Metagenomic analysis of rhizospheric soil microbial communities associated with *Striga* infested maize field

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Declaration

I declare that this dissertation, entitled "Metagenomic analysis of rhizospheric soil microbial communities associated with *Striga* infested maize field", is the outcome of the original research that was carried out by me under the supervision of Professor Olubukola Oluranti Babalola. I, the undersigned, therefore state that this dissertation, submitted to North-West for the award of Master of Science in Biology in the Faculty of Natural and Agricultural Sciences, has never before been submitted by me or anyone else in whole or in part for the award of any degree. All information from published literature has also been appropriately acknowledged.

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Date: …………………………………………………………

Supervisor's name: Professor Olubukola Oluranti Babalola

Signature:

Date: ……………………………………………………………

Dedication

I dedicate this dissertation to the Almighty God.

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General Abstract

The most problematic weed in sub-Saharan Africa (SSA), often known as witchweed, is an obligate root hemiparasite of grasses that significantly reduces and severely damages food crops. In this study, the structure and functional diversity of the microbial communities in the *Striga hermonthica*-infested maize rhizosphere were compared to those in unaffected soil. We hypothesize that microbial structure and diversity are influenced by *Striga* infestations. To evaluate the microbial diversity in the soil and the availability of biocontrol organisms in the infested soil using shotgun sequencing in this study, we compared soil samples from *Striga*-infested soil with those from bulk soil. The major bacteria phyla observed in all samples were Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Acidobacteria, Chloroflexi, Verrucomicrobia, and Cyanobacteria. The archaea phyla were Crenarchaeota and Euryarchaeota and the predominant phyla of fungi included Ascomycota and unclassified Fungi. The alpha diversity analysis showed that there was no significant difference in the abundance of microbiomes between infested and uninfected soil, while the beta diversity analysis showed a significant difference at $p > 0.05$. Additionally, the major nutrient pathways relating to plant growth-promotion properties that were identified were those involving carbohydrates, phosphorus, nitrogen, and secondary metabolism. There have also been notable traces of functional categories present in the maize rhizosphere despite the invasion of this parasite. For the biological management of *Striga* for sustainable agriculture, mapping out strategies to isolate novel biocontrol agents and exploring genes that can be used to effectively control this weed will be helpful.

Keywords: Plant-microbe interactions, biocontrol agents, SEED subsystem, shotgun metagenomics, parasitic weed

Chapter 1

General Introduction

1.0 Background information

Most people in the world, especially in Africa, rely on maize as their primary source of nutrition (Alori et al. 2019). With roughly 3.1 million acres of land, South Africa produces close to eight million tons of maize crop each year (Du Plessis 2003). Increased yields and better management techniques are required to meet the growing demand for maize (Sessitsch et al. 2012). However, many biotic and abiotic restrictions, including *Striga* weed, are limiting maize outputs. This causes food scarcities for a hundred million people and amplifies economic problems, thus resulting in yearly losses of more than $10 billion economic value (Dafaallah 2019; Akaogu et al. 2019; Kamara et al. 2020). When compared to other *Striga* species, *Striga hermonthica* has the most damaging effect on grain crops, particularly maize (Dafaallah 2019; Oula et al. 2020a). Natural substances, such as amino acids, sugars, mucilage, nucleosides, and organic acids that are passively secreted by maize roots aid in attracting microorganisms from the bulk soil to the rhizospheric environment (Edwards et al. 2015).

Interactions of different plant species, root exudates, soil properties, and many other elements occur in the rhizosphere, a special ecological niche that determines the composition of microbial communities (Mendes et al. 2013; Singh et al. 2004). In comparison to those in the bulk soil, organic compounds released by roots can sustain large amounts of microbial biomass and intensive metabolic activity, as well as support the more active and unique microbial communities emerging in the rhizosphere. In the rhizosphere, the relationship between plants and microbes regulates crucial biogeochemical processes, notably the nutrient cycle and greenhouse gas emissions. While certain pathogenic microorganisms threaten plant health, numerous helpful microorganisms in the rhizosphere provide plants with mineral nutrients and phytohormones, and boost a plant's defenses against phytopathogens (Mendes et al. 2013; Marschner et al. 2004). Therefore, it is critical to assess the functional capacity of rhizosphere microbial communities in order to establish the relationship between the activities of the microbial community in the soil and plant development and health (Li et al. 2014).

Shotgun metagenomics is now recommended above other methods for this purpose since it allows for the functional profiling of a microbial community in its environment (De Tender 2017). According to this study's hypothesis, the functional diversity of rhizospheric microbiomes will be dominant in the soil even in spite of the invasion of the parasitic *Striga* there. The shotgun metagenomics technique was used for the first time in this study to examine the functional diversity of rhizospheric microbial communities in the root of a maize plant infected with *Striga hermonthica*.

1.1 Problem statement

Maize is cultivated in almost all countries across the world, with the cultivated fields occupying an area of approximately 160 million hectares (Da Silva et al. 2017). Maize in sub-Saharan Africa is considered the most important crop with the greatest yield potential among all the other agricultural crops (Cairns et al. 2013; Prasanna 2012). *Striga hermonthica* is an obligate root hemiparasite of grasses in sub-Saharan Africa causing devastating crop losses annually. It, commonly attacks cereals, including maize (*Zea mays L.)* (Spallek et al. 2013). Ejeta and Butler (1993) and Musyoki et al. (2015) rated *Striga* spp. as a major biotic threat to cereal production in sub-Saharan Africa, with yield losses ranging from 30% to 90%. These figures, however, exclude its huge impact on agricultural commodities (Parker 2009). The impacts of *Striga* spp. have been estimated to cause about 100 million people to suffer food insecurity, while also resulting in approximately $ 10 billion losses in economic damage annually (Ejeta 2007; Waruru 2013). *Striga* constitutes an unrelenting constraint on food productivity. Given the significant crop yield losses owing to *Striga* infestations, there is a need to analyse the microbiomes that are present in the rhizosphere that can be used as biocontrol agents against *Striga* infestations.

1.2 Justification

Although several methods, such as cultural methods, chemical control, host resistance and biological control, have all been used in the control of this parasitic weed, none can individually reduce *Striga* infestations to a non-economic level (Kountche et al. 2019). However, the integration of two or more methods has proved to be successful over time. On the other hand, the negative effect of chemicals on the soils has allowed for an increase in the development of bioinoculants. They benefit plant growth by improving the production of plant growth-promoting hormones, reducing plant pathogens, and increasing the nutrient uptake (Magallon-Servín et al. 2020b). One of the most promising strategies for identifying the microbiome associated with most agricultural crops is metagenomics. Shotgun metagenomics is required in order to gain an overview of the rhizospheric microbiome associated with *Striga*-infested maize fields. As a result, the focus of this study is on the microbial communities associated with the rhizospheric soils of *Striga*-infested maizefields.

1.3 Aim and Objectives

1.3.1 Aim

The aim of the study is to analyse the microbial communities associated with *Striga*-infested maizefields.

1.3.2 Objectives

The specific objectives of this study include the following:

I. Determine the taxonomic and structural diversity of the microbial communities present in the rhizosphere of an infested maizefield;

II. Determine the functional diversity of the microbial communities present in the rhizosphere of an infested maizefield;

III. Evaluate the functional profiles of the rhizospheric microbiome.

Chapter two

Striga hermonthica: a highly destructive pathogen in maize production

2.0 Abstract

Maize is a major cereal crop in sub-Saharan Africa (SSA). Subsistence farmers mostly but unknowingly plant it in areas where the parasite *Striga hermonthica's* action often reduces the harvest. This parasite is an obligate root hemiparasite of grasses commonly known as witchweed and is the most challenging weed in SSA that causes significant constraints and devastating losses to food production annually. Several *Striga* control strategies have been examined over the past decades, such as cultural practices, biological control by parasitic fungi and bacteria, and the use of chemicals to manage *Striga* in cereal. In addition, the integration of two or more methods to control *Striga* weed has proven to be more effective than using a single control method. Due to the residual effect of chemicals on soils, there has been a rising need to develop a more environmentally friendly approach to combat this parasitic weed. Using bioinoculants in the control of this root parasitic weed is economically safe, socially acceptable, and environmentally friendly compared to chemical methods. Constraint to maize production, the deleterious effect of *S. hermonthica* on crops, and the methods used in combating this parasitic weed and its prospects were discussed in this study.

Keywords

Striga hermonthica, biological control, plant-microbe interactions, bioinoculants, pests and diseases management, food security.

2.1 Introduction

Maize (*Zea mays* L.) is considered a very significant cereal crop and probably has the greatest yield potential among crops in sub-Saharan Africa (SSA) as it occupies more than 50% of aggregate land given entirely to crop production (Gasura et al. 2019). Due to the ease of processing, high yield potential, and low cost of production, maize is considered very significant in ameliorating food insecurity in SSA (Stanley et al. 2021a). However, there are several biotic and abiotic constraints limiting its production. This includes the parasitic weed; *Striga,* causing food scarcity faced by 100 million people, thereby increasing economic challenges that amount to more than 10 billion USD losses yearly (Dafaallah 2019; Oula et al. 2020b). *Striga hermonthica* among diverse *Striga* species causes the most devastating loss to cereal crops including maize. Crop losses due to *Striga* cause yield reduction of up to 100%, excluding the high effect on the agricultural product (Akaogu et al. 2019; Kamara et al. 2020).

S. hermonthica, generally known as purple witchweed, is a challenging weed to crops in SSA (Gowda et al. 2021). It is a hemiparasite of grasses belonging to the family *Orobanchaceae.* This species of *Striga* causes a destructive harvest reduction annually and attacks crops, such as sorghum, sugarcane, finger millet, pearl millet, rice, cowpea, and maize, causing a decimating effect on the yield of crops (Mudereri et al. 2020; Stanley et al. 2021a). This parasitic weed depends solely on its host plant for nutrients and growth, which results in substantial damage to crops, such as chlorosis, wilted silk, thin stalk, reduced height, and total loss of crops in farmlands with high invasion (Mbuvi et al. 2017a; Menkir et al. 2020b). The invasion of this parasite is prominent in places with characteristic poor soils and intense cultivation with poor management practices (Gowda et al. 2021).

Several methods, such as the traditional, chemical, and biological methods, and host resistance have been used to combat this parasitic weed. Traditional practices including hand pulling, crop rotation, intercropping, trap or catch crop planting, push-pull technology, and soil fertility improvement (nitrogen fertilization) are used. Chemical management practices, for example, weedicide (imazapyr), genetic control, resistant crop varieties, suicidal germination, biological control agents, such as fungi and bacteria, and certain insects have been used (Parker and Riches 1993; Aly 2007; Sauerborn et al. 2007; Naoura et al. 2021). None of these methods have been able to eradicate the incidence of *S. hermonthica* alone, however, the incorporation of two or more methods have proven effective in eradicating it completely. The adverse effect of chemical compounds on soils has led to increased use of microbial inoculants, which are advantageous to plant development by enhancing its secretion of plant growth hormones, increasing nutrient uptake, and reducing plant pathogens (Magallon-Servín et al. 2020a). Therefore, the main objective of this review is to elucidate the challenges and economic importance of *Striga* weed on the maize plant and review eco-friendly bioinoculants for combating the weed in SSA.

2.2 Maize plant production and its importance

Maize is a perennial crop that belongs to the family *Poaceae* (*Gramineae*) (Saboor et al. 2021). This crop is produced globally, and it is also one of the top three important cereals after rice and wheat. Maize is cultivated on over 40 million hectares in SSA countries and constitutes more than 25% of daily caloric intake in both southern and eastern Africa (Acevedo-Siaca and Goldsmith 2020). It serves as a staple food and feed for livestock and is a means of livelihood and food security for 70% of the South African population (Mxolisi 2021). Modern maize grain contains 4% oil, 73% starch, 9% protein, and 14% other nutrients comprising fiber (Maqbool et al. 2021). It is also a primary source of carbohydrates (Enghiad et al. 2017), which have a beneficial influence on health (Bernardo et al. 2019; Munekata et al. 2020). Maize is also used in the food, paint, paper, biodiesel, textile, and adhesives industries (Chukwudi et al. 2021).

2.2.1 Constraints to maize production

Several constraints, including abiotic conditions, such as climate change, drought and salinity stress, and biotic conditions, such as pests and diseases, affect maize production. Climate change is a global phenomenon known for its impact on the earth. It significantly affects precipitation, wind, and temperature, which has been a subject of concern to researchers over the past decades (Manuel et al. 2021). Climatic change results from human actions that have disrupted nature over time. These activities have caused the reduction in natural resources needed for agriculture and have led to the ruin of infrastructures and consequently increased the cost of living (Manuel et al. 2021).

Climate variables, such as precipitation and temperature have recently become increasingly unpredictable, resulting in extended drought and temperature variations that are beyond the optimal range. With changing weather patterns, crop susceptibility to abiotic stress poses a new challenge to maintaining crop output growth (Bhusal et al. 2021). Plant height, leaf area, shoot fresh weight, shoot dry weight, kernel weight, and grain yield per plant were all observed to be severely reduced as a result of the combined heat and drought stress. In a research involving individual application of each of two stresses, drought stress had a more severe effect than heat stress according to Hussain et al. (2019). Salinity stress is usually caused by the high concentration of NaCl, which stimulates abiotic stress in plants. It retards plant growth and productivity, resulting from ion toxicity and osmotic stress (Munns and Tester 2008; Bhusal et al. 2021).

Maize production is a prevalent form of agriculture, including mixed cropping and livestock production in Southern Africa. However, the need for food increases because of the population increase, which needs to be aligned with production practices (Tui et al. 2021). Soil fertility has continuously declined because of its continuous land use and lack of necessary inputs, which has caused a deficiency in both macronutrients, including nitrogen, phosphorus, and calcium, and essential micronutrients, such as copper and zinc causing poor yield of maize (Iseghohi et al. 2020).

Striga hermonthica is a foremost biotic constraint to the production of maize in SSA, adversely impacting the sustenance of people and significantly increasing food insecurity and poverty (Dawud et al. 2017). This weed has forced farmers to abandon their land. Researchers have attempted to reduce its effect on agriculture by producing quality breeds that are resistant to abiotic stress (drought, low nitrogen) and bioinoculants to combat *Striga* infestation (Tofa et al. 2021; Adnan et al. 2020; Laing and Shimelis 2019; Samwel et al. 2021).

2.3 Biology of *Striga*

Striga spp. are annual, photosynthetically active, root hemiparasitic plants that feast on other plants for growth (Rich 2020c; Spallek et al. 2013). Its growth pattern is complex, intimately associated with its host. A single *Striga* plant can yield up to 500,000 seeds capable of remaining in the soil for 20 years under optimum conditions (Lobulu et al. 2019). *Striga* life cycle with that of its host operates simultaneously and requires mechanisms that harmonize the lifecycles of both the host and the parasitic plant. Its lifecycle usually involves; germination, host attachment, haustoria formation, penetration and establishment of vascular connections, accretion of nutrients, flowering, and production of seeds (Yacoubou et al. 2021; Stanley et al. 2021a). These seeds germinate only in the presence of host-derived chemical signals, strigolactones (SL), which are usually accompanied by a period of pre-conditioning that requires warm weather and moist soil (Ejeta 2007; Bouwmeester et al. 2007; Marro et al. 2021; Kountche et al. 2019; Parker 2012). A haustorium is formed, which connects the host plant to the parasite. This allows for the flow of water and nutrients between the *Striga* plant and the host plant, damaging the latter. However, this parasite cannot survive on its own at any stage but solely depends on the host plant for survival and growth (Cimmino et al. 2018) (Figure 2.1). It takes about 10 weeks for *Striga* spp. to complete its life cycle after egression and this completion is usually after the host plant has been harvested (Yacoubou et al. 2021). *Striga hermonthica* is a high crossing species, having noticeable heterogeneity within an area (Mrema et al. 2017a). This unique characteristic of dispersal has added to its genetic modification. It has facilitated the spreading of the parasitic plant depending on the availability of dispersal agents (Menkir et al. 2020b). Within the population of *Striga hermonthica*, genetic diversity are primarily present for host-specific actions (Mandumbu et al. 2019). The variation process occurring within the parasite explains the resistivity potential at its accommodating stage. A typical example can be found in a particular cultivar of crops planted in the presence of *S. hermonthica* species. The attributes mentioned above are some of the reasons why the parasite is a great threat to the plant host.

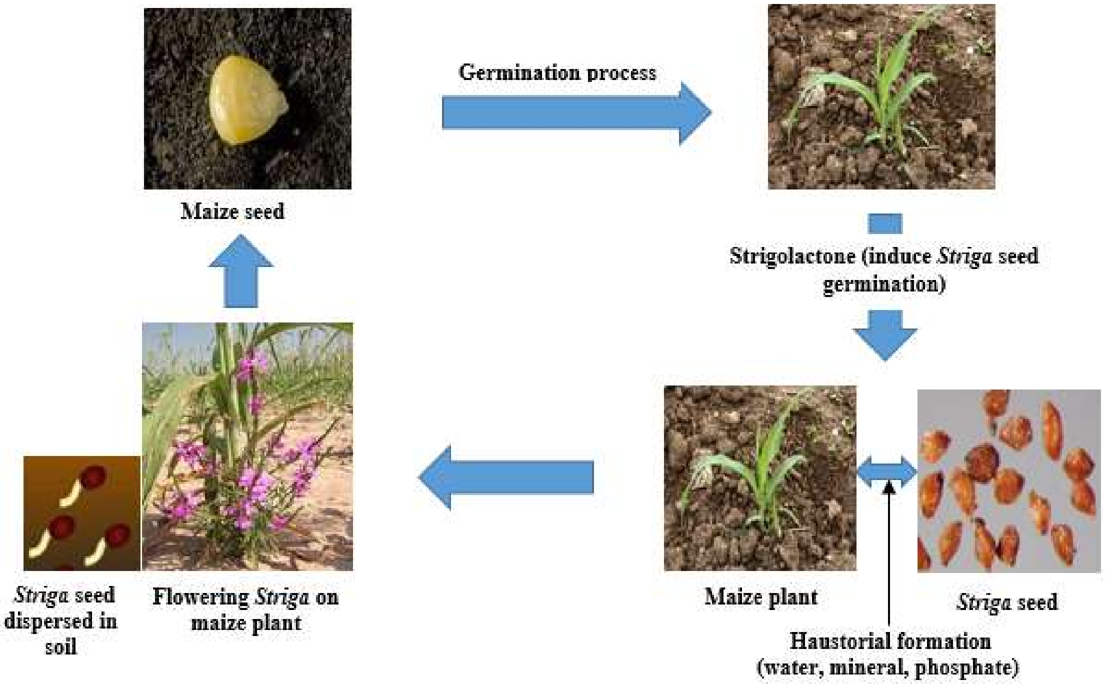


Figure 2.1: The *Striga*-maize cycle.

