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Fungal community structure variability between the root rhizosphere and endosphere in a granite catena system in Kruger National Park, South Africa

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Fungi colonise various substrates such as organic matter (dead or alive) from plants or animals. These fungi can be specialists (i.e. belonging to a substrate) or generalists (i.e. surviving on different types of organisms). Fungi fulfil various functions in specialised niches, for example, acting as plant pathogens, helping in plant growth from the root systems or decomposing organic matter and fertilising soil. Species are specialised to occur in only one niche, or others can utilise or occur in various niches. For example, certain species occur only within certain plant tissues (endophytes), on the exterior surface of the plants growing above the ground (epiphytes) or below the ground in the sphere surrounding the roots (rhizosphere). Different soil types or conditions can favour certain species. This study used environmental sequencing to characterise the fungal communities associated with the root exterior and interior of *Sida cordifolia*, a plant growing across the varying soil conditions of the catena system. Fungal rhizosphere communities between three commonly occurring plant species – *S. cordifolia, Melhania acuminata* (both Malvaceae) and *Kyphocarpa angustifolia* (Amaranthaceae) – in one of the soil types were also studied to compare and contrast the fungal rhizosphere communities of these herbs. Molecular Operational Taxonomic Units co-occurred between niches, soil conditions and the rhizospheres of three plants at the same location, whilst others were restricted to only one niche or plant species. Results showed that soil conditions in a catena can influence the associations of fungal species between different catena zones, on the outside and inside of the roots, and that these communities also differ between plant species.

Conservation implications: This study showed that complex and sensitive fungal communities are associated with plant roots in different zones of the catena. This is most likely also true between different habitats and soil types on a larger scale. This study emphasises the need to also manage a catena system on the micro-ecological scale whilst framing conservation and management plans of the Kruger National Park.

Keywords: Fungi; Sodic soil; Grazing lawn; Rhizosphere; Root endophytes; Catena.

Introduction

Soils harbour a great diversity of fungal species that have various ecological functions (Bridge & Spooner 2001; Havlicek & Mitchell 2014). Saprophytic fungi break down dead organic matter and, in turn, fertilise the soil (Setala & McLean 2004). Certain plant fungal pathogens are specifically adapted to infect plants through roots and to spread or survive in soils, while some pathogens affecting tissues of plants growing above the soil also have the ability to survive in soils. Propagules of more specialised below-ground fungi, such as mycorrhiza that form specialised root associations benefitting plant health, can also be found in surrounding soils (Moore, Robson & Trinci 2011). Similarly, propagules of various fungi occurring in different niches and substrates above ground can also be found in soils (Aylor 2003; Taylor & Bruns 1999).

Soil conditions can permit specific fungal species to grow and produce fruiting structures. Soil qualities such as pH and nutrient concentrations, soil types, temperature and humidity may influence optimal growth conditions for species (Rousk et al. 2010). Furthermore, types of vegetation in a particular area such as grasslands and forests, which harbour their own unique fungal communities, serve as inoculum sources for associated soils (Talbot et al. 2014).

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Similarly, propagules of coprophilous fungi (those closely associated with animal dung) can also be isolated from soil (Richardson 2001).

Fungi occurring asymptomatically within plant tissues are named endophytes and they play diverse roles (Rodriguez et al. 2009). These vary from highly symbiotic relationships, for example, conferring traits such as herbivore resistance to their hosts or boosting growth, to detrimental as is the case with latent plant pathogens. Other fungi do not have any specific known function, or simply have the ability to overcome plant resistance and infect the tissues (Rodriguez et al. 2009).

Endophyte communities can differ between plant tissues; for instance, some endophytes are better specialised to infect leaves (Khare, Mishra & Arora 2018). Roots also often harbour very specialised endophytes that have functions beneficial to their host (Sabra et al. 2018). Root endophytes can include fungi that infect through aerial tissues or from surrounding soils and often play beneficial roles for plant health (Glynou et al. 2016; Rodriguez et al. 2009). However, certain fungi have the ability to occur across various plant tissues.

Plant pathogens have varying biological compositions (Agrios 2005). As already mentioned, latent pathogens can reside inside plant tissues without causing disease symptoms until triggered by specific stimuli such as stress conditions (Slippers et al. 2007). Other pathogens do not follow this strategy and can only cause disease symptoms. Certain pathogens are also adapted to only infect aerial tissues or below-ground tissues such as roots. However, survival strategies of pathogens may enable them to survive in other substrates such as soil or water (Hargreaves et al. 2015; Havlicek & Mitchell 2014; Lareen, Burton & Schafer 2016).

The rhizosphere is an interface consisting of a zone of soil that is associated with the roots (Hiltner 1904). Often this area contains exudates of the plant, which select for specific fungal communities to be present (Lareen et al. 2016; Sasse, Martinoia & Northen 2018). This area has an important function where fungi, as well as other microbial organisms, can facilitate the movement of nutrients (Mommer, Kirkegaard & Van Ruijven 2016; Schippers, Bakker & Bakker 1987). Other functions include protection against organisms feeding on roots, or plant pathogens (Moore et al. 2011).

Grazing lawns are unique ecological phenomena (McNaughton 1984). Areas of grazing lawns contain natural vegetation significantly different from the surrounding area. The vegetation has a high nutritional value and productivity, attracting animals that in turn fertilise the soil again (Verweij et al. 2006). It is also known that these areas are self-sustaining although the mechanisms behind this phenomenon are still unknown (Archibald 2007; Hempson et al. 2015). Grazing lawns can form part of a catena system, such as the one forming the topic of this special issue. A catena consists of a serial soil group sequence from foothills to the crest, and that originated from the same geological material (Brady & Weil 2002).

The Kruger National Park (KNP) is one of the largest game reserves in Africa (Carruthers 2017). It covers the north-eastern part of South Africa and now forms part of the Great Limpopo Transfrontier Park, linking it with the Gonarezhou National Park in Zimbabwe, and the Limpopo National Park in Mozambique. Recently, four research 'supersites' have been identified and established in KNP, with each of these supersites representing catenas with distinct geological, ecological and climatic features (Rughöft et al. 2016; Smit et al. 2013). Such a site represents an ideal opportunity to determine if fungal communities will be uniform over niches and soil conditions within the system, or if the catena will harbour a complex and differing fungal community.

