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MOLECULAR PROFILING OF COWPEA RHIZOSPHERIC MICROBES USING METAGENOMICS SEQUENCING

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Abstract

There have been several studies investigating the compositions and structure of microbes residing in the legume root rhizosphere/nodules. Although most of these microbes do not form associations with the rhizosphere/nodules of legumes themselves, but they are usually in great number in the rhizosphere with various activities. The aim of this study was to use metagenomics high-throughput technology (NovaSeq 600 sequencing system) to describe the diversity of microbial communities and functional activities associated with cowpea (*Vigna unguiculata* L. Walp) root rhizosphere/nodules with respect to the differences in the season of collection, i.e., winter and summer rhizosphere soil samples. On the hierarchy of family, *Sphingobacteriaceae*, *Cytophagaceae*, *Flavobacteriaceae* and *Cyclobacteriaceae* were more prominent in WS while *Planctomycetaceae*, *Conexibacteriaceae*, *Streptomycetaceae*, *Bradyrhizobiaceae*, *Micrococcaceae* and *Nocardioidaceae* were more abundant in SS at least at 3% =/> in relative abundance. This shows that, there was more diversity of plants growth promoting microbes in SS than WS. This is in conjunction with the results at the functional profiles where the sequence counts for most of the metabolic activities most especially nitrogen, phosphorus and sulphur metabolisms in SS were higher than WS. Although, potassium and iron acquisition metabolisms were more prominent in WS than SS. The presence of these families of microbes were significantly influenced by the soil physiochemical elements and also difference in the season of collection. Therefore, the structural diversity and functional profiles of microbes were more prominent in the summer than winter soil samples.

Keywords: Functional profiles, soil richness, genomic materials, agricultural metabolisms, cowpea rhizosphere soil and exudate constituents.

Introduction

Cowpea is usually an annual herbaceous plant that exists in different forms of growth. The growth of cowpea is commonly sprawling, climbing and vertical, always unspecified when the conditions are favourable. It is characterised with a tough taproot which has horizontal roots spread in the soil surface. The cowpea plant is normally associated with nodulating bacterial species (*Bradyrhizobium* spp.), which makes it a suitable crop for cultivation during summer and in marginal regions of Southern Africa. It also grows well in areas with higher rainfall and cooler temperatures.

Nonetheless, cowpea thrives best in the summer season and does not tolerate much of soils that are cold (Gerrano *et al*., 2015). The presence of microbes in facilitating the growth and development of crops such as cowpea is very critical and the microbial abundance is decided via a number of factors such as plant species, abiotic and biotic environmental factors and the nature of plant root chemical constituents.

The plants roots frequently interact with the soil environment and the rhizosphere, where microbial compositions and activities are significantly enhanced by the favourable conditions provided by the roots for microbial growth and reproduction (Zhang *et al*., 2013, Zou *et al*., 2013).

Plant roots release chemicals into the soil environment and are commonly referred to as exudates. The chemical compounds that are typically found in root exudates alter the physiochemical properties of the soil, which in turn affects the populations of soil microorganisms in the vicinity of the root surface (Dakora and Phillips, 2013).

Furthermore, soil microbial communities play crucial roles in enhancing the health of plants, soil richness, carbon and other nutrients cycling. Various genera of bacteria, fungi and archaea are important soil compositions. They contribute actively in different biotic roles in the soil ecological niche in order to ensure a dynamic nutrients decompositions for crop growth, development and yield (Grzywacz *et al*., 2014). These soil microbes are responsible for stimulating plant development and growth, this is done via metabolization and mobilization of soil nutrients. This leads to production of several regulators that promote plant development and yield, protect plant from plant diseases and infections, enhance soil texture and structure, and biologically remediate the soil by removing the heavy metals that are toxic to microflora and fauna in the soil as well as degrading xenobiotic compounds such as pesticides (Ahemad, 2019; Gupta *et al*., 2015).

In order to clarify the composition and functionality of the microbe community in a sample, a number of studies that have previously used 16S rRNA sequencing technique have employed approaches based on particular or targeted functional genes (Poretsky *et al*., 2014).

However, despite that these methods are very useful; they do not give the wholesome image of the microbial community present in a sample physiologically. Because of the priority given to understanding total genomic materials present in a sample, there have been several advancements in the genomic techniques such as the latest culture independent method described as metagenomics. Because it uses high-throughput sequencing, which allows researchers to identify and characterize the whole community of microbes both compositionally and functionally, this technology has proven to be quite beneficial (Thomas *et al*., 2012, Ventosa *et al*., 2015). The microbial community composition and functional activities engaged in several agriculturally relevant

metabolisms found in the cowpea rhizosphere soil (taken from two different seasons), and in the bulk soil were investigated in this work using a metagenomics approach based on high-throughput sequencing.

Material and Method

Site and Collection of Samples

The site for soil sampling was at Iresa-pupa, Surulere local government, Ogbomosho, Oyo State Nigeria. The collections of the soil samples were done at two different seasons, which were at both raining (winter) and dry (summer) seasons. The cowpea roots were gently shaken to get rid of soil that is not firmly attached to roots and the remaining adhered soil to the root was used as rhizospheric soil. The control which was the soil outside the cowpea field and was regarded as the bulk soil. The soil samples collected were named WS, SS and BS as winter sample, summer sample and bulk soil respectively. A 2-mm sized sieving mesh was used to sieve the soil collected. The soil sieved was thoroughly mixed in a big container in order to ensure a properly mixed replicate soil sample. Approximately 2 kg of sieved soil was collected and transported from the field to the Microbial Biotechnology Laboratory, Department of Science Laboratory Technology, Federal polytechnic Ayede, Oyo state, on ice at 4 °C, then stored at – 80 °C for genomic DNA extraction.

Physicochemical Analysis, Genomic DNA Extraction and Metagenomics Sequencing of Soil Samples

Soil physicochemical properties including organic carbon, total carbon, organic matter, ammonium, potassium, calcium, total nitrogen and phosphorus were determined according to the method of (Enagbonma and Babalola, 2023). The metagenomics DNA was extracted in triplicate from 0.25 g of the composite sample using the Power Soil DNA Isolation Kit (USA, MOBIO, CA, USA) following the manufacturer's instructions. The DNA extracts were pooled, and pair-end libraries (2 × 150 bp paired-end chemistry) were prepared with the Nextera DNA Flex Library Preparation Kit (Illumina) prior to high-throughput sequencing on the NovaSeq 6000 platform.

