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Metagenomic Study of Bacteria Diversity and Functional Profile in Bulk and Rhizosphere Soils of Fusarium-Wilt Infected Plantain (*Musa paradisiaca*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Soil contains a great diversity of microorganisms, including bacteria which are known to be drivers of soil ecosystem functions. This study was aimed at investigating the bacterial communities in bulk and rhizosphere soil of Fusarium wilt-infected plantain (*Musa paradisiaca*). Physicochemical analysis revealed that electrical conductivity (312 µS/cm), cation exchange capacity (15.62 meq/100g), phosphate (0.16 mg/kg), nitrogen (1.53 mg/kg), moisture (19.45 mg/kg), potassium (1.39 mg/kg), magnesium (0.61 mg/kg), clay (60 %), and silt (35%) were higher in bulk soil than rhizosphere soil. The 16S rRNA metagenomic sequences quantified a total of 89341 bacterial taxonomic units from bulk soil which consist of 10 phyla, 13 classes, 16 orders, 18 families, 21 genera, and 19 species. A total of 88034 bacterial taxonomic units which comprised of 9 phyla, 13 classes, 23 orders, 22 families, 26 genera, and 25 species, were found in rhizosphere soil. The most

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abundant phyla in bulk soil are Actinobacteria (31%), Proteobacteria (26%), and Gemmatimonadetes (17%) Acidobacteria (17%) and Planctomycetes (3%) while the prominent phyla in rhizosphere soil are Actinobacteria (63%) Proteobacteria (24%), Acidobacteria (7%) and Planctomycetes (3%). The major functional profiles of bacterial communities in both bulk and rhizosphere soils are metabolism of amino acids, carbohydrates, terpenoids, polyketides, cofactors, and xenobiotic degradation. Alpha diversities among the bacterial community were higher in Simpson's reciprocal index for both bulk and rhizosphere soils. This study opens up new frontiers in expanding metagenomics studies on environmental samples which would capture and contribute to the identification of soil bacteria useful to ecosystem functions.

Keywords: Fusarium wilt; plantain; metagenomics; rhizosphere; soil.

1. INTRODUCTION

Fusarium wilt, a devastating disease caused by the fungus *Fusarium oxysporum*, poses a significant threat to a wide range of crops, including plantains and bananas (Agrios, 2015). The disease is characterized by the wilting and death of infected plants, leading to substantial economic losses [1]. The fungus enters the plant through the roots and colonizes the xylem vessels thereby blocking the flow of water and nutrients. Disease progression results in the collapse of leaves at the petiole, the splitting of the pseudostem base and eventually plant death [2,3]. To fully comprehend the dynamics of Fusarium wilt infection, a deep understanding of the microbial communities in both bulk soil and the rhizosphere is essential. The rhizosphere, the region of soil influenced by plant root exudates, harbours a distinct microbial community that can either promote or suppress the growth of pathogenic organisms [4].

The rhizosphere microbiome, a diverse community of microorganisms residing in the soil surrounding plant roots, plays a pivotal role in plant health by promoting growth, suppressing pathogens, and enhancing stress tolerance [5]. Some rhizosphere microbes also act as biocontrol agents, producing antifungal compounds or competing with pathogens for resources, thereby, reducing plant disease incidence and severity [5]. Studies have also shown that rhizosphere microbiomes can also play a role in *Fusarium* wilt disease suppression [6,7]. For example, one study found that a specific strain of *Pseudomonas fluorescens* was able to suppress Fusarium wilt in tomato plants [6]. Other studies have shown that the diversity of the rhizosphere microbiome is correlated with Fusarium wilt disease suppression [7].

Metagenomics, a powerful approach utilizing high-throughput sequencing of environmental DNA, facilitates a comprehensive exploration of microbial communities in complex ecosystems. By analyzing the genetic material in soil samples, researchers can gain insights into the resident microbial communities' taxonomic composition and functional capabilities [8,9]. There is sparse information on *Fusarium oxysporum f. sp. cubense* (Foc), the fungus, responsible for Fusarium wilt in plantain (*Musa paradisiaca*) in Nigeria, especially in the Port Harcourt metropolis, Rivers States. The aim of this study is to investigate the underlying microbial communities residing in the bulk and rhizosphere soil of Fusarium wilt-infected plantain. Thus, there is a need for a comparative metagenomic analysis of bulk and rhizosphere soil from Fusarium wilt-infected plants to provide valuable insights into the microbial communities associated with this disease.

2. MATERIALS AND METHODS

2.1 Soil Sampling

Soil samples were collected from a five-acre plantain farm in Choba, Port Harcourt Nigeria. Bulk and rhizosphere soil samples were collected from five symptomatic on the same farm in a completely randomized design. Bulk soil at a 0.5 m distance from the individual plant was collected at 20 cm depth using a soil core ring. To collect rhizosphere soil, plantain roots 10 cm long (measured from the root tip) were sampled from an individual plant. The roots were shaken by hand to remove any loose soil, leaving only strongly adhered soil, which was considered the rhizosphere soil. The soil samples were placed in sterile polythene bags and transferred to microbiology laboratory.

2.2 Analysis of the Physicochemical Properties of the Soil Samples

The soil samples were first air-dried. Soil samples were sent to the Department of Crop

and Soil Science, University of Port Harcourt, Nigeria for pH measurement and nutrient analysis. Briefly, soil pH was measured with a pH meter following the soil being mixed using water (1:5 w/v) for 30 min. Temperature was recorded using a digital thermometer, and available phosphorus was determined using the Bray 2 method. Copper, zinc, iron, and Manganese were determined using the dilute double acid method. Meanwhile, potassium, calcium, and magnesium were determined using ammonium acetate extraction.

Mechanical analysis (slit, slay, coarse, fine sand), determination of organic matter, and cation exchange capacity (CEC) were also determined. Briefly, CEC was determined using the ammonium acetate method, whereas organic matter was measured using dry combustion.

2.3 Genomic DNA Extraction from the Garden soil and Metagenomics

The garden soil samples were transferred to a microcentrifuge tube for genomic DNA extraction. For genomic DNA extraction, bulk soil was ground in a sterile mortar and pestle and sieved through a 2-mm sieve before being transferred to a microcentrifuge tube. Total soil DNA was extracted using a DNeasy Power Soil Kit (Qiagen, Germany) following the manufacturer's protocol. The quantity and quality of the extracted DNA were verified using a Qubit 2.0 Fluorometer (Thermo Scientific, USA).

