Fungal diversity and community composition of wheat rhizosphere and non-rhizosphere soils from three different agricultural production regions of South Africa

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HIGHLIGHTS

- Fungal diversity and richness decreased from non-rhizosphere to rhizosphere soil in wheat.
- Ascomycota and Basidiomycota dominated rhizosphere and non-rhizosphere soils.
- *Fusarium*, *Phoma* and *Colletotrichum* were the pathogenic genera detected in wheat rhizosphere.
- *Trichoderma*, *Aureobasidium* and *Acaulospora* were the most dominant beneficial genera detected.

ABSTRACT

Understanding complex interactions among plant genotypes, environmental conditions and microbiome structure provides crucial information for sustainable farming practices towards disease control in agriculture. In this study, fungal diversity and composition in wheat rhizosphere and non-rhizosphere soils were investigated. Special emphasis was placed on pathogenic and beneficial genera. Wheat rhizosphere and non-rhizosphere soil from three different wheat growing regions were analyzed using Illumina high-throughput sequencing. The analysis showed a significant decline in the fungal diversity and richness from non-rhizosphere to rhizosphere soils. Ascomycota and Basidiomycota were the dominant fungal phyla detected in both rhizosphere and non-rhizosphere soils across the three test sites. Genera known to include wheat pathogens detected included *Fusarium*, *Phoma* and *Colletotrichum* genera while, beneficial groups included *Trichoderma*, *Aureobasidium* and *Acaulospora*. The presence of *Fusarium* was observed to be inversely proportional to that of *Aureobasidium,* a well-known antagonist of the *Fusarium* spp. This information could provide new opportunities to explore the potential of manipulating natural fungal antagonistic microorganisms for use in controlling soil-borne pathogenic fungi in wheat.

Keywords: Fungal communities; *Triticum sativum L.);* High-throughput ITS sequencing; Microbiome; Soil microbiology

1. Introduction

Current models predict that the world's population will reach 9 billion by 2050 and that crop yields will need to increase by 70-100% to sustain the population growth (Reid and Greene, 2012). Wheat, along with maize and rice provides 50% of human calories and are critical food sources in regions with rapid population growth such as Asia, Africa and the Middle East (Donn, 2015). However, global wheat production has slowed to 0.9% per year. At this level the 2050 targets will not be met and an increase to 1.5% per year should be the target (Fischer and Edmeades, 2010).

In South Africa (SA), wheat (*Triticum sativum* L.) is considered an important cereal crop and it ranks second after maize in terms of the area under cultivation (533 000 ha) with production ranging from 1.3 to 2 million tons per year (DAFF, 2016). Major wheat production areas in SA are the Free State, the Western Cape and Northern Cape Provinces. Other provinces such as Gauteng and North-West are ranked as minor producers of wheat (Nhemachena and Kirsten, 2017). As with global downward trends, the Free State Province has shown a drop (up to 50%) in annual wheat production (Daff, 2016). Production in other irrigation and winter wheat areas also declined, but not as drastically. This calls for strategic intervention to increase production to at least 2.7 million tons per year in order to feed the country's growing population and avoid food price increases (Fischer and Edmeades, 2017).

Several factors have been attributed to the decline in SA wheat production including unpredictable weather conditions and drought, poor soil fertility and occurrence of pests and diseases (Scot, 1990). Due to poor soil quality most of the local wheat producers resort to fertilizer use (FAO, 2005). This together with lime can increase the total variable input costs to as much as 17 to 30% in wheat production (DAFF, 2010). Additionally, root and crown diseases in wheat also negatively impact

on wheat germination and yield losses in South Africa (Lamprecht et al., 2006). Economically important diseases of wheat include take-all caused by *Gaeumannomyces graminis* (Sacc.) Arx & Olivier var. *graminis*, crown rot caused by *Fusarium pseudograminearum* O'Donnell & Aoki, common root rot caused by *Rhizoctonia solani (*Burgess et al., 2001; Kuzdralinsk et al., 2014) etc. The prevalence of these soil-borne pathogenic fungi in wheat differs from one geographic region to another (Kuzdralinsk et al., 2014). Disease control of these pathogens is reasonably successful through different cultivation practices such as the use of disease resistant cultivars, crop rotation, use of pathogen-free seeds, appropriate planting date and plant density as well as by chemical and biological control methods. Cultivation practices have been reported to influence the plant and soil microbiome (Sergaki et al., 2018) and therefore it is imperative to understand how microbial communities differ from one production area to the other. This will help in the development of a more effective holistic plant protection strategy. In many cases, diseases are associated with plant microbiome imbalances or shifts which makes exploitation of the entire microbiome a desirable strategy. More so, the plant microbiome has been known to be one of the key determinants of plant health and productivity (Berg et al., 2016).

The study of root-associated microbial communities has been important in understanding their ecological role in natural environments (Cavaglieri et al., 2009). Soil microorganisms that establish positive interactions with plant roots play a key role in agricultural environments and are promising for their potential use in sustainable agriculture (Di Cello et al., 1997). The rhizosphere community contains multiple species of microbes that exert beneficial effects on plant growth and health (Zhao et al., 2018). These include nitrogen-fixing bacteria, mycorrhizal fungi, plant growth-promoting rhizomicrobes (PGPR), biocontrol microbes and protozoa. On the other

hand, soil-borne pathogens colonize the rhizosphere causing plant diseases through breaking the protective microbial “shield” and overcoming the plant’s innate defense mechanisms (Mendes et al., 2013). The complexity and diversity of microbes in the rhizosphere are essential for maintaining homeostasis in the soil ecosystem (Garbeva et al., 2004; Raaijmakers et al., 2009). Therefore, understanding the fungal population dynamics of the wheat rhizosphere is the first step towards successful manipulation of microbial communities to reduce plant pathogen pressure and increase yield (Bever, 2012). To our knowledge, the studies on soil fungi in wheat focused on fungal diversity across different farming systems (Hagn et al., 2003; Sommermann et al., 2018; Abdelfattah et al., 2016). However, no studies have been conducted to investigate fungal diversity with special emphasis on plant pathogenic and beneficial communities in the wheat rhizosphere at the seedling stage in SA. Therefore, the aim of our study was to assess the fungal composition and diversity of wheat rhizosphere in three wheat growing regions of SA and to further understand the dynamics in the pathogenic and beneficial fungal populations in these production areas.

