Diversity and seasonality of fungal communities in soil from the Succulent Karoo biodiversity hotspot, South Africa

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HIGHLIGHTS

• Fifty nine fungal endophytes were identified from mesembs in the Succulent Karoo.

• The genus *Fusarium* was the most abundant in samples from the flowering season.

• The genera *Alternaria* and *Cladosporium* were equally most abundant in the dry season.

• *Fusarium*, *Paecilomyces* and *Talaromyces* largely contributed to seasonality observed.

• Rare genera were *Neophaeosphaeria*, *Periconia*, *Preussia*, *Schizothecium* and *Truncatella*

ABSTRACT

The fungal rhizosphere communities associated with soil surrounding Aizoaceae plants in the Namaqua National Park in the Succulent Karoo biodiversity hotspot, South Africa, were analysed by culture-dependent and culture-independent methods. The keratinophilic fungal Order, Onygenales, was the most abundant Order observed in the total fungal population. Fungal communities observed during the dry and flowering seasons differed significantly. The Orders Ascosphaerales and Chaetothyriales were the major contributors to the observed seasonality of fungal communities. No isolates belonging to these Orders were obtained in the culture-dependent experiments, while isolates belonging to the Orders Hypocreales and Eurotiales were abundant in culture. Seasonal differences were most pronounced for the Order Ascosphaerales, which contributed less than 0.1% to the total population during the dry season, but more than 20% to the flowering season population. This study provides the first next generation sequencing analysis of the remarkable soil-associated fungal richness and diversity in the Succulent Karoo, the most biodiverse arid ecoregion in the world.

KEYWORDS

Aizoaceae, Ascosphaerales, Chaetothyriales, conservation, Namaqua National Park, Onygenales.

1. Introduction

Soil is essential to entire ecosystems; it sustains biodiversity and performs irreplaceable and often fundamental, complex functions (Nannipieri *et al.*, 2003; Janion-Scheepers *et al.*, 2016). The soils of the Succulent Karoo have unique features including textural water barriers, differential infiltration of rainwater, water-absorbing clay minerals and nocturnal distillation, which together alter storage, conductivity, water permeation and water supply to plants (Mucina *et al.*, 2006; Francis *et al.*, 2007). The chemical and physical properties of soil in the Succulent Karoo are highly

variable due to weathering of the soil mantle and this variability is thought to increase the overall biodiversity of the ecosystem (Francis *et al.*, 2007; Bourne *et al.*, 2017).

Soil microorganisms, such as those acting as symbionts and decomposers, for example, play critical roles in global processes such as nutrient cycling (Van der Heijden *et al.*, 2008; Bates *et al.*, 2012; McGuire *et al.*, 2015). While some studies (e.g. Suryanarayanan *et al.*, 2005; Loro *et al.*, 2012) have indicated that fungal diversity is lower in arid regions than in tropical biomes, other studies have revealed that fungal diversity in these areas is higher than initially expected (Grishkan and Nevo, 2010; Sterflinger *et al.*, 2012; McGuire *et al.*; 2013).

The Namaqua National Park is a protected reserve inside the Succulent Karoo biome, South Africa, known to be the most diverse arid ecoregion in the world. The Succulent Karoo is also one of only two arid biodiversity hotspots recognised by Conservation International (CI) (Myers *et al.*, 2000). The Namaqua National Park is home to a diverse, locally adapted flora which includes more than 6000 plant species, more than any other arid region in the world (SANParks, 2013). Of these, the plant family Aizoaceae comprises more than 1 800 species, 63% of southern Africa's succulent flora (Chesselet *et al*., 2002).

In this study, we investigated the fungal communities in the rhizospheric soils of Aizoaceae plants in the Namaqua National Park using both culture-dependent and culture-independent methods. The objectives of this study were to estimate fungal diversity and to evaluate the effects of seasonality by comparing fungal diversity in soil during the dry and flowering (wet) seasons. To our knowledge, this is the first study on the fungal inhabitants of soils in the Succulent Karoo biodiversity hotspot.

2. Materials and methods

2.1. Study site and sampling

The study area is located close to the Skilpad camp (30°09'19"S; 17°43'55"E) in the Namaqua National Park, South Africa, and samples were obtained under the auspices of South African

National Parks permit number CRC/2015/010--2014. The region is designated as warm-temperate with a mean annual temperature of 16.8ºC and a summer aridity index value of >4.8. Seasonal, unimodal winter rainfall occurs from May to September with mean annual precipitation of 240mm across the entire park (Mucina *et al.*, 2006; SANParks, 2013).