2.4 16S rRNA Gene Amplification using Illumina Hi-seq 2500 PE Platform

The prokaryotic hypervariable V3–V4 region from the 16S rRNA gene was amplified using the primers set 341-F (5′ – CCTACGGGNBGCASCAG – 3′) and 805-R (5′-GACTACNVGGGTATCTAATCC- 3′). PCR reactions were carried out with Phusion High-Fidelity PCR Master Mix (New England Biolabs, UK). The same volume of 1 × loading buffer (containing SYB green) was mixed with PCR products, and electrophoresis was operated on 2% agarose gel for detection. PCR products were mixed in equidensity ratios, and a mixture of the PCR products was purified with a QIAquick Gel Extraction Kit (Qiagen, Germany).

Sequencing libraries were generated using NEBNext Ultra DNA Library Pre Kit for Illumina following the manufacturer's recommendations. The library quality was assessed on the Qubit 2.0 Fluorometer (Thermo Scientific, USA) and sequenced using the Illumina Hi-seq platform, generating 250 bp paired-end reads.

2.5 Bioinformatic Analysis of the 16S rRNA Sequences

The datasets of the 16S rRNA sequences of the soil samples were uploaded on the European Galaxy server (https://usegalaxy.eu/ [9]. In this server, various analyses were carried out on the dataset such as quality control, taxonomic classification, functional profiling and alpha diversity index.

2.5.1 Quality control of the sequences

Quality control on the raw reads of the 16SrRNA sequences was carried out using Fast QC and multiple QC tools. FastQC provides information on various parameters, such as the range of quality values across all bases at each position. MultiQC allows summarizing the output of different outputs from FastQC. After quality control, the sequences were then filtered on a minimum average read quality score of 9, according to the recommendations from Nygaard *et al.* [10]. After filtering, the sequences were analyzed again using FastQC and MultiQC to see if the anomalies detected had been corrected.

2.5.2 Taxonomic classification of the 16S rRNA sequences

Taxonomic classification was performed using the kraken2 tool [11]. This tool uses the minimizer method to sample the k-mers (all the read's subsequences of length *k*) in a deterministic fashion to reduce memory consumption and processing time. In addition, it masks low-complexity sequences from reference sequences by using dustmasker**.** After assigning the corresponding taxa to each sequence, the data was visualized using the Krona pie chart tool [12].

2.5.3 Alpha diversity and functional profile

The alpha diversity was estimated based on Shannon, Simpson, Simpson's reciprocal index, Berger Parker, and Fisher's index. Alpha diversity figures were plotted using the PhyloSeq packages [13]. This method enables the mapping of gene abundance profiles, which was predicted from Tax4Fun. The bacterial OTUs were imported into Tax4Fun, and the functional genes were identified from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [14].

3. RESULTS

3.1 Physicochemical Properties of Bulk and Rhizosphere Soil of Fusarium Wilt Infected Plantain

Physicochemical properties of bulk and rhizosphere soil of fusarium wilt infected plantain are presented in Table 1. The bulk and rhizosphere soils are brown in colour, have slight acidic pH of 6.3 and temperature of 28 ^{o}C. The textural class of bulk and rhizosphere soils was loamy sand. The particle size of rhizosphere soil (89.3 mg/kg) was higher than that of bulk soil (76.5 mg/kg). Similarly, organic matter (20.42 mg/kg), nitrate (1.33 mg.kg), iron (13.73 mg/kg), calcium (0.26 mg/kg), copper (0.131 mg/kg), zinc (0.313 mg/kg), manganese (0.36 mg/kg), fine sand (15.24 mg/kg), air porosity (61.02 %), and bulk density (0.766 g/cm^{3}) were also higher in rhizosphere than bulk soil.

3.2 Composition of Bacterial Community in Bulk and Rhizosphere Soil of Diseased Plantain using 16S rRNA Metagenomics

All bacteria found in the bulk and rhizosphere soil of fusarium wilt infected plant were taxonomically identified by amplifying and sequencing the complete 16S rDNA genes. A total of 89341 bacterial taxonomic units which consist of 10 phyla, 13 classes, 16 orders, 18 families, 21 genera, and 19 species, were generated from bulk soil (Table 2). However, a total of 88034 bacterial taxonomic units which comprised of 9 phyla, 13 classes, 23 orders, 22 families, 26 genera, and 25 species, were isolated from rhizosphere soil (Table 2).

3.2.1 Relative abundance of phyla

The result in Table 3 shows the most abundant phyla in bulk and rhizosphere soil. The most abundant phyla in the bulk soil are Actinobacteria (31%), Proteobacteria (26%), and Gemmatimonadetes (17%) while the least phyla were Bacteriodetes (0.3%) and Chloroflexi (0.7%). On the other hand, the dominant phyla in rhizosphere soil are Actinobacteria (63%), Proteobacteria (24 %), and Acidobacteria (7 %). The least phyla in rhizosphere were Bacteriodetes (0.3 %) and Armatimonadetes (0.05%) (Table 3).

3.2.2 Relative abundance of class

Different classes of bacteria in bulk and rhizosphere soil are presented in Table 4. The dominant classes in bulk soil are Alphaproteobacteria (21%), Actinomycetia (20%), and Gemmatimonadetes (17%). Chlamydiia (0.06%), and Ktedonobacteria (0.2%) were the least classes. The dominant classes in rhizosphere soil are Alphaproteobacteria (20%), Rubrobacteria (14%), and Acidobacteriia (7%). Chthonomonadetes (0.05%), and Ktedonobacteria (0.2 %) were the least classes.

3.2.3 Relative abundance of order

The relative abundance of orders in bulk and rhizosphere soil are shown in Table 5. Gemmatimonadales (17 %), Geodermatophilales (11 %), and Acidimicrobiales (11 %) are the most abundant orders in bulk soil. The least orders are Parachlamydiales (0.06%) and Ktedonobacterales (0.2%). Streptomycetales (30 %), Rubrobacterales (14), and Solirubrobacterales (9 %) were the most abundant orders in rhizosphere soil while Acidothermales (0.3%) and Bacteroidales (0.3%) were the least orders.

