Received: 25 January 2017 | Revised: 25 May 2017 | Accepted: 10 June 2017
DOI: 10.1002/jobm.201700043

RESEARCH PAPER

Journal of Basic Microbiology

Ecological guild and enzyme activities of rhizosphere soil microbial communities associated with Bt-maize cultivation under field conditions in North West Province of South Africa

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Funding information
National Research Foundation of South Africa, Grant numbers: 84168, 88967; Professional Development Program

Insecticidal proteins expressed by genetically modified Bt maize may alter the enzymatic and microbial communities associated with rhizosphere soil. This study investigated the structure and enzymatic activity of rhizosphere soil microbial communities associated with field grown Bt and non-Bt maize. Rhizosphere soil samples were collected from Bt and non-Bt fields under dryland and irrigated conditions. Samples were subjected to chemical tests, enzyme analyses, and next generation sequencing. Results showed that nitrate and phosphorus concentrations were significantly higher in non-Bt maize dryland soils, while organic carbon was significantly higher in non-Bt maize irrigated field soil. Acid phosphatase and β-glucosidase activities were significantly reduced in soils under Bt maize cultivation. The species diversity differed between fields and Bt and non-Bt maize soils. Results revealed that Actinobacteria, Proteobacteria, and Acidobacteria were the dominant phyla present in these soils. Redundancy analyses indicated that some chemical properties and enzyme activities could explain differences in bacterial community structures. Variances existed in microbial community structures between Bt and non-Bt maize fields. There were also differences between the chemical and biochemical properties of rhizosphere soils under Bt and non-Bt maize cultivation. These differences could be related to agricultural practices and cultivar type.

KEYWORDS
Bt maize rhizosphere soil, enzyme activities, microbial community structure, next generation sequencing

1 | INTRODUCTION

Maize is one of the world's most important agricultural crops and it is a staple food for many developing countries such as South Africa. In 1997, genetically modified (GM) maize expressing insecticidal Cry proteins (Bt toxins) were among the first GM plants to be approved in South Africa. By 2013, South Africa had 2.3 million hectares of GM crops under cultivation, of which the majority was maize (representing 78% of the GM crops under cultivation) [1]. This crop either have resistance to insect pests or tolerance to broad range of

Abbreviations: DL, dryland; DLBt, dryland Bt; DLNBt, dryland non-Bt; IL, irrigated land; ILBt, irrigated Bt; ILNBt, irrigated non-Bt.

herbicides, or both [2]. The most dominant types of GM cultivars are insect-resistant (Bt maize) and herbicide-tolerant (Roundup Ready® soybean). However, new GM cultivars have been developed that offer stacked traits (herbicide tolerance plus resistance to multiple insect pests) and increased stress tolerance (e.g., salt stress or drought tolerant varieties) [2]. This rapid and widespread adoption of GM crops has led to a dramatic shift in the agricultural landscape and has raised concerns about the impact of agricultural biotechnology on non-target microorganisms in the soil environment. Although some GM crops can provide a variety of benefits, there may also be negative impacts on the environments especially to non-target soil microorganisms such as bacteria and fungi [3].

Soil bacterial communities are relevant and good indicators for monitoring potential impacts of different agricultural practices such as farming practices, fertilizer applications as well as pesticide applications on the ecosystem functions. Soil microorganisms are a very important part of the environmental ecosystems, which could adjust energy flow and play a pivotal role in growth and development of agricultural crops [4]. They are also involved in soil biochemical processes such as enzyme production that are responsible for catalytic reactions necessary for organic matter decomposition, energy transfer, environmental quality, and crop productivity [5,6]. In addition, soil enzymes also play important roles in the nutrient cycling and are good indicators of soil quality [6,7]. Numerous studies have investigated the soil microbial properties using broad-scale or integrative methods such as enzyme activities, microbial biomass, and microbial diversity associated with Bt maize. Typically, the results of such studies have shown significantly positive, negative, and/or sometimes transitory effects of Bt maize on essential microbial properties [6,8–11]. However, the impacts of Bt maize may be masked by "functional redundancy" where overall soil functions are unaffected but microbial community composition is altered and key functions mediated by specific microbial populations are affected. Therefore, in-depth studies on the soil microbial communities associated with field grown Bt and non-Bt maize are essential to understand the microbial processes and changes in the chemical and biochemical processes in soil. Currently, metagenomic analysis of microbial ecology, such as next generation sequencing (NGS) based on 16S rRNA gene profiling, has been the focus of several environmental studies including soil [12]. Such profiling analyses provides extensive information on community structure and composition [13]. In addition, phylogenetic and functional analyses of microorganisms can be determined at community level [14]. Our aim was to study the structure and enzymatic activities of rhizosphere soil microbial communities associated with field grown Bt and non-Bt maize. Such profiling analyses provides extensive information on community structure and composition [13]. In addition, phylogenetic and functional analyses of microorganisms can be determined at community level [14]. Our aim was to study the structure and enzymatic activities of rhizosphere soil microbial communities 16S rRNA gene profiling, has been the focus of several environmental studies including soil [12] associated with field grown Bt and non-Bt maize.

2 | MATERIALS AND METHODS

2.1 | Study fields

The study was conducted in two localities in the North West Province of South Africa, where maize is intensively cultivated. These localities are situated between latitudes (26°22′45″S and 26°44′0″S) and longitudes (26°48′23″E and 27°4′52″E) and comprised of established fields under dryland (DL) and irrigation (IL) conventional cultivation where Bt maize had been grown. Transgenic Bt maize expressing the Cry1Ab protein (event MON 810) and a near-isogenic non-Bt line were used. Cultivars used under DL cultivation comprised of DKC 80-12 B and DKC 80-10 (Monsanto), while for IL PAN 6236B and PAN 6126 from Pannar were used.

2.2 | Rhizospheric soil sampling

Soil samples were randomly collected from the rhizosphere of both Bt and non-Bt maize in all study fields. Sampling was done in a W-shaped pattern in all fields to obtain representative samples. A total of 16 soil samples were collected from the rhizosphere of Bt maize (8 each from DL and IL), while 14 soil samples were collected from non-Bt maize (7 each from DL and IL) rhizosphere. All maize plants were at the maturing stage at the time of sample collection. These samples were collected aseptically as described by Dick et al. [15] and immediately transported in ice to the laboratory for further analyses.

2.3 | Determination of soil enzymatic activities

The activities of acid phosphatase (EC 3.1.3.2) and β-glucosidase (EC 3. 2.1.21) were assayed using 1 g of soil with the appropriate substrates and incubated for 1 h (37 °C) at an optimal pH as described by Tabatabai [16] and Dick et al. [15], respectively. Urease (EC 3.5.1.5) enzyme activity was estimated according to Kandeler and Gerber [17]. This method was based on the estimation of urea hydrolysis in soils. Briefly, this method involves mixing 5 g of soil with a urea solution and incubating it for 2 h at 37 °C. Enzyme activities were assayed in duplicate with one control, to which substrate was added after incubation.