Sampling was performed before (dry season) and after the annual rainfall (flowering season, abbreviated flr) in May 2014 and October 2014 respectively. Five plots of 200m^2 were sampled during both seasons. Each plot was subdivided into 10 subplots of 20m^2 each. Three soil samples containing the top 10cm of soil at the base of common Aizoaceae plants were collected from each subplot using a soil corer. The soil corer was sterilised with a 3.5% sodium hypochlorite solution between each sample to prevent cross-contamination. Soil samples were stored at 4ºC for a maximum of 96h during transport and then stored at -80ºC in the laboratory. Subsequently, the 30 soil samples from each plot were pooled, mixed thoroughly and sieved with a pore size of 4mm to obtain five representative composite soil samples (one from each plot) from each season.

2.2. Culture dependent sample processing

Fungal isolations were performed using the methods of Waksman (1916) and Warcup (1950) onto nutrient poor (water agar and potato carrot agar) and nutrient rich (potato dextrose agar (PDA) and malt extract agar (MEA)) agar (Biolab, Merck, Darmstadt, Germany) in 90mm Petri dishes. In order to characterize the culturable fungal diversity in the soil morphologically distinct colonies were sub-cultured and purified on PDA. DNA from each morphotype was extracted using the ZR Fungal DNA MiniPrep^{TM} kit (Zymo Research Corp, Irvine, CA, USA) according to manufacturer's instructions. The internal transcribed spacer (ITS) region ITS1-5.8S-ITS2 of the fungal rRNA gene complex was amplified using the universal fungal primers ITS1 and ITS4 (White *et al.*, 1990). Each 25µl PCR mix contained the following: 2µl of 10x DreamTaq DNA buffer (Thermo Fisher Scientific, Waltham, MA), 2µl of 25µM dNTP's (Promega Corp., Madison, WI), 0.5µl (0.2µM) of each primer (Sigma-Aldrich, St. Louis), 0.2µl (5U/µl) DreamTaq DNA polymerase (Thermo Fisher

Scientific Inc., Wilmington, DE, USA), 2µl template DNA and 17.3µl of ddH_2O. The PCR reactions were performed using the following conditions: initial denaturation at 94°C for 2min, 30 cycles of denaturation at 94°C for 30s, annealing at 52°C for 30s and elongation at 72°C for 45s, final elongation at 72°C for 7min. PCR amplicons were sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase (Applied Biosystems, Paisley, UK) using both forward and reverse primers.

Sequences were analysed with BioEdit and compared to those in the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) database and those published on MycoBank (www.mycobank.org) (Crous *et al.*, 2004) using the BLASTn algorithm (Altschul *et al.*, 1990). If ITS region sequence similarity was >97% the most similar reference sequence from above databases was used to assign identity. An identical name was assigned to sequences sharing less than 5% nucleotide difference and these species were treated as a single operational unit. The global fungal nomenclature database www.indexfungorum.org was used to ensure that the most current name of fungal species was used and to eliminate synonymy.

2.3. Culture independent sample processing

Total DNA from 0.25g of each composite soil sample was extracted using the ZR Soil Microbe DNA MiniPrep™ kit (Zymo Research Corp., Irvine, CA, USA) according to manufacturer's instructions. DNA was extracted in triplicate and the three replicate subsamples pooled to minimize spatial heterogeneity. Purity and concentration of DNA was quantified spectrophotometrically with a NanoDrop 2000 (NanoDrop, Thermo Scientific Inc., Wilmington, DE, USA) at wavelengths of A260 and A280. The ITS1 region of metagenomic DNA were amplified using fungal-specific forward and reverse primers with barcode sequences attached: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACCTGCGGARGGATCA-3' and 5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAGATCCRTTGYTRAAAGTT-3'.

The PCR reaction mixture consisted of 2.5µl of genomic DNA template, 5µl (1µM) of each primer and 12.5µl of 2x KAPA HiFi HotStart ReadyMix (Mg^{2+}, DNA polymerase, PCR Buffer, dNTPs) (KAPA Biosystems Inc., Wilmington, MA, USA). PCR conditions were: 95ºC for 3min, followed by 25 cycles of 95ºC for 30s, 55ºC for 30s, 72ºC for 30s and finally an extension step at 72ºC for 5min. The quality of the sequence library was assessed using a Qubit@ 2.0 Fluorometer (Thermo Scientific Inc., Wilmington, MA, USA). The libraries were then sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). Barcode and primer sequences were removed. Bioinformatics pipeline QIIME v. 1.6.0 (Caporaso *et al.*, 2010) was used for sequence processing. Sequences of unsuitable length (<200 or >1000bp) were removed during quality filtering. The UNITE/QIIME 12_11 ITS reference database (http://unite.ut.ee/) was used for sequence analysis with 97% sequence similarity employed in the UCLUST algorithm (Edgar, 2010) and chimeras removed using the UCHIME algorithm (Edgar *et al.*, 2011). Suitable sequences were classified into OTU's.