2.4 Economic importance of *Striga*

Striga species happens to be a significant and most challenging living constraint in the production of cereal, especially maize. It is mainly found in the high-temperate regions or parts of SSA and the Middle East of the Asian continent (Table 2.1). The parasitic effect causes significant deterioration in the yield and growth of food crops. The harmful effect of the parasite can be apparent even before the parasite emerges from the soil, which has made the control discouraging to farmers. It has been calculated that over 50 million hectares of tillable soils under cereals cultivation, including maize, have been infested by *Striga* spp. (Dafaallah 2019).

Grain losses resulting from this parasite have been reported to be up to 75%, depending on the host affected, average weather conditions, varieties cultivated, degree of the infestation, and the nature of the soil (Anitha et al. 2020).

Table 2.1: Some *Striga* species found across countries in sub-Saharan Africa.

***Striga* species**	**Host Plant**	**Countries found**
S. gesnerioides	Wild legumes, cowpeas	Botswana, Burundi, Angola, Central African Republic, Burkina Faso, Cameroon, Ethiopia, Democratic Republic of Congo, South Africa, Sierra Leone, Zimbabwe, Tanzania, Gambia, Kenya, Mali, Ghana, Malawi, Nigeria, Somalia, Zambia, Mozambique, Uganda, Angola, Ruanda
S. aspera	Finger millet, sugarcane, rice, sorghum, maize, wild grasses	Central African Republic, Burkina Faso, Guinea, Cameroon, Gambia Bissau, Ethiopia, Niger, Côte d'Ivoire, Nigeria, Sudan, Mali, Ghana, Senegal.
S. hermonthica	Maize, sorghum, millet, rice, Sugarcane	Ethiopia, Angola, Burundi, Kenya, Niger, Senegal, Tanzania, Sudan, Namibia, Uganda, Togo, Sudan, Nigeria, Democratic Republic of Congo, Cameroon, Central African Republic, Eritrea, Gambia, Djibouti, Gambia Bissau, Côte d'Ivoire, Chad, Zimbabwe, South Africa
S. gastonii		Central African Republic, Chad
S. asiatica	Rice, sorghum	Botswana, Kenya, South Africa, Democratic Republic of Congo, Malawi, Angola, Mozambique, Sudan, Zambia, Tanzania, Zimbabwe, Lesotho, Namibia, Madagascar, Zanzibar, Burundi
S. forbesii	Maize, rice, sugarcane, sorghum	South Africa, Botswana, Ethiopia, Malawi, Angola, Kenya, Mozambique, Sudan, Tanzania, Swaziland, Zimbabwe, Zambia, Uganda

S. dalzielii		Nigeria, Guinea, Mali
S. brachycalyx		Ghana, Democratic Republic of Congo, Nigeria, Côte d'Ivoire, Burkina Faso
S. elegans		Angola, Kenya, Mozambique, Botswana, Ethiopia, Malawi, Namibia, South Africa, Zimbabwe, Swaziland, Zambia, Tanzania
S. angolensis		Angola
S. aequinoctialis		Angola, Siera Leone, Guinea, Liberia
S. angustifolia	Sugarcane, sorghum	Zimbabwe, Malawi, Zambia, Tanzania
S. chrysantha		Central African Republic

Source: (Atera et al. 2011; Timko et al. 2007; Sakadzo et al. 2021)

The decrease in grain harvest of enhanced cultivars has improved to 90%, as reported by Anitha et al. (2020). The losses produced yearly have been estimated to be more than USD10 billion and farmers who record up to 80% losses because of parasite invasion were eventually forced out of their farms (Dafaallah 2019). However, if adequate measures are not taken, the attack of *Striga* on farmland will escalate and become a challenge to the production of crops.

2.5 Host resistance

Most of the species of *Striga* are found in Africa. *S. hermonthica*, *S. aspera*, and *S. asiatica* infest the grass family (Poaceae) which includes rice, sorghum, maize, finger and pearl millets while *S. gesneriodies* parasitize dicotyledonous plants which includes members of the Euphorbiaceae, Convolvulaceae, and Solanaceae. The most important host for *S. gesneriodiies* is cowpea (Timko et al. 2012; Muchira et al. 2021). The management of *Striga* through the use of crop-resistant varieties has been reported in several crops including maize and has reduced the emergence of *Striga* and the production of *Striga* seeds. It is regarded as the most suitable method of reducing *Striga* effects on its host for a longer period of time. A mutation at LOW GERMINATION STIMULANT 1 (LGS1) causes both quantitative and qualitative alterations in the SL content of root exudates, significantly reducing the action of the germination stimulant of *Striga* without having a negative impact on productivity (Gobena et al. 2017). Also, TIS108, a MAX1-specific inhibitor, decreases the synthesis of 4DO and orobanchol, allotting resistance to *Striga* without significantly altering the morphology of rice (Ito et al. 2022). Butt et al. (2018) reported that CRISPR/Cas9 system in translational research can be used for target improvement of plant architectural trait. The study showed that targeted engineering of CCD7 could improve crop yield and lower risk of *Striga* infection. The apocarotenoid metabolite Zaxinone also regulates the growth and strigolactone biosynthesis in rice (Wang et al. 2019a). Host resistance against *Striga* consists of two wide categories namely, tolerance and resistance. Tolerance is when the host plant is able to prolong parasitic infection with minified negative consequences while resistance results in *Striga* reduction level in the host plant. Host resistance has not been fully accomplished to date, only partial resistance has been reported in several hosts (Mwangangi et al. 2021). The evaluation and the emergence count of *Striga* have been used as a tool for resistance and tolerance in modifying maize against *S. hermonthica* (Shayanowako et al. 2018; Stanley et al. 2021a; Timko et al. 2012). *Striga* resistance is characterized into two; the pre-attachment stage, which occurs before host attachment, causing degraded germination process, and the post-attachment stage where *Striga* is already attached to the host (Rodenburg et al. 2016; Mallu et al. 2022). The use of genetic engineering and gene editing tools can be used as a tool to evaluate for resistance against *Striga* by improving the strigolactone outline of the host plant. Genetic resistance can either be adopted autonomously or as part of an integrated management system (Jamil et al. 2021; Muchira et al. 2021; Mallu et al. 2022; Kavuluko et al. 2021).

Striga resistant varieties and hybrids with several forms of resistance have been developed over the years in which notable advancement has been recorded. According to Akaogu et al. (2019), inbred TZdEI 352 could serve as a valuable parent for hybrid development in *Striga* prevalent agro-ecologies of SSA. Grain yield enhancement under *Striga* infestation was achieved by genomic selection, which also led to an increase in yield performance in the optimal environment for bi-parental maize production. However, under drought conditions, genomic selection was inefficient in increasing grain output (Badu-Apraku et al. 2019). This resulted from a limited genetic base to work with, which led to a lack of significant genetic variances and heritabilities. *Striga* emergence was substantially reduced, and production was improved using hybrids developed over three breeding periods. The identification of maize inbred lines and hybrids with a higher level of field resistance to *S. hermonthica* without compromising the yield potential and other traits under non-infested conditions were successful by selecting multiple traits using a stringent field technique combined with the appropriate weight assigned to traits in the selection index (Menkir and Meseka 2019). Quality Protein Maize (QPM) produced higher yield of output in comparison to non-QPM maize genotype for *S. asiatica* susceptibility in both an agar gel assay and pot test (Nyakurwa et al. 2018). In addition, the use of rhizotron in the study of *S. hermonthica* has shown the existence of post-attachment resistance using cultivar KSTP94 (resistant). A limited level of *Striga* attachment was observed as compared to CML144 (susceptible maize inbred lines) (Yoneyama et al. 2015; Mutinda et al. 2018). This was similar to the study conducted by Amusan et al. (2008) where ZD05 exhibits post-attachment resistance. Furthermore, 30 maize inbred lines for *S. asiatica* resistance using agar gel assay and in pots showed reduced *Striga* attachment and emergence (Gasura et al. 2019). Although a lot of resistant varieties have been developed, none has immunity against this persistent parasitic weed. This could be due to climate change and development of resistant strains, which may have caused the evolvement of the weed to be resistant to previously susceptible control measures. Climate change has been reported to increase the incidence of pest and diseases (Skendžić et al. 2021). *Striga* populations may vary in their virulence as well; it's feasible that possessing a variety of resistance mechanisms may offer some protection against changing parasite populations.

Recently, a wide genomic study of *S. hermonthica* showed that recognized loci and genes could be a very good source for breeding *Striga*-resistant maize cultivars (Adewale et al. 2020; Badu-Apraku et al. 2020). However, further research employing various mapping populations is required to validate the markers discovered so that marker-assisted breeding for *Striga* resistance in tropical maize can become a reality in *Striga* endemic areas of SSA. This will also provide information about genetic mechanisms in maize resistance to *Striga* infestation and the functional genes that are responsible for resistance. KSTP94 is a good genetic source for breeding as it resists the infestation of both the pre-attachment and post-attachment stages. Similarly, the use of genetic engineering can be used to produce and store high resistance diversity (Wakabayashi et al. 2019; Wakabayashi et al. 2020).

2.6 *Striga* management strategies

Several *Striga* management strategies include cultural practices, chemical control, and the utilization of bioinoculants for the regulation of *Striga* (Figure 2.2). Nevertheless, their application is contingent upon the accessibility of resources and skills among subsistent farmers. These applications are discussed below.

2.6.1 Cultural management

Various cultural management strategies have been proposed to manage *Striga* on maize farms. These strategies aid in minifying *Striga* seed in the soil, enhancing soil prolificacy, maturation, and slowing down the sprouting and growth of seedlings (Silberg et al. 2021). The strategies include: intercropping of cereals with legumes (Jamil et al. 2021; Lee and Thierfelder 2017; Mutyambai et al. 2019), water management (Dawud 2017b; Goldwasser and Rodenburg 2013), mixed cropping, crop rotation (Kuyah et al. 2021), a combination of trap crops, nitrogen fertilizers (Tadesse 2018), trap cropping (Garba et al. 2017; Goldwasser and Rodenburg 2013), cover cropping (Randrianjafizanaka et al. 2018; Rich 2020b), Push-pull technology (Ndayisaba et al. 2020; Niassy et al. 2022), and fertilization (Dawud 2017b; Jamil et al. 2011). In push-pull technology, the *desmodium* root secretes compounds that promote the development of parasitic *Striga* weeds but prevent them from adhering to maize roots through radical growth inhibition. This causes depletion of *Striga* seed bank (Khan et al. 2002; Khan et al. 2010; Ndayisaba et al. 2020). Early planting preceding rainfall also minimizes *Striga* plague in the semi-desert regions, which often occurs a few months after cultivation (Mrema et al. 2017b). Smallholder farmers do not easily accept these strategies because of limited resources, technical know-how, time, and labor. Proper fertilizer application, rate, and timing still pose a problem to maize farmers in the developing world.

Removing *Striga* weeds with hands or tools is the most affordable cultural control method. This method is preferably adopted before the flowering of *Striga* weed to obviate its multiplication in the soil. Nevertheless, it is arduous, lengthy, and less efficient in mitigating crop damage (Mahuku et al. 2017; Rich 2020a; Goldwasser and Rodenburg 2013). Recently, cover cropping and the use of chemicals with a variety of *Striga*-resistance was established to alleviate the impingement of *Striga* on maize and rice (Rodenburg et al. 2020; Abdallah et al. 2015). The use of integrated management strategies to reduce the impact of *Striga* and increase the yield of maize demands a perception of the advantages and the disadvantages attainable in the field.

2.6.2 Chemical control strategies

Chemical compounds that imitate strigolactone actions present a rising advancement in *Striga* management because these compounds can allow the sprouting of the parasitic weed without a host. This method is known as suicidal germination (Babalola and Odhiambo 2008; Zwanenburg et al. 2016). Previously, this method was not achievable because of its high cost, appropriately applied protocol, and easy-to-use formulation accompanied by strigolactones produced through several chemical processes, which are unstable in the field. However, when subjected to chemical formulations, simple strigolactones are now attainable on the field. This strategy is attractive as it reduces *Striga* seed bank quickly. Even though several methods have been developed for the suicidal killing of this parasite, its application under field conditions is minimal because they are quite expensive, lack of properly implemented protocol, and formulation that is simple to utilize (Kountche et al. 2019; Zwanenburg et al. 2016; Samejima et al. 2016). According to Ito et al. (2011), TIS108 suppressed SL levels in rice seedlings and did not reduce plant height in comparison with TIS13, which greatly reduces plant height. It was also able to minify the germination of *Striga*. Most recently, it was reported that SL analog MP16 was able to cause maximum reduction of *Striga* emergence (97%) under greenhouse evaluation and Nijmegen-1 was able to cause 60% and 40% reduction of *Striga* emergence in sorghum and pearl millet fields, respectively as compared to the standard GR-24. These SL analogs could be used to ameliorate suicidal germination technology to eradicate *Striga* seed bank in SSA (Jamil et al. 2022).

Using imazapyr-resistant (IR) to coat maize seeds, and the use of imidazolinone herbicides, including pyrithiobac, imazapic, imazaquin, and imazapyr, has reduced *Striga* growth through all the farming seasons, causing sporadic acceleration in maize production outputs (Kanampiu et al. 2018; Menkir et al. 2010; Chikoye et al. 2011; Habimana et al. 2014).

However, the constant use of chemicals on the soil has led to a decline in the quality of soil, pollution of water, greenhouse gas emissions, and declined in soil biodiversity (Wang et al. 2018; Vasco et al. 2021; Basu et al. 2021b).

2.6.3 Use of bioinoculants as a biocontrol agent

Exploring the use of microorganisms is an efficient strategy to combat environmental degradation while enhancing sustainable agriculture and soil health (Babalola et al. 2007). The quest for an eco-friendly approach to reduce the adverse effect of agrochemicals on plants and soils has led to the discovery of microbial formulations to improve crops (Raklami et al. 2019; Jabborova et al. 2020). Bioinoculants, also known as microbial or soil inoculants, are agricultural improvements that use beneficial microorganisms to promote plant health (Olanrewaju et al. 2019; Pascale et al. 2020; Devi et al. 2020; Vannier et al. 2019; Hashem et al. 2019). These microorganisms include bacteria and fungi.

The application of soil microorganisms, such as rhizobacteria and Arbuscular mycorrhizal (AM) fungi have significantly decreased cultivation, production, and attachment of *Striga* (Babalola 2010a; Olanrewaju et al. 2017; Lanfranco et al. 2018). Studies have shown that AM fungi can inhibit parasite infection (Odelade and Babalola 2019; Bari et al. 2021; Ramji Moturu et al. 2018; Xie et al. 2010; López-Ráez et al. 2012; Bouwmeester et al. 2007; Lendzemo et al. 2005). Though AM fungal inoculation reduced the quantity of *Striga* and biomass, there was no corresponding rise in cereal grain yield (Lendzemo et al. 2005), AM fungi have been reported to enhanced cereal growth and performance against *Striga* and facilitates plant assimilation of phosphorus (P), water, and micronutrients from the soil. The inoculation of AM fungi on maize cultivars decreased the incidence of *S. hermonthica* and increased nitrogen (N) and phosphorus (P) uptake (Samejima and Sugimoto 2018; Bonfante and Genre 2010). *Bacillus subtilis, Bacillus amyloliquefaciens,* and *Burkholderia phytofirmans* have been able to reduce *Striga* infestation in sorghum by 47% in a screen test (Mounde et al. 2015). Enzymes, such as xylanases, pectinase, and amylases from *Bacillus* species and *Streptomyces* species can directly cause the decay of *Striga* seeds. In contrast, isolates of *Rhizobium* can solubilize insoluble phosphate and make it available to plants (Neondo et al. 2017). Moreover, soil microorganisms can combat *Striga* by producing amino acids like methionine, leucine, and tyrosine or release secondary metabolites including trichothecenes and sesquiterpenoids "β-lactone" derivatives, and anthranilic acid, which could cause strigolactone perception to be hampered (Tyc et al. 2017).

Additionally, evidence have shown that strigolactone deterioration by fungi could cause a decline in *Striga* growth, enhancing the action of soil microbes for germination (Masteling et al. 2019; Takahashi et al. 2021; Huang et al. 2014; Boari et al. 2016). Applying soil microorganisms, such as plant growth-promoting rhizobacteria (PGPR) and AM fungi is a more effective and environment-friendly method to eradicate *Striga* (Samejima and Sugimoto 2018). Notwithstanding, several factors such as; the dynamics of PGPR, which changes on host plants, different modes of actions because all rhizobacteria do not have the same mechanism of operation for plant growth promotion can affect the effectiveness of these methods, especially in the field. In addition, isolates that are effective for plant growth-promoting abilities *in vitro*, might not necessarily be effective under field conditions. This is due to the fact that the PGPR mechanism has not been fully understood (Basu et al. 2021b).

Myco-herbicides are fungi-based herbicides that can prevent *Striga* from destroying cereal crops reducing its abundance in the soil. These myco-herbicides are produced from the fungus *Fusarium* *oxysporum* (Bàrberi 2019; Rebeka et al. 2013; Zimmermann et al. 2016). Many *F. oxysporum* strains yield some amino acids (e.g., L-tyrosine and L-leucine). They can only destroy *Striga* and not maize crops because they securely balance the free amino acid system. The use of soil microorganisms to convert methionine discharged by *F. oxysporum* strains to ethylene can be used to cause suicidal germination for the seeds of *Striga* (Rubiales et al. 2018; Nzioki et al. 2016). *F. oxysporum*, disintegrated to produce *F. oxysporum f. sp. Strigae* (FOS) specifically inhibits *S. asiatica* and *S. hermonthica's* appearance (Mrema et al. 2018; Mrema et al. 2020; Shayanowako et al. 2020). A simple method of fungi delivery using a toothpick was developed, and the incorporation of FOS caused an improvement ranging from 40%-50% in maize production (Nzioki et al. 2016). However, myco-herbicides are usually affected by environmental factors, especially temperature and humidity, and therefore demand proper formulations (Abdessemed et al. 2020).

It is paramount to examine the compatibility of the applied microorganisms with the host, consider a suitable inoculum medium, consistency, and the maintenance of their activities in infested soil before considering the method to adopt. These microorganism-based biocontrol approaches still demand thorough field examination under different climatic conditions (Mohammadi 2019; Müller-Stöver et al. 2016).