2.4 | Chemical analysis

Standard chemical analyses of the soil were performed by the Agricultural Research Council-Institute for Soil Climate and Water (ARC-ISCW). The pH of the soil was determined as described by McLean [18] with potassium chloride (pH [KCℓ]) by means of a calibrated pH meter (Radiometer PHM 80, Copenhagen). Ammonium (NH_4^+-N) concentrations were measured by means of the ammonia-selective electrode method [19] and organic carbon was determined by the Walkley–Black method of Nelson and Sommers [20]. The anions nitrate (NO_3^--N), nitrite – (NO_2^--N), and phosphate – (PO_4^--P) were determined according to the method of Sonnevelt and Van den Ende [21]. The P-Bray 1 was determined according to the procedure of Bray and Kurtz [22].

2.5 | Genomic DNA extraction

The Macherey–Nagel Nucleospin Soil DNA Extraction kit (Macherey–Nagel, Germany) was used to extract DNA from rhizospheric soil samples as described by the manufacturer. DNA quantity and quality were determined by using a NanoDrop 1000 Spectrophotometer (Thermo FischerScientific, CA, USA).

2.6 | Illumina MiSeq sequencing

Microbial genomic DNA from Bt and non-Bt maize soil samples were normalized to concentration $\leq 10\ ng\ \mu l^{-1}$. Sequencing library preparation guide was followed (Illumina Inc.). Locus-specific primers 341F (5′-CCTACG GGNG GCWGCAG-3′) and 805R (5′-GACTACHVGGGTATC-TAATCC-3′) [9], targeting the hypervariable V3–V4 region (≈460 bp) of the bacterial 16S rRNA gene were used. Illumina forward and reverse overhang adapters (Illumina, Inc., CA, USA) were attached to the 5′-end of forward and reverse primers, respectively. All polymerase chain reaction (PCR) components and protocols were exactly as reported in the library preparation guide (Illumina, Inc.). Sequencing run on the Illumina MiSeq, de-multiplexing and secondary analyses of the reads were performed using the MiSeq reporter software (Illumina, Inc.).

Raw data from Illumina sequencing of the 16S rRNA gene were processed on the Galaxy GVL 4.0.0 pipeline (http://galaxy-qld.genome.edu.au/galaxy) as previously described [23]. To improve the quality of next generation sequencing data and eliminate the effect of random sequencing errors, some unreliable data from the libraries were deleted, such as average q-value below 25, singletons, and reads shorter than 200 bp. Sequences were classified into operational taxonomic units (OTUs) with 97% similarity for the 16S rRNA gene after excluding chimeric sequences by using the UCHIME method. Taxonomic information of sequences by the Ribosomal Database Project (RDP) classifier for the 16S rRNA gene were assigned at confidence cutoff of 0.5.

2.7 | Statistical analyses and bioinformatics

The data sets obtained from both chemical and biochemical analyses of both Bt and non-Bt maize soil samples were analyzed with the Statgraphics software package version 5 (Statistical Graphics Corporation, USA). Redundancy analysis (RDA) was performed to measure chemical and enzymatic properties that influence microbial community variations. The significant correlations of the parameters were examined by a Monte Carlo permutation. The triplot was generated by CANOCO 4.5 (Biometrics Wageningen, the Netherlands). Graphs were generated by CanoDraw 4.0 (Biometrics Wageningen).

The Alpha-diversity parameters were calculated for each field under Bt and non-Bt maize cultivation comprising of OTUs richness, Shannon Weiner (H′), Evenness, Inverse Simpson indexes, Chao1 richness estimator, and the rarefaction curve at 0.03 using gplot package of R on the relative abundance of each taxon. A principal coordinate analysis (PCoA) was carried out based on weighted beta diversity. In addition, a Venn diagram was constructed using the following online site (http://bioinfogp.cnb.csic.es/tools/venny/ date of access: 10 June 2016). All multivariate and community analyses were conducted using the gplot and vegan, packages of R based on the relative abundance of each taxon.

TABLE 1 Mean values of chemical properties of DL and IL under Bt and non-Bt maize fields.

Parameters	pH (KCℓ)	Organic carbon (%)	NO_3^+ (mg kg^{-1})	NO_2^- (mg kg^{-1})	NH_4^+ (mg kg^{-1})	P (mg kg^{-1})
Dryland fields						
DLNBt ($n = 8$)	6.2^a	1.0^a	6.1^a	0.4^a	2.4^a	41.2^a
DLBt ($n = 7$)	6.3^a	0.9^a	4.1^b	0.4^a	2.4^a	31.7^b
Irrigated fields						
ILNBt ($n = 8$)	6.4^a	1.4^a	29.5^a	0.5^a	2.8^a	52.2^a
ILBt ($n = 7$)	6.6^a	1.2^b	26.4^a	0.6^a	2.8^a	82.7^a

Fields under DL and IL conditions with different combinations of superscript alphabetic letters in the same column indicate significant difference between each other.

3 | RESULTS

3.1 | Chemical properties of Bt and non-Bt maize rhizosphere soil under DL and IL

In Table 1, the mean values of soil chemical characteristics comprising of Bt and non-Bt maize samples under DL and IL conditions are shown. Results of Bt and non-Bt maize fields under DL conditions showed a slightly acid pH, whereas fields under IL conditions of Bt and non-Bt maize soils indicated a slightly acid to neutral pH (Table 1). Nitrate (NO_3^+) and phosphorus (P) concentrations were significantly higher ($p < 0.05$) in non-Bt maize soils under DL conditions compared to Bt maize soil. There was no significant difference ($p > 0.05$) in values of nitrite (NO_2^-), ammonium (NH_4^+), and organic carbon (C) between Bt and non-Bt maize fields under DL conditions. No significant difference ($p > 0.05$) in values of nitrate (NO_3^+), nitrite (NO_2^-), ammonium (NH_4^+), and phosphorus (P) were showed between Bt and non-Bt maize fields under IL conditions (Table 1). However, non-Bt maize soil under IL conditions did show a significantly higher ($p < 0.05$) organic carbon (C) percentage compared to Bt maize soil.

3.2 | Biochemical properties of Bt and non-Bt maize rhizosphere soil under DL and IL

The average activities of the enzymes assayed are presented in Fig. 1. Results illustrated that there were significant differences in acid phosphatase and β-glucosidase activities between Bt and non-Bt maize soil samples under DL and IL conditions, while urease showed no significant differences. Significantly higher acid phosphatase ($p < 0.05$) and β-glucosidase activities ($p < 0.05$) were recorded for soils under non-Bt maize cultivation of DL and IL conditions.

