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Full Length Article

Seasonal Variation Drives Microbial Diversity in Cowpea Rhizosphere in a Semi-Arid Region

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Abstract

The microbial communities' diversity patterns of cowpea rhizospheric soil and the driving factors responsible for their composition and structure are crucial for agricultural sustainability and food security. In this study, shogun sequencing was used to determine the microbial structure and diversity of cowpea rhizosphere during two planting seasons (winter and summer). The microbial communities' composition and structures in the two seasons were found to be distinct from each other. The bacterial distributions in the summer sample, with 98.06% relative abundance, were higher than the winter sample, with 87.69%, while, eukaryal distributions in winter with 11.11% relative abundance was higher than summer with 1.24%. Bacteroidetes, Proteobacteria, Firmicutes and Ascomycota were dominant during winter, while Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia, Planctomycetes, Acidobacteria, Ascomycota and Crenarchaeota were dominant during summer. Variations were observed in the soil physical and chemical properties of both seasons with summer sample having high organic carbon and total carbon, organic matter, pH, and total nitrogen. Differences in seasonal temperature and soil physical and chemical parameters account for the differences in microbial composition and diversity of the two season. This study showed that edaphic factors strongly influence the abundance and diversity of rhizosphere community during the two seasons.

Keywords: Plant-microbe interactions; Soil ecology; Leguminous plants; Microbial diversity; Seasonal variation

Introduction

Microorganisms are ubiquitous, yet their diversity and distributions are not consistent across different environments. Soil microorganisms provide important ecosystem services, such as improving soil structure and aggregation, as well as nutrient and water recycling (Wang *et al.* 2018). The physical and chemical qualities of soil are influenced by plant roots, soil, and microbial interactions, which determine rhizosphere microbiological traits (Bhople and Sharma 2020). Soil environmental parameters, such soil texture, moisture, pH, temperature, nutrients and biotic factors are shown to affect the distribution of microbial community (Regan *et al.* 2014; Wang *et al.* 2018; Akinola *et al.* 2021).

Plants can directly alter the rhizosphere environment by secreting carbohydrates, polysaccharides, vitamins and other substances through their roots, which drive microbial activity in the area. As a result, the rhizosphere is a centre of intensive microbial activity driven heavily by plant root secretions (Stringlis *et al.* 2018). The quality and quantity of chemicals produced by plant roots determine the diversity of rhizosphere microbial activity (Bhople and Sharma 2020). The rhizosphere soil is hence regarded as the most versatile and dynamic ecological niche on the planet (Mommer *et al.* 2016; Mueller *et al.* 2019). Rhizosphere microorganisms can help to increase soil quality and crop yield. Majority of the microbial community involved in the rhizosphere process is unculturable (Sneha *et al.* 2021). Advanced analytical techniques, such as amplicon and shotgun sequencing, are used to evaluate microbial diversity, allowing for more detailed analyses of soil microbial population and activity (Fadiji and Babalola 2020; Nwachukwu and Babalola 2022).

Leguminous plants have become increasingly important as a source of balanced nutrients over the years. Among the various leguminous plants cultivated, cowpea (*Vigna unguiculata* L. Walp.) have displayed some interesting traits of adaptation to different environmental stresses, which makes them economically and agronomically expedient. In addition, cowpea cultivation has contributed enormously to the income of farmers and peasants and enhances diets across South America, Africa and Asia (Hall 2012; Singh 2014). Cowpea plants have several environmental benefits over other plants because of their ability to survive in varied types of environments including semi-arid regions, with little or no input required (Hall 2012). Its richness in nutrition is significant because it

contains a high level of protein and low fat, which has been proven to prevent various metabolic and related cardiovascular infections. Thus, all the cowpea parts above the ground are usually used as a multipurpose crop ranging from its leaves to other parts, such as green beans, mature beans, green pods and can also be transformed to more processed foodstuff ingredients, such as flour (Xiong *et al.* 2013; Kapravelou *et al.* 2015).