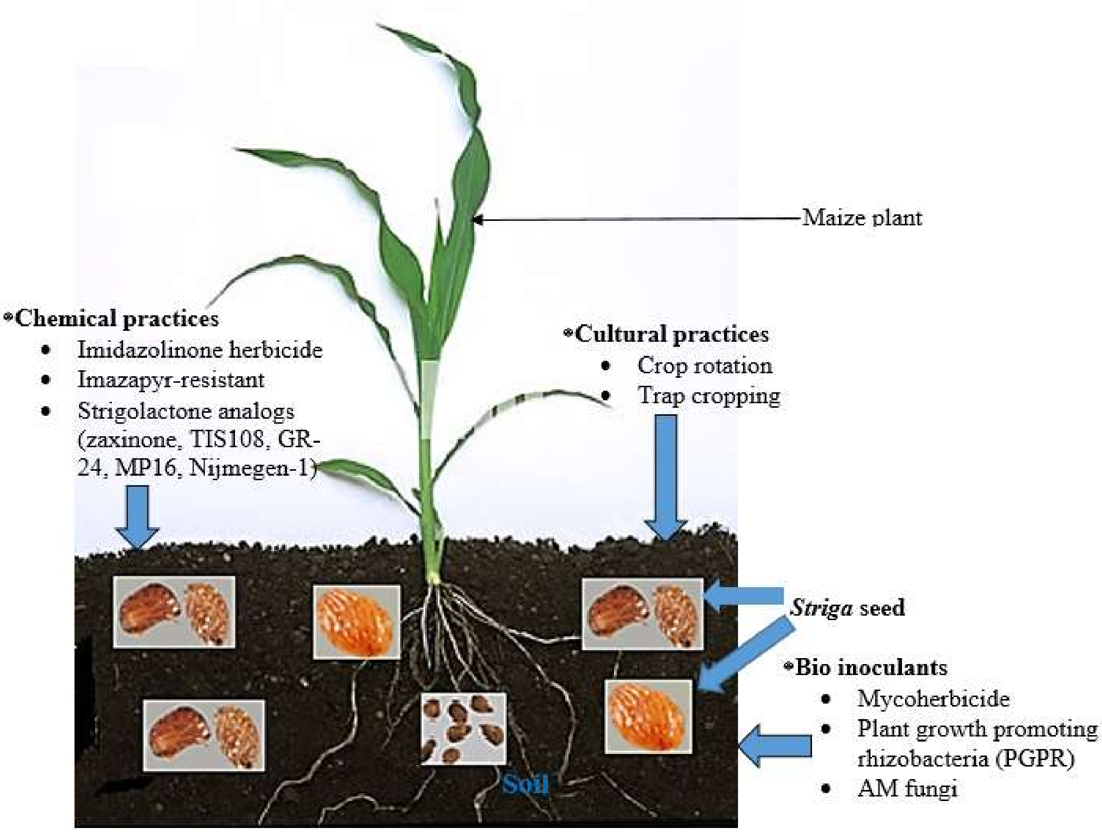


Figure 2.2: Methods of combating *Striga* in maize plant

2.6.4 Integrated management of *Striga*

Striga control with a single method is ineffective. However, incorporating two or more methods has caused significant output (Jamil et al. 2021; Sibhatu 2016). For example, combining host resistance with *F. oxysporum* caused a considerable reduction of *Striga* (Shayanowako et al. 2020; Mrema et al. 2020). *Striga*-resistance was reduced due to the coating of the seed of imazapyr-resistant hybrid maize (Menkir et al. 2020a). Furthermore, cereal-legume crop rotation combined with germination stimulants can significantly reduce the parasite seeds in the soil (Jamil et al. 2021; Kountche et al. 2019). According to an experiment carried out by Abdullahi et al. (2022), the combination of P fertilizer, resistant variety with *Brandyrhizobium* has been able to reduce *Striga gesnerioides* and increased the grain yield of cowpea. The use of *Striga*-resistant and herbicide-resistant maize with legumes suppressed *Striga* seed germination and reduced *Striga* seedbank in the soil (Kanampiu et al. 2018). It has also been suggested that the introduction of self-destructive development with a genetic resistant variety is the best approach for *Striga* seed bank death (Jamil et al. 2018; Zarban et al. 2021).

2.7 Metagenomics for surveillance of *Striga* infested soil

The macro- and microfora in soil are incredibly rich and diversified, and it is a living, incredibly complex, naturally occurring unconsolidated mineral and organic material with a dynamic ecosystem. The role of these microorganisms include; biological control of diseases, pests, and weeds, elemental cycling, fixing carbon and nitrogen, mineralizing decomposing organic matter, improving soil aggregation and stability, and nutrient bioavailability to plants (Nwafor and Chibuike 2015; Costa et al. 2015; Rashid et al. 2016; Trivedi et al. 2016). *Striga* weeds thrive on poor soil, however, the parasite can be inhibited by the biotic and abiotic factors that are present in the soil in spite of the presence of the invasive pathogen and susceptible host (Baker and Cook 1974; De Corato 2020; Sakadzo et al. 2021). Due to the long-term deleterious effect of chemicals on the soil (Prashar and Shah 2016; Alengebawy et al. 2021), there is a need to explore the microbial diversity of *Striga*-infested soil in other to discover biocontrol microorganisms and antagonists that can effectively combat this root parasitic plant using metagenomic tools.

In the recent past, laboratory culturing methods were used to examine soil microbial populations, in which only 1% of the microorganisms can be cultured using culture-based techniques (Coats and Rumpho 2014). Scientists have recently been able to predict the structure and makeup of microbial communities due to advancements in next-generation sequencing technology, which allow the production of megabases of sequence data in a short time and allows for better comprehension of microbial diversity through the use of DNA genome sequencing (Coats and Rumpho 2014). While culture-based techniques have made it possible to isolate microorganisms for in-depth research, molecular approaches like metagenomics are increasingly making it possible to identify microbes (Turner et al. 2013a). Diverse applications have been reported using these NGS technologies, including single-gene targeted sequencing, whole-genome sequencing, and shotgun metagenome sequencing, to study the microbial population in a culture-independent context (Kim et al. 2013;

Zhou et al. 2021). The use of metagenomics sequencing technology is an intriguing method for identifying suitable biocontrol bacteria and detecting their antagonists against *Striga* (Matsumoto et al. 2021; Zhou et al. 2021).

2.8 Conclusion and future prospect

Several efforts have been made in the past decades to develop eco-friendly approaches to combat the decimating effect of *Striga* weed on cereal crops. These approaches include maize varieties having resistance to *Striga* in SSA, the toothpick procedure of fungi inoculation, and an integrated management system is accepted as the only alternative to control this parasitic weed. However, further research is required to provide a sustainable and effective solution to combat the *Striga* problem. There is a need to understand the host-parasite interaction and host resistance. More so, there is a need to develop grains with permanent resistance status. Applying bioinoculants by employing modern tools like genomic engineering, and gene editing is a significant, cost-effective, and eco-friendly approach to *Striga* management. Furthermore, crop rotation and intercropping with an artificial host can eradicate the parasites in the soil. Moreover, farmers' willingness, planning, labor, input, and commitment are vital for the efficacy of seed coating and the introduction of FOS. In addition, the similarities between soil and climatic attributes, and the dissemination of knowledge to smallholder farmers are necessary. Before field trials, chemical effect on the soil and their residual effect on the environment must be considered. To explore these approaches with the intent of regulating the challenges accompanied by it might yield a significant output of *Striga* control.

Chapter 3

Microbial communities in the soil rhizosphere

3.0 Abstract

The rhizosphere of a plant serves several useful purposes to benefit the microorganisms that are inhabited there. The extensive functional capacities of the rhizospheric microbial communities have been clarified by several studies, but our knowledge of the precise mechanisms underpinning the influence of rhizospheric microbiome assemblies is still restricted. Furthermore, little is understood about the numerous advantageous properties of the rhizospheric microbiome. The biotic and abiotic variables that influence the rhizospheric microbiome and its microbiomic features that are advantageous to plant growth and resistance to disease are outlined in this review. We place special emphasis on the way in which rhizospheric microbiome assemblies are impacted by plant root metabolites, and also look into the way in which the microbiome affects plant growth, yield, and resistance to diseases. In conclusion, we present a fresh viewpoint and recommend a cutting-edge technique by demonstrating how the creation of a synthetic microbial community can offer a useful strategy for understanding the interactions between microorganisms and plants.

3.1 Introduction

There is a highly complex microbiotic community, namely, the root microbiota and the rhizosphere, that is present in the soil that adheres closely to terrestrial plant roots (Khan et al. 2021a). It is as a result of the potential benefits of this community that the simulated integration of microbiomes in agricultural soils has recently gained attention (Kumar and Dubey 2020). The soil accommodates a dynamic ecosystem, constantly changing, and also causing factors in its environment to shift. On the other hand, the functioning of the microbiome exhibits patterns that are strongly influenced by the environment (Jansson and Hofmockel 2020). The microbial communities either adapt to these shifting environmental conditions and continue to survive, or fail to adapt and therefore, deteriorate (Wu et al. 2019). The low success rate in the application of the majority of lab-scale synthetic microbiome technologies can be attributed to this variable reaction. It is, therefore, essential to gather knowledge on the wide range of difficulties that can be overcome by microbes in that particular environment in order to increase the rate of absorption of the designated microbiome under different soil conditions. In this study, we review the soil rhizospheric microbiome and its association with plant growth promotion and sustainable agriculture.

3.2 The plant microbiome hotspot in the rhizosphere

Despite the enormous diversity of microorganisms in the soil, they are concentrated in small quantities that make up only 1% of the total soil volume (Odelade and Babalola 2019). These microhabitats, where microorganisms gather in clusters to form biofilms or colonies, are distinguished in terms of biogeochemical processes occurring at speeds that are faster than those occurring in bulk soil (He et al. 2021). A soil has been defined as a microbial hotspot by Kuzyakov and Blagodatskaya (2015). These hotspots include the following: the area of soil adhering to the living roots that is influenced by the plant root exudates, otherwise known as the rhizosphere; the detritusphere, the area of soil where the plant litter decomposes and where the organic matter is turned over; the soil aggregate surfaces; and the biopores, which are the spaces created by burrowing fauna and the penetration of deep roots (Nadarajah 2022). These areas supply labile and resistant organic materials for bioprocesses, and they are also significant in terms of the conditions that they create to restrict microbial activity, such as nitrogen nutrition, oxygen availability, and soil moisture (Lazcano et al. 2021). Activities including respiration, gas exchange, nutrient and moisture usage, and various biological processes are concentrated in the soil as a result of the availability of the localized labile carbon and other easily usable nutrients (Singhal et al. 2021; Valadares et al. 2020). Rhizodeposition, whereby plant roots secrete a variety of substances (e.g. sugars, organic acids, amino acids, polysaccharides, vitamins, and other secondary metabolites), of low and high molecular weights, into the surrounding soil, is the primary process that underlies the development of such unique rhizospheric characteristics (Meryem et al. 2022). Nearly 10% to 16% of nitrogen and 11% of the total photosynthetically-fixed carbon are present in these rhizodeposits (Tian et al.

2020). These exudates exert a significant impact on the way in which the rhizosphere develops since they change the chemistry of the soil immediately surrounding the plant roots and act as a substrate for the growth of specific soil microorganisms (Cordovez et al. 2019). The nutritional state of the plant, its stage of growth, and even the distance in relation to the position of the root, all affect the qualitative and quantitative components of the plant root exudates (Pantigoso et al. 2020; Astolfi et al. 2020). As a result, there is competition in the rhizosphere that drives the selection of the particular rhizospheric microbial populations by plants. Surprisingly, about five percent (5%) of the microorganisms present in the rhizosphere contribute to plant development, and particularly in limited environments, plants automatically choose these helpful microbes that promote their survival and growth, (Chukwuneme et al. 2021b; Ahmed et al. 2019; Gupta et al. 2021). Microorganisms from the rhizosphere can also penetrate plant roots, internal tissues and the ground surface (Omomowo and Babalola 2019; Zhang et al. 2019b). As a result, colonies of microorganisms that live on or inside plants and engage in mutually beneficial symbioses are frequently linked to them. The microbiome, or the complete genome of the microbial population, is crucial in facilitating the host's ability to absorb nutrients, maintain metabolic function, and withstand biotic and non-biotic challenges (Adedeji et al. 2020; Nwachukwu and Babalola 2021). Therefore, the first step in enhancing a host plant's distinctive characteristics is to establish the core specific microbiome that supports its growth.

3.3 Importance of the plant microbiome in agricultural growth

From an ecological standpoint, plants are more than just isolated organisms since they coexist with the plant microbiota, which influence plant productivity and growth (Table 3.1). Only five percent (5%) of microorganisms have been cultivated by applying the existing techniques; thus, the microbial richness of the soil and the presence of rhizospheric microbiomes have been severely underestimated (de Faria et al. 2021). Beneficial species, including biocontrol agents, other plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi, nitrogen-fixing bacteria, and creatures harmful to plant growth, such as pathogenic microbes, make up the plant-associated microbiomes.

The variety of microbes found in the core plant microbial biome, notably bacteria, has been demonstrated by next-generation sequencing techniques using the 16S rRNA gene (Bharti and Grimm 2021; Yang et al. 2021). In several analyses of clone libraries collected from the rhizospheres of 14 distinct plant types, Hawkes *et al*. (2007) discovered that over 1200 bacterial phyla were connected to plants, with the Proteobacteria phylum being the most prevalent. By identifying and responding to environmental cues, these groups of plants and microbes adapt their growth and development to specific environmental perturbations. According to Gopal and Gupta (2016), the ecological functions of plant-associated microbes, such as biofertilization, defense against pathogens, and tolerance in respect of abiotic stresses, have a significant impact on the overall fitness of the plant.

Table 3.1: Variable plant responses to microbial inoculation

Microbial inoculants	**Crops**	**Increased Yield**	**References**
Arbuscular mycorrhizal fungus	Several crops	Up to 19%	(Chareesri et al. 2020)
Bacillus subtilis, *Bacillus aryabhattai*, and *Paenibacillus polymyxa*	Wheat	Up to 17%	(Hussain et al. 2020)
Enterobacter sp PR 14	Rice, sorghum, millet	Up to 38.13%, 30.75%, and 16.36% (dry weight)	(Sagar et al. 2020)
Bacillus cereus LPR2 and silver nanoparticle	Maize	Up to 70%	(Kumar et al. 2020)
Bacillus sp and nanozeolite	Maize	Up to 29.8%	(Chaudhary et al. 2021)
Bacillus aryabhattai	Wheat	Negative increase of 12%	(Farahat et al. 2020)
Pseudomonas putida and *Cardisoma armatum*	Maize	55.7%	(Agbodjato et al. 2021)

Asospirillum	Betel vine	195.6 cm (length)	(Chitra et al. 2020)
Arbuscular mycorrhizal and Trichoderma viride	Onion	Up to 17.5%	(Metwally and Al-Amri 2020)
Pseudomonas palleroniana and *Pseudomonas jesenii*	Chickpea	Up to 100.65% (dry weight)	(Rawat et al. 2019)
Enterobacter asburiae	Quinoa	Up to 79.64% (dry weight)	(Mahdi et al. 2020)
Enterobacter hormoaechei	Okra	75.6%	(Roslan et al. 2022)

3.4 The function of the plant microbiome in nutrient uptake

The earlier findings of the symbiotic connections between mycorrhizal fungi and legume rhizobia led to research into plant microbiomes. Rhizobia absorb nitrogen from the atmosphere in a form that legumes can use and rely on their hosts for photosynthesis and some of the requirements necessary for the fixation of nitrogen (Pahari et al. 2021). Mycorrhizal fungi increase the total root surface area and release enzymes that improve the ability of the roots to absorb nutrients. In addition to nutrient transfer, the mycorrhizal fungi also enhance the structure of the soil by forming uniform soil clusters (Muneer et al. 2020). According to a research on signaling crosstalk and the cellular responses of the two symbionts, the legume-rhizobium relationship uses mechanisms developed to verify the existing endosymbiotic connections with the arbuscular mycorrhizal fungi (Álvarez et al. 2022). Nevertheless, accomplishing the ancient ideal of creating *in planta* systems-of-change grains into independent nitrogen-fixing plants by establishing a legume symbiotic pathway in them depends on an understanding of the underlying distinctions between the two responses (Geurts et al. 2012).

Other free-living rhizobacteria and nitrogen-fixing endophytes belonging to the genera *Azospirillum, Achromobacter, Pseudomonas, Bradyrhizobium, Burkholderia*, *Bacillus,* and

Azotobacter were found to improve crop biomass (Igiehon and Babalola 2018; Rodríguez-Rodríguez et al. 2021; Rajkumar and Kurinjimalar 2021). The plant microbiome also includes a number of fungi (including *Penicillium, Aspergillus, Fusarium, Cephalosporium*, and *Chaetomium*) and phosphate-solubilizing bacteria (including *Bacillus, Aerobactor, Alcaligenes*, and *Pseudomonas*) (Alori et al. 2017). They can mineralize organic phosphorus by releasing different phosphatase enzymes, and also improve the dissolvable inorganic phosphorus (P) by discharging CO_2, OH, or protons, and organic acid anions, such as malate, citrate, and oxalate (Mohanram and Kumar 2019). Additionally, rhizospheric bacteria aid in the absorption of trace minerals, including zinc (Zn) and iron (Fe). Through the production of organic acid anions or siderophores, the ferric ion (Fe3+) is transferred to the cell surface, where it is reduced to a dissolvable ferrous ion (Fe2+) (Gong et al. 2019). The bacteria-produced siderophores, as well as the fungi-produced siderophores, incorporate enterobactin, pyoverdine, ferrioxamines, and ferrichromes (Basirat et al. 2011). Graminaceous and dicotyledonous plant species have both been reported to benefit from the promotion by fluorescent pseudomonads of iron feeding via siderophores (Shirley et al. 2011). It is also known that rhizospheric bacteria (such as *Pseudomonas, Curtobacterium, Stenotrophomonas, Plantibacter,* and *Streptomyces*) can mobilize zinc (Zn) by making the medium more acidic through the formation of gluconic acid (Babalola 2010a; Odelade and Babalola 2019). Moreover, the rhizospheric microbiome is crucial for the breakdown of organic matter, which increases the fertility of the soil and eventually increases the productivity of the plant. Bacteria such as *Cellulomonas* sp., *Pseudomonas* sp., *Cytophaga* sp., *Streptomyces* sp., *Chryseobacterium gleum*, and *Sporocytophaga* sp., and lignocellulolytic fungi, are known to break down plant biomass and release nutrients that are used by both the bacteria and the fungi for their own nutrition, as well as for the plant (Mohanram and Kumar 2019). Therefore, even in nutrient-deficient soils, the plant microbiome aids in plant growth.

3.5 Plant microbiome role in host immunity and pathogen defense

Pathogen-repelling microorganisms in the rhizosphere compete for nutrients and space, produce antibiotics or hydrolytic enzymes, and fend off infections (Tarkka et al. 2021). Ammonia, phenazine-1-carboxylic acid (PCA), oligomycin A, pyoluterin, butyrolactones, pyrrolnitrin, and other moieties are some of the antimicrobial metabolites produced by microbes (Budak et al. 2022). *Pseudomonas fluorescens* produces the antibiotic, 2,4-diacetylphloroglucinol (DAPG), which is used in controlling soil-borne pahogens such as *Meloidogyne incognita* and *Fusarium oxysporum* (Meyer et al. 2016). By means of the synthesis of extracellular cell wall-degrading enzymes, such as -1,3 glucanase and chitinase, bacteria are reported to infect and breakdown the fungal plant disease spores (Whipps 2001; Yadav et al. 2017). Numerous antibiotic compounds with varied levels of antibacterial activity are produced by the majority of microbial biocontrol strains. Agrocin 84, an antibiotic to closely related strains, and broad-spectrum antibiotics, called polyketides, are produced by *Agrobacterium radiobacter* (Raaijmakers et al. 2010). By securing the available iron and denying its availability to harmful microorganisms, bacterial iron chelators efficiently contribute to the biocontrol of pathogens in that they limit their proliferation. *Bacillus subtilis* siderophores considerably reduce the *Fusarium* wilt of pepper plants induced by *Fusarium oxysporum* (Yu et al. 2011). *Penicillium citrinum*, *Aspergillus niger*, and *Trichoderma harzianum* siderophores were found to be efficient biological control agents and to improve chickpea growth (*Cicer arietinum*) (Janardan et al. 2011).

