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Comparative Insights into the Microbial Diversity and Community Structure of *Striga hermonthica*-Infested Maize Rhizosphere

Olumayowa Mary Olowe [1], Ayansina Segun Ayangbenro [1], Akinlolu Olalekan Akanmu [1], Funso Raphael Kutu [2], Jude J. O. Odhiambo [3] and Olubukola Oluranti Babalola [1,*]

[1] Food Security and Safety Focus Area, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho 2735, South Africa
[2] School of Agricultural Sciences, University of Mpumalanga, Private Bag X11283, Mbombela 1200, South Africa
[3] Department of Plant and Soil Sciences, Faculty of Science, Engineering and Agriculture, University of Venda, Thohoyandou 0950, South Africa
* Correspondence: olubukola.babalola@nwu.ac.za

Abstract: The damaging competition between crops and parasitic weeds has a negative impact on agricultural productivity; however, the impact of disturbance on the soil's microbial community has received less attention. Hence, this study investigates the microbial composition and diversity of the maize rhizosphere infected with *Striga hermonthica* using a shotgun sequencing approach from two maize-growing fields (Eruwa, Nigeria and Mbuzini, South Africa). The rhizosphere soil DNA was extracted from infested soil using a Nucleospin soil genomic DNA extraction kit and sequenced on an Illumina platform. The dominant phyla were Actinobacteria, Bacteroidetes, Deinococcus-Thermus, Acidobacteria, Chloroflexi, Cyanobacteria, Planctomycetes, Verrucomicrobia, Chlorobi, Proteobacteria, Firmicutes, Nitrospirae, Thermotogae, Synergistetes, Ascomycota, Euryarchaeota, and Crenarchaeota. Bacteria phyla were observed to be of higher proportion in the rhizosphere soil samples obtained from *Striga*-infested maize field in Eruwa (Es) than those recovered from Mbuzini (Ms). The alpha diversity of microbial communities indicated insignificance differences ($p > 0.05$) between the five taxonomical groups (phylum, class, order, family, and genus), while the beta diversity produced a significant ($p = 0.01$, $R = 0.52$) difference in the microbial diversity of the infested soil. In summary, the study sheds light on the diversity and composition of the microbiome of *Striga hermonthica*-infested soil, which influences the microbial functions in the management and sustenance of plant health against parasitic weeds.

Keywords: parasitic weed; microbial distribution; shotgun sequencing; plant health; infested soil

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1. Introduction

Striga hermonthica is a hemiparasite of grasses of the *Orobanchaceae* family that affects crops in Sub-Saharan Africa (SSA). These species of *Striga* attack crops, such as maize, sorghum, finger millet, rice, and pearl millet, thereby causing major annual harvest yield reductions and decreasing crop productivity [1]. It depends entirely on its host plants for nutrients and growth, which results in significant crop damage, such as wilted silk, reduced height, thin stalks, total crop loss, or death in farmlands with a heavy infestation. The infestation of the parasitic weed is particularly pronounced in the region with poor management practices, typical poor soil, and intensive farming. The most prevalent and agronomically important *Striga* species in Sub-Saharan Africa are *Striga hermonthica*, *S. aspera*, *S. forbesii*, and *S. asiatica* [2,3]. They parasitize a wide range of cereal crops, such as *Zea mays*, *Sorghum bicolor*, *Eleusine coracana* L., and *Oryza* spp. L., and *Pennisetum glaucum* L. In addition, dicots, such as *Vigna unguiculata* L., *Nicotiana tabacum* L., *Vigna subterranean* L., and *Ipomea batata* L. are parasitized by *Striga gesnerioides* [4].

Striga spp. were identified as the main biotic constraints to the production of cereal in SSA with a significant reduction in quality and yield ranging from 30% to 90% [5,6]. According to Ejeta [7] and Scholes and Press [8], it was estimated that approximately 100 million people are faced with food insecurity due to the impacts of *Striga*, resulting in an annual economic loss of about USD 1 billion.

Many studies have shown the damaging impact of competition between crops and weeds on agricultural output; however, the microbiological component is given less attention [9–11]. A wide diversity of microorganisms, including beneficial, harmful, and neutral microbes, may interact with plant roots simultaneously in the rhizosphere during plant growth and development. Meanwhile, an extensive study has been conducted to determine how disturbances affect the composition of the soil microbial population [12,13].

Seasonal fluctuations may differ in terms of temperature, soil nutrient concentration, and rainfall. In contrast to seasons with low temperatures and soil moisture, such as spring, seasons with high temperatures and soil moisture, such as summer, enhance microbial activities and nutrient cycling [14]. Considering cultivated agricultural soils, Lin, et al. [15] showed that changes in environmental conditions had a significant impact on the diversity and composition of bacteria, particularly on the dominating bacterial population. In addition, it was found that Chloroflexi and Actinobacteria were significantly higher in summer, whereas Acidobacteria was significantly higher in spring [16]. It is therefore necessary to understand the effect of season on soil microorganisms in agricultural soil infested with *S. hermonthica*.

In addition, several researchers have reported the impact of land use types on soil properties, such as organic carbon, soil pH, and soil water content, which are closely related to the structure and diversity of microbial communities [17–19]. Grasslands for animal feed and cropland for yields are two common types of land use in agriculture. Decline in soil microbial diversity and related soils have been linked to crop management methods, such as the use of inorganic fertilizers, intense tillage, and monoculture [20]. However, various studies have revealed varied impacts of cropland and grassland management on certain microorganisms because of differences in soil characteristic functions, experimental sites, and setups [21]. Based on the results of the high-throughput sequencing, Liu, et al. [22] observed significant levels of α diversity in the farmland for both bacteria and fungi. In another study, the abundance of Verrumicrobiota increased in cropland and decreased in grasslands in European soils [23]. In addition, Sui et al. [17] revealed that microbial community structures are affected by the agricultural cultivation of wetlands by altering the pools of available soil carbon, nitrogen, and phosphorus.

The recent development in next-generation DNA sequencing (NGS) technology, including metagenomics analysis, has given the opportunity to enhance our understanding of the composition and function of soil microbial communities. The composition and diversity of fungi in soil and plant samples have been studied using molecular techniques, such as internal transcribed spacer (ITS), whereas bacteria have been studied using 16S rRNA. However, to examine the total microbial community in an environment, shotgun metagenomics has turned out to be more advantageous than other metagenomics techniques [24]. It allows the taxonomic and functional classification of the total microbial community in a particular environment. Currently, there exists a paucity of knowledge regarding the effects of seasonal variations and land use patterns on the soil microbial community in the parasitic weed infested maize fields. We, therefore, postulate that increased microbial diversity will improve the functions of the soil ecosystem and its tolerance to both seasonal changes and other associated effects of land use disturbances. However, a decrease in diversity could result in the loss of ecological systems, which would decrease the stability of the system. To ensure the sustainable provision of ecosystem services, soil microbial diversity is therefore important. Thus, we reveal the microbial diversity and composition within the maize rhizosphere in the *Striga hermonthica*-infected field using a shotgun metagenomics sequencing approach. This improved ecological understanding will open up avenues to develop practical control strategies for *S. hermonthica*.

2. Materials and Methods

2.1. Soil Sample Collection

Rhizosphere soil samples were collected from maize-cultivated fields in Eruwa, Oyo state, Nigeria (7°28′2.034″ N 3°28′21.671″ E) and Mbuzini, Mpumalanga Province, South Africa (25°55′30.90″ S 31°56′11.70″ E). Maize has been cultivated on the two sampled fields in the two locations for more than a decade, and they both had a history of *Striga hermonthica* infestation. The rhizosphere soil samples were collected in January 2022 during the dry season in Eruwa, Nigeria, with an average temperature of 33 °C and cumulative rainfall of 8 mm, while in Mbuzini, Mpumalanga Province of South Africa, rhizosphere soil samples were collected in March 2022 with an average temperature of 21 °C and cumulative rainfall of 38 mm. The soil samples were obtained randomly in three replicates at four different points on the *Striga*-infested sites, while uninfected soil samples (bulk) were also taken at a depth and diameter of 15 cm and 8 cm, respectively (Figures 1 and 2). Each sample was divided into two parts. Before analyzing the physicochemical characteristics, one part was air-dried and homogenized, while the other part was kept at −80 °C for DNA extraction.

