Fungal diversity associated with the mycorrhizosphere soil of *Brachycorythis conica* subsp. *transvaalensis,* a critically endangered and endemic terrestrial orchid from South Africa

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Abstract

The Albertina Sisulu orchid, *Brachycorythis conica* subsp. *transvaalensis* is a critically endangered terrestrial orchid with a single population remaining in the Gauteng Province of South Africa. For the conservation of this endemic orchid, several strategies are being implemented such as protection of habitat, identifying pollinators and *in vitro* propagation. For symbiotic germination, it is essential to identify the mycorrhizal associates of this orchid using non-destructive sampling. In this study, high-throughput sequencing was used to catalogue and compare the diversity of fungi associated with the mycorrhizosphere of this orchid and non-mycorrhizosphere soils collected from the same coordinates. Bioinformatics and statistical analyses of the data showed that, despite the substantial overlap in the community composition of fungi associated with these two soil types, several exclusive fungal species were identified from the mycorrhizosphere of the orchid. These included an assortment of potential orchid mycorrhizal species from the orders Agaricales, Cantharellales and Sebacinales. This study provides the first insight into the soil fungal diversity associated with the mycorrhizosphere of this critically endangered orchid. In the future, data from this study can be used for optimising conservation measures and isolation of suitable mycorrhizal species required for *in vitro* symbiotic germination of this orchid.

Keywords: Agaricales, Albertina Sisulu orchid, Cantharellales, orchid mycorrhizae, Sebacinales

1. Introduction

The plant-associated microbial diversity includes beneficial and pathogenic organisms in addition to many others whose specific roles remain unknown (Berendsen et al. 2012; Berg et al. 2015). Most plant organs are colonized by different microbial communities, with the highest diversity of beneficial microorganisms in and around the roots (Baldrian 2017; Berendsen et al. 2012). Most plant species are associated with mycorrhizae which is a symbiotic association between plant roots and fungi (Brundrett and Tedersoo 2018; Strullu-Derrien et al. 2014). In addition to assisting plants with mineral nutrient and water uptake, mycorrhizae improve disease and stress tolerance (Babikova et al. 2013; Jung et al. 2012; Pozo and Azcón-Aguilar 2007). Certain plant taxa such as orchids have an obligate symbiosis with mycorrhizal fungi and cannot survive without these fungal associations in nature.

Orchids produce small, wind-dispersible seeds. Due to their small size, these seeds lack endosperm tissue that can serve as a nutrient source for the developing embryo during germination. To acquire nutrients, the germinating orchid embryo forms an obligate association with one or more mycorrhizal fungi (Rasmussen et al. 2015; Smith and Read 2010). In mature photosynthetic orchids a continued mutually beneficial interaction is highly likely, where orchids provide the mycorrhizal fungi with carbohydrates in exchange for mineral nutrients (Dearnaley et al. 2012). Non-photosynthetic mycoheterotrophic orchids, on the other hand, rely on their mycorrhizal associates for all their nutrients throughout their life cycle (Leake 2005).

Most orchid mycorrhizal fungi are from the phylum Basidiomycota, while a few are from the Ascomycota (Dearnaley 2007). Classically, orchids were known to exclusively associate with fungi from the 'Rhizoctonia' complex (Dearnaley et al. 2012). However, recent microbiome studies using high-throughput sequencing showed that symbiotic fungal species that associate with orchids are more diverse and include taxa from Thelephoraceae, Serendipitaceae, Atractiellomycetes among others (Jacquemyn et al. 2015; Kottke et al. 2008; Kottke et al. 2010; Martos et al. 2009; Martos et al. 2012; McCormick et al. 2018; Oja et al. 2015; Suárez et al. 2006; Suárez et al. 2008; Valadares et al. 2021). The community composition of orchid mycorrhizal fungi is substantially influenced by the host plant species, life stages, and various ecological factors (Dearnaley et al. 2012; Li et al. 2021; Ventre Lespiaucq et al. 2021) and usually includes both unique and cosmopolitan fungal species (Herrera et al. 2019; Valadares et al. 2021; Yaun et al. 2010).

About 500 orchid species have been identified in South Africa of which nearly 94 % are endemic (Johnson and Bytebier 2015). Echoing global trends, this South African orchid biodiversity is threatened by climate change, illegal collection, habitat destruction and encroachment by invasive plant species (Ballantyne and Pickering 2012; Herrera et al. 2019; Johnson and Bytebier 2015; Swarts and Dixon 2009; Wraith et al. 2020). In the list of threatened plant species published by the South African National Biodiversity Institute (SANBI) at least 70 orchid species were marked as critically endangered

while 140 are of conservation concern (SANBI 2020). Conservation approaches such as seed banks, tissue culture, restoration and maintaining native ecosystems are being implemented. However, the specificity of orchids towards their insect pollinators and mycorrhizal fungi complicate these conservation initiatives (Swarts and Dixon 2009).

Habitat destruction has endangered several orchid species in South Africa, such as *Brachycorythis conica* subsp. *transvaalensis* (Chinsamy et al. 2011; Raimondo et al. 2013; SANBI 2020). This orchid is characterized by sweet-scented white flowers with pink flecks (Fig. 1A) and a unique tuberous root system which lacks distinct lateral roots (Fig. 1B). This orchid was formally described in 1955, the same year Albertina Sisulu (a South African anti-apartheid activist), together with the African National Congress Women's League, launched the freedom charter (Hankey 2016). As a result, to honour Albertina Sisulu's contribution to the anti-apartheid struggle, the common name of this orchid was named after her.

Since its discovery, a few populations of this orchid were reported from Limpopo and Mpumalanga Provinces. However, the only surviving population of this orchid (about 68 plants) is located in the Krugersdorp area, Gauteng Province (Peter et al. 2019; Raimondo et al. 2013). This population is threatened by construction projects which have been temporarily halted by community initiatives (Hankey and Cooper 2018). To protect the remaining population of *B. conica* subsp. *transvaalensis*, several conservation measures have been implemented, such as restriction of access to its habitat, eradication of invasive species, *in vitro* seed germination, as well as identifying its pollinators and mycorrhizal symbionts (Hankey and Cooper 2018; Peter et al. 2019).