3.3 | Bacterial diversity and richness between Bt and non-Bt maize rhizosphere soil under DL and IL

The similarity based OTUs, species richness and diversity were shown in Fig. 2 under DL and IL fields. A total of 306,672 and 238,594 valid reads were obtained from Bt and non-Bt maize fields under DL conditions, respectively (Supporting Information Table S1), with number of sequences ranging from (33,850 to 68,201) and (25,790 to 51,605) at 3% distance, respectively. In Bt and non-Bt maize fields under IL conditions has a total of 326,952 and 216,489 valid reads, respectively (Supporting Information Table S1). The number of sequences ranged from (28,462 to 55,258) and (24,486 to 41,408) between Bt and non-Bt maize fields. The results indicate that Bt maize fields under DL and IL conditions had the highest number of species present, compared to non-Bt maize fields (Supporting Information Table S1). All rarefaction curves approached a plateau, indicating that the number of sequences obtained was sufficient to describe the bacterial diversity within these soil

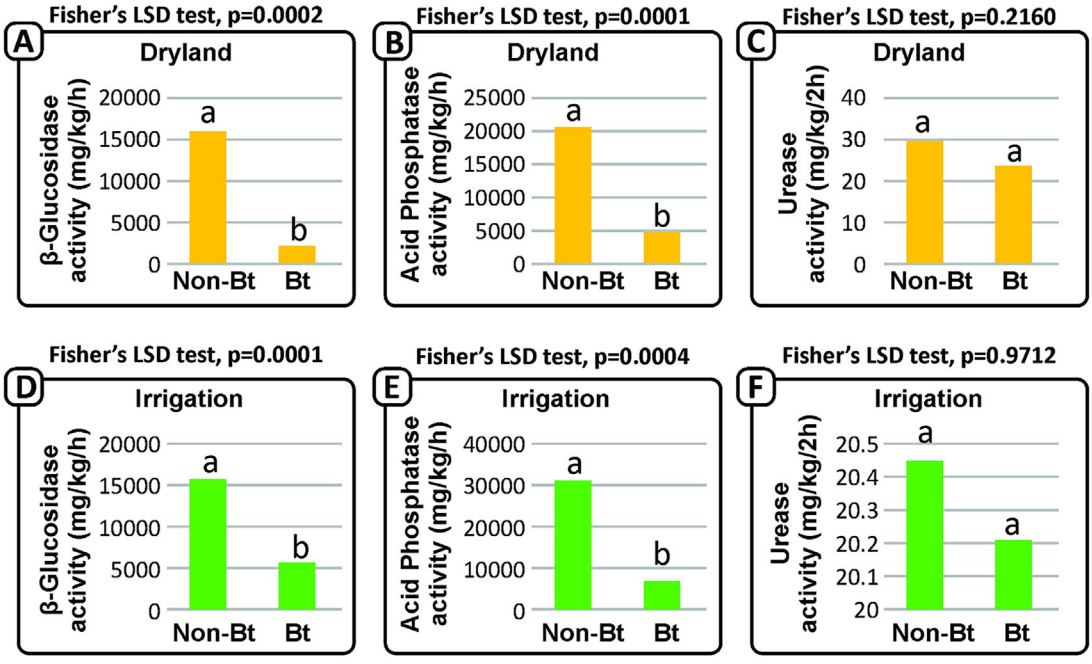

FIGURE 1 Activity of β-glucosidase (A, D), acid phosphatase (B, E), and urease (C, F) under dryland and irrigated conditions of Bt and non-Bt maize fields. The data are expressed as the means of two Replications. Different letters (a, b) indicates a significant difference at $p \leq 0.05$

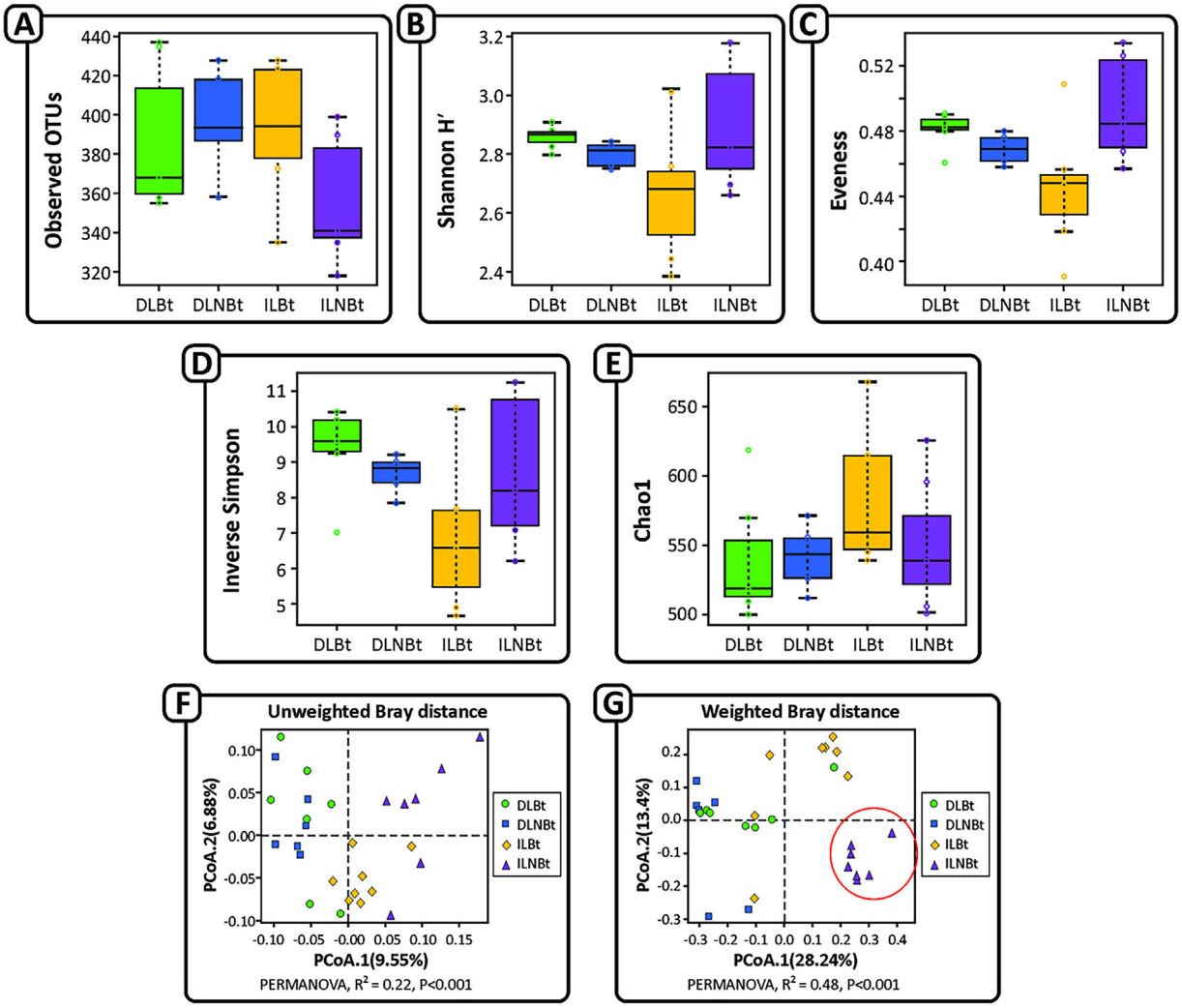

FIGURE 2 Similarity based OTUs and species richness estimates of the Bt and non-Bt maize dryland (DL) and irrigated (IL) fields. (A) Observed OTUs, (B) Shannon–Weiner index (H′), (C) evenness, (D) inverse Simpson, and (E) Chao1 richness estimator. (F and G) Principal coordinate analyses (PCoA) of unweighted and weighted Bray–Curtis distance matrix showing microbial differences between Bt and non-Bt bacterial communities of dryland and irrigated fields. Relative abundance of OTUs obtained from clustering at 97% sequences similarity were used to compute PCoA. DLBt and DLNBt represent the dryland Bt and non-Bt maize samples, while ILBt and ILNBt represent the irrigated Bt and non-Bt maize samples