Figure 1. Map of geographical location of sampling sites in Eruwa, Oyo State Nigeria.

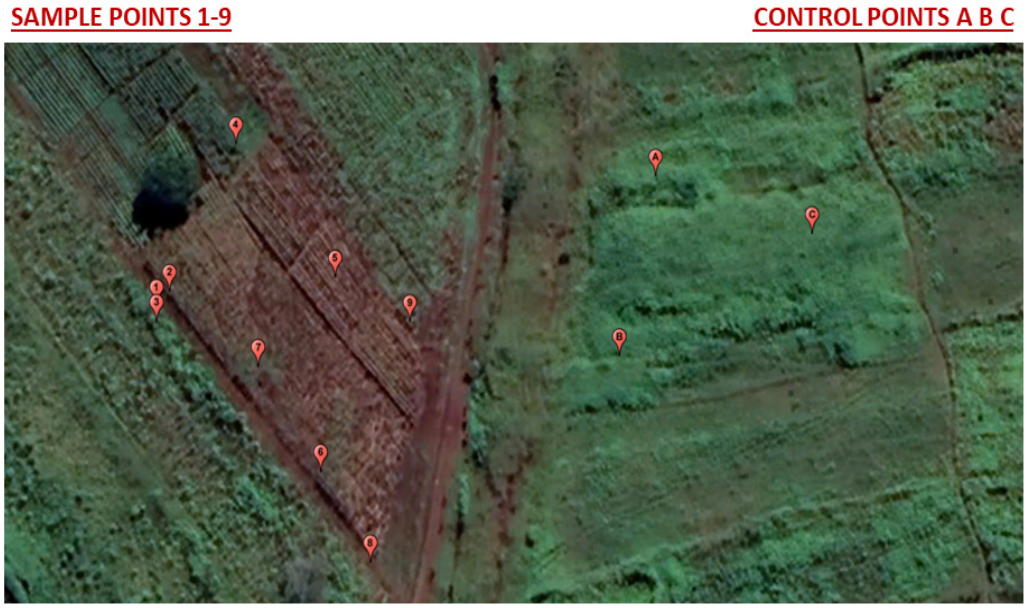


Figure 2. Map of geographical location of sampling sites in Mbuzini, Mpumalanga Province, South Africa.

2.2. Analysis of the Physicochemical Properties of Soil

A total of 500 grams of soil were taken from an air-dried sample and then sieved through a 2 mm porosity sieve to eliminate dirt. After combining the samples with distilled water at a ratio of 1:2.5 soil, the pH of the mixtures was measured using a pH meter (Jenway, Bibby Scientific Ltd., Stone, Staffs, UK). The organic matter (OM) was determined by loss of ignition method [25]; organic carbon (Org-C) was quantified by the method of Walkley and Black [26]; and total carbon and nitrogen were determined using the dry combustion method [27]. The exchangeable N-NH_4 and N-NO_3 were calculated in 1 M KCl solution in accordance with Kachurina, et al. [28], and the absorbance was measured spectrophotometrically (Helios Epsilon, Thermo Scientific, USA) at 260 nm and 220 nm. Using Bray No. 1 solution as the extractant, the phosphorus content of the soil was calculated [29]. The sulfate content of the soil was measured as described by Calvo, et al. [30], while sodium and potassium were determined by flame photometer (model FP6410, Drawell International Technology Limited, Shanghai, China) [31].

2.3. DNA Extraction of Soil Samples and Library Preparation

The rhizosphere soil DNA was extracted from a 500 mg soil sample using a Nucleospin soil genomic DNA extraction kit according to manufacturer's instructions. The Illumina sequencing platform was used for shotgun whole-genome sequencing at Novogene AIT Genomics Singapore Pte Ltd. A Nextera DNA flex library kit was used to prepare libraries containing 20–50 ng of DNA. The samples were then fragmented simultaneously, and adapter sequences were added. The concentration of the libraries was measured using the Qubit® dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA), and the average library size was determined using an Agilent 2100-Bioanalyzer. The libraries were pooled and diluted to 0.6 nM before being sequenced for 300 cycles on the Illumina NovaSeq 6000 platform.

2.4. Metagenome Sequence Annotation and Statistical Analysis

The uploaded raw metagenome sequences on a metagenomics rapid annotation subsystem technology (MG-RAST) server were subsequently subjected to quality filtering using SolexaQA to trim low-quality reads, and the metagenome datasets were dereplicated [32]. The quality control process involves the elimination of the artificial sequences,

the filtering of ambiguous bases, the determination of a minimum read size, and length filtering. Subsequently, the sequences produced were annotated using BLAT against the M5NR database, the allowing for non-redundant integration of several datasets [33,34]. More than 90% of the taxonomic classifications were assigned along with other domains in the SEED subsystems database on the MG-RAST platform. The unclassified reads related to the microbial domain were retained for statistical analysis after sorting and agglomerating the replicates' mean abundance values. The normalization option on the MG-RAST was chosen to reduce the effects of the experimental error. The identified microbiomes were organized based on the taxonomic classification, and the unclassified sequence reads were retained for statistical analysis. The relative abundance of the taxa was calculated in percentages following an independent analysis of the 24 sequences using MG-RAST. The average relative abundance values from the three replicates for each sample site (Es1, Es2, Es3 Ec and Ms1, Ms2, Ms3 Mc) were used for the statistical analysis. The accession number for these sequences may be obtained on the NCBI SRA collection under the Bioproject accession number PRJNA888840 for soil samples from Eruwa (Es) and PRJNA889583 for Mbuzini (Ms).

After the data was modified, the Shinyheatmap was used to plot the relative abundance graph of microbial communities at the phylum level [35]. PAST version 3.20 was used to calculate the Shannon diversity and Pielou Evenness indices for each sampling site, and the Kruskal–Wallis test was used to compare the indices between the sites. Primary coordinate analysis using a Euclidean distance matrix was used to determine the beta diversity, and one-way analysis of similarities was used to evaluate the variances in the community structure between the locations (ANOSIM) [36].

3. Results

3.1. Physicochemical Properties of Striga Hermonthica-Infested Maize Rhizosphere and Bulk Soils

The outcomes of a comparison of the physicochemical characteristics between the two sampling sites, (Eruwa, Nigeria and Mbuzini, South Africa) are shown in Table 1. The *S. hermonthica*-infested soil samples revealed that the pH in soil samples Es2 and Es3 was basic in comparison to Es1 and Ec (bulk soil), which were acidic, and that soil samples Ms1, Ms2, Ms3, and Mc (bulk) were all acidic. When compared to soil samples E3 and Ec (bulk), available phosphorus, organic carbon, organic matter, total nitrogen, and total carbon were more significant ($p \leq 0.05$) in soil samples E1 and E2, while potassium, sodium, ammonium, and sulfate were more significant ($p \leq 0.05$) in soil samples Ms1 and Ms2 compared to M3 and Mc (bulk) (Table 1).