Bacillus and *Pseudomonas*, two types of rhizobacteria, may also serve as inducers to promote resistance to diseases in certain plants. The pyochelin siderophores, siderophores pyoverdine, and salicylic acid (SA), generated by *Pseudomonas aeruginosa,* can increase resistance to diseases caused by *Colletotrichum lindemuthianum* on bean and *Botrytis cinerea* on bean and tomato plants, and on tobacco plants with a mosaic virus (Bigirimana and Höfte 2002; Höfte and Bakker 2007). Similar to this, *Serratia marcescens* 90-166 produces a catechol-type siderophore that makes cucumbers resistant to a variety of bacterial, viral, and fungal diseases, including *Fusarium* *oxysporum*, *Colletotrichum orbiculare*, *Pseudomonas syringae*, the cucumber mosaic virus, and *Erwinia tracheiphila* (Press et al. 2001). For pathogen defense, the rhizobacterial interaction may activate the jasmonic acid- and ethylene-signaling pathway or the salicylic acid-dependent signaling transduction system (Ton et al. 2002). The increased number of enzymes, including polyphenol oxidase, peroxidase, chitinase, and ammonia phenylalanine lyase, increased phytoalexin synthesis, and the increased synthesis of genes associated with stress are all characteristics of plants with this type of induced resistance (Yi et al. 2013; Santos et al. 2018). As a result, in addition to influencing the plant immune system, several microbial interactions in the rhizosphere provide improved biocontrol against diseases.

3.6 Influence of biotic and abiotic stresses on the soil rhizosphere

There are typically a number of symbiotic, reciprocal, and competing microbial interactions in the rhizosphere microbiome (Li et al. 2021) (Fig 3.1). The most prevalent bacterial taxa in the rhizosphere are *Proteobacteria, Firmicutes*, and *Bacteroidetes*, while the most prevalent fungal invaders are *Ascomycota* and *Basidiomycota* (Trivedi et al. 2021). These successful colonizers have all created cooperative coexisting communities or have engaged in resource competition. Microbes are subjected to various biotic and abiotic stimuli in the rhizospheric environment that might have several outcomes in respect of altering the community structure and eventually affecting the health of the linked plants (Goss-Souza et al. 2020; Wu et al. 2019). The physico-chemical characteristics of the rhizospheric soil, the variety of root exudates produced by plants, and the secondary metabolites produced by other soil bacteria are the main determinants of community organization (Wu et al. 2019; Goss-Souza et al. 2020). The formation of the root microbiome is driven by nutritional stress. Although there are large quantities of phosphorus (P) in the biosphere, only inorganic orthophosphate can be utilized by plants. Phosphate starvation responses (PSRs), activated by phosphate stress, control several genes involved in the synthesis of primary and secondary metabolites (organic acids, glucosinolates) and influence the composition of the root microbiome, which eventually relieves phosphate stress (Li et al. 2022). Rhizospheric communities are altered by pesticide residues and other environmental pollutants. Atrazine inoculation significantly enriches three OTUs: *Halobacillus* uncultured, *Bacillus decolorationis*, and *Cesiribacter* sp. JJ02. According to Xu et al. (2018a) this is evidence of a direct impact.

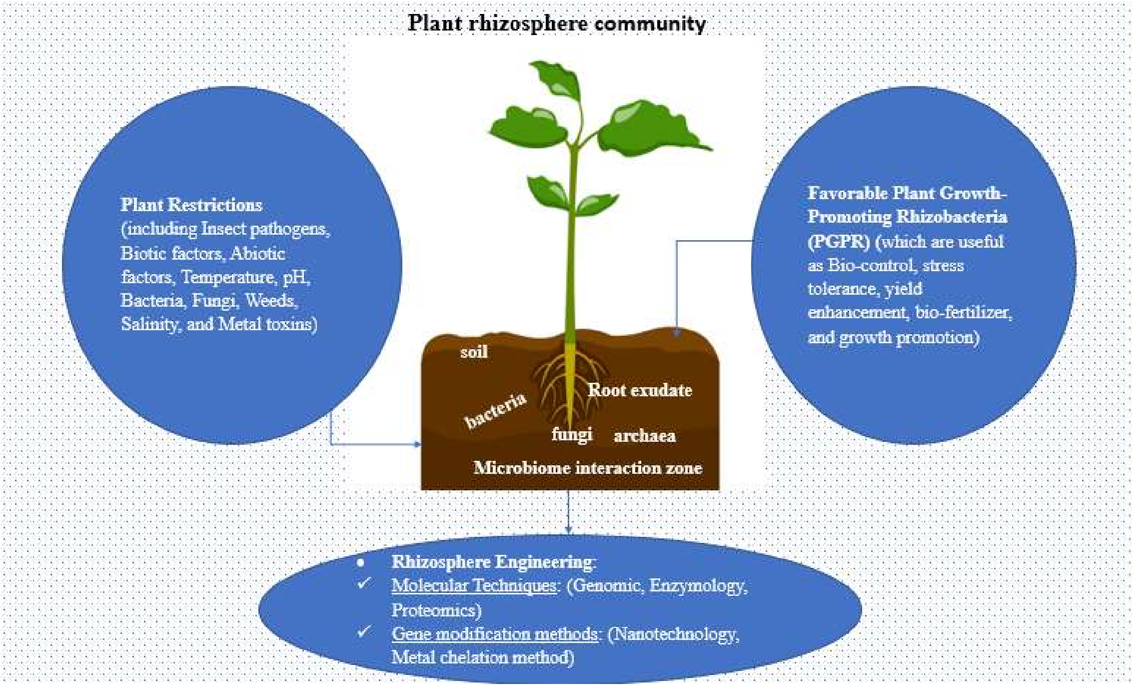


Fig 3.1: A plant rhizosphere community

Furthermore, glyphosate applied to leaf surfaces can be transferred by maize to the soil, greatly increasing the amount of *Fusarium* on the maize roots (Kremer and Means 2009). Importantly, pesticides can modify plant exudation, which can change the microbiota in the rhizosphere. For instance, after being treated with diclofop-methyl, rice seedlings greatly enhance the root exudation of amino acids, organic acids, and fatty acids. This alters the richness and diversity of the rhizosphere's microorganisms and raises the relative amounts of the *Massilia* and *Anderseniella* taxa (Chen et al. 2017; Qian et al. 2018). Other environmental pollutants such as single-walled carbon nanotubes (Chen et al. 2022b), antibiotics (Wang et al. 2019b), chiral insecticides (Liu et al. 2020), plastic film residues (Wang et al. 2021b), and Ag nanoparticles (Feng et al. 2021) also affect the microbial communities in the soil. Generally, the rhizospheric microbiome assembly must depend on the connections between the roots and the microbiota that are mediated by biochemistry.

The precise methods for the construction of the rhizospheric microbial community are, however, still unknown because of how complicated these biochemically mediated linkages are. How specific plant root signals and exudates attract the microbiome to the plant's rhizosphere is unknown. Future investigation will be necessary to pinpoint the process through which plants control microbiome assembly.

3.7 Microbiome engineering using a plant-based approach

Under controlled conditions, specified exudates that attract bacteria can be secreted by plants. These exudation patterns have been altered by applying a variety of manipulation techniques, the most popular of which include genetic engineering, plant breeding, and manipulations based on metagenomics (Arif et al. 2020; Dries et al. 2021; Kumar and Dubey 2020). Engineering techniques are used to develop plant cultivars that are more resilient to biotic and abiotic stresses, such as infections and drought, in that they attract and maintain a healthy microbiome.

According to a study that evaluated plant cultivars with different levels of wilt resistance, several disease-suppressing bacteria were prevalent in the resistant rhizosphere cultivars (Kwak et al. 2018). Using the quantitative trait locus (QTL) mapping technique, the host genome can be searched for particular gene pools linked to unique behavioral features. Once a suitable gene pool has been identified in the plant genome, modern technologies for genome editing, such as site-specific zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered repeatedly interspaced short palindrome repeats of the (CRISPR)/CRISPR-associated protein 9 (Cas9) can be used to alter the characteristics for enlisting the preferred microbiome in the rhizosphere (Kaul et al. 2021; Kumar and Dubey 2020). By using these tools, important traits included in the metabolic and biosynthetic processes at the translational and transcriptional levels can be targeted. The most sophisticated technique for deleting or introducing crucial genes across numerous plants is CRISPR/Cas9. This method was employed in a study to create a tomato plant resistant to the pathogen, *Pseudomonas syringae* pv. tomato (Pto) DC3000, which causes the bacterial speck disease (Ortigosa et al. 2019). Furthermore, by inhibiting the TaMLO gene (linked to the colonization of fungal infections) present in wheat protoplasts, Shan et al. (2014) effectively created a cultivar of wheat resistant to powdery mildew disease. The CRISPR/Cas9 technology has been applied to genome editing to achieve much more, and there is a great deal more to come (Shan et al. 2014).

To summarize, advanced plant genome editing techniques and plant breeding strategies are potential means to gather advantageous alleles that can act as selection drivers to direct the development of a dynamic microbiome. These modification tactics are still hindered by technical issues such as changing the structure of the root exudate, competition from native soil microorganisms, environmental stress factors, the host genotype, and a lack of *in situ* editing tools. Despite this, the application of cutting-edge synthetic biology and omics techniques to *in situ* microbiome engineering can help healthier microbiomes establish themselves and thrive.

3.8 Biotic and abiotic stressors adapted by an engineered microbiome

Understanding the complexity of microbiome-mediated protection will enable long-term solutions to maintain healthy management methods that consider the interactions of the microbiome with the plants, soils, and environment. In terms of plant health, it has become clear that the soil microbiome is a crucial element in developing systemic defenses against insects and pathogens in plants (Pineda et al. 2017). For the purpose of enhancing plant fitness, some research has proficiently created the SynComs, *in vitro* and pot test (Tsolakidou et al. 2019). This technique still has a long way to go before it can be adequately applied in the field and under various soil conditions.

An important environmental problem is the salinity of agricultural soil systems because this issue has already harmed over 840 million hectares of arable land and is expected to do even more harm (Rath et al. 2019). Salinity effects could be reduced by introducing halo-tolerant synthetic microbiomes into saline soils. Although most microorganisms are halo-sensitive, some halophytes associated with plants can be thought of as possible targets for the construction of synthetic microbiomes. Inoculated halotolerant rhizobacteria have been shown to increase the native microbial community's resistance to salt stress, which in turn improves plant stability and growth in unfavorable saline environments (Zilaie et al. 2022). It is recommended that engineered microbiome techniques be adopted in regions with irrigation systems using saline water.

One of the biggest challenges to agricultural output is drought. To ensure successful crop development in the face of scarce water resources, plant stress tolerance must be increased (Liu et al. 2019). With the exception of a few model plants, some bacteria associated with roots were shown to be able to increase plant tolerance to drought stress (Kour et al. 2019; Hone et al. 2021), but their method for doing so with regard to a particular host is still poorly understood (Trivedi et al. 2020). For instance, the model plant, *Sorghum bicolor*, secretes particular metabolites that promote the abundance and activity of monoderm bacteria, which in turn modify the composition of the root-associated microbiome and benefit the improvement of *Sorghum bicolor* plants under drought stress (Xu et al. 2018b). This could be a model for creating SynComs from the microbiomes associated with the plant to boost crop output in desert regions with inadequate irrigation and low precipitation.

Conclusion

The future development of bioproducts will depend on a better knowledge of how microorganisms influence crop growth and resistance to disease. In this study, we look back on the intricate relationships between plants and bacteria in the rhizospheres of plants and consider the root microbiome assembly patterns that promote plant development and resistance to disease. The significance of the plant root microbiome in fostering plant development and resistance to disease is emphasized by the research findings, which also demonstrate the intricate relationships that exist between root microorganisms and plants. Additionally, a review of the current studies deepens our understanding of microbiological engineering. In its turn, the use of the reductionist idea in the early microbiome studies serves to speed up the creation of microbial products. However, the effectiveness of microbial products is still significantly influenced by environmental variables and the colonization of microbiomes. It is still challenging to pinpoint the specific mechanisms of the colonization and assembly of the rhizosphere microbiome. The mechanisms can be better understood through further research, which will subsequently lead to a breakthrough in the use of beneficial microbiomes in real-world agricultural development. The study of the microbial phenotypes necessary for colonization and persistence in the rhizosphere environment, as well as the development of more sophisticated technologies and methodologies to disclose the intricate mechanisms of rhizosphere microorganism assembly, are potential future directions. Also, we need to focus more on the functional analysis of the plant microbiome.

Chapter 4

Microbial community structure and functional diversity in maize rhizosphere as influenced by *Striga hermonthica* infestations

4.0 Abstract

The most problematic weed in sub-Saharan Africa (SSA) is known as witchweed (*Striga hermonthica*). It is an obligate root hemiparasite of grasses that causes major limitations and catastrophic losses to food crops. This study compares the microbial community structure and diversity in the *Striga hermonthica*-infested maize rhizosphere to uninfected soil. We hypothesize that microbial structure and diversity are influenced by *Striga* infestations. In this study, using shotgun sequencing, we compared soil samples from *Striga*-infested soil with bulk soil to investigate the microbial diversity in the soil and the availability of biocontrol organisms in the infested soil. The major bacterial phyla observed in all samples were Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Acidobacteria, Chloroflexi, Verrucomicrobia, and Cyanobacteria. The archaea phyla were Euryarchaeota and Crenarchaeota, and the predominant fungi phyla included Ascomycota and unclassified (Fungi). The alpha diversity analysis showed no significant difference in the infested and uninfected soil, while the beta diversity analysis showed a significant difference at $p > 0.05$. The major nutrient pathways relating to the plant growth-promotion properties that were identified were the carbohydrate, phosphorus, nitrogen, and secondary metabolic pathways Additionally, despite the invasion of this parasite, there have been notable traces of functional groups of microorganisms present in the maize rhizosphere. Mapping out strategies to isolate novel biocontrol agents and to explore genes that could be helpful in effectively controlling this weed will help in the biological control of *Striga* for sustainable agriculture.

Keywords: Plant microbe interactions, biocontrol agents, SEED subsystem, shotgun metagenomics, parasitic weed

4.1 Introduction

Given that it makes up more than half of the total area used for crop production in sub-Saharan Africa (SSA), one of the most significant cereal crops, maize (*Zea mays* L.), is thought to produce the best yields (Gasura et al. 2019; David et al. 2022). On over 3.1 million hectares of land, South Africa annually produces close to eight million tons of maize (Fadiji et al. 2021a). The human population consumes the majority of the white maize crop (Du Plessis 2003; Fadiji et al. 2021a). Several contraints affect maize production. They include abiotic stresses (climate, salinity, and drought) and biotic stresses (pests, diseases, and weeds). *Striga hermonthica*, among other species of *Striga,* is the most economically important biotic constraint to maize production in SSA (David et al. 2022; Dawud 2017a; Kamara et al. 2020). It causes a loss of over $10 billion, resulting in food shortages for 100 million people. *Striga*-related crop losses could result in output reductions of up to 100% (Dafaallah 2019; Kamara et al. 2020).

Purple witchweed, also known as *S. hermonthica*, is in the *Orobanchaceae* family. This *Striga* specie infects crops such as sugarcane, sorghum, pearl millet, rice, cowpea, finger millet, and maize, drastically reducing harvests every year and thus depleting crop productivity (Mudereri et al. 2020; Stanley et al. 2021b; Gowda et al. 2021). This parasitic weed has no root of its own but attaches to the roots of the host plant before entering its vascular system and eventually capturing minerals, water, and photosynthates for growth. It has a strong phytotoxic effect on its host (Gressel et al. 2004; Yonli et al. 2005; Khan et al. 2002). The presence of this parasite causes significant crop damage, including wilted silk, chlorosis, thin stalks, and complete crop loss in farms where, as a result of overcultivation and inadequate management practices, the parasite is prevalent (Mbuvi et al. 2017b; Menkir et al. 2020c).

The conventional, chemical, biological, and host resistance techniques were used previously to manage *Striga* infestations. Intercropping, hand weeding, planting of trap or catch crops, crop rotation, soil fertility improvement, and push-pull technologies (nitrogen fertilization) are all examples of conventional farming techniques. Suicidal germination, genetic control, imazapyr, the application of weedicides (chemical methods), the planting of resistant crop varieties, and the use of biological control agents, such as bacteria, fungi, and certain insects, have all been used as management approaches (Aly 2007; Naoura et al. 2021; Chikoye et al. 2020; Babalola 2010a; Babalola 2010b; Babalola and Odhiambo 2008). None of these techniques has been successful in completely eliminating *S. hermonthica* cases on its own, but a combination of two or more techniques has been demonstrated to be effective. As a result of the negative impact of chemical compounds on soils, the use of microbial inoculants, which benefit plant growth by promoting the release of plant growth hormones, increasing nutrient uptake, and lowering plant infections, is becoming increasingly common (Magallon-Servín et al. 2020b; David et al. 2022; Oula et al. 2020a; Babalola et al. 2007).

Considering the lasting negative impact of fertilizers on soil, it is necessary to investigate the microbial diversity of soil that is infested with *Striga* in order to find biocontrol agents and antagonists that by using metagenomic approaches, can successfully fight this root parasite plant (Prashar and Shah 2016; Alengebawy et al. 2021). Owing to recent developments in next-generation sequencing technology (NGS), which allow for the production of megabases of sequence data in a short period of time and for a better understanding of microbial diversity on the basis of DNA genome sequencing, scientists can now forecast the composition and organization of microbial communities. Although culture-based methods have allowed for the isolation of microbes in intensive studies, molecular methods such as metagenomics are rapidly contributing to the identification of microorganisms (Coats and Rumpho 2014; Turner et al. 2013b; Fadiji et al. 2021a). These NGS technologies have a variety of recognized applications, including single-gene-targeted shotgun metagenome and whole genome sequencing, that can be used under culture-dependent conditions to examine the microbial population (Kim et al. 2013; Zhou et al. 2021). A thrilling technique for choosing the best biocontrol bacteria and finding those that are *Striga* antagonists is the metagenomic sequencing technology (Zhou et al. 2021; Matsumoto et al. 2021). We hypothesize that the soil of a *Striga*-infested maizefield will be microbially less diverse and possibly contain biocontrol microorganisms that differ from those in an uninfected maizefield. In this study, we use a shotgun metagenomic technique to examine the rhizospheric microbial communities associated with *Striga*-infested maize soil.