Bioinformatics Analysis of the Sequenced Reads

The MG-RAST (version 3.3.6) server was used for automated annotation and analysis of the metagenome sequence raw data of rhizospheric soil samples from bulk soil and cowpea.

The data was subjected to QC (Quality Control), this included filtering of the quality of the sequence by removing the read of sequence that contain nucleotide that are ambiguous, filtering of the read sequence length (mean sequence reads length that are longer than standard deviation of 2.0 were removed) and de-replication (identifying redundant or highly similar genomes and retaining only the best-quality representative from each group). The hierarchical classification option was utilized as the data type of SEED Subsystems (Calhoun *et al*., 2018), as the annotation source for the taxonomic and functional annotation of metagenome data, with parameters of identity cut-off – 60%, maximum e-value of 1e-5, and minimum alignment length of 60, measured in bp for amino acids.

Comparative Analysis of the Metagenomics Reads among the Three Samples

Sequence read data from raining, dry, and bulk soil samples were compared using alpha diversity for comparative metagenome analysis. This was done by calculating the Shannon-Wiener and Simpson's diversity indices using the MG-RAST's "diversity" function, evenness, and Chao (Kieft and Simmons, 2015).

Determination by percentages of the relative abundance of microbial diversity was done. The structural community of the

microbes of both hierarchical class and family in the three soil samples was pictured by PCA (i.e principal component analyses) via Bray Curtis dissimilarity matrices through the *Canoco 5* software for their statistical representation in the three soil samples (Guo et al., 2013). Furthermore, the most significant factor i.e the physicochemical parameters shaping the compositions and structures of the microbial communities in the soil samples was also determined on a canonical correspondence analysis (CCA) *Canoco 5* software (Guo *et al*., 2013).

Functional Analysis of Metagenomics Reads

Metagenomics functions in terms of the agricultural metabolisms (nitrogen, phosphorus, potassium, sulphur and iron acquisitions) present in the three soil samples were analysed compositionally. To identify the potential for each metabolism in each soil sample, the presence of various genes, processes, transporters and enzymes linked to each of these metabolisms were determined using the sequence counts. The online tool Morpheus, available at https://software.OR broadinstitute.org/morpheus/, was used to create the heatmap that shows the compositions, distributions, and richness of each metabolism per soil sample as well as the genes, transporters, enzymes, and processes connected to each metabolism in the soil samples (Xia *et al.,* 2019).

Results

Physical and Chemical Characteristics of the Soil Samples

The soils analysis indicated that both WS and BS were neutral in nature (pH 7.62 and 7.67 ± 0.01 respectively) while SS was slightly basic (pH 8.03 ± 0.01). Whereas, the organic carbon (Org C) was 0.61%, 1% and 0.56%; organic matter (OM) was 0.69%, 1.14% and 0.72%; ammoniacal nitrogen (N-$NH4^{+}$) was 11.74, 10.20 and 13.30; total carbon was 0.707, 1.390 and 0.603 mg/kg; total nitrogen was 0.081, 0.096 and 0.062 mg/kg; phosphorus was 112.38, 21.88 and 83.31 mg/kg; calcium was 796, 3460 and 703 mg/kg and potassium was 547, 401 and 211 mg/kg in WS, SS and BS respectively.

Taxonomic Comparisons of species composition among the Three Soil Samples

Only a small number of the classified sequences were found to have a relative abundance >1%, belonging to the classes *Sphingobacteria*, *Actinobacteria*, *Cytophagia*, *Alphaproteobacteria*, *Flavobacteria*, *Betaproteobacteria*, *Bacilli*, *Gammaproteobacteria*, *Planctomycetacia*, *Bacteroidia*, *Solibacteres*, and *Verrucomicrobiae*. The average relative abundances at the family hierarchy are shown (Figure 1).

Each family of microorganisms has a different makeup and level of abundance. As illustrated in Fig. 1, the relative abundance of microbial compositions at the family level for *Sphingobacteriaceae*, *Cytophagaceae*, unclassified (derived from *Sphingobacteriales*), *Flavobacteriaceae*, and *Cyclobacteriaceae* present in the WS were 15%, 8%, 5%, 5%, and 4%, respectively. Both of these microbial levels of family had significant compositions.