Table 1. Chemical analysis of the *Striga hermonthica*-infested and bulk rhizosphere soil samples from Eruwa, Nigeria and Mbuizi, South Africa.

	pH	E.C	Potassium	Sodium	Available Phosphorus	Organic Carbon	Organic Matter	Total Nitrogen	Total Carbon	Nitrate	Ammonium	Sulfate
Es1	6.8 ^{c}	14.0 ^{g}	0.1 ^{g}	1.0 ^{h}	50.0 ^{a}	11.4 ^{c}	19.7 ^{c}	1.3 ^{c}	57.0 ^{c}	0.3 ^{f}	0.01 ^{f}	0.0 ^{e}
Es2	7.5 ^{a}	54.0 ^{d}	0.4 ^{e}	1.1 ^{f}	33.8 ^{d}	17.4 ^{a}	29.10 ^{a}	1.9 ^{a}	87.0 ^{a}	0.8 ^{e}	0.02 ^{e}	0.0 ^{e}
Es3	7.1 ^{b}	18.0 ^{f}	0.20 ^{f}	1.2 ^{e}	41.7 ^{b}	12.6 ^{b}	21.6 ^{b}	1.4 ^{b}	63.0 ^{b}	0.3 ^{h}	0.01 ^{g}	0.0 ^{e}
Ec	6.1 ^{d}	11.0 ^{h}	0.1 ^{h}	1.1 ^{g}	40.3 ^{c}	6.9 ^{d}	11.9 ^{d}	0.8 ^{d}	34.0 ^{d}	0.3 ^{g}	0.01 ^{f}	0.0 ^{e}
Ms1	5.6 ^{e}	195.0 ^{a}	569.0 ^{a}	9.6 ^{b}	2.4 ^{e}	1.02 ^{h}	0.1 ^{e}	0.1 ^{g}	1.1 ^{h}	56.6 ^{a}	5.2 ^{d}	17.8 ^{a}
Ms2	5.4 ^{f}	71.0 ^{c}	248.0 ^{c}	11.9 ^{a}	0.5 ^{h}	2.8 ^{e}	0.0 ^{h}	0.2 ^{e}	3.0 ^{e}	42.2 ^{b}	15.3 ^{a}	4.4 ^{c}
Ms3	5.4 ^{f}	101.3 ^{b}	338.3 ^{b}	9.1 ^{c}	1.2 ^{f}	1.6 ^{f}	0.04 ^{g}	0.1 ^{f}	1.8 ^{f}	40.6 ^{c}	8.1 ^{b}	7.9 ^{b}
Mc	5.3 ^{g}	38.0 ^{e}	198.0 ^{d}	5.9 ^{d}	0.7 ^{g}	1.1 ^{g}	0.1 ^{f}	0.1 ^{h}	1.2 ^{g}	22.9 ^{d}	6.3 ^{c}	1.6 ^{d}

Means with different letters along the row are significantly ($p \leq 0.05$) different. Es—Eruwa rhizosphere sample, Ec—Eruwa bulk soil sample, Ms—Mbuzini rhizosphere sample, Mc—Mbuzini bulk soil sample.

3.2. Metagenome Dataset of the Striga Hermonthica-Infested Soil across the Sampling Sites

The raw sequences uploaded for analysis on MG-RAST server showed the mean value of samples from the rhizosphere of *Striga-infested* maize from Eruwa, Nigeria were Es1—3,728,213.3, Es2—29,591,867, Es—333,321,075, and Ec—4,130,496, and those from Mbuzini, South Africa were Ms1—3,318,161.3, Ms2—5,201,988, Ms3—4,989,910.67, and Mc—5,029,225 (Supplementary Table S1). The quality of retained mean sequences after

the quality control (QC) assessment were recorded as Es1—2,946,553.3, Es2—23,440,439.7, Es3—27,489,973.5, and Ec—274,899,73.5, for Eruwa, Nigeria and Ms1—2,628,894.3, Ms2—4,361,224.5, Ms3—4,112,527.33, and Mc—4,192,873.67 for Mubizini, South Africa for the rhizosphere and bulk samples (Supplementary Table S1).

3.3. Distribution of the Major Microbiome Phyla in the Infected Soil Samples

The major bacteria, fungi, and archaea, and their abundance, are presented in Figure 3. Except for Actinobacteria and Gemmatinonadetes, the majority of the phyla are more prevalent in the rhizosphere soils from Eruwa (Es1, Es2, Es3, and Ec) than in the rhizosphere soil from Mbuzini (Ms1, Ms2, Ms3, and Mc).

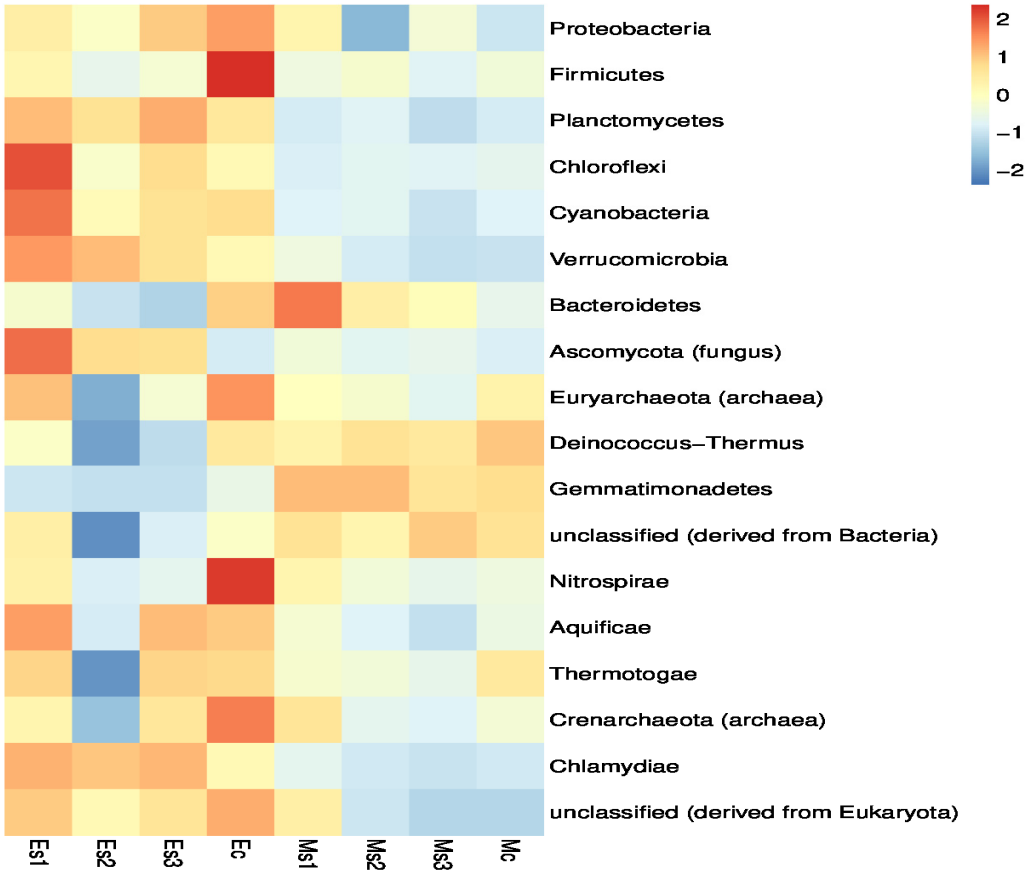


Figure 3. The predominant microbiome phyla of *Striga hermonthica*-infested soil as revealed by heatmap. Es—Eruwa rhizosphere sample, Ec—Eruwa bulk (control) sample, Ms—Mbuzini rhizosphere sample, Mc—Mbuzini bulk (control) sample.

Actinobacteria, Bacteroidetes, Deinococcus-Thermus, Acidobacteria, Chloroflexi, Cyanobacteria, Planctomycetes, Verrucomicrobia, Chlamydiae, Aquificae, Spirochaetes, Chlorobi, Deferribacteres, proteobacteia, fermicutes, Nitrospirae, Thermotogae, and Synergistetes are the main bacteria phyla, while Archaea and fungal phyla consisted of Euryarchaeota, Crenarchaeota, and Ascomycota, respectively (Figure 3). In soil samples from Eruwa, Nigeria, and Mbuzini, South Africa, which had been infested by *Striga hermonthica*, and in the bulk soil sample, the microbial community was found to be more abundant, including Es1, Es2, Es3, and Ec (bulk soil), as well as Ms1, Ms2, Ms3, and Mc (bulk soil), as shown in Figure 2. Moreover, treatment Ec (bulk soil) had the highest bacterial phyla distribution as depicted by the principal component analysis (Figure 4).