4.2 Materials and methods

4.2.1 Site description and soil sampling

For this study, samples from the *Striga*-infested maize rhizosphere were collected from a farmland located at Mbuzini ($25^0$55’30.9” S; $31^0$56’11.7” E) in Mpumalanga province, South Africa. After the farmers had granted access, soil samples of the *Striga*-infested maize rhizosphere were collected from three locations on the same farm and were labelled as SA, SB, SC, from various points on the infested field and from the bulk soils (BS) on the uninfected field. With the use of a spade, the infested rhizosphere soil was collected from an area, 2 x 4m^2 , and at a depth of 20 cm, and a diameter of 6 cm around the plant, as earlier suggested by Zhang et al. (2019a). The samples were subsequently transported to the laboratory in a cooler box filled with ice packs and stored at -20^0C for subsequent analysis.

4.2.2 DNA Extraction and Metagenome Sequencing

The DNA components of the soil samples were extracted according to the manufacturer’s instructions from five grams (5 g) of soil by using the NucleoSpin Soil kit (Macherey-Nagel GmbH, Duren, Germany). To prepare the sample, 250mg of soil sample was transferred into a NucleoSpin® bead tube type A containing the ceramic beads and 700 µl buffer SL1 was added to the tube. An additional 150 µl enhancer SX was added in other to adjust the lysis conditions. The NucleoSpin® bead tube was attached horizontally to a vortexer and the samples were vortexed at maximum speed under room temperature (25 ^{0}C) for 5 minutes. To remove precipitation contaminants, The samples were centrifuged for 2 minutes at 11,000 x g. 150 µl buffer SL3 was added and vortexed for 5 seconds. It was thereafter incubated for 5 minutes at 4 ^{0}C and centrifuged at 11,000 x g for 1 minute. To filter the lysate, the NucleoSpin® inhibitor removal column (red ring) was placed in a 2 ml collection tube. 700 µl of clear supernatant from the previous step was loaded into the filter and centrifuged for 1 minute at 11,000 x g. The NucleoSpin® inhibitor removal column was discarded. To adjust the binding condition, 250 µl buffer SB was added and vortexed for 5 seconds. To bind the DNA, NucleoSpin® soil column (green ring) was placed in a 2 ml collection tube. 500 µl of sample was loaded into the column and centrifuged at 11,000 x g for 1 minute. The flow-through was discarded and the column was placed back into the collection tube. The remaining samples were loaded into the column and centrifuged at 11,000 x g for 1 minute. The flow through was also discarded and the column was placed back into the collection tube. To wash and dry the silica membrane, it was washed four (4) times with SB and SW2 and vortexed for 2 seconds and also centrifuged for 30 seconds at 11,000 x g, the flow through was discarded and the column was placed back into the collection tube. It was centrifuged for another 2 minutes at 11,000 x g to get a dry silica membrane. To obtain the elute DNA, the NucleoSpin® soil column was placed into a new microcentrifuge tube, 100 µl of buffer SE was added and incubated for a minute at room temperature while the lid was open. The lid was closed, and the tube was centrifuged at 11,000 x g for 30 seconds. By applying the Illumina Sequencing Technology, a shotgun genome sequencing was performed on the samples at NovogeneAIT Genomics Singapore Pte Ltd., Singapore. The concentration of DNA was determined with the aid of the Qubit® dsDNA HS kit (Life technologies). The DNA libraries were prepared according to the recommended protocol using the NEBNext® Ultra^{Tm} IIDNA library prep kit. The library was diluted to 2 ng/µl and then, after having been quantified by the Qubit2.0 fluorometer, was detected by the Agilent 2100 Bioanalyzer for its insert size. Q-PCR was performed to ensure the library quality (effective concentration > 3 nM). Subsequent to its pooling in accordance with its effective concentration and expected data volume, a 300-cycled sequencing of the paired end was performed on the Novaseq 6000 platform.

4.2.3 Data analysis of sequences

The generated metagenome sequences were uploaded to the metagenomics rapid annotation subsystems technology (MG-RAST) platform. The uploaded data were pre-processed using

SolexaQA in order to trim the low-quality reads, and followed by de-replication, where Artificial Duplicate Reads (ADRs) were removed from the datasets (Meyer et al. 2008). Thereafter, the DRISEE (Duplicate Read Inferred Sequencing Error Estimation) was used to screen for sequencing errors owing to measurements of ADRs and reads similar to those for model organisms, such as fly, mouse, cow, and human. All were screened using a Bowtie aligner (Kent 2002). After determining the read quality, the sequences on the M5NR database were annotated against other databases using a BLAST-like alignment (BLAST-algorithm) (Wilke et al. 2012). The taxonomy categorization tool (> 65%) was filtered, and other domains were screened out in the SEED subsystems database on MG-RAST. The SEED subsystems were also used to profile the functional categories of the microbiomes. The mean abundance values of the replicates were sorted, agglomerated, and the unclassified reads were kept for statistical analysis. The sequences were subsequently deposited in the NCBI SRA database, with BioProject accession number PRJNA882739.

4.2.4 Statistical Analysis

The Simpson Diversity, Shannon Diversity, and Pielous Evenness indices for each sampling site were evaluated using PAST version 3.20 (Hammer et al. 2001), and the differences in the indices between the sites were investigated using the Kruskal-Wallis test (Carrell and Frank 2015). The one-way analysis of similarities (ANOSIM) was used to evaluate the variations in the community structure and the functions within the sites, and the principal coordinate analysis, based on a Euclidean distance matrix, was used to describe the beta diversity (Carrell and Frank 2015). To show how the generated phyla microbial communities and the various functional categories were distributed throughout the maize fields, principal component analysis with CANOCO 5 (Microcomputer Power, Ithaca, 148 NY) was conducted. ClustVis was used to plot the relative abundance graph of the microbial communities at the phylum level and at Level 1 of the functioning groups after the dataset had been adjusted (Metsalu and Vilo 2015). The Shinyheatmap was used to plot the relative abundance graph at the class, order, family, and genus levels, and also along the various functional pathways (Babicki et al. 2016).

4.3 Results

4.3.1 Overall analysis of sequenced data from soil samples collected from various locations

The sequence reads for the three (3) samples from the *Striga*-infested rhizosphere were SA (1,285,898,222), SB (1,614,800,990), SC (1,374,665,496), and the bulk soil (BS) read was (1,558,169,695). After quality control (QC), the sequence reads were SA (852,568,380), SB (1,182,825,510), SC (1,036,401,918), and BS (1,224,529,204), with the mean G + C content of 65%, 65%, 62%, and 66%, respectively. Sequences that corresponded with protein identified in the samples after quality control checks were SA (1,261,942), SB (2,000,684), SC (1,729,561) and BS (2,252,580), respectively (supplementary Table 1). The richness and diversity of the microbial communities were assessed using rarefaction analysis through MG-RAST (Fig. 4.1).

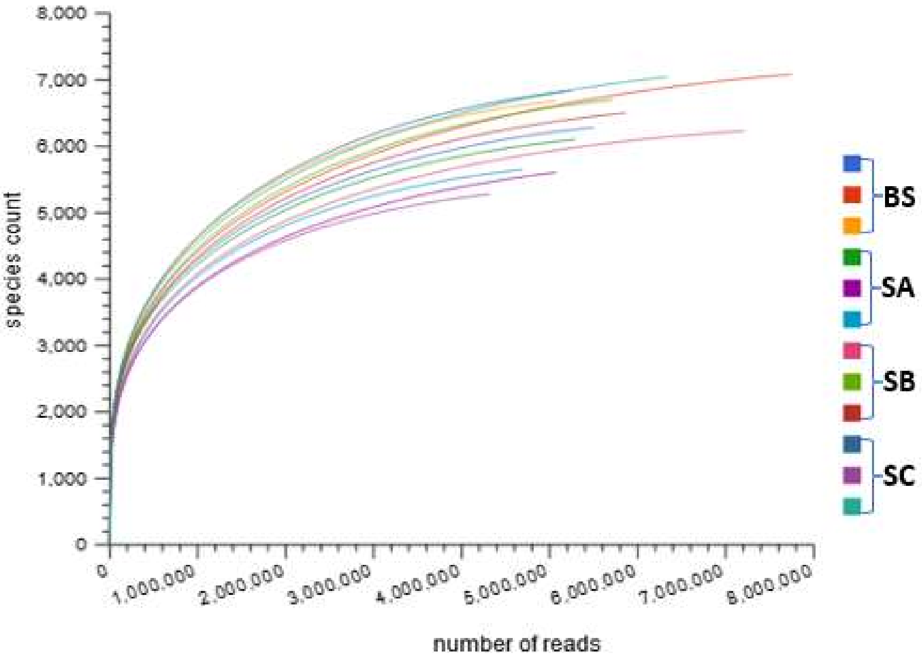


Figure 4.1: The richness and diversity of the microbial communities showing the saturation of the sequences. The BS sample is from the bulk soil; SA, SB and SC are the *Striga*-infested maize samples.

4.3.2 Distribution of soil samples at the phylum level across the various sites

The Refseq database revealed the dominant phyla in the samples. The bulk soil (BS) samples were more enriched than those taken from the *Striga*-infested maize soil (Figure 4.2). The prominent rhizospheric bacterial phyla observed included: Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Acidobacteria, Chloroflexi, Verrucomicrobia, and Cyanobacteria.

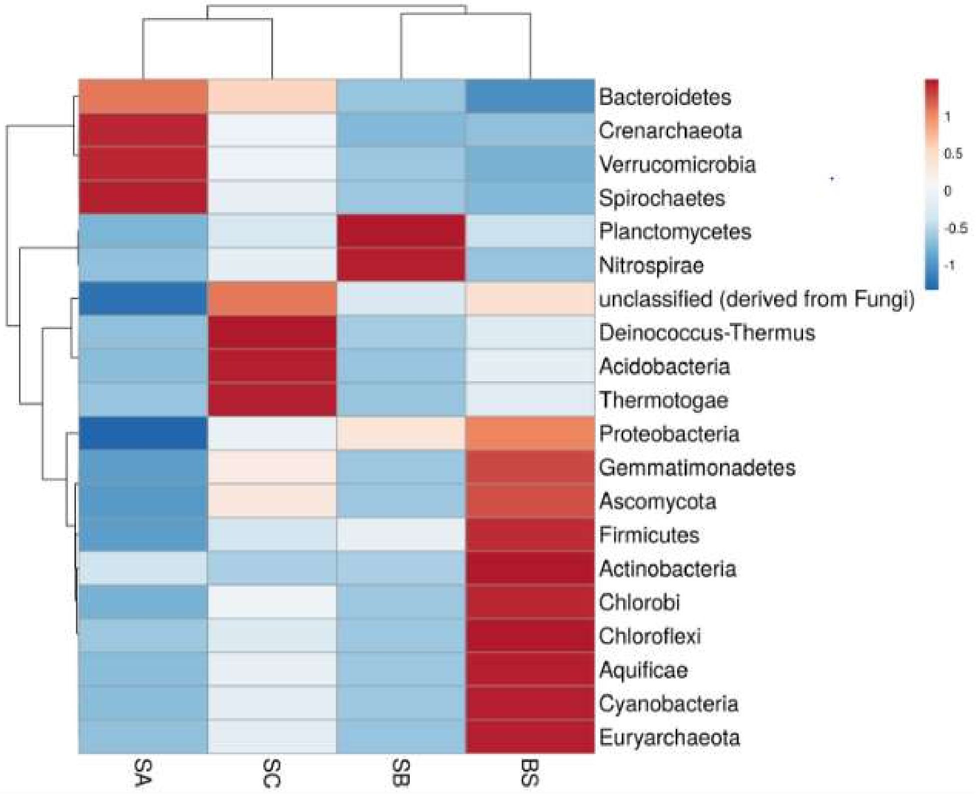


Figure 4.2: The bulk soil and *Striga*-infested maize soil samples from three separate locations on the same farm were compared for microbial phylum abundance. Based on the relative levels of abundance, the scale bar depicts a gradient of color saturation. [The BS sample is from the bulk soil; SA, SB and SC are the *Striga*-infested maize samples].

The occurrence of phyla, such as Gemmatimonadetes, Deinococcus-Thermus, Chlorobi, and Aquificae*,* amounted to less than 1%. The predominant phyla of archaea were Crenarchaeota and Euryarchaeota*,* while the predominant phyla of fungi included unclassified (Fungi) and Ascomycota. Of the phyla observed, the bulk soil (BS) was found to have the highest concentration of microorganisms compared to that in the *Striga*-infested soils (SA, SB, and SC), with no significance ($p > 0.05$) across all of the sampling sites. Using PCA, a comparison was made between the samples from the bulk soil and from the *Striga*-infested rhizosphere (SA, SB, SC) to determine the abundance of the detected microbial phyla, with the bulk soil having the highest concentration (Fig. 4.3).

4.3.3 The structural makeup of the microbial communities from various sites

The bulk soil had the highest abundance of prominent phyla, including Firmicutes, Actinobacteria, Chlorobi, Cyanobacteria, Euryarchaeota, and Gemmatimonadetes. Acidobacteria were found to be dominant in *Striga*-infested soil, SC (7.6%), followed by BS (4.3%), and SB (3.3%), with the lowest concentrations being recorded for SA (3.1%), with no significant differences being apparent ($p > 0.05$) (Fig. 4.2).

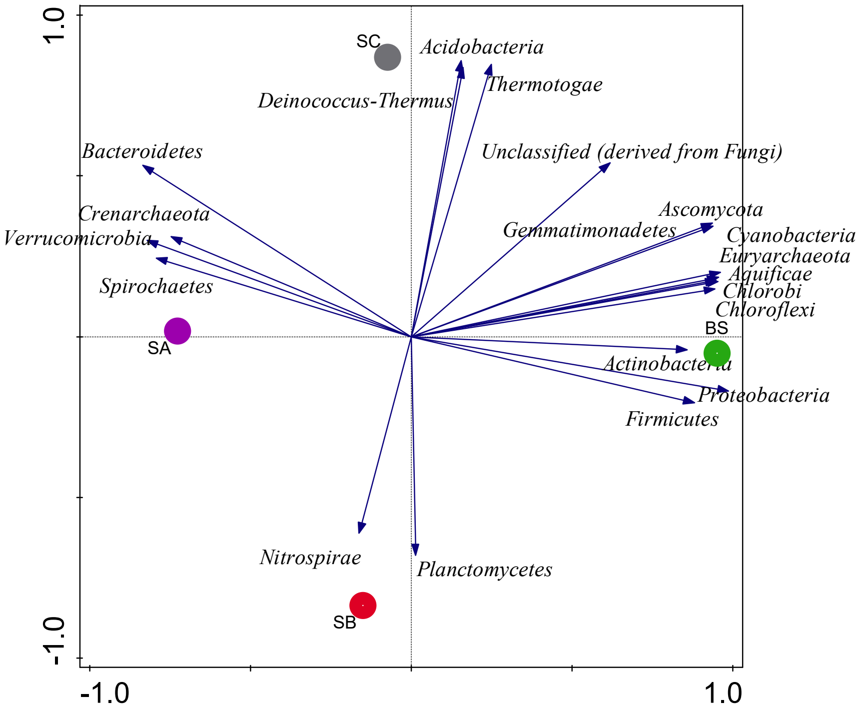


Figure 4.3: PCA graph of the distribution of microorganisms in the samples. The vector arrow indicates the effects of the microbe metagenomes. Axis 1 (63.7%) and Axis 2 (21.4%) outline the variations based on CANOCO.

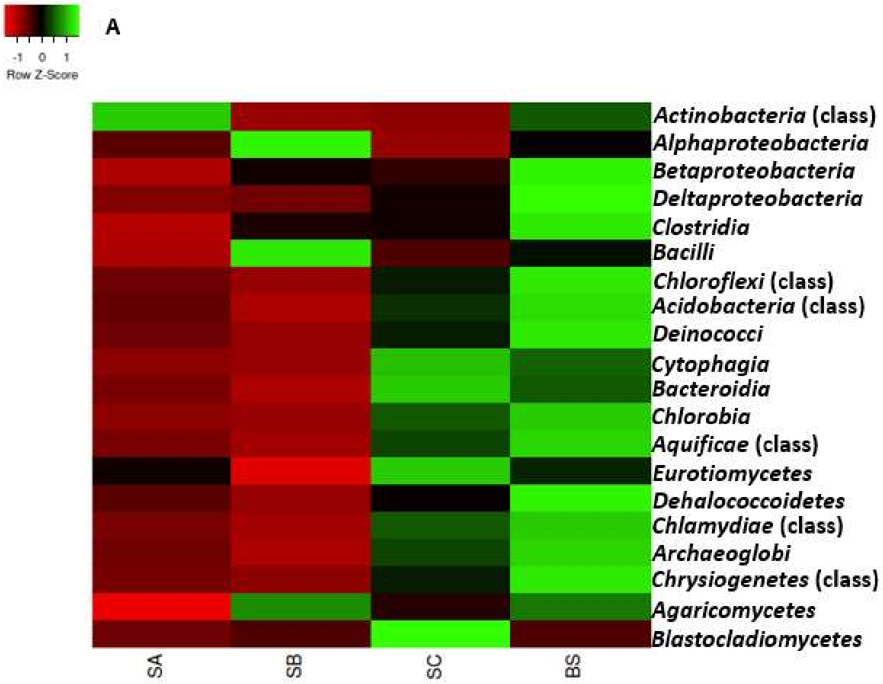
As shown in Figure 4.2, the research found that at a 4.2% level, bacteroidetes dominated at SA. This was followed by SC (3.6%), SB (2.1%) and BS (1.6%). Proteobacteria proved to be dominant in the following order: BS (60.2%), SB (53.3%), SC (48.2%). Ascomycota dominated at BS (0.6%) and was followed at SC (0.5%), at SB (0.3%), and the least dominant of all, at SA (0.2%).

