dominant bacterial taxa, (e.g. *Candidatus koribacter*, *Acidobacterium*, *Arthrobacter*) and functional categories (clustering-based subsystems metabolism, carbohydrates metabolism, and amino acids and derivatives) in each of the soil samples (Figure 4).



**Table 3.** Diversity indices for bacterial communities from rhizosphere and bulk soils.

	Diversity Indices	F3B	F3R	F4B	F4R	p-value (F3; F4)
Bacterial taxonomic composition	Simpson	0.952	0.951	0.934	0.935	F3p-value = 0.51; F4p-value = 0.83
	Shannon	3.173	3.172	3	2.999	
	Evenness	0.885	0.884	0.744	0.743	
Functional category	Simpson	0.925	0.926	0.9339	0.9229	F3p-value = 0.51; F4p-value = 0.51
	Shannon	2.862	2.869	2.846	2.842	
	Evenness	0.625	0.629	0.615	0.613	

Note: F3B = Ventersdorp bulk soil, F4B = Mafikeng bulk soil, F4R = Mafikeng rhizosphere, and F3R = Ventersdorp rhizosphere.

Secondary metabolism, photosynthesis, potassium metabolism and dormancy and sporulation were significantly different between the rhizosphere and bulk soils in Mafikeng and Ventersdorp (Figure 4). Dormancy and sporulation, photosynthesis, and secondary metabolism were more abundant in the Ventersdorp bulk soil than the rhizosphere soil (Figure 4(A)). However, potassium metabolism was more in the Ventersdorp rhizosphere (Figure 4(A)). Research studies on maize crops have shown that increased potassium metabolism arises from stimulation of root growth or elasticity of root hair by bacteria for plant growth and development (dos Santos et al. 2020; Meena et al. 2014; Singh et al. 2010). The maize plants would have poorly formed roots without sufficient potassium availability, grow slowly, generate lower crop yields and increase susceptibility to disease and pests (Meena et al. 2014).

Photosynthesis, potassium metabolism, and secondary metabolism were more abundant in the Mafikeng bulk soil while dormancy and sporulation were more abundant in the Mafikeng rhizosphere (Figure 4(B)). Environmental periodic stresses hinder bacterial growth. Most bacteria induce an irregular cell division when subjected to growth-restricting stress to create a resilient, metabolically dormant daughter cell that germinates when exposed to favorable environmental conditions and initiates rapid growth to restore the bacterial community (Bressuire-Isoard et al. 2018).

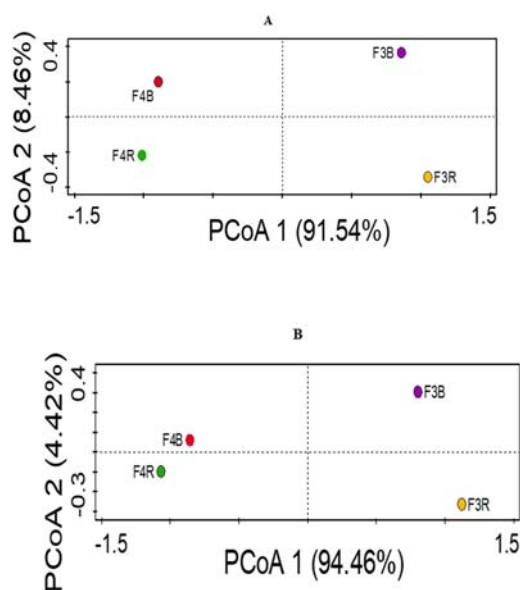
Furthermore, 100% of the combined PCoA axis 1 and 2 explained bacterial community variation (Figure 5(A)). Also, 98.88% of the combined PCoA axis 1 and 2 explained functional category variation (Figure 5(B)). The PCoA plot showed that the maize rhizosphere formed no cluster with the bulk

soil samples, suggesting that bacterial composition and the functional categories were different between the soil samples at Mafikeng and Ventersdorp. The aggregation of diverse bacterial communities occurs due to the selection pressure of the maize roots that continuously release exudates containing carbohydrates and amino acids into the rhizosphere. The influence of host plants on the bacterial diversity in the rhizosphere microbes has been observed in different crops including peas and wheat (Hamel et al. 2018). Hamel et al. (2018) reported that in an analysis of high-frequency pea production, there was an increase of the bacterial diversity in pea rhizosphere with soil mineral nitrogen level than in the bulk soil. Also, metagenomic analysis of wheat plants demonstrated that there was high bacterial diversity in the rhizosphere than in bulk soil (Velázquez-Sepúlveda et al. 2012). In addition, during the initial stages of maize growth, several studies based mainly on selected rhizospheric isolates also reported higher bacterial diversity and stated that the rhizosphere of small plants is a more unpredictable ecosystem than the rhizosphere of mature plants (Rodriguez et al. 2017). García-Salamanca et al. (2013) observed in accordance with the assumption that the rhizosphere is more nutrient-rich than bulk soil; the levels of activity of alkaline phosphatase,  $\beta$ -glucosidase and dehydrogenase enzymes from bacterial cells in the maize rhizosphere were higher than the same enzymatic activity tested in bulk soil.