2.4. Statistical analysis

Statistical analyses were conducted in R v.3.1.2 (R Development Core Team, 2014) and PAST 3.14 software (Hammer *et al*., 2001). Differences in fungal communities between seasons in both culture-dependent and culture-independent studies were visualised by nonmetric multidimensional scaling (NMDS) ordination using the Jaccard and Bray-Curtis dissimilarity indices for binary (presence / absence) and abundance data, respectively (Taguchi and Oono, 2005). Permutational multivariate analysis of variance (PERMANOVA) was performed to complement NMDS results and analysis of similarity (ANOSIM) was used to determine if relative abundance of fungi differed between the seasons studied. Krona Tools (https://github.com/marbl/Krona/wiki/KronaTools) were used to construct Krona charts providing a diagrammatic view of every population (Ondov *et al.*, 2011).

3. Results

3.1. Fungi cultured from soil

Fungi cultured from Namaqua National Park soil samples were classified into 22 fungal genera. Nineteen of these genera were classified into seven Orders in the Phylum Ascomycota (Fig. 1a). Twenty-four isolates belonged to the Order Eurotiales, 14 isolates to Order Hypocreales and nine to Order Pleosporales. In total, 61 different fungal species were obtained: 24 species were isolated from the dry season samples and 45 species from the flowering (wet) season. Eight of the 61 species (*Alternaria alternata*, *Aspergillus aureolus*, *Cladosporium cladosporioides*, *C. oxysporum*, *Fusarium oxysporum*, *F. solani*, *Talaromyces pinophilus* and *Trichoderma koningiopsis*) were isolated during both seasons. *Fusarium oxysporum* was the most frequently isolated species, at almost 20% relative abundance (RA) (Table S1). It was also the most frequently isolated species when the flowering season was considered separately. *Cladosporium cladosporioides* was most frequently isolated during the dry season and was also found to be the second most abundant species (at almost 10%) when the total population from both seasons was considered (Table S1).

Seasonality of fungal communities in soil samples was evident from the clustering of dry season samples and flowering season samples observed by non-metric multidimensional scaling (NMDS) (Fig. S1). The clustering indicated that differences in fungal communities between the two seasons sampled were much larger than differences within each season, both concerning relative abundance (Fig. S1a) and presence / absence data (Fig. S1b). These results were confirmed with permutational multivariate analysis of variance (PERMANOVA) where $p < 0.0086$, $F = 18.25$ and $r^2 = 0.21$ were calculated with the Bray-Curtis index and $p < 0.0067$, $F = 10.45$ and $r^2 = 0.42$ with the Jaccard index employed. The fungal population associated with different soil samples from the dry season in the Namaqua National Park was more homogenous than those of the flowering season, as observed by the close clustering of dry season samples (Fig. S1).

3.2. Fungal phylotypes observed in soil using culture independent methods

In culture-independent studies, only 61.4% of reads could only be classified as known fungal species, indicating the possibility of novel taxa. The remaining 66859 reads could be classified to at least class level, with 28896 (43% of total reads) acquired from soil samples taken during the dry season in comparison with 37693 (57%) from soil sampled during the flowering season.

3.3. Comparison of fungal Orders revealed in soil by culture dependent and culture independent methods for both seasons combined

Culture-independent studies distinguished 56 fungal Orders (Table 1, Fig. 1b). Only 10 of these Orders were represented by isolates obtained by culture-dependent methods (Fig. 1a). Fifty of these fungal Orders had relative abundance figures of less than 5% when both sampling seasons were considered. The Order Onygenales was found to be most abundant in culture-independent studies (almost 19% of the total population), yet no isolate of this fungal Order was obtained from any sample using culturing methods. Seasonality was observed with 10 and 11 Orders observed only from dry and flowering season samples, respectively (Table 1).

In culture-dependent analyses, most isolates belonged to the Order Hypocreales, closely followed by Eurotiales (both >30% of total population), when results from both seasons were combined (Fig. 1a). Hypocreales was the third most abundant fungal Order observed in culture-independent studies, whereas Eurotiales comprised only 3% of the total population observed from culture-independent studies (Table 1). These discrepancies are indicative of the very well-established species bias when culturing methods were considered alone. Interestingly, the Order Agaricales from Phylum Basidiomycota was the second most abundant in culture-independent studies, although only a single species (*Coprinopsis erythrocephala*) belonging to this Order was obtained by culture-dependent methods (Table S1).