2. Materials and Methods

2.1. Site description and soil collection

A total of 240 rhizosphere and 60 non-rhizosphere soil samples were collected from three different wheat growing agro-ecological regions in SA i.e. Napier, Western Cape Province (Site A), Pretoria, Gauteng Province (Site B) and Bethlehem, Free State Province (Site C). Rhizosphere soil was sampled from four different cultivars and the cultivars were specific for each region. The wheat cultivars grown included ‘Kariega’ and ‘SST88’ (Site A), ‘Kariega’ (Site B) and ‘Eland’ (Site C). From the 240 rhizosphere soil samples, 80 were collected at 20 m spacing in each site, from each

cultivar. Eight replicate plots were randomly selected for sampling within each location. From each plot, we collected 30 rhizosphere samples that were evenly distributed across the sampling site. The non-rhizosphere soil samples comprised of 20 samples per site. Soil was collected in the field between rows approximately 40 cm away from the sampled plants with a clean auger (washed and disinfected with 70% ethanol between sampling) from a soil depth of approximately 10 cm. Approximately 10 g of rhizosphere soil were collected (from each plantlet at the 4-5 leaf stage with the crown root system fully developed) by uprooting the whole plant and gently shaking off all the access soil from the roots. Only the soil in direct surrounding the roots was gently shaken and brushed off to represent the root zone soil.

Soil samples were placed inside marked zipper bags and transported to the laboratory in cooler boxes for storage at 4 °C to minimize the development of commensals. Soil samples were processed within two days. Each prepared soil sample was stored in an ultralow temperature freezer at −80 °C for DNA extraction.

2.2. DNA extraction and sequencing

Total community DNA was extracted from 0.25g soil using the MoBio PowerSoil™ DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA concentration in each sample was determined using Nanodrop ND-2000 UV–VIS Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) prior to further analysis.

The fungal ITS region was amplified with illumine Miseq using ITS1 (TCCGTAGGTGAACCTGCGG) and ITS2 (GCTGCGTTCTTCATCGATGC) (White et al., 1990). Sequencing was performed at Molecular Research DNA (MR DNA)

(www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines.

2.3. Data processing and bioinformatic analysis

Sequence data were processed using MR DNA analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater). The sequences from the Illumina were joined and depleted of bar codes. Sequences with ambiguous base calls and those <150 bp (Abdelfattah et al., 2016) were discarded. Noises, singletons and chimeras were removed from sequences. Generated operational taxonomy units (OTUs) were defined by clustering at a similarity threshold of 97%.

Normalization of the OTU table was done with rarefaction to an even depth of 1 000 sequences in order to maintain homogeneity of samples. The indices were then calculated from the rarefied OTU table (Smalla et al., 2001). Alpha diversity was calculated using Chao1 and Shannon-Weaver indices. Principle coordinates analysis (PCoA), based on distance matrix of Bray-Curtis estimation, was used to evaluate similarities or differences between samples. The alpha diversity indexes relative to the samples were represented by box plots and statistically compared using a standard t-test. We further tested for significant differences in the relative abundance of taxonomic groups using one-way ANOVA and Tukey's multiple comparison test. Test results with $p < 0.05$ were considered statistically significant. All statistical analyses were executed in R version 3.4.3 (R Core Team, 2017).

3. Results

3.1. Fungal richness and diversity

Fungal communities were characterized by next-generation sequencing of nuclear ribosomal internal transcribed spacer-1. A total of 1 598 148 valid reads were generated from 14 soil samples at 3% distance after paired-end alignments, quality filtering, and deletion of chimeric and singletons. Sequences ranged from 34 767 to 432 036 per sample, with a median read count of 114 153 per sample. The high-quality reads were clustered into 1 268 microbial OTUs at 97% similarity after the removal of OTUs that were unassigned or not assigned to the target species.

The species richness and diversity of the soils from the three sites is presented in Figure 1. The t-test revealed a significant difference ($p<0.05$) on the species richness (Chao) and diversity (Shannon-Weaver) between rhizosphere and non-rhizosphere soil samples (supplementary Table 1). The OTU richness was significantly lower ($p<0.05$) in the rhizosphere soil (cv. SST88 at Site A- 774.1 ± 19.01, cv. Kariega at Site A- 847.4 ± 41.32 and cv. Kariega at Site B- 929.9 ± 27.22 and cv. Eland at Site C- 753.9 ± 28.14,) soil compared to non-rhizosphere (Site A- 800.2 ± 23.65 and Site B- 946.2 ± 21.97, Site C- 736.9 ± 28.98) as shown in Figures1 A and B, respectively. Similarly, the fungal diversity was lower in rhizosphere soil samples (cv. Kariega at Site A- 3.085 ± 0.10, cv. Eland at Site C- 3.93 ± 0.43, cv. SST88 at Site A- 4.41 ± 0.38 and cv. Kariega at Site B- 4.49 ± 0.02) compared to non-rhizosphere (Site A- 3.58 ± 0.18, Site B- 4.03 ± 0.31 and Site C- 4.65 ± 0.27) soil samples across the three sites. Rhizosphere soil from cv. Kariega at Site B had a significantly ($p<0.05$) higher fungal diversity of 4.49 ± 0.02 while rhizosphere from cv. Kariega at Site A had the lowest fungal diversity of 3.085 ± 0.10. In addition, significant differences ($p<0.05$) in fungal diversity and species richness was observed between the rhizosphere soils of cv.