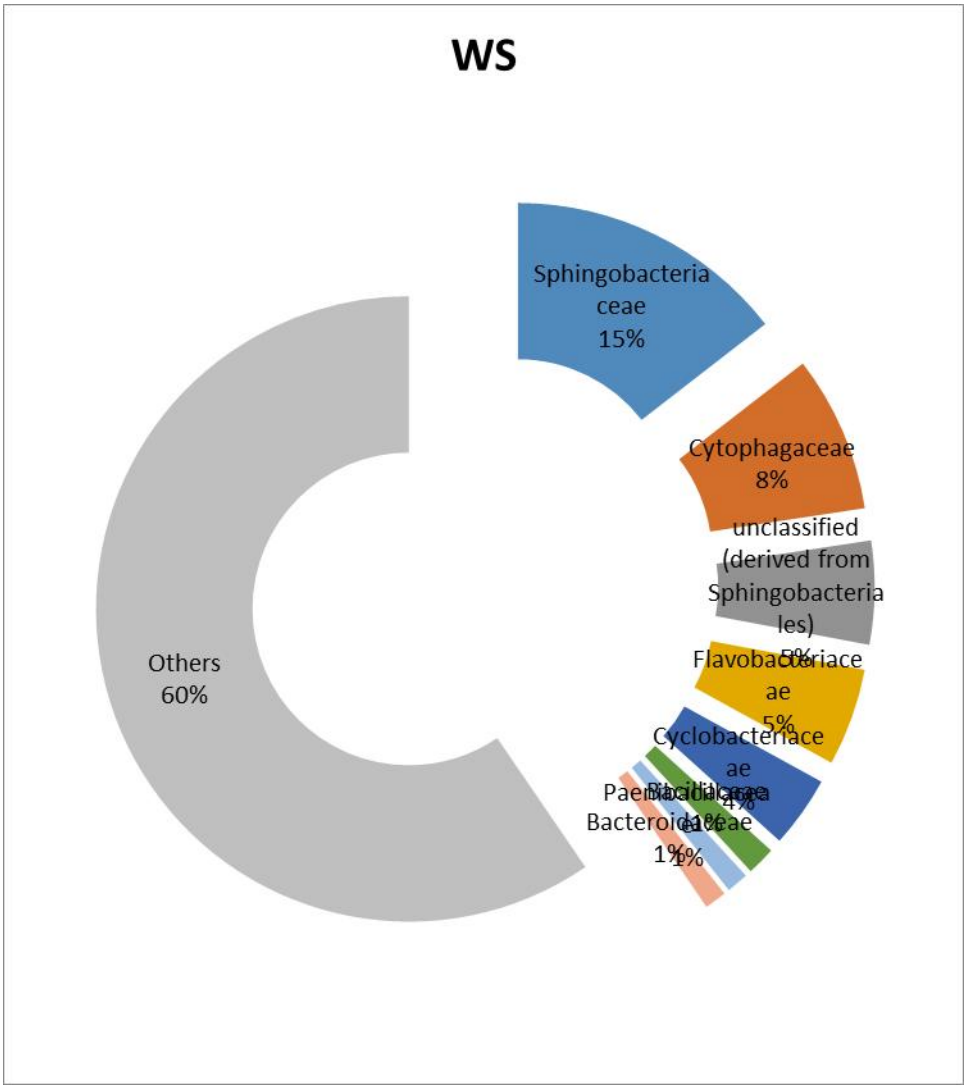


Figure 1: Relative abundance of the winter sample (WS) at family level.

In the soil sample collected during the summer season (SS), *Planctomycetaceae* (6%), *Conexibacteriaceae* (4%) and *Streptomycetaceae* (4%), were the microbial family with the high relative abundances. While others with substantial abundances were in the family of *Micrococcaceae* (3%), *Nocardioidaceae* (3%), *Bradyrhizobiaceae* (3%), *Mycobacteriaceae* (2%), *Solibacteraceae* (2%) and *Gemmatimonadaceae* (2%) as indicated in Figure 2.

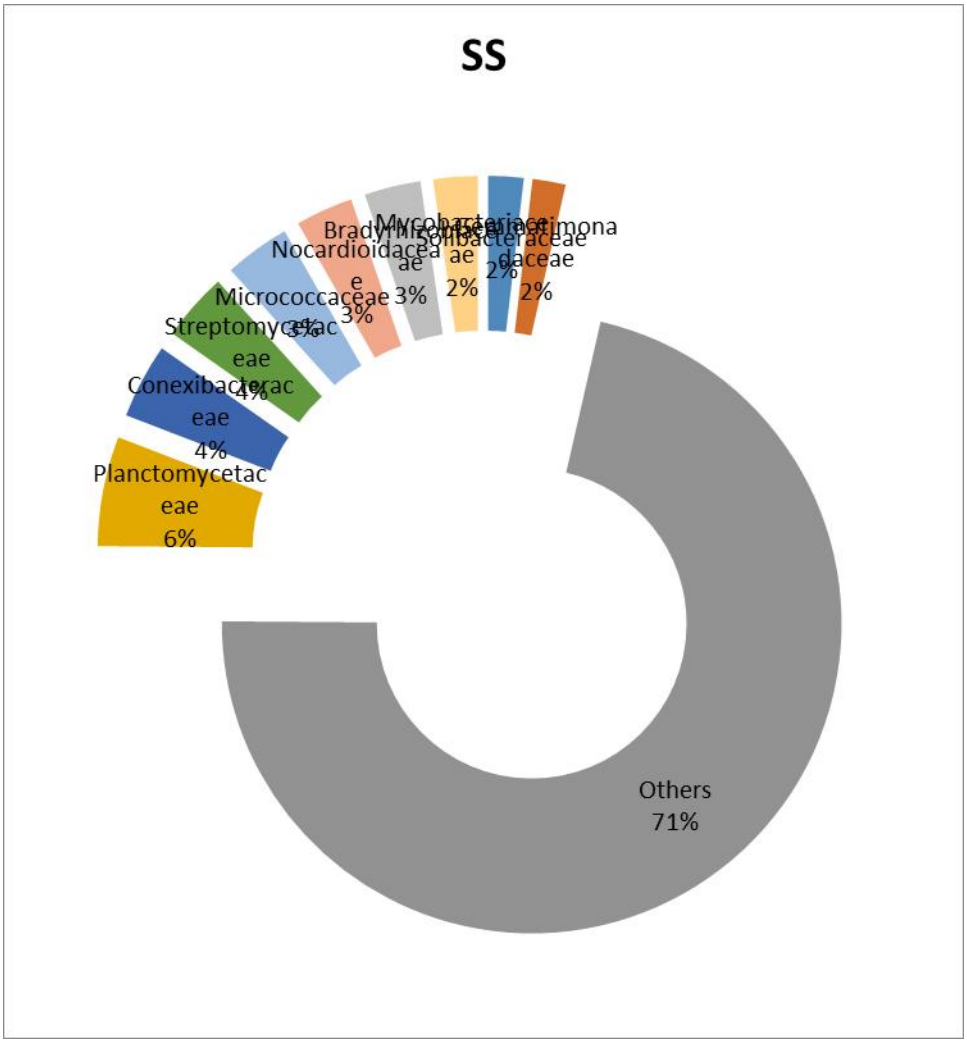


Figure 2: Relative abundance of the summer sample (SS) at family level.

In the family delineation of the soil samples collected from the bulk soil (BS), the predominant families were: Conexibacteraceae (6%), *Planctomycetaceae* (4%), *Streptomycetaceae* (4%) and *Nocardioidaceae* (4%) (Figure 3). While other families with tangible relative abundances were *Rubrobactereraceae* (3%), *Micrococcaceae* (3%), *Geodermatophilaceae* (3%), *Bradyrhizobiaceae* (2%) and *Mycobacteriaceae* (2%).

