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Original article

Maize rhizosphere modulates the microbiome diversity and community structure to enhance plant health

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ABSTRACT

Metagenomic has been explored in investigating microbiome diversity. However, there is limited available information on its application towards securing plant health. Hence, this study adopts the metagenomic approach to unravel the microbiome diversity associated with healthy (LI and MA) and Northern corn leaf blight (NCLB) infected (LID and MAD) maize rhizosphere in the maize growing field at Lichtenburg and Mafikeng, North-West province of South Africa. The extraction of whole DNA from the respective healthy and diseased rhizosphere soils was conducted and sequenced using shotgun metagenomics. A total of 12 bacteria, 4 archaea and 2 fungal phyla were found as predominant across the fields with the use of the SEED subsystem database. The most predominant bacteria phyla included *Proteobacteria, Dienococcus-Thermus, Gemmatimonadetes, Chlorobi, Cyanobacteria, Planctomycetes, Verrucomicrobia, Acidobacteria, Firmicutes, Chloroflexi* and *Bacteroidetes.* Archaea consisted of *Euryarchaeota, Thaumarchaeota, Crenarchaeota* and *Korachaeota,* while *Ascomycota* and *Basidiomycota* were the dominant fungal phyla. Microbial abundance and diversity were higher in the rhizosphere of healthy maize (LI and MA) rhizosphere as compared to the NCLB diseased (LID and MAD), in the order LI > MA > LID > MAD. At phylum and genus level, alpha diversity index showed no significant ($p > 0.05$) difference in the abundance of the microbial community of healthy and NCLB infected maize rhizosphere, while beta analysis produced a significant ($p = 0.01$) difference in the microbial diversity in the soil. Taken together, the study revealed that the abundance of microbial diversity in the maize rhizosphere influences the efficacy of the rhizosphere microbiome to modulate microbial functions towards managing and sustaining plant health.

1. Introduction

Maize is a food and industrial crop that is cultivated in more than 160 countries and on every continent except for Antarctica (Rahmawati et al., 2021). Its global relevance is hinged on its economic, nutritional, environmental, and cultural impacts (Tanumihardjo et al., 2020). It is a cereal of choice in many households and is especially preferred in Southern and Eastern Africa, Central America, and Mexico (Ranum et al., 2014, Mangani et al., 2019). As a result of the population expansion, maize consumption in the developing countries has been predicted to double by 2050, while its output is projected to peak by 2025. Therefore, considering its position as the basis of food security in some of the world's poorest regions of Africa, Asia, and Latin America, there arises the need to investigate the factors limiting the production of this important cereal as to ensure the sustainability of its productions.

Despite the management systems in place, diseases impairing maize health, productivity and contamination of grains have been recorded across the globe (Akanmu et al., 2020, Enebe and Babalola, 2021, Ahmad et al., 2012). In South Africa, bacteria stalk rot, black bundle disease, late wilt, charcoal-rot, common rust, downy mildew and northern corn leaf blight among others, have been reported (Schoeman et al., 2018, Okoth et al., 2017). Northern corn leaf blight (NCLB) is a disease characterised by grey-green borders surrounding the canoe-shaped lesions which range between 3and 15 cm long and form large-blighted areas at

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advanced stage till the entire leaves becomes blighted. It is an important foliar maize disease caused by the ascomycete *Setosphaeria* turcica and anamorph *Exserohilum turcicum* [(Pass.) K.J. Leonard & Suggs] (Welz and Geiger, 2000, Vieira et al., 2014). NCLB has been reported as the most prominent leaf diseases of maize in South Africa which is more prevalent under irrigation farming. An average yield loss of 15 to 30 % has been reported, and up to 50 % yield loss has been documented while for every 10 % increase in disease severity, 2–8 % potential yield reduction exist (Craven et al., 2020).

Since plants are sessile creatures with roots that anchor them in the earth, soil thereby plays an important role in the growth, development, and health of maize plants. Securing the integrity of soil is thereby vital and strategic to attaining the optimum level of health and yield. This is because biodiversity services including climate stability, water, energy, and food security rely on its functioning (Babalola et al., 2020, Enagbonma et al., 2020, Odelade and Babalola, 2019). As in other microbes, the causative pathogens of plant diseases have also been recovered from the plant phyllosphere, endosphere, and rhizosphere (Dong et al., 2019, Igiehon et al., 2019). However, the vast majority of microbes are found in the rhizosphere which is the major site of interaction between the plants (via the roots) and the microbes. The rhizosphere thus plays the host to a wide range of biological activities which predetermine the health status of plants. This array of microorganisms include both the beneficial and the potentially pathogenic ones. Therefore, it is essential to understand the dynamics of the criteria for plants recruitment of beneficial microbes which to a large extent restricts the pathogens (Glick and Gamalero, 2021). Studies have shown that microbe-microbe and plant–microbe communication which occur in the rhizosphere via the exchange of numerous signal molecules has a significant effect on microbial behavior (Akanmu et al., 2021). The disease suppressive soils, therefore, operate on a natural principle of competition for dominance and survival. This leads to stiff microbial competition that eventually determines the chances for development or sustenance of plants' health or diseased conditions (Enebe and Babalola, 2021, Omomowo and Babalola, 2019).

The naturally occurring rhizosphere microbiomes have been estimated to contain hundreds to thousands of different microbes with plant growth promoting bacteria (PGPB), and plant Growth promoting fungi (PGPF) as the most abundant (Dlamini et al., 2022, Akanmu et al., 2021). Both PGPB and PGPF benefits the growth and development of plants in diverse ways, including the enhancement of plant growth, facilitating the provision of mineral and fixed nitrogen for plants' use, including the enhancement of seed germination, root and shoot length, production of secondary metabolites, increasing plant tolerance to environmental stresses, and protection of plants from a wide range of phytopathogenic microbes (Glick and Gamalero, 2021, Olowe et al., 2020). Investigation into the microbiome since the last decade have to an extent unravelled the complexity and structure of microbial communities, although the knowledge of the organization of such complex communities and their interdependency among themselves and with the biotic and abiotic environment is still at its infancy (Sergaki et al., 2018). Some molecular tools including; Phospholipid Fatty Acid (PLFA) (Liu et al., 2017), Fatty Acid Methyl Ester (FAME) (Cavigelli et al., 1995), Denaturing Gradient Gel Electrophoresis (DGGE) and Terminal Restriction Fragment Length Polymorphism (*T*-RFLP) (Marschner, 2007) have earlier been applied to the study of microbial communities in soil. However, the advent of metagenomics enables the sequencing of the whole environmental DNA using 454 pyrosequencing and 16S-rRNA sequencing (Amplicon) in the diversity study of microbiome. While these methods lack the temerity of deeper insights into the functions of the soil organisms, the culture-independent high-throughput sequencing (HTS) and shotgun metagenomics have improved over this limitation (Prigigallo et al., 2016, Fadiji et al., 2021b). With the shotgun metagenomics reported to unravel the functional diversity of microbiomes in a specified environment (Fadiji et al., 2021a, Fadiji and Babalola, 2020).