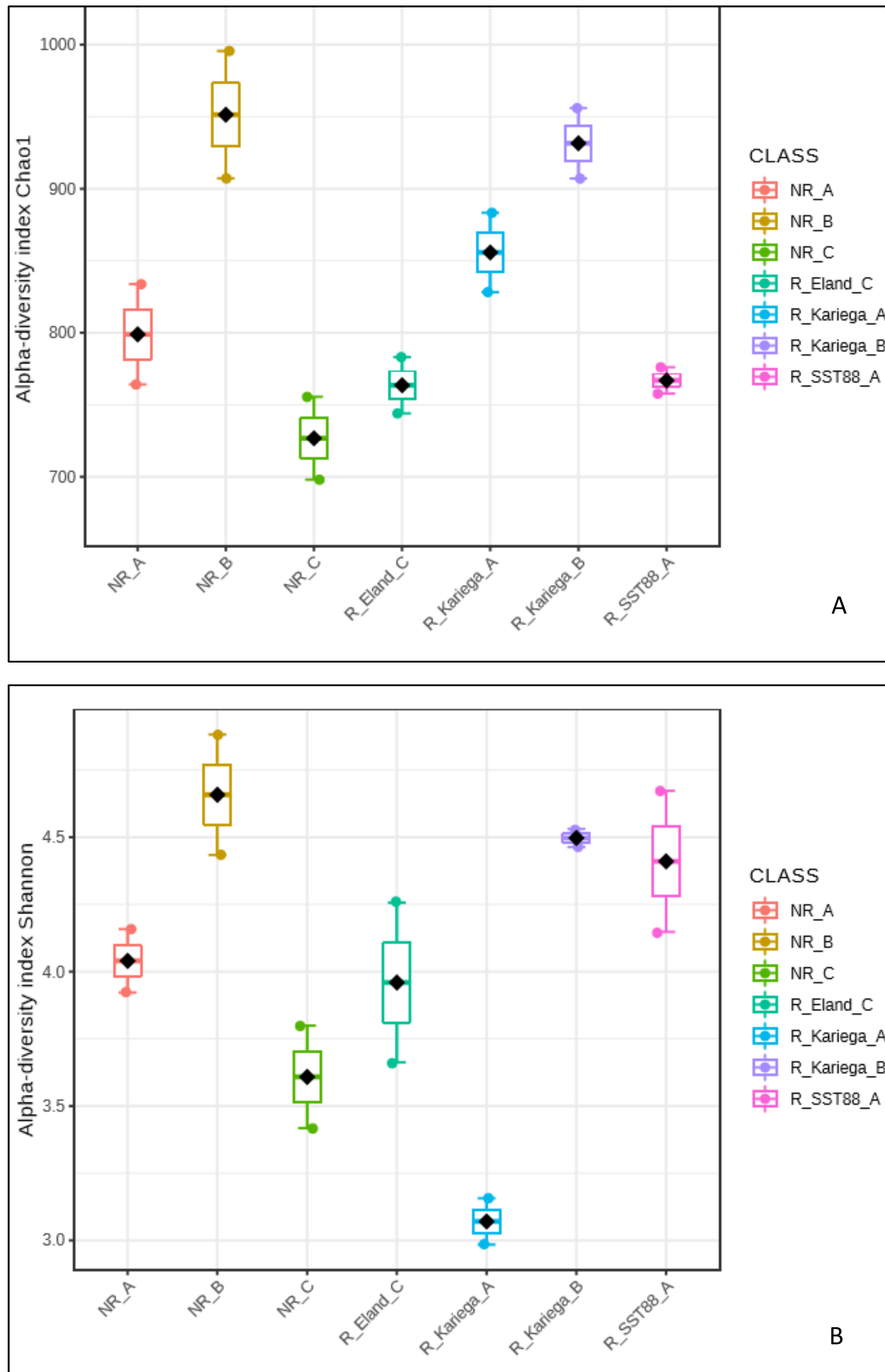

Fig. 1. Alpha diversity of the estimated OTU richness (Chao1 (A)) and diversity (Shannon (B)) indexes of the 16S rRNA gene libraries for clustering at 97% identity for wheat rhizosphere and non- rhizosphere soil. NR_A, Non- rhizosphere soil at Site A; NR_B, Non- rhizosphere soil at Site B; NR_C, Non- rhizosphere soil at Site C; R_Eland_C, Eland rhizosphere soil at Site C; R_Kariega_A, Kariega rhizosphere soil at Site A; R_Kariega_B, Kariega rhizosphere soil at Site B; R_SST88_A, SST88 rhizosphere soil at Site A.

Kariega and cv. SST88 grown at Site A. The cv. Kariega rhizosphere soil showed significantly ($p<0.05$) lower species richness and diversity compared to cv. SST88 rhizosphere soil. Meanwhile, significant differences ($p<0.05$) were also observed in species richness and diversity between cv. Kariega grown in Site A and Site B. Similar to the observations in the rhizosphere soil samples, the highest alpha diversity in non-rhizosphere soils was observed at Site B (4.65 ± 0.27) while Site C (3.58 ± 0.18) had the lowest alpha diversity.

The effect of wheat cultivar and site on relative abundances of observed OTUs showed significant differences with a PERMANOVA test statistic for cultivar abundances of the OTUs. A significant effect ($p<0.05$) was found for both cultivar and site, although no significant interaction could be determined for the two (supplementary Table 2). Based on Bray-Curtis dissimilarity analysis, the beta diversity of the fungal communities between three sites were significantly different ($p<0.01$). About 41.5% of observed variation could be explained by the first two principle coordinates (Figure 2). The rhizosphere and non-rhizosphere OTUs clustered together according to sampling site in principle component analysis. In addition, the separation of the different sampling sites was clearly visible. The three cultivars (cv cv. SST88, cv. Kariega and cv. Eland) were generally well separated although, minimal separation was observed in fungal communities in the rhizosphere of cv. SST88 and cv. Kariega at Site A (Figures 2 and 3).