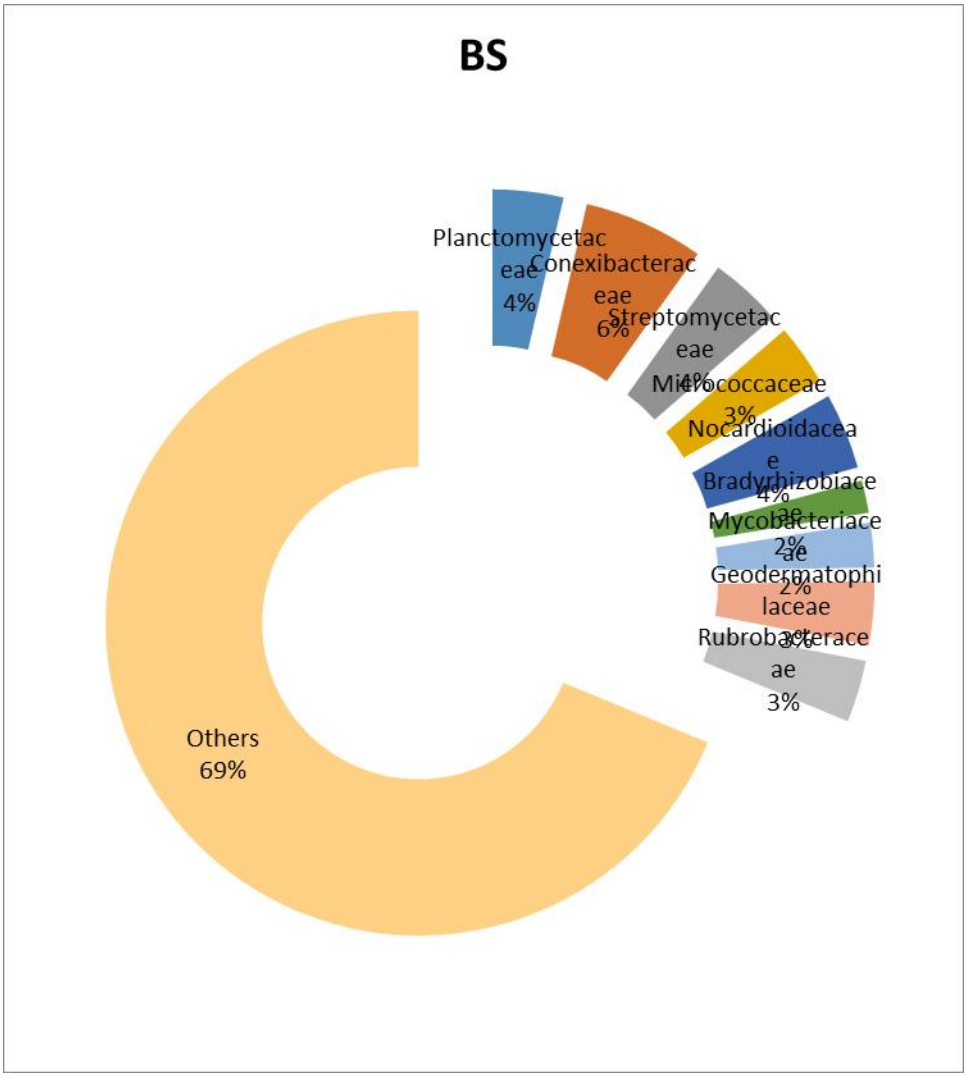


Figure 3: Relative abundance of the bulk sample (BS) at family level.

The taxonomic results obtained from the MG-RAST were analysed using *Canoco 5 software* in order to have the understanding of the microbial structures in all the soil samples (WS, SS and BS) collected.

The structural diversity of the microbes at family level was exhibited via principal component analysis (PCA). The result presented in Figure 4 indicated the significance in the distribution of microbes throughout the three soil samples especially between WS and SS.

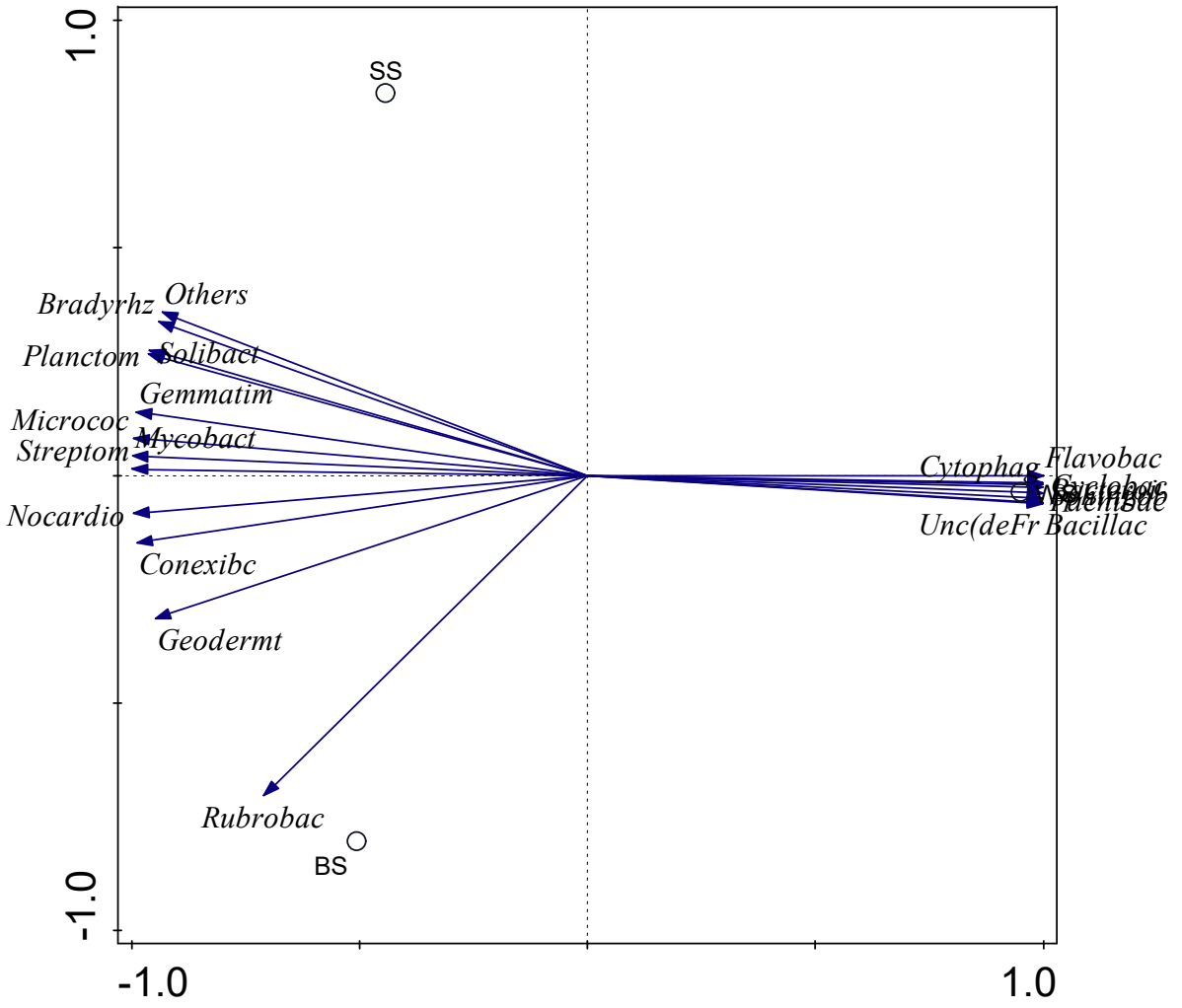


Figure 4: Principal Component Analysis (PCA) of microbial community structures in the WS, SS and BS at the family level.