3.2.4 Relative abundance of family

Relative abundances of the families in bulk and rhizosphere soil are illustrated in Table 6. Gemmatimonadaceae (17 %), Geodermatophilaceae (11%), and Caulobacteraceae (11 %), are the dominant family in bulk soil while Simkaniaceae (0.06%), and Ktedonosporobacteraceae (0.2%) are the least genera. The relative abundances of bacteria in rhizosphere soil revealed that Streptomycetaceae (30 %), and Baekduiaceae (14 %), Conexibacteraceae (9 %) are the most abundant family while Chthonomonadaceae (0.05%), Gordoniaceae (0.3%), and were the least families.

3.2.5 Relative abundance of genus

Relative abundances of the genera in bulk and rhizosphere soil are shown in Table 7. Gemmatirosa (17 %), Geodermatophilus (11 %), and Brevundimonas (11%) are the dominant genera in bulk soil while Simkania (0.06 %), Ktedonosporobacter (0.2%), and Muribaculum (0.3%) are the least genera. The relative abundances of bacteria in rhizosphere soil showed that Gemmatirosa (17 %), Geodermatophilus (11%), and Brevundimonas (11 %) are the most abundant genera while Simkania (0.06% (58) and Ktedonosporobacter (0.2%) were the least.

3.2.6 Relative abundance of species

Relative abundances of the bacteria species in bulk and rhizosphere soil are presented in Table 8. *Gemmatirosa kalamazoonesis* (17%), *Geodermatophilus obscurus* (11%), and *Brevundimonas* sp. (11 %) are the dominant species in bulk soil while *Aquihabitans* sp. (0.4%) and *Hyphomicrobium nitrativorans* (1%) and are the least species. The relative abundances of bacteria species in rhizosphere soil showed that *Streptomyces* sp. (30%), *Candidatus Koribacter versatilis* (8%), and *Sphingomonas lutea* (3%) are the most abundant species while *Muribaculum gordoncarteri* (0.3%) and *Skermania piniformis* (0.3%) are the least species in rhizosphere soil.

Table 1. Physicochemical analysis of bulk and rhizosphere soil of fusarium wilt infected plantain

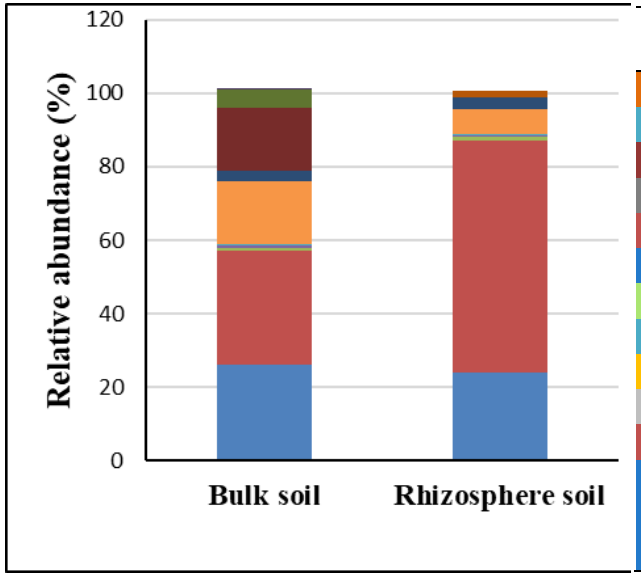
Parameters	Bulk soil	Rhizosphere soil
pH	6.31± 4.26	6.33± 1.34
Temperature (^oC)	28.3± 1.80	28.5± 5.28
Soil colour	Slight brown	Deep brown
Texture	Silty Loamy	Loamy sand
Particle size (mg/kg)	76.5± 1.66	89.3± 3.30
Electrical conductivity (µS/cm)	312± 0.71	187± 0.00
Alkalinity (mg/kg)	11.81± 1.11	11.75± 1.11
Cation Exchange Capacity (CEC) (meq/100g)	15.62± 4.90	14.37± 0.00
Organic matter (%)	18.00± 6.22	20.42± 3.11
Nitrate (mg/kg)	0.791± 1.11	1.33± 0.22
Phosphate (mg/kg)	0.156± 0.01	0.116± 1.10
Nitrogen (mg/kg)	1.531± 1.25	0.954± 0.73
Moisture (wt%)	19.45± 0.99	16.45± 4.05
Potassium (mg/kg)	1.394± 0.51	1.255± 0.12
Iron (mg/kg)	10.44± 3.70	13.73± 3.33
Magnesium (mg/kg)	0.612± 7.40	0.485± 0.05
Calcium (mg/kg)	0.182± 0.15	0.264± 5.6
Copper (mg/kg)	0.019± 2.22	0.131± 9.66
Zinc (mg/kg)	0.272± 0.05	0.313± 2.00
Manganese (mg/kg)	0.102± 1.11	0.36± 1.11
Clay (%)	60.00 ± 3.07	57.20 ± 4.20
Coarse sand (%)	1.55 ± 0.43	1.88± 1.33
Silt (%)	35.0 ± 1.08	32.0 ± 4.12
Fine sand (%)	13.930 ± 0.49	15.24 ± 1.80
Air porosity (%)	56.80 ± 1.10	61.02 ± 1.22
Bulk density (g/cm^3)	0.651± 0.01	0.766± 0.02

Values are means of duplicates: ± Standard error of the mean

Table 2. Taxonomic units of bulk and rhizosphere soil

Taxonomic unit	Bulk soil	Rhizosphere soil
Total number of Bacteria	89341	88034
Phylum	10	9
Class	13	13
Order	16	23
Family	18	22
Genus	21	26
Species	19	25

Table 3. Relative abundance of the most representative phyla in bulk and rhizosphere soil



Phylum	Bulk soil		Rhizosphere soil	
	%	(total)	%	(total)
Nitrospirae	0	0	2	1497
Armatimonadetes	0	0	0.05	47
Chlamydiae	0.06	58	0	0
Firmicutes	5	4110	0	0
Gemmatimonadetes	17	15358	0	0
Planctomycetes	3.0	2570	3.0	2853
Acidobacteria	17	14754	7	5799
Bacteriodetes	0.3	274	0.3	265
Chloroflexi	0.7	593	0.4	391
Cyanobacteria	1	953	1	879
Actinobacteria	31	27671	63	55393
Protoeobacteria	26	23000	24	20910

