

Metagenomics Profiling of *Vigna Unguiculata* (L.) Walp. Rhizosphere Microbiome Under Hydrocarbon Stress in a Microcosm

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Research Article

Keywords: contamination, cowpea, diversity, hydrocarbon, metagenomics

Posted Date: September 9th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7427109/v1>

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Additional Declarations: No competing interests reported.

Abstract

Hydrocarbon compounds are among the dominant soil contaminants and abiotic stressors affecting plant growth and soil communities. Recently, however, plant-microbe interaction has been exploited to alleviate the abiotic stress and remove the contaminants from soil, which offers a cheaper, safer, and eco-friendly alternative to currently available methods. In this study, microbial community profiling of the rhizosphere of *Vigna unguiculata* (L.) Walp. was conducted using a culture-independent approach with a view to unraveling plant-induced community changes. Soil samples were collected from rhizosphere and non-rhizosphere hydrocarbon-contaminated soils, from which 16S rDNA and 18S ITS were extracted, purified, and sequenced using Illumina Miseq sequencing. A total of 1191 and 1373 16S rDNA and 341 and 484 ITS operational taxonomic units (OTU) with a 97% similarity cutoff obtained from non-rhizosphere and rhizosphere soil were analyzed, respectively. Cowpea sustained richer and more diverse bacterial species, with 335 more variant species than the bulk soil, which had only 153 dissimilar species. Fewer but more diverse fungal species were detected in the non-rhizosphere, with only 94 OTUs out of 578 OTUs found in both the soils. Eight bacterial phyla, including Proteobacteria, Acidobacteriota, Actinobacteriota, Cyanobacteria, Patescibacteria, Chloroflexi, Firmicutes, and Bdellovibrionota, were predominant in both the soil samples. Cowpea rhizosphere was colonized by more Ascomycota in contrast to the non-rhizosphere, where Basidiomycota and other classified and unclassified fungal species were in abundance. Members of the genera *Sphingomonas* (~ 20%), species in Saccharimonadales, Curvularia, and *Paraconiothyrium* were the dominant species in the rhizosphere. In the non-rhizosphere, Acidobacteria (~ 20%), KCM-B-112 (in the Acidithiobacillaceae), *Aspergillus*, and *Lepidosphaeria* were the predominant. Beta diversity indices indicated significant differences in the community composition of the soils and a great divergence, especially between rhizosphere and non-rhizosphere bacterial species. Unnamed bacterial species, which account for 35% of the community, were more in the rhizosphere. The dissimilarity in the community membership and its structure is an indication of the special role played by the cowpea in shaping its rhizosphere. Therefore, a deeper understanding of the rhizosphere's ecological complexity will enhance bioremediation outcomes and also inform sustainable agricultural practices, especially in contaminated soil.

Introduction

The world's most precious ecosystem is soil. It is one of the most diversified environments on earth and the most complicated ecosystem in nature since it is home to a wide variety of different organisms that interact and contribute to the biogeochemical cycles that support life. There is no other place in nature where species are as closely clustered together as in soil communities, but this richness is seldom understood since it is underground and entirely hidden from human sight (Food and Agriculture Organization; FAO, 2015). Small invertebrates, viruses, actinomycetes, bacteria, and fungi can all be found embedded within soil particles. In areas with ideal soil conditions, plant roots and fungal mycelia also grow well. In addition to providing support for higher plants and animals, soil also produces food and biomass, filters and transfers water, nutrients, and pollutants, and stores carbon (Ragnarsdóttir and Banwart, 2015). In addition to providing for fundamental human needs like food, clean water, and air, soils are an important source of biodiversity (Keesstra *et al.*, 2016). Agricultural production, sustainability, and environmental protection are significantly influenced by the state and quality of soils and, as a consequence of both, has bearing on plant, animal and human health (FAO, 2015).

A major challenge confronting soil is degradation. It is a natural or human-induced process that reduces the soil's functional capacity. According to the Europeans' "Toward Thematic Strategy for Soil Protection," one of the critical cause of soil degradation is contamination (Vacha, 2017). Soil contamination is a worldwide problem as many contaminants have been detected even in the areas thought to be free of contaminants. Interestingly, soil is an environment with "memory" since contaminants can accumulate and be discovered in the soil over time due to persistence and resistance to decomposition (Azoury *et al.*, 2013). Among the major sources of soil contaminants is crude petroleum. With increased usage of petroleum hydrocarbons for energy and raw materials, several pollutants have been released into the environment; damaging soil, surface water, and groundwater (Ossai *et al.*, 2019). The ability of the soil to filter, buffer, and transform both inorganic and organic contaminants is compromised by petroleum hydrocarbon contamination (FAO,

2018). In addition, it can affect aquatic life, obstruct plant metabolism, reduce the variety and richness of soil flora and fauna, and harm plant growth (Salam and Idris, 2019).

Numerous species can be found in the soil environment, including bacteria, fungi, invertebrates, and plants, which are crucial to the cycling of nutrients and food production (Khan *et al.*, 2018a). The main forces behind organic matter mineralization in soil are microorganisms, which are also capable of converting petroleum contaminants into less harmful forms. Based on the above principle, contaminated soils are often reclaimed through a process called bioremediation (Jain *et al.*, 2022; Ossai *et al.*, 2022). Although this type of microbial activity can occur in bulk soil, *in-situ* plant growth have the potential to increase microbial contributions to bioremediation in the soil by stimulating microbial biomass in the rhizosphere (Bell *et al.*, 2014). One of the Earth's most intricate environments is the rhizosphere and plays a crucial role in plant growth and productivity, soil fertility, carbon sequestration, and the degradation of environmental pollutants (Alotaibi *et al.*, 2021). However, the success of these processes depends on the particular microorganisms, plant species and prevailing soil conditions (Ismail *et al.*, 2021).