3.4. Comparison of fungal Orders in soil from culture dependent and culture independent methods for dry season samples

More than 80% of fungal isolates cultured from soil samples collected during the dry season belonged to the Orders Eurotiales (39%), Capnodiales (26%) and Hypocreales (25%). The Order Hypocreales accounted for 27% of the OTUs in the culture-independent study, making it the most abundant during the dry season, followed by Onygenales (21%) and Agaricales (16%). The Orders Eurotiales and Capnodiales accounted for less than 7% each of the total fungal population in culture-independent studies from dry season soil samples (Fig. 2a).

Table 1. Relative abundance of fungal Orders obtained by culture-independent (Illumina MiSeq NGS) methods from soil sampled from the Namaqua National Park, South Africa, during the dry (Dry) and flowering (Flr) seasons, as well as both seasons combined (Total).

Fungal Order	RA Total	RA Dry	RA Flr
Onygenales	18.917%	20.743%	17.528%
Agaricales	15.165%	16.269%	14.324%
Hypocreales	14.215%	26.678%	4.728%
Ascosphaerales	11.399%	0.055%	20.033%
Chaetothyriales	8.973%	1.315%	14.801%
uncultured Ascomycota	5.595%	7.222%	4.357%
Pleosporales	3.614%	0.997%	5.605%
Eurotiales	3.087%	4.357%	2.120%
uncultured Basidiomycota	2.951%	3.205%	2.758%
Capnodiales	2.879%	6.492%	0.129%
Xylariales	2.638%	0.000%	4.647%
Mucorales	1.490%	3.097%	0.266%
Dothideales	0.932%	2.114%	0.032%
Incertae sedis	0.841%	0.388%	1.185%
Diversisporales	0.802%	0.003%	1.409%
Jahnulales	0.772%	0.709%	0.819%
Pezizales	0.532%	0.083%	0.875%
Cantharellales	0.422%	0.073%	0.688%
Sordariales	0.353%	0.017%	0.608%

Helotiales	0.211%	0.093%	0.300%
Cortciales	0.171%	0.377%	0.013%
Mortierellales	0.168%	0.388%	0.000%
Hymenochaetales	0.162%	0.028%	0.263%
Polyporales	0.142%	0.280%	0.037%
Saccharomycetales	0.141%	0.097%	0.174%
Ostropales	0.126%	0.007%	0.216%
Botryosphaeriales	0.118%	0.132%	0.108%
Thelephorales	0.109%	0.000%	0.192%
Sebacinales	0.094%	0.204%	0.011%
Rhizophlyctidales	0.070%	0.118%	0.034%
Russulales	0.060%	0.000%	0.105%
Boletales	0.043%	0.083%	0.013%
Atractiellales	0.037%	0.087%	0.000%
Blastocladiales	0.030%	0.021%	0.037%
Pertusariales	0.025%	0.000%	0.045%
Harpellales	0.024%	0.055%	0.000%
Kickxellales	0.022%	0.052%	0.000%
Tremellales	0.018%	0.031%	0.008%
Verrucariales	0.016%	0.000%	0.029%
Neocallimastigales	0.013%	0.031%	0.000%
Pucciniales	0.012%	0.028%	0.000%
Amphisphaeriales	0.010%	0.024%	0.000%
Entomopthorales	0.009%	0.003%	0.013%
Peltigerales	0.009%	0.000%	0.016%
Teloschistales	0.009%	0.000%	0.016%
Geastrales	0.007%	0.017%	0.000%
Glomerellalles	0.007%	0.017%	0.000%
Arachnomycetales	0.006%	0.000%	0.011%
Hysterangiales	0.004%	0.010%	0.000%
Malasseziales	0.003%	0.000%	0.005%
Ustilaginales	0.003%	0.000%	0.005%
Atheliales	0.001%	0.003%	0.000%

* *RA* = Relative Abundance of Fungal Orders (most abundant to least abundant in the total population) reported as a percentage of either the total population or the dry/flowering season populations, respectively.

3.5. Comparison of fungal Orders in soil from culture dependent and culture independent methods for flowering season samples

Most fungal isolates cultured from the flowering season samples belonged to the Order Hypocreales (44%), followed by the Orders Eurotiales (28%) and Pleosporales (18%). In culture-independent studies, the Ascosphaerales was the most abundant fungal Order observed in soil samples, at 20% RA (Fig. 2b). In this study, no isolates from Order Ascosphaerales could be cultured. Additionally, no fungi belonging to the next two most abundant Orders (Onygenales (18%) and Chaetothyriales (15%)) observed in culture-independent study could be isolated in the culture-dependent study. As was the case with dry season samples, more than 80% of isolates obtained in culture-dependent studies from the flowering season belonged to only three fungal Orders.