Table 4. Relative abundance of the most representative class in bulk and rhizosphere soil

Class	Relative abundance (%)			
	Bulk soil		Rhizosphere soil	
	%	(total)	%	(total)
Alphaproteobacteria	21	19077	20	17770
Deltaproteobacteria	4.0	3923	0.2	652
Gammaproteobacteria	0.0	0.0	3.0	2488
Actinomycetia	20	17851	39	34161
Acidobacteriia	17	14754	7.0	5799
Gemmatimonadetes	17	15358	0.0	0.0
Bacteroidia	0.3	274	0.3	265
Planctomycetia	2.0	1066	3.0	289
Tepidiformia	0.5	425	0.4	391
Ktedonobacteria	0.2	168	0.2	168
Acidimicrobiia	11	9820	0.0	0.0
Bacilli	5.0	4110	0.0	0.0
Pseudanabaenales	1.0	953	0.0	0.0
Chlamydiia	0.06	58	0.0	0.0
Thermoleophilia	0.0	0.0	9.0	8281
Chthonomonadetes	0.0	0.0	0.05	47
Nitrospira	0.0	0.0	2.0	1497
Rubrobacteria	0.0	0.0	14	12686

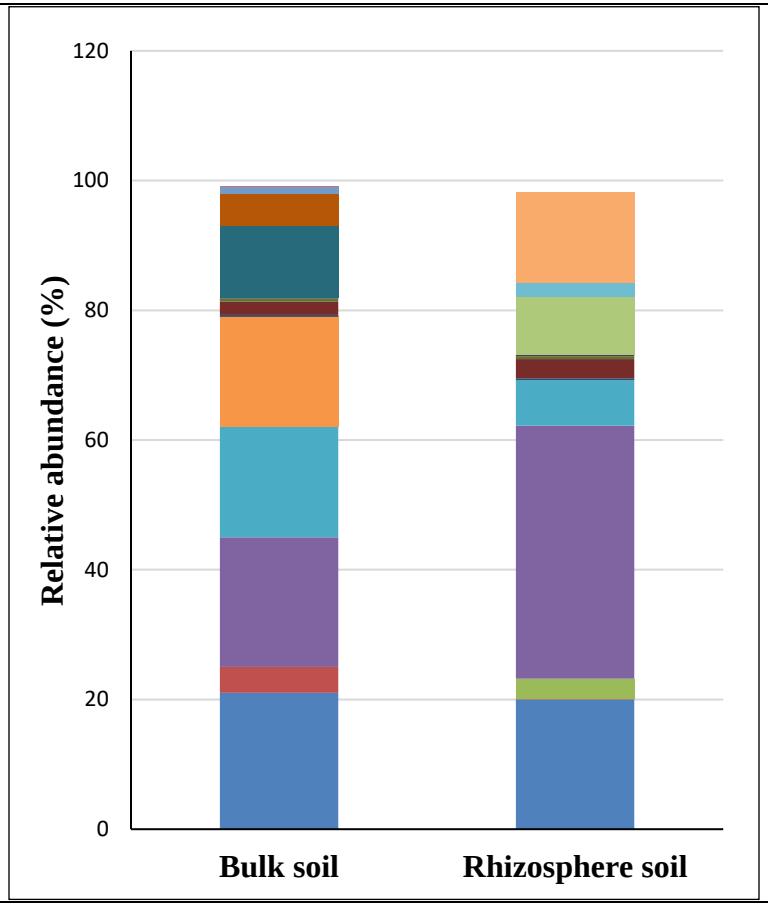


Table 5. Relative abundance of the most representative order in bulk and rhizosphere soil

Order	Relative abundance (%)			
	Bulk soil		Rhizosphere soil	
	%	total	%	total
Hyphomicrobiales	6.0	4981	6.0	5021
Rhodospirillales	5.0	4454	7.0	6234
Caulobacterales	11	9642	3.0	2354
Myxococcales	4.0	3923	0.0	0.0
Acidimicrobiales	11	9820	6.0	5522
Propionibacteriales	9.0	7985	2.0	2035
Geodermatophilales	11	9866	0.0	0.0
Tepidiformales	0.5	425	0.4	391
Ktedonobacterales	0.2	168	0.0	0.0
Gemmatimonadales	17	15358	0.0	0.0
Bryobacterales	9.0	7914	0.0	0.0
Acidobacteriales	8.0	6840	6.0	5522
Bacillales	5.0	4110	0.0	0.0
Isosphaerales	3.0	2458	3.0	2299
Bacteroidales	0.3	274	0.3	265
Parachlamydiales	0.06	58	0.0	0.0
Sphingomonadales	0.0	0.0	5.0	4161
Rhodospirillales	0.0	0.0	7.0	6234
Xanthomonadales	0.0	0.0	2.0	1380
Enterobacterales	0.0	0.0	1.0	1108
Nitrospirales	0.0	0.0	2.0	1497
Streptomycetales	0.0	0.0	30	26115
Jatrophihabitantales	0.0	0.0	2.0	1965
Catenulisporales	0.0	0.0	2.0	1640
Streptosporangiales	0.0	0.0	1.0	1087
Micromonosporales	0.0	0.0	0.9	805

Order	Relative abundance (%)			
	Bulk soil		Rhizosphere soil	
	%	total	%	total
Corynebacteriales	0.0	0.0	0.3	284
Acidothermales	0.0	0.0	0.3	230
Rubrobacterales	0.0	0.0	14	12686
Solirubrobacterales	0.0	0.0	9.0	8281
Chthonomonadales	0.0	0.0	0.05	47