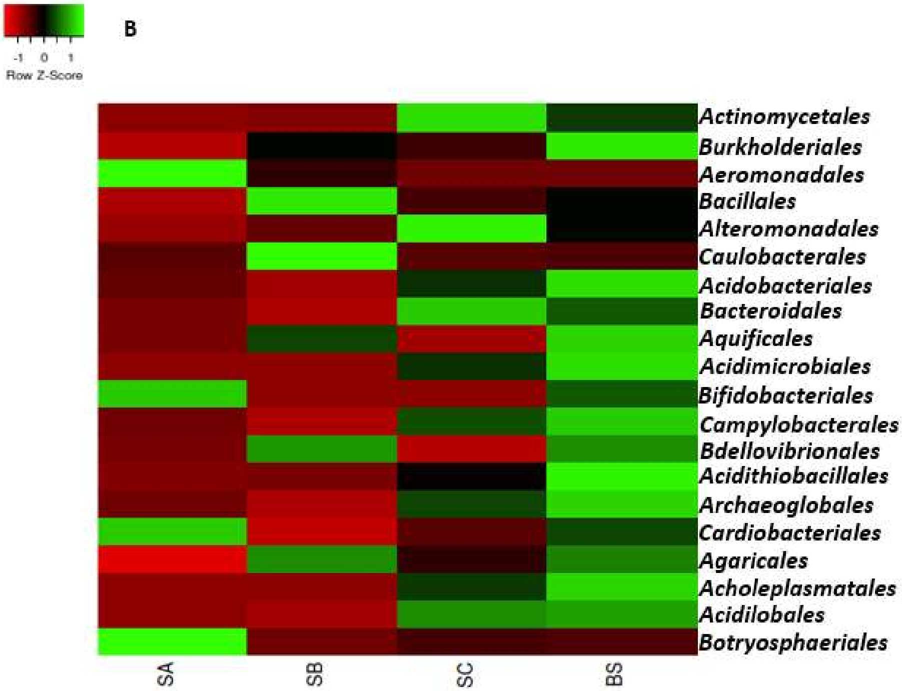
At class level, *Bacilli, Actinobacteria, Alphaproteobacteria, Actinobacteria, Agaricomycetes* and *Blastocladiomycetes* were more prevalent in *Striga*-infested soil as opposed to their presence in the bulk soil, while the concentrations of *Betaproteobacteria, Deltaproteobacteria, Clostridia, Chloroflexi, Acidobacteria, Bacteroidia, Chlorobia,* and *Archaeoglobi* were greatest (they were the most abundant) in both the bulk soils and the infested soils (Fig 4.4A). Furthermore, at order level, *Actinomycetales, Aeromonadales, Bacillales,* and *Bacteroidales* were dominant in the infested soil even though the bulk soil had larger concentrations of microbes in it, including *Burkholderiales, Acidobacteriales,* and *Acidimicrobiales* (Fig. 4.4B).

At family level, *Acidobacteriaceae, Acidothermaceae, Archaeoglobaceae, Acidaminococcaceae, Brachyspiraceae*, amongst others, *Acidilobaceae* were observed to be dominant in the bulk soil, while *Acetobacteraceae, Actinomycetaceae,* and *Acidimicrobiaceae* were dominant in the *Striga*-infested soils (Fig. 4.4C). Also, at the genus level, as opposed to their presence in the bulk soil, *Bacillus, Aspergillus, Basidiobolus,* and *Allomyces* were dominant in the infested soils (Fig. 4.4D). *Bacillus* has been used as a biocontrol against *Striga*. Additionally, *Burkholderia,* which is more abundant in the bulk soil, has been used as a biological control against this parasitic weed.

4.3.4 Diversity estimation of microbial communities acquired

The Evenness and Shannon indices calculated for the phylum and genus microbial community levels showed no significant difference ($p > 0.05$) (Table 4.1). On the basis of the Euclidean distance matrix, the PCoA was used to analyze the composition of the microbial community. The PCoA plot revealed that the BS sample evidently differed (ANOSIM, $P= 0.01$, $R= 0.58$) from those samples taken from the infested soils (SA, SB, SC) (Fig. 4.5).


A
-1 0 1
Row Z-Score
Actinobacteria (class)
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Clostridia
Bacilli
Chloroflexi (class)
Acidobacteria (class)
Deinococci
Cytophagia
Bacteroidia
Chlorobia
Aquificae (class)
Eurotiomycetes
Dehalococcoidetes
Chlamydiae (class)
Archaeoglobi
Chrysiogenetes (class)
Agaricomycetes
Blastocladiomycetes
SA
SB
SC
BS


B
-1 0 1
Row Z-Score
Actinomycetales
Burkholderiales
Aeromonadales
Bacillales
Alteromonadales
Caulobacterales
Acidobacteriales
Bacteroidales
Aquificales
Acidimicrobiales
Bifidobacteriales
Campylobacterales
Bdellovibrionales
Acidithiobacillales
Archaeoglobales
Cardiobacteriales
Agaricales
Acholeplasmatales
Acidilobales
Botryosphaeriales
SA
SB
SC
BS

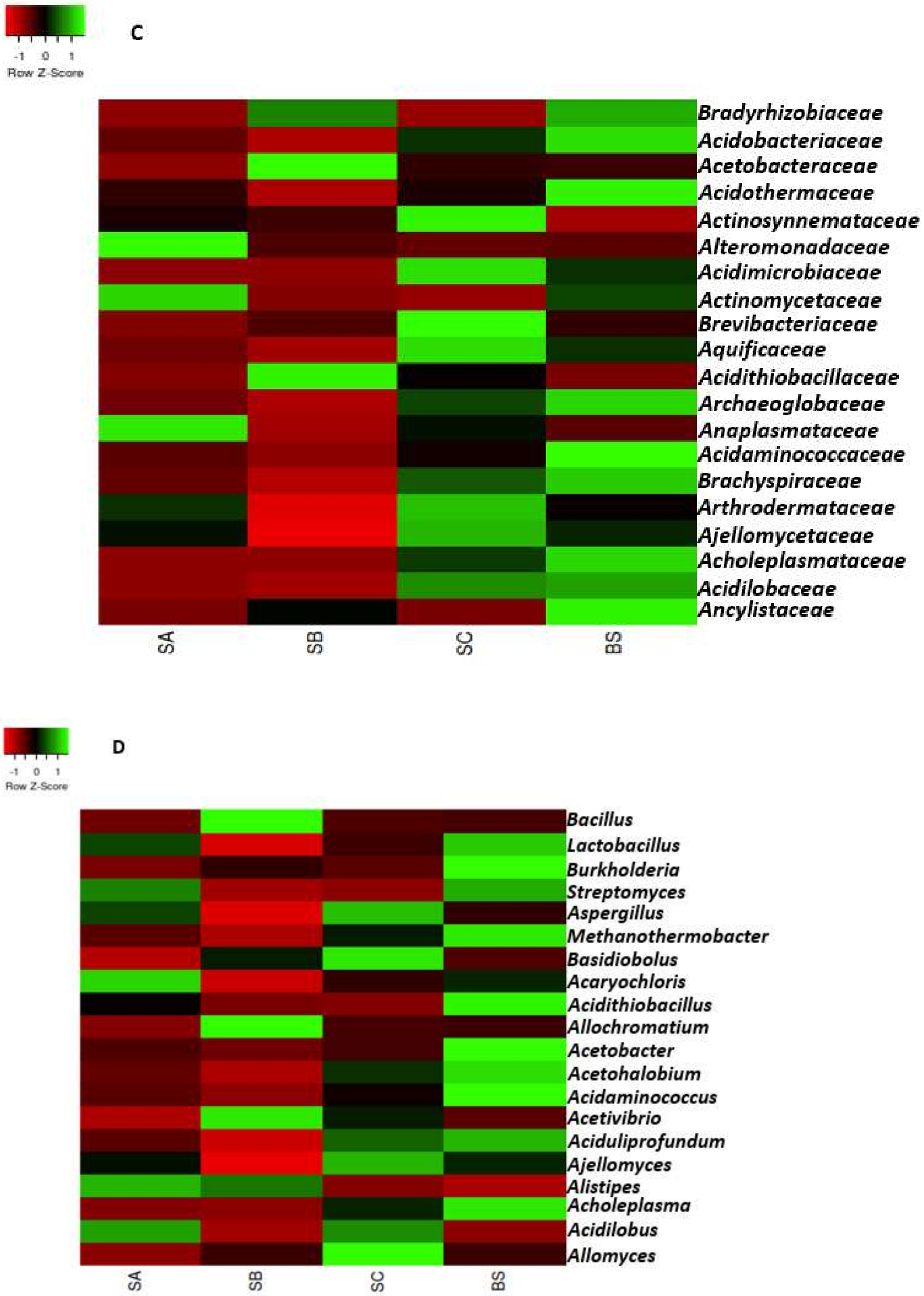


Figure 4.4: Microbial community heatmap at (A) class, (B) order, (C) family, and (D) genus levels. The scale bar represents the color saturation gradient based on the relative abundance levels, with a z-score-transformed relative abundance of the microorganisms. The BS sample is from the bulk soil; SA, SB and SC are the *Striga*-infested maize samples.

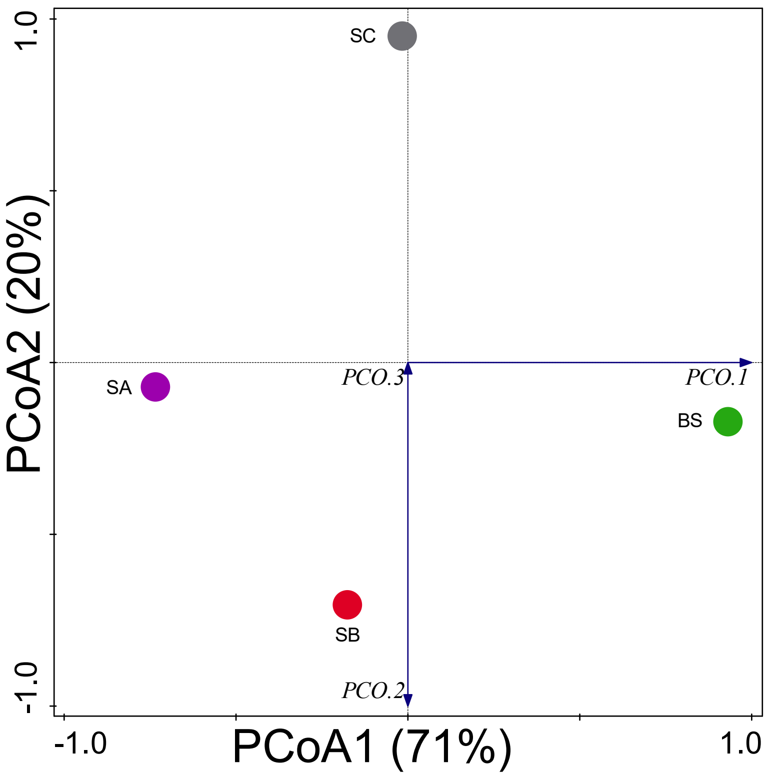


Figure 4.5: PCoA graph of the microbial communities in the soil based on the Bray-Curtis differences. The BS sample is from the bulk soil; SA, SB, and SC are the *Striga*-infested maize samples.

Table 4.1: Diversity and evenness present in the rhizosphere of the *Striga*-infested soil and bulk soil

Taxon	Statistics	SA	SB	SC	BS	*p*-Value
Phylum	Simpson_1-D	0.683±0.08	0.639±0.06	0.690±0.067	0.729±0.060	0.45
	Shannon_H	1.598±0.057	1.503±0.057	1.687±0.057	1.792±0.057	
	Evenness e^H/S	0.1301±0.106	0.1183±0.094	0.1422±0.099	0.1579±0.080	
Genus	Simpson_1-D	0.978±0.025	0.970±0.027	0.932±0.026	0.980±0.028	0.17
	Shannon_H	4.269±0.21	4.187±0.21	3.838±0.21	4.317±0.21	
	Evenness e^H/S	0.629±0.093	0.577±0.10	0.407±0.10	0.658±0.10	

Mean ± standard error (n = 3). *P-value* based on the Kruskal-Wallis test. Samples SA, SB and SC, are from the *Striga*-infested maize rhizosphere, while BS is from the bulk soil.

4.3.5 Functional analysis of rhizospheric microbiomes connected with maize plant

The SEED subsystem Level 1 results revealed 28 major functional categories attributable to the community of microorganisms in the respective locations (Fig. 4.6). These categories include: clustering-based subsystems (CBS), miscellaneous (MIS), carbohydrates (C), protein metabolism (PM), sulfur metabolism (SFM), metabolism of aromatic compounds (MAC), photosynthesis (PSTH), membrane transport (MT), secondary metabolism (SM), phages, prophages, transposable elements, and plasmids (PPTEP), phosphorus metabolism (PhosM), stress response (SR), iron acquisition and metabolism (IAM), fatty acids, lipids, and isoprenoids (FALI), dormancy and sporulation (DAS), amino acids and derivatives (AAD), regulation and cell signalling (RCS), potassium metabolism (POTM), respiration (RES), and nitrogen metabolism (NM). There was no significant difference ($p > 0.05$) in the identified functions. The distribution of the identified functional categories for the SA, SB, SC, and BS locations was obtained using PCA (Fig. 4.7). The distribution showed that eight (8) functional categories dominated at Location SB, seven (7), at Location SC, six (6), at Location SA, while seven (7) functional categories dominated the bulk soil (BS).

The function, NULL, was observed to be highest in the SEED subsystem Level 2 for gene annotation across all samples (Fig. 4.8). The relative abundance of this NULL protein in the samples was prominent in the following order: 21.7% (SB) > 21.3% (SA) > 21.2% (SC) > 21.1% (BS).

4.3.5.1 Alpha and Beta diversity evaluation of the functional groups across the sampling sites

The Evenness index and the Shannon index (Table 4.1) were used to assess the functional group diversities at Level 1 of the SEED subsystem, and it was found that they did not significantly differ from one another ($p > 0.05$). The Kruskal-Wallis test was used to estimate the degree of diversity differences in samples from each location, and no significant difference was found (p > 0.98). The PCoA showed that the abundance levels of the 28 functional categories were evenly distributed across all sites (Fig 4.9). In addition, there was a significant difference across all sample locations using the one-way ANOSIM (P = 0.01 and R = 0.58).

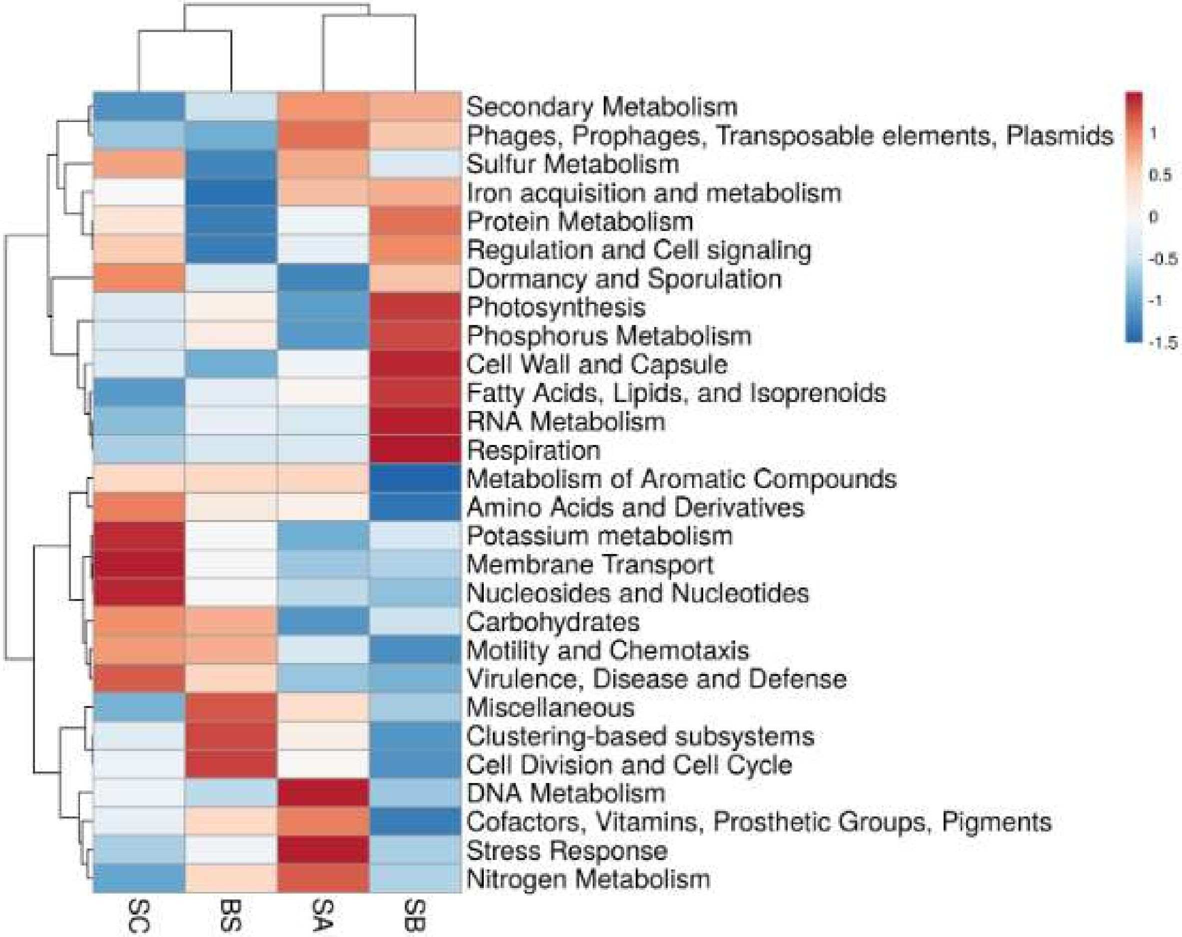


Figure 4.6: Key metabolic sequences in samples from the maize plant collected at various locations. The multiple colors show the relative abundance levels, using the scale bar as an indication. Samples SA, SB, and SC are from the *Striga*-infested rhizosphere, while BS is from the bulk soil.

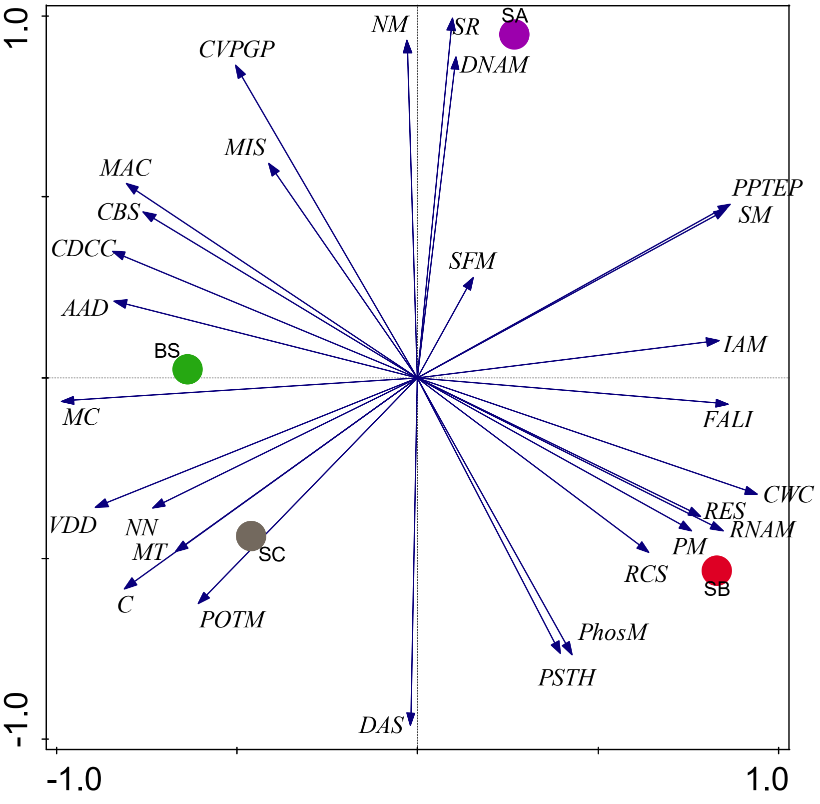


Figure 4.7: The vector shows the distribution of each metabolic activity in the PCA graph that displays the functional analysis of the microbiomes. Axis 1 (73.9%) and Axis 2 (16.6%) explain the variations based on Euclidean distances. Samples SA, SB, and SC are from the *Striga*-infested rhizosphere, while BS is from the bulk soil.