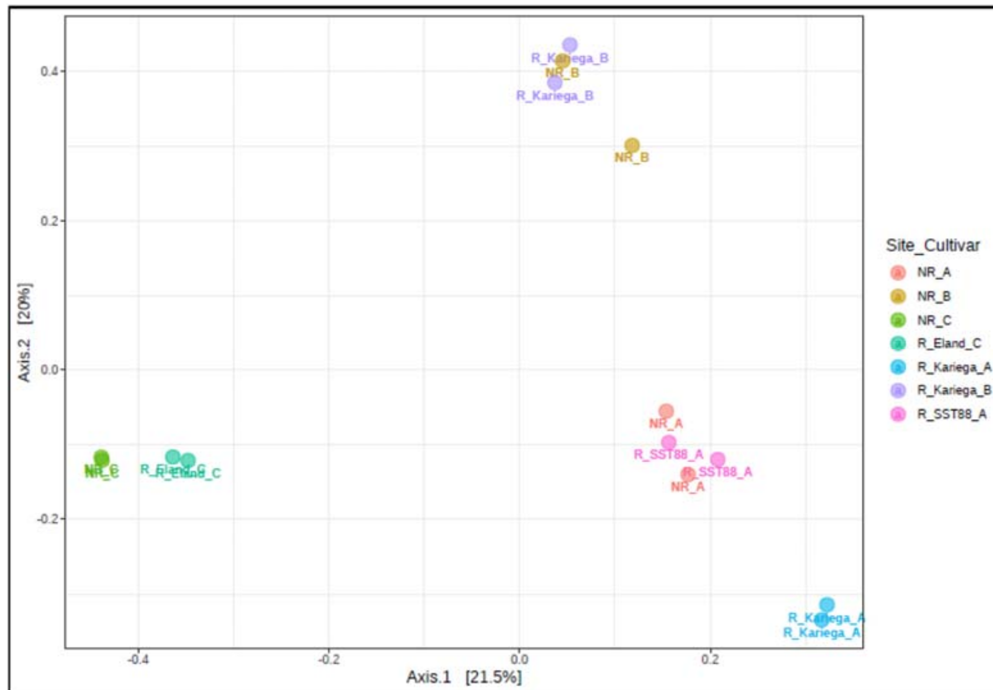

Fig. 2. Principal component analysis of the fungal community composition on wheat rhizosphere and non-rhizosphere soil collected from different regional sites in South Africa, according to illumina sequencing data. NR_A, Non- rhizosphere soil at Site A; NR_B, Non- rhizosphere soil at Site B; NR_C, Non- rhizosphere soil at Site C; R_Eland_C, Eland rhizosphere soil at Site C; R_Kariega_A, Kariega rhizosphere soil at Site A; R_Kariega_B, Kariega rhizosphere soil at Site B; R_SST88_A, SST88 rhizosphere soil at Site A.

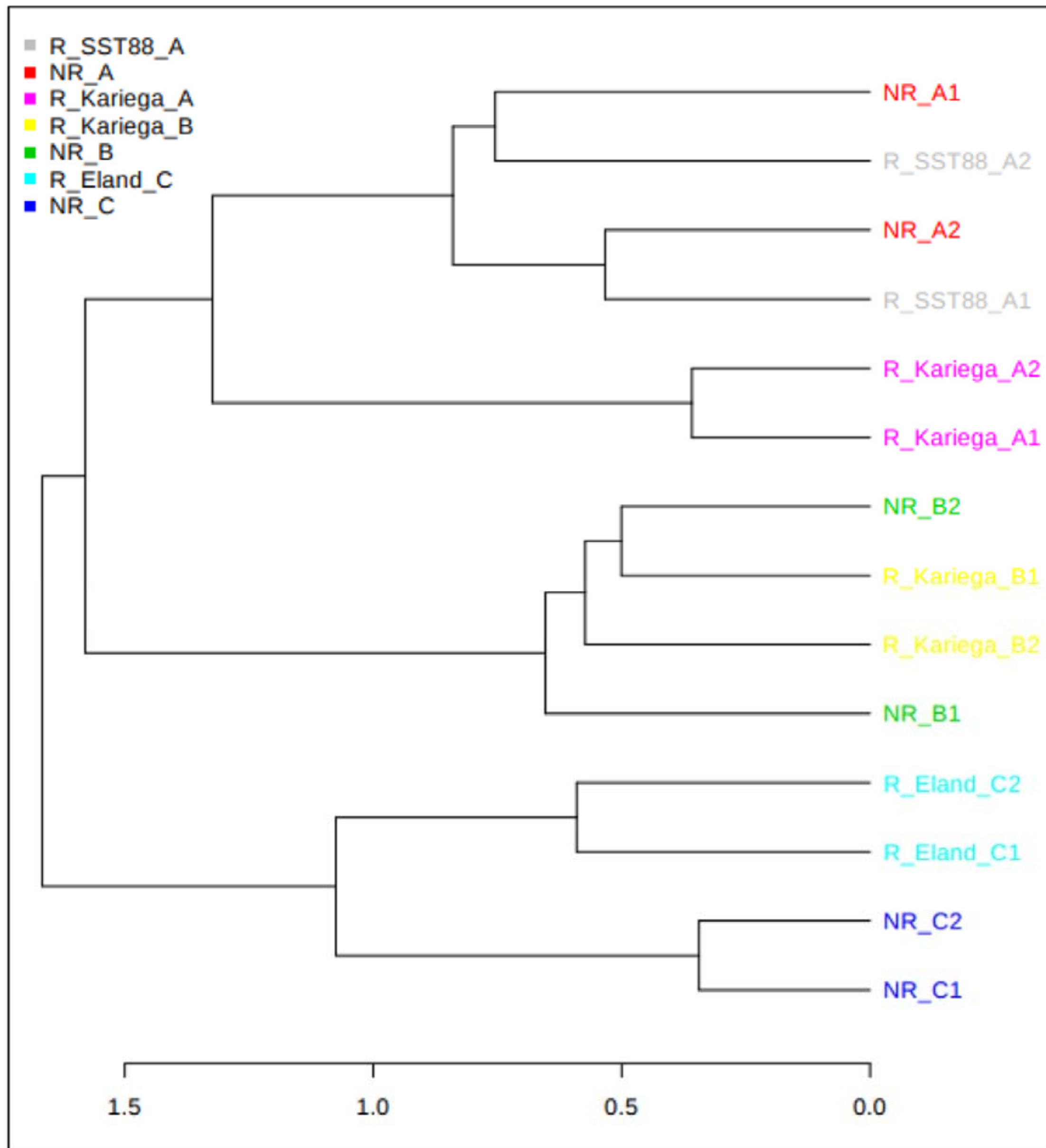

Fig. 3. Dendrogram from hierarchical cluster analysis (using Bray- Curtis Index) of fungal composition at OUT level for rhizosphere and non- rhizosphere soil of wheat. NR_A, Non- rhizosphere soil at Site A; NR_B, Non- rhizosphere soil at Site B; NR_C, Non- rhizosphere soil at Site C; R_Eland_C, Eland rhizosphere soil at Site C; R_Kariega_A, Kariega rhizosphere soil at Site A; R_Kariega_B, Kariega rhizosphere soil at Site B; R_SST88_A, SST88 rhizosphere soil at Site A.