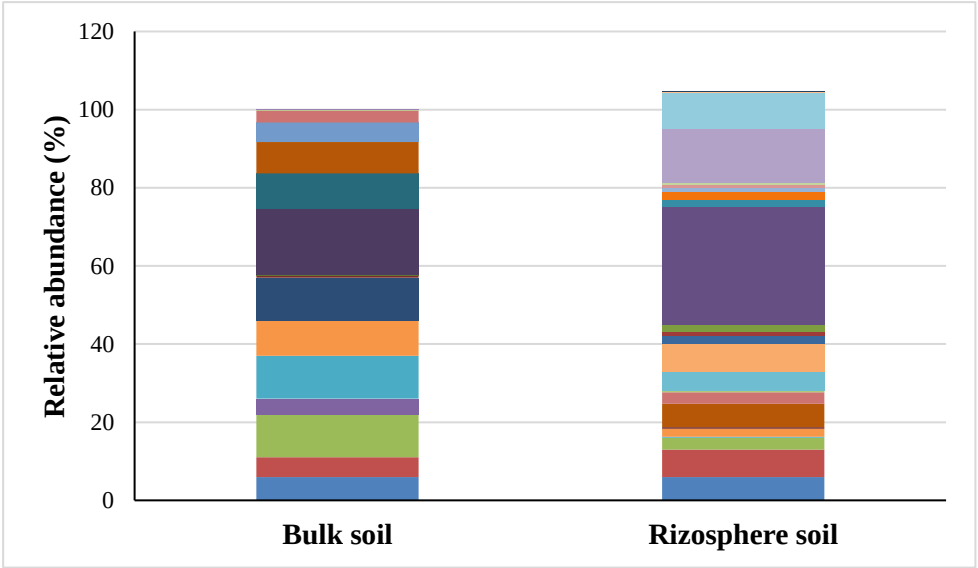


Table 6. Taxonomy and relative abundances of the most representative family in bulk and rhizosphere soil

Family	Relative abundance (%)			
	Bulk soil		Rhizosphere soil	
	%	total	%	total
Hyphomicrobiaceae	6.0	4981	3.0	2634
Devosiaceae	0.0	0.0	2.0	1442
Methylobacteriaceae	0.0	0.0	1.0	945
Sphingomonadaceae	0.0	0.0	5.0	4161
Caulobacteraceae	11	9642	3.0	2354
Rhodanobacteraceae	0.0	0.0	2.0	1380
Bruguierivoracaceae	0.0	0.0	1.0	1108
Acidobacteriaceae	8.0	6840	6.0	5522
Isosphaeraceae	3.0	2458	3.0	2299
Nitrospiraceae	0.0	0.0	2.0	1497
Tepidiformaceae	0.5	425	0.4	391
Muribaculaceae	0.3	274	0.3	265
Chthonomonadaceae	0.0	0.0	0.05	47
Streptomycetaceae	0.0	0.0	30	26115
Nocardioidaceae	5.0	4834	2.0	1452
Jatrophihabitantaceae	0.0	0.0	2.0	1965
Catenulisporaceae	0.0	0.0	2.0	1640
Thermomonosporaceae	0.0	0.0	1.0	1087
Micromonosporaceae	0.0	0.0	0.9	805
Gordoniaceae	0.0	0.0	0.3	284
Baekduiaceae	0.0	0.0	14	12686
Conexibacteraceae	0.0	0.0	9.0	8281
Acetobacteraceae	0.0	0.0	7.0	6234
Planococcaceae	5.0	4110	0.0	0.0
Geodermatophilaceae	11	9866	0.0	0.0
Nocardioidaceae	5.0	4834	0.0	0.0
Actinopolymorphaceae	4.0	3151	0.0	0.0
Iamiaceae	11	9661	0.0	0.0
Simkaniaceae	0.06	58	0.0	0.0
Ktedonosporobacteraceae	0.2	168	0.0	0.0
Bryobacteraceae	9.0	7914	0.0	0.0
Gemmatimonadaceae	17	15358	0.0	0.0
Kofleriaceae	3.0	2615	0.0	0.0
Sandaracinaceae	1.0	1308	0.0	0.0
Azospirillaceae	5.0	4454	0.0	0.0

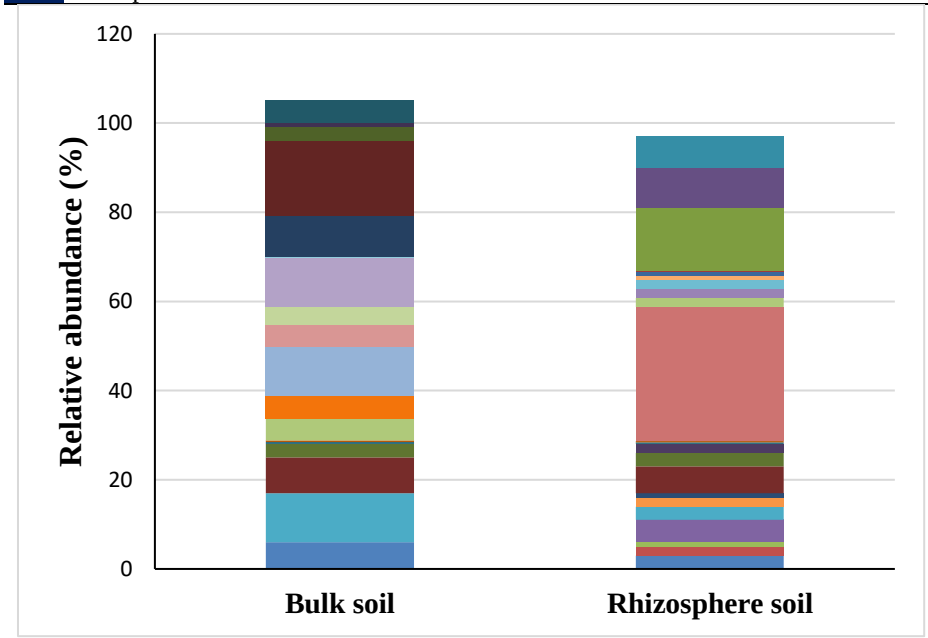


Table 7. Taxonomy and relative abundances of the most representative genera in bulk and rhizosphere soil