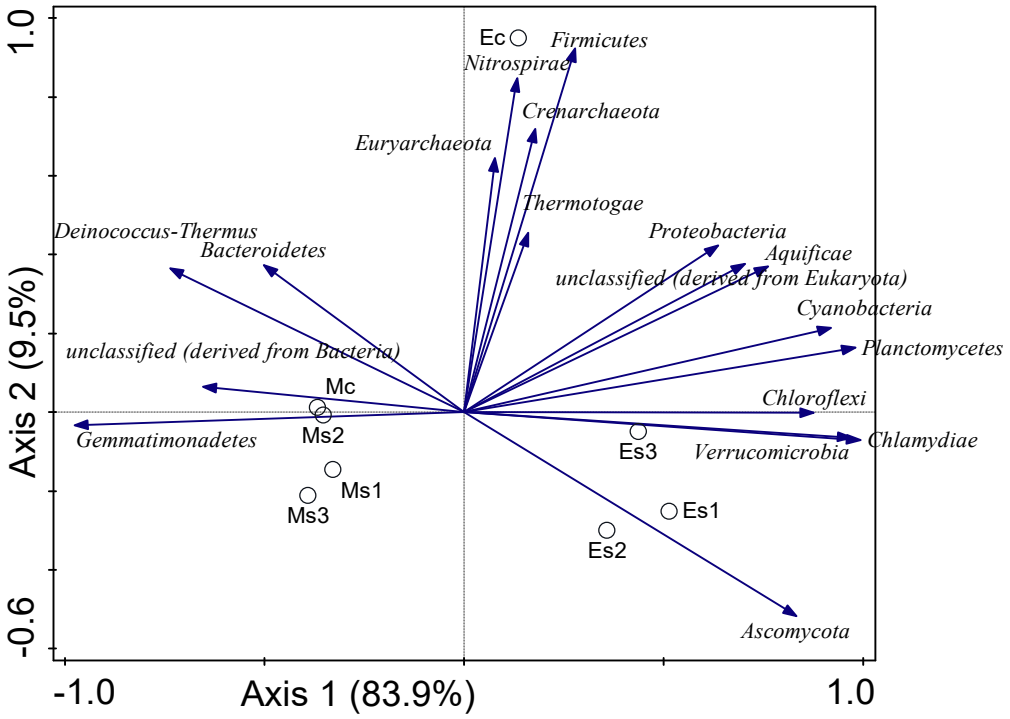


Figure 4. The PCA graph of average soil microbial community phyla of *Striga hermonthica*-infected soil. Es—Eruwa rhizosphere sample, Ec—Eruwa bulk (control) sample, Ms—Mbuzini rhizosphere sample, Mc—Mbuzini bulk (control) sample.

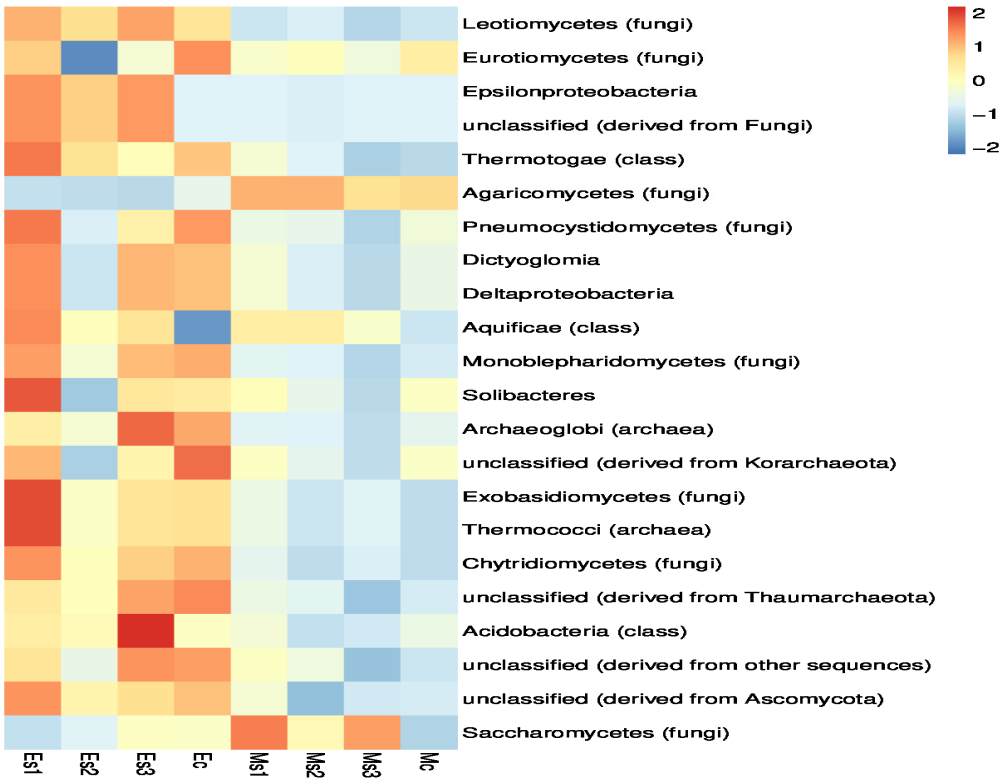
Bacterial phyla were observed to be of higher proportion in the rhizosphere soil samples obtained from *Striga*-infested maize field in Eruwa (Es) than those recovered from Mbuzini (Ms). The bacterial phyla include Proteobacteria (Es1 = 39.11%, Es3 = 40.80%, and Ec = 42.04%), *Firmicutes* (Es1 = 5.55%, Es3 = 5.23%, and Ec = 7.10%), Planctomycetes (Es1 = 4.30%, Es2 = 3.80%, Es3 = 4.50%, and Ec = 3.6%), Choroflexi (Es1 = 4.51%, Es2 = 3.38%, Es3 = 3.85%, and Ec = 3.55%), Cyanobacteria (Es1 = 3.22%, Es2 = 2.53%, Es3 = 2.76%, and Ec = 2.80%), Verrucomicrobia (Es1 = 2.83%, Es2 = 2.69%, Es3 = 2.44%, and Ec = 2.15%), Chlamidae (Es1 = 0.14%, Es2 = 0.09%, Es3 = 0.010%, and Ec = 0.11%), Bacteroidetes (Ms1 = 2.71%, Ms2 = 2.37%, Ms3 = 2.29, and Mc = 2.11%), Deinococcus-Thermus (Es1 = 0.84%, Es2 = 0.87%, Es3 = 0.86%, and Ec= 0.90%), Gemmatimonadetes (Es1 = 1.47%, Es2 = 1.47%, Es3 = 1.22, and Ec = 1.30%), and unclassified bacteria (Es1 = 0.34%, Es2 = 0.33%, Es3 = 0.35%, and Ec = 0.35%). Euryarcheota (Es1= 0.89%, Es3 = 0.81%, and Ec = 0.91%) (Ms1 = 0.82%, Ms2 = 0.81, and Mc = 0.84%) showed higher abundance in both infected soil samples collected from both Eruwa and Mbuzini, while Crenarcheota (Es1 = 0.16%, Es3 = 0.16, and Ec = 0.18%) were more predominant and showed higher abundance in infected soil from Eruwa. Ascomycota (Es1 = 1.41%, Es2 = 0.97%, and Es3 = 0.95%) was also observed to have higher proportion in *Striga*-infested soil from Eruwa (Es) (Figure 3).