3.2. *Relative abundance*

In the rhizosphere soils, the phylum Ascomycota (41.6- 60.4%) was the most abundant irrespective of sampling site. The second most dominant phylum was Basidiomycota (10.7-25.4%) (Figure 4). Although the fungal composition was similar between the different cultivars, their relative abundance was different. The abundance of the Ascomycota phylum in the rhizosphere soils in different sites were as follows, cv. Kariega at Site A- 41.6%), cv. SST88 at Site A- 60.4%, cv. Kariega at Site B- 54.5% and cv. Eland at Site C- 47.1% while that of the Basidiomycota phylum were cv. Kariega Site A- 25.4%, cv. SST88 at Site A- 21.7% and cv. Eland at Site C- 25.5%.

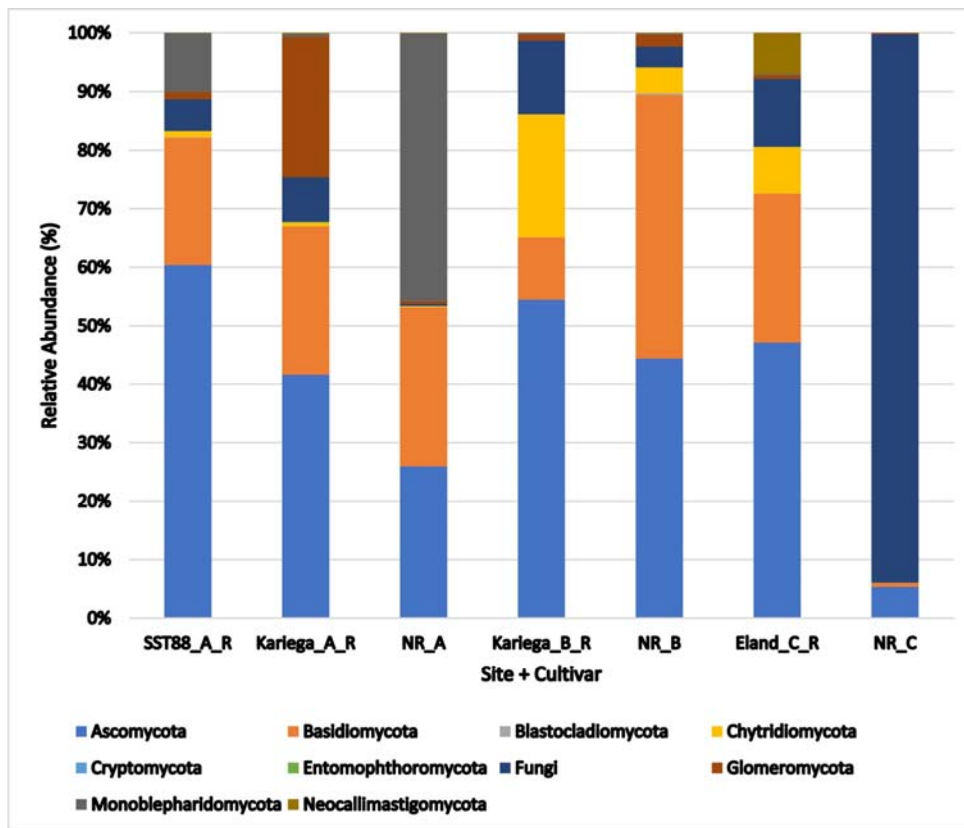

Fig. 4. Overall relative abundance of fungal phyla of wheat on cv. SST88 and Kariega rhizosphere (R), and non-rhizosphere (NR) soil at Site A, Kariega rhizosphere (R) and non- rhizosphere soil at site B and Eland rhizosphere and non-rhizosphere soil at Site C. NR_A, Non- rhizosphere soil at Site A; NR_B, Non- rhizosphere soil at Site B; NR_C, Non- rhizosphere soil at Site C; Eland_C_R, Eland rhizosphere soil at Site C; Kariega_A_R, Kariega rhizosphere soil at Site A; Kariega_B_R, Kariega rhizosphere soil at Site B; SST88_A_R, SST88 rhizosphere soil at Site A.

Across all sites the rhizosphere soils, the Ascomycota phylum was generally dominated by fungal member classes of Sordariomycestes, Dothideomycetes, Eurotiomycetes and Orbiliomycetes with *Fusarium*, *Aureobasidium* and *Colletotrichum* being the most dominant genera. Basidiomycota were dominated by member classes Agaricomycetes and Tremellomycetes. Similar to the rhizosphere, fungi belonging to the phyla Ascomycota (Site A- 25.5%, Site B- 44.5% and Site C- 5.3%) and Basidiomycota (Site A- 22.3%, Site B- 45.1% and Site C- 0.7% were observed in higher relative abundances in the non-rhizosphere soil. Meanwhile, Monoblepharidomycota was more dominant in the non-rhizosphere soil of Site A (45.6%). And the unclassified fungi, mainly from the Mucoromycotina class dominated Site C (93.7%) soil.