3.6. Seasonal trends observed in populations of soil fungi

Sufficiency of the sampling effort was assessed, and sampling completeness calculated as 85% and 80% when Chao 2 and Jackknife 1 were used as estimators, respectively (Chao, 1987; Colwell and Coddington, 1994). Non-metric multidimensional scaling (NMDS) ordination separated the fungal populations observed in the dry season from those observed in the flowering season. This holds true when both relative abundance data (Fig. S2a) and presence / absence data (Fig. S2b) were evaluated, indicating that fungal communities differed significantly in the two seasons sampled. These results were confirmed with PERMANOVA where values of $p < 0.0467$, $F = 2.04$ and $r^2 = 2.35$ were calculated with the Bray-Curtis index and $p < 0.0086$, $F = 3.54$ and $r^2 = 1.676$ with the Jaccard index employed. ANOSIM values for this study indicated dissimilarity in fungal populations between seasons with $R = 0.34$ ($p < 0.015$) when the Bray-Curtis index was used and $R = 0.748$ ($p < 0.0071$) when the Jaccard index was used.

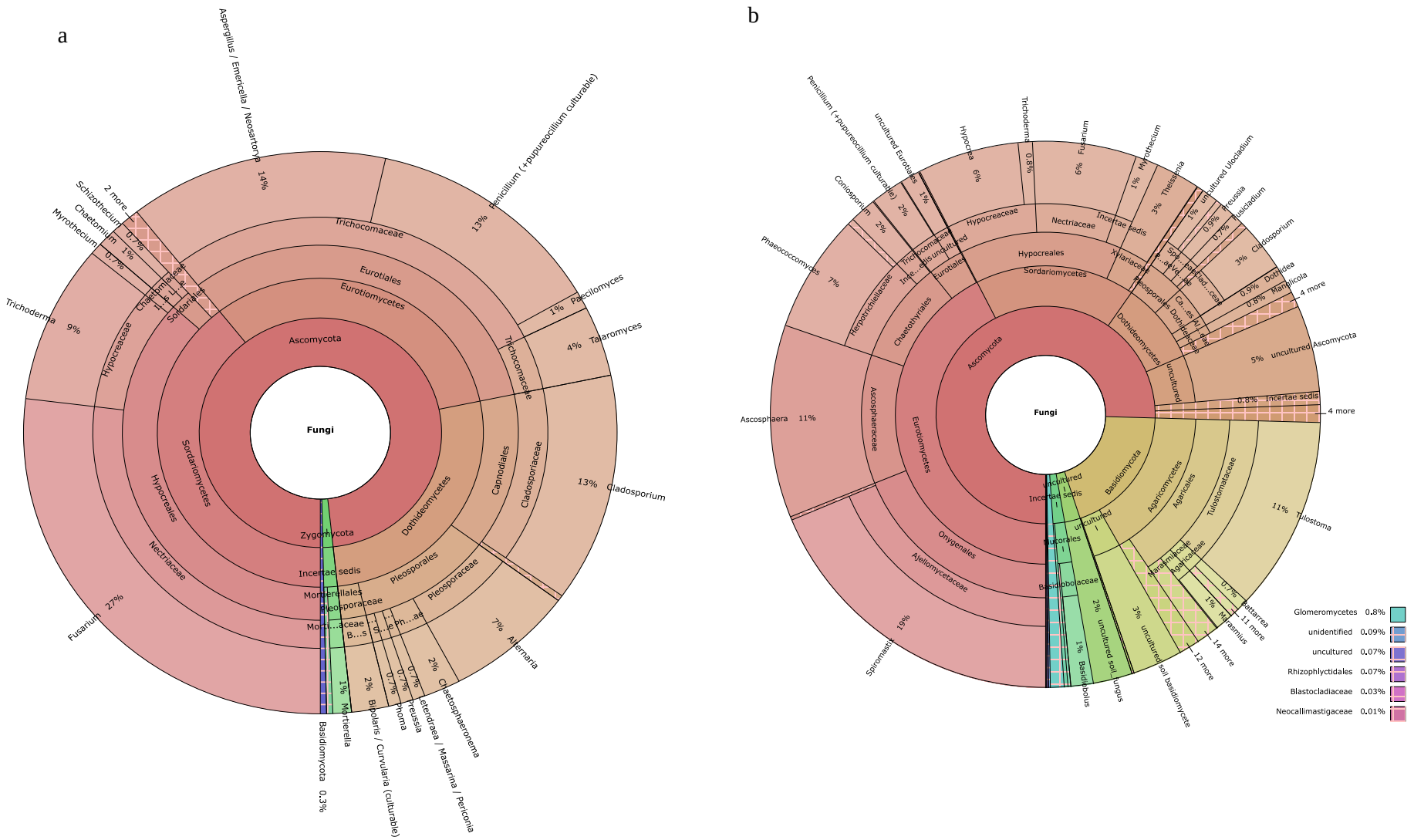

Fig. 1. Krona charts indicating the taxonomic identification and relative abundance of fungi obtained by (a) culture-dependent and (b) culture-independent methods from soil samples acquired in the Namaqua National Park, South Africa, during dry and flowering seasons (combined).

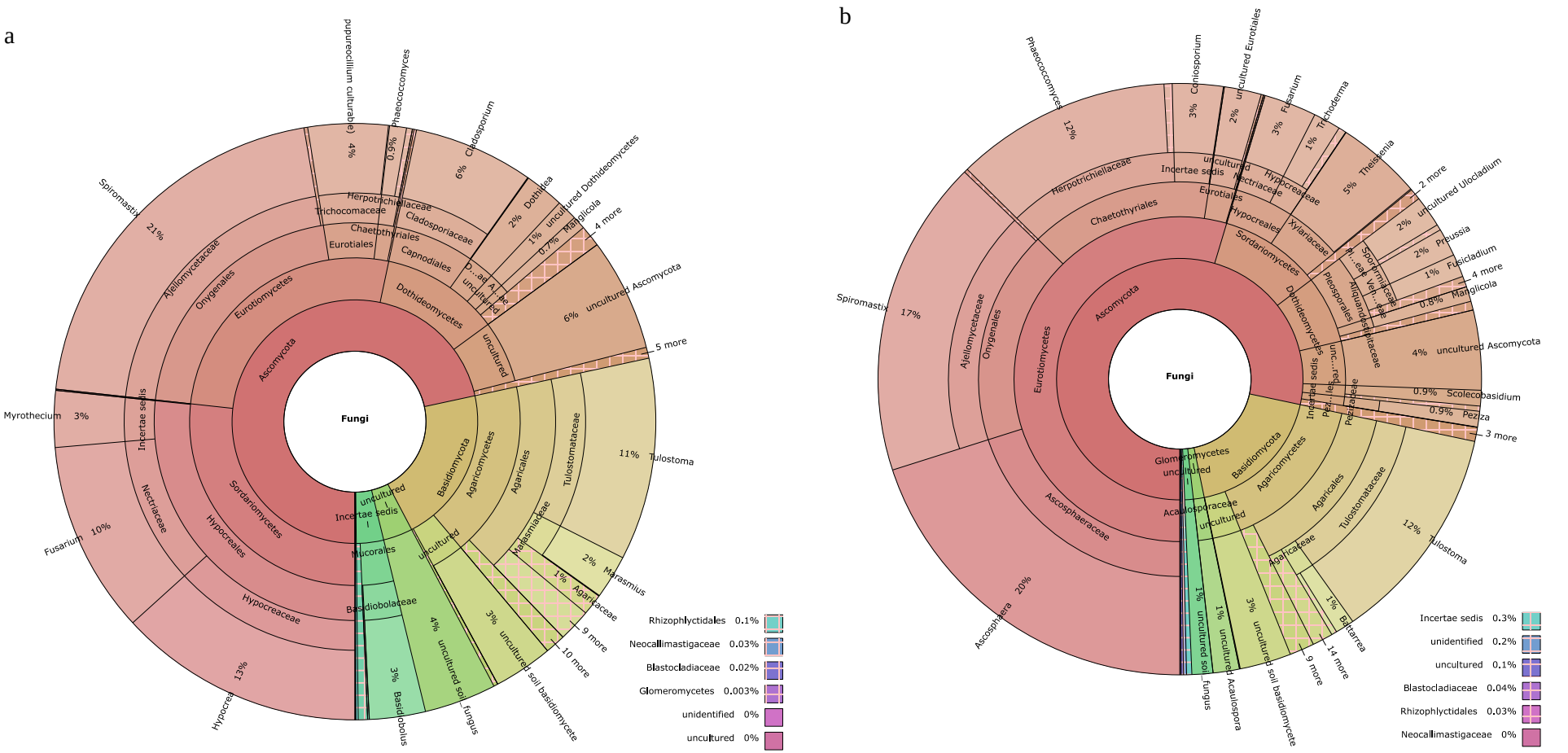

Fig. 2. Krona charts indicating the taxonomic identification and relative abundance of fungi obtained by culture-independent methods from soil samples acquired in the Namaqua National Park, South Africa, (a) during the dry season and (b) during the flowering season.