It is critical to understand the diversity of bacteria that live on the cowpea plant, as well as how different external factors affect them. This is due to the influence of environmental variables on the composition and structure of microbes, as well as their diverse responses to these external factors, which drive the differences in microbial distributions across topographical regions (Zhang *et al.* 2017). Increasing evidence have suggested that soil pH is a crucial factor regulating the composition and structure of the communities of bacteria and archaea (Wang *et al.* 2015), whereas the diversity of plants regulates the structural communities of fungi residing in the soil over wide-ranging topographical regions (Chen *et al.* 2017). This suggests that microbes usually respond to external factors differently, thus, it is important to know the diversity of the microbes inhabiting the cowpea rhizosphere under different seasons for agricultural improvement and sustainability. Therefore, we hypothesize that the microbial diversity and structure varied with different seasons and are influenced by soil abiotic factors. Culture-independent metagenomics was used to access the diversity of microbes inhabiting cowpea rhizosphere during summer and winter season in a semi-arid region of South Africa.

Materials and Methods

Area of study and sampling of rhizosphere soil

The North-West University Farm in Molelwane, South Africa provided the samples of cowpea rhizospheric soil. The area has a semi-arid tropical savannah climate, with mean annual rainfall of about 571 mm in the summer. The soil in Mafikeng is predominantly hutton in nature, characterized by a red colour, and hard site when dry, with a slope of 0–4% having low permeability (Materechera 2014). In the winter, temperatures range from 3 to 21°C, while in the summer, temperatures range from 17 to 31°C. The winter soil samples were collected during the winter-peak period in July 2018, while the summer samples were collected in December 2018. Soil samples were collected during the flowering stage and soil adhering to the roots were carefully collected. We established 10 by 10 m plots on the field in three replicates and the distance between each plot and the next was greater than 15 m and these were regarded as comprehensive replicates (Ren *et al.* 2017). The soils were collected by taking three replicate soil samples from each plot on the field, storing them in a cooler box with ice packs and transporting them to the laboratory for storage at -20°C until used for metagenomic DNA extraction. The soil samples were sieved with a 2 mm sieve and air-dried before being analysed for physical and chemical characteristics.

Determination of physical and chemical properties of the soil samples

LECO CR-12 C analysing machine was used to determine the total carbon and organic carbon for the soil samples as described by Dhillon *et al.* (2015). The organic matter (OM) of the soil was determined by ash drying (Science and McKeague 1978). The pH of the soil was performed in a soil to water slurry of ratio 2:1. The available nitrate and ammonium in soil was analysed by measuring the absorbance using a colorimeter at 520 and 660 nm, respectively as described by Laverty and Bollo-Kamara (1988). Similarly, a modified method of Kelowna extraction was employed for measuring both potassium and phosphorus available in the soil (Qian *et al.* 1994) and the amount of sulphate and calcium present was done by calcium chloride extraction method (Science and McKeague 1978).

DNA extraction from the cowpea soil samples, amplification and NovaSeq 6000 Illumina sequencing

The extraction of the entire DNA from the cowpea rhizosphere soil was conducted using the Power Soil DNA Isolation Kit (MOBIO, USA) following the kit's protocol. The library preparations were conducted using Nextera DNA Flex library preparation kit (Illumina). The evaluation of the initial/preliminary DNA concentration was performed using Qubit® dsDNA HS Assay Kit (Life Technologies).. The concentrations of the samples were measured using the Qubit® dsDNA HS Assay Kit after cleaning them with the DNEasy PowerClean Pro Cleanup Kit (Qiagen) (Life Technologies, the USA). After that, 20–25 ng of DNA was used to make the libraries. At the same time, the samples were fragmented, and adapter sequences were introduced. The adapter sequences were used in a limited-cycle PCR and unique indices were added to the samples. The libraries' equimolar ratios of 0.7 n*M* were pooled and Illumina's NovaSeq 6000 platform was used to sequence the produced libraries paired end for 300 cycles. For all samples, the raw sequences were deposited in the NCBI SRA dataset with the BioProject accession number PRJNA588152.

Taxonomic classification and data analysis

The taxonomic characterizations of the metagenomic reads were determined using the Metagenomic Rapid Annotation using Subsystem Technology (MG-RAST) server version 3.3.6 (Meyer *et al.* 2008) for the classification of the microbial taxonomic nomenclature. The data sets were uploaded to the server at http://www.mg-rast.org. On the

MG-RAST server, the sequences were reviewed for quality. ambiguous base-filtering, which removes sequences with more than 5 ambiguous base pairs with a 15 phred score cutoff, species-specific host-filtering, and length filtering, which removes sequences with lengths more than 2 standard deviations from the mean. Following the QC step, sequence annotation was performed on the M5NR database using the BLAST-like alignment tool (BLAT) method (Kent 2002), which allows for nonrebundant inclusion of various databases (Wilke *et al.* 2012). The RDP database was used for taxonomic classification, whereas the SEED subsystems database was used to define functional categories. The metabolic pathways were discovered using the parameters of an e-value of 1e5, a maximum alignment length of 15 bp and a minimum identity of 60%. Any sequences that were not annotated received no further analysis.