Significant ($p<0.05$) differences in the relative abundance of specific fungal classes were observed between the rhizosphere of cv. Kariega and that of cv. SST88 at Site A (supplementary Figure 1). For example, Pezizomycetes, Sordariomycetes, Tremellomycetes, Mucoromycotina and Monoblepharidomycetes were 4.8, 3, 2.4, 3.9 and 22 times higher in cv. SST88 than in cv. Kariega at Site A, respectively. While in Glomeromycetes, Mortierellomycotina and Leotiomycetes the relative abundance differences were 19, 9.11 and 3 times higher in cv. Kariega than in cv. SST88 at Site. A more pronounced variation of the two cultivars was observed at family level. The pair wise comparisons showed that, the Acaulosporaceae family was significantly ($p<0.05$) higher in the rhizosphere of cv. Kariega (23%) compared to that of cv. SST88 (0.35%) at Site A. However, the abundance of the Nectriaceae family was significantly ($p<0.05$) higher in the cv. SST88 rhizosphere (22.5%) compared to the cv. Kariega (3.5%) rhizosphere.

3.3. Pathogenic and beneficial fungal general

We further assessed the abundance of fungal genera pathogenic (*Colletotrichum, Cladosporium, Fusarium, Aspergillus*, *Alternaria*, *Cateractispora*, *Penicillium*, *Sclerotinia*, and *Phoma*) and beneficial (*Aureobasidium, Trichoderma and Acaulospora)* to wheat at the three sites. Amongst the pathogenic fungi, the *Fusarium* genus (0.92%-21,1%) was the most abundant across all the sites. Overall, no significant ($p>0.05$) changes were observed in the abundance of *Fusarium*, *Colletotrichum*, *Cladosporium*, *Aspergillus* and *Cateractispora* from the non-rhizosphere to the rhizosphere soils. Meanwhile, a significant ($p<0.05$) increase was observed in the abundance of *Aureobasidium* from the non-rhizosphere to the rhizosphere soil. The pairwise comparisons showed significantly ($p<0.05$) higher abundances of *Fusarium* (21.1%) in the rhizosphere of cv. SST88 compared to non-rhizosphere soil at Site A. In contrast, the abundances of *Fusarium* decreased significantly ($p<0.05$) from the non-rhizosphere soil to the rhizosphere of cv. Kariega at the same site. As a general observation, the abundance of *Fusarium* was inversely proportional to that of *Aureobasidium*. The *Colletotrichum* genus was present at abundances above 3% in both the rhizosphere of cv. Kariega and non-rhizosphere soils at Site B while, in the other two sites, the presence of *Colletotrichum* was almost negligible (<0.5%). The *Cateractispora*, *Penicillium*, *Sclerotinia*, and *Phoma* genera were significantly ($p<0.05$) enriched in the rhizosphere of cv. Kariega at Site A when using the pairwise comparisons. The opposite was noticed in the rhizosphere of cv. SST88 at the same site. *Acaulospora* was noticed to be enriched in the rhizosphere of most of the sampled soils except for the rhizosphere of cv. Eland at Site C, where it decreased substantially.

Table 1. Pathogenic and beneficial fungal genera that were affected by wheat rhizosphere at three sites in South Africa. "+" denotes fungi with high relative abundance in rhizosphere soil than in non- rhizosphere soil. "-" denotes fungi with lower relative abundance in rhizosphere than in non- rhizosphere soil. "x" denotes no changes in relative abundance between rhizosphere and non- rhizosphere soil. Wheat cultivars (SST88, Kariega and Eland); NR, Non- rhizosphere; R, Rhizosphere.

		Site A						Site B			Site C		
		SST88			Kariega			Kariega			Eland		
		NR	R		NR	R		NR	R		NR	R	
Pathogenic fungi	*Phoma*	0,125	0,06	-	0,125	0,14	+	0,61	0,655	+	0,06	0,345	+
	Alternaria	0,035	0,055	+	0,035	0,065	+	0,075	0,28	+	0,005	0,005	x
	Colletotrichum	0,05	0,13	+	0,05	0,04	-	3,29	3,61	+	0,015	0,185	+
	Alternaria	0,035	0,055	+	0,035	0,065	+	0,075	0,28	+	0,005	0,005	x
	Bipolaris	0	0	x	0	0	x	0,48	0,985	+	0	0,1	+
	Cladosporium	0,405	0,98	+	0,405	0,98	+	4,735	4,01	-	0,135	2,24	+
	Fusarium	10,41	21,085	+	10,41	3,28	-	1,97	2,785	+	0,915	1,545	+
	Ramulispora	0	0	x	0	0,01	+	0,015	0,645	+	0	0	x
	Paraphaeosphaeria	0,02	0,415	+	0,02	0	-	0,015	0,01	+	0	0,02	+
	Leptosphaeria	0,025	0,03	+	0,025	0	-	0	0,02	+	0	0,04	+
	Sclerotinia	0,03	0,01	-	0,03	0,04	+	0,035	0,045	+	0	0,09	+
	Paraphaeosphaeria	0,02	0,415	+	0,02	0	-	0,015	0,01	-	0	0,02	+
	Aspergillus	1,225	1,435	+	1,225	0,15	-	0,55	0,2	-	0,005	0,365	+
	Penicillium	0,75	0,79	-	0,75	2,24	+	0,505	0,685	+	0,19	3,525	+
	Cateractispora	0,79	0,135	-	0,79	1,525	+	0	0,035	+	0,01	0,005	-
Beneficial fungi	*Aureobasidium*	3,455	8,175	+	3,455	12,25	+	9,155	16,51	+	1,945	7,365	+
	Acaulospora	0,015	0,365	+	0,015	23,35	+	0,08	0,335	+	0,015	0,135	+
	Trichoderma	0,105	0,6	+	0,105	0,76	+	0,46	0,165	-	0,535	3,66	+

4. Discussion

The present study assessed microbial diversity and composition in wheat rhizosphere and non- rhizosphere soils from three different wheat growing sites (regions) through Illumina sequencing of the ITS1 and ITS2 regions. Special emphasis was placed on understanding the dynamics of pathogenic and beneficial fungal populations at the vegetative growth stage. At this stage, the root crown zone is developed enough to help with acquisition of nutrients and water thus determining the wheat's survival during winter (Cook, 1991). Furthermore, the invasion of cereal's crown and root tissues by soil-borne pathogens is more prevalent at this stage (Weisi, 1987; Zillinsky, 1983). In this study, diverse and significantly different fungal populations were detected in the rhizosphere of the different cultivars tested. Similar observations were made with non-rhizosphere soils in the three different growing sites. Microbial diversity and richness play a key role in the sustainable development of soil health, ecosystem function and crop production (Kennedy and Smith, 1995). Fungal genera comprising of major wheat root and crown rot pathogens as well as beneficial (disease control and facilitate nutrient availability) microbes were also detected. This information is valuable in the development of sustainable disease management strategies such as those that employ natural or artificially applied biological control agents (Chandrashekara et al., 2012).