4. Discussion

Most studies investigating soil-inhabiting fungi have focused on mesic environments and tropical biomes, with studies in arid environments located in the Southern Hemisphere and focussing on biodiversity hotspots being especially rare (McGuire *et al.*, 2013; Tedersoo *et al.*, 2014). To date, the study investigating plants most closely related to Aizoaceae and conditions of the arid Succulent Karoo was that of Colemann-Derr *et al.* (2016) where the fungal community associated with the succulent plant *Agave*, native to the arid environments of Mexico, was investigated. In both the study on *Agave* and the current study Phylum Ascomycota, the largest fungal Phylum (McLaughlin *et al*., 2001), accounted for 75% of the total population.

Fungal isolates belonging to the Orders Hypocreales and Eurotiales were the most frequently cultured in this study. Fungi from these two Orders have been shown to have a positive correlation with soil temperature, with more fungal isolates cultured from soil obtained in warmer conditions (Devi *et al.*, 2012). In the current study, however, an 11% decrease in abundance of cultured fungal isolates belonging to the Order Eurotiales from the dry to the warmer flowering season was observed. This was mainly due to more isolates of *Aspergillus* and *Penicillium* being cultured from dry season samples. These genera are able to endure xeric conditions and are frequently isolated from soil (Kornikłowicz-Kowalska and Kitowski, 2013; Oliveira *et al.*, 2013; Porras-Alfaro *et al.*, 2014).

The relative abundance of the Order Capnodiales was higher in the dry season when both culture dependent and culture independent results from the current study are considered. The genus *Cladosporium* was in large part responsible for this finding since most isolates cultured in the culture dependent study and observed in the culture independent study were members of this genus. Many fungal species that belong to the Orders Capnodiales and Dothideales are able to withstand xerotolerant conditions (Gunde-Cimerman *et al.*, 2009). Fungi that belong to the Order Dothideales were also observed to be significantly more abundant in the dry season in soil from the Namaqua National Park in comparison with the flowering season.

Culture-independent results revealed that fungi from the Order Onygenales were the most abundant in the fungal population when results from both seasons were considered together. No isolate from this Order could be cultured in this study. The Onygenales are a group of fungi that require the presence of keratin for growth (Solé *et al.*, 2002). This may explain why the fungus could not be cultured, since keratin was absent from the growth media used. The most abundant genus observed within this Order was *Spiromastix* from the family Ajellomycetaceae. This thermally dimorphic fungal family exists in mycelial form in soil but changes to a yeast-like form in their mammalian hosts (Dukik *et al.*, 2017). Ajellomycetaceae has previously been implicated in human infections, notably of immuno-compromised individuals in South Africa (Dukik *et al.*, 2017).

The largest difference between relative abundance observed for the culture-independent study was seen with the Order Ascosphaerales that comprised only 0.05% of the total population observed from dry seasons samples but contributed more than 20% RA of the total flowering season population. The presence and abundance of Ascosphaerales might be explained by their association with bees that are present and active during the flowering season in Namaqualand (Maxfield-Taylor *et al.*, 2015). Infection with genus *Ascosphaera* is fatal in adult bees, while larval bees become mummified (Maxfield-Taylor *et al.*, 2015). The presence of *Ascosphaera* in Namaqualand, a region recognised as a centre of bee diversity and endemism, especially of oligolectic bees (Mayer and Kuhlmann, 2004), is therefore of great concern and requires in depth future research.

The same trend, although less pronounced, was observed for the Order Chaetothyriales, with 1.3% and 14.8% of the total population belonging to this order in samples from the dry and flowering seasons, respectively. Fungi from the Order Chaetothyriales are known for favouring xeric conditions and for their ability to colonize extreme environments. Most species in the Order Chaetothyriales have yet to be cultured (de Hoog *et al.*, 2011). *Phaeococcomyces*, from the family Herpotrichiellaceae, was the most abundant genus observed from the Order Chaetothyriales in this study. These lower eukaryotes are known as poikilotolerant melanised fungi, with the ability to survive complete desiccation for prolonged periods, allowing them to thrive in arid environments