In the present study, we used high-throughput sequencing to catalogue the fungal diversity associated with the mycorrhizosphere soil of *B. conica* subsp. *transvaalensis* and compared it with non-mycorrhizosphere soil collected from the same coordinates. We hypothesised that soil types would influence the fungal community composition and richness, and that the orchid's mycorrhizosphere soil would contain a diverse range of mycorrhizal fungal species.

2. Methods and Materials

2.1 Collection of soil samples

Due to the current conservation status of *B. conica* subsp. *transvaalensis*, collection of live plant samples was not feasible. Therefore, mycorrhizosphere soil samples from the orchids were used in the present study.

In Apr 2018, six soil samples (3 samples × 2 soil types) were collected near the Walter Sisulu National Botanical Garden, Krugersdorp (26°04'31.4"S, 27°49'02.3"E). Soil was collected from the mycorrhizospheres of three *B. conica* subsp. *transvaalensis* plants that were about 30 metres apart.

From each orchid, one soil sample was collected. After removing the topsoil and plant litter, a 12 cm^2 soil core was extracted 10 cm away from the orchid at a depth of 10 cm. Three non- mycorrhizosphere soil samples were randomly collected from a site 50 m to the north of this orchid population where no orchids have been previously observed.

2.2 Soil sample preparation and extraction of environmental DNA

All the soil samples were dried at room temperature (21-23 °C) for two weeks. Approximately 50 g of each soil sample was pulverized using a Retsch grinding jar attached to a Qiagen TissueLyser II for 2 min at 20 frequency/sec. After each pulverization step, the grinding jars were surface sterilized using 4 % (v/v) sodium hypochlorite solution and 4N hydrochloric acid. Thereafter, the jars were thoroughly rinsed with sterile distilled water and dried using a blow dryer.

DNA was extracted from 0.5 g of each soil sample using the Mo-Bio PowerSoil® DNA Isolation Kit following the manufacturer's protocols. All DNA samples were stored at -20 °C until the preparation of the fungal amplicon library.

2.3 Preparation of amplicon library

Each soil DNA sample was amplified in triplicate using two sets of primers targeting the complete Internal Transcribed Spacer (ITS1 region -5.8S gene-ITS2 region) and the total fungal diversity was amplified using primers ITS1F and ITS4 (Gardes and Bruns 1993; White et al. 1990). For detecting Tulasnellaceae, each DNA sample was separately amplified using primers ITS1 and ITS4-Tul (Taylor and McCormick 2008). Each 25 µl PCR reaction included 5mM 5 × Promega GoTaq Flexi Buffer, 2.5 mM Promega $MgCl_2$, 0.1 mM Promega dNTPs, 1.5 mM Amresco BSA, 1U Promega GoTaq Hot Start Polymerase, 0.2 mM of each primer, 2 µl template DNA, and the final volume was made up with PCR grade water. PCR conditions were 96 °C for 2 min, followed by 30 cycles of 94 ˚C for 30 sec, 60 ˚C for 40 sec (ITS1F + ITS4) / 54 ˚C for 40 sec (ITS1 + ITS4-Tul), 72˚C for 1 min, and final extension for 72 ˚C for 10 min. PCR products were verified using gel electrophoresis.

2.4 Pooling of amplicons and amplicon sequencing

For each soil sample, three separate PCR replicates for each primer pair were pooled into a single sample. Thereafter, 25 µL of each pooled PCR product was cleaned using Agencourt AMPure XP PCR purification beads (Beckman Coulter Genomics, USA). Amplicon library preparation and Illumina MiSeq sequencing were outsourced to Inqaba Biotechnical Industries (Pty) Ltd, SA. The raw Illumina data was deposited in the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/docs) under the accession number PRJNA693177.

2.5 Analyses of high-throughput sequencing data

The Illumina MiSeq sequencing data was demultiplexed by Inqaba Biotechnical Industries (Pty) Ltd. The ITS 1 region was used for further analyses using Quantitative Insights into Microbial Ecology 2 (QIIME2) v2020.8 (Bolyen et al. 2019). The plugin 'q2-dada2' (Callaham et al. 2016) was used for

filtering, trimming, denoising and deletion of singletons and chimeras. During filtering, sequences shorter than 200 bp with more than 6 bp homopolymers and a Phred quality score below 30 were discarded from the analysis. The 'q2-vsearch' plugin (Rognes et al. 2016) was used for the *de novo* assembly of the reads at a 98 % sequence similarity. Taxonomy was assigned to Operational Taxonomic Units (OTUs) using the plugin 'qiime feature-classifier' (Bokulich et al. 2018). The UNITE fungal ITS database v8.2 (Abarenkov et al. 2020) was used as the reference for assigning taxon names to the OTUs.

2.6 Statistical analyses of microbiome data

The species richness, Shannon, and Simpson diversity indices were calculated to compare the soil fungal diversity among the two sample types, mycorrhizosphere and non-mycorrhizosphere soils. The number of different taxa per sample was used to calculate the species richness. A Principal Coordinate Analysis (PCoA) was used to visualize the fungal community composition in different soil types. PCoA was computed using an abundance matrix, using Bray-Curtis dissimilarity. These statistical analyses were performed using the pipeline available through Calypso v8.84 (Zakrzewski et al. 2017). To see if community composition of soil fungi varied statistically among different soil types, we used a permutational multivariate analysis of variance (PERMANOVA) using the 'adonis' function of the 'vegan' package of R version 4.1.0 (R Core Team 2020). Krona plots were generated with Krona tools V2.7.1 (Ondov et al. 2011)

3. Results

3.1 Fungal diversity associated with soil samples

A total of 182 797 raw reads were obtained from high-throughput sequencing of environmental DNA extracted from mycorrhizosphere and non-mycorrhizosphere soil samples. After quality filtering, 162 222 (88.75 %) reads were used for downstream analyses. A substantial portion of these reads were recovered from the mycorrhizosphere of three orchids (92 004 reads). A total of 100 fungal OTUs were identified after *de novo* assembly of the filtered reads recovered from both soil types. The majority of these OTUs were represented by Ascomycota (69 %) and Basidiomycota (25 %). The remaining OTUs were from Mucoromycota (4 %), and Mortierellomycota (2 %) (Fig. 2A and 3A, B).