Fungal diversity along with species richness (OUT numbers) declined from the non-rhizosphere to the rhizosphere soils across the three sites. Similar trends were previously observed in other plants such as Arabidopsis (Shakya et al., 2013), rice (Lundberg et al., 2012; cotton Qiao et al., 2018) and Populus (Edwards et al., 2015). This observation was attributed to differences in the microbial community composition between rhizosphere soils and the surrounding non-rhizosphere soils. Wang et al.

(2017) also reported higher fungal diversity in non-rhizosphere compared to rhizosphere soil in wheat during the flowering stage under conservative tillage farming systems. The narrowing of the fungal populations in the rhizosphere was mainly attributed to the selection pressure exerted by plant roots on the surrounding soil microbial community. The current study demonstrated the ability of wheat roots at the vegetative stage to enrich and promote the dominance of specific fungal groups including Ascomycota, Basidiomycota and unclassified fungi under the subdivision Mucoromycotina. Similar trends were also observed in previous studies by Lauber et al. (2008) and Nemergut et al. (2008). They reported that Ascomycota and Basidiomycota were the dominant fungi in vegetated soils. Fungi within the Ascomycota phylum grow quickly and become dominant during the early stages of plant growth because of their ability to use the carbon resources immediately released by roots into the soil (Hannula et al., 2012). Most of the plant pathogens are members of the Ascomycota phylum (Carris et al., 2012). On the other hand, Basidiomycota consists mainly of saprotrophic yeasts which are the primary agents of plant litter decomposition and their hyphal networks grow throughout the soil interface. They represent highly dynamic channels through which nutrients are readily distributed (Crowther et al., 2012). This trend was observed in all cultivars and in non-rhizosphere soil across all sites except for non-rhizosphere soil of Site C, where Mucoromycotina was mostly abundant.

Wheat cultivars differed in recruiting other fungal communities especially in different geographical settings which were characterised by different environmental conditions. For instance, the phyla Chytridiomycota did not differ significantly between the rhizospheres of cv. SST88 and cv. Kariega at Site A. However, the same phyla significantly increased in abundance in the rhizosphere of cv. Kariega at site B

compared to Site A. Additionally, different cultivars also showed enrichment of different fungal groups at the same site. For instance, cv. SST88 and cv. Kariega at Site A enriched different fungal groups namely Monoblepharidomycota and Glomeromycota, respectively. In the meantime, cv. Kariega at Site B enriched Chytridiomycota while Neocallimastigomycota and Chytridiomycota were enriched by cv. Eland at site C. A study done by Mommer et al. (2016) indicated that plants often select their rhizobiomes *via* root exudation. Furthermore, Wardle (2002); Wagg et al. (2014); Hannula et al. (2017) suggested that fungal populations are strongly influenced by plant genotype as they affect nutrient availability and cycling processes. Moreover, geographic locations were observed to be another factor affecting microbial populations ((Igiehon and Babalola, 2018; Philippot et al., 2013). Previous studies have reported that geographical location as well as crop management practices can affect soil microbial community composition and consequently the composition of rhizosphere microbial communities (Göre and Bucak, 2007; Sapkota et al., 2017; Soman et al., 2017).

In this study, differences in fungal communities between two wheat cultivars (cv. Kariega and cv. SST88) grown on the same site were noticed. Three fungal orders i.e. Hypocreales, Agaricales and Mortierellales were abundant in the rhizosphere of cv. SST88 while Lobulomycetales, Rhizophlyctidales and Agaricales were abundant in the rhizosphere of cv. Kariega at Site A. This was not surprising since host genotype is a major factor in determining the composition of root communities (Sapkota et al., 2015). This observation highlights the importance of investigating microbial diversity and community composition of different cultivars during the process of developing disease control strategies. Notably, fungal diversity in the rhizosphere was strongly influenced by cultivar in all the three sites. For instance, differences in rhizosphere

fungal diversity were observed between cultivars cv. SST88 and cv. Kariega at Site A. It has been reported that different genotypes of the same plant species when grown in the same soil are often associated with distinct microbial communities (Berendsen et al., 2012; Berg and Smalla, 2009). Studies on chickpea showed that different cultivars produced different arrays of root phytochemicals (Cruz et al., 2012; Ellouze et al., 2012) resulting in different soil fungal communities (Yang et al., 2012; Ellouze et al., 2013).

Further variations in fungal diversity were observed between rhizosphere and non-rhizosphere soils in the three sites. Essel et al. (2019) demonstrated diversity indices where fungal OTUs were less in the wheat rhizosphere compared to non-rhizosphere soil under different tillage and crop rotation systems. These diversity variations were well defined at Site C where the diversity of non-rhizosphere soil was 0.72 times higher that of the rhizosphere soil. The current study noted that lower fungal diversity in non-rhizosphere soils was associated with the enrichment of certain fungi groups. For example; Mucorales, classified under the Mucoromycotina subphylum was more abundant (93.7%) in non-rhizosphere soil compared to rhizosphere soil (11.52%) at Site C. As a result, Site C had the lowest fungal diversity on non-rhizosphere soil.