With the exception of Agaricomycetes (fungi) (Ms1 = 1.47%, Ms2 = 1.47%, Ms3 = 1.22%, and Mc 1.30%) and Saccharomycetes (fungi) (Ms1 = 0.009%, Ms2 = 0.007%, Ms3 = 0.008%, and Mc = 0.005%), the class revealed that there were more fungi in Eruwa than Mbuzini. Eurotiomycetes (fungi) (Es1 = 3.01%, Es3 = 2.80%, and Ec = 3.12%) (Ms1 = 2.80%, Ms2 = 2.85%, and Mc= 2.92%) and Pneumocystidomycetes (fungi) (Es1 = 0.35%, Es3 = 0.32%, and Ec = 0.34%) (Ms1 = 0.29%, Ms2 = 0.29%, and Mc = 0.30%) showed higher abundance in both infected soil samples collected from both Eruwa and Mbuzini. Class bacteria showed higher abundance from *Striga*-infested and bulk soil samples recovered from both Eruwa and Mbuzini, with the exception of Epsilonproteobacteria (Es1 = 1.56%, Es2 = 1.31%, Es3 = 1.55%, and 0.48%), Thermotogae (class) (Es1 = 0.57%, Es2 = 0.51%, Es3 = 0.47%, and 0.53%) and Acidobacteria

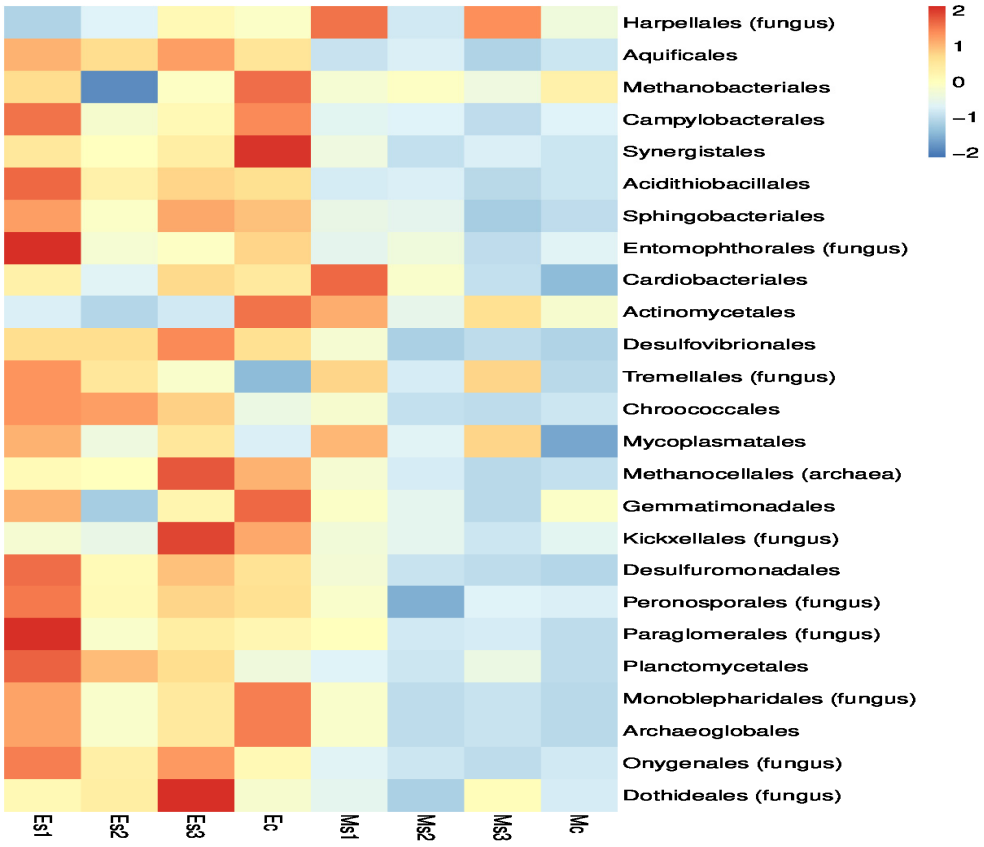
(class) (Es1 = 0.01%, Es2 = 0.01%, Es3 = 0.02%, and 0.01%). Class Archaea consisting of Archaeoglobi (Es1 = 0.06%, Es2 = 0.06%, Es3 = 0.07%, and Ec = 0.05%), unclassified (derived from Korarchaeota) (Es1 = 0.05%, Es2 = 0.04%, Es3 = 0.04%, and Ec = 0.53%), Thermococci (archaea) (Es1 = 0.03%, Es2 = 0.02%, Es3 = 0.03%, and Ec = 0.03%) and unclassified (derived from Thaumarchaeota) (Es1 = 0.02%, Es2 = 0.02%, Es3 = 0.02%, and Ec = 0.02%) were observed to show more abundance in the soil sample from Eruwa (Es) (Figure 5a).

Other bacteria were more predominant in infested rhizosphere soil and bulk samples (Es and Ec) obtained from Eruwa compared to soil samples from Mbuzini (Ms and Mc). The Order bacteria were observed to be more predominant and showed more abundance in soil samples obtained from Eruwa, with the exception of Methanobacteriales (Ms1 = 2.0%, Ms2 = 2.0%, Ms3 = 2.0%, and Mc = 2.1%) and Actinomycetales (Ms1 = 0.22%, Ms2 = 0.18%, Ms3 = 0.21%, and Mc = 0.19%). In addition, Mycoplasmatales (Es1 = 0.07% and Es3 = 0.07%) and (Ms1 = 0.07% and Ms3 = 0.07%) was in abundance in both infected soil samples collected from both Eruwa and Mbuzini. The order fungi consisting of Entomophthorales (Es1 = 0.40%, Es2 = 0.31%, Es3 = 0.32%, and Ec = 0.35%), Kickxellales (Es1 = 0.03%, Es2 = 0.03%, Es3 = 0.03%, and Ec = 0.03%), Peronosporales (Es1 = 0.006%, Es2 = 0.005%, Es3 = 0.006%, and Ec = 0.006%), Paraglomerales (Es1 = 0.004%, Es2 = 0.002%, Es3 = 0.003%, and Ec = 0.003%), Monoblepharidales (Es1 = 0.003%, Es2 = 0.003%, Es3 = 0.003%, and Ec = 0.003%), Onygenales (Es1 = 0.0005%, Es2 = 0.0004%, Es3 = 0.0005%, and Ec = 0.0004%), and Dothideales (Es1 = 0.0003%, Es2 = 0.0003%, Es3 = 0.0004%, and Ec = 0.0002%) were observed to be more predominant and showed more abundance in the infected rhizosphere soil samples obtained from Eruwa (Es) compared to Mbuzini (Ms). The fungi that were in abundance in both sampling sites include Harpellales (Es1 = 5.7%, Es2 = 5.9%, Es3 = 6.1%, and Ec = 6.0%) (Ms1 = 6.6%, Ms2 = 5.8%, Ms3 = 6.5%, and Mc = 6.0%) and Tremellales (Es1 = 0.1% and 0.1%) and (Ms1 = 0.1% and 0.1%). Methanocellales (archaea) (Es1 = 0.07%, Es2 = 0.07%, Es3 = 0.08%, and Ec = 0.07%) were also observed to have a higher proportion in *Striga*-infested soil from Eruwa (Es) (Figure 5b).