The study focuses on characterisation of the fungal communities from the root ecto- and endo-environments of one plant species which occurred within the different soils and soil conditions of the grazing lawn zone and the neighbouring hillside zone of the catena (Theron, Van Aardt & Du Preez 2020; Vermeulen, Casson & Swart 2020). Whether different plants in the catena will have similar specialised rhizosphere fungal communities was also investigated, as it would add to the complexity to properly conserve and manage the catena site should variations be observed.

Materials and methods

Study site and sampling

The study was conducted at the Southern Granite Supersite catena close to the Stevenson-Hamilton Memorial (Smit et al. 2013) in April 2017 (autumn), during which time it still rained frequently. *Sida cordifolia* (Malvacae, Malvales) or Flannel Weed is an invasive herbaceous plant (Jain et al. 2011) and grows across the grazing lawn zone (Sterkspruit soil type, high clay content, mean sodium concentration 3802 mg/kg, pH mean 6.4) and hillside zone (Clovelly soil type, high sand percentage [90%], mean sodium concentration 1062 mg/kg, pH mean 5.85) of the catena (Sandoval-Denis, Swart & Crous 2019; Theron et al. 2020; Vermeulen et al. 2020). This plant was selected for sampling for rhizosphere and endophytic fungal communities from the two zones (Vermeulen et al. 2020). Two additional native herbaceous species, namely, *Melhania acuminata* (also Malvaceae) and *Kyphocarpa angustifolia* (Amaranthaceae, Caryophyllales) were collected from the hillside zone for rhizosphere comparisons (Vermeulen et al. 2020). Ten plants from the grazing lawn and 10 plants from the hillside zones were excavated for each plant species, respectively. The plants occurred randomly throughout the two sites. The plant and soil samples were transferred aseptically to paper bags, and kept cold while being transported to the laboratory.

Illumina sequencing and analysis

The plant roots were submerged in sterile water and shaken to dislodge soil from the rhizosphere that was attached to the root surfaces. Consequently, the roots were surface sterilised in a standard sodium hypochlorite (3%), ethanol (70%) and sterile distilled water series. The roots were cut into small

pieces, freeze dried and pulverised with 2-mm-diameter metal beads in a Qiagen TissueLyser II cell disrupter (Qiagen, Germantown, USA). The soil suspension was dried so that only the soils remained, and were subsequently also lysed. For each sample, 0.1 g of the pulverised plant and soil samples were used. The genomic Deoxynuceic Acid (DNA) from the soil suspension and sterile root samples was extracted by using the Soil and Plant II Nucleospin® Kits (MACHERY-NAGEL GmbH and Co KG, Duren, Germany), respectively, as per the user manual. The DNA concentrations, determined by using a Nanodrop LITE spectrophotometer (Thermo Scientific, USA), were all adjusted to 10 ng/µL using sterile water. The Internal Transcribed Spacer 2 (ITS2) region was targeted for the Illumina sequencing study using primers ITS3 and ITS4 fitted with Illumina adapters following the protocol described in Tonjock et al. (2019). Generated amplicons were pooled on the basis of plant, zone, rhizosphere or root endophyte categories, normalised and sequenced using an Illumina MiSeq (Illumina, USA) based on the procedure described by Tonjock et al. (2019), using MiSeq v3 reagents and as paired 300 bp reads, at the Next Generation Sequencing Facility at the Department of Health Sciences, University of the Free State, South Africa.

Sequences were run through a quality control pipeline as described by Tonjock et al. (2019) with the final step being taxonomic assignment against the UNITE database at 99% similarity (Nilsson et al. 2011) and did not differ from results obtained at 97% similarity. In this pipeline, Chimeric sequences were identified, using usearch 6.1.544 (Edgar 2010) as the chimera detection method (Edgar 2010), and filtered out of the quality trimmed reads by using identify_chimeric_seqs.py and filter_fasta.py commands, respectively. Taxonomic assignments were verified according to current taxonomic classifications. Molecular Operational Taxonomic Unit (MOTU) tables were normalised. Prior to the analysis, the OTU-table was normalised using normalize_table.py in QIIME with the CSS normalisation option (Paulson et al. 2013) and all analyses were carried out using R (www.r-project). Shared and unique MOTUs between communities were plotted using the venn function in gplots (https://CRAN.R-project.org/package=gplots). The DNA reads were deposited in Genbank under the bioproject accession no. PRJNA624016 (accession numbers: *S. cordifolia* roots grazing lawn SAMN14564480, *S. cordifolia* roots slope SAMN14564481, *S. cordifolia* rhizosphere grazing lawn SAMN14564484, *S. cordifolia* rhizosphere slope SAMN14564483, *M. acuminata* rhizosphere slope SAMN14564482, *K. angustifolia* rhizosphere slope SAMN14564485).

Ethical considerations

Ethical approval for the multidisciplinary project as a whole was obtained from the Interfaculty Animal Ethics Committee at the University of the Free State (UFS-AED2019/0121). SANParks permit numbers for collection of soil for lab analyses and vegetation for identification purposes are, respectively, SK069, SK2095 and SK054.

Results

Illumina sequencing and analysis

After quality control analyses, a substantial number of reads remained (Table 1). The majority of the MOTUs that could be assigned with names in the pipeline (Table 2, Figures 1–6) belonged to Ascomycetes (41 of 54), while the remainder 12 MOTUs belonged to Basidiomycetes and one to Zygomycete. No other types of fungi were detected by using the particular experimental design, DNA extraction protocol and Polymerase Chain Reaction (PCR) setup. A large percentage of MOTUs that could not be named by the pipeline were detected. The Ascomycete and Basidiomycete groups included diverse orders. The number of named MOTUs per niche (namely, endophytes vs. rhizosphere communities of *S. cordifolia* in two zones, and rhizosphere communities of different plant species in one zone) varied between 16 and 26, with the lowest number being that of the grazing lawn endophytes of *S. cordifolia* and the highest being that of the rhizosphere community of *K. angustifolia*.