Based on soil types, 74 fungal OTUs were detected from the mycorrhizosphere soil of *B. conica* subsp. *transvaalensis*, whereas non-mycorrhizosphere soil contained 72 OTUs. Among these, 48 OTUs were mutually shared between the two soil types (Fig. 2B). Orchid mycorrhizosphere and non- mycorrhizosphere soils included 28 and 26 exclusive fungal OTUs, respectively (Fig. 2B).

Fungal species richness, as demonstrated by the Shannon and Simpson indices, were not significantly influenced by the soil type ($P > 0.05$). In the PCoA plot the data points clustered by soil

types without any overlap (Fig. 4). In addition, a PERMANOVA comparing soil types also suggested it being a significant factor influencing fungal diversity ($P < 0.04$).

3.2 Community composition of fungi associated with the mycorrhizosphere of B. conica subsp. transvaalensis

The proportion of Basidiomycota was higher in the mycorrhizosphere of the orchid (Fig. 2 C and D), while the non- mycorrhizosphere soil included a higher percentage of 'unidentified fungi' (Fig. 3A, B). The Basidiomycota included some unclassified fungi from known orchid mycorrhizal taxa in the order Sebacinales (unidentified) and the families Entolomataceae and Psathyrellaceae, and Tulasnellaceae (Fig. 3A, and 5).

The orchid mycorrhizosphere soil contained several exclusive fungi from the Ascomycota. The diversity of fungi from the Pleosporales was higher in the orchid's mycorrhizosphere (30%) than in non- mycorrhizosphere soils (19 %; Figs. 3A, B and 5).

4. Discussion

In the present study, high-throughput sequencing was used for cataloguing and comparing the fungal diversity associated with the mycorrhizosphere of *B. conica* subsp. *transvaalensis* and non-mycorrhizosphere soil. Analyses of the sequence data showed that there was a substantial overlap in fungal OTUs in the two soil types, yet there were also striking differences and more than 20 fungal taxa were unique in each soil type. The orchid's mycorrhizosphere included an assortment of fungi from the Agaricales, Cantharellales, and Sebacinales which are taxonomically related to previously described orchid mycorrhizal fungi (Dearnaley et al. 2012; Jacquemyn et al. 2017; Kottke et al. 2008; Martos et al. 2009; Selosse et al. 2010; Suárez et al. 2006; Suárez et al. 2008; Valadares et al. 2021; Waterman et al. 2011). The fungal species found in both soil types are members of the microbiome that naturally occurs in the grassland ecosystem from where both soil types were collected.

Earlier research showed that the majority of orchid mycorrhizal fungi reside in the Basidiomycota (Jacquemyn et al. 2017; Kottke et al. 2008; Valadares et al. 2021). This is in line with the results of the present study where a majority of the taxa identified as orchid mycorrhizal fungi belonged to this phylum. These included undescribed taxa from the orders Agaricales (*Clitopilus* and *Coprinellus)* and Cantharellales (unidentified Tulasnellaceae). The undescribed Sebacinales was simultaneously detected in both the soil types in this study. Fungi from this order form symbiotic associations with a wide variety of plants (Cannon and Kirk 2007; Kottke et al. 2008). However, in this study the read count for this undescribed Sebacinales was higher in the mycorrhizosphere of the orchid, suggesting a potential symbiotic association with *B. conica* subsp. *transvaalensis*.

Fungi from the Pleosporales (Ascomycota) are frequently detected from the roots of various species of orchids (Jacquemyn et al. 2017; Schweiger 2019). It is still unclear whether these fungi are symbionts or endophytes in the orchid roots. In the current study, Pleosporales was one of the most common fungal orders recovered from both soil types. Among these, at least eight taxa were exclusively identified from the mycorrhizosphere of the orchid. These are unidentified species of *Coniothyrium*, *Pyrenochaeta*, *Dictyosporiaceae*, *Keissleriella*, *Phaeosphaeriaceae*, *Dictyosporium heptasporum* and *Pseudocoleophoma bauhiniae*. Most of these genera are either known as plant pathogens or saprophytes (Zhang et al. 2009). However, fungal species in the genera *Coniothyrium* and *Pyrenochaeta* have also been identified as endophytes from orchids (Novotna et al. 2018; Tan et al. 2012). Some of the Pleosporales exclusively detected from the mycorrhizosphere soil might live in symbiosis with *B. conica* subsp. *transvaalensis* in a similar manner as has been described for other saprophytes and plant pathogens from the orders Agaricales and Cantharellales (Andersen and Rusmussen 1996; Selosse et al. 2010). To confirm this hypothesis, infection trials would be required.

Previously, Waterman et al. (2011) catalogued the diversity of mycorrhizal fungi associated with various South African orchids. The sampling areas of the present study to that of Waterman and co-workers were distinct. Nonetheless, orchids from both studies belonged to the subfamily Orchidoideae. According to Waterman et al. (2011), fungal preferences for orchids are largely preserved, even among closely related clades. A comparison of the results from this study with that of Waterman and co-workers include both overlapping (Tulasnellaceae and Sebacinales) and distinct fungal taxa. It is also possible that the distinct root architecture of *B. conica* subsp. *transvaalensis* might influence the spectrum of soil fungi that can associate with it and explain the presence of the taxa that were not observed by Waterman and co-workers.

South Africa houses a diverse range of terrestrial orchids. However, research on their associated mycorrhizal fungi is scarce. Through this study, we identified various potential orchid mycorrhizal fungi in the mycorrhizosphere of *B. conica* subsp. *transvaalensis* using short-read amplicon sequencing. However, we could not achieve species-level identity for many of these putative orchid mycorrhizal fungi. This is due to the constraints of the short-read sequencing technique and the fungal reference database used in this study (Hibbett et al. 2016; Lücking et al. 2020; Nilsson et al. 2019; Xu 2016).