fields (Supporting Information Fig. S1). Alpha diversity estimates shown in Fig. 2, illustrated that the mean of the OTUs richness and Chao1 richness estimator of the non-Bt soils population under DL conditions were greater (Fig. 2A and E), than DL non-Bt maize soils population. In contrast, under IL conditions, Bt maize soil populations had the higher richness (Fig. 2A and E), while non-Bt maize soils had the lowest richness (Fig. 2A and E) (Supporting Information Table S1). Furthermore, the mean of the evenness, Shannon and Simpson indexes showed that DL Bt maize soils population exhibited the highest diversity (Fig. 2B–D), compared to non-Bt maize soils. While under irrigated conditions non-Bt maize exhibited the highest diversity, compared to IL non-Bt maize soils population (Fig. 2B–D). Overall, the OTUs (or species) are more evenly distributed in DL Bt maize soils (mean evenness value of 0.48) than in DL non-Bt maize soils (mean evenness value of 0.46) (Fig. 2C). However, under IL conditions non-Bt maize soil showed the highest evenly distribution species (mean evenness value of 0.48), compared to Bt maize soils (mean evenness value of 0.45) (Fig. 2C).

Tukey HSD tests for differences in OTUs diversity measures between DLBt/DLNBt and ILBt/ILNBt maize soils populations indicated that the differences found were not significant ($p > 0.05$).

These results indicate that soils with a large number of species showed a degree of evenness (equitability) among species abundance. If compared to fields that displayed low species richness, indicating that many individuals belonging to the same species were detected.

3.4 | Relationship between bacterial communities among DL and IL Bt and non-Bt maize rhizosphere soil

To obtain an overall view on the identified linkages between DL and IL Bt and non-Bt maize soil samples, Bray–Curtis distance's principal coordinates analysis (PCoA) plots of the OTUs distributions (at 97% 16S rRNA sequence similarity) based on unweighted (absence/present of taxa) and weighted (absence/present and relative abundance of taxa) are shown in Fig. 2F and G. Permutational analysis of variance (PERMANOVA) of unweighted (PERMANOVA, $R^2 = 0.22$, $p < 0.001$) and weighted (PERMANOVA, $R^2 = 0.48$, $p < 0.001$) Bray–Curtis distance matrixes suggests that the differences between Bt and non-Bt maize soils of DL and IL conditions are not largely influenced by Bt maize (Fig. 2F and G). Nevertheless, the PCoA plots of both weighted and unweighted Bray–Curtis distance similarity matrices suggest that there are some differences between the OTUs richness and abundance between certain Bt and non-Bt maize fields under dryland and irrigated conditions (Fig. 2F and G). For example, the DLBt, DLNBt, and ILBt soil samples were dispersed between each other, while ILNBt soil sample clustered separately together (weighted measures) (Fig. 2G). These results suggest that some of the bacterial species in DLBt, DLNBt, and ILBt field samples were similar across fields, compared to ILNBt soil samples.

3.5 | Bacterial taxonomic community composition

3.5.1 | Soil bacterial community composition between Bt and non-Bt maize rhizosphere soil under DL and IL cultivation

Dryland (DL) and irrigated (IL) Bt and non-Bt maize soils showed similarities in bacterial community composition at the phylum level with 36 bacterial phyla identified from both fields. Both fields of Bt maize soils represented 33 bacterial phyla respectively, while non-Bt maize soils under DL conditions represented 32 bacterial phyla and IL conditions 34 bacterial phyla. The Bt and non-Bt maize soil samples for both fields were predominated by members of the phyla Actinobacteria (14.4–37.0%), Proteobacteria (14.4–30.4%), and Acidobacteria (11.7–24.4%) (Fig. 3). Furthermore, results indicated that Actinobacteria (Bt = 36.99% and non-Bt = 30.44%) was the dominant phylum under DL fields. In contrast, Proteobacteria (30.35%) were predominant in soil under Bt maize conditions of IL, while non-Bt maize soil were dominated by Acidobacteria (24.37%) (Fig. 3).

The Venn diagrams in Fig. 4 illustrates the distribution of the soil bacterial communities between Bt and non-Bt maize soils under DL and IL conditions and the total shared richness. The number of species present in soils under Bt and non-Bt maize cultivation of DL were 303 and 297, respectively. Under IL condition, the number of species present in Bt maize soil is 310 and in non-Bt maize soil 305. Furthermore, results

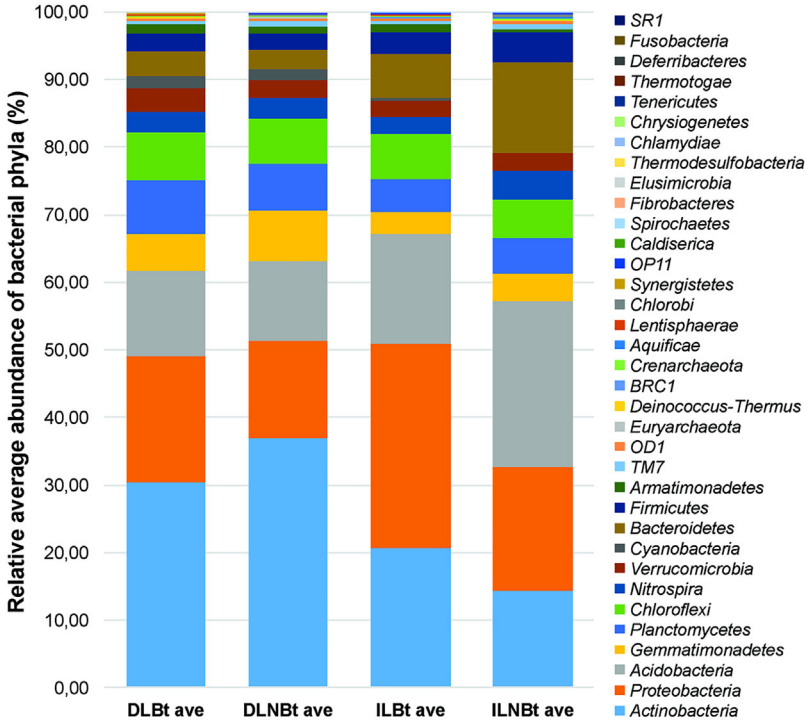

FIGURE 3 Relative average abundance of bacterial phyla present in dryland and irrigated fields of bacterial communities of Bt and non-Bt maize

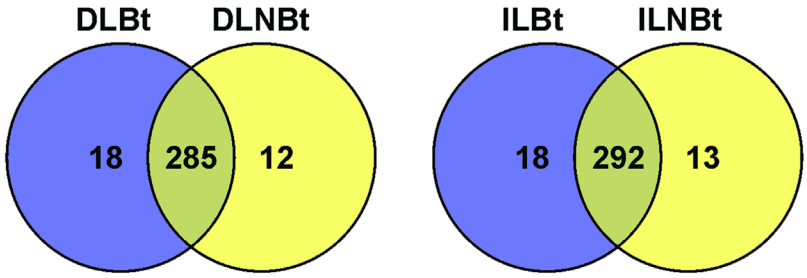

FIGURE 4 Venn diagrams signifying the number of unique and shared species between Bt and non-Bt maize DL and IL field soils at 3% distance level. DLBt and DLNBt represent the dryland Bt and non-Bt maize samples, while ILBt and ILNBt represent the irrigated Bt and non-Bt maize samples

showed that the number of species shared between DL Bt and non-Bt maize soils were 285, whereas IL Bt and non-Bt maize soils shared 292 species between each other (Fig. 4). Results also indicate that within Bt maize soils under DL and IL conditions *Arthrobacter*, *Gp*, *Rubrobacter*, and *Sphingomonas* were the most dominant genera present among both fields (Fig. 5). While under non-Bt maize cultivation of DL and IL conditions, *Gp* and *Rubrobacter* were the dominant genera among both fields. However, it was interesting to note that *Sphingomonas* and *Arthrobacter* were not detected in non-Bt maize soils under DL and IL conditions, respectively. These results indicate that more than 90% of soil microorganisms found in Bt and non-Bt maize soils under DL and IL conditions were similar (Fig. 4).