Four MOTUs occurred in all of the niches or were present in both types of soils, but showed interesting shifts in frequency (Table 2, Figures 1, 2, 4 and 5). An MOTU with the assigned name of *Alternaria* was dominant in all of the rhizosphere communities, but was present only in low numbers as a root endophyte. Similarly, *Fusarium* was present with frequency >1% as an endophyte and in all of the rhizosphere samples, but shifted to a lower presence in the *S. cordifolia* rhizosphere in the slope vegetation. *Rhodotorula* was always present in frequencies higher than 1% as an endophyte in *S. cordifolia* in both soils, but had low frequencies in the rhizospheres of all plants. Interestingly, in *S. cordifolia* rhizosphere samples, it was present as a root endophyte in the slope but not present in the slope rhizosphere. The only MOTU present across all niches and soils, but in low numbers, was one presented as *Microdiplodia*.

For the rhizosphere communities, there was a high degree of MOTU overlap, and also numbers of unique MOTUs (Table 2, Figures 1–6). Fifty MOTUs were shared between rhizosphere soils of *S. cordifolia* in the grazing lawn and slope conditions, while there were 57 unique MOTUs in the sodic grazing lawn site, and 63 in the non-sodic slope site (Figure 3). For the comparisons between the rhizospheres of the three plant

TABLE 1: Summary of number of reads before and after quality control analyses.

Samples	Number of reads before QC	Number of reads after QC
Sida cordifolia sodic roots grazing lawn	51 033	30 997
Sida cordifolia roots non-sodic slope	52 237	34 715
Melhania acuminata rhizosphere non-sodic slope	599 941	148 777
Sida cordifolia rhizosphere non-sodic slope	332 552	175 961
Sida cordifolia rhizosphere sodic grazing lawn	243 299	108 389
Kyphocarpa angustifolia non-sodic slope	362 745	186 865

QC, quality control.

TABLE 2: Summary of the most prominent Molecular Operational Taxonomic Units that were assigned names from the rhizospheres and roots of three plant species in two zones of the catena.

Molecular Operational Taxonomic Units	Slope	Grazing lawn	Grazing lawn	Slope	Slope	Slope
	Endophyte	Endophyte	*Rhizosphere*	*Rhizosphere*	*Rhizosphere*	*Rhizosphere*
	Sida cordifolia	*Sida cordifolia*	*Sida cordifolia*	*Sida cordifolia*	*Kyphocarpa angustifolia*	*Melhania acuminata*
Amphisphaeriales, Pestalotiopsidaceae, '*Pestalotiopsis*'	**2**	0.6	-	-	0.02	-
Botryosphaeriales, Botryosphaeriaceae, '*Botryosphaeria*'	***57***	-	0.03	-	-	0.01
Botryosphaeriales, Botryosphaeriaceae, '*Diplodia*'	0.13	-	-	-	-	-
Botryosphaeriales, Phyllostictaceae, '*Guignardia*'	-	-	-	0.02	-	-
Botryosphaeriales, Botryosphaeriaceae, '*Lasiodiplodia*'	**9**	-	-	-	-	0.014
Botryosphaeriales, Botryosphaeriaceae, '*Microdiplodia*'	0.07	0.05	0.04	0.09	0.09	0.04
Botryosphaeriales, Botryosphaeriaceae, '*Sphaeropsis*'	-	-	-	-	0.18	-
Cantharellales, Ceratobasidiaceae, '*Ceratobasidium*'	-	-	-	0.1	-	-
Cantharellales, Ceratobasidiaceae, '*Rhizoctonia*'	-	-	0.014	-	-	-
Capnodiales, Mycosphaerellaceae, '*Mycosphaerella*'	-	-	-	-	0.6	-
Capnodiales, Teratosphaeriaceae, '*Teratosphaeria*'	-	0.19	-	-	0.03	-
Chaetothyriales, Herpotrichiellaceae, '*Exophiala*'	0.04	**1.1**	-	0.02	-	-
Diaporthales, Diaporthaceae, '*Diaporthe*'	0.08	-	-	-	-	-
Diaporthales, Diaporthaceae, '*Phomopsis'*	0.05	-	-	-	-	0.013
Dothideales, Dothioraceae, '*Aureobasidium'*	0.11	0.12	-	-	0.013	0.012
Erysiphales, Erysiphaceae, '*Erysiphe'*	-	-	0.014	-	-	-
Eurotiales, Aspergillaceae, '*Paecilomyces'*	-	-	-	-	0.05	-
Eurotiales, Aspergillaceae, '*Penicillium*'	-	-	0.018	0.06	0.06	-
Hypocreales, Nectriaceae, '*Fusarium*'	**4**	**19**	**9**	0.07	**3**	**9**
Hypocreales, Nectriaceae, '*Gibberella*' (now *Fusarium*)	-	0.014	0.014	-	-	0.1
Hypocreales, Nectriaceae, '*Haematonectria*' (now *Fusarium*/*Neocosmospora*)	-	-	-	-	-	-
Incertae sedis, '*Coniosporium'*	-	-	0.014	-	-	-
Medeolariales, Dermateaceae, '*Dermea'*	-	-	-	-	0.4	0.7
Myrmecridiales, Incertae sedis, '*Myrmecridium*'	-	0.05	-	-	-	-
Onygenales, Ajellomycetaceae, '*Spiromastix'*	-	-	-	-	**2**	-
Pleosporales, Pleosporaceae, '*Alternaria*'	0.09	0.02	***76***	***86***	***71***	***46***
Pleosporales, Corynesporascaceae, '*Corynespora*'	-	-	-	0.04	-	-
Pleosporales, Pleosporaceae, '*Curvularia*'	0.13	0.07	-	-	-	-
Pleosporales, Didymellaceae, '*Epicoccum*'	-	0.07	-	-	-	-
Pleosporales, inc. sed., '*Fusculina*'	0.78	0.02	-	-	-	-
Pleosporales, Lophiostomataceae, '*Lophiostoma*'	0.15	-	-	-	0.015	-
Pleosporales, Didymellaceae, '*Phoma*'	0.06	0.3	-	-	-	-
Pleosporales, Sporormiaceae, '*Preussia*'	-	-	-	-	0.02	0.02
Pleosporales, Acrocalymmaceae, '*Rhizopycnis*'	0.01	***29***	0.04	0.03	-	-
Saccharomycetales, inc. sed., '*Candida*'	-	-	-	0.05	0.03	0.1
Saccharomycetales, Saccharomycodaceae, '*Hanseniaspora*'	-	-	0.03	-	-	0.1
Saccharomycetales, Saccharomycetaceae, '*Lodderomyces*'	-	-	-	0.02	0.03	0.1
Sordariales, Chaetomiaceae, '*Chaetomium'*	0.07	-	0.014	0.02	**1.5**	-
Sordariales, Chaetomiaceae, '*Humicola*'	**4.5**	**6**	0.03	-	-	-
Togniniales, Togniniaceae, '*Phaeoacremonium*'	0.13	0.16	-	-	-	-
Agaricales, Agaricaceae, '*Agaricus*'	-	-	0.03	0.04	0.03	0.02
Agaricales, Amanitaceae, '*Amanita*'	-	-	0.09	0.2	0.04	0.05
Agaricales, Agaricaceae, '*Leucoagaricus*'	-	-	-	-	-	0.2
Agaricales, Hymenogastraceae, '*Phaeocollybia*'	-	-	-	-	-	0.1
Boletales, Boletacceae, '*Boletus*'	-	-	**11**	**10**	**12**	**8**
Cystobasidiales, Cystobasidiaceae, '*Occultifur*'	-	-	-	-	0.05	0.02
Polyporales, Ganodermataceae, '*Ganoderma*'	-	-	-	0.02	0.05	0.8
Polyporales, Polyporaceae, '*Neolenthinus*'	-	-	0.1	-	-	-
Sporidiobolales, Incertae sedis, '*Rhodotorula*'	**3**	***23***	0.16	-	0.13	0.013
Sporidiobolales, Sporobolomycetaceae, '*Sporobolomyces*'	-	-	-	-	0.02	0.7
Tremellales, Tremellaceae, '*Cryptococcus*'	-	-	-	-	0.13	0.1
Tremellales, Tremellaceae, '*Dioszegia*'	-	-	-	0.3	-	-
Mucorales, Cunninghamellaceae, '*Gongronella*'	-	-	-	-	0.4	-
Motu Totals	**20**	**16**	**17**	**18**	***26***	***23***