Genus	Relative abundance (%)			
	Bulk soil		Rhizosphere soil	
	%	total	%	total
Devosia	2.0	1442	0.0	0.0
Microvirga	1.0	945	0.0	0.0
Rhodoplanes	3.0	2634	1.0	1031
Sphingomonas	5.0	4161	0.0	0.0
Phenylobacterium	1.0	1283	0.0	0.0
Caulobacter	1.0	1071	0.0	0.0
Dyella	2.0	1380	0.0	0.0
Sodalis	1.0	1108	0.0	0.0
Candidatus_Koribacter	5.0	4504	0.0	0.0
Edaphobacter	1.0	1018	0.0	0.0
Tepidiforma	0.4	391	0.5	425
Muribaculum	0.3	265	0.3	274
Chthonomonas	0.05	47	0.0	0.0
Aquisphaera	2.0	2299	3.0	2458
Nitrospira	2.0	1497	0.0	0.0
Streptomyces	30	26115	0.0	0.0
Nocardioides	2.0	1452	5.0	4834
Jatrophihabitans	2.0	1965	0.0	0.0
Catenulispora	2.0	1640	0.0	0.0
Thermomonospora	1.0	1087	0.0	0.0
Dactylosporangium	0.9	805	0.0	0.0
Skermania	0.3	284	0.0	0.0
Acidothermus	0.3	230	0.0	0.0
Baekduia	14	12686	0.0	0.0
Conexibacter	9.0	8281	0.0	0.0
Acidibrevibacterium	7.0	6234	0.0	0.0
Filomicrobium	0.0	0.0	2.0	1901

Genus	Relative abundance (%)			
	Bulk soil		Rhizosphere soil	
	%	total	%	total
Methyloligella	0.0	0.0	1.0	1180
Nitrospirillum	0.0	0.0	5.0	4454
Haliangium	0.0	0.0	3.0	2615
Sandaracinus	0.0	0.0	1.0	1308
Gemmatirosa	0.0	0.0	17	15358
Paludibaculum	0.0	0.0	9.0	7914
Candidatus_Koribacter	0.0	0.0	8.0	6840
Jeotgalibacillus	0.0	0.0	5.0	4110
Geodermatophilus	0.0	0.0	11	9866
Actinopolymorpha	0.0	0.0	4.0	3151
Actinomarinicola	0.0	0.0	10	9299
Brevundimonas	0.0	0.0	11	9642
Ktedonosporobacter	0.0	0.0	0.2	168
Simkania	0.0	0.0	0.06	58

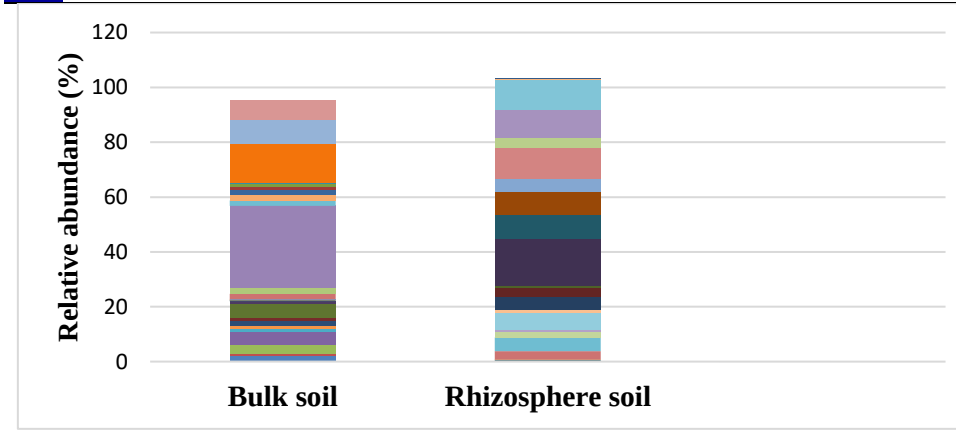
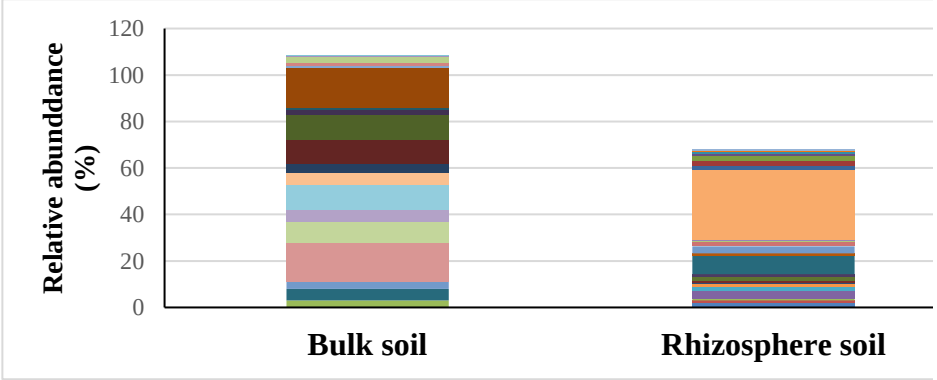


Table 8. Taxonomy and relative abundances of the most representative species in bulk and rhizosphere soil

Species	Relative abundance (%)				Species	Relative abundance (%)			
	Bulk soil		Rhizosphere soil			Bulk soil		Rhizosphere soil	
	%	total	%	total		%	total	%	total
Devosia sp.	0.0	0.0	2.0	1442	*Gemmatirosa kalamazoonesis*	17	15358	0.0	0.0
Microvirga sp.	0.0	0.0	1.0	945	*Paludibaculum fermentans*	9.0	7914	0.0	0.0
Rhodoplanes sp.	3.0	2634	1.0	1031	*Jeotgalibacillus malaysiensis*	5.0	4110	0.0	0.0
Sphingomonas lutea	0.0	0.0	3.0	2736	*Geodermatophilus obscurus*	11	9866	0.0	0.0
Sphingomonas rhizophila	0.0	0.0	2.0	1425	*Nocardioides* sp.	5.0	4834	0.0	0.0
Phenylobacterium zucineum	0.0	0.0	1.0	1283	*Actinopolymorpha singaporensis*	4.0	3151	0.0	0.0
Caulobacter sp.	0.0	0.0	0.5	404	*Actinomarinicola tropica*	10	9299	0.0	0.0
Caulobacter mirabilis	0.0	0.0	0.8	667	*Brevundimonas* sp.	11	9642	0.0	0.0
Dyella caseinilytica	0.0	0.0	2.0	1380	*Candidatus Filomicrobium_marinum*	2.0	1901	0.0	0.0
Sodalis sp.	0.0	0.0	1.0	1108	*Methyloligella* sp.	1.0	1180	0.0	0.0
Candidatus Koribacter versatilis	5.0	4505	8.0	6840	*Hyphomicrobium nitrativorans*	1.0	869	0.0	0.0
Edaphobacter sp.	0.0	0.0	1.0	1018	*Leptolyngbya* sp.	1.0	953	0.0	0.0
Aquisphaera giovannonii	3.0	2299	3.0	2458	*Muribaculum gordoncarteri*	3.0	274	0.0	0.0
Nitrospira japonica	0.0	0.0	2.0	1497					
Tepidiforma bonchosmolovskayae	0.0	0.0	0.4	391					
Muribaculum gordoncarteri	0.0	0.0	0.3	265					
Aquihabitans sp.	0.4	362	0.0	0.0					
Streptomyces sp.	0.0	0.0	30	26115					
Nocardioides baekrokdamisoli	0.0	0.0	2.0	1452					
Jatrophihabitans sp.	0.0	0.0	2.0	1965					
Catenulispora acidiphila	0.0	0.0	2.0	1640					
Thermomonospora curvata	0.0	0.0	1.0	1087					
Dactylosporangium vinaceum	0.0	0.0	0.9	805					
Skermania piniformis	0.0	0.0	0.3	284					
Acidothermus cellulolyticus	0.0	0.0	0.7	230					