The heatmap plot depicted the relative percentage of each bacterial genus within each field (Fig. 6). As shown in Fig. 6, some soil genera in DLBt, DLNBt, and ILBt maize fields overlapped, while ILNBt formed a separate cluster (Fig. 2F and G). A similar trend was observed in the PCoA (Fig. 2F and G). These fields gathered by decreasing order of similarity in soil genera. The relative abundance for each bacterial genus was depicted by color intensity with the legend indicated in the figure on the right. In this study, a total of 24 genera and a group named "Other" (with OTU abundance percentage of less than 5%) were identified between DL and IL Bt and non-Bt maize field samples in the present study (Fig. 5). The four most abundant genera identified in DL Bt maize soil include, *Rubrobacter* (overall average 15.2%), *Gp* (14.4%), *Arthrobacter* (13.6%), and *Sphingomonas* (10.2%), while under DL non-Bt maize soil *Rubrobacter* (17.0%), *Arthrobacter* (13.98%), *Gp* (13.3%), and *Skermanella* (8.1%) were the top four genera. Under IL Bt

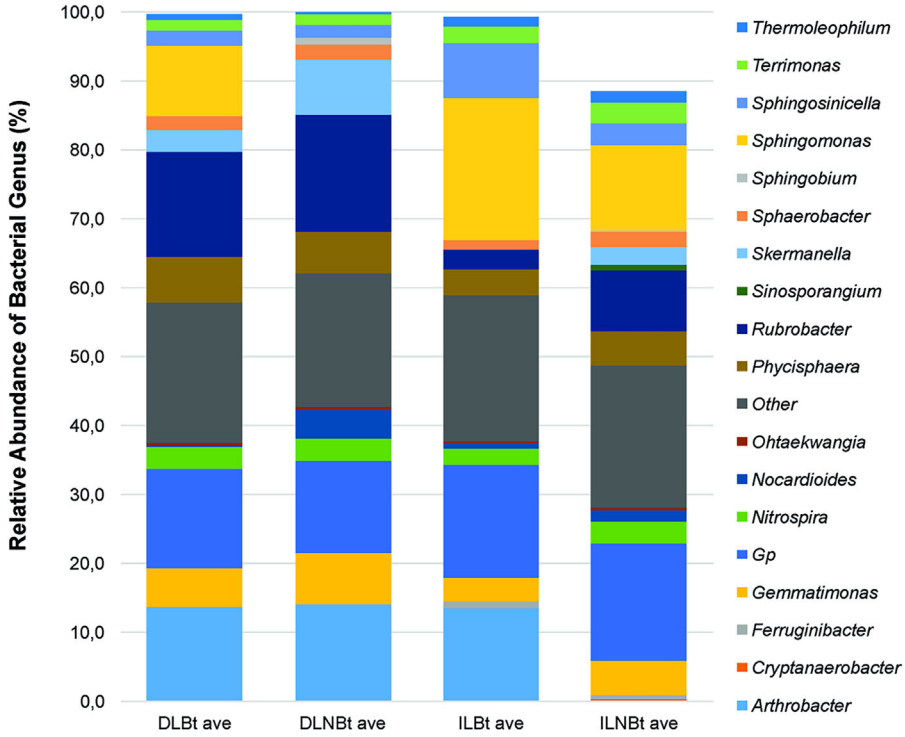

FIGURE 5 Relative abundance of predominant bacterial composition in the four treatments

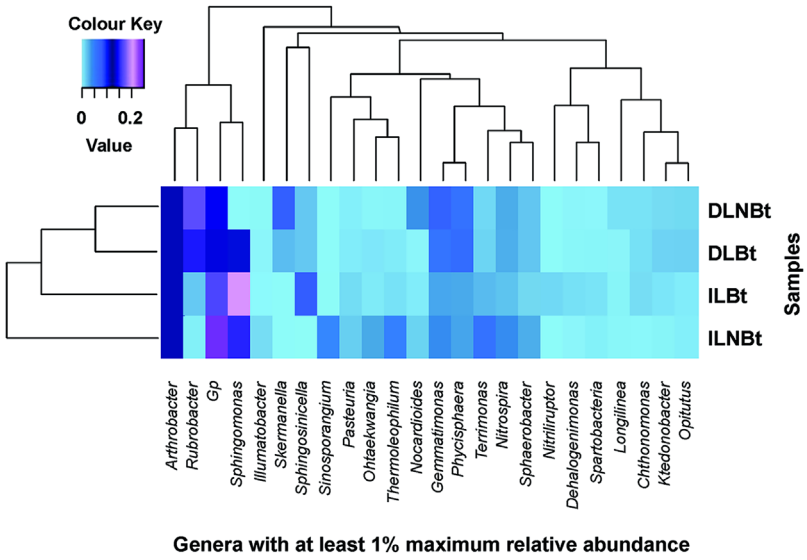

FIGURE 6 Effect of Bt maize on non-target soil organisms. Heat map of weighted Bray–Curtis with hierarchal clustering of bacterial distribution of different communities from the dryland and irrigated Bt and non-Bt maize soil samples at the genus level. The relative abundance for each bacterial genus were depicted by color intensity (clustering on the *X*-axis) with each field (*Y*-axis clustering). The higher values are represented by darker colors whereas lower ones are represented by lighter colors. DLBt and DLNBt represent the dryland Bt and non-Bt maize samples, while ILBt and ILNBt represent the irrigated Bt and non-Bt maize samples

maize field samples *Sphingomonas* (overall average 20.5%), *Gp* (16.4%), *Arthrobacter* (13.4%), and *Sphingosinicella* (7.9%) were the four most abundant genera. Whereas *Gp* (17.0%), *Sphingomonas* (12.3%), *Rubrobacter* (8.8%), and *Gemmatimonas* (5.1%) were the top four genera under IL non-Bt maize field samples. However, there were some variances between DL and IL Bt and non-Bt maize soil fields in the relative abundance of these major genera. In DL and IL Bt maize fields' soil samples, the bacterial pairs of *Sinosporangium*/*Sphingobium* as well as *Skermanella*/*Sphingobium* were not present, respectively. Furthermore, DL non-Bt maize field was the only soil where *Sphingomonas* was absent.