The structural profile of microbial communities is often an indicator for defining soil quality and fertility. It as well aids the response of the microbes to both biotic and abiotic modifications (Enagbonma et al., 2020, Babalola et al., 2020, Ojuederie et al., 2019). Interestingly, the abundance and diversity of microbes in the maize rhizosphere of both healthy and NCLB infected maize have not been fully explored, thereby indicating the possibility of other useful but undiscovered organisms or microbial roles that could be productively engaged towards crop improvement. To the best of our knowledge, this is one of the foremost report in which shotgun metagenomic approach was used to investigate the community structure of rhizosphere microbial community in maize plant infected with NCLB. Therefore, the understanding of the abundance and diversity of microbial profiles associated with the rhizosphere soils of healthy and NCLB diseased maize plants is strategic to ensure sustainable and secured maize productions. Hence, we hypothesized that abundance and diversity of rhizosphere microbiome positively impacts disease management in healthy maize compared to the NCLB infected maize.

2. Materials and methods

2.1. Field history

The maize rhizosphere soil samples used in this study were collected from the maize cultivated sites at Lichtenberg (25°59′40.2″ S, 26°31′44.2″ E) and Mafikeng (25°47′19.1″S 25°37′05.1″E) which are two different locations within the North-West Province of South Africa. The province during summer has the temperature range of 17° to 31 °C (62° to 88 °F) and in winter 3° to 21 °C (37° to 70 °F). The annual total rainfall is estimated at an average of 360 mm (about 14 in) which majorly occur during summer (October and April). In the two farms sampled, maize cultivar WE 3127 was cultivated. Agricultural management systems employed include application of NPK fertilizers (150 N, 75 P2O5, and 75 K2O all in kg ha − 1), artificial irrigation, mechanized land tilling systems and machinery-assisted weed control measures. Based on the documented records and information gathered from the farmers, the field in Mafikeng (MA), which is the agricultural farm of the North-West University occupies approximate 20 ha and has been under rotational cultivation of maize and cowpea since 1989. Also, the farm sampled at Lichtenberg (LI) has been under continuous maize cultivation for about 10 years. The choice of the locations was influenced by the occurrence of Northern corn leaf blight (NCLB) disease in some parts of the farms. Identification of NCLB diseased plants was based on the symptoms occurrence on the leaves, such as elliptical, grey-green lesions of 3–15 cm long, tan with distinct dark zones of fungal sporulation, which turns to a blight that becomes severe in its advanced stage (Vieira et al., 2014). From each of the farms, soil samples were collected from the rhizosphere of both the healthy and the NCLB infected plants. This was carried out about 8 weeks after planting the maize to allow adequate interaction of plant root and soil microoganisms within the rhizosphere.

2.2. Sample collection

The sample collection was conducted in January 2021. Each of the two farms (MA & LI) were demarcated into two regions. In each region, about 50 g of rhizosphere soil samples considered sufficient

for DNA extraction and physicochemical analysis (Bai et al., 2014, Akinola et al., 2021) were collected between 5 and 20 cm in-depth from each healthy (15 samples) and NCLB infected (15 samples) maize plants rhizosphere. The respective soil samples collected per region were pooled into two replicates and labelled accordingly as either sample from healthy or NCLB diseased maize plants. This makes a total of four samples obtained from each farm site. The maize plants sampled were carefully uprooted and shaken to remove loose soils around the roots, while soils that were tightly bound to the root were collected in sterile bags, transferred to cooler boxes containing ice (4^{O}C) and immediately transported to the laboratory. All the samples were stored at -20^{O}C until analysis. Representative samples were prepared for DNA extraction and high throughput sequencing. Analysis of the soil samples collected shows higher values of soil pH (LI – 6.95, LID- 6.76) and Organic Carbon (LI – 1.02 %, LID – 0.89 %) in both the rhizosphere of healthy and diseased maize plants at Lichtenburg compared with those collected at Mafikeng pH (MA – 5.17, MAD – 5.37), Organic carbon (MA – 0.26, MAD – 0.25) (Supplementary Table 1), These were carried out according to the procedure described by (Walkley and Black, 1934, Muwawa et al., 2010).

2.3. Extraction of DNA and shotgun sequencing

The rhizosphere soils collected were subjected to the DNA extraction protocol of the Qiagen DNeasy PowerMax Soil Kit (USA). Shotgun metagenomic sequencing was conducted on the extracted DNA at the Molecular Research LP, Texas, USA. Nextera DNA library preparation Flex kit (Illumina) was employed in the library preparation, following standard procedure. Evaluation of the initial DNA concentrations in the prepared samples was carried out using the Life Technologies Qubit® dsDNA HS Assay Kit produced by Carlsbad, USA. The samples were further cleaned with DNEasy PowerClean Pro Cleanup Kit (Qiagen) and the concentrations of the samples after the cleanup were again checked using the Qubit® dsDNA HS Assay Kit (Life Technologies, the USA). The library was then prepared using 20–25 ng of DNA, after which the samples were concurrently fragmented and an adapter sequence added. After that, the adapters were employed in a limited-cycle PCR to introduce unique indices into each sample. The final library concentrations were determined using the Qubit® dsDNA HS Assay Kit (Life Technologies) after the libraries were prepared, and the average size of the library was determined using the Agilent 2100 Bioanalyzer (Agilent Technologies). The libraries were pooled and diluted to 0.6 nM before being sequenced for 300 cycles paired-end on the NovaSeq system (Illumina) 6000 system.

2.4. Metagenome's annotation and data analysis

Each metagenome sequences were uploaded to the metagenomics rapid annotation online service (MG-RAST) (Meyer et al., 2008), which performed quality checking on the raw data. This included the use of Trimmomatic v 0.33 software with default parameters to remove adaptor and low-quality reads from the sequenced data (Bolger et al., 2014). The artificial sequences were removed, ambiguous bases filtered, a specified minimum read size was specified, and length filtering was part of the quality control process. Following quality control, the generated sequences were annotated using BLAT (Kent, 2002) against the M5NR database (Wilke et al., 2012), which permits non-redundant integration of several datasets. The SEED database was used to do taxonomic profiling, with criteria such as a 10^{5}% e-value cut-off and a minimum 60 % sequence similarity to a subsystem. On the MG-RAST, the data normalization option was selected to reduce the impact of experimental error. The identified microbiome table was grouped by taxon, and the unclassified sequence reads were saved for statistical analysis. After an independent examination of the 8 sequences using MG-RAST, the relative abundance of the taxa in percentages was determined. For statistical analysis, the average values of the relative abundance of the two replicates for each sample site (LI, LID, MA & MAD) were utilized. The accession number for these standard sequences may be obtained on the NCBI SRA collection bio project number PRJNA821718.