MOTU, Molecular Operational Taxonomic Unit.

Molecular Operational Taxonomic Units were arranged alphabetically according to family and within families. Numbers in bold are higher than 1, whilst numbers in bold and italics are higher than 20.

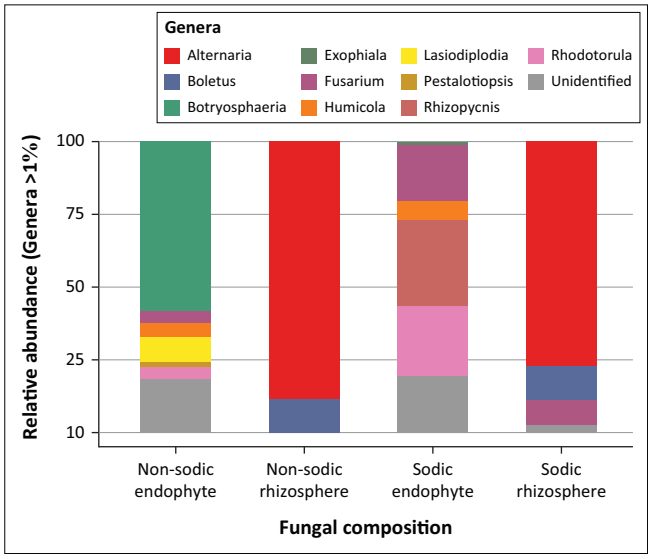


FIGURE 1: Percentage relative abundance of fungi (up to genus level) with a frequency higher than 1% at 97% level of sequence similarity. Results were obtained for endophytes and rhizosphere fungi *Sida cordifolia* growing in the grazing lawn and slope zones, from the grazing lawn and slope zones, respectively.

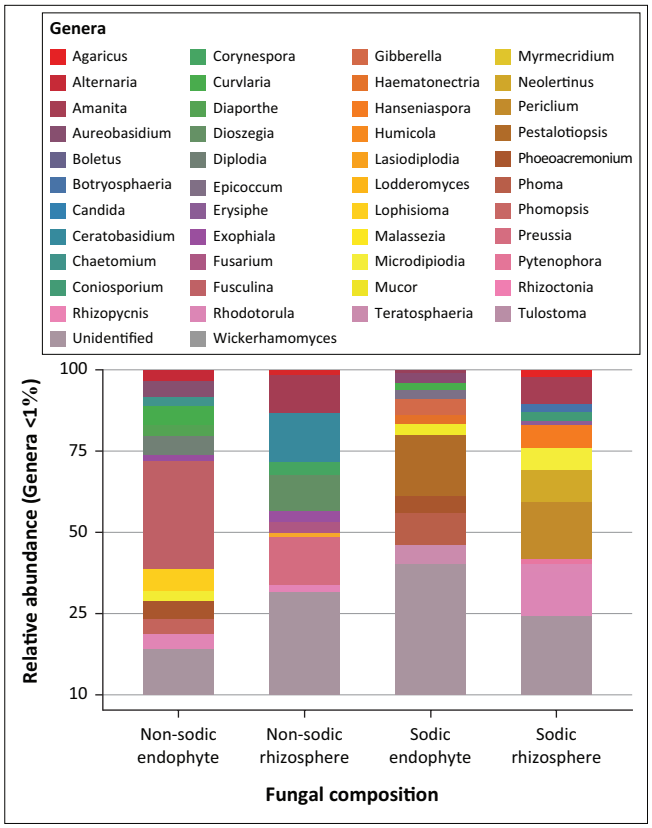
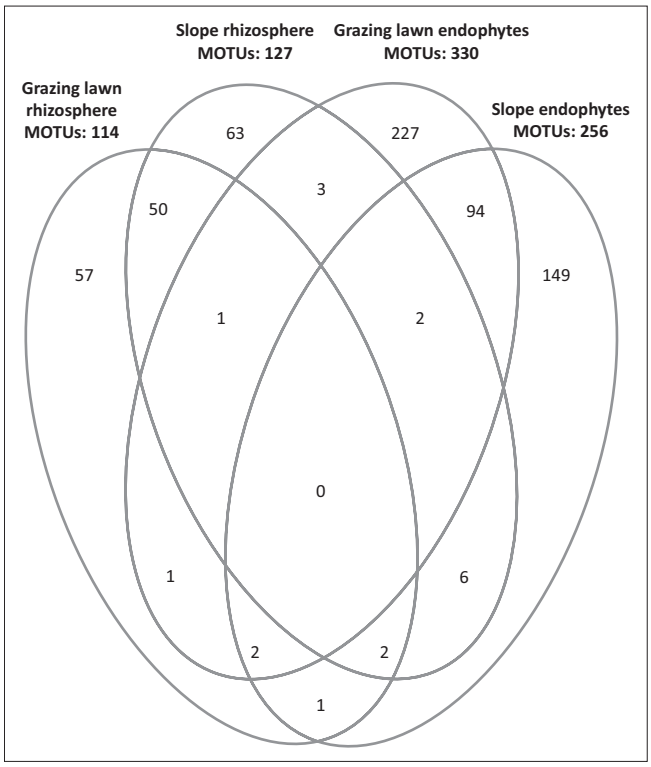