Statistical Analysis

For each sample, the Simpsons, Pielou Evenness and Shannon diversity indices were calculated and the Kruskal-Wallis test was used to compare the indices. PAST version 3.20 was used for statistical analysis (Hammer *et al.* 2001). Similarly, a one-way ANOVA was employed to test for significant difference among the soil samples at $P < 0.05$ (Guo *et al.* 2015). The determination of microbial compositions and structural diversity in the three soil samples were performed using relative abundances in percentages. The visualization of the compositional structure of the microbial community were statistically conducted via principal components analyses (PCA) centred on the matrices of Bray Curtis dissimilarity in the Canoco 5 software (Microcomputer Power, Ithaca, USA). ClustVis (https://biit.cs.ut.ee/clustvis/) was used to generate the heatmap (Metsalu and Vilo 2015). Each row in the heatmap represents the microorganism and each column a sample. Samples were ordered according to the Euclidean ranked relative abundances.

Results

Physicochemical characteristics of the soil samples

As shown in Table 1, there were differences in soil parameters between the two soil samples. The results showed that summer sample (SS) had the highest concentrations of organic carbon (Org. C) and organic matter (OM) of 1 and 1.14%, while winter sample (WS) had 0.61 and 0.69% of Org. C and OM, respectively. The ammonium ($N\text{-}NH_4$) level was high in winter compared to summer, while total carbon and nitrogen were higher in summer compared to winter samples (Table 1).

Metagenomics sequencing datasets

The raw reads were 19,368,970 and 10,429,936 for winter and summer samples, respectively. After quality control in MG-RAST, the retained mean sequences were 13,261,514 and 8,982,497 with GC content of 42 ± 10 and 64 ± 10% for winter and summer samples, respectively (Table 2).

Microbial compositions and distribution in the soil samples

The three domains, Bacteria, Archaea and Eukarya were represented in each sample. Bacteria domain was the most dominant in all samples accounting for 87.69 and 98.06% in winter and summer soils, respectively. Eukarya and archaea account for 11.11 and 0.49% in winter sample and 1.24 and 0.57% in summer sample, respectively. The dominant phyla in winter sample were Bacteroidetes (70%), Proteobacteria (8%), Firmicutes (7%), and Ascomycota (6%) (Fig. 1). Other less dominant phyla include Verrucomicrobia, Actinobacteria, Cyanobacteria, Planctomycetes, Acidobacteria, Chlorobi, Basidiomycota, Blastocladiomycota, Chytridiomycota, Thaumarchaeota, Euryarchaeota, Crenarchaeota, Korarchaeota and Nanoarchaeota (Fig. 1). In the summer, the dominant phyla were Proteobacteria (43%) and Actinobacteria (34%), while Bacteroidetes, Firmicutes, Verrucomicrobia, Planctomycetes, Acidobacteria, Ascomycota, Crenarchaeota, Cyanobacteria, Chlorobi, Basidiomycota, Blastocladiomycota, Chytridiomycota, Thaumarchaeota, Euryarchaeota and Korarchaeota, were less dominant (Fig. 1).

Diversity indices of the microbial structure in the cowpea rhizospheric soil

The alpha diversity indices in the microbial structure, calculated using Evenness, Shannon and Simpson's indices at the phylum level, revealed no significant differences between the two seasons (Table 3). The Shannon index for the winter season was 0.4952, while that of summer was 0.6975. The Bray-Curtis similarity and distance indices between WS and SS were also not significant (Table 3).

The principal component analysis of the microbial structure using CANOCO 5 varied significantly in the two seasons. Different bacterial phyla were found dispersed throughout the two seasons with disparity for the choice of the soil sample. Firmicutes, Bacteroidetes, Chlorobi were the phyla significantly richer and dispersed in winter, while Proteobacteria, Acidobacteria, Planctomycetes, Verrucomicrobia, Actinobacteria and Cyanobacteria were significantly dispersed in summer. All the fungal phyla (Ascomycota, Basidiomycota and Glomeromycota) were found significantly dispersed only in winter.