The Shinyheatmap was used to plot the relative abundance graph of rhizosphere microbiome communities at the phylum level after the dataset was adjusted (Khomtchouk et al., 2017). The Shannon diversity and Pielou Evenness indices for each of the sampling sites were assessed using PAST version 3.20, and the indices between the sites were compared using the Kruskal–Wallis test. The beta diversity was defined using primary coordinate analysis based on a Euclidean distance matrix, and the variations in community structure among the locations were assessed using one-way analysis of similarities (ANOSIM) (Carrell and Frank, 2015).

3. Results

3.1. Metagenomic sequencing and processing

The sequences uploaded for analysis on MG-RAST server showed the mean values of samples from rhizosphere of healthy maize in Lichtenburg (LI) (1,615,034,280) and Mafikeng (MA) (725,518,084,5), while the sequence count for rhizosphere soils from NCLB diseased plants in Lichtenburg (LID) and Mafikeng (MAD) were 526,054,117 and 1,105,047,462 respectively. However, the quality of retained mean sequences after the Quality Control (QC) assessment recorded LI = 1,165,623,372 and MA = 673, 172,171 for samples from healthy rhizosphere, and LID = 487,655, 198,5 and MAD = 1,025,190,816 for those of NCLB diseased plant rhizosphere soils (Supplementary Table 2). The rarefaction generated in the MG-RAST analysis depicts the abundance and diversity of the microbial communities in the rhizosphere (Fig. 1).

3.2. Composition of the major microbiome phyla across the fields

A total of 12 bacteria, 4 archaea and 2 fungal phyla were found as predominant across the fields with the use of the Subsystem database. Except in the cases of *Actinobacteria* and *Crenarachaeota*, all other phyla were more abundant in the rhizosphere soils from healthy maize (LI and MA), than those of diseased maize (LID and MAD).

The most predominant bacteria phyla included *Proteobacteria, Dienococcus-Thermus, Gemmatimonadetes, Chlorobi, Cyanobacteria, Planctomycetes, Verrucomicrobia, Acidobacteria, Firmicutes, Chloroflexi* and *Bacteroidetes.* Archaea consisted of; *Euryarchaeota, Thaumarchaeota, Crenarchaeota* and *Korachaeota* while *Ascomycota* and *Basidiomycota* were the major fungal phyla observed. The abundance of microbial community was found to be more abundant in soil samples from healthy plants than diseased in both locations, with LI, MA, LID and MAD as the decreasing order recorded (Fig. 2), although no significant $p > 0.05$ difference was recorded in the microbial variations across the site (Supplementary Table 3). Meanwhile, the principal component analysis distribution of the identified microbial phyla, as indicated in Fig. 2, showed LI as the treatment with the largest dispersion (Fig. 3).

3.3. Composition and abundance of microbial communities

A higher proportion of bacteria phyla were recorded from the rhizosphere of healthy maize samples than those of diseased maize, especially for the samples recovered from Lichtenburg (LI) consisting of *Proteobacteria* (41.36 %), *Bacteroidetes* (4.20 %),

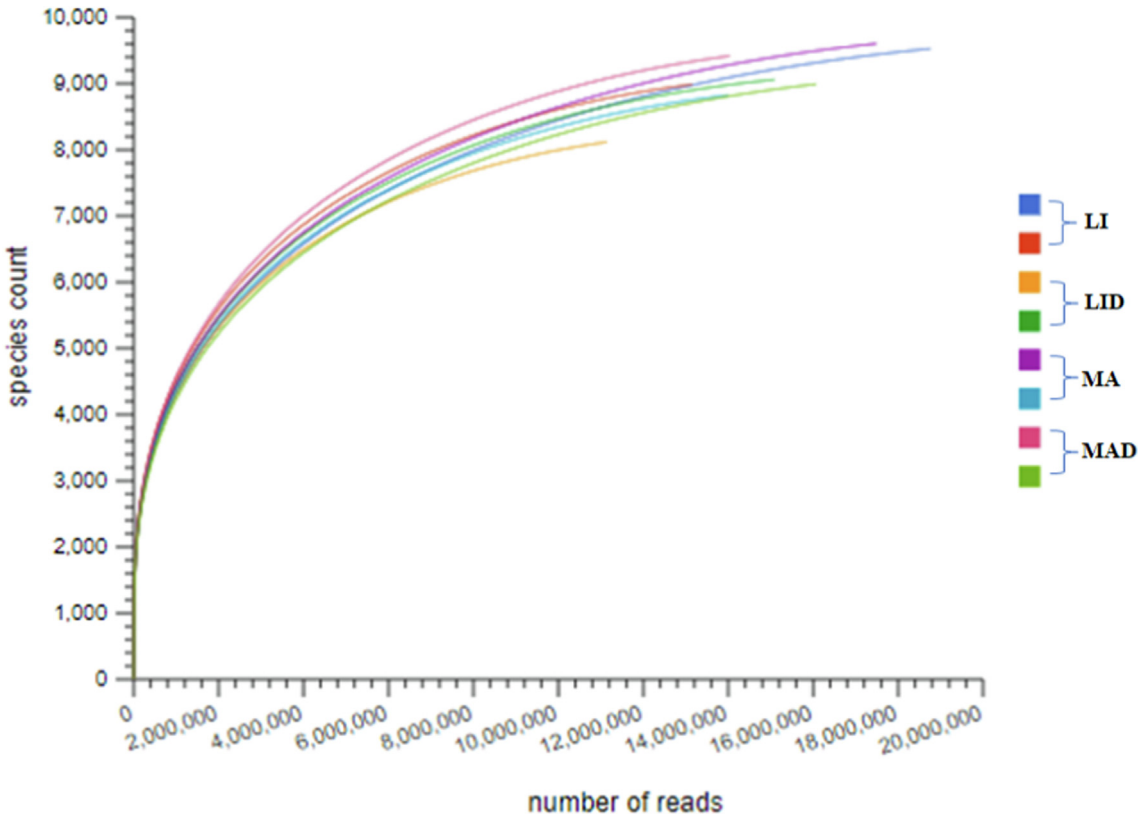

Fig. 1. Rarefaction curve showing the species richness sequence in the studied site. LI- rhizosphere soil from healthy maize in Lichtenburg field, LID- rhizosphere soil from diseased maize in Lichtenburg field, MA- rhizosphere soil from healthy maize in Mafikeng field, MAD- Mafikeng.