In the future, studies involving *B. conica* subsp. *transvaalensis* should consider isolating its symbiotic fungi from the roots and tubers for direct application in the conservation of this orchid. However, this is not a simple endeavour, as destructive sampling of this critically endangered orchid is not feasible, and isolating orchid mycorrhizal is often challenging (Zhu et al. 2008). Enrichment of orchid mycorrhizal fungi for isolations could be achieved by baiting the soil with orchid seeds, which are rarely available (Brundrett et al. 2003; Phillips et al. 2011; Yang et al. 2020; Zi et al. 2014). Besides this, shifting focus to other, more abundant orchids in the area, such as *Habenaria epipactidea* for isolating mycorrhizal fungi directly from the roots might be beneficial. It is not known how specific the

interaction between orchids and their mycorrhizal fungi in the region are, but earlier studies on terrestrial orchids suggested a degree of non-specificity between orchids and their fungal partners (Taylor et al. 2003; Warcup 1971). Testing the efficacy of mycorrhizal fungi isolated from orchids growing in the same region for germination of B. conica subsp. transvaalensis may thus yield positive results for the conservation of this orchid. In addition, we propose using long-read sequencing of the mycorrhizosphere -associated mycobiome of *B. conica* subsp. *transvaalensis* to more closely identify the taxonomic identity of fungi involved in the interaction.

5. Conclusion

In this study, we investigated the fungal diversity associated with the mycorrhizosphere of *B. conica* subsp. *transvaalensis*, a critically endangered South African terrestrial orchid. This orchid lacks a well-defined root system, yet when comparing the fungal diversity between mycorrhizosphere and non- mycorrhizosphere soils, we identified both overlapping and exclusive taxa. Furthermore, a significant portion of the mycorrhizosphere fungal diversity included previously undescribed fungi. It is reasonable to assume that some of the identified fungi are symbiotically associated with the plants. However, their symbiotic relationships with this orchid will remain unknown until live plant sampling becomes feasible. Overall, data from this work will be useful in the future for optimizing conservation efforts.

Declaration of Competing Interest

The authors state that they have no known competing financial interests or personal connections that may seem to have influenced the work described in this publication.

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Data accessibility

The high-throughput sequencing data generated in this study is available at the NCBI Sequence Read Archive (https://submit.ncbi.nlm.nih.gov/ subs/sra/) under the accession number PRJNA693177.

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Figure legends

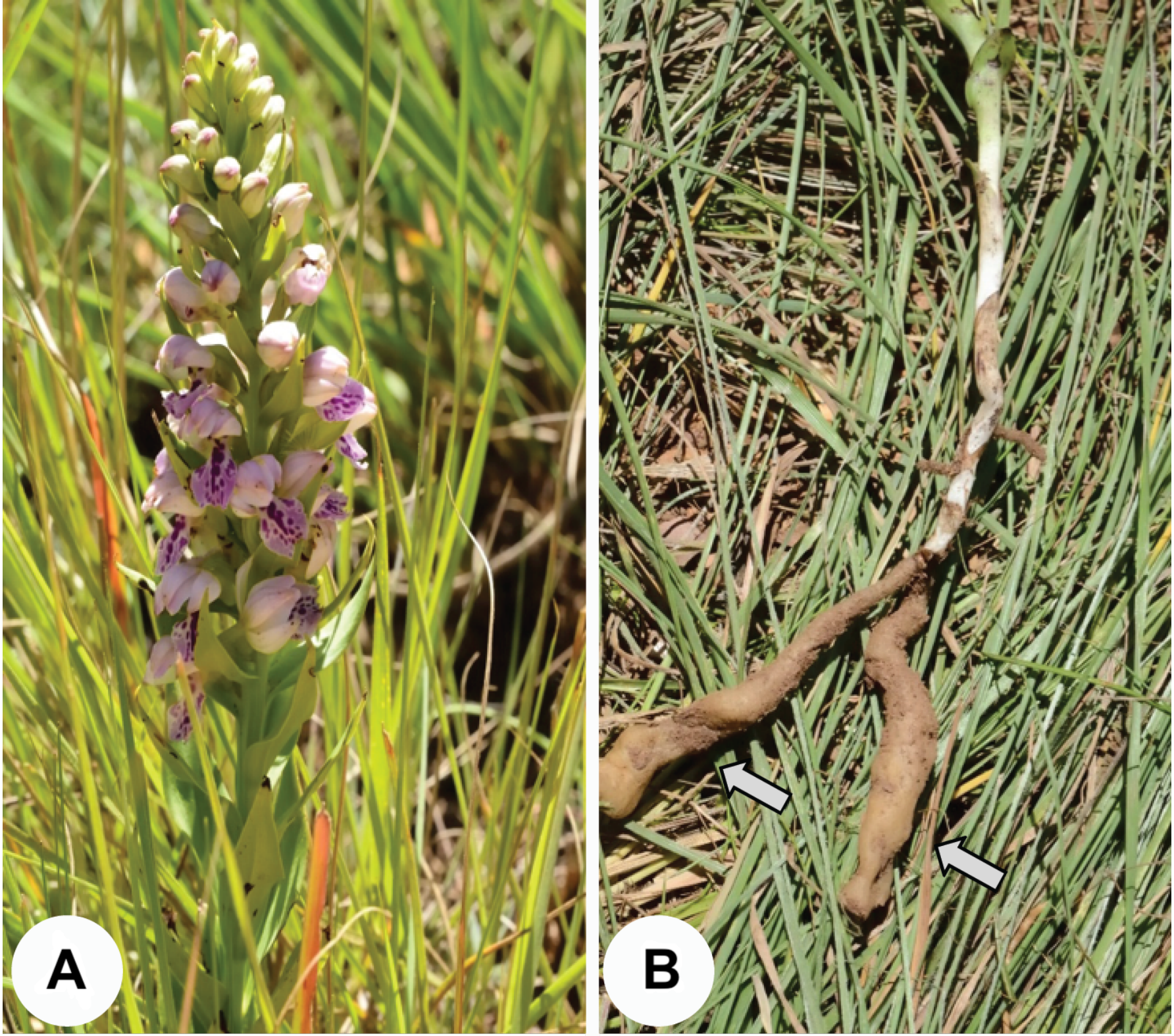
Figure 1. *Brachycorythis conica* subsp. *transvaalensis.* (A) Above-ground plant with inflorescence, and (B) subterranean tuberous structure (indicated by arrows) lacking a lateral root system.