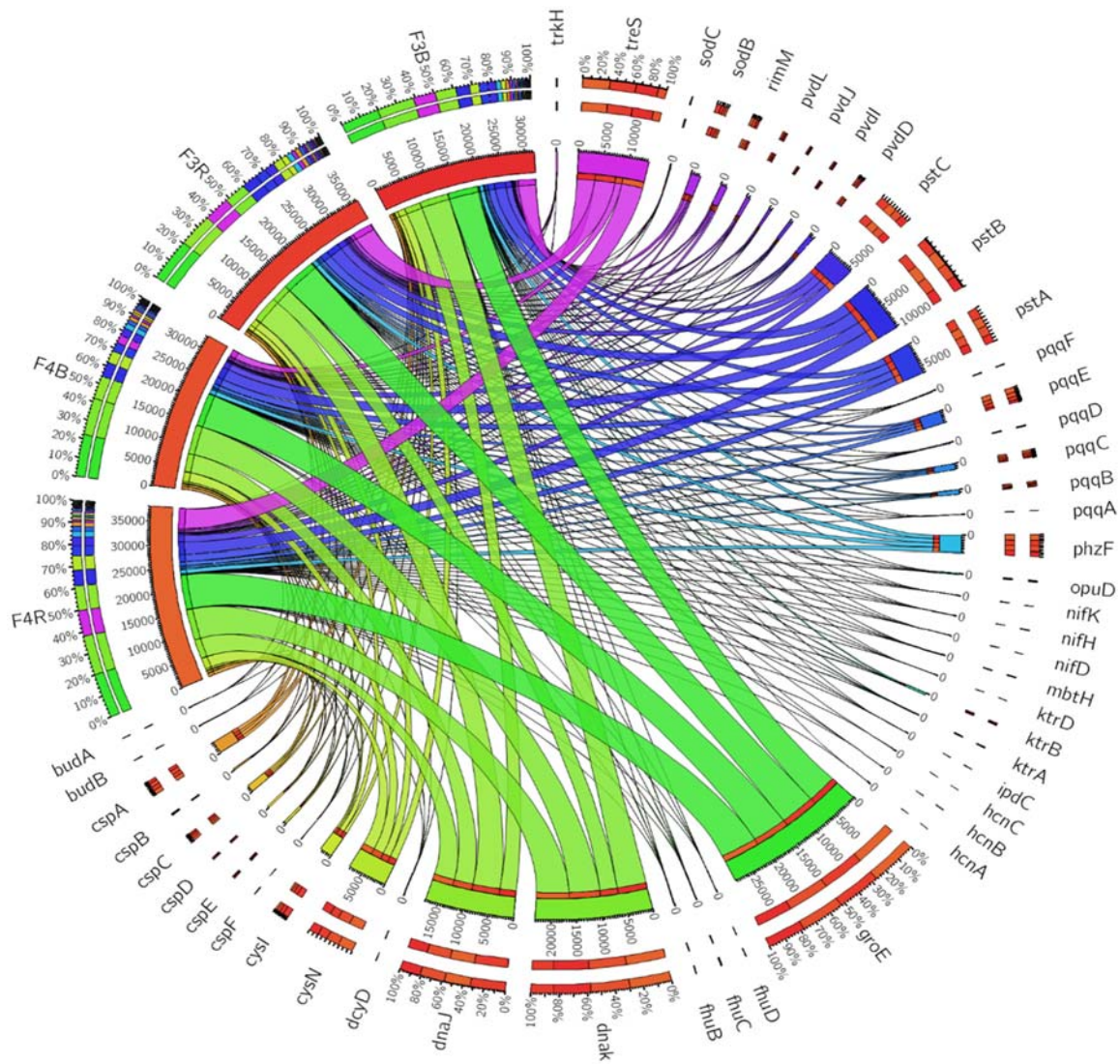
### The maize rhizosphere: a hotspot for survival

The plant-beneficial genes (Figure 6) can enhance the availability of nutrients, improve the ability to break down aromatic and toxic compounds, reduce the impact of abiotic stressors and inhibit plant pathogens in maize rhizosphere and bulk soil samples in Mafikeng and Ventersdorp. *treS*, *trkH*, *sodC*, *rimM*, *pvdII*, *phzF*, *groE*, *dnaJK*, *cysCIN*, *cspABCDEF*, *pqqBDEF*, and *pstABC* genes were more in the maize rhizosphere soils than the bulk soils from Mafikeng and Ventersdorp. These functional genes mainly emerge from prevalent bacterial communities in the rhizosphere than bulk soils due to high plant-derived organic exudate concentrations (Li et al. 2014; Vieira et al. 2020). The abundance of the genes in the rhizosphere determines the ability of bacteria to increase the resistance of maize-plants to various types of stress (Hartman and Tringe 2019). In addition, essential antibiotic-resistant genes from the genera *Burkholderia*, *Mycobacterium*, and *Bacillus* have been identified in previous studies trying to verify their abundant distributions in the maize rhizosphere (Li et al. 2014).