Firmicutes (4.82 %), *Cyanobacteria* (2.34 %), *Planctomycetes* (2.68 %), *Verrucomicrobia* (2.17 %), *Gemmatimonadetes* (1.21 %), *Deinococcus-Thermus* (0.77 %) and *Chlorobi* (0.41 %), while *Acidobacteria* (4.32 %),

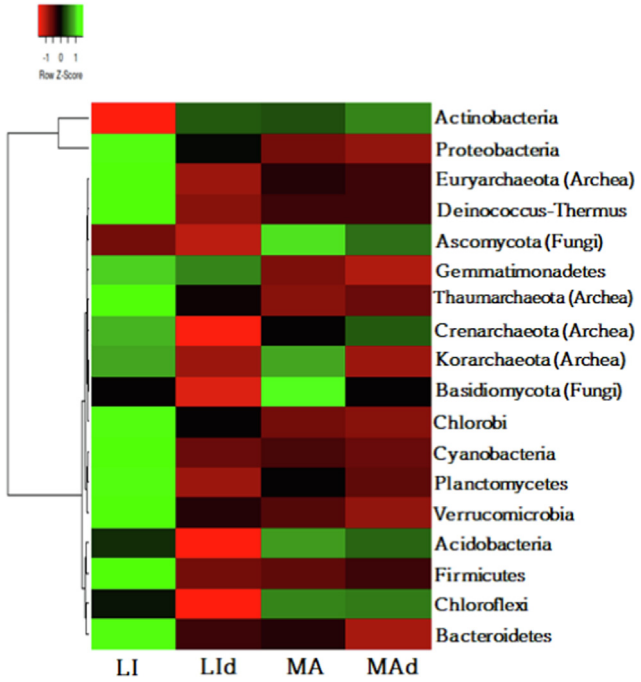

Fig. 2. The heatmap shows the major phyla of soil microbial communities associated with maize plants. LI, rhizosphere soil from the healthy plant at Lichtenburg; LID rhizosphere soil from the diseased plant at Lichtenburg site, MA, rhizosphere soil from the healthy plant at Mafikeng; LID rhizosphere soil from the diseased plant at Mafikeng site.

Actinobacteria (MAD = 47.28 %), *Chloroflexi* (MA = 2.98 %) were more abundant in Mafikeng (MA) samples. Ascomycota (LI = 0.36 %, LID = 0.26 %) and *Basidiomycota* (0.04 %, 0.03 %) were the most predominant fungal phyla which showed respective abundance in healthy than diseased samples recovered from Lichtenburg. A similar observation was recorded in the phyla *Ascomycota* (MA = 0.76 %, MAD = 0.68 %) and *Basidiomycota* (MA = 0.05 %, MAD = 0.04 %) from Mafikeng samples. The most abundant archaea phyla recorded across the sites were *Euryarchaeota, Crenarchaeota, Thaumarchaeota,* and *Korarchaeota,* which were mostly more abundant in the rhizosphere of the healthy samples (LI and MAD), except *Crenarchaeota* in Mafikeng (MA) (Supplementary table 3).

The class level showed higher abundance of bacteria in LI than MA, except in *Actinobacteria* (class) (44.07 %) and *Solibacteres* (2.44 %). Similarly, the predominant bacteria class from LID include *Alphaproteobacteria* (16.57), Deltaproteobacteria (4.83 %), *Gammaproteobacteria* (5.45 %) while the most predominant in MAD are Actinobacteria (class) (44.81 %), Betaproteobacteria (8.27 %), and *Planctomycetacia* (1.85 %). The fungi class *Sordariomycetes* (MA = 0.31 %, MAD = 0.34 %), *Eurotiomycetes* (MA = 0.25 %, MAD = 0.23 %), *Dothideomycetes* (MA = 0.13 %, MAD = 0.12 %) were more prevalent in Mafikeng. The dominant archea class *Methanomicrobia* (0.28 %), *Thaumarchaeota* (class) (0.14 %) and *Thermococci* (0.08 %) were recorded in LI, while Halobacteria expressed similar level of abundance in the two locations (Fig. 4a).

Generally, there were more dominant bacteria order recorded in LI and MA than LID and MAD. Also, Lichtenburg samples (LI and LID) showed higher abundance except in *Actinomycetales* (MA = 39.15 %, MAD = 40.29 %), *Solibacterales* (MA = 2.44 %%, MAD = 2.34 %), *Rhodospirillales* (MA = 2.04 %, MAD = 1.99). Archea order; *Methanosarcinales* (LI = 0.15 %, LID 0.13 %), *Methanomicro-*

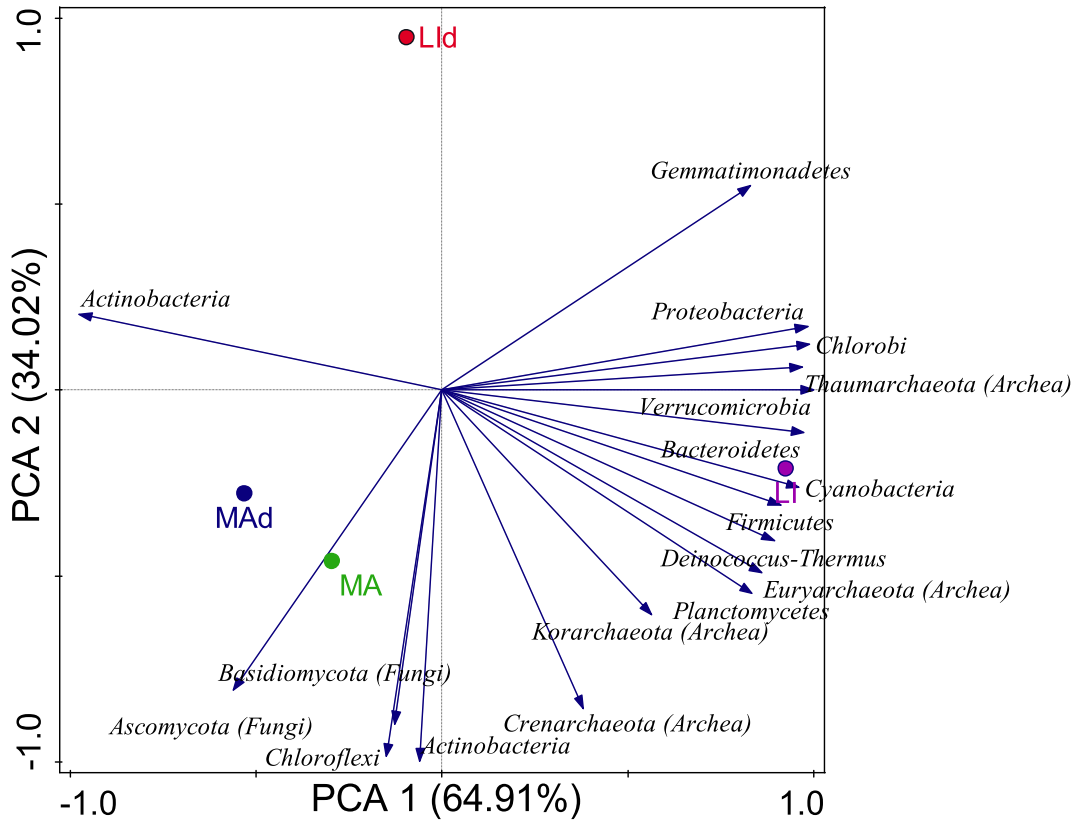

Fig. 3. The PCA graph of average soil microbial community phyla. LI, rhizosphere soil from the healthy maize in Lichtenburg; LID, rhizosphere soil from the diseased maize in Lichtenburg. MA rhizosphere soil from the healthy maize in Mafikeng, MAD, rhizosphere soil from the diseased maize in Mafikeng.

biales (LI = 0.11 %), and *Thermococcales* (LI = 0.08 %, LID = 0.07 %) were the most dominant while there was no significant variation in the abundance of *Halobacteriales*, *Thermoproteales*, *Methanobacteriales* and *Desulfurococcales* across the respective healthy and diseased plants rhizosphere from the two locations. The fungal order expressed higher abundance in MA and MAD as shown in *Eurotiales* (0.22 %, 0.20 %), *Sordariales* (0.12 %, 0.17 %), Hypocreales (0.15 %, 0.13), and *Pleosporales* (0.13 %, 0.12 %) respectively, while *Desulfurococcales*, *Saccharomycetales*, *Agaricales* and *Ustilaginales* recorded similar level of abundance in the two locations (Fig. 4b).