In the past two decades, reclamation of polluted soil using plant-microbe interaction have received a special attention considering its cost-effectiveness, eco-friendliness and passiveness (Velmourougane *et al.*, 2017). Although plants and microbes may both breakdown petroleum hydrocarbons independent of one another, research indicates that the rhizosphere effect is the main mechanism underlying the degradation process (Frick *et al.*, 1999). Various activities taking place in the rhizosphere (root exudation, microbial population growth, horizontal gene transfer and desorption of contaminants) make the degradation of organic pollutants more rapid (Correa-Garcia *et al.*, 2018). In the recent past, it has been established that plant-bacterial synergism increased the rate of hydrocarbon degradation in rhizosphere compared to bulk soil and that more degradation was promoted by larger microbial population brought about by higher plant density (Hazaimah *et al.* 2019; Gagnon *et al.*, 2020; Ismail *et al.*, 2024). While using cowpea to establish a rhizosphere in a hydrocarbon contaminated soil, previous studies reported its biostimulation potentials (Ismail *et al.*, 2019), its ability to influence physicochemical and biological properties of the rhizosphere (Farouq *et al.*, 2022) and also its role in the reclamation of crude oil perturbed soil (Ismail *et al.*, 2022). Despite that, the vast diversity, composition and interrelationships of rhizosphere microbiome are not yet explored as the case is for most plant-microbe interactions so far reported (Harris *et al.*, 2020). Consequently, the present study sought to explore the cowpea rhizosphere community composition and dynamics with a view to elucidating the plant's influence on microbial diversity.

Materials and Methods

Experimental site

The experiments were carried out at the botanical garden of Usmanu Danfodiyo University Sokoto (UDUS), Nigeria (13.1246° N, 5.1994° E) in a microcosm.

Sample collection

The seeds of *V. unguiculata* were procured and then identified to be IT07K-318-33 cultivar at the UDUS herbarium. The Kaduna Refinery and Petrochemical Company (KRPC) provided the Bonny light crude oil. From the UDUS Botanical Garden, pristine soil samples were obtained.

Experimental setup

Pristine soil samples (6000g) were weighed into plastic pots (120 mm × 80 mm × 60mm) and thoroughly contaminated with Bonny light crude oil at a rate of 11340mg/Kg. The pots were grouped into two and replicated 4 times. To allow volatile chemicals to escape and for microbial adaption, the setup was left alone for two weeks. After that, viable cowpea seeds were put into one of the two sets in order to create rhizosphere soil (CR). For non-rhizosphere control, the other set

remained unplanted (CN). The treatments were irrigated every other day with equal volume of distilled water. Purposive sampling technique was used in collecting soil samples for metagenomics analysis.

DNA extraction, amplification, sequencing and Library Preparation

Using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.), microbial DNA was extracted from soil samples in accordance with the manufacturer's instructions. The PCR was conducted using the marker region of the bacteria, 338F-806R, and amplified by (95°C for 2 min, followed by 25 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and final extension for 5 min) using primers 338F 5'-barcode- ACTCCTACGGGAGGCAGCA -30 and 806R 5'-GGACTACHVGGGTWTCTA. PCR reaction was performed in triplicate 20 µL mixtures containing 4 µL of 5 × FastPfu buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu Polymerase, and 10 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using AxyPrep DNA Gel Extraction Kit (Axygen Bioscience, Union City, CA, US.) according to the manufacturer's instructions and quantified using QuantiFluor™-ST (Promega, US). Library was constructed using Linked 'Y' adapter; the adapter dimer were removed by using beads; Using PCR amplification for libraries concentration and single-stranded DNA fragments were generated using hydroxides. Sample libraries were pooled in equimolar and paired-end sequence (2 × 250/300 bp) on an Illumina MiSeq platform according to the standard protocols.

Data analysis

Raw FASTA files were demultiplexed, quality-filtered using QIIME (version 1.9.1) with the following criteria. Based on overlap relationship, paired-reads were subjected into a single read. The merged reads were used for OTU clustering, taxonomy classifying and community diversity assessment. Sequence reads were trimmed by software Trimmomatic (Bogler *et al.*, 2014); after trimming, reads were assembled by Flash software (Magoc and Salzberg, 2011) for further analysis by MOTHUR 1.33.0 (Kozich *et al.*, 2013). Unique sequences were aligned to the reference SILVA database by default settings, and chimeric sequences were removed. Sequences passing these screens were classified using a Ribosomal Database Project naïve Bayesian rRNA classifier with a confidence of 80%. At each taxonomic level, the proportion of sequence identities were calculated as a percent of all sequences classified in that sample. OTUs (operational taxonomic units) were classified at similarities of 97% after normalized to 7369 sequences per sample. The α -diversity indices, including observed operational taxonomic units (OTUs), Chao1, abundance-based coverage estimator (ACE), Simpson's and Shannon's, were calculated using MOTHUR. Bacterial community structure, based on the OTU composition was performed by principal coordinates analysis (PCA) conducted in R (Version 3.1 .2).

Statistical analysis

Statistical Analysis of Metagenomic Profiles (STAMP) software version 2, was used to assign distinct taxonomic levels for each of the metagenomes obtained (Parks et al. 2014). The relative proportion difference between the two treatments' taxonomic distributions was evaluated using a two-sided Fisher's exact test, the Newcombe-Wilson confidence interval method, and Benjamini-Hochberg FDR (for correction).

Results

Bacterial abundance and diversity

Culture-independent bacterial analysis of rhizosphere and non-rhizosphere soil was carried out with a view to understanding bacterial community structure and dynamics. A total of 1191 and 1373 operational taxonomic units (OTU) with 97% similarity cutoff obtained from 131468 and 115555 16SrDNA sequence reads of non-rhizosphere and rhizosphere soil were obtained and analyzed respectively. The bacterial community richness based Chao and ACE indices in the rhizosphere and non-rhizosphere soils was 1373 (Chao = 1385.78; ACE = 1389.40) and 1206 (Chao = 1270.55;

1272.77) different species (Table 1). The diversity indices in the non-rhizosphere and rhizosphere soils estimated using Shannon (4.83 and 5.68) and Simpson (Inv. Simpson) (28.22 and 111.32) respectively were also presented.

The bacterial community composition of the two soil samples showed that 1038 OTUs were present in both rhizosphere and non-rhizosphere soil. The rhizosphere soil contained 335 more species whereas, the non-rhizosphere had only 153 as shown in Fig. 1. Eight major bacterial phyla were identified to form the most abundant bacterial taxa in the soil. The bacterial phyla include Proteobacteria, Acidobacteriota, Actinobacteriota, Cyanobacteria, Patescibacteria, Chloroflexi, Firmicutes, and Bdellovibrionota as shown in Fig. 2. Different bacterial genera including *Sphingomonas*, *Stenotrophobacter*, *Saccharimonadales*, *Planktothrix*, *Mycobacterium*, *Microvirga*, *Paracoccus*, *Bryobacter*, *Nocardioides* and *Bacillus* formed majority of the bacterial community as shown in Fig. 2.