3.5.2 | Correlation between environmental parameters and microbial community

The average values of the dominant soil chemical and enzymatic activities used in the RDA analysis are presented in Table 1 and Fig. 1. Based on the results obtained, it is evident that pH (KCℓ) was the dominant chemical parameter in Bt maize field samples, while nitrate, organic carbon, phosphorus, nitrite and ammonium were the predominant parameters in non-Bt maize field samples under DL conditions (Fig. 7). Furthermore, results showed that non-Bt maize field samples were positively associated with acid phosphatase, β-glucosidase and urease activities. However, a negative association was observed between Bt maize field samples and enzyme activities (Figs. 1 and 7). Results also indicated an association between chemical parameters and enzymatic activities. Ammonium was positively associated with urease and acid phosphatase (Fig. 7), while β-glucosidase was positively associated with organic carbon, phosphorus, nitrate and nitrite. A negative association was also apparent between pH (KCℓ) and all the enzymatic activities assayed (Fig. 7).

Relative abundance in Bt maize soils of some genera, *Ohtaekwangia* and *Nitrospira* were correlated with pH (KCℓ), while the relative abundance in non-Bt maize soils of some genera, *Skermanella*, *Arthrobacter*, and

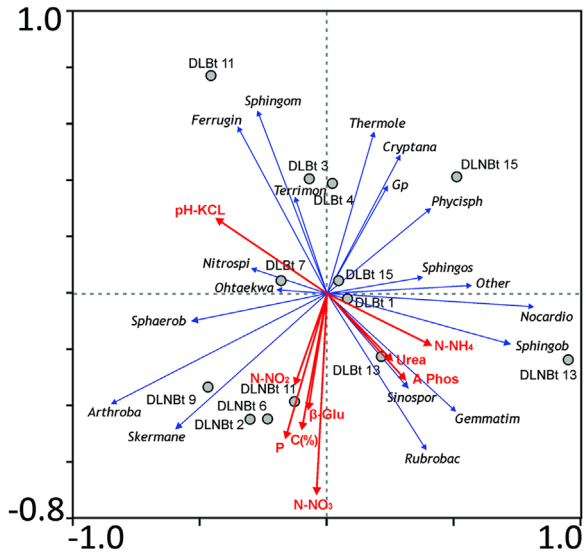

FIGURE 7 RDA triplot of dominant genera as affected by selected environmental variables. Genera are indicated by blue vectors and chemical and biochemical variables are represented by red vectors under DL conditions

Sphaerobacter were correlated with nitrate, phosphorus, carbon, and nitrite (Table 1 and Fig. 7). Some other non-Bt maize genera such as *Sphingobium* and *Nocardioides* were correlated with ammonium (Fig. 7). The relative abundance of most genera in non-Bt maize fields were strongly correlated to acid phosphatase, β-glucosidase, and urease enzyme activities (Fig. 7). Acid phosphatase, β-glucosidase, and urease enzyme activities were relatively high and showed a positive association with the relative abundance of most genera in non-Bt maize fields as compared to the Bt maize field (Figs. 1 and 7).

Results obtained indicated that ammonium, pH (KCℓ), phosphorus, and nitrite had a strong positive association with Bt maize field samples, whereas organic carbon and nitrate had a strong positive association with non-Bt maize field samples under IL conditions (Fig. 8). Furthermore, results showed that non-Bt maize field samples were positively associated with acid phosphatase, β-glucosidase, and urease activities, while Bt maize field samples had a strong negative association with enzyme activities (Figs. 1 and 8). In addition, acid phosphatase, β-glucosidase, and urease activities were strongly associated with organic carbon in the non-Bt maize field. These enzymes were also positively associated with nitrate content of the IL non-Bt maize field, but to a lesser extent. Ammonium and pH (KCℓ) was moderately associated with urease activity (Fig. 8), while phosphorus and nitrite concentrations showed a negative association with the enzyme activities (Fig. 8).

Relative abundance in non-Bt maize field samples of some genera, *Skermanella* was associated with pH (KCℓ) and ammonium concentration, while *Gp*, *Terrimonas*, and *Sphaerobacter* were strongly associated with organic carbon (Fig. 8). *Nitrospira*, *Rubrobacter*, and *Nocardioides* were strongly associated with nitrate concentrations. The relative abundance in IL Bt maize field of the soil microbial genus, *Sphingomonas* and *Arthrobacter* was positively associated with nitrite, phosphorus, ammonium, and pH (KCℓ), but to a lesser extent. *Sphingosinicella* and *Ferruginibacter* were negatively associated with all six chemical parameters in the IL Bt maize field. The relative abundance of most genera in non-Bt maize field was strongly correlated to acid phosphatase, β-glucosidase, and urease enzyme activities. A similar trend was observed in non-Bt maize field samples of DL (Fig. 7). Results indicated that both non-Bt maize field under DL and IL conditions were positively associated with enzyme activities, while Bt maize fields were negatively associated with these enzymes.

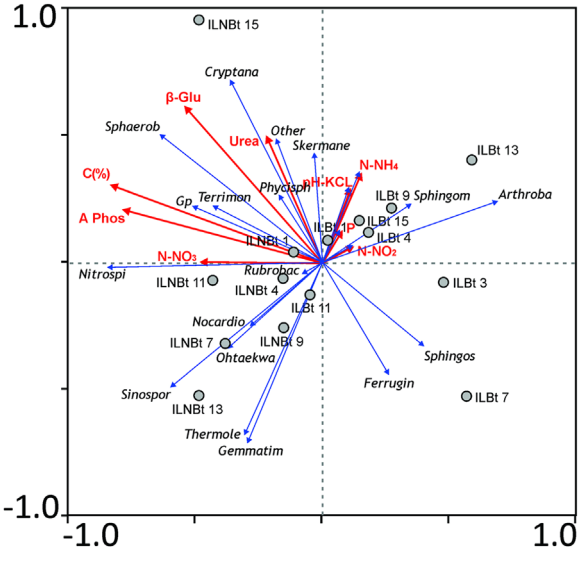

FIGURE 8 RDA triplot of dominant genera as affected by selected environmental variables. Genera are indicated by blue vectors and chemical and biochemical variables are represented by red vectors under IL conditions

4 | DISCUSSION

Soil microbial communities perform multifarious processes, which have major agricultural and ecological importance. In agriculture, it is pertinent to maintain healthy soils by regulating the physico-chemical and biochemical cycles along with the soil microbial communities present in soil [10]. Unfortunately, planting of Bt crops, could affect soil processes, due to the genetic modification and perhaps the Bt-toxin.

Soil chemical composition do not only contribute to plant nutrition but it also plays a role in microbial activities and overall soil fertility [24]. For the represented study, nitrate, phosphorus, and organic carbon levels were significantly higher under non-Bt maize soil, except for phosphorus under IL Bt maize soil. This is similar to what Griffiths et al. [25] and Liu et al. [26] previously observed for GM and non-GM maize soils. As explained by Powell et al. [27], differences in chemical properties between Bt and non-Bt maize soils under DL and IL might be a result of the difference in nutrient utilization by soil bacteria. In addition, agricultural practices and application of fertilizers could also contribute to the differences observed in chemical properties of these conventional fields [28]. These findings are further substantiated by the redundancy analyses (RDA) which indicate that organic carbon and nitrate were strongly associated with non-Bt maize field samples under DL and IL conditions (Figs. 7 and 8). However, these correlations were not statistically significant. High organic carbon content favors soil structure; water-holding capacity; root penetration, and adsorption of microorganisms and nutrients [29]. These factors in turn contribute to conditions that further favor microbial activity and nutrient cycling, making the aim of achieving sustainable ecosystems more viable [29].