At family level, the most dominant bacteria family associated with LI include *Streptomycetaceae* (10.16 %), and *Bradyrhizobiaceae* (4.20 %), LID; *Nocardioidaceae* (3.23 %), *Sphingomonadaceae* (2.74 %), MA; *Solibacteraceae* (2.44 %), *Conexibacteraceae* (3.60 %) and *Pseudonocardiaceae* (3.37 %), MAD; *Burkholderiaceae* (3.59 %) and *Mycobacteriaceae* (4.42 %). The archea family; *Methanosarcinaceae* (0.13 %), *Nitrosopumilaceae* (0.10 %), *Thermococcaceae* (0.08 %), *Cenarchaeaceae* (0.04 %) were dominant in LI and *Sulfolobaceae* (0.05 %) in MA. The dominant fungal families were; *Trichocomaceae* (MA = 0.22 %, MAD = 0.20 %), *Nectriaceae* (MA = 0.15 %, MAD = 0.13 %), and *Phaeosphaeriaceae* (MA- 0.08 %, 0.07 %) (Fig. 4c).

At genus level, the bacteria found dominant in the rhizosphere samples from health maize than those of diseased maize include *Streptomyces* (LI = 10.16 %, MA = 7.50 %), *Candidatus Solibacter* (LI = 2.31 %, MA = 3.60 %), *Conexibacter* (LI = 3.26 %, MA = 3.60 %), *Bradyrhizobium* (LI = 1.96 %, MA = 1.86 %). The general *Nocardioides* (LI = 2.10 %, LID = 1.95 %), *Rhodopseudomonas* (LI = 1.39 %, LID = 1.21 %), *Gemmatimonas* (LI = 1.26, LID = 1.00) and *Pseudomonas* (LI = 1.04, LID = 0.93) were more dominant in Lichtenburg while Mycobacterium (MA = 4.27, MAD = 4.42), *Candidatus Solibacter* (MA = 2.44 %, MAD = 2.34 %), *Conexibacter* (MA = 3.60 %), *Burkholderia* (MA = 2.69 %, MAD = 2.43 %), Frankia (MA = 3.35 %, MAD = 3.33 %), *Methylobacterium* (MA = 1.27 %, MAD = 1.34 %) and *Rhodococcus* (MA = 1.64 %, MAD = 1.56 %) dominated the Mafikeng field. Furthermore, except for *Sulfolobus* (MA = 0.04 %), the archea genus consisting of *Methanosarcina* (0.10 %), *Nitrosopumilus* (0.10 %), *Cenarchaeum* (0.04 %) and *Pyrococcus* (0.04 %) were more abundant in LI. However, the fungi genus showed higher prevalence in MA; *Gibberella* (0.10 %), *Neosartorya* (0.09 %), *Penicillium* (0.03 %), *Phaeosphaeria* (0.08 %) and *Nectria* (0.05 %), while general *Aspergillus* (0.07 %), *Neurospora* (0.07 %) and *Chaetomium* (0.06 %) were more associated with diseased maize plant in Mafikeng field (MA) (Fig. 4d).

3.4. The diversity study of the microbial communities of the healthy and diseased rhizosphere samples

The alpha and beta diversity indexes measured by Simpson, Shannon, and Evenness at both phylum and genus level showed no significant ($p > 0.05$) differences among the healthy and diseased rhizosphere samples collected in two farm sites (Table 1). As revealed in the PCoA analysis, the composition of soil microbial community across the treatments and sites showed a more significant difference between samples LI and LID, compared to MA and MAD (Fig. 5). Analysis of similarity (ANOSIM) revealed that variations and diversity of the soil microbial communities across the sampled sites differed significantly ($p = 0.01$).

4. Discussion

For centuries, maize has remained a crop of choice across the globe owing to its nutritive values and its essential impacts on human and animal diets. To further harness the benefits of this important cereal and ensure its safe and secured consumption,