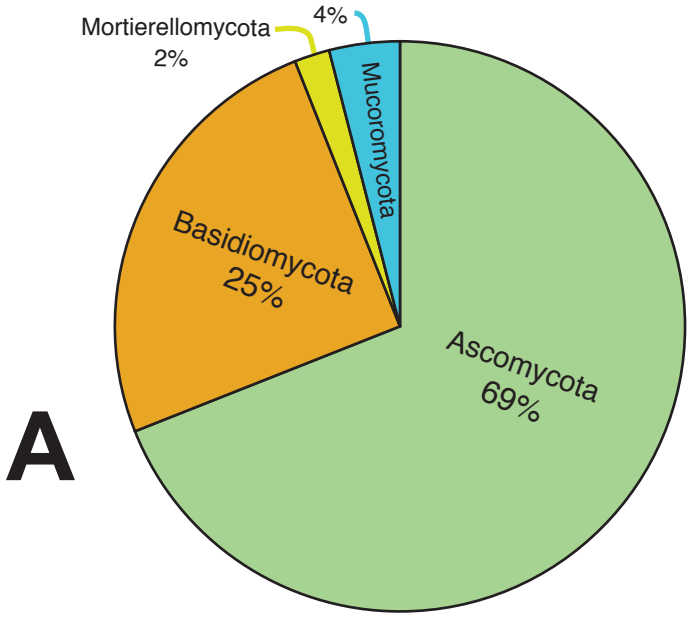
Figure 2. Graphical representations of fungal taxa identified from the mycorrhizosphere of *Brachycorythis conica* subsp. *transvaalensis* and non-mycorrhizosphere soils. (A) From both soil types together; (B) shared and unique taxa between the two soil types; (C) fungal phyla detected from mycorrhizosphere soil with percentage of predicted mycorrhizal and non-mycorrhizal taxa; and (D) fungal phyla detected from non-mycorrhizosphere soil with percentages of predicted mycorrhizal and non-mycorrhizal taxa.

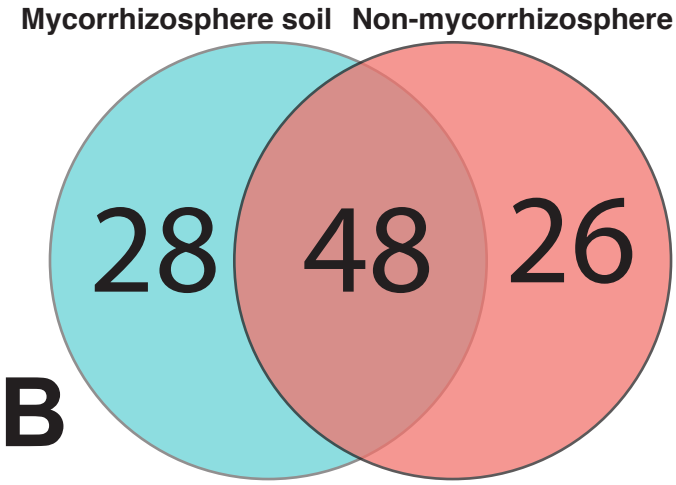
Figure 3. Krona plots showing the diversity of fungal genera (where available) detected from high-throughput sequencing of soil samples collected from the (A) mycorrhizosphere of *Brachycorythis conica* subsp. *transvaalensis* and (B) non-mycorrhizosphere soils.

Figure 4. Box plots of (A) species richness, (B) Shannon, and (C) Simpson diversity indexes of soil fungal communities associated with the mycorrhizosphere of *Brachycorythis conica* subsp. *transvaalensis* and non-mycorrhizosphere soils. (D) Principal Coordinates Analysis of soil fungal communities associated with mycorrhizosphere and non-mycorrhizosphere soil.

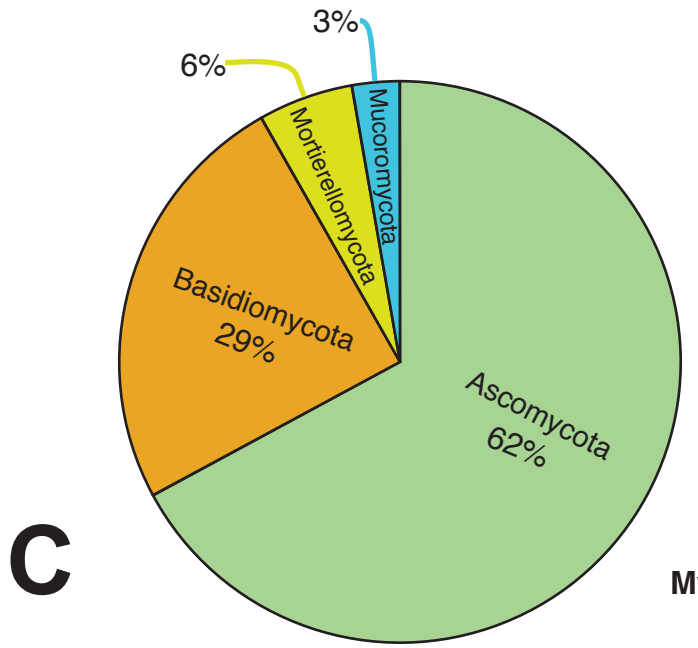
Figure 5. Distribution of fungal taxa (up to species level, where available) detected from the mycorrhizosphere of *Brachycorythis conica* subsp. *transvaalensis* and non-mycorrhizosphere soils. Taxa exclusively detected from the mycorrhizosphere = blue bars, non-mycorrhizosphere soil = pink bars and present in both soil types = blue and pink bars. Orchid mycorrhizal fungal orders are highlighted in pink = Agaricales, yellow = Cantharellales, and blue = Sebacinales.