The distribution of the soil physical and chemical properties is presented in Fig. 2. Organic matter (OM), organic carbon (Org. C), calcium (Ca), total carbon, and pH were distributed in the summer sample, while total P and K were distributed in winter sample. To demonstrate

Table 1: Physicochemical parameters analysis of the soil samples

Sample	Org. C (%)	OM (%)	N-NH_4 (mg/kg)	Total C (mg/kg)	Total N (mg/kg)	pH	P (mg/kg)	Ca (mg/kg)	K (mg/kg)
Winter Sample	0.617 ± 0.006	0.7 ± 0.01	11.74 ± 0.01	0.707 ± 0.002	0.083 ± 0.002	7.62 ± 0.01	112.38 ± 0.15	796 ± 3.00	547 ± 0.0
Summer Sample	0.99 ± 0.01	1.14 ± 0.15	10.27 ± 0.07	1.36 ± 0.04	0.095 ± 0.005	8.04 ± 0.01	21.84 ± 0.04	3461 ± 1.528	402 ± 1.00

Values are mean ± standard deaviation. Note: Org. C = Organic carbon; OM = organic matter; N-NH_4 = ammonium; Total C = total carbon; Ca = calcium; P = phosphorus; K = potassium

Table 2: Sequence information of uploaded data on the MG-RAST server

Analysis statistics	Winter sample (WS)	Summer sample (SS)
Uploaded count (bp)	3,050,611,052	1,836,993,861
Uploaded sequences count	19,368,970	10,429,936
Uploaded mean sequence length (bp)	158 ± 43	176 ± 70
Uploaded mean GC (%)	42 ± 10	64 ± 11
Artificial duplicate reads: Sequence count	5,720,406	1,115,181
Post QC: count	2,138,130,444	1,615,794,893
Post QC: sequences count	13,261,514	8,982,497
Post QC: mean sequence length (bp)	161 ± 42	180 ± 67
Post QC: Mean GC (%)	42 ± 10	64 ± 10

Values are mean ± standard deaviation. Note: bp = basepairs

Table 3: Alpha diversity of the microbial phyla showing comparisons among the two soil samples

Alpha diversity	Winter sample	Summer sample
Variance	1.09E+11	5.78E+10
Correlation	1.0	0.08527
Simpson-1-D	0.4952	0.6975
Shannon-H	1.199	1.591
Coeff. Var	333.0666	249.5767
Similarity and distance indices (Bray-Curtis)	1.0	0.20905
Evenness-e^H/S	0.1441	0.2135

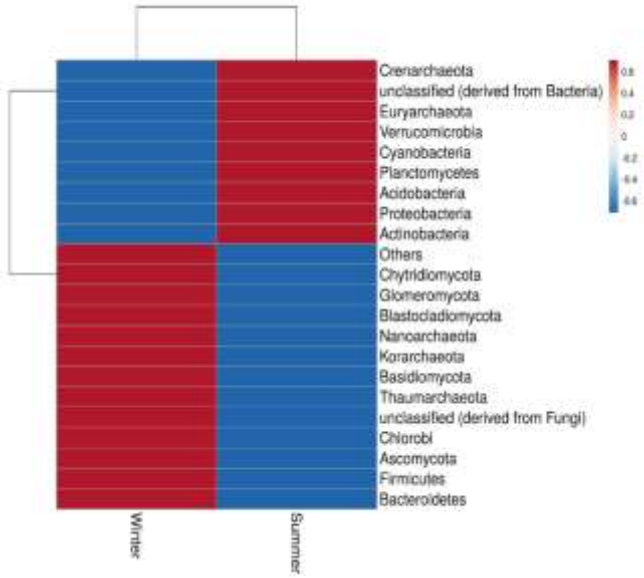

Fig. 1: Heatmap of the microbial structure and relative abundance in cowpea rhizospheric soil during the winter and summer seasons

the distribution of the various phyla across the seasons, the CCA was used. All parameters have significant effect on the distribution of Proteobacteria in all seasons (Fig. 2). In addition, organic matter, organic carbon, calcium, total carbon, total nitrogen, potassium, phosphorus and ammonium have an influence on the diversity of Cyanobacteria, Actinobacteria, Acidobacteria, Verrucomicrobia, Planctomycetes, Euryarchaeota and Crenarchaeota during summer. Similarly, OM, organic C, Ca, total C, total N, K, P and N-NH_4 influenced the diversity of Firmicutes, Bacteroidetes, Chlorobi, Ascomycota, Basidiomycota, Glomeromycota, and Thaumarchaeota during winter (Fig. 2). In the PCA analysis, the vector lengths depict the strength of dominance of the microbial metagenomes and the physical and chemical parameters during each season. Axis 1 and Axis 2 explained 57.3 and