Table 1: Bacterial community structure estimators (Alpha diversity)

Treatment	Reads	Community abundance indices (97% level of significance)							
		OTU	Chao	Richness	Shannon	ACE	Evenness	Coverage	Simpson
Non-rhizosphere	131468	1191	1270.55	1206	4.83	1272.77	0.68	0.9990	0.035431
Rhizosphere	115555	1373	1385.78	1373	5.68	1389.40	0.79	0.9996	0.008983

Based on bacterial abundance, results presented in Fig. 3 shows the similarity and abundance of members in the bacterial community. Members of the genera *Sphingomonas* (~ 20%) and *Saccharimonadales* were the dominant species in the rhizosphere. In the non-rhizosphere, *Acidobacteria* (~ 20%) and KCM-B-112 (in the *Acidithiobacillaceae*) were the predominant. However, *Kallotenue* and *Blastocatella* were among the less prevalent species in the rhizosphere and non-rhizosphere communities respectively. Out of the more than 100 taxonomic units identified, Figure 4 shows the 30 most abundant species encountered in both of the soil samples. All the 30 species belong to the phyla Acidobacteriota, Actinobacteriota, Bdellovibrionota, Chloroflexi, Cyanobacteria, Firmicutes, Patescibacteria and Proteobacteria.

Results of the bacterial community beta diversity indices are presented in Table 2. Bray-Curtis and UniFrac (weighted and unweighted) indices as measures of species diversity or its relatedness between rhizosphere and non-rhizosphere soil were used. Bray-Curtis dissimilarity metrics was 0.628 indicating a difference between the two communities. Using the unique fraction (UniFrac) to highlight phylogenetically distant species, the weighted and unweighted values were 0.289 and 0.300 respectively which indicate a great divergence between rhizosphere and non-rhizosphere bacterial species.

Table 2: Bacterial community structure estimators (Beta diversity)

Estimate	Bray-Curtis	UniFrac	
		Weighted	Unweighted
Beta diversity	0.628	0.289	0.300

Fungal abundance and diversity

The ITS sequencing of the fungal 18S rRNA revealed the presence of 341 and 484 OTU from 87952 and 128988 reads of the non-rhizosphere and rhizosphere soil respectively (Table 3). The fungal community richness were estimated at 341 and 494 in the non-rhizosphere and rhizosphere respectively with Chao and ACE indices reaching 356 and 351.37 in non-

rhizosphere; and 504 and 500.69 in rhizosphere respectively; which pointed out richer fungal species in the rhizosphere than non-rhizosphere soil. The Shannon and Simpson diversity indices were also estimated with values of 4.28 and 4.00 (Shannon) and 0.03 and 0.05 (Simpson) in the non-rhizosphere and rhizosphere respectively; indicating more diversity in the non-rhizosphere soil.

Table 3: Fungal community structure estimators (Alpha diversity)

Sample	Reads	Community abundance indices (97% level of significance)							
		OTU	Chao	Richness	Shannon	Simpson	ACE	Evenness	Coverage
Non-rhizosphere	87952	341	356	341	4.28	0.03	351.4	0.7342	0.99
Rhizosphere	128988	484	504	494	4.00	0.05	500.7	0.6453	0.99

The results of fungal community composition showed that the rhizosphere and non-rhizosphere soils of cowpea had 247 OTU in common. Only 94 OTU were distinctly different in the non-rhizosphere soil as compared to 237 OTU in the plant rhizosphere (Fig. 5). At phylum level, the cowpea rhizosphere was colonized by more Ascomycota in contrast to the non-rhizosphere where Basidiomycota and other classified and unclassified fungal species were in abundance (Fig. 6a). In the cowpea rhizosphere, fungi of the genus *Curvularia* accounted for more than 20% of the total fungal species present followed by *Paraconiothyrium* (~ 15%) and many unclassified species (~ 18%). In the non-rhizosphere however, *Aspergillus* and *Lepidosphaeria* formed the most abundant genera making approximately 16% and 12% respectively. *Curvularia* was 10 fold more abundant in the rhizosphere than non-rhizosphere whereas the abundance of *Aspergillus* in the rhizosphere is about a quarter of the non-rhizosphere. Other prominent genera in the rhizosphere include *Fusarium*, *Alterneria*, *Humicola* and *Candida*. In the non-rhizosphere soil, some of the most abundant genera were *Fusarium*, *Colletotrichum*, *Thialavia* and *Rhexothecium*. Significant number of the fungal genera in both rhizosphere (~25%) and non-rhizosphere (~40%) soil were either unclassified or unspecified as shown in Figure 6b. Hierarchical clustering (Figure 7) of the fungal communities in rhizosphere and non-rhizosphere showed that the vast majority of species were members of Ascomycota whereas Chytridomycota, Zoopagomycota and Blastocladiomycota formed insignificant proportion especially in the cowpea rhizosphere. Figure 8 shows the overall 30 most abundant species in which the majorities are Ascomycota and ≤ 20% are unclassified. Others include *Curvularia* (12%), *Aspergillus* (11%), *Paraconiothyrium* (7%), *Lepidosphaeria* (7%) and *Fusarium* (7%). *Malassezia* (< 1%) was the only fungal genus in the phylum Basidiomycota that made up of the list of the 30 most abundant species.

Results in Table 4 showed that the fungal species in the cowpea rhizosphere differed from those in the non-rhizosphere soil as estimated using Bray-Curtis (0.675). It also showed that the most abundant species in both rhizosphere and non-rhizosphere soil were closely related (weighted unifracs = 0.851) whereas, some species observed in the rhizosphere were not present in the non-rhizosphere and *vice versa*.