(Sterflinger *et al.*, 2012; Nai *et al.*, 2013). *Phaeococcomyces chersonesos* (the most abundant species from this study), *Sarcinomyces petricola*, *Knufia chersonesos* and *Knufia petricola* are recognised as synonyms for the same species (Tsuneda *et al.*, 2011; Nai *et al.*, 2013). Due to its significance as part of an early diverging fungal lineage, this species has been proposed as a model organism for studying symbiosis as well as pathogenicity (Nai *et al.*, 2013). The Herpotrichiellaceae are weak fungal competitors and are typically found in habitats where there is limited competition with other microorganisms (Chizhikova *et al.*, 2016). It has been reported that fungi from the Order Chaetothyriales have the ability to thrive on recalcitrant carbon, in the form of organic material resistant to decomposition (Nai *et al.*, 2013).

A similar trend as observed for the Order Ascosphaerales in the culture-independent study was observed for the Order Diversisporales. Only 0.003% of the total population observed from dry season samples were members of the Order Diversisporales: c.f. 1.4% of the total population observed from flowering season samples. Fungi from the Order Diversisporales are known as hypogeous arbuscular mycorrhizal taxa (AMF) and classified in the ancient Phylum Glomeromycota. In this study, the abundance of AMF increased significantly in the flowering season after rainfall. AMF are known to respond directly to changes in soil moisture (Hawkes *et al.*, 2011), such as the rainfall events that initiate the flowering season in Namaqualand. Members of the Phylum Glomeromycota have the ability to rapidly respond to plant root exudates and other rhizodeposits (Philippot *et al.*, 2013) that are produced at the start of the flowering season.

The results of this study highlight the importance of seasonality in fungal community compositions. While most genera observed in this study were not limited to a specific season, there were statistically significant differences observed in species richness and diversity of the fungal communities sampled during the dry and the flowering season from soil in Namaqualand, as well as with the species involved in each community and the key contributors in abundance of each community. No single genus dominated the fungal population observed in either of the seasons or both seasons combined. The fungal population observed during the dry season was more diverse

and species rich than that of the flowering season, whilst certain species, as previously described, dominated the flowering season to a higher degree than during the dry season.

The precipitation events that occur in Namaqualand create optimal conditions for Aizoaceae to release seed from their fruit capsules and establish new connections with their fungal co-inhabitants. Fungi, via their hyphal networks and with their ability to access water and nutrients unavailable to other organisms such as bacteria, have high community plasticity coupled with the ability to rapidly respond to changes in soil moisture content in arid environments (Kaisermann *et al.*, 2015; Velez *et al.*, 2016). Due to the limited current knowledge on how soil fungal communities respond to temperature and precipitation changes (Solomon *et al.*, 2007) and even more inadequate knowledge of fungal phenology (Matulich *et al.*, 2015), confident predictions on the impact of climate change on fungal biodiversity are not possible, particularly for biodiversity hotspots such as the South African Succulent Karoo where environmental conditions in different seasons are vastly different (Matulich *et al.*, 2015).

To our knowledge, the only other study on the seasonality of fungal communities associated with succulent plants in an arid environment was done on the monocot plant, *Agave* (Colemann-Derr *et al.*, 2016), where it was observed that seasonality significantly influenced the fungal community. The results of the current study, where seasonality affected the diversity, richness and abundance of fungi associated with Aizoaceae, are consistent with these observations. The results of our study highlight the need to include temporal factors when undertaking environmental sampling, so as to eliminate inconsistencies associated with single time point studies (Kivlin *et al.*, 2016).

Declarations of interest

None.

Contributions of authors

Zelda Pieterse: Laboratory work and writing the manuscript; Theresa Aveling: Field work and writing the manuscript; Adriaana Jacobs: data analysis and writing the manuscript; Don Cowan: writing the manuscript and securing funding.

Statement of relevance

The Succulent Karoo biodiversity hotspot in South Africa, is the most biodiverse arid ecoregion in the world. It is home to the mostly endemic plant family, the Aizoaceae. The microbial populations in the soil that interact with Aizoaceae have not previously been investigated by next generation sequencing. In this study fungal rhizosphere communities associated with soil surrounding Aizoaceae plants in the Namaqua National were sampled by both culture-dependent and culture-independent methods to determine the major role players in seasonality. during the months of May (dry season) and October (flowering season) and identified by morphological and molecular methods. Seasonal differences were most pronounced for the Order Ascosphaerales, which contributed less than 0.1% to the total population during the dry season, but more than 20% to the flowering season population. This study provides the first next generation sequencing analysis of the remarkable soil-associated fungal richness and diversity in the Succulent Karoo and offer new insights into the dynamic nature of fungal populations in arid environments and the role of seasonality.

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