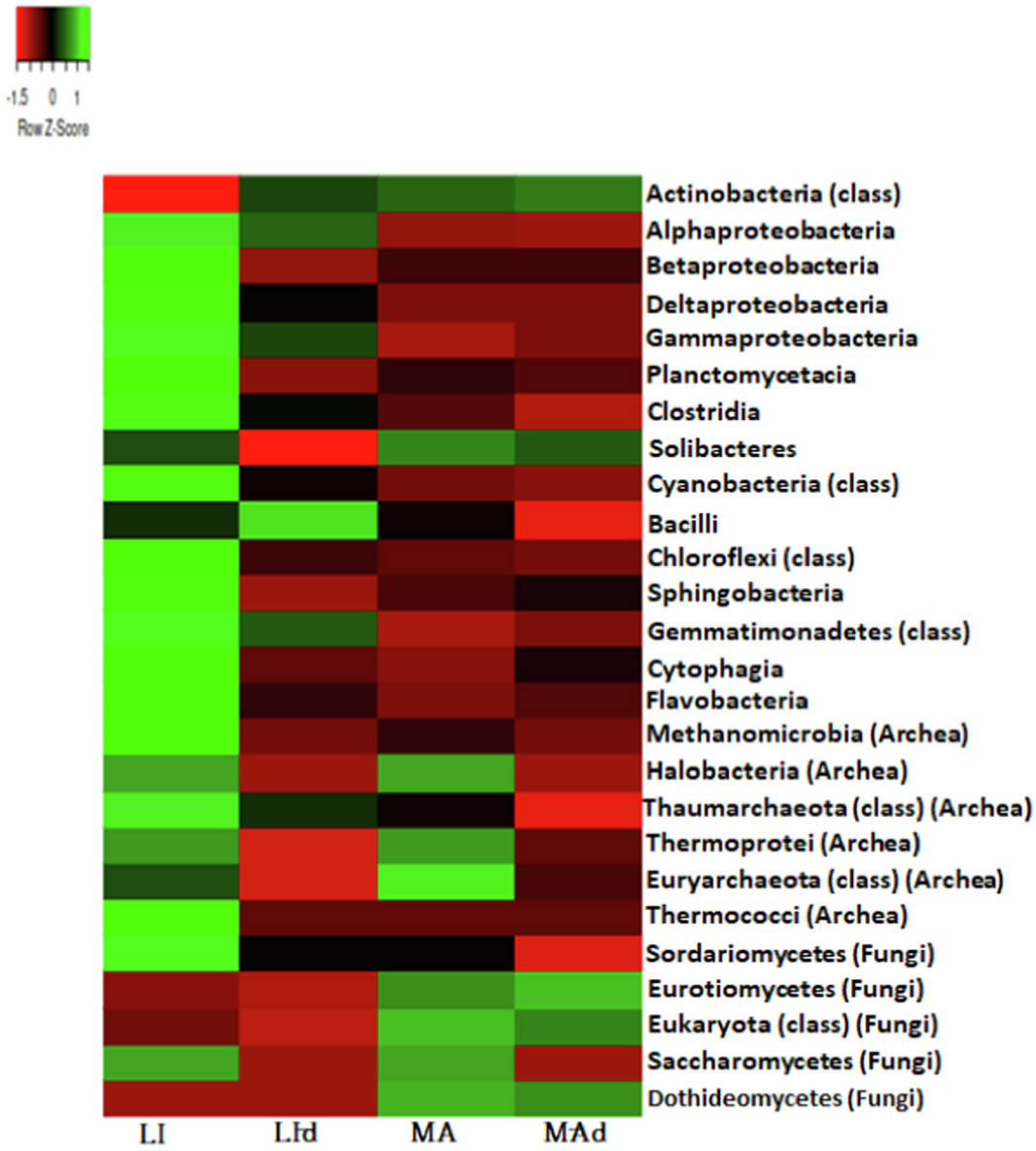

Fig. 4a. The heatmap shows the major class of soil microbial communities associated with maize plants. LI, rhizosphere soil from the healthy plant at Lichtenburg; LID rhizosphere soil from the diseased plant at Lichtenburg site, MA, rhizosphere soil from the healthy plant at Mafikeng; LID rhizosphere soil from the diseased plant at Mafikeng site.

there arises the need to understand the associated community structure and microbiome diversity which impacts its health and productivity (Tanumihardjo et al., 2020). Plant rhizosphere is considered the most complex ecosystem that inhabits various organisms including; bacteria, fungi, nematodes, herbivores, and arthropods among others. It is, therefore, the most active section of the soil's frontier, where the plant–microbe activities affect a wide range of processes, including plant health (Akanmu et al., 2021). The current investigation employed a shotgun metagenomics approach to investigate the composition and diversity of the rhizosphere microbial communities of the healthy and Northern corn leaf blight (NCLB) diseased maize plants, to profile the associated microbes towards further exploration in enhancing plant health.

The higher abundance of the microbes recorded at the phylum level, in the rhizosphere of healthy maize compared to those of NCLB diseased suggests that the gap in the abundance could contribute to the differences in the resistance of the plants to diseases. This was verified in a report which considered the biological activities required for maintaining healthy soil and suppressing plant diseases to be controlled by the soil microbial population (An et al., 2011), while the reduction in soil microbial diversity is associated with the emergence of soil-borne plant disease (Zhao et al., 2021). More so, the diversity and abundance of microbes across the locations studied, especially dominance of bacteria phyla *Proteobacteria*, *Bacteriodetes, Firmicutes, Cyanobacteria, Planctomycetes, Verrucomicrobia, Gemmatimonadetes, Deinococcus-Thermus* and *Chlorobi* in Lichtenburg (LI) could be as a result of the variations in the factors influencing the microbial population and distributions in the soil horizons (Bhattarai et al., 2015). Most members of the genera such as *Streptomyces, Candidatus Solibacter, Conexibacter* and *Bradyrhizobium* were prevalent in the rhizosphere of healthy compared to diseased maize, and they have been reported for their positive influence on maize growths (Fadiji et al., 2021b, Enebe and Babalola, 2019). Some of the specialised attributes of this group vary from the production of bioactive secondary

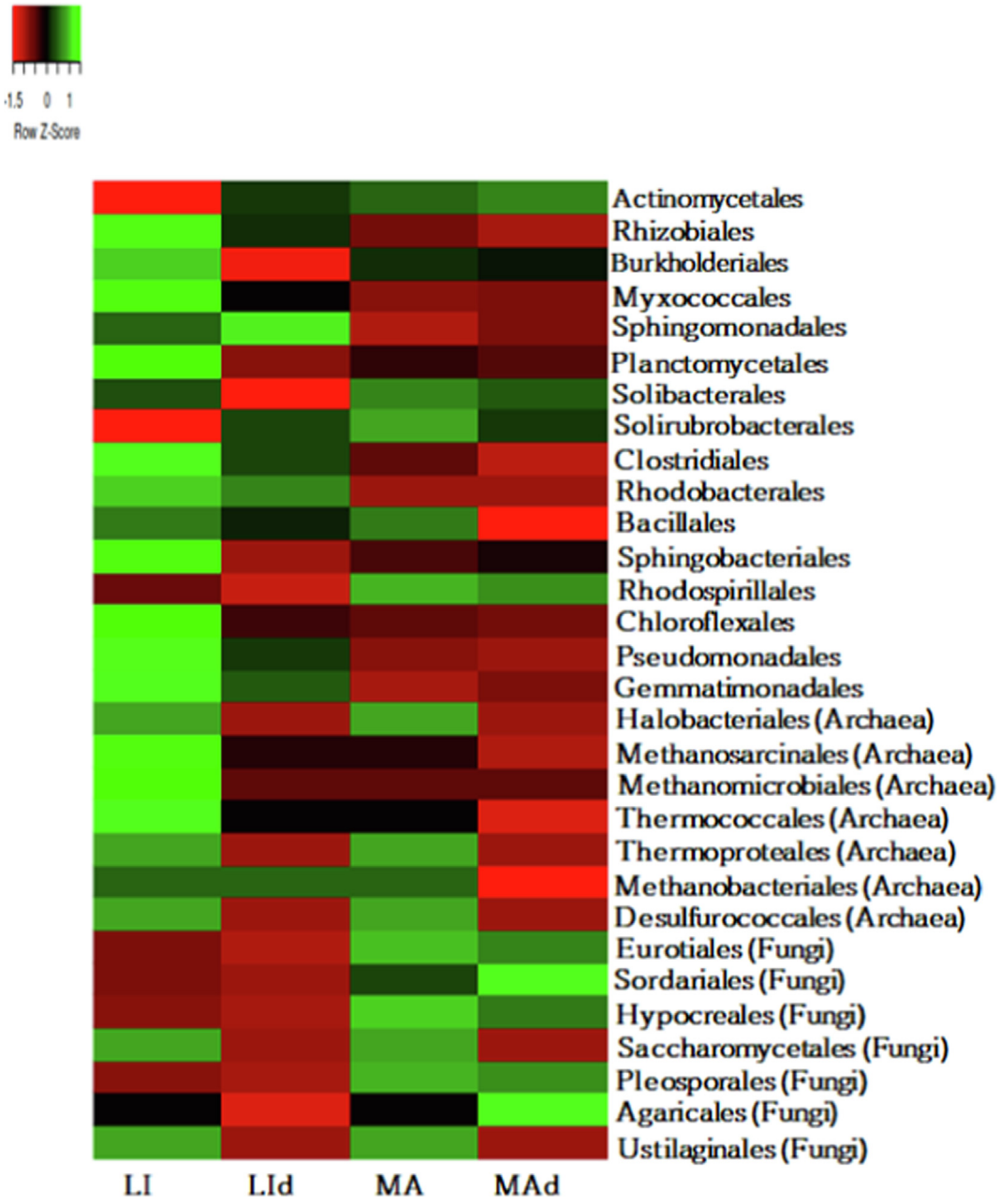

Fig. 4b. The heatmap shows the major order of soil microbial communities associated with maize plants. LI, rhizosphere soil from the healthy plant at Lichtenburg; LID rhizosphere soil from the diseased plant at Lichtenburg site, MA, rhizosphere soil from the healthy plant at Mafikeng; LID rhizosphere soil from the diseased plant at Mafikeng site.