Table 4: Fungal community structure estimators (beta diversity)

Estimate	Bray-Curtis	UniFrac	
		Weighted	Unweighted
Beta diversity	0.675	0.851	0.506

Discussion

Metagenomics of soil samples from contaminated cowpea rhizosphere and non-rhizosphere soil was investigated. The results showed that there were 1191 and 1373 OTU with 97% similarity cutoff obtained in the non-rhizosphere and rhizosphere soil respectively. The OTU which denotes a group of closely related sequences which are 97% similar, is used to categorize bacteria into taxonomically similar groups (Qian *et al.*, 2020). In microbial ecological studies, variation is assessed within (alpha diversity) and between (beta diversity) communities of the same or different samples respectively. In this study, alpha diversity indices including Chao1 and ACE showed that the bacterial species richness and abundance was high. The higher Chao1 and ACE values observed especially in the rhizosphere soil might be due to the cowpea growth on the soil. The bacterial richness and population were enhanced by the presence of cowpea plant as indicated by higher diversity in the rhizosphere soil. Studies by Allamin *et al.* (2020) have reported an increase in bacterial richness and abundance in petroleum oily sludge remediation using *C. cajan*. In addition, the work of Sivaram *et al.* (2020) have also demonstrated an unprecedented species richness and abundance in a study centered on characterization of bacterial communities colonizing the rhizosphere of plants with C3 and C4 photosynthetic pathways grown in soil contaminated with PAHs. Their studies strengthen the observation made in this study that presence of cowpea facilitated the existence of a rich and high bacterial population in the soil.

Similarly, the bacterial richness and evenness as estimated using Shannon and Simpson indices indicated high bacterial abundance. As Shannon index gives more weight to rare species, Simpson index puts more emphasis on common species (Qian *et al.*, 2020). Based on the values obtained, there were diverse bacterial taxa in the rhizosphere (evenness = 0.786) whereas, the non-rhizosphere have abundant bacteria with common properties in the soil sample. This agrees with the work of Kumar *et al.* (2018) who observed that soil samples obtained from the rhizosphere of plant grown in oil contaminated site showed a higher richness in the microbial flora when compared to that of unplanted samples. This is further highlighted by the presence of more OTUs in the rhizosphere than non-rhizosphere soil as shown in Fig. 1.

Investigation into the bacterial community composition shows that 8 bacterial phyla including Proteobacteria, Acidobacteriota, Actinobacteriota, Cyanobacteria, Patescibacteria, Chloroflexi, Firmicutes, and Bdellovibrionota formed more than 95% of the bacterial community. Patel *et al.* (2016) showed that perturbation of soil leads to increase in the richness of proteobacteria in the environment. Yamamoto *et al.* (2018) have also shown that proteobacteria and actinobacteria were the dominant bacterial taxa in the rhizosphere of *Glaux maritima* and *Salicornia europaea*. These findings may support the claim that Gram negative bacteria and actinomycetes predominate plant rhizosphere as observed using culture-based techniques (Kennedy and Luna, 2005). This is also supported by the work of Chaudhari *et al.* (2020) who observed similar trends in pea rhizosphere using metagenomics approach. Acidobacteriota which form a significant part of the bacterial community especially in the non-rhizosphere soil have also been reported as diverse and abundant bacterial group. Dunbar *et al.* (2002) have shown that the members of the phylum acidobacteriota are particularly abundant in soil habitats and may represent over 50% of the total bacterial community. Their presence might have been favored by the acidic nature of the soil environment (Farouq *et al.*, 2022) as majority of the species (although still uncultured) are acidophilic.

Results in this study show that presence of cowpea caused a shift in the diversity and abundance of major bacterial taxa in the community. The relative abundance of proteobacteria and acidobacteriota decreased considerably in the cowpea rhizosphere. The two bacterial phyla have been identified to be resistant to severe hydrocarbon contamination and the decrease in their relative abundance observed in the rhizosphere might have resulted to lowering the contaminants concentration due to biodegradation. Studies by Yu *et al.* (2020) have shown the predominance of proteobacteria and acidobacteriota in highly contaminated soil. Shifting of the relative abundance of these major taxa is often noted upon contamination with petroleum hydrocarbons (Khan *et al.*, 2018; Auti *et al.*, 2019). Conversely, increase in relative abundance of the remaining 6 bacterial phyla was observed in the rhizosphere. Increase in abundance of Actinobacteria might be related to accumulation of PAHs which are not readily degraded. Earlier studies by Uhlik (2012) have shown that actinobacteria are involved in the degradation of aromatic hydrocarbons in a long-term contaminated soil. Firmicutes which formed the phylum for most Gram positive bacteria did not highly vary between the rhizosphere and non-

rhizosphere (Fig. 3) and their relative abundance have been reported (Sutton *et al.* 2013; Allamin *et al.*, 2020) even though at low level (Khan *et al.*, 2018b; Kumar *et al.*, 2019). This may justify the need for culture-independent ecological studies considering the fact that Firmicutes are considered as the most abundant soil bacteria which might turned to be otherwise. The work of Li *et al.* (2020) has also reported the enrichment of Patescibacteria in the rhizosphere of ryegrass than non-rhizosphere during the cleanup of PAHs contaminated soil; a case similar to one observed in this study.

In addition, the decrease in the abundance of proteobacteria might be correlated with increase in the relative abundance of bacteria in the phylum Bdellovibrionota. The phylum Bdellovibrionota is composed of obligate predators that can consume some Gram negative bacteria inhabiting various environments (Li *et al.*, 2021). Although literature on biodegradation of hydrocarbons by members in the phylum Bdellovibrionota is still sketchy, their presence in the rhizosphere have been reported (Jiménez *et al.*, 2020; Wu *et al.* 2021). As the phylum still remain enigmatic taxon (Waite *et al.*, 2020), the role played by some members of the phylum in bioremediation is likely since recent studies have highlighted their possibility to partake in nutrient recycling and promote plant growth. Wu *et al.* (2021) have reported a positive correlation between increased soil nutrients and abundance of rhizosphere bacterial communities (including Bdellovibrionota) in soil with tea and Chinese chestnut intercropping. This, although not explicitly portrayed, albeit their predatory role, may suggest their contribution in plant growth promotion. Likely, secretion of acidic root exudates might have encourage their relative abundance, because some studies have indicate that abundance of Bdellovibrionota was enhanced by the presence of acidic substrates in both bulk and rhizosphere soil of *Hypericum* spp. (Noviana, 2021).

The occurrence of chromogenic bacterial phyla like cyanobacteria and chloroflexi was also observed more in the rhizosphere soil. Majority of member species in the phyla are autotrophic and their presence in hydrocarbon polluted environment have been reported (Khan *et al.*, 2018b; Kumar *et al.*, 2019). Dagher *et al.* (2019) have also reported Chloroflexi as one of the dominant taxon in the rhizosphere of four plants where they attributed the diversity of the rhizosphere microbiome to plant growth and their species in hydrocarbon contaminated sediments. Studies by Khan *et al.* (2018b) have also reported the presence of Cynobacteria in contaminated rhizosphere soil. The presence of these taxa in hydrocarbon contaminated soil might not be surprising considering the fact that their biodegradation potentials have since been reported (Das and Chandran, 2011).