It was shown by PCA how different microbes at family level were dispersed throughout WS, SS and BS. The results indicated that the following family of microbes were highly concentrated in the winter sample (WS): *Cytophagaceae*, *Paenibacilliceae*, *Bacilliceae*, *Flavobacteriaceae*, *Sphingobacteriaceae* and *Cyclobacteriaceae*. Whereas the following family of microbes were found distributed throughout both SS and BS: *Bradyrhizobiaceae*, *Planctomycetaceae*, *Solibacteraceae*, *Gemmatimonadaceae*, *Micrococcaceae*, *Mycobacteriaceae*, *Streptomycetaceae*, *Nocardioidaceae*, *Conexibacteraceae*, *Geodermatophilaceae* and *Rubrobactereraceae* (Figure 4).

Also, (Figure 5) unveiled the influences of the physicochemical parameters on the microbial-community structures at the family level. The effects of physicochemical parameters on the structures of microbes were revealed through visualization of canonical correspondence analysis (Figure 5)

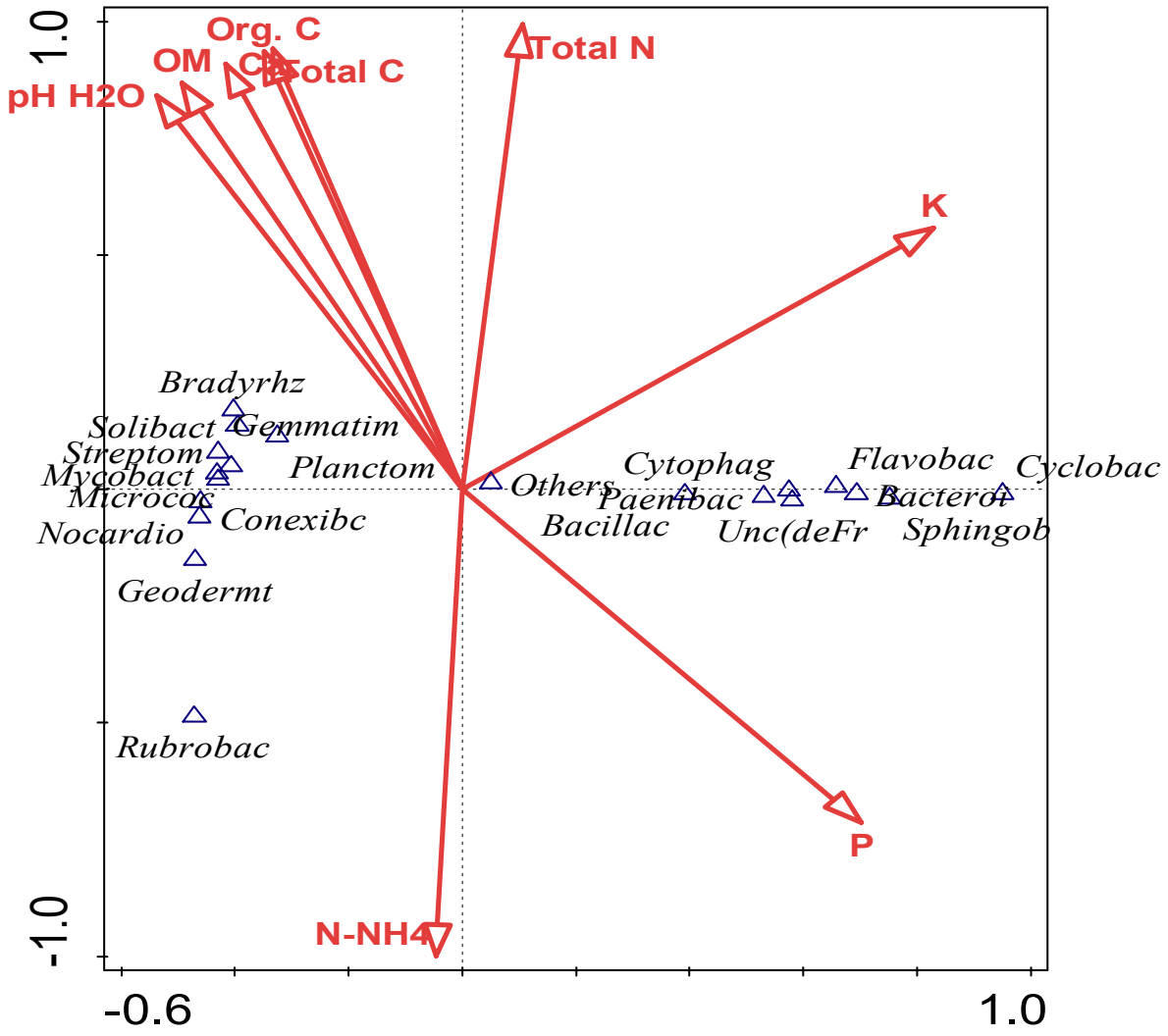


Figure 5: Canonical correspondence analysis (CCA) illustrating the physicochemicals' effects on the family-level microbial-community structures in the soil samples.