metabolites, antimicrobial, promoting plant growths and most importantly antibiotics that suppressed the expression of pathogenic organisms as in the case of Streptomyces (Procópio et al., 2012).

The shotgun metagenomic approach also enabled the observation of *Ascomycota* and *Basidiomycota* as the major fungal phyla observed in both locations though with higher abundance in Mafikeng and samples from healthy plants. This result is consistent with the earlier reports of fungal phyla associated with maize fields (Enebe and Babalola, 2020, Fadiji et al., 2021b). The fungal genus *Gibberella, Neosartorya, Penicillium, Phaeosphaeria, Nectria* dominated the healthy rhizosphere samples (LI and MA), whereas *Aspergillus, Neurospora, Chaetomium* were more associated with diseased maize (LID and MAD) with higher abundance in MAD. Several species of *Aspergillus* are common soilborne pathogens of maize (Olawuyi et al., 2014), and their presence in the South African agricultural soil has been described as largely unexplored (Visagie and Houbraken, 2020). Studies showed that strains of *Neurospora crassa*, which is a species in the genus, have been collected in the tropical and subtropical geographical regions. Also, *N. crassa* has been documented as an endophyte of Scots pine (*Pinus sylvestris*), and the fungus possesses the ability to switch to a pathogenic state when its interaction with the host is disrupted (Kuo et al., 2014). Furthermore, despite *Chaetomium* being another most dominant fungal genera in the diseased maize field, some members of *Chaetomium* genera have been shown to demonstrate antimicrobial properties, and were found effective as biological control agents against Phytophtora nicotianae strain causing root rot in citrus (Hung et al., 2015), wheat leaf rust (Puccinia recondita) and rice blast (Magnaporthe grisea) (Park et al., 2005), while it

Fig. 4c. The heatmap shows the major family of soil microbial communities associated with maize plants. LI, rhizosphere soil from the healthy plant at Lichtenburg; LID rhizosphere soil from the diseased plant at Lichtenburg site, MA, rhizosphere soil from the healthy plant at Mafikeng; LID rhizosphere soil from the diseased plant at Mafikeng site.

enhances the copper stress tolerance in maize seedling (Abou Alhamed and Shebany, 2012).

After the prokaryotes and eukaryotes, archaea have been classified as the third domain of life (Jung et al., 2020). Although at lower abundance in the rhizosphere compared to bacteria and fungi, but most of the predominant archaea phyla, *Euryarchaeota, Crenarchaeota, Thaumarchaeota,* and *Korarchaeota* recovered from this study have earlier been reported as members of rhizosphere microbiome of rice, maize, and Scot pine among others (Catão et al., 2013, Elkins et al., 2008, Fadiji et al., 2021b). However, archaea genera *Methanosarcina, Sulfolobus, Nitrosopumilus, Cenarchaeum, Thermococcus* and *Pyrococcus* are microbes living in extreme environments and playing a vital role in exploring biological resources, some of which include mercury methylation (Ma et al., 2019), indole acetic acid production (White, 1987), growth promotion and disease resistance (Song et al., 2019). However, plants maintained a peculiar mode of interaction with archaea since the rhizosphere generates the natural habitats for both aerobic and anaerobic archaea (Lecomte et al., 2018).

As revealed in the principal component analysis (PCA), PCA 1 contributed nearly 65 % to the total variations indicating a higher abundance of up to two-third of the microbial phyla in location LI, while the principal coordinate analysis (PCoA) revealed>70 % variation in the microbial communities of the healthy and NCLB diseased maize, with higher disparity recorded in Lichtenburg (LI and LID). It can be deduced from this observation that microbial diversity in soils evolves and plays an essential role in crop production sustainability by enriching the soil and reducing the biotic and abiotic stressors (Chaparro et al., 2012, Jat et al., 2021). The measure of alpha diversity such as Simpson, Shannon and Evenness at phylum and genus level were not significant, revealing the close index of the microbial community at each level. Also, the microbial disparity across the rhizosphere soils of healthy and diseased maize suggests that the microorganisms could have developed specific-associated functions in plant ecosystems.