The *ipdC* genes in the maize rhizosphere and bulk soils in Mafikeng and Ventersdorp had poor representation due to less plant growth-promoting bacteria (Jijón-Moreno et al. 2015). The *ipdC* genes encode Indole-3-pyruvate decarboxylase enzymes that produce IAA for different functions in



**Figure 5.** Principal coordinates analysis (PCoA) of (A) bacterial structure and composition and (B) functional categories of maize rhizosphere and bulk soils.



**Figure 6.** Circos software plot of genes involved in plant-beneficial function observed in the maize rhizosphere and bulks soils. (F3R = Ventersdorp rhizosphere; F3B = Ventersdorp bulk; F4R = Mafikeng rhizosphere; and F4B = Mafikeng bulk).

plants (Sugawara et al. 2015). This includes the physiological processes, including embryogenesis, organogenesis, vascular differentiation, root and shoot development, trophic growth and fruit production (Etchells et al. 2016). In plant-bacteria symbiotic relationships, ACC deaminase activity is carried out by *dycD*, and *rimM* genes. The *ipdC* genes were more abundant in the Mafikeng (F4R) rhizosphere compared to Mafikeng (F4B) bulk soil. ACC is an intermediate precursor that reduces plant ethylene, obstructing the nodulation process (Pandey and Gupta 2019).

The *hcnABC*, *nifDHK*, and *ktrABD* genes were poorly represented in maize rhizosphere and bulk soils from Mafikeng and Ventersdorp. These genes promote plant growth and development synthesizing antimicrobial compounds (*hcnABC*), potassium transport (*ktrABD*, *trkH*), nitrogen fixation and nitric oxide synthesis (*nifDHK*) (Gründling

2013; Rijavec and Lapanje 2016; Zhang et al. 2016). The *budAB* genes that aid in acetoin and butanediol synthesis were observed in our samples, although they were poorly represented. *FhuABCD* and *mbtH* genes help with sequestration of iron by the production of siderophore, while the *phzF* genes promote phenazine production (Egamberdieva and Lugtenberg 2014). Siderophores and phenazine produced serve as antibiotics and prevent pathogenic diseases in plants (Egamberdieva and Lugtenberg 2014; Stephan et al. 2019). We observed genes that alleviate stresses such as drought and the winter season, such as *cspABCDEFI* (Yu et al. 2017) and *groE*, *dnaJK* (da-Silva et al. 2017). These genes promote cold-shock and heat-shock protein production and were more abundant in the maize rhizosphere soils than in the maize bulk soils from Mafikeng and Ventersdorp. The *pstABC* and *pqqABCDE* genes were also well presented in our Ventersdorp and Mafikeng rhizosphere soils than in the bulk soils in our study. These genes are responsible for phosphate uptake and solubilization (Li et al. 2017).

**Table 2.** Forward selection of environmental variables that best describes variation in structural composition based in SEED subsystems between rhizosphere and bulk soil samples.

Name	Explains %	Contributions%	Pseudo-F	P
Total C (%)	88.6	88.6	15.6	0.31
N-NH (mg/kg)	6.5	6.5	1.3	0.666
pH (H2O)	4.8	4.8	<0.1	1

## Conclusion

The bacterial community structures and functional potentials were significantly shaped by the rhizosphere.

Differences in dominant bacterial communities were recorded between the rhizosphere and bulk soils. Rhizosphere microbial communities are recruited from the assortment of microbes in bulk soils through the signaling of root exudates. Plant species determine the quantity and quality of exudation which are then used by rhizospheric microbes for the benefit of the plants. Functional prospects such as sulphur metabolism, iron acquisition metabolism, metabolism of aromatic compound, respiration, membrane transport, cell wall and capsule carbohydrates metabolism, amino acids and derivatives, and respiration were more abundant in maize rhizosphere soils than in the bulk soils. Bacterial community structures are stabilized by the establishment of plants, and interactions with the rhizosphere of these plants additionally influence them. The metabolic pathways of plant-beneficial genes increased in the maize rhizosphere compared to the bulk soil samples in the present study. A good understanding of the genes that encode valuable metabolic pathways crucial for plant development and the shifting metabolic pathways of microbial genes will enable the farming industry to enhance environmental and economic sustainability by making more informed soil management decisions.

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### Disclosure statement

No potential conflict of interest was reported by the author(s).

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### Compliance to ethical standards

Human and animal rights: No human subjects or livestock were included in this research.

Informed consent: The scientists certify that this study adhered to ethical and professional standards.

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