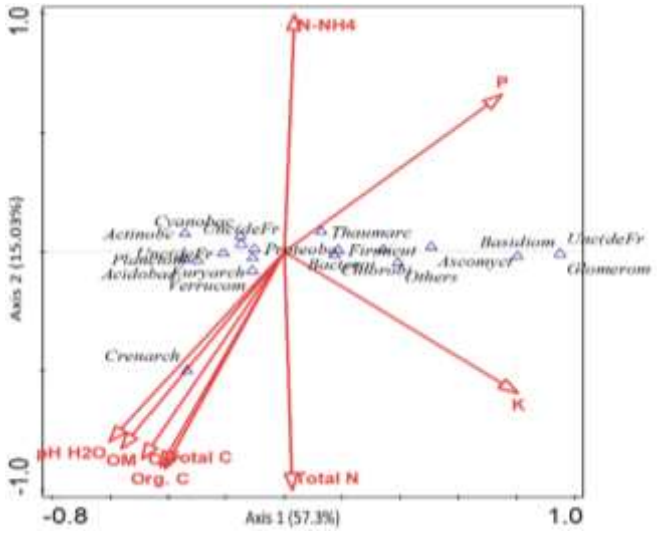

Fig. 2: Distribution of soil properties and their effect on the microbiome of cowpea rhizosphere during winter and summer
Note: Org. C = Organic carbon; OM = organic matter; N-NH_4 = ammonium; Total C = total carbon; Ca = calcium; P = phosphorus; K = potassium

15.03% variation, respectively. The longest vector lengths on the PCA analysis graph show the phylum that predominated in each sample.

Discussion

The hypothesis that the microbial diversity and structure varied with different seasons and are influenced by soil abiotic factors was tested in the study. Culture-independent metagenomics was used to access the diversity of microbes inhabiting cowpea rhizosphere during summer and winter season in a semi-arid region of South Africa. Although there were variations in the distribution and abundances of microorganisms during the two seasons, they were not statistically difference using diversity indices.

Edaphic factors, such as moisture and temperature, have been linked directly with humidity, precipitation and air temperature during different seasons (Siles *et al.* 2016). This results in significant differences in soil moisture and temperature observed during different seasons. These variations have been identified as key drivers of the soil microbial community, as well as soil physical and chemical properties, over the course of a year (Zhou *et al.* 2015). These could be veritable predictors of soil health (Myrold *et al.* 2014). In this study, the composition, structural diversity and the influence of edaphic factors on the cowpea rhizospheric soil during summer and winter growing seasons was investigated. The three main domains, namely archaea, bacteria, and fungi, were present in the two seasons with varied abundances. The variation in the cowpea rhizosphere microbiome of the two seasons is attributed to changes in soil physical and chemical properties as well as changes in weather condition. Several studies have also reported seasonal variations to be a significant factor in shaping microbial communities present in samples (Yang *et al.* 2014; Guo *et al.* 2015; Wang *et al.* 2015). These variations in the compositions and structure of the microbes can be attributed to varied temperature in the environmental conditions of the two seasons. Similarly, there have been reports emphasizing environmental changes to influence a pattern of decrease and increase of microbial diversity across various seasons (Sundqvist *et al.* 2013; Wang *et al.* 2015). Furthermore, during winter season the soil usually experiences fluctuating conditions, such as unbalanced nutrients and unstable viscosity and instability in the heat energy (Jansson and Taş 2014), which accounts for variation in microbial structure and diversity.

In this study, the domain bacteria account for majority of organisms found in both season. In the summer rhizosphere soil, the phyla Proteobacteria and Acidobacteria were found to be prominent, while the winter soil was dominated by Bacteroidetes. The relative abundance of the fungal domain was higher in winter season compared to summer. The relative abundance of Ascomycota was distinctively higher in winter season. Reports have shown that several species of fungi have the ability to form spores (Harrison and Ivanov 2017). Hence, these species form propagules bag-like, which enable them to thrive even in harsh environmental conditions like the winter season (Davison *et al.* 2015). Bacterial and fungal compositions between the two seasons did not differ significantly possibly because the identified unexpected dispersal agents were substantially not dominant (Egan *et al.* 2014). Similarly, the results showed that different phyla were found dispersed in different seasons. The microbial structure in these soil samples show the disparity in the choice of where each phylum was found based on their abilities to thrive in such soil samples. This means that there were some microbes that have the ability to survive high temperature, such as during summer, while others can only thrive in the low temperature

environments, such as the winter season, while other microbes have the ability to thrive in both seasons due to their ability to adapt and survive different weather conditions. This is similar to the report of Jansson and Taş (2014), which showed that several microbial species could develop adaptive features to survive and thrive in unfavourable conditions, such as low and high temperature, both in diversity and functions. For instance, native microbes develop various adaptive strategies, such as dormancy or produce some specific protein products to survive in harsh temperatures.