The most abundant species identified were *Sphingomonas*, *Stenotrophobacter*, *Saccharimonadales*, *Planktothrix*, *Mycobacterium*, *Microvirga*, *Paracoccus*, *Bryobacter*, *Nocardioides* and *Bacillus*. The presence of these species is an indication of their hydrocarbon biodegradation ability. Previous studies have frequently listed species including *Sphingomonas*, *Stenotrophobacter*, *Paracoccus*, *Nocardioides* and *Bacillus* as part of bacterial communities during phytoremediation of polluted environments using metagenomics approaches. The role of *Sphingomonas* in hydrocarbon biodegradation and plant growth promotion have been established (Asaf *et al.*, 2020). *Saccharibacteria* which was also rich in the soil have been reported to form part of rhizosphere community. Although still uncultured, genomic data have shown that the species possess very small genomes and cell sizes, and the organisms are thought to live symbiotically with other microorganisms depending on co-metabolism; even though genes for degrading complex carbon compounds have been identified (Lemos *et al.*, 2019; Ruthi *et al.*, 2020).

To understand the difference in bacterial communities between rhizosphere and non-rhizosphere soil, the beta diversity indices suggest a strong divergence between the two treatments. Obviously, results have shown the enrichment of some species in the rhizosphere particularly unnamed bacterial species which accounts for 35% of the community. The dissimilarity in the community membership and its structure is an indication of special role played by cowpea in shaping its rhizosphere. Chaudhari *et al.* (2020) have made similar observation where the rhizosphere was having higher bacterial diversity than the bulk soil of *Pisum sativum*. They propose that pea plants shaped their rhizosphere microbiome, in order to meet its requirements for nutrient uptake and stress amelioration. This argument may support the findings in this study considering the similarity of the situations (i.e. plant type and abiotic stress).

It is evident from the findings that the cowpea rhizosphere had more abundant and richer fungal species compared to the non-rhizosphere. Furthermore, the alpha diversity indices also hinted out a more diverse species in the rhizosphere than non-rhizosphere. The disparity observed in the fungal abundance is linked to the ability of cowpea to secrete exudates which helped to maintain the abundance and diversity. In the non-rhizosphere soil however, hydrocarbon toxicity might have caused the decrease in the fungal abundance and diversity. It has been demonstrated that plants like *Arabidopsis thaliana* and *Medicago truncatula* are able to maintain soil fungal abundance and diversity which is mediated largely through root exudation (Shukla *et al.*, 2011). In the non-rhizosphere, absence of exudates which limits soil organic matter upon which majority of fungal species depend, the species were less diverse especially that fungi tolerate less soil disturbance than other microbial populations (Bhattarai *et al.*, 2015). In a comparative study using fabaceae and poaceae, it was observed that fungal community in the fabaceae rhizosphere was five-folds more abundant and had significantly different rhizosphere communities than poaceae (Turner *et al.*, 2013). Tardif *et al.* (2016) demonstrated that contamination level had a more pronounced effect on the fungal communities than on the bacterial communities. However, fungal consortium provides a greater spectrum of bioremoval enzyme activity, demonstrated rapid degradation of crude oil by special fungal species belonging to several genera (Al-Zaban *et al.*, 2020).

On the other hand, the fungal community structure was significantly influenced by cowpea growth in which members of Ascomycota dominated the rhizosphere. The variation observed between the two treatments is based on the fact that Ascomycota are often mycorrhizal, forming symbiotic relationships with plant roots, which are more abundant in the rhizosphere. This association allows for mutualistic benefits, such as nutrient exchange and enhanced plant growth. In return, the plant provides carbohydrates to the fungi, supporting their growth. On the other hand, the Basidiomycota are often saprotrophic, decomposing organic matter and thriving in environments with abundant organic carbon. Additionally, they may be more tolerant of harsher environmental conditions, such as reduced nutrient availability, which could be more prevalent in non-rhizosphere soil. Recent studies by Liu *et al.* (2022) reported higher occurrence of Ascomycota in rhizosphere soils which was significantly influenced by tobacco growth and caused the change in the soil microbial composition as compared to bulk soil samples.

The present study provides a comprehensive insight into the fungal community composition in the cowpea rhizosphere and non-rhizosphere soil, revealing significant differences in the abundance and distribution of various fungal genera. The dominance of *Curvularia* in the rhizosphere, accounting for over 20% of the total fungal species, is a particularly noteworthy finding. This enrichment of *Curvularia* in the rhizosphere, being 10-fold more abundant than in the non-rhizosphere, suggests a strong adaptation and potentially a beneficial interaction with the cowpea plant. *Curvularia* species are known to exhibit diverse ecological roles, including plant growth promotion, biocontrol of pathogens, and decomposition of organic matter (Kumar & Kumar, 2019; Gouda *et al.*, 2016). Some *Curvularia* species can produce plant hormones like auxins and gibberellins, facilitating root development and nutrient uptake (Khan *et al.*, 2021). Furthermore, certain *Curvularia* strains have demonstrated antagonistic activity against soilborne pathogens, potentially contributing to disease suppression in the rhizosphere (Sharma *et al.*, 2020). The ability of the fungal species to degrade and enhance hydrocarbon phytoremediation has been reported also (Ani *et al.*, 2021; Hamad *et al.*, 2021; Alshammary *et al.*, 2025).

The relatively high abundance of *Paraconiothyrium* (~15%) in the cowpea rhizosphere is also interesting. *Paraconiothyrium* species have been reported as endophytes with potential roles in plant growth promotion and stress tolerance (Khiralla *et al.*, 2020). Some *Paraconiothyrium* strains have exhibited the ability to solubilize phosphate, enhancing phosphorus availability for the plant (Osman *et al.*, 2021). These findings suggest that *Paraconiothyrium* may contribute to nutrient mobilization in the rhizosphere, supporting cowpea growth. Until recent past (Ramdass and Rampaersad, 2021), when *Paraconiothyrium* was reported as hydrocarbon degrader, its association with plants or dead plant litters (Tennakoon *et al.*, 2022; Dai *et al.*, 2023) has prevailed.