FIGURE 2: Percentage relative abundance of fungi (up to genus level) with a frequency lower than 1% at 97% level of sequence similarity. Results were obtained for endophytes and rhizosphere fungi from *Sida cordifolia* growing in the grazing lawn and slope zones, respectively.

species in the slope site, 144 MOTUs were shared between all, while the native *M. acuminata* and *K. angustifolia* plants had 183 and 178 MOTUs, respectively; unique MOTUs were compared to the 63 unique MOTUs of the invasive *S. cordifolia* (Figure 6). In total, *M. acuminata* had 482 MOTUs, *K. angustifolia* had 451 and *S. cordifolia* had 291 MOTUs.



MOTU, Molecular Operational Taxonomic Units.

FIGURE 3: Venn diagram of the fungal Molecular Operational Taxonomic Units obtained from the outside (rhizosphere) and inside (endophytes) of *Sida cordifolia* roots growing on the grazing lawn and slope zones, respectively.

When comparing the number of MOTUs of the *S. cordifolia* rhizospheres and root endophyte samples in the grazing lawn and slope sites (Figure 3), no MOTU was shared between all the rhizosphere and root samples. Very low numbers were shared between the rhizosphere and root collections from both sites. However, more MOTUs were shared within the rhizosphere samples for the two sites, as well as between the root samples, as previously stated. The rhizosphere samples had almost half of the number of MOTUs that the root samples had.

The most dominant MOTUs in the rhizosphere (>1% frequency) for all the plant species and in both zones (Table 2, Figures 2 and 4) were assigned as *Alternaria* and *Boletus*. *Fusarium* was also dominant in the rhizospheres of *K. angustifolia*, *M. acuminata* and for S. *cordifolia* in the grazing lawn zone, while it was less dominant in the hill slope zone (Table 2, Figures 2 and 4). *K. angustifolia* had the most diverse rhizosphere community. Molecular Operational Taxonomic Units (<1% frequency) that were shared included *Candida*, *Lodderomyces*, *Agaricus*, *Amanita* and the plant pathogenic genus *Ganoderma*. The remainder of the MOTUs occurred only in one plant species or zone, or were shared between species or the endophyte and rhizosphere niches.

Endophyte profiles from *S. cordifolia* roots were quite different from the rhizophere profiles of the same plant (Table 2, Figures 2 and 3). Profiles of the dominant MOTUs (>1%) included different fungi than those present in the rhizosphere,

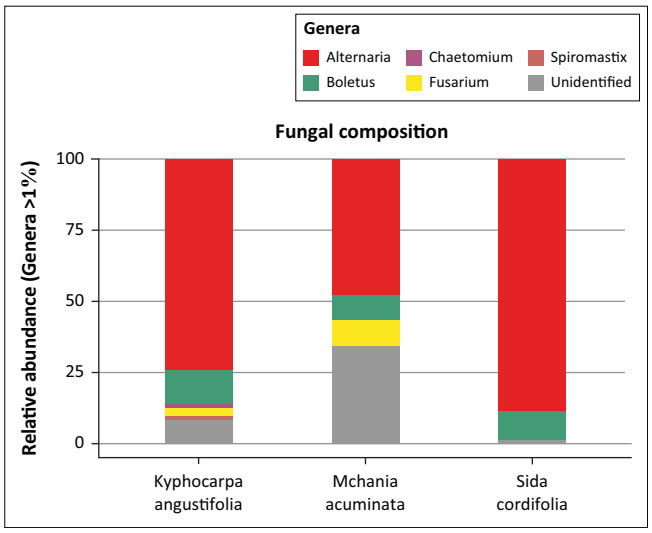


FIGURE 4: Percentage relative abundance of fungi (up to genus level) with a frequency higher than 1% at 97% level of sequence similarity. Results were obtained for rhizosphere fungi from three plant species growing in the slope zone.