Similarly, when the relationship between environmental changes and microbial community structure was examined, it was discovered that the microbial community was closely linked to environmental changes, such as low and high temperatures. This suggests that the hypothesis of Bass Becking, which states, "everything is everywhere" can be applied. However, environmental properties are a significant factor that shape the community composition and structure of the microbial phyla (Burns *et al.* 2015). This is consistent with the findings from this study, which indicated that differences in the seasons was an important environmental factor, which potentially affected the communities of microbes in both seasons.

The main drivers of microbial structure are the available nutrients in the soil as observed in this study. Soil nutrients and their availabilities (such as nitrogen, phosphorus, potassium, carbon and calcium) are the determining factors responsible for the microbial composition and structure (Rashid *et al.* 2016). The distributions of these parameters may also be largely dependent on the type of exudation mechanisms. The availability of soil nutrients in shaping microbial richness and distributions is a critical feature to take into consideration. The distribution and richness of organic carbon, organic matter, total carbon, total nitrogen, potassium, phosphorus, calcium and ammonium were significantly correlated with the difference in the distributions and structure of the microbial phyla present in the samples and this has been reported by a number of studies (Shen *et al.* 2013; Lin *et al.* 2015; Wang *et al.* 2015).

Litter and root exudates are the bases for plants and soil microbial communities' interactions (Knelman *et al.* 2012; Cui *et al.* 2018). Plants have the ability to define the sources of organic carbon, organic matter, total carbon, calcium, total nitrogen, potassium, phosphorus and ammonium and modify the soil environments physically and chemically and hence indirectly disturb the structure of the microbial communities present in the soil ecosystem (Landesman *et al.* 2014; Li *et al.* 2018). The impacts of these nutrients in structuring the microbial communities may be ascribed to the disorderliness in the stoichiometric stability of the elements and homeostatic reactions through the microbes (Cui *et al.* 2018). The elemental balance in the soil samples is hence a good microbial composition and structure variation predictor. As a result, the apparent diversity patterns of various microbe phyla between the two seasons underlined the importance of these ecological factors in changing microbial community distribution. The results of this study further showed that the phyla of archaea preferred nutrients, such as, available phosphorus, organic matter, calcium and organic carbon, while fungi preferred available phosphorus and potassium in the summer and winter seasons. The ability of archaea to decompose litter is lower than both bacteria and fungi and hence, they are very sensitive to soil nutrient variations (Singh *et al.* 2012). As a result, the difference between the two seasons in bacterial, fungal and archaeal changes is directly proportional to the impact of environmental conditions on microbial populations. Furthermore, the outcomes of the result from this study showed that the preferential distinct separation in the compositional structure of bacterial, fungal and archaeal phyla in the two seasons could be attributed to their responses to environmental influences.

The pH of any soil has usually been considered as a critical factor in determining the microbial-community diversity (Hu *et al.* 2013; Wang *et al.* 2015; Li *et al.* 2018). There is every indication from our results that pH and moisture contents have great impacts on some of the bacterial and archaeal phyla dispersed during the summer. This shows that these phyla required moderately alkaline environment to thrive in the summer. The pH values of the soil samples analysed are close to neutrality and this has been favourably considered for several of the microbial population (Shen *et al.* 2013).

Conclusion

It was found that seasonal variation drives microbial community structure and diversity in a semi-arid soil. The outcomes from this study indicated that edaphic characteristics were the major driving features influencing the variations in the microbial community composition and structure. Furthermore, this study was able to identify specific driving factors among the phyla of bacteria, fungi and archaea, which suggest why there were disparities in responses of the microorganisms to the changes in the soil environment of the cowpea ecosystem.

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Author Contributions

All authors contributed equally to the manuscript.

Conflict of Interest

All authors declare no conflicts of interest.

Data Availability

The datasets used has been deposited in the NCBI database under the Bioproject accession number PRJNA588152.

Ethics Approval

Not applicable to this manuscript.

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