In contrast to the rhizosphere, the non-rhizosphere soil was dominated by *Aspergillus* and *Lepidosphaeria*. The higher abundance of *Aspergillus* in the non-rhizosphere aligns with its known saprophytic lifestyle and ability to thrive in diverse soil environments (Bennett, 2010). *Aspergillus* species are efficient decomposers of organic matter and play a crucial role in nutrient cycling in the soil ecosystem. The lower abundance of *Aspergillus* in the rhizosphere compared to the non-rhizosphere could be due to competition with rhizosphere-competent fungi or plant-mediated effects on soil chemistry. Studies have shown that *Paraconiothyrium* is capable of producing secondary metabolites including macrolides, terpenoids, polyketides, phenolics, and furanone of which, many have been reported to be inhibitory to other fungal species including *Aspergillus* (Wang *et al.*, 2023; Ngo *et al.*, 2014). This might explain the decrease in non-rhizosphere abundance of the *Aspergillus* spp. The presence of *Lepidosphaeria*, while less extensively studied, suggests a preference for non-rhizosphere conditions, potentially related to its specific nutritional requirements or tolerance to environmental stressors prevalent in the bulk soil.

The presence of other prominent genera such as *Fusarium*, *Alternaria*, *Humicola*, and *Candida* in the rhizosphere and *Fusarium*, *Colletotrichum*, *Thialavia*, and *Rhexothecium* in the non-rhizosphere further highlights the complexity of the fungal communities in these two distinct soil compartments. *Fusarium* is a ubiquitous genus containing both pathogenic and beneficial species (Gordon, 2017). The presence of *Alternaria* in the rhizosphere might reflect its potential role as a saprophyte or a weak pathogen, while *Humicola* is known for its cellulolytic activity and contribution to organic matter decomposition (Singh, 2011). *Candida*, typically known as yeast, suggests the presence of readily available carbon sources in the rhizosphere. *Colletotrichum*, known primarily as a plant pathogen, might be present as an opportunistic colonizer of plant debris in the non-rhizosphere. *Thialavia* and *Rhexothecium* are relatively less studied genera, and their roles in the soil ecosystem warrant further investigation.

A significant proportion of fungal genera in both the rhizosphere (~25%) and non-rhizosphere (~40%) soil remained unclassified or unspecified. This highlights a critical gap in our understanding of fungal diversity in soil ecosystems. Metagenomic approaches, while powerful, often encounter limitations in taxonomic assignment, particularly for less well-characterized fungal groups. Advances in fungal genomics, coupled with improved taxonomic databases and phylogenetic analyses, are crucial for resolving the identity and ecological roles of these unclassified fungi (e.g., Blackwell, 2011; Hibbett *et al.*, 2007). Future studies employing targeted isolation and cultivation techniques, combined with advanced molecular tools, are needed to characterize these unknown fungal taxa and elucidate their contributions to soil processes.

The observed differences in fungal community composition between the rhizosphere and non-rhizosphere soil likely reflect the influence of root exudates, nutrient availability, and other plant-mediated factors. Root exudates, containing a variety of organic compounds such as sugars, amino acids, and organic acids, serve as a selective substrate for specific microbial groups, shaping the rhizosphere microbiome (e.g., Bais *et al.*, 2006). Furthermore, the depletion of certain nutrients in the rhizosphere, due to plant uptake, can also influence the composition of the fungal community. Understanding the specific interactions between cowpea plants and rhizosphere fungi requires further investigation, including experiments involving gnotobiotic systems and targeted manipulation of root exudate composition.

Conclusion

This study presents some convincing evidences that *Vigna unguiculata* (L.) Walp. exerts a strong influence on rhizosphere microbial community dynamics under hydrocarbon stress, promoting a more taxonomically rich, diverse, and functionally specialized microbiome compared to the non-rhizosphere soils. The cowpea rhizosphere demonstrated a significant enrichment of bacterial taxa such as *Sphingomonas*, *Saccharimonadales*, and *Paracoccus*, as well as fungal genera including *Curvularia* and *Paraconiothyrium*, which are known for their potential roles in hydrocarbon degradation and plant-growth promotion. Notably, unnamed bacterial taxa—constituting a significant proportion of the community—were more prevalent in the rhizosphere, highlighting the possible existence of novel, uncultured organisms shaped by cowpea

root exudates and hydrocarbon-mediated selective pressures. Beta diversity analyses confirmed a strong divergence in community composition between rhizosphere and bulk soil, reinforcing the concept of plant-mediated microbiome sculpting under environmental stress. The microbial architecture in the rhizosphere suggests a highly adapted consortium capable of mitigating abiotic stress while possibly enhancing nutrient availability and phytoremediation efficiency. These findings highlight the pivotal role of plant-microbe interactions in remediating contaminated environments and suggest cowpea as a robust candidate for rhizoremediation strategies targeting petroleum-polluted soils. Future research should prioritize the isolation and functional characterization of the dominant and unclassified taxa identified, alongside metabolomic profiling of root exudates and gene expression analyses to decipher molecular interactions strengthening this plant-microbe synergy. Ultimately, a deeper understanding of the rhizosphere's ecological complexity will not only enhance bioremediation outcomes but also inform sustainable agricultural practices in marginal and contaminated lands.

Declarations

Acknowledgements

The authors acknowledge the support of Tertiary Education Trust Fund (TETFUND) Nigeria, for supporting the research.