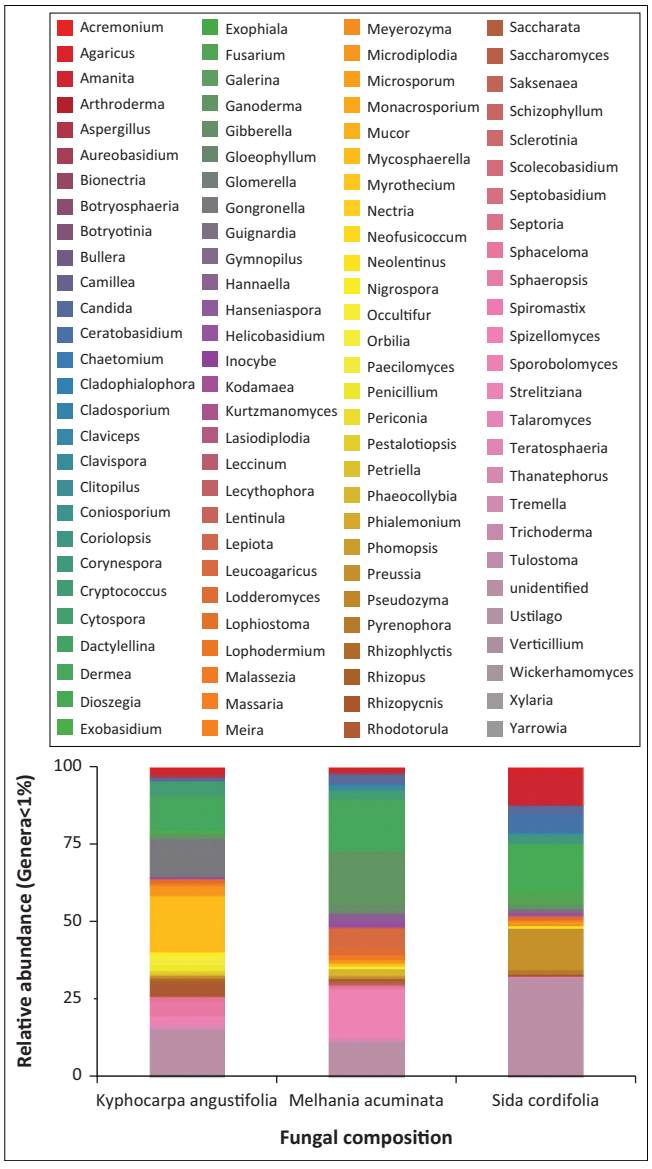
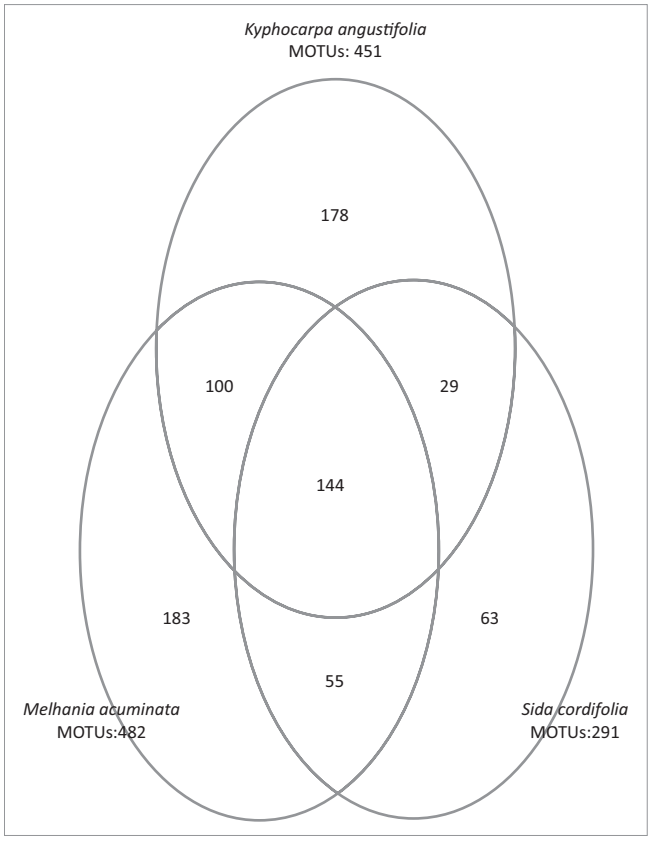


FIGURE 5: Percentage relative abundance of fungi (up to genus level) with a frequency lower than 1% at 97% level of sequence similarity. Results were obtained for rhizosphere fungi from three plant species growing in the slope zone.

while the endophyte patterns between the grazing lawn and slope vegetation zones were also different. However, MOTUs assigned as *Humicola*, *Fusarium* and *Rhodotorula* were present in both zones at a frequency >1%. Some MOTUs were only found as endophytes, including those assigned as *Pestalotiopsis* and *Phaeoacremonium*, members of Pleosporales (*Curvularia*, *Fusculina*) and *Myrmecridium*. In most cases, endophyte MOTUs could be found in the rhizosphere, including those of other plants, albeit at different levels of frequency. *Microdiplia* was the only MOTU found in similar levels as an endophyte and in the rhizospheres.

Comparisons between the rhizosphere and root endophyte communities in the soils of the grazing lawn and slope vegetation zones showed distinct differences in presence or absence, or shifts in frequency (Table 2, Figures 2 and 4). Out of 27 detected MOTUs, 19 were unique to the rhizosphere soils, while only four were unique to the internal tissues of the roots. Some MOTUs, such as *Alternaria*, *Boletus*, *Microdiplodia*, *Chaetomium*, *Penicillium*, *Agaricus* and *Amanita*, occurred in the rhizosphere of both zones at similar levels, while there were shifts in the frequencies of others, such as those of *Fusarium*.

For the endophyte community, *Fusarium* and *Humicola* were present in roots of both zones at frequencies >1%. *Humicola* were absent from all rhizosphere soils but were found in the rhizosphere of *S. cordifolia* in the grazing lawn zone. *Microdiplodia*, *Aureobasidium*, *Phoma*, *Curvularia* and *Fusculina* were present at frequencies <1% in roots from both zones.



MOTU, Molecular Operational Taxonomic Units.

FIGURE 6: Venn diagram of the fungal Molecular Operational Taxonomic Units obtained from the rhizosphere of three plant species growing in the slope zone.

Lasiodiplodia and *Botryosphaeria* (both in the Botryosphaeriaceae) were only present in the hill slope zone and at frequencies higher than 1%, while other MOTUs with frequencies <1% were also unique to a zone. Molecular Operational Taxonomic Units showing shifts in frequency, included *Pestalotiopsis* that was more frequent in the hill slope zone, and *Exophiala* and *Rhyzopycnis* that were more frequent in the grazing lawn roots.