Bacterial families are predominant and showed high abundance in the *Striga*-infested rhizosphere and bulk soil samples recovered from Eruwa (Es). Meanwhile, Nannocystaceae (Ms = 0.051%, Ms2 = 0.062%, Ms3 = 0.060%, and Mc = 0.63%) showed more abundance in *Striga*-infested and bulk soil samples obtained from Mbuzini (Ms). In addition, *Thermaceae* was observed to be abundant in Es1 = 0.027%, Es3 = 0.023%, and Ec = 0.026%, Ms1 = 0.024%, Ms2 = 0.023%, and Mc = 0.024%, Heliobacteraceae, Es3 = 0.14%, Ec = 0.13%, and Ms1= 0.13% and Ms3= 018%, unclassified (derived from Thermotogales) Es1 = 0.048%, Ec = 0.062%, and Ms1 = 0.052% and Ms2 = 0.049%, Hahellaceae Es1 = 0.045%, Es3 = 0.042%, Ec = 0.051%, and Ms1 = 0.044%, Ms2 = 0.043%, and Mc = 0.044%, and Rickettsiaceae Ec= 0.032%, Ec = 0.048%, and Ms1 = 0.031%, Ms = 0.031%; that is, they showed abundance in the soil samples from both sites. All fungal families present showed high abundance in soil samples from Eruwa, compared to Mbuzini except for Xylariaceae (fungus) (Ms1 = 0.20%, Ms2 = 0.23%, Ms3 = 0.23%, and Mc = 0.25%). Ferroplasmaceae (archaea) was more predominant in soil samples Ms1 = 0.21%, Ms2 = 0.23%, Ms3 = 0.23%, and Mc = 0.24% recovered from Mbuzini, while Nitrosopumilaceae (archaea) was more abundant in Eruwa soil samples (Ms1 = 0.87%, Ms2 = 0.65%, Ms3 = 0.70%, and Mc = 0.72%) when compared to Eruwa (Es) (Figure 5c).



(**a**)



(**b**)

Figure 5. *Cont.*

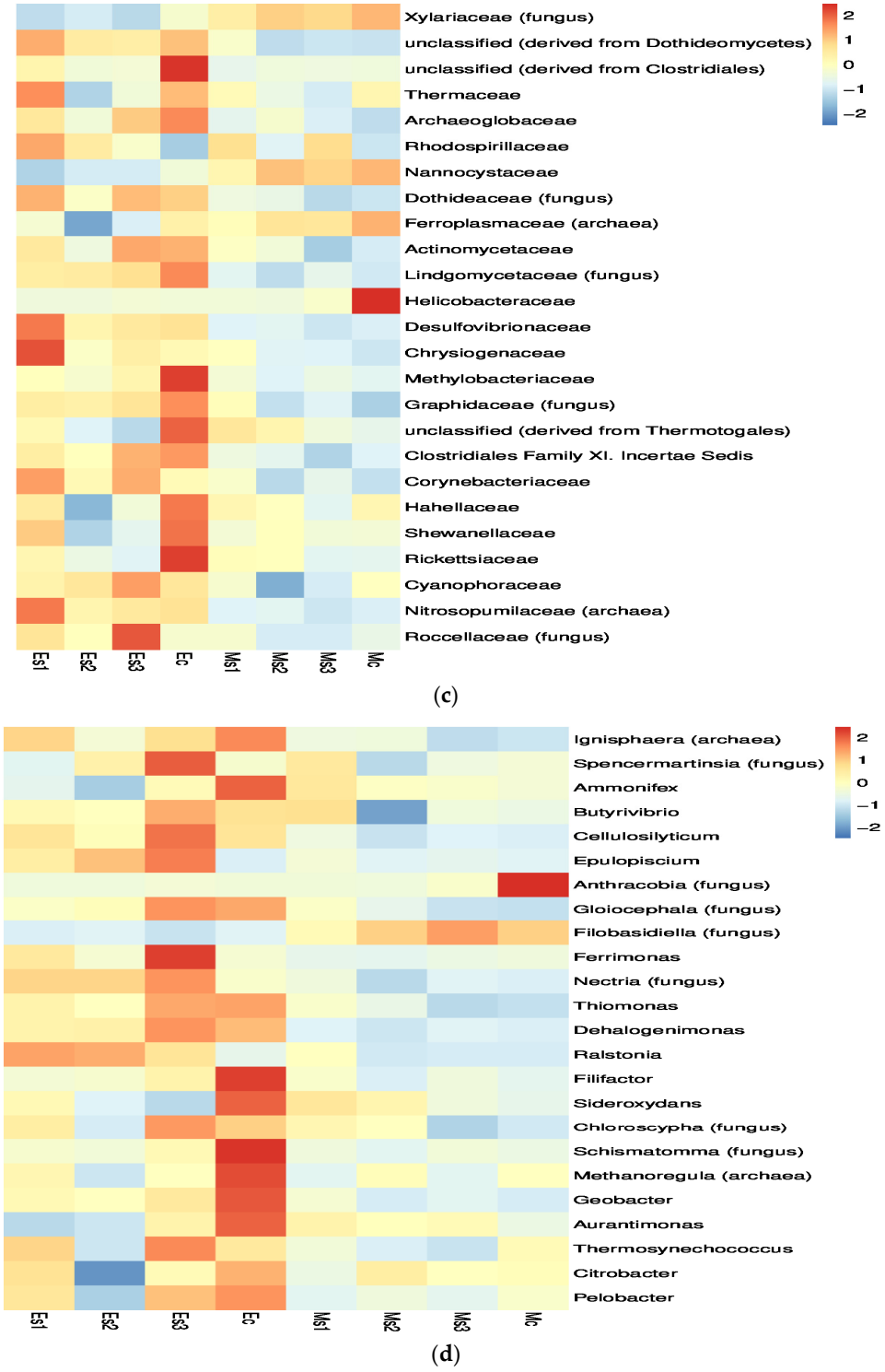


Figure 5. (**a**) The main microbiome class (**b**) order (**c**) family and (**d**) genus of *Striga hermonthica*-infested soil as revealed by heatmap. Es—Eruwa rhizosphere sample, Ec—Eruwa bulk (control) sample, Ms—Mbuzini rhizosphere sample, Mc—Mbuzini bulk (control) sample.

Generally, there were more predominant bacterial genera in infested and bulk rhizosphere soil samples from Eruwa (Es1, Es2, Es3, and Ec), with the exception of *Epulopiscium* (Ms1 = 0.81%, Ms2 = 0.65%, Ms3 = 0.67%, and Mc = 0.64%) obtained from soil samples from Mbuzini. In addition, there were bacteria genera that showed abundance in some of the soil samples obtained from both sampling sites, such as, *Ammonifex* (Es3 = 0.003% and Ec = 0.004%)

(Ms1 = 0.003% and Ms2 = 0.003%), *Butyrivibrio* (Es1 = 0.23%, Es2 = 0.22%, Es3 = 0.24%, and Ec = 0.23%) (Ms1 = 0.24%), *Ralstonia* (Es1 = 0.08%, Es2 = 0.08%, and Es3 = 0.07%) (Ms1 = 0.06%), *Filifactor* (Es1 = 0.05%, Es2 = 0.05%, Es3 = 0.05%, and Ec = 0.07%) (Ms1 = 0.05%), *Sideroxydans* (Es1 = 0.05%, Esc = 0.06%) (Ms1 = 0.05%, Ms2 = 0.05%, and Ms3 = 0.04%), *Aurantimonas* (Es3 = 0.03%, and Ec = 0.03%) (Ms1 = 0.03% and 0.03%), *Citrobacter* (Es1 = 0.02%, Ms3 = 0.02%, and Ec = 0.02%) (Ms2 = 0.02%, Ms3 = 0.02%, and Mc = 0.02%), and *Pelobacter* (Es1 = 0.02%, Es3 = 0.02%, and Ec = 0.02%) (Mc= 0.02%). Most of the fungal genus showed abundance in both sampling sites, except *Nectria* (fungus) (Es1 = 0.23%, Es2 = 0.23%, Es3 = 0.26%, and Ec = 0.20%) and *Schismatomma* (fungus) (Es1 = 0.09%, Es2 = 0.09%, Es3 = 0.09%, and Ec = 0.12%), which were more abundant in rhizosphere soil samples obtained from Eruwa. Similarly, *Filobasidiella* (Es1 = 0.36%, Es2 = 0.42%, Es3 = 0.45%, and Ec = 0.42%) obtained from Mbuzini, and *Ignisphaera* (archaea) showed more abundance than those from Eruwa (Es1 = 0.012%, Es3 = 0.018%, and Ec = 0.020%) soil samples, while *Methanoregula* (archaea) showed similar abundance in from both sampling sites (Es1 = 0.08%, Es3 = 0.08%, and Ec = 0.10%) (Ms2 = 0.08% and Mc = 0.08%) (Figure 5d).