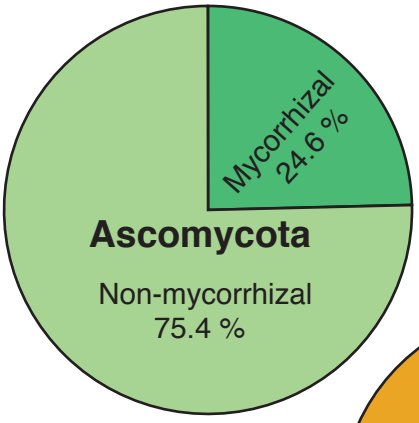

A
B

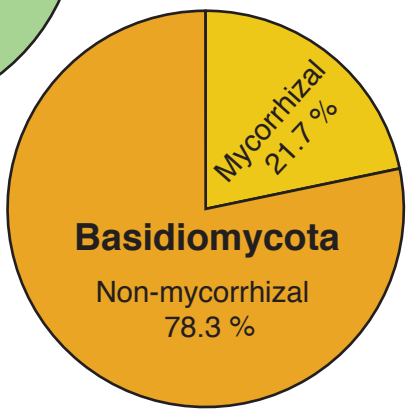




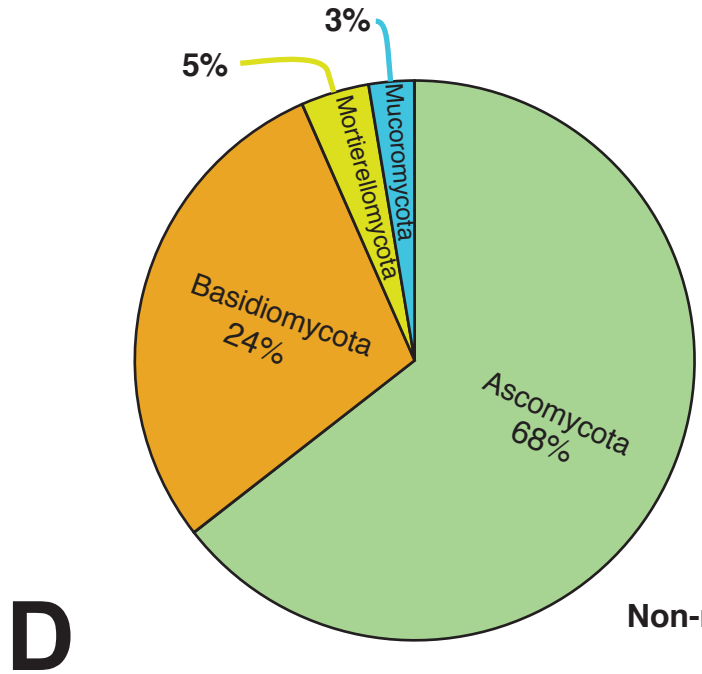
B

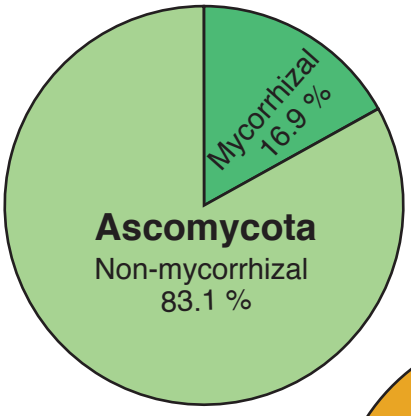


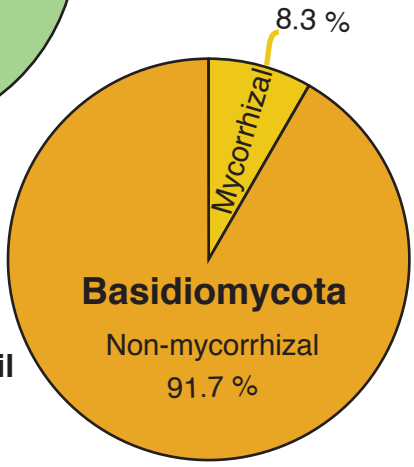




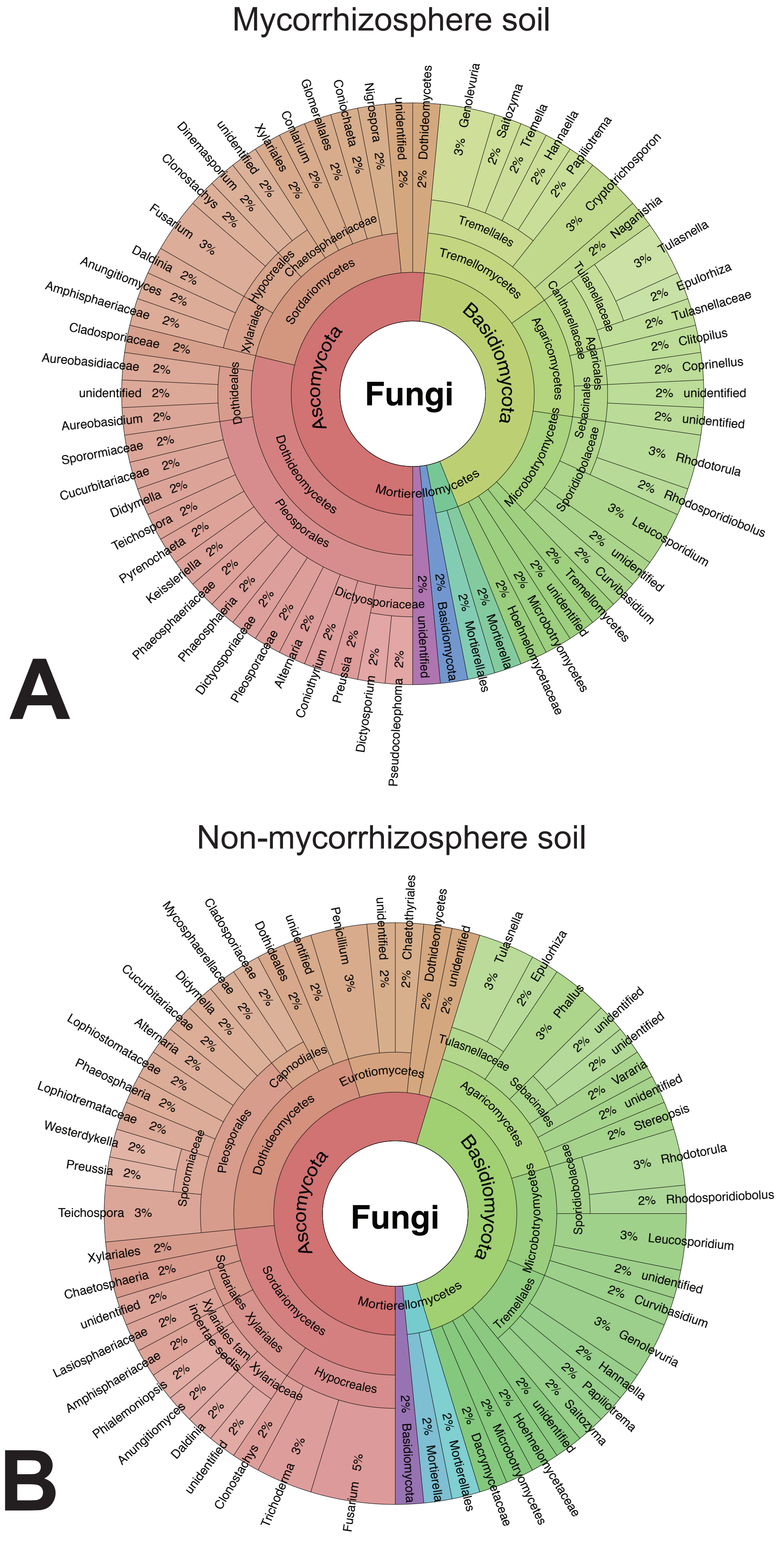
Mycorrhizosphere soil

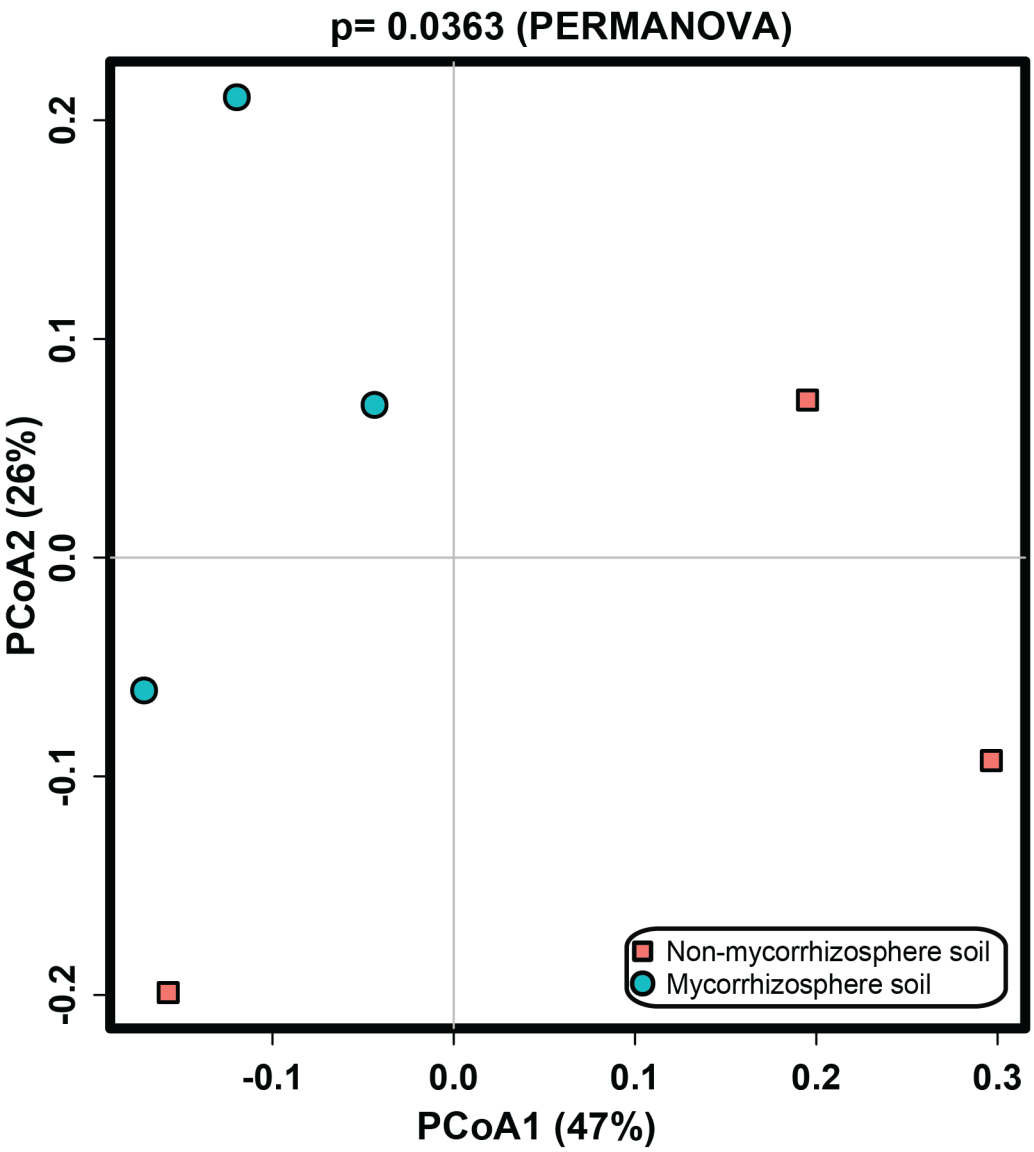


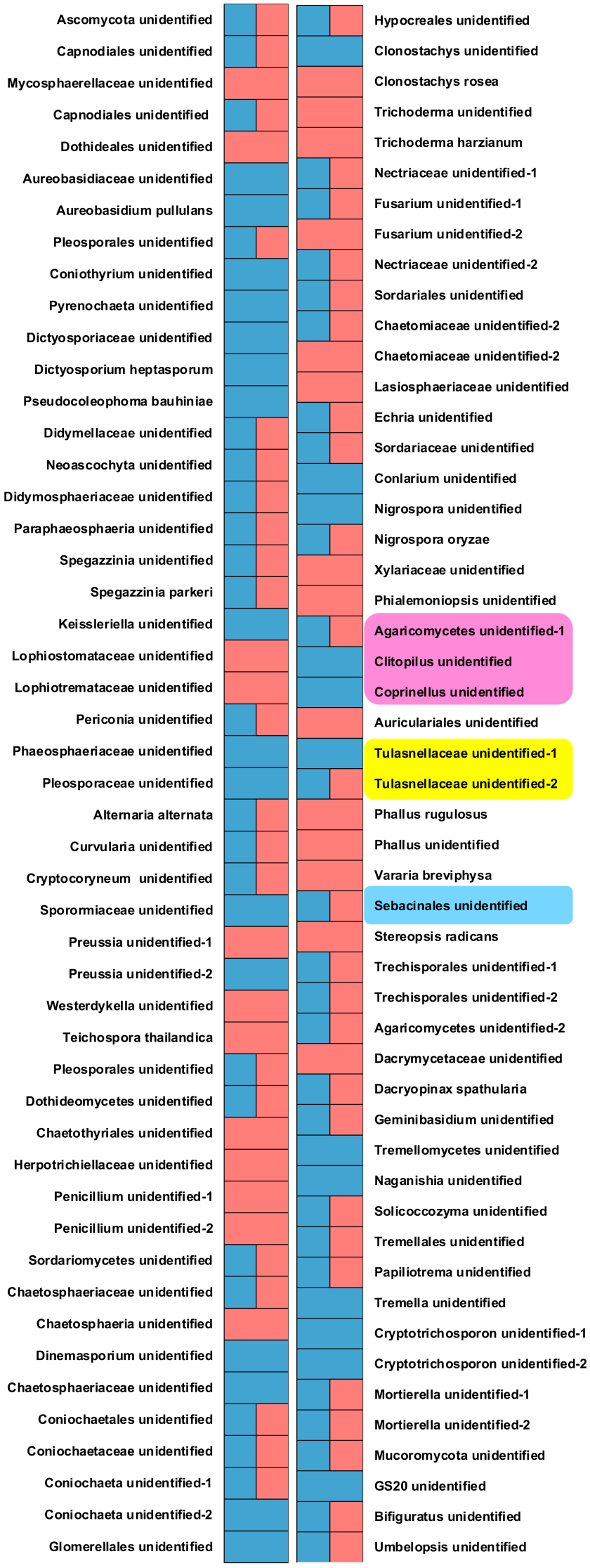




Non-mycorrhizosphere soil


Mycorrhizosphere soil
A
Fungi
Ascomycota
Basidiomycota
Mortierellomycetes
Sordariomycetes
Dothideomycetes
Tremellomycetes
Agaricomycetes
Microbotryomycetes
Xylariales
Hypocreales
Chaetosphaeriaceae
Dothideales
Pleosporales
Dictyosporiaceae
Tremellales
Cantharellaceae
Tulasnellaceae
Agaricales
Sebacinales
Sporidiobolaceae
Dinemasporium 2%
unidentified 2%
Xylariales 2%
Conlarium 2%
Glomerellales 2%
Coniochaeta 2%
Nigrospora 2%
unidentified 2%
Dothideomycetes 2%
Genolevuria 3%
Saitozyma 2%
Tremella 2%
Hannaella 2%
Papiliotrema 2%
Cryptotrichosporon 3%
Naganishia 2%
Tulasnella 3%
Epulorhiza 2%
Tulasnellaceae 2%
Clitopilus 2%
Coprinellus 2%