Many studies have successfully matched presence and absence of soil enzymes to soil ecological processes, hence, they are accepted as indicators of soil microbial activities and fertility [30–32]. In our study, the activities of β-glucosidase and acid phosphatase in soil were significantly higher under non-Bt maize soil samples compared to Bt maize soil. The significant increase of these soil enzymes in non-Bt maize soils could be attributed to the positive association with organic carbon (Figs. 7 and 8), which also plays an important role in the soil nutrient cycle [29]. Enzyme activities of soils are usually correlated with their organic carbon and available nitrate contents [33]. Similar results were obtained by Dick and Tabatabai [34] and Frankenberger and Dick [35], who also found organic carbon to be positively related to enzyme activities. Higher levels of organic carbon stimulate microbial activity, and therefore enzyme synthesis.

Furthermore, the lower soil enzyme activity shown in soils of Bt maize under DL and IL conditions indicated that some of the bacterial species were perhaps inhibited and did not participate in the metabolic activities of the soil [36]. In previous studies involving Bt crops, there was no consistent trend between quantity levels of soil enzymes of GM and non-GM plants, nevertheless, there were differences among seasons and crop varieties [37,38]. These results are not consistent with our findings.

Overall, alpha diversity was higher under DL Bt and IL non-Bt maize soils, respectively. However, soil bacterial richness were greater under DL non-Bt and IL Bt maize soils, respectively (Fig. 2A and E). The higher soil bacterial richness in DL non-Bt maize soil samples is not unexpected due to the high contents of essential nutrients in this soil. The observation of no significant differences between the DLBt/DLNBt and ILBt/ILNBt maize OTU richness of Bt and non-Bt maize soil populations suggest that the Bt maize (including cultivar type) do not have any effect on the soil species richness in these fields populations. Analysis of the DL and IL Bt and non-Bt maize soil microbiota indicated dominance by the members of the bacterial phyla Actinobacteria, Proteobacteria, and Acidobacteria (Fig. 3). With Actinobacteria being the most dominant phyla present in both Bt and non-Bt maize soil under DL conditions, Proteobacteria and Acidobacteria were the major phyla present in Bt and non-Bt maize soil under IL, respectively. All of these phyla contain taxa commonly found within the soil community [4]. These results were consistent with those of Barriuso et al. [39] and Newman et al. [40], who also found the above mentioned bacterial phylum's to be the predominant groups in the rhizosphere of Bt maize. The reason why the phylum composition of the soil bacterial community differed under IL conditions could be due to the water supply in the fields and type of maize variety used. Similar results as these were obtained by Baumgarte and Tebbe [41], Fang et al. [42], and Barriuso et al. [38] who also found plant cultivars, soil structure, and environmental factors (plant growth phase) to have an impact on soil bacterial microbial community structures. In IL Bt maize soil, the genus *Sphingomonas* belonging to the phylum Proteobacteria was found to be dominant. It is known that this particular genus can respond to labile carbon sources and are considered r-selected, fast growing, and opportunistic bacteria [39]. The detection of *Sphingomonas*, is in agreement with reports from Bumunang et al. [43] and Dohrmann et al. [3] who also found this genus to be the most commonly found in maize soil. Therefore, it had been suggested that this species can be used as a key indicator in monitoring GM effects on soil maize communities [43]. However, in Bt and non-Bt maize fields under DL conditions, the genus *Rubrobacter* was the most enriched genus, originating from the phylum Actinobacteria, one of the major groups, which are mostly gram-positive microbes and plays an important component of soil communities. Although unexplained, *Sphingomonas* and *Arthrobacter* were not detected in DL and IL non-Bt maize soils, respectively. The results of this study showed subtle variations in soil bacterial community composition between DL and IL Bt and non-Bt maize soils. The largest difference in relative abundance was observed for *Sphingomonas* and *Arthrobacter*. Based on our results, we conclude that variances in soil microbial communities and differences observed in enzymes were probably a result of farming practices and environmental factors. This is in accordance to a recent study in our laboratory where variations in diversity and abundance of endophytes associated with the phyllosphere of Bt and non-Bt maize could not be conclusively linked to Bt genetic modification of the maize plant [44].

In conclusion, it is recommended that future studies should expand number of samples collected to include pre-sowing, pre-harvest, and post-harvest data as such approach will give more insights into the potential impacts of the genetic modification of the Bt maize. Nevertheless, the findings of our study has provided an insightful snapshot of the ecological guild and enzymatic activities of rhizosphere soil microbial communities in soil under Bt and non-Bt maize cultivations. However, further investigations are needed to elucidate whether these differences were transient, seasonal, or over a period of time.

ACKNOWLEDGMENTS

This work is based on the research supported in part by the National Research Foundation of South Africa through the grants awarded to Rasheed Adeleke and Deidre van Wyk with grant unique numbers 84168 and 88967, respectively. The authors acknowledge the Agricultural Research Council's sponsorship of van Wyk DAB through the Professional Development Program. The authors sincerely thank Obinna Ezeokoli for assisting with bioinformatics analyses.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