3.2.7 Functional genes encoded in bulk and rhizosphere soil

The bacterial community functional profiles in bulk and rhizosphere soil are presented in Fig. 1. The major functional profiles associated with the bacterial communities in bulk and rhizosphere soils are, metabolism of amino acids, carbohydrates and energy, xenobiotic degradation, metabolism of terpenoids and polyketides, and metabolism of cofactors and vitamins.

3.2.8 Alpha diversity index of soil bacterial communities in bulk and rhizosphere soil

Bacterial diversity and richness of the bacteria community in bulk and rhizosphere soil is shown in Fig. 2. The result revealed that the diversities among the bacterial community were higher in Simpson's reciprocal index for both bulk and rhizosphere soils while the least was found in Berger Parker alpha diversity index.

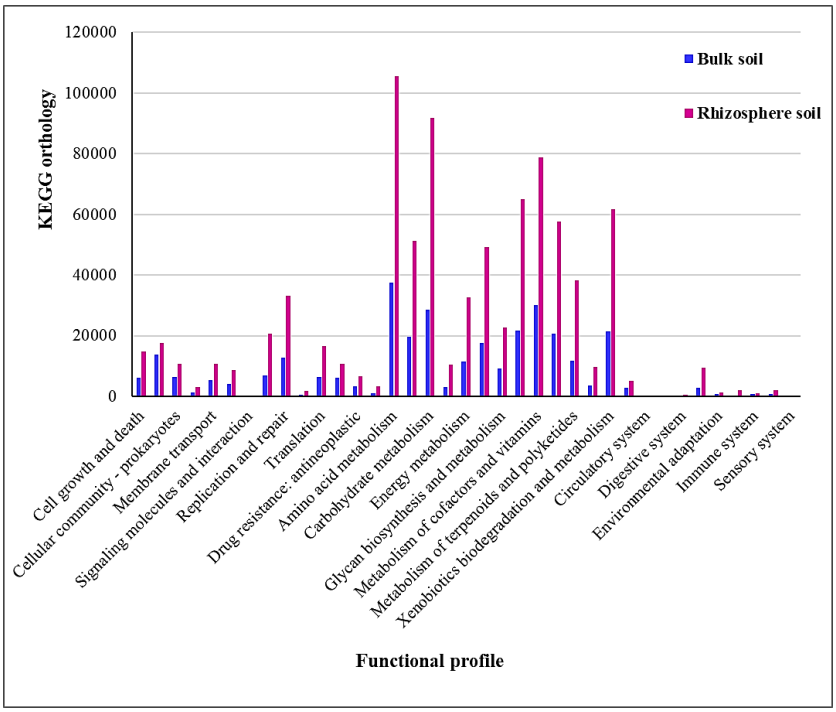


Fig. 1. Functional characteristics of bacterial communities in bulk and rhizosphere soil

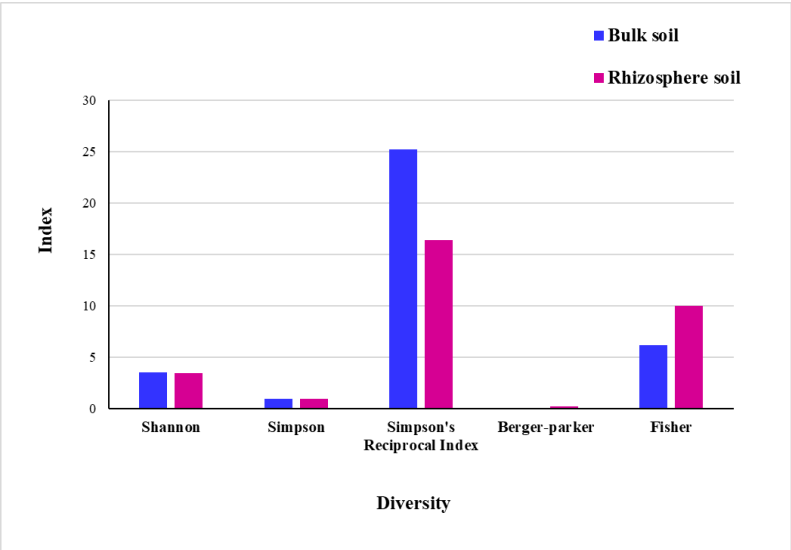


Fig. 2. Alpha diversity index of bacterial communities in bulk and rhizosphere soil

4. DISCUSSION

4.1 Physicochemical Properties of the Soil Samples

Soil physicochemical characteristics are important indicators of soil quality. The soil samples in had a pH of about 6.3. Soil with such pH conditions enhances the nutrient availability. The optimum pH range for most plants was between 5.5 and 7.5 [10]. Moisture content and temperature of the soil were 21.5% and 25.7^{o}C respectively. At every stage of plant growth, including the whole plant, tissue cell, and subcellular level, temperature and moisture have an effect on the growth and development of plants. They make nutrients available and promote the growth of microbes [11]. The electrical conductivity of bulk (312 µS/cm) and rhizosphere 187 µS/cm) soil, which is used to assess the soil's quality. It measures the number of ions in soil solution. In addition to improving soil structure and enhancing nutrient and water retention, soil organic matter plays a crucial role in supporting a rich and diverse soil biota [12].