Table 4.2: The examined functional categories of microbiomes for evenness and diversity at Level 1 of the SEED subsystem from each location

Level 1	SA	SB	SC	BS	p-Value
Shannon_H	2.903±0.11	2.921±0.11	2.898±0.11	2.879±0.11	0.99
Evenness_e^H/S	0.6512±0.09	0.6629±0.09	0.6475±0.09	0.6356±0.09	

Mean ± standard error (n = 3). *P-value* based on the Kruskal-Wallis test. Samples SA, SB, and SC are from the *Striga*-infested rhizosphere, while BS is from the bulk soil.

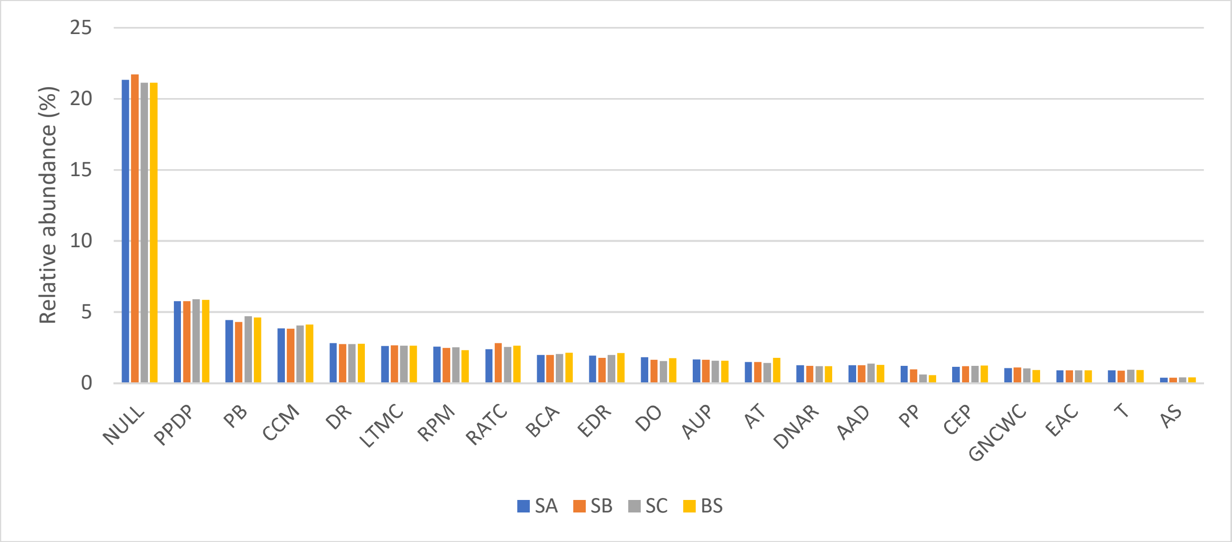


Figure 4.8: Functional categories obtained at SEED subsystem Level 2. Samples SA, SB, and SC are from the *Striga*-infested rhizosphere, while BS is from the bulk soil.

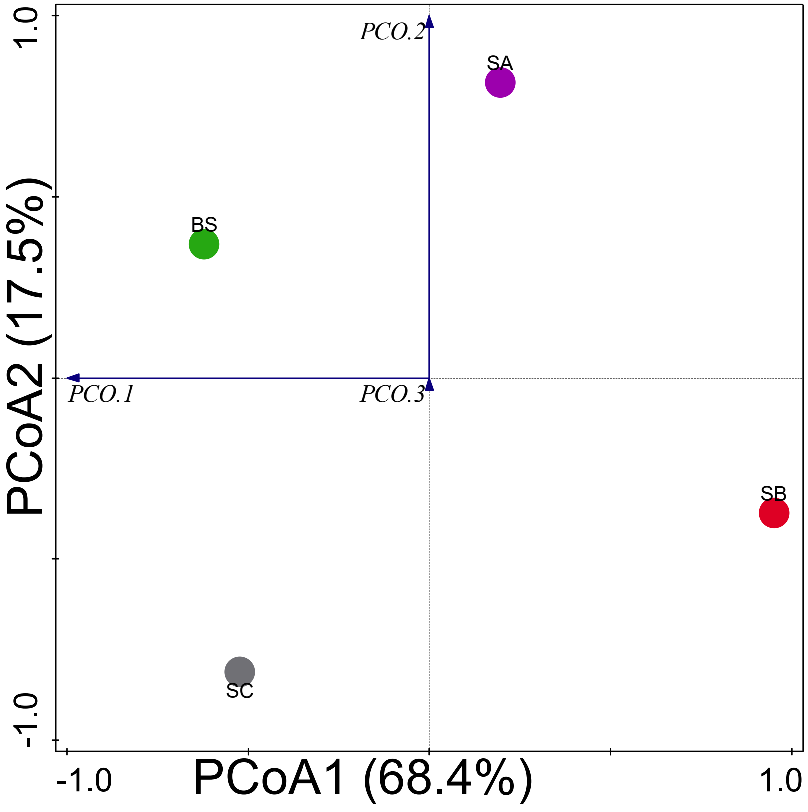


Figure 4.9: Based on Euclidean dissimilarities, the PCoA graph shows the functional groups for all the microbiomes from each location that were discovered at the SEED subsystem level. Samples SA, SB, and SC are from the *Striga*-infested rhizosphere, while BS is from the bulk soil.

4.4 Discussion

Maize is a major cereal crop in South Africa, and depending on the farmer's preferences, it can be grown by using a wide range of agricultural techniques. As a result of growing worries about the effects of inorganic fertilizers and chemicals on the environment and human health, the use of bioinoculants in sustainable agriculture is becoming more and more popular around the world. However, considering that samples from the bulk soil locations presented with the highest relative abundance of microorganisms, as seen in this research, it was inferred that the *Stiga* infestation influences microbial structure and is enriched with microorganisms that can be exploited to combat parasitic *Striga.* This agrees with the findings of De Corato (2020) that despite the virulence of plant diseases, there are still microorganisms in infested soils that can be used to fight them.

Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Acidobacteria, Chloroflexi, Verrucomicrobia, Cyanobacteria, Euryarchaeota, Crenarchaeota, and Ascomycota were the major microbial phyla observed in this study. These organisms had previously been reported in related studies to be actively engaged in a variety of activities in the rhizosphere of maize (Li et al. 2014; Liu et al. 2018; Wen et al. 2016; Correa-Galeote et al. 2018; de Quadros et al. 2012).

Proteobacteria are engaged in a variety of processes, including the cycling of nitrogen, carbon, and sulfur, required for the cycling of nutrients in plants (Delmont et al. 2018). *Alphaproteobacteria* can function in difficult environments such as those with limited quantities of nutrients, while surviving with *Caulobacterales*, a member of this phylum, and are able, in spite of inadequate nutrient conditions, to induce nitrogen fixation (Bijlani et al. 2021). Until sufficient nutrients are available, they can perform tasks, which include shielding the plants from toxins and phagocytosis (Wang et al. 2020).

Firmicutes are a significant phylum of bacteria that can be employed as biofertilizers because of their critical function in promoting plant growth, the biocontrol of plant diseases (Lee et al. 2022; Wang et al. 2021a; Mamphogoro et al. 2020), and the phytoremediation of heavy metals. For example, *Bacillus altitudinis* AMCC1040, effective in the control of root knot nematodes that cause ginger bark-cracking disease in ginger planting areas, were found in Firmicutes, Actinobacteria, and Gemmatimonadetes (Wang et al. 2021a). This phylum's members possess key characteristics that are crucial to crop production, making it possible to use these organisms as biofertilizers and microbial inoculants for sustainable agriculture (Mattoo et al. 2021). The ability of Actinobacteria to solubilize phosphorus, zinc, and potassium, as well as their synthesis of iron-chelating chemicals and phytohormones, such as indole acetic acid, makes them crucial for application as bioinoculants and biofertilizers (Lasudee et al. 2021).

The presence of *Bacillus, Burkholderia,* and *Aquificales* was observed in the metagenomes of this study in which *Bacillus* was found to be more abundant in *Striga*-infested soil. However, *Burkholderia*, and *Aquificales* were more dominant in bulk soil. These microorganisms are useful as microbial inoculants because they play a significant role in conferring plants with some level of resistance to abiotic stresses, such as drought and high temperatures, unsatisfactory saline conditions, metal toxicity, and cold stress injury (Basu et al. 2021a; Khan et al. 2021b; Babalola and Akindolire 2011). In a screen test, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, and *Burkholderia phytofirmans* successfully reduced the *Striga* infestation on sorghum by 47% (Mounde et al. 2015). The PCA distribution in this research revealed that microbiomes are more dominant in bulk soil. This is, however, contrary to the findings of Fadiji et al. (2021b) to the effect that there is a high abundance of the microbiome in maize rhizosphere. This could be as a result of the infestation of *Striga hermonthica* in the soil. Nevertheless, there were still some notable microbiomes, such as *Bacteroidetes, Verrucomicrobia,* and *Crenarchaeota,* which, despite the *Striga* invasion, are linked to biological control treatments present in the soil (Cernava et al. 2019; Mamphogoro et al. 2020).

The SEED subsystem was also used in profiling the functional categories of the microbiomes. Different metabolisms, including catabolism and anabolism, are included in Level 1, while the other levels highlight the metabolic and gene pathways (Overbeek et al. 2014). While the prevalence of microbiomes differs throughout the locations in this study, the functional differences found among the locations at subsystems Level 1 are not statistically different ($P > 0.05$). From the results obtained from the *Striga*-infested soils, eight (8) functional categories were present at Location 2 (SB), seven (7) at Location 3 (SC) and six (6) at Location 1 (SA), while the bulk soil had seven (7) functions. This supports the idea that functional similarity, and not taxonomic similarity, determines the way in which microorganisms are categorized (Burke et al. 2011). This research also shows that the higher functional level of the genes, rather than that of the "species", might be the critical level to address the assembly and organization of microbial communities.

Other investigations have also shown that the functional levels of some of the microorganisms depend mostly on the environment and the type of hosts they inhabit rather than on their taxonomic categorization (Hong et al. 2019; Hardoim et al. 2015; Chen et al. 2022a; Dastogeer et al. 2020).

Our findings from the alpha diversity analysis reveal that nearly all of the subsystems are present in the samples from all of the locations since the functions displayed by the metagenomes at all of the sites approached the theoretical limit of 2.81 (Dinsdale et al. 2008). Additionally, a low evenness score was found (approximately 0.99, Table 4.2), which suggests that limited numbers of metabolic processes (including respiration, clustering-based subsystems, and protein metabolism) are prominent at each location.

This study was able to demonstrate the various major metabolisms and the distinctive traits of the identified functional categories in the metagenomes. Additionally, our findings show that none of the functional groups attributed to microorganisms from any of the sampling locations varied significantly ($P > 0.05$). The PCoA map demonstrated clear separations ($R = 0.58$) between each location (Fig. 4.8). An additional assessment was made using ANOSIM, which found a significant variation in the functional categories of the microbiomes among the samples (P-values = 0.01). Additionally, PCA was used to evaluate the claim that despite the invasion of parasitic *Striga,* the functional diversity of the rhizospheric microbiome will predominate in the soil. The results show that the functional diversity of the microbial community was evenly distributed across the samples. However, there were notable functional paths, such as the clustering-base system (CBS), amino acid and derivatives (AAD), co-factor, vitamins, prosthetic groups and pigments (CVP). There were also some nitrogen metabolic functions, that were more enriched in the bulk soil. This could be that as a result of the *Striga* infestation, the microorganisms in the rhizosphere have likely lost their functions (Spallek et al. 2013). However, there are notable functional pathways in the infested soil that can be observed from the PCA graph. For example, phosphorus metabolism (PhosM) was found to be dominant at location SB, secondary metabolism (SM), iron acquisition and metabolism (IAM), and sulfur metabolism (SFM), at Location SA, while potassium metabolism (POTM) was found to be more dominant at Location SC. Our findings also show that each *Striga*-infested rhizosphere is associated with a few major functional gene categories. In samples from locations SB, SC, and SA, respectively, dominant sequences related to stress response, fatty acids, lipids, and isoprenoid metabolism, phage, and prophage, and carbohydrate metabolism were found (supplementary Table 2). Owing to the prevalence of microbial activities in the rhizosphere of *Striga*-infested soil, the microbial communities in the infested rhizosphere are able to help plants obtain carbon through the various metabolic pathways, such as the TCA cycle, glycolysis, and gluconeogenesis, which are also present in the rhizosphere of the *Striga*-infested maize plant (supplementary Fig. 1) (Chukwuneme et al. 2021a).

Nitrogen is a crucial macronutrient for the maintenance of life, including plants. Nitrogen-fixing bacteria provide N_2 to plants for their metabolic processes in the form of fixed molecules, such as nitrate, and amino acids, ammonia and urea. Increases in plant biomass and other physiological properties are facilitated by sufficient N_2 in the soil (Beatty et al. 2016). In our study, ammonia assimilation, allantoin utilization, denitrification, nitrogen fixation, ammonification, nitrate and nitrite, coupled with nitrilase, were sequences connected to the metabolic activities involved in the nitrogen cycle.

The bulk soil was observed to accommodate more dominant nitrogen-fixing bacteria than those present in the infested rhizosphere; this could possibly be because of the effect of the *Striga* infestation, which resulted in the restriction of nitrogen-fixing bacteria in the rhizosphere of the maize plant. Allantoins are purine-derived ureides that mostly travel from the root nodules to the aerial parts of plants. The utilization of allantoin guarantees that nitrogen will be cycled and made available for plant growth. In fact, allantoin has been linked to nutrient cycling and stress tolerance (Baral and Izaguirre-Mayoral 2017). It has been reported that allantoin is used in the development of nodules in soybean (Tajima et al. 1977; Pélissier et al. 2004). In this study, allantoin was seen to be dominant at Location SC (supplementary Fig. 2). The abundance of other well-represented nitrogen metabolism routes was found to be highest in the rhizosphere samples, which is consistent with the results of Mendes et al. (2014), who investigated nitrogen metabolism pathways in soybean rhizospheres and bulk soils. However, our study found that ammonia assimilation and nitrogen fixation are more prevalent in bulk soils than in the rhizosphere.

Additionally, secondary metabolism-related sequences dominated in the SB samples. At a more fundamental level, auxin-associated traits that have been shown to promote plant development, such as auxin production (supplementary Fig. 5) were found (Hong et al. 2019). Sequences related to sulfur metabolism were widely dispersed among the samples and did not significantly vary ($p > 0.05$). Another crucial macronutrient for plants is phosphorus (P). P, which includes ATP, pyrophosphate, phospholipids, and nucleic acids, is a crucial biomolecule involved in energy metabolism. Phosphorus fertilizer is frequently applied to soils since it is generally scarce in the soil and also readily absorbed by plants (Stigter and Plaxton 2015). Alkylphosphonate utilization, high affinity phosphate transportation, P uptake (cyanobacteria), phosphate metabolism, phosphoenolpyruvate phosphomutase, phosphate-binding DING proteins (supplementary Fig. 4), and phosphonate metabolism were the phosphorus metabolism pathways found in the samples.

However, the research showed that there was no significant difference ($p > 0.05$) between the samples.

Furthermore, many of the sequences in the SB samples were related to iron uptake and metabolism. The metabolic pathways, siderophore *achromobactin*, *bacillibactin* siderophore, siderophore pyochelin, siderophore assembly kit, siderophore *enterobactin*, siderophore *staphylobactin*, siderophore pyoverdine, siderophore [alcaligin-like], and the iron siderophore sensor and receptor system were used to further support this (supplementary Fig. 5). An essential nutrient, iron, is involved in the physiological processes, such as respiration, photosynthesis, gene regulation, and oxygen transport, in plants and soil microbes (Inoue et al. 2020). It has also been linked to the production of siderophore, which enhances plant growth (Hong et al. 2019; Marag et al. 2018). Additionally, it has been shown that siderophore production results in the plant developing systemic resistance to diseases (Dube et al. 2019). Furthermore, it was discovered that clustering-based subsystems predominated in all the samples, particularly those from the BS locations. There have been reports that multiple functioning genes with unknown functions can be found in clustering-based subsystems (Castañeda and Barbosa 2017). This demonstrates that the rhizosphere of a *Striga* infestation contains a number of important genes, the activity of which has not yet been fully elucidated.

4.5 Conclusions

Using shotgun metagenomic sequencing, this work examined the rhizospheric microbial populations and the functional pathways of *Striga*-infested maize plants and bulk soil. Our results show that there are microorganisms present in the rhizosphere of *Striga*-infested maize soil that can be used to inhibit the *Striga* parasite, promote plant health, and improve soil health. Our research has also demonstrated that the *Striga hermonthica* infestation has an impact on the functional diversity of the rhizospheric microbiome in maize plants. However, there have been notable functional categories present in the *Striga*-infested rhizosphere, despite the infestation. Additionally, our study also indicated that functional genes are highly prevalent across the samples but that their functions are unknown. Going forward, it would be intriguing to investigate, by performing metabolomic analyses, the metabolic capabilities of the microbial community in the rhizosphere of *Striga*-infested soil.

Chapter 5

General conclusions and recommendations

5.1 General conclusions

Over the past few decades, numerous initiatives have been undertaken to create environmentally friendly solutions to combat the devastating impact of *Striga* weeds on grain harvests. These strategies include the adoption of maize cultivars in SSA that are resistant to *Striga.* The sole alternative option is the toothpick method of fungal inoculation and an integrated management system which are recognized as the means to effectively control this parasitic plant. The complex interactions between the maize plant and the microbiomes in its rhizosphere were also examined in this study. The research results highlight the importance of the plant root microbiome in promoting development of the plant and resistance to disease. They also highlight the complex interactions that exist between root microbes and their host plants.

Using shotgun metagenomic sequencing, this study investigated the rhizospheric microbial populations and functional pathways pertaining to *Striga*-infested maize plants and bulk soil. Taken together, our findings show the existence of microorganisms that can be employed to inhibit the *Striga* parasite, increase plant health, and enhance soil health in the rhizosphere of *Striga*-infested maize soil. Our studies also reveal that the functional diversity in the maize rhizosphere is impacted by the infestation of *Striga hermonthica*. Despite the infestation, there were observable functional categories in the *Striga*-infested rhizosphere. Furthermore, our research showed that highly abundant functional genes with unidentified roles were to be found in the *Striga*-infested samples.

5.2 General recommendations

Understanding the host-parasite interactions and host resistance and developing grains with long-lasting resistance are all necessary for the control of *Striga* weed. Additionally, it is important to educate smallholder farmers on the connections between climatic and soil characteristics. Also, when conducting field tests, account should be taken of the impact of chemicals on the soil and their persistent effects on the environment. In the future, it would be interesting to use metabolomic analysis to assess the metabolic profile of the rhizosphere microbial communities in *Striga*-infested soil.