[1] Iversen M, Grønsberg IM, van den Berg J, Fischer K. Detection of transgenes in local maize varieties of small-scale farmers in Eastern Cape, South Africa. PLoS ONE 2014;9:e116147. https://doi.org/10.1371/journal.pone.0116147.
[2] Cheeke TE. Effects of the cultivation of genetically modified Bt crops on non-target soil organisms. In: Cheeke TE, Coleman DC, Wall DH, editors. Microbial ecology in sustainable agroecosystems. Boca Raton Florida: CRC Press; 2012. p. 153–227.
[3] Dohrmann AB, Küting M, Jünemann S, Jaenicke S. Importance of rare taxa for bacterial diversity in the rhizosphere of Bt-and conventional maize varieties. ISME J 2013;7:37–49.
[4] Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH. Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 2013;11:789–99.
[5] Carpenter JE. Impact of GM crops on biodiversity. GM crops 2011;2:7–23.
[6] Zhang J, Bo G, Zhang Z, Kong F. Effects of straw incorporation on soil nutrients, enzymes, and aggregate stability in tobacco fields of China. Sustainability 2016;8:710. https://doi.org/10.3390/su8080710.
[7] Pajares S, Gallardo JF, Masciandaro G, Ceccanti B. Enzyme activity as an indicator of soil quality changes in degraded cultivated Acrisols in the Mexican Trans-volcanic Belt. Land Degrad Dev 2011;22:373–81.
[8] Chen ZH, Chen LJ, Zhang YL, Wu ZJ. Microbial properties, enzyme activities and the persistence of exogenous proteins in soil under consecutive cultivation of transgenic cottons (*Gossypium hirsutum* L.). Plant Soil Environ 2011;57:67–74.
[9] Griffiths BS, Caul S, Thompson J, Birch ANE. Soil microbial and faunal community responses to maize and insecticide in two soils. J Environ Qual 2006;35:734–41.
[10] Ondreičková K, Mihálik D, Ficek A, Hudcovicová M. Impact of genetically modified maize on the genetic diversity of rhizosphere bacteria: a two-year study in Slovakia. Pol J Ecol 2014;62:67–76.
[11] Herlemann DP, Labrenz M, Jürgens K, Bertilsson S. Transitions in bacterial communities along the 2000km salinity gradient of the Baltic Sea. ISME J 2011;5:1571–9.
[12] Lemos LN, Fulthorpe RR, Triplett EW, Roesch LF. Rethinking microbial diversity analysis in the high throughput sequencing era. J Microbiol Meth 2011;86:42–51.
[13] Kakirde KS, Parsley LC, Liles MR. Size does matter: application-driven approaches for soil metagenomics. Soil Biol Biochem 2010;42:1911–23.
[14] Cowan D, Meyer Q, Stafford W, Muyanga S. Metagenomic gene discovery: past, present and future. Trends Biotechnol 2005;23:321–9.
[15] Dick RP, Breakwell DP, Turco RF, Doran JW. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Doran JW, Jones AJ, editors. Methods for assessing soil quality. Madison, WI: Soil Science Society of America; 1996. p. 247–71.
[16] Tabatabai MA. Soil enzymes. In: Weaver RW, Angle JS, Bottomley PS, editors. Methods of soil analysis, part 2. Madison, WI: Soil Science Society of America; 1994. p. 775–833.
[17] Kandeler E, Gerber H. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol Fert Soils 1988;6:68–72.
[18] McLean EO. Soil pH and lime requirement. In: Weaver RW, Angle JS, Bottomley PS, editors. Methods of soil analysis, part 2. Madison, WI: Soil Science Society of America; 1982. p. 199–224.
[19] Banwart WL, Tabatabai MA, Bremner JM. Determination of ammonium in soil extracts and water samples by an ammonia electrode 1. Commun Soil Sci Plant Anal 1972;3:449–58.
[20] Nelson DW, Sommers L. Total carbon, organic carbon, and organic matter. In: Weaver RW, Angle JS, Bottomley PS, editors. Methods of soil analysis part 2. Madison, WI: Soil Science Society of America; 1982. p. 199–224.
[21] Sonneveld C, van ven Ende J. Soil analysis by means of a 1:2 volume extract. Plant Soil 1971;35:505–16.
[22] Bray RH, Kurtz LT. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci 1945;59:39–46.
[23] Afgan E, Coraor N, Chilton J, Baker D. Enabling cloud bursting for life sciences within Galaxy. Concurr Comp-Pract E 2015;27:4330–43.
[24] Meliani A, Bensoltane A, Mederbel K. Microbial diversity and abundance in soil: related to plant and soil type. Am Plant Nutr Fertil Technol 2012;2:10–8.
[25] Griffiths BS, Caul S, Thompson J, Birch ANE. Microbial and microfaunal community structure in cropping systems with genetically modified plants. Pedobiologia 2007;51:195–206.
[26] Liu N, Zhu P, Peng C, Kang L. Effect on soil chemistry of genetically modified (GM) vs. non-GM maize. GM Crops 2010;1:157–61.
[27] Powell JR, Levy-Booth DJ, Gulden RH, Asbil WL. Effects of genetically modified, herbicide-tolerant crops and their management on soil food web properties and crop litter decomposition. J Appl Ecol 2009;46:388–96.
[28] Esperschütz J, Gattinger A, Mäder P, Schloter M. Response of soil microbial biomass and community structures to conventional and organic farming systems under identical crop rotations. FEMS Microbiol Ecol 2007;61:26–37.
[29] Meena VS, Maurya BR, Bohra JS, Verma R. Effect of concentrate manure and nutrient levels on enzymatic activities and microbial population under submerged rice in alluvium soil of Varanasi. Crop Res Int J 2013;45:1–9.
[30] Baćmaga M, Borowik A, Kucharski J, Tomkiel M. Microbial and enzymatic activity of soil contaminated with a mixture of diflufenican+mesosulfuron-methyl+iodosulfuron-methyl-sodium. Environ Sci Pollut R 2015;22:643–56.
[31] Cardoso EJBN, Vasconcellos RLF, Bini D, Miyauchi MYH. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? Sci Agric 2013;70:274–89.
[32] Luna L, Pastorelli R, Bastida F, Hernández T. The combination of quarry restoration strategies in semiarid climate induces different responses in biochemical and microbiological soil properties. Appl Soil Ecol 2016;107:33–47.
[33] Taylor JP, Wilson B, Mills MS, Burns RG. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biol Biochem 2002;34:387–401.
[34] Dick WA, Tabatabai M. Kinetic parameters of phosphatases in soils and organic waste materials. Soil Sci 1984;137:7–15.

[35] Frankenberger WT, Dick WA. Relationships between enzyme activities and microbial growth and activity indices in soil. Soil Sci Soc Am J 1983;47:945–51.
[36] Beura K, Rakshit A. Bt cotton influencing enzymatic activities under varied soils. Open J Ecol 2013;3:505.
[37] Icoz I, Saxena D, Andow DA, Zwahlen C. Microbial populations and enzyme activities in soil in situ under transgenic corn expressing cry proteins from *Bacilus thuringiensis*. J Environ Qual 2008;37:647–62.
[38] Shen RF, Cai H, Gong WH. Transgenic Bt cotton has no apparent effect on enzymatic activities or functional diversity of microbial communities in rhizosphere soil. Plant Soil 2006;285: 149–59.
[39] Barriuso J, Valverde JR, Mellado RP. Effect of Cry1Ab protein on rhizobacterial communities of Bt-maize over a four-year cultivation period. PLoS ONE 2012;7:e35481. https://doi.org/10.1371/journal.pone.0035481.
[40] Newman MM, Hoilett N, Lorenz N, Dick RP. Glyphosate effects on soil rhizosphere-associated bacterial communities. Sci Total Environ 2016;543:155–60.
[41] Baumgarte S, Tebbe CC. Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Mol Ecol 2005;14:2539–51.
[42] Fang M, Kremer RJ, Motavalli PP, Davis G. Bacterial diversity in rhizospheres of non-transgenic and transgenic corn. Appl Environ Microbiol 2005;71:4132–6.
[43] Bumunang EW, Jordaan K, Barros E, Bezuidenhout C. Analysis of rhizobacterial community in field grown GM and non-GM maize soil samples using PCR-DGGE. J Agric Technol 2015;11:831–8.
[44] Mashiane R, Ezeokoli TE, Adeleke R, Bezuidenhout C. Metagenomic analyses of bacterial endophytes associated with the phyllosphere of a Bt maize cultivar and its isogenic parental line from South Africa. World J Microbiol Biotechnol 2017;33:80. https://doi.org/10.1007/s11274-017-2249-y.

SUPPORTING INFORMATION

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How to cite this article: van Wyk DAB, Adeleke R, Rhode OHJ, Bezuidenhout CC, Mienie C. Ecological guild and enzyme activities of rhizosphere soil microbial communities associated with Bt-maize cultivation under field conditions in North West Province of South Africa. *J Basic Microbiol*. 2017;57:781–792.
https://doi.org/10.1002/jobm.201700043