unidentified 2%
unidentified 2%
Rhodotorula 3%
Rhodosporidiobolus 2%
Leucosporidium 3%
unidentified 2%
Curvibasidium 2%
Tremellomycetes 2%
unidentified 2%
Microbotryomycetes 2%
Hoehnelomycetaceae 2%
Mortierella 2%
Mortierellales 2%
Basidiomycota 2%
unidentified 2%
Pseudocoleophoma 2%
Dictyosporium 2%
Preussia 2%
Coniothyrium 2%
Alternaria 2%
Pleosporaceae 2%
Dictyosporiaceae 2%
Phaeosphaeria 2%
Phaeosphaeriaceae 2%
Keissleriella 2%
Pyrenochaeta 2%
Teichospora 2%
Didymella 2%
Cucurbitariaceae 2%
Sporormiaceae 2%
Aureobasidium 2%
unidentified 2%
Aureobasidiaceae 2%
Cladosporiaceae 2%
Amphisphaeriaceae 2%
Anungitiomyces 2%
Daldinia 2%
Fusarium 3%
Clonostachys 2%
Non-mycorrhizosphere soil
B
Fungi
Ascomycota
Basidiomycota
Mortierellomycetes
Dothideomycetes
Eurotiomycetes
Sordariomycetes
Capnodiales
Pleosporales
Sporormiaceae
Sordariales
Xylariales
Xylariales fam incertae sedis
Xylariaceae
Hypocreales
Agaricomycetes
Tulasnellaceae
Sebacinales
Microbotryomycetes
Sporidiobolaceae
Tremellales
Mycosphaerellaceae 2%
Cladosporiaceae 2%