Discussion

A catena system, such as the Stevenson–Hamilton granite supersite in the KNP, represents an ideal system to study the fungal communities that are associated with plant roots in different soil types and conditions and from different plant species. Within only approximately 500 m, a range of soil types and conditions are found that was also reflected by differences in the plant community (Theron et al. 2020). Similarly, marked differences in the fungal communities existed in the plant–soil interface. Unique MOTUs and shifts in frequencies were observed between the inside (endophytes) and outside (rhizosphere) communities of *S. cordifolia* roots, and also between two different soils. Furthermore, differences were observed between the rhizosphere soils of three different plant species in one zone, although the dominant MOTUs were relatively similar.

Fungi specialised to occur in different substrates, for example, soil compared to plant tissues, are different because they require different dissemination strategies and may also have different functional roles (Bayman et al. 1997; Carroll 1988; Hardoim et al. 2015, Moore et al. 2011; Talbot et al. 2014; Yang et al. 2018). The root rhizosphere is also known to usually harbour selected species either from the general soil environment or the root, and are often specialised according to the plant species (Berlanas et al. 2019; Mohanram & Kumar 2019). Nonetheless, levels of overlap do occur (Tedersoo et al. 2016). Our results thus reflected previous literature and represent the first such study for the KNP. It also represents the first evidence regarding how fungal communities differ within a catena system.

Alternaria and *Fusarium* include species that are endophytic, saprobic in soils and pathogenic or beneficial to plants, and that occur in numerous habitats (Leslie & Summerell 2006; Woudenberg et al. 2013). Such cosmopolitan fungi can be dominant or omnipresent in communities because of their ability to easily colonise or disperse, as opposed to more specialised fungi that may not always have a rapid growth rate and that have evolved with their hosts or niche (Vujanovic et al. 2006). While such dominant MOTUs of *Alternaria* and *Fusarium* were detected, shifts in their frequencies occurred between different substrates (e.g. *Alternaria* was dominant in rhizospheres but less so as an endophyte) and soils (*Fusarium* was one of the dominant groups in all collections except in the slope rhizosphere of *S. cordifolia; Fusarium* was also almost five times as dominant as a root endophyte of *S. cordifolia* in the grazing lawn area compared to the slope). Plants that were selected were all healthy, and the presence of *Alternaria* within plants was latent and in low numbers, and the higher abundance of *Fusarium* could be because of non-pathogenic *Fusarium* species. An interesting result was that other fungi that can occur both in plants and soils, for example, *Curvularia*, *Penicillium* and *Aureobasidium* (Domsch, Anderson & Gams 1980; Haas et al. 2016) were selectively detected, and others were not detected at all, such as cladosporiod fungi.

Knowledge regarding the ecological functions of fungi associated with different soil types and those associated with plants is virtually non-existent for natural and pristine ecosystems in South Africa. The ecological functions of these fungi can only be guessed from the existing literature. Intensive studies are needed to elucidate the functions of the various fungi characterised in this study, and how changes will impact them and their associated hosts. The more rare fungal groups could be more specialised and sensitive, but without more surveys it will be difficult to identify them as important in the ecosystem or if they are promoting plant and soil health (Frac et al. 2018), as opposed to just being able to infect at a low frequency.

Sida cordifolia is not native to South Africa, but a declared invasive weed (Morris, Witkowski & Coetee 2011). It was chosen because of its ability to grow across the different soils of the catena system. Nonetheless, the communities involved with the rhizospheres and roots in the two sites of the catena showed telling variation. The invasive plant *S. cordifolia* appeared to attract only half of the number of MOTUs present in its rhizosphere compared to those in the native relative *M. acuminata*, and the other native plant *K. angustifolia*. However, more extensive sampling will have to be performed to really prove this statistically.

Various factors could possibly explain the differences observed between *S. cordifolia* and the two native herbs. Certain plants have been shown to have a stronger rhizosphere effect than others (Gomes et al. 2003), and what has been observed in this study could merely be such an effect. No studies have been conducted for comparing the microbial rhizosphere community of invasive plants, or even non-native plants, with those of native plants in the same area. Nonetheless, it could be hypothesised that the ability to attract a functional rhizosphere microbial community that benefits the plant will aid the establishment or invasiveness of a plant (Coats & Rumpho 2014), similar to what has been found with other types of microbes such as mycorrhiza (Nuñez & Dickie 2014; Rodríguez-Echeverría et al. 2009). Furthermore, it has been shown that some invasive plants can change the soil microbiome around their roots to their benefit (Dawkins & Esiobu 2017; Policelli et al. 2019). What has also been found in other groups of plant-associated microbes, such as endophytes, was that the endophytic community of non-native plants were composed of endophytes with wide host ranges and large geographical locations (cosmopolitan fungi), while the native plants had more specialised and diverse endophytes including those with narrow host ranges (Clay et al. 2016; Hoffman & Arnold 2008; Newcombe et al. 2009). Whether the native plant

species in the KNP have closer evolved endophytic and rhizosphere fungi compared to introduced plants will have to be studied further.

Many of the MOTUs detected in all the samples were assigned names of genera known to include plant pathogens, such as *Fusarium* and *Alternaria*. However, these genera include a wide diversity of species with diverse ecological roles and life strategies, including pathogens, non-pathogens or latent pathogens (pathogens that can hide as endophytes) and endophytes. It is difficult to assess if the DNAs detected from the samples represented pathogenic species or not based on the limited sequence data, especially because the ITS regions commonly used in Next Generation Sequencing (NGS) studies, are known to be incapable of differentiating between species of these genera (Schoch et al. 2012).