Conflict of Interest

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

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Figures

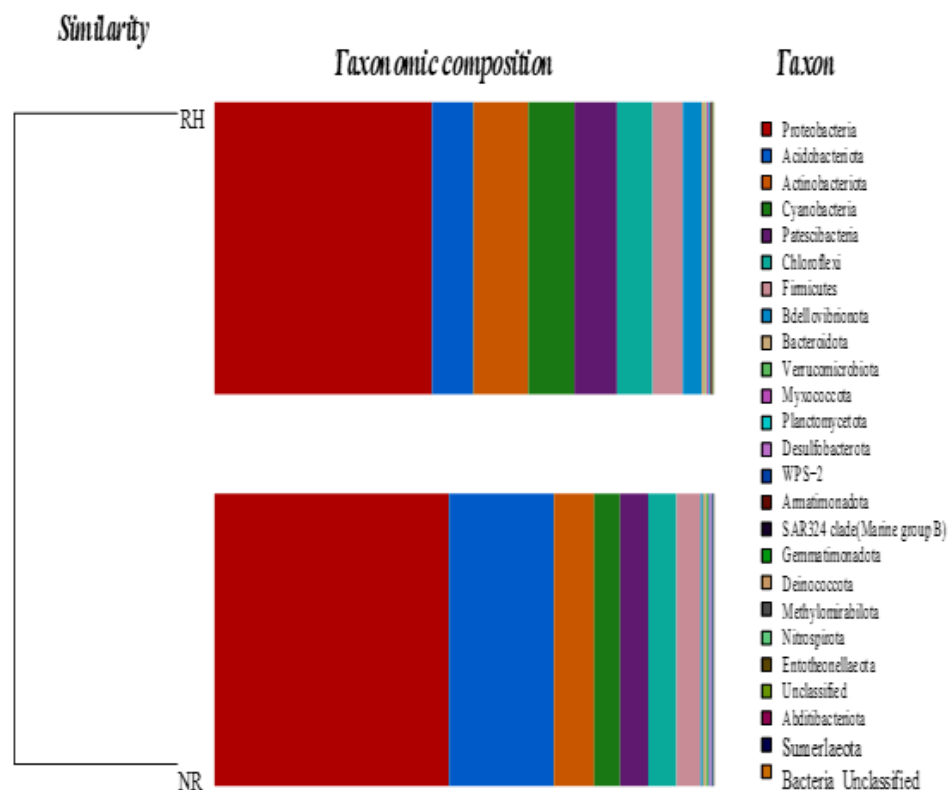


Figure 3

Taxonomic similarity of bacterial communities in rhizosphere and non-rhizosphere soil

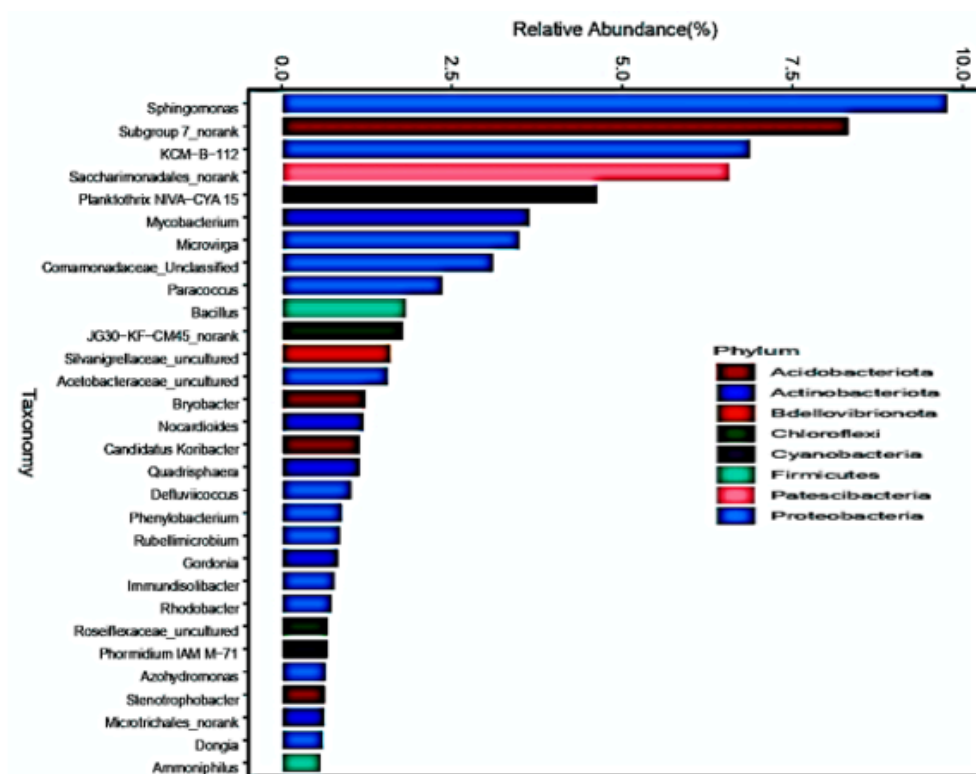


Figure 4

Most abundant bacterial species (30) in rhizosphere and non-rhizosphere soil

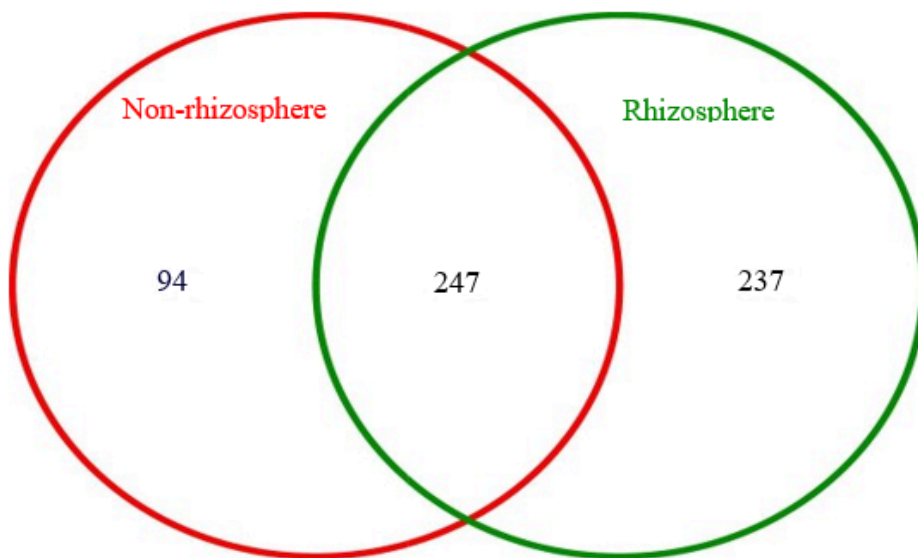


Figure 5

Fungal population. OTUs abundance and distribution in rhizosphere and non-rhizosphere soil