3.4. Diversity Indices of the Microbiome of the Striga-Infested Sampling Sites

The microbial community of *Striga*-infected rhizosphere soil and bulk samples of the two sampling sites were evaluated using Pielou evenness and Shannon diversity. With Kruskal–Wallis one-way analysis of variance, alpha diversity of microbial communities indicated insignificant differences ($p > 0.05$) between the five taxonomical groups (phylum, class, order, family, and genus) (Table 2). In addition, the PCoA analysis revealed the composition of the *Striga*-infected soil and bulk samples sites showed a more significant difference between Es1, Es2, Es3, and Ec compared to Ms1, Ms2, Ms3, and Mc (Figure 6). The PCoA inference was validated by analysis of similarity (ANOSIM) (p =0.01, R = 0.52).

Table 2. Alpha diversity indices of microbiomes across the sampling sites.

		Es1	Es2	Es3	Ec	Ms1	Ms2	MS3	Mc	*p*-Value
Phylum	Simpson_1-D	0.767	0.75	0.755	0.74	0.73	0.719	0.711	0.714	0.998
	Shannon_H	1.936	1.834	1.88	1.836	1.77	1.733	1.694	1.709	
	Evenness_eˆH/S	0.289	0.2609	0.273	0.2613	0.24	0.2357	0.227	0.23	
Class	Simpson_1-D	0.672	0.658	0.641	0.657	0.79	0.77	0.797	0.783	0.976
	Shannon_H	1.651	1.603	1.564	1.684	1.94	1.878	1.981	1.929	
	Evenness_eˆH/S	0.2084	0.199	0.1911	0.215	0.28	0.262	0.29	0.275	
Order	Simpson_1-D	0.793	0.772	0.777	0.792	0.74	0.764	0.732	0.756	0.998
	Shannon_H	1.952	1.884	1.894	1.949	1.88	1.915	1.845	1.892	
	Evenness_eˆH/S	0.2817	0.263	0.266	0.281	0.26	0.272	0.253	0.265	
Family	Simpson_1-D	0.852	0.843	0.845	0.851	0.87	0.875	0.87	0.874	1
	Shannon_H	2.256	2.234	2.237	2.272	2.38	2.386	2.378	2.349	
	Evenness_eˆH/S	0.382	0.374	0.375	0.388	0.43	0.435	0.432	0.491	
Genus	Simpson_1-D	0.815	0.768	0.76	0.887	0.85	0.857	0.861	0.849	0.991
	Shannon_H	2.314	2.152	2.143	2.605	2.41	2.438	2.434	2.323	
	Evenness_eˆH/S	0.405	0.344	0.341	0.541	0.45	0.458	0.456	0.408	

p-Value was based on Kruskal–Wallis one-way analysis of variance. Es—Eruwa rhizosphere sample, Ec—Eruwa bulk (control) sample, Ms—Mbuzini rhizosphere sample, Mc—Mbuzini bulk (control) sample.

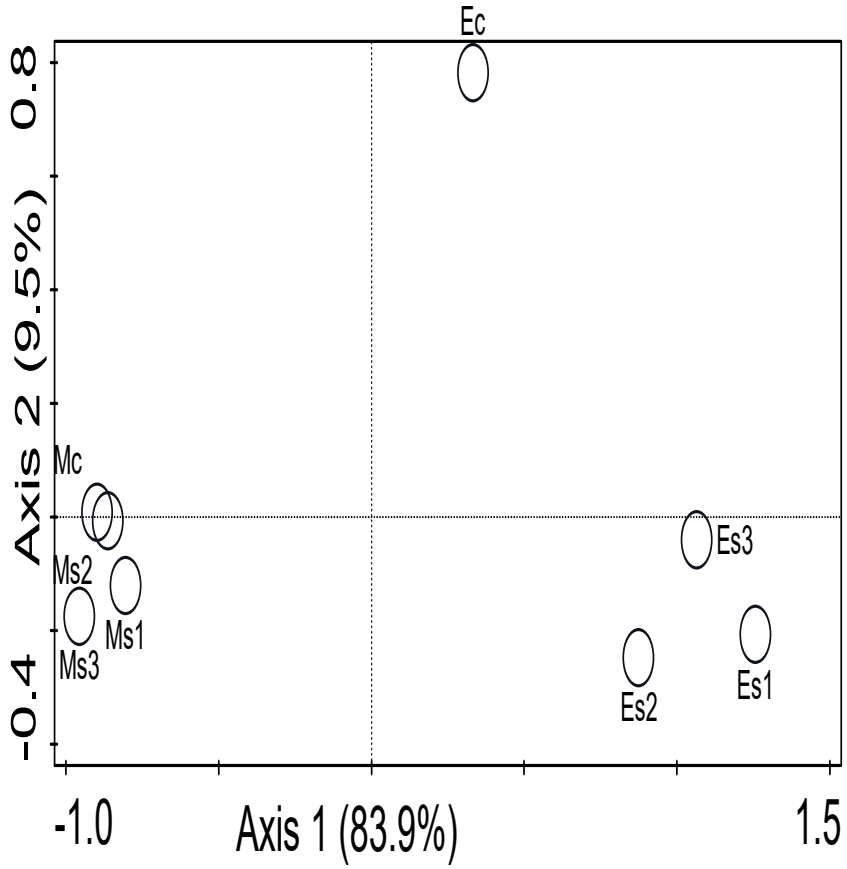


Figure 6. PCoA graph of the soil microbial community based on Bray–Curtis dissimilarities. Es—Eruwa rhizosphere sample, Ec—Eruwa bulk (control) sample, Ms—Mbuzini rhizosphere sample, Mc—Mbuzini bulk (control) sample.

3.5. Influence of Environmental Variables on the Microbial Community

Canonical correspondence analysis (CCA) was used to depict the relationship between the measured physicochemical factors and the relative abundances of the microbiome phyla. Based on their impacts on the dispersion of microbes between the two locations and a significant test utilizing forward selection of environmental factors, four physicochemical parameters—available phosphorus, nitrate, sulfate, and pH—were selected (Figure 7). The CCA established that the microbiome structure of the two sampling sites was influenced by soil physicochemical variables with the CCA permutation test = 0.00. Chloroflexi, Planctomycetes, and Aquifica were positively correlated with available phosphorus but negatively correlated with pH, nitrate, and sulfate. However, Chlamydiae, Ascomycota, Acidobacteria, and Verrucomicrobia were positively correlated with available phosphorus and were negatively correlated with nitrate, pH, and sulfate. Likewise, Gemmatimonadetes, Crenarchaeota, Nitrospirae, Firmicutes, Deinococcus-Thermus, Bacteroidetes, and Proteobacteria were positively correlated with nitrate and sulfate but negatively correlated with available phosphorus and pH (Figure 7). The forward selection of environmental variables was used to test the factors that best explain the variation in the microbial community. CCA showed that available phosphorus significantly (p = 0.006) contributed 86.0% of the variation, pH insignificantly (p = 0.002) contributed 78.5% variation, sulfate insignificantly (p = 0.084) contributed 35.7%, while pH insignificantly (p = 0.034) contributed 66.8% of the variation of the microbial community in the two sampling sites (Table 3).