Cation exchange capacity (CEC) is a measure of soil's ability to hold and exchange cations, and nutrients, such as calcium, magnesium, and potassium, for plant uptake [13]. It has an impact on the pH, structural stability, nutrient availability, fertilizer, and associated soil amelioration reaction [14]. Calcium, magnesium and sulphur are the secondary nutrients in soils. Calcium is required for cell wall formation; it maintains cellular pH; Magnesium is required for chlorophyll formation and ribosome formation; Sulphur is required for nitrogen metabolism and protein formation [15].

4.2 Metagenomics and Bacterial Diversity in Garden Soil

In this study, the predominant bacteria phyla in both bulk and rhizosphere soil were Actinobacteria, Proteobacteria and Acidobacteria while Bacteriodetes and Chloroflexi were the least represented. Although, the bacterial communities in rhizosphere soil were higher than that of the bulk soil at specie level, the common bacterial species in both soils were *Candidatus Koribacter versatilis, Gemmatirosa kalamazoonesis, Paludibaculum fermentans, Jeotgalibacillus malaysiensis, Geodermatophilus obscurus, Actinomarinicola tropica, Nocardioides* sp., *Brevundimonas* sp., and *Muribaculum gordoncarteri*. The rhizosphere zones of plants are nutrient-rich areas that serve as hotspots for microbial communities, which can undergo significant changes upon pathogen invasion [16]. According to [17], [18], and [19] differences in bacterial communities observed in bulk and rhizosphere soil was as a result of “rhizosphere effect” due to the recruitment and accumulation of specific microorganisms in the rhizosphere.

Many high-throughput sequencing investigations have revealed that whereas a small percentage of microbial taxa have relative abundances more than 1%, a sizable portion of microorganisms belonging to uncommon taxa have relative abundances less than 0.1% [20,21]. Predominant microorganisms play significant ecological roles, whereas less predominant species contribute to little soil metabolic activities [22]. Nonetheless, some research has shown that the less predominant species take part in transformation of soil nutrients, redox reactions, and bioremediation [23,24,25].

The phylum Proteobacteria constitutes the largest and phenotypically most diverse bacteria. Members of this phylum are found in diverse classes (alpha-, beta-, gamma-, and epsilonproteobacteria). Bacteria such as nitrogen-fixing bacteria, ammonia-oxidizing bacteria, cellulose-decomposing bacteria, nitrifying bacteria, and denitrifying bacteria, are members of the proteobacteria family, which is important for nitrogen recycling and improves plant growth, yield, and fruit/seed quality [26]. They are also carbon monoxide oxidizers, which are composed of mesophilic and neutrophilic bacteria [27].

Actinobacteria are common phyla that breakdown plant residues *in vitro* [28]. They play multifunctional roles in plant growth and yield, nutrient cycling, soil quality, crop productivity, and plant health, making them not only the eco-friendly solution for agriculture but also for humans [29,30,31]. In fact, many actinobacterial strains are reported as potassium and/or phosphate solubilizing bacteria, symbiotic or free-living diazotrophs [32], plant growth promoting and biocontrol agents (anti-virus, antifungal etc.), mitigators of abiotic stress and plant probiotics [33].

Acidobacteria because of their preponderance and ubiquity in the soil, speculations have been made regarding their dynamic roles in vital ecological processes viz., regulation of

biogeochemical cycles, decomposition of biopolymers, exopolysaccharide secretion, and plant growth promotion. These bacteria are expected to have genes that might help in survival and competitive colonization in the rhizosphere, leading to the establishment of beneficial relationships with plants [34].

Bacteroidetes actively participate in global nutrient cycling including carbon, nitrogen, and phosphate) to positive or adverse effects on host growth and development. According to Jamil et al. [16], understanding the composition, diversity, function, and network structure of the bulk and rhizosphere microbiome in Fusarium wilt-infected soils in relation to soil physicochemical such as pH, macro–micronutrient content, and mineral content properties is an important key to controlling the spread of soil-borne disease.

4.3 Functional Profile

The major functional profile associated with bacterial community in bulk and rhizosphere soils were metabolism of amino acids, carbohydrates and energy, xenobiotic degradation, metabolism of terpenoids and polyketides, and metabolism of cofactors and vitamins. Although thousands of distinct bacterial species are found in the soil, their proportions vary extensively, the abundance of key species could influence microbial community function. The rhizosphere is an assembly point for microbes that emit carbon dioxide or respire acid, which aids in the dissolution of insoluble minerals and increases the uptake of phosphorus and other mineral elements by plants [35, 36].

The two main types of nutrients that give the body the energy it needs are lipids and carbohydrates. However, the use of proteins or amino acids as fuel is restricted to circumstances where consumption of fats or carbohydrates is insufficient to provide the required energy [37]. Important characteristics linked to microbial colonization, plant root growth, symbiotic relationships, and disease in the rhizosphere have also been demonstrated to be altered by amino acids [38]. In addition, amino acid production and other organic chemicals found in abundance near plants are often beneficial to microbes. In mutualistic relationships, some may alter plant metabolism to obtain nutrients, whereas in commensals, benefits are not reciprocated and pathogens might inflict harm to the plant [42,43]. Polyketides are often employed antibiotics that impede the growth of bacteria and fungi. In pathogen stress, the expression of polyketide cyclase genes is markedly elevated [41]. The results revealed that Fusarium-wilt infection could change the assembly of bulk and rhizosphere-associated microbial communities and attract particular disease-controlling microbes.

4.4 Alpha Diversity of the Bacterial Community

Alpha diversities of the bacterial community were found to be higher in bulk soil than rhizosphere soil. This is may be due to fine particles associated with high nutrient availability [44]. In addition, a variety of soil nutrients are released from plant roots and rhizosphere by exudation and deposition [42], enabling large populations and species diversity of microbes in rhizosphere soils as compared to bulk soils [43].

5. CONCLUSION

The result of the study helped to reveal the diverse bacterial communities in fusarium wilt infected bulk and rhizosphere soil as well as their functional profile using metagenomics studies. The study also demonstrated that the bacterial community composition and diversity differ between bulk and rhizosphere soil of FW-infected soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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