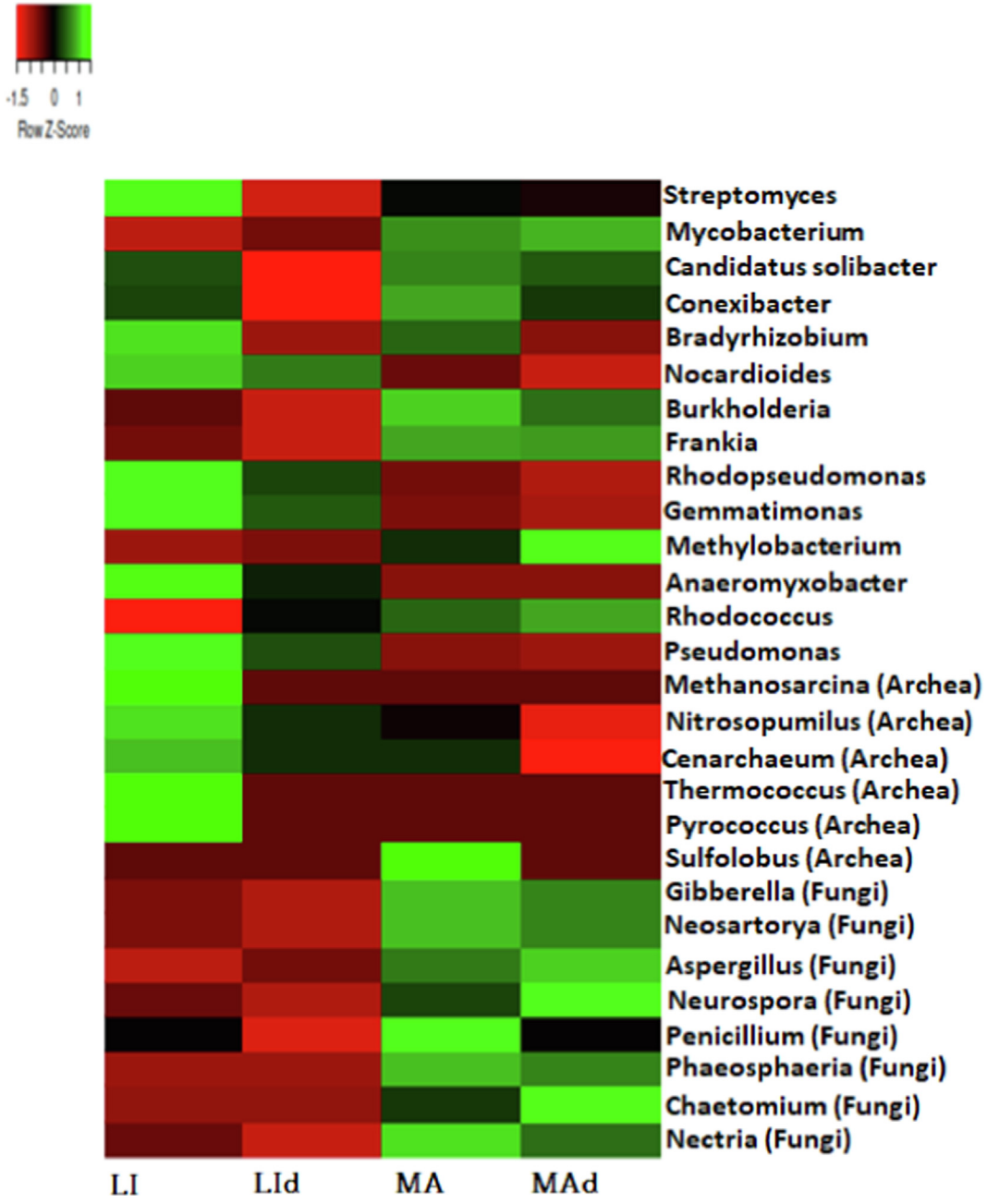

Fig. 4d. The heatmap shows the major genus of soil microbial communities associated with maize plants. LI, rhizosphere soil from the healthy plant at Lichtenburg; LID rhizosphere soil from the diseased plant at Lichtenburg site, MA, rhizosphere soil from the healthy plant at Mafikeng; LID rhizosphere soil from the diseased plant at Mafikeng site.

Table 1
Evaluation of the evenness and diversity of rhizosphere microbial community from each sample.

		LI	LID	MA	MAD	p-value
Phylum	Simpson_1-D	0,72±0.06	0,66 ± 0.06	0,67 ± 0.06	0,67 ± 0.06	0.93
	Shannon_H	1,71 ± 0.23	1,47 ± 0.22	1,52 ± 0.21	1,57 ± 0.21	
	Evenness_e^H/S	0,31 ± 0.03	0,24 ± 0.10	0,25 ± 0.1	0,27 ± 0.09	
Genus	Simpson_1-D	0,87 ± 0,07	0,91 ± 0,09	0,89 ± 0,08	0,89 ± 0,07	0.48
	Shannon_H	2,46 ± 0,30	2,60 ± 0,36	2,52 ± 0,29	2,51 ± 0,41	
	Evenness_e^H/S	0,36 ± 0.14	0,42 ± 0.23	0,39 ± 0.13	0,38 ± 0,14	

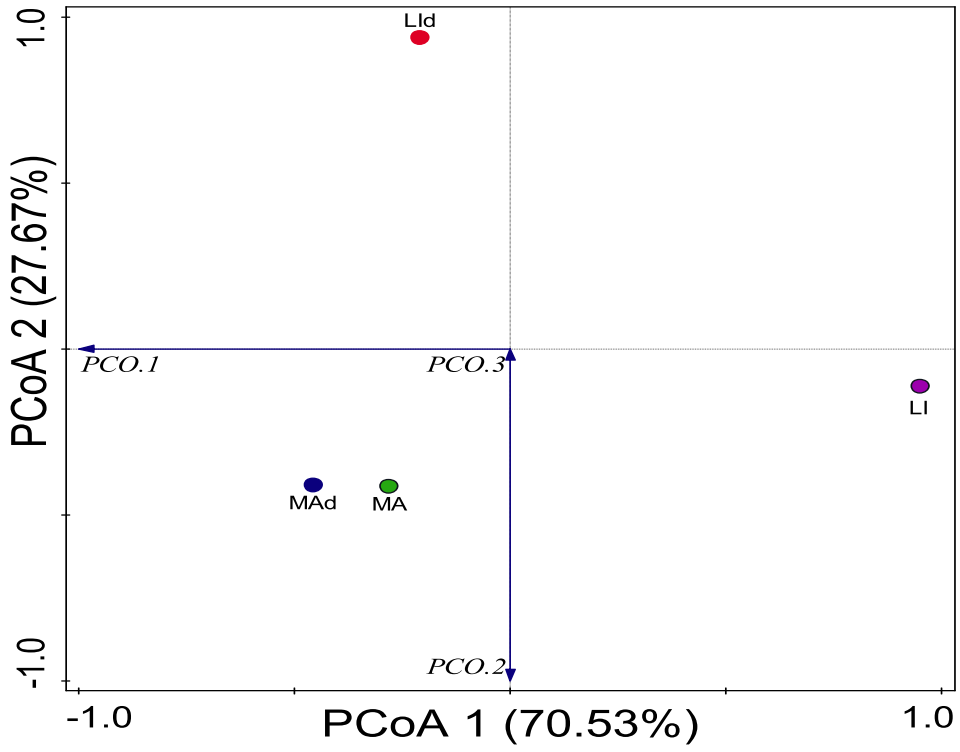

Fig. 5. PCoA graph of the soil microbial community based on Bray-Curtis dissimilarities. LI, rhizosphere soil from the healthy maize in Lichtenburg; LID, rhizosphere soil from the diseased maize in Lichtenburg. MA rhizosphere soil from the healthy maize in Mafikeng, MAD, rhizosphere soil from the diseased maize in Mafikeng.

5. Conclusion

The shotgun metagenomic study of healthy and northern corn leaf blight diseased maize rhizosphere unveiled an interesting feature that revealed the participation of an array of microorganisms, especially the yet-uncultured in the soil–plant-microbe interaction. Microbial abundance and diversity were found as more associated with the healthy rhizosphere, and this was observed to positively influence the rhizosphere microbiome in modulating the microbial functions towards the management of plant health. Since securing the integrity of soil is essential towards attaining the optimum level of health and yield, it is therefore, opined that further investigation into the microbiome of healthy plant rhizosphere would provide novel insights into the microbe's contributions to the stability of the microbiome, and this can be employed in sustainable management of northern corn leaf blight in maize.

6. Author's contribution

OOB conceived of the concepts. OOB and SPD collected the data and developed the manuscript. AOA and AEF are postdocs in the team; they evaluated the work. OOB supervised all co-authors. The authors have prudently read the final manuscript and have decided that the manuscript be published.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.103499.

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