An interesting result is that members of other important pathogen groups have been detected. Molecular Operational Taxonomic Units in the Botryosphaeriaceae and an MOTU from the Teratosphaeriaceae were detected as root endophytes. These fungi are not recorded as soil fungi and are mostly known from aerial tissues of woody plants, where they have latent pathogenic life stages before causing various disease symptoms (Crous, Wingfield & Groenewald 2009; Finlay & Clay 2007; Marsberg et al. 2014; Pillay et al. 2013; Sieber 2007; Slippers & Wingfield 2007). While their presence in the rhizosphere could be attributed to the presence of spores, the prominence of especially two MOTUs of the Botryosphaeriaceae (*Botryosphaeria* and *Lasiodiplodia*) from the roots of the herbaceous plant *S. cordifolia* is interesting. An MOTU that was prominent in all of the rhizosphere samples had the assigned name of *Boletus* (Boletaceae). The MOTU was not present in the root endophyte samples. Members of Boletaceae are important ecto-mycorrhizal partners of plant roots and can easily be seen by the production of mushroom-like fruiting bodies (Goldman & Gryzenhout 2019). South Africa does not have reported indigenous ectomycorrhizal fungi, while the only indigenous bolete species is *Phaeogyroporus sudanicus* or the bushveld bolete (Goldman & Gryzenhout 2019; Gryzenhout 2010; Tonjock et al. 2020; Van der Westhuizen & Eicker 1994). While the fungus is known to be ectomycorrhizal (Thoen & Ducousso 1990, as *Phlebopus sudanicus*), its host range throughout South Africa, including the KNP, has not really been fully established. This bolete has been observed in the KNP by the first author, and it is thus possible that the MOTU could represent this species, especially so because there are no *Phaeogyroporus* sequences currently present in the UNITE database (https://unite.ut.ee/). The absence of the MOTU from inside surface sterilised roots could indicate that it does not have ectomycorrhizal associations with the three plant species.

Ecto-mycorrhizal fungi such as boletes are more easily observed than other groups of mycorrhiza because they produce visible fruiting bodies above soil. The detection of a bolete sequence raises the question if other native ectomycorrhizal partners to indigenous plants in the KNP do not in actual effect exist. This is especially so because extensive surveys for the presence of such fungi have not yet been conducted in the KNP. Furthermore, species of some plant genera that are known to have ectomycorrhizal partners, such as *Albizia*, *Terminalia* and *Combretum* in neighbouring countries such as Zimbabwe (Tsamba et al. 2015) and even up to central Africa (Eneke, Njoh & Egbe 2018; Härkönen, Niemelä & Mwasumbi 2003; Härkönen et al. 2015), occur in the KNP. Related species such as *Colophospermum mopane* occur in the KNP, which belong to Detariodeae (Fabaceae). This family includes keystone genera known to have ecto-mycorrhizal partners in the iconic and widespread Miombo woodlands that are spread from Zimbabwe further north into Central Africa (Palgrave 2015). In fact, members of these named genera are present in the catena (Theron et al. 2020). Studying these special fungi is vital because they play an integral role in the health and resilience of their associated plants, and should they disappear for some reason, it will have grave consequences on plant survival in the KNP.

Other macrofungal MOTUs (fungi that can be seen without the need of a microscope) that were detected from all of the rhizosphere samples included the mushroom genera *Agaricus* and *Amanita*. *Agaricus* is known to be saprophytic while many species of *Amanita* are known ecto-mycorrhizal plant partners (Goldman & Gryzenhout 2019). However, certain *Amanita* species, including South African native species such as *A. veldiei*, are saprophytes (Goldman & Gryzenhout 2019). An MOTU assigned as *Ganoderma* was present in the rhizosphere samples of the three plant species from the slope. *Ganoderma* species are common throughout South Africa and include wood rotting bracket fungi that often are pathogens of plants (Goldman & Gryzenhout 2019; Tchoumi et al. 2019). A number of species of these genera occur in South Africa (Tonjock et al. 2020), and conducting more detailed surveys in the KNP to ascertain what species occur in the park will prove to be useful.

For the data analysis pipeline used in this study, the UNITE database (Nilsson et al. 2019) was used for taxonomic assignment. This database is better curated than general databases such as Genbank, and widely used for fungal environmental sequencing (Nilsson et al. 2019). However, taxonomic assignments must always be verified because the assignment process may not truly reflect the correct taxonomic identity even at the genus level, or may not reflect current taxonomies (Tonjock et al. 2019). The ITS region is also known to not always distinguish species in some genera, although it is still widely used as the barcode and minibarcode for fungi (Schoch et al. 2012). In practice, complicated taxonomic groups such as *Phoma* and *Epicoccum* cannot be distinguished based only on ITS data in routine ITS-based environmental sequencing studies (Tonjock et al. 2019). The conclusions from results of this study must thus be made with care considering that even generic names may not be accurate. However, in some cases, associations can be made if

members of a particular family or genus have similar ecological attributes, such as the Boletaceae. In other cases, such as for *Fusarium* (Leslie & Summerell 2006), different species often have certain ecological roles (e.g. pathogen or latent pathogen or non-pathogenic, or pathogen with saprophytic survival phase). It is thus possible that an MOTU assigned as *Fusarium* (or previously used sexual names such as *Gibberella* and *Haematonectria*) from different niches and substrates may in fact represent different *Fusarium* species with different ecological roles.

Conclusion

Results from this study revealed a complex fungal community associated with the plant–soil interface within a catena system. This is despite the fact that the two sites occurred within a 500 m area. Results from other studies showed the effect of the different geological and hydrological processes on the plant and animal communities, while the biological communities also affect each other (Janecke 2020; Janecke & Bolton 2020; Theron et al. 2020; Vermeulen et al. 2020). This study also showed the need to include fungi in studying these interactions more thoroughly. Fungi provide fundamental ecosystem services as key saprophytes, plant degraders, pathogens and those providing benefits to the plant as mycorrhiza, endophytes or rhizosphere partners.

The complexity of fungal communities differing already in a small component of the entire ecosystem of the catena indicates that such a system with its differing communities will be sensitive to change and disturbances, both in the short and long runs. Similar results have also been obtained by looking at the bacterial communities (Vermeulen et al. 2020). This is despite the fact that the overall animal and plant communities may appear more uniform and thus do not indicate the importance to specifically also define differences of microbial communities on a micro-ecological scale. The same will be true for fungal communities associated with other plant species and soil types of the KNP.

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Authors' contributions

The authors directly participated in the study design, execution and interpretation of the research.

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Data availability

Data are available from the corresponding author, on request.

Disclaimer

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