Dothideales 2%
unidentified 2%
Penicillium 3%
unidentified 2%
Chaetothyriales 2%
Dothideomycetes 2%
unidentified 2%
Tulasnella 3%
Epulorhiza 2%
Phallus 3%
unidentified 2%
unidentified 2%
Vararia 2%
unidentified 2%
Stereopsis 2%
Rhodotorula 3%
Rhodosporidiobolus 2%
Leucosporidium 3%
unidentified 2%
Curvibasidium 2%
Genolevuria 3%
Hannaella 2%
Papiliotrema 2%
Saitozyma 2%
unidentified 2%
Hoehnelomycetaceae 2%
Microbotryomycetes 2%
Dacrymycetaceae 2%
Mortierellales 2%
Mortierella 2%
Basidiomycota 2%
Fusarium 5%
Trichoderma 3%
Clonostachys 2%
unidentified 2%
Daldinia 2%
Anungitiomyces 2%
Phialemoniopsis 2%
Amphisphaeriaceae 2%
Lasiosphaeriaceae 2%
unidentified 2%
Chaetosphaeria 2%
Xylariales 2%
Teichospora 3%
Preussia 2%
Westerdykella 2%
Lophiotremataceae 2%
Phaeosphaeria 2%
Lophiostomataceae 2%
Alternaria 2%
Cucurbitariaceae 2%
Didymella 2%


p= 0.0363 (PERMANOVA)
PCoA2 (26%)
0.2
0.1
0.0
-0.1
-0.2
-0.1
0.0
0.1
0.2
0.3
PCoA1 (47%)
Non-mycorrhizosphere soil
Mycorrhizosphere soil


Ascomycota unidentified
Capnodiales unidentified