Certain pathogenic fungi belonging to the Ascomycota phylum, known to cause major disease in wheat were observed in this study. Plant pathogenic fungal genera such as *Phoma*, *Colletotrichum* and *Fusarium* were observed in high abundance across all the three sites. Soil- borne pathogens identified included; *Fusarium solani, F. oxysporum, F. equiseti, F. pseudograminearum, F. avenaceum, Fusarium sp, Phoma tropica, Phoma sp, Giberella zeae*. Pathogenic fungi from the *Fusarium spp* are reported to cause economically important diseases on wheat in South Africa (SA)

namely *Fusarium* crown rot, *Fusarium* Head blight, *Fusarium* wilt and *Fusarium* root rot (Lamprecht et al., 2006). *Fusarium* was most abundance in cv. SST88 Site A compared to the other cultivars. Furthermore, *Fusarium* was the most abundant pathogenic genus observed in all the rhizosphere and non- rhizosphere samples. This observation can be attributed to the production of wheat under dry-land farming systems in SA (DAFF, 2016) where the occurrence of *Fusarium* was previously reported to be the main cause of major diseases in wheat. Diseases caused by fungi in the genera *Bipolaris and Fusarium* are favored by dry soil conditions (Wegulo et al., 2013).

Fungal species known to have biological control of *Fusarium* pathogens were observed in conjunction with pathogens in this study. Fungal genera known to have a beneficial effect namely; *Aureobasidium*, *Acaulospora* and *Trichoderma* were observed in the current study. Han et al. (2016) and Berendsen et al. (2012) reported that *Aureobasidium* and *Trichoderma* represent genera that contain antagonistic species which could potentially be used in the suppression of plant pathogens and ultimately the control of soil-borne diseases in food crops. Previous studies on the efficacy of fungal biological control agents focused more on *Trichoderma,* a genus with species well known for their biological control properties (Gajera et al., 2013; Lecomte et al., 2016; Mukherjee et al., 2012). Although the *Trichoderma* genus was observed in this study in both rhizosphere and non- rhizosphere soils across the three sites, it was however, present in lower abundances of <1%. Interestingly, *Aureobasidium* another potential biological control group (Wachowska and Głowacka, 2014) was observed in higher abundances of between 1% and 16%.

Fungi belonging to the *Aureobasidium* genus are mostly saprotrophic and polymorphic fungi (Gniewosz and Duszkiewicz-Reinhard, 2008) and some of their

species can be used as biological control agents to protect wheat plants from pathogenic *Fusarium species* (Wachowska et al., 2013). *Aureobasidium pullulans* was reported to be commonly found on the surface or inside the tissue of cereal kernels where they suppress the growth of phytopathogens (Wachowska and Głowacka, 2014). In the present study, *A. pullulans* was the only species detected under the Aureobasidium genus and its abundance was observed to be inversely proportional to that of the *Fusarium* species in both the rhizosphere and non-rhizosphere soils. The relative abundance of *A. pullulans* was also observed to increasing from the non-rhizosphere to the rhizosphere soils across all the three sites.

Wachowska and Głowacka (2014) also observed an antagonistic effect of *A. pullulans* against *Fusarium culmorum* known to cause winter wheat spikes. Furthermore, Wachowska et al. (2015) demonstrated that *A. pullulans* could be used in an integrated approach to protect wheat grain from being colonized by pathogenic *Fusarium* species. The antagonistic effect of *A. pullulans* was reported to be related to production of extracellular enzymes as well as grain phytochemicals (Castoria et al., 2001). Root pathogens such as *Fusarium* species are highly efficient colonizers of the host plant's rhizosphere and rhizoplane thus, having the potential to significantly influence populations of other rhizosphere colonizers including microorganisms that can help in the biological control of other root pathogens.

Other beneficial groups observed in this study included fungi belonging to the Glomeromycota genus. *Acaulospora*, a member of the Glomeromycota genus was detected in high abundances (24.0%) in the rhizosphere of cv. Kariega at Site A. On the other note, the same fungi were detected in low frequencies (>2%) in cv. SST88 at the same site as well as in other cultivars from other sites. The low abundance of *Acaulospora* has also been reported previously (Cui et al., 2018). A study by Singh

and Adholeya (2013) revealed lower abundance of arbuscular mycorrhizas (AM) fungi in agricultural fields. The Glomeromycota family comprises of arbuscular mycorrhizas which are characterized by the formation of unique structures, arbuscules and vesicles (Brundrett, 2002). Fungi of the *Glomeromycota* family have been previously reported to be associated with wheat roots (Cui et al., 2018). Khaitov and Teshaev (2015) suggested that soil properties are an important factor influencing the AM root colonization and they further reported the colonization of the rhizosphere by *Glomeromycota* in the two cotton varieties tested. The AM fungi is of agricultural importance as it helps plants to capture soil nutrients such as phosphorus, sulfur and nitrogen as well as other important micronutrients including magnesium, iron and manganese (Brundrett, 2002). The AM fungal species distribution was reported to be strongly dependent on the location (Castillo et al., 2016) and these findings agree with the findings of the current study.

In conclusion, the current study indicated that the soil fungal diversity and composition of wheat rhizosphere and non-rhizosphere soil differs from one production region to the other. Wheat cultivar also affect fungal composition in the rhizosphere. The rhizosphere soils were abundant of pathogenic populations compared to the non-rhizosphere soil. Important wheat known fungal pathogenic genera detected in both the rhizosphere and non-rhizosphere soils included *Fusarium*, *Phoma*, *Colletotrichum* and *Paraphaeosphaeria* and their presence was inversely proportional to *Aureobasidium*, *Acaulospora* and *Trichoderma* which are deemed as beneficial fungi in wheat production. The findings of the current study can serve as a baseline study for future development of fungal based antagonistics on soil health, plants growth and biocontrol research and gives an outline on the pathogenic fugal populations occurring in the major wheat production areas of SA.

Author Contributions

All authors were involved in the planning of the experiment and statistical design. M.P.G, M.B and N.S did the soil sampling and DNA extraction. Sequencing was done at MR DNA facility. Statistical analysis, data analysis, structuring and interpretation of the paper was done by M.P.G, M.B and N.S. More interpretation and editing was done by M.B, N.S and L.K. L.K. and N.L supervised the project and corrected the final version of the manuscript. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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