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Supplementary data

Supplementary figures

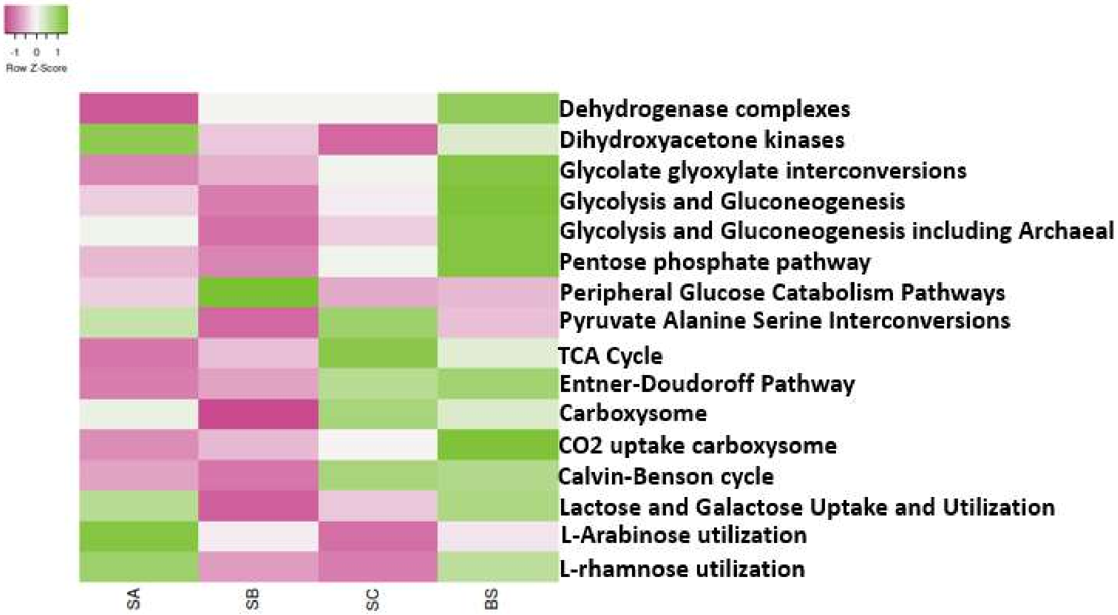

Figure 1: Heat map displaying the relative prevalence of the metabolic pathways for carbohydrates in samples from different locations. The scale bar depicts a color saturation gradient based on relative abundances of the microbiome taxa that have been z-score transformed. Samples SA, SB and SC, are from *Striga*-infested rhizosphere, while BS is the bulk soil.

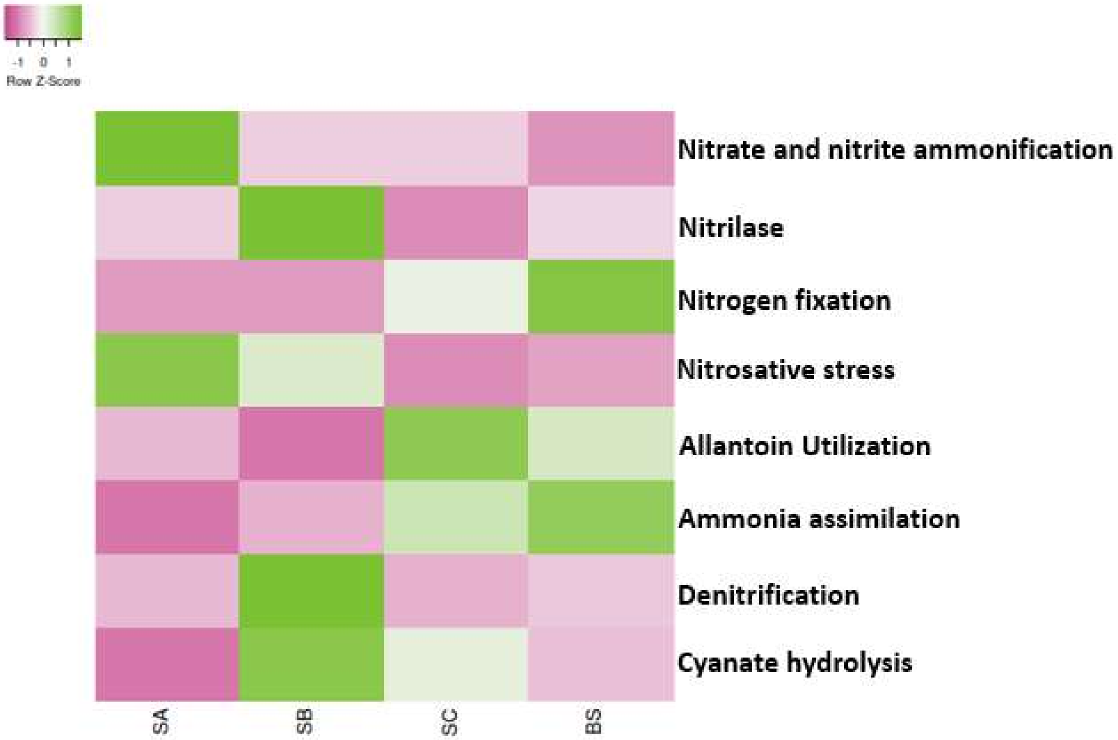

Figure 2: Heat map displaying the relative prevalence of the metabolic pathways for nitrogen in samples from different locations. The scale bar depicts a color saturation gradient based on relative abundances of the microbiome taxa that have been z-score transformed Samples SA, SB, and SC, are from *Striga*-infested rhizosphere, while BS is the bulk soil.

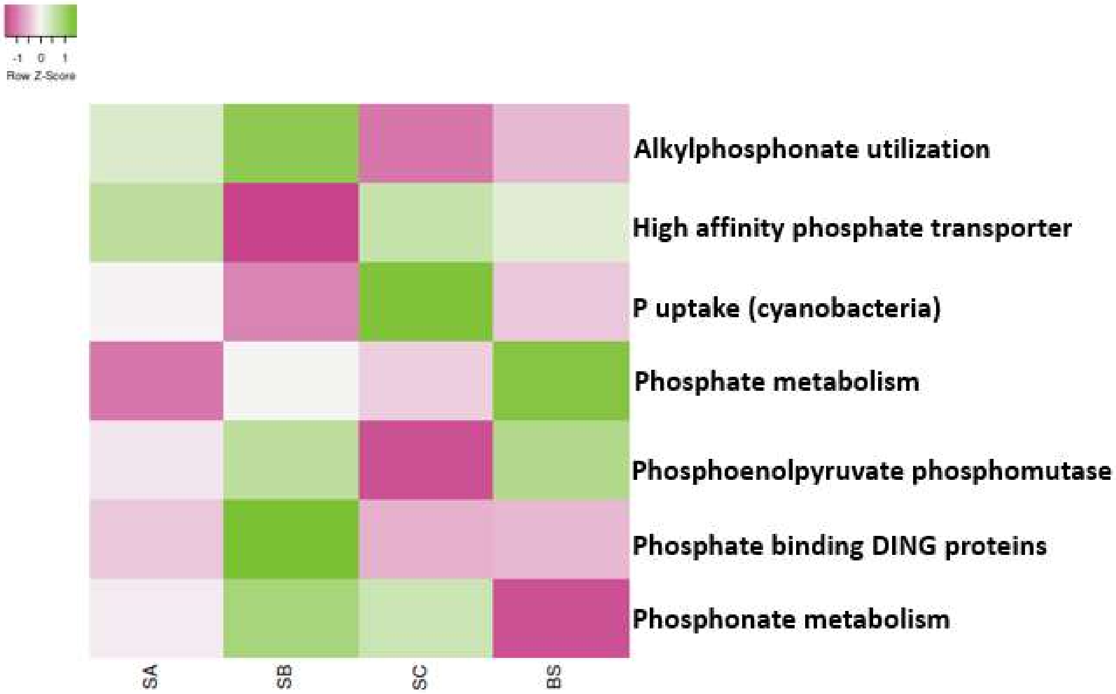

Figure 3: Heat map displaying the relative prevalence of the metabolic pathways for Phosphorus in samples from different locations. The scale bar depicts a color saturation gradient based on relative abundances of the microbiome taxa that have been z-score transformed. Samples SA, SB and SC, are from *Striga*-infested rhizosphere, while BS is the bulk soil.

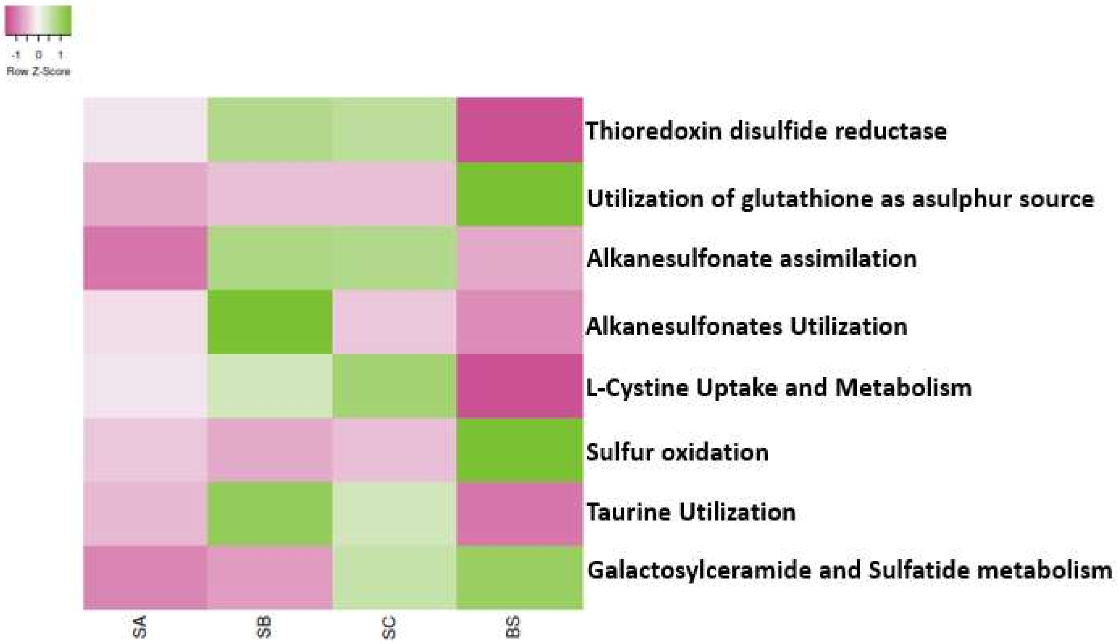

Figure 4: Figure S2C: Heat map displaying the relative prevalence of the metabolic pathways for Sulfur in samples from different locations. The scale bar depicts a color saturation gradient based on relative abundances of the microbiome taxa that have been z-score transformed. Samples SA, SB and SC, are from *Striga*-infested rhizosphere, while BS is the bulk soil.

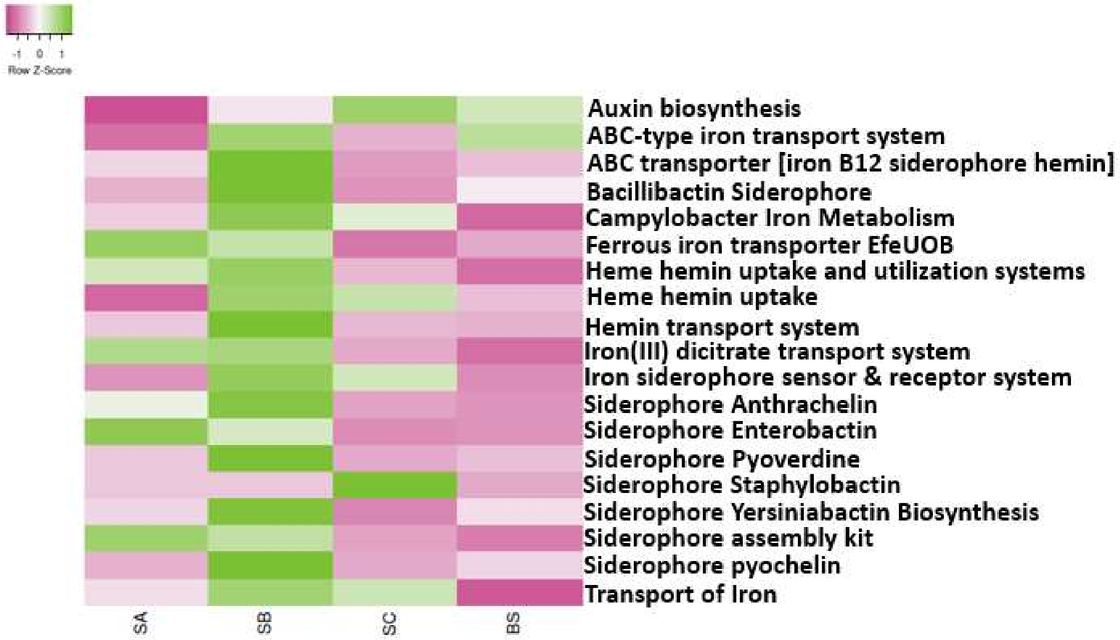

Figure 5: Heat map displaying the relative prevalence of the metabolic pathways for Secondary and Iron in samples from different locations. The scale bar depicts a color saturation gradient based on relative abundances of the microbiome taxa that have been z-score transformed. Samples SA, SB and SC, are from *Striga*-infested rhizosphere, while BS is the bulk soil.

Supplementary tables

Table 1: Average percentage of sequences across all locations.

Sampling locations	**SA**	**SB**	**SC**	**BS**
Uploading Information				
Bp count	1285898222	1614800990	1374665496	1558169695
Sequence count	5017434	6263815	5296027	6104445
Mean sequence length	256±29	258±28	259±27	256±29
Mean GC content (%)	65±8	65±8	62±11	66±7
Post QC Information				
Bp count	852568380	1182825510	1036401918	1224529204
Sequence count	852568380	1182825510	1036401918	1224529204
Mean sequence length	256±29	257±28	259±27	256±29
Mean GC content (%)	65±8	65±8	62±11	66±7
Aligned reads				
Identified protein features	1261942	2000684	1729561	2252580
Identified rRNA features	2554	2889	3821	3223
Processed reads				
Predicted protein features	2572473	3669824	3521996	4559885
Predicted rRNA features	6326	7572	9537	8612

The values in the table represent the averages of the three replicates from each location. Bp = base pair, GC = guanine and cytosine, and QC = quality control. Samples SA, SB and SC, are from *Striga*-infested rhizosphere, while BS is the bulk soil.

Table 2: Average percentage of sequences related to prominent microbiome metabolisms across all locations

Functions	**SA**	**SB**	**SC**	**BS**	***p*-Value**
Amino Acids and Derivatives	10.03±0.08	10.08±0.05	10.18±0.22	10.17±0.13	0.89
Carbohydrates	13.54±0.39	13.14±0.80	13.39±0.69	13.90±0.18	0.89
Cell Division and Cell Cycle	0.92±0.01	0.87±0.06	0.95±0.01	0.92±0.002	0.19
Cell Wall and Capsule	3.86±0.10	3.95±0.18	3.89±0.10	3.78±0.07	0.74
Clustering-based subsystems	12.82±0.21	12.64±0.39	12.61±0.34	12.95±0.09	0.89
Cofactors, Vitamins, Prosthetic Groups, Pigments	5.84±0.02	5.69±0.15	5.76±0.03	5.81±0.08	0.46
DNA Metabolism	4.51±0.09	4.38±0.09	4.42±0.14	4.39±0.02	0.62
Dormancy and Sporulation	0.14±0.00	0.18±0.03	0.14±0.02	0.14±0.01	0.62
Fatty Acids, Lipids, and Isoprenoids	2.66±0.05	2.67±0.08	2.94±0.26	2.75±0.02	0.67
Iron acquisition and metabolism	0.97±0.23	1.49±0.82	0.88±0.26	0.68±0.09	0.79
Membrane Transport	4.12±0.08	4.51±0.49	4.03±0.10	4.17±17	0.73
Metabolism of Aromatic Compounds	1.97±0.06	1.92±0.11	2.41±0.47	2.09±0.02	0.25

Miscellaneous	6.50±0.08	6.49±0.08	6.64±0.05	6.59±0.03	0.50
Motility and Chemotaxis	1.11±0.07	1.32±0.24	0.92±0.10	1.06±0.04	0.28
Nitrogen Metabolism	1.24±0.05	1.15±0.04	1.15±0.01	1.17±0.02	0.41
Nucleosides and Nucleotides	3.21±0.03	3.04±0.16	3.20±0.02	3.20±0.05	0.68
Phages, Prophages, Transposable elements, Plasmids	1.54±0.46	1.39±0.34	0.96±0.01	0.90±0.03	0.31
Phosphorus Metabolism	1.28±0.03	1.34±0.05	1.30±0.02	1.31±0.01	0.84
Photosynthesis	0.10±0.00	0.08±0.02	0.08±0.01	0.10±0.00	0.43
Potassium metabolism	0.55±0.06	0.60±0.11	0.60±0.09	0.50±0.02	0.92
Protein Metabolism	7.41±0.28	7.19±0.61	7.81±0.37	7.77±0.05	0.51
RNA Metabolism	3.51±0.11	3.38±0.04	3.51±0.15	3.29±0.03	0.17
Regulation and Cell signaling	1.22±0.14	1.23±0.20	0.16±0.13	1.04±0.03	0.88
Respiration	4.15±0.18	4.00±0.33	4.13±0.29	4.35±0.04	0.62
Secondary Metabolism	0.28±0.01	0.29±0.01	0.32±0.03	0.30±0.00	0.35
Stress Response	2.62±0.05	2.61±0.04	2.51±0.01	2.54±0.02	0.11
Sulfur Metabolism	1.02±0.01	1.13±0.10	1.14±0.13	1.07±0.03	0.68
Virulence, Disease, and Defense	2.87±0.03	3.28±0.36	2.99±0.05	3.08±0.10	0.19

Mean ± standard error (n= 3), p-values based on Kruskal–Wallis test. Samples SA, SB, and SC are from *Striga*-infested rhizosphere, while BS is the bulk soil.

Appendix

PPDP – Plant-Prokaryote DOE project

CCM – Central Carbohydrate metabolism

LTMC – Lysine, Threonine, methionine, and cysteine

DO – Di- and Oligosaccharides

AUP – Arginine; Urea cycle, polymines

AAD – Aromatic amino acids and derivatives

PB – Protein biosynthesis

DR – DNA repair

RPM – RNA processing and modification

RATC – Resistance to antibiotics and toxic Compounds

BAC – Branched - chain amino acid

EDR – Electron donating reactions

AT – ABC transporters

DNAR - DNA replication

PP – phages, prophages

CEP – Capsular and extra cellular polysaccharides

GNCWC – Gram – Negative Cell Wall Components

EAC – Electron accepting reactions

T – Transcription

AS – ATP synthases