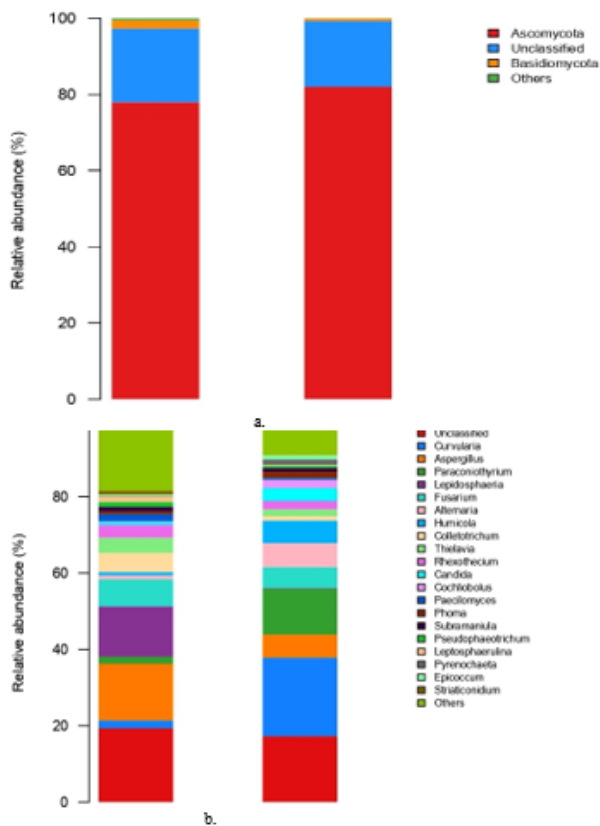


Figure 6

Bar plot of bacterial communities at (a) phylum and (b) genus level

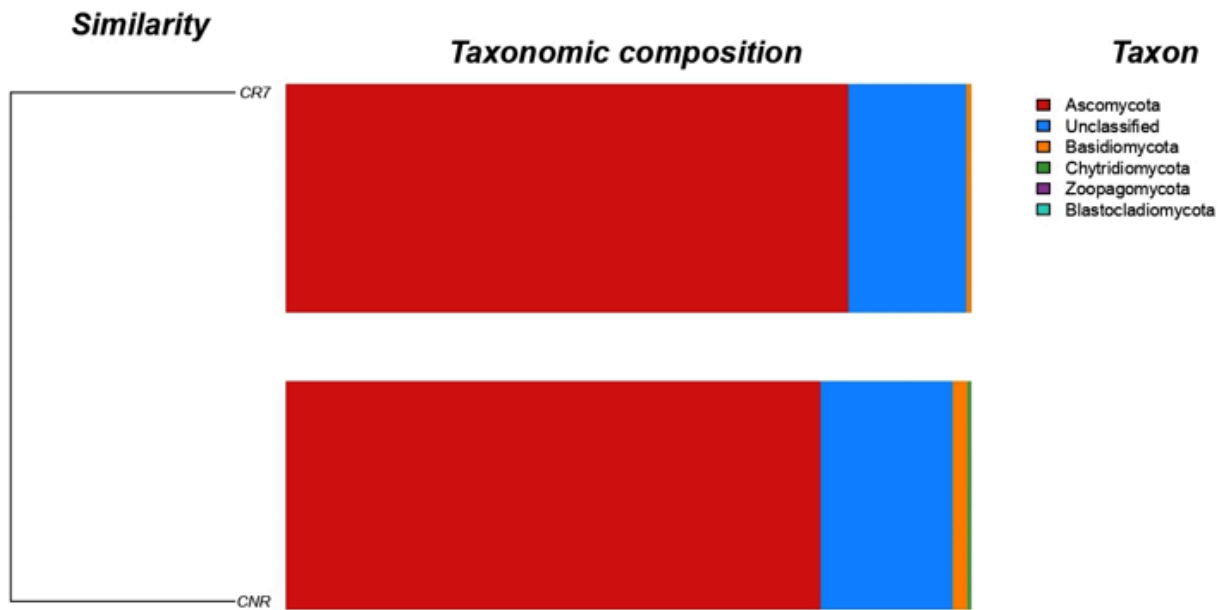


Figure 7

Taxonomic similarity of fungal communities in rhizosphere and non-rhizosphere soil

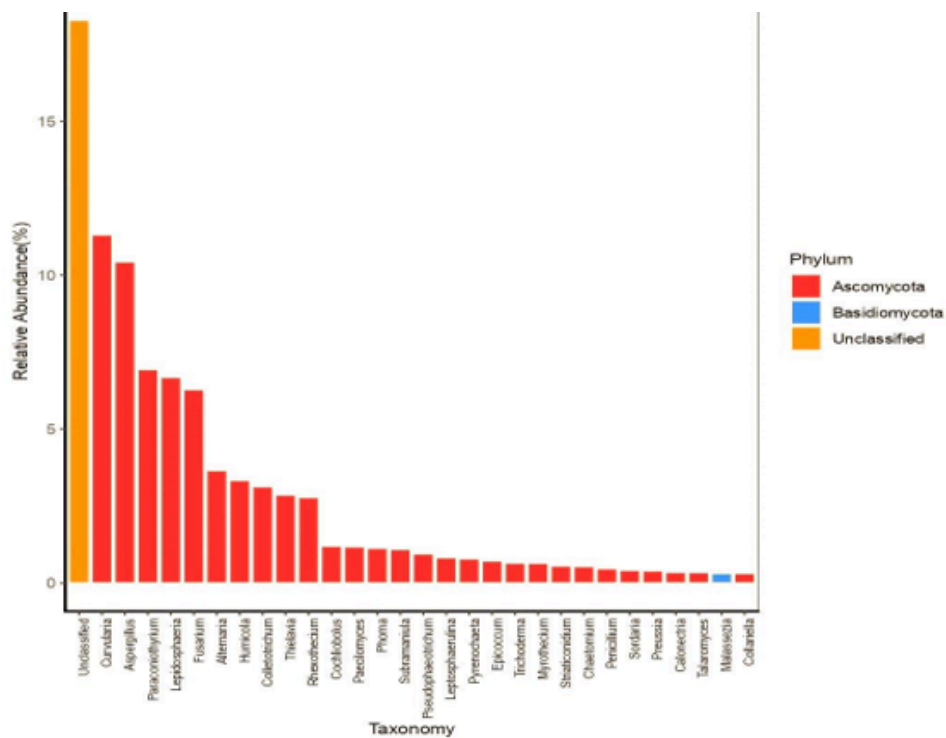


Figure 8

Most abundant fungal species (30) in rhizosphere and non-rhizosphere soil