The results from CCA indicated how the richness and distributions of the parameters had a substantial impact on the microbial structures at the family level. From (Figure 5), the distribution of OM, organic C, Ca, total C, total N, K, P and N-NH_4 in WS and its richness in P and K significantly influenced the diversity of *Flavobacteriaceae*, *Bacteroidaceae*, *Bacilliceae*, *Cytophagaceae*, unclassified (derived from *Sphingobacteriales)*, *Cyclobacteriaceae*, and *Paenibacciliceae*, in it. Also, the microbial diversity of both SS and BS were influenced by the distributions of OM, organic C, Ca, total C, total N, K, P and N-NH_4 and these favoured the presence of *Bradyrhizobiaceae, Planctomycetaceae,* Conexibacteriaceae, Streptomycetaceae, *Solibacterceae*, *Mycobacteriaceae, Gemmatimonadaceae*, *Micrococcaceae*, *Nocardioidacae*, *Geodermatophilaceae* and *Rubrobactereraceae* in both samples. However, WS richness in OM, organic C, Ca, total C and total N significantly influenced *Bradyrhizobiaceae, Planctomycetaceae, Streptomycetaceae, Solibacterceae*, *Mycobacteriaceae, Gemmatimonadaceae* and *Micrococcaceae* while BS richness in N-NH_4 significantly influenced *Rubrobactereraceae*, *Nocardioidacae*, *Geodermatophilaceae* and *Conexibacteriaceae*.

Functional Profiles of the Three Soil Samples (WS, SS and BS)

Looking at the various metabolisms at the level of SEED subsystem, amino acids and derivatives and carbohydrates were dominant in both SS and BS than WS, but carbohydrates was more dominant in BS than SS (Figure 6). While SS was marginally greater than BS, WS had higher levels of dominance for cell division, cell cycle, and cell wall and capsule than both SS and BS. Both SS and BS were shown to have higher levels of clustering-based subsystems and cofactors, vitamins, prosthetic groups, and pigments than WS.

DNA metabolism and dormancy and sporulation were both observed higher in WS than SS and BS, however, the former was higher in SS than BS while the latter was slightly higher in BS than SS. Fatty acids, lipids and isoprenoids was clearly dominant in SS than WS but slightly more abundant than BS. Both SS and BS were found to have more dominant membrane transport, metabolism of aromatic compounds, motility and chemotaxis, nucleosides and nucleotides, and stress response compared to WS; however, SS had slightly higher levels of membrane transport, motility and chemotaxis, and nucleosides and nucleotides than BS. The observations from (Figure 6) showed that phages, prophages, transposable elements, plasmids, protein and RNA metabolisms, respiration and virulence, disease and defense were all more dominant in WS compared to the other two soil samples (SS and BS), but SS had more pronounced virulence, disease, and defense as well as protein and RNA metabolisms and respiration was slightly higher in SS than BS. Secondary metabolism was considerably higher in BS than both SS and WS but higher in WS than SS.

Table 1: Biological processes, protein products and genetic transporters involved in agricultural metabolisms at level 3

Metabolisms	Biological processes, protein products and genetic transporters
Nitrogen	Allantoin Utilization, Amidase clustered with urea and nitrile hydratase functions, Ammonia assimilation, Cyanate hydrolysis, Denitrification, Nitrate and nitrite ammonification, Nitric oxide synthase, Nitrogen fixation and Nitrosative stress
Phosphorus	Alkyl phosphonate utilization, High affinity phosphate transporter and control of PHO regulon, P uptake (cyanobacteria), Phosphate metabolism, Phosphoenolpyruvate phosphomutase and Phosphonate metabolism
Sulfur	Alkanesulfonate assimilation, Alkanesulfonates Utilization, Galactosylceramide and Sulfatide metabolism, Inorganic Sulfur Assimilation, Sulfur oxidation, Taurine Utilization, Thioredoxin-disulfide reductase and Utilization of glutathione as a sulphur source
Potassium	Glutathione-regulated potassium-efflux system and associated functions, Hyperosmotic potassium uptake and Potassium homeostasis
Iron acquisition	ABC-type iron transport system, ABC transporter [iron.B12.siderophore.hemin], *Campylobacter* Iron Metabolism, Encapsulating protein for DyP-type peroxidase and ferritin-like protein oligomers, Ferrous iron transporter EfeUOB, low-pH-induced, Heme, hemin uptake and utilization systems in Gram Negatives, Heme, hemin uptake and utilization systems in Gram Positives, Hemin transport system, Iron(III) dicitrate transport system Fec, Iron Scavenging cluster in *Thermus*, Iron acquisition in *Streptococcus*, Iron acquisition in *Vibrio*, Iron siderophore sensor & receptor system, Siderophore Pyoverdine, Siderophore Yersiniabactin Biosynthesis, Siderophore assembly kit and Transport of Iron

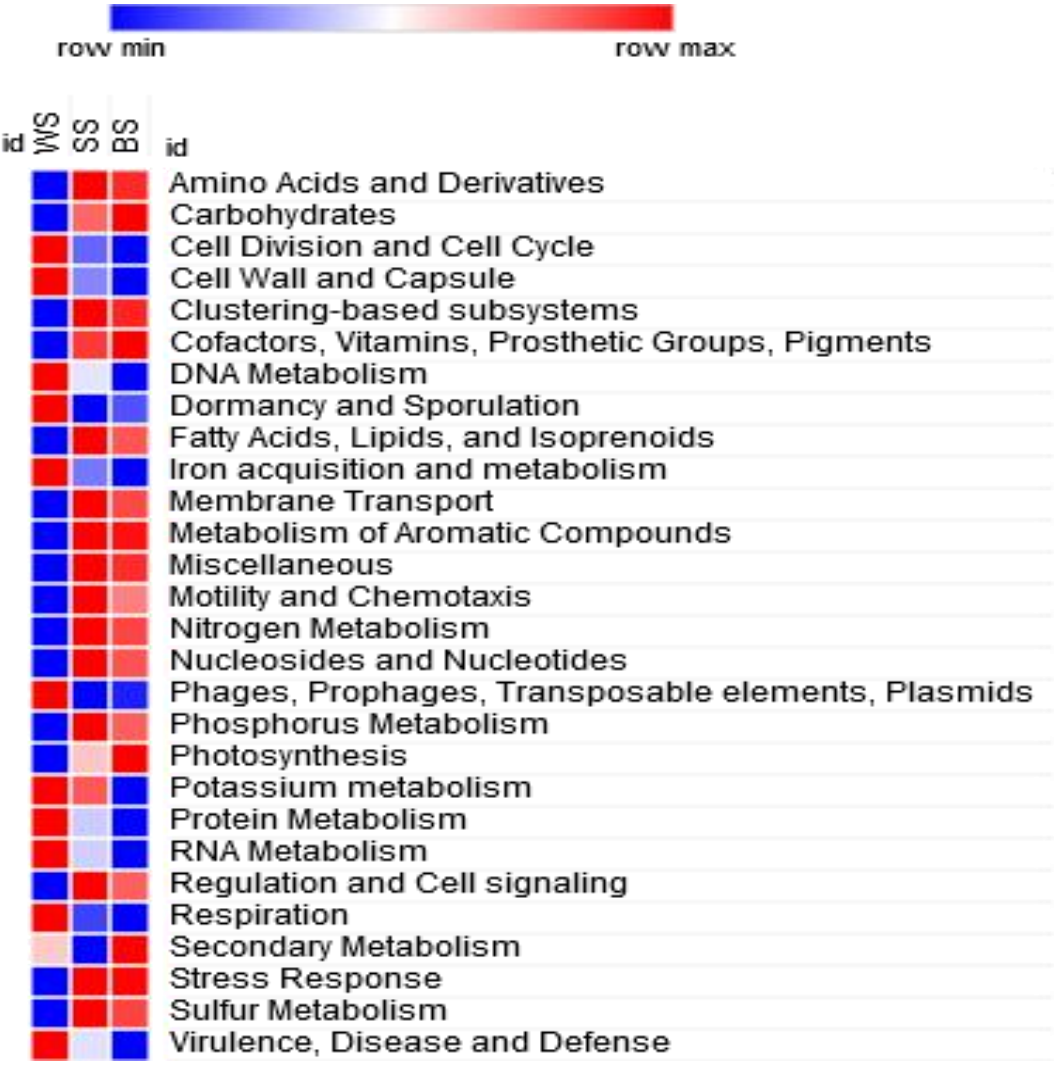


Figure 6: Comparison of the functional metabolisms for the microbial metagenomes of the three soil samples (WS, SS and BS).

Additionally, a number of protein products, biological processes, and genetic transporters—including alkyl phosphonate utilization, high affinity phosphate transporter and control of PHO regulon, P uptake (*cyanobacteria*), phosphate metabolism, phosphoenolpyruvate phosphomutase, and phosphonate metabolism—are involved in the metabolism of phosphorus in the rhizosphere soil of cowpeas that were collected. In the soil samples analyzed, various proteins, biological processes, and genetic transporters were associated with iron metabolism. These include the ABC-type iron transport system, ABC transporters [iron, B12, siderophore, hemin], *Campylobacter* iron metabolism pathways, encapsulating proteins for DyP-type peroxidase and ferritin-like oligomers, the low-pH-induced ferrous iron transporter EfeUOB, as well as heme and hemin uptake and utilization systems in both Gram-negative and Gram-positive bacteria. Other identified mechanisms were the hemin transport system, the Fe (III) dicitrate transport system, the iron-scavenging cluster in Thermus, and iron acquisition pathways in *Streptococcus* and *Vibrio*. Additionally, siderophore-related systems such as the iron siderophore sensor and receptor system, pyoverdine, yersiniabactin biosynthesis, siderophore assembly kit, and overall iron transport

processes were also linked to iron acquisition. Additionally, Table 1 highlights several protein products, biological processes, and genetic transporters associated with sulfur metabolism. These include alkanesulfonate assimilation and utilization, galactosylceramide and sulfatide metabolism, inorganic sulfur assimilation, sulfur oxidation, taurine utilization, thioredoxin-disulfide reductase activity, and the use of glutathione as a sulfur source. Similarly, the protein products, biological processes, and genetic transporters linked to potassium metabolism included the glutathione-regulated potassium-efflux system and related functions, hyperosmotic potassium uptake, and potassium homeostasis. Auxin biosynthesis and degradation, which are connected to secondary metabolism, were among the other essential protein products, biological processes, and genetic transporters related to agricultural advancements discovered in the soil samples.

Discussion

The result from this research indicated the importance of soil exudates in terms of physicochemicals present in the soil and their effects on the composition and structures of native microbes. These physicochemical elements were at different levels of richness and distributions in the soil samples collected and hence, this has great influence on the microbial communities (Figure 5). There are different combined factors such as structure of the soil, environmental and availability of soil nutrients greatly influencing the native communities of microbes and their activities and these are potentially soil health determinants (Salam and Obayori, 2019). Nutrients such as nitrogen, sulphur, phosphorus, iron, and potassium are essential in soils, as plants require them in relatively large quantities for proper growth and development. These nutrients and others are rapidly depleting in the soil due to rigorous and poor management of agricultural practices, thus, this has prompted dependency on artificial, synthesised fertilizers from chemicals which are also affecting the soil ecosystem (Sharma *et al*., 2013, Rashid *et al*., 2016). Only sequences belonging to the *Bradyrhizobiaceae* family were detected in both the SS and BS samples in high relative abundance at the family level. This clearly suggests that the *Bradyrhizobiaceae* family was a member of the cowpea rhizobial symbionts, as reported by (Bejarano *et al*., 2014).

From this study there were other non-rhizobial family of microbes that were considerably high in the soil samples especially in WS and they are: *Planctomycetaceae*, *Conexibacteriaceae*, *Streptomycetaceae*, *Micrococcaceae*, *Mycobacteriaceae*, *Sphingobacteraceae*, *Cytophagaceae*, *Flavobacteriaceae*, *Solibacteraceae*, *Nocardioidaceae*, *Cyclobacteriaceae* and *Gemmatimonadaceaea*. Given that the range of flavonoids a legume exudes is thought to be one factor influencing host specificity, it is plausible that variations in exudation patterns contribute to the selection of distinct bacterial populations (De Meyer *et al.*, 2015). **From this study we observed that** soil parameters were a significant factor that influenced the microbial compositions and structure of cowpea rhizosphere collected. Overall, certain microbial groups that appeared in low relative abundance in soil sample WS were found to be significantly higher in soil sample SS, and vice versa. Previous studies have shown that the physicochemical richness of soil plays a major role in shaping the composition of endophytic bacterial communities associated with plant roots (Bulgarelli *et al*., 2015; Lebeis, 2014).

Furthermore, a broad analysis of the functional diversity of the three soil metagenomes revealed that core metabolic processes related to nitrogen, potassium,

phosphorus, sulphur, and iron acquisition were well represented. This was evidenced by the annotated sequences of protein products, biological processes, and transport systems associated with these key agricultural metabolisms (Table 1). The most important nutrient for plant growth and productivity is nitrogen (N_2). Even though the atmosphere contains roughly 78% Nitrogen, developing plants cannot use it. By employing a variety of protein products, biological processes, and genetic transporters, nitrogen fixing bacteria transform nitrogen into ammonia, converting atmospheric nitrogen (N_2) into forms that plants can use (Stefan *et al*., 2014). Metagenomic analysis of WS, SS, and BS revealed the presence of pathways such as ammonia assimilation, denitrification, nitrate and nitrite ammonification, nitrogen fixation, allantoin utilization, and cyanate hydrolysis. These processes constitute major components of nitrogen metabolism and are known to depend heavily on the diverse assemblages of microorganisms present in the environment (Costa *et al*., 2015). According to Fox *et al*. (2014), sulphur is a crucial component of the sulphur-containing amino acids cysteine and methionine and is also necessary for the creation of the CoA enzyme and vitamins.

From our results, several complexes of protein products, genetic transporters and biological processes were annotated for sulphur metabolism namely: sulphur oxidation, alkanesulfonate assimilation, inorganic sulphur assimilation, L-Cystine uptake and metabolism, Galactosylceramide and Sulfatide metabolism and Thioredoxin-disulfide reductase. This implies that oxidation of sulphur in the sulphur metabolism is very important. This is in conjunction with the work of Yousuf *et al*. (2014), which confirmed that, microbial sulphur oxidation is a dynamic process occupying an important place in the biogeochemical cycle of sulphur, and that majority of sulphur in soil is bound to organic molecules and is not readily available to plants unless saprobes present in the soil degrade such organic molecules (Gahan and Schmalenberger, 2014). Phosphorus (P), the second most important nutrient limiting plant growth after nitrogen, is abundantly present in soils in both organic and inorganic forms (Khan *et al*., 2014). According to this study, phosphorus metabolism is frequently associated with alkylphosphonate usage, high affinity phosphate transporter, modulation of Phosphate Regulon (Pho), phosphate and phosphonate metabolisms, and phosphoenol pyruvate phosphomutase. This means that metabolising phosphate and phosphonate is very critical in phosphorus metabolism. There have been confirmations that the insoluble P is present as an inorganic mineral such as apatite or as one of several organic forms including inositol phosphate (soil phytate), phosphomonesters, and phosphotriesters in the soil. These phosphate and phosphonate are solubilised to release phosphorus into the soil for plant via several of these protein products, biological processes and genetic transporters (Glick, 2012, Olanrewaju *et al*., 2017).

Iron is an essential nutrient for nearly all life forms, as virtually all microorganisms—except for certain species of *Lactobacillus*—require it for survival and growth (Saha *et al*., 2016). Also, we identified some protein products, transport system and biological processes which are linked to iron acquisition; ABC-type iron transport system, Heme, hemin uptake and utilization systems in gram negatives, Heme, hemin uptake and utilization systems in gram positives, Hemin transport system, Iron (III) dicitrate transport system, Iron siderophore sensor, receptor system, siderophore pyoverdine, siderophore assembly kit and transport of iron. In the aerobic environment, iron occurs principally as Fe^{3+} and is likely to

form insoluble hydroxides and oxyhydroxides, thus making it generally inaccessible to both plants and microorganisms (Jing *et al*., 2014). Therefore, siderophores—low-molecular-mass iron chelators with high association constants for complexing iron, is secreted by bacteria in order to get iron. Iron (Fe^{3+}) in the Fe^{3+} siderophore complex on the bacterial membrane of both gram-positive and gram-negative rhizobacteria is reduced to Fe^{2+}, which is then released from the siderophore via a gating mechanism connecting the inner and outer membranes. It is possible for the siderophore to be recycled or destroyed during this reduction process (Saha *et al*., 2016).

Thus, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation (Fahad, 2012). In addition, it further means that the activities of inducing systemic resistance against plant pathogens are part of the metabolic activities in the soil of WS, SS and BS. Potassium (K) is the third major essential macronutrient for plant growth. In the metabolism of potassium from our result, Glutathione-regulated potassium-efflux system and associated functions, Hyperosmotic potassium uptake and potassium homeostasis were the transport systems, protein products and biological processes involved. This implies that, metabolic activities of balancing K, making K available for and it uptake by plant are all processes going on in the soil samples analysed (Verma *et al*., 2015).
Interestingly, from Table 1, we observed several sequences annotated which are associated with aromatic compounds metabolism that revealed protein products, transport system and biological processes (anaerobic benzoate metabolism, benzoate catabolism, benzoate transport and degradation cluster and chlorobenzoate degradation) linked to the metabolism of aromatic compounds. It has been shown that pathways for degrading aromatic compounds are the main and crucial source of exchange of factor metabolism for microbes that can utilise various aromatics as the major source of carbon and energy (Díaz *et al*., 2013). Benzoate is the chemical active ingredient in several pesticides which are not commonly degradable in the environments where they are applied, however microbes can metabolise it and use it as source of carbon and energy (Meneghine *et al*., 2017). Furthermore, the results of compositional, structural and functional profiles were more prominent in the soil sample collected during summer than winter season. Ultimately, these results is in correlation with (Gerrano *et al*., 2015) which states that cowpeas are much less tolerant of cold soils but grow best during summer.

Conclusion

In conclusion, the metagenome sequence analysis-based approach proved to be helpful in identifying microbial diversity and its functional attributes to the cowpea rhizosphere soil samples collected during winter (WS), summer (SS) and as bulk soil (BS). In this study, we analyzed the cowpea rhizosphere bacterial communities collected across different seasons, along with bulk soil samples. Overall, the abundance and diversity of microbial communities were higher in the rhizosphere soil of SS compared to WS, a pattern closely linked to seasonal variation. Interestingly, certain plant growth-promoting microorganisms appeared more abundant in the rhizosphere soil of WS than in SS, suggesting that seasonal differences and variations in soil physicochemical properties play a key role in shaping the bacterial community structure between the two soil types. For microbial function annotation, a number of metabolic activities were observed in the rhizosphere soil of the samples with more concentration on the most important metabolisms which were the nitrogen,

phosphorus, potassium, sulphur and iron acquisition. Furthermore, a lot of genes, transport systems, enzymes and processes were linked to these metabolisms. Generally, the structural and functional profiles of the native microbes were more profound in the cowpea rhizosphere soil sample collected during (SS) than the winter sample (WS).

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