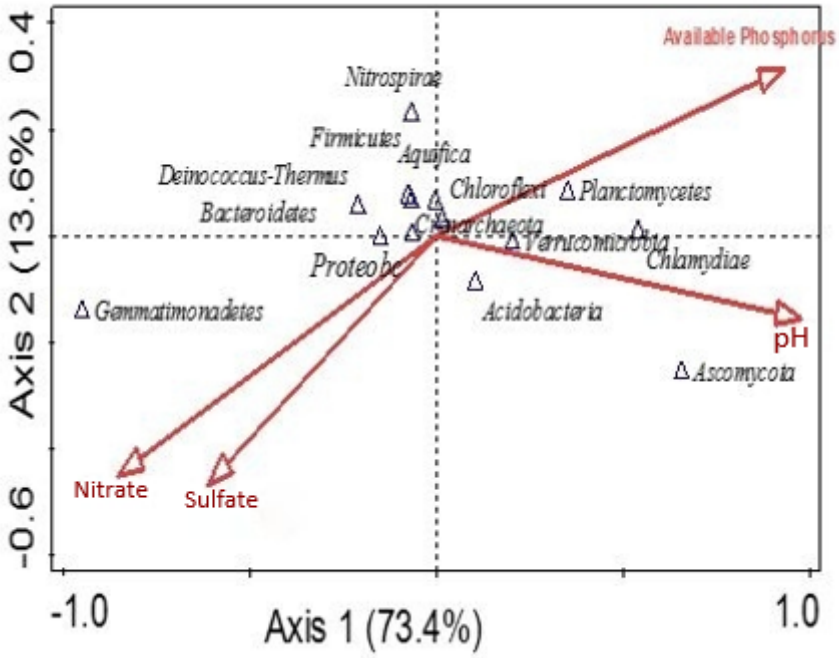


Figure 7. The forward selection of environmental variables predicts the variance and influence on the microbial diversity as explain by canonical correspondence analysis (CCA).

Table 3. Impact of environmental parameters on the microbiome composition of soil sample as depicted by the forward selection of environmental variables.

Physicochemical Parameter	Contribution %	Pseudo F	*p*-Value
Available Phosphorus	86.0	22.0	0.006
pH	78.5	15.2	0.002
Nitrate	66.8	9.4	0.034
Sulfate	35.7	2.9	0.084

4. Discussion

Soil microbiomes significantly boost their genetic potential, aiding in nutrient uptake, promoting plant growth, and increasing their ability to withstand abiotic challenges. *Striga hermonthica* is an important yield-limiting factor of a number of staple crops, especially in developing nations such as Nigeria and South Africa. The two infested sites, which are located in the two countries, have some notable differences in their microbial communities. There are a few bacterial phyla in common, their relative abundances vary, and the similarities are mostly confined to the family level and higher. This emphasizes that different microbiomes with associated climatic and anthropogenic activities represent significant challenges to adaptation. Some phyla, such as Proteobacteria, Crenarchaeota, and Firmicutes are more abundant in Eruwa than in Mbuzini, and vice versa, suggesting a certain evolutionary conservatism in microbial niches. It is possible that many of these differences are due to climatic conditions and land use types on soil properties. Here, in this study, we employed shotgun sequencing approach to unveil the microbial diversity and composition within the maize rhizosphere infected with *Striga hermonthica* from two locations with different weather conditions and land use types.

The diversity and composition of the microbiome from the two locations are strongly influenced by soil pH, and it has been discovered that pH was primarily responsible for driving microbial community in agricultural soils [37,38]. Some rhizosphere soil samples obtained from Eruwa (Es) showed that they had alkaline pH values, while others were acidic (Es1 = 6.8, Es = 7.5, Es3 = 7.1, and Ec = 6.1). This indicates that the soil pH is in the optimal range (pH 6–8) for microbial growth [39], and those soil samples of Mbuzini are

all acidic (Ms1 = 5.6, Ms2 = 5.4, Ms3 = 5.4, and Mc = 5.3) (Table 1). The soil acidification in Mbuzini may be a result of the intensive use of nitrogen fertilizers, which could be the main cause of the decrease in soil pH [40]. Our result is in line with those earlier reported by Fierer and Jackson [38] and Rousk, et al. [41], in which soil pH played a significant role in defining microbial diversity by influencing the homeostasis of microbes or regulating the availability of soil nutrients.

Furthermore, it has been established that the proliferation of soil microbiome is influenced by soil nutrient status [42]. It is observed that the microbial community composition is sensitive to the levels of nitrogen, phosphorus, and potassium in soil [43]. Moreover, increasing N input suppresses the soil microorganisms in unmanaged ecosystems [44]. In this study, the high significant impact of organic carbon on soil samples obtained from Eruwa was in agreement with the results of other studies that showed that OC had the greatest effect on soil bacterial community structure [45,46]. The presence of organic carbon also indicates that microbial communities exhibit a uniform distribution pattern regardless of soil water content and depth [47]. Furthermore, the majority of bacteria, fungi, and Actinomycetes are demonstrated to be positively correlated with soil organic carbon concentration [48]. For available phosphorus, the result indicates that the available phosphorus had a stronger influence on the microbial community composition of the soil samples recovered from Eruwa (Es1 and Es3), which is in accordance with the studies of Yao, et al. [49] and Fierer and Jackson [38]. This finding implies that the availability of available *p* in acidic soils is a more significant mediator of microbial community composition.

The composition of the microbiome of the soil samples from Eruwa and Mbuzini based on their abundances are as follows. Proteobacteria showed the largest relative abundance in Eruwa ((Es1 = 39.11%, Es3 = 40.80%, and Ec = 42.04%) (Figure 2). This is in line with the results of other studies suggesting the dominance of Proteobacteria [50,51]. The microbial phyla include Proteobacteria, Firmicutes, Planctomycetes, Choroflexi, Cyanobacteria, Verrucomicrobia, Gemmatimonadetes, and unclassified bacteria Chlamidae, Euryarcheota, Crenarcheota, and Ascomycota comprise most of the microbiome of soil samples from Eruwa, with differences in abundance and composition at lower taxonomic levels, which may be a result of the soil nutrients, environmental condition, and anthropogenic activities [52–55], thereby influencing soil microbial colonization and thus changing the soil microbial community occupancy. Ascomycota is the dominant fungal phylum, and most Ascomycota are saprophytic [56], which can decompose recalcitrant substances, such as lignin and keratin, and thus improve soil quality.

There was no significant difference in the richness and diversity of the microbiome of *Striga*-infested soil from both sites, revealing a close index of the microbial community at each level. This is not in agreement with Dube, et al. [57], who reported that there was higher bacterial diversity of land under intensive agricultural production for over 50 years compared to uncultivated land. The principal component analysis (PCA), PCA 1, contributed nearly 83.9% to the total variations, indicating a higher abundance of microbial phyla in soil sample Ec (bulk soil) from Eruwa. Likewise, the principal coordinate analysis (PCoA) contributed 83.9% variation in the microbial communities of the soil samples from Eruwa, with higher disparity recorded in Eruwa (Es1, Es2, Es3, and Ec). It can be deduced from this observation that microbial diversity in soil plays an essential role in crop production sustainability by enriching the soil and reducing the biotic and abiotic stressors, such as *S. hermonthica* [58,59].

5. Conclusions

In this study, shotgun metagenomics was employed to determine the composition and diversity of the microbiome of *S. hermonthica*-infested maize rhizosphere soils from Eruwa, Nigeria and Mbuzini, South Africa, in order to establish a link between the microbiome and physicochemical characteristics of the *S. hermonthica*-infested soil that might have been impacted by weather conditions and human activities. Our findings established that differences in the microbial community structure of the two sampling sites are more likely

from climatic conditions and the type of land use practiced on the two sampling sites. Therefore, this study will help in further understanding of the microbial functions in the management of abiotic and biotic stresses and the sustenance of plant health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13053260/s1, Table S1: Diversity evaluation of the shotgun metagenome analysis from the rhizosphere of Striga-infested maize from Eruwa, Nigeria and Mbuzini, South Africa.

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Conflicts of Interest: The authors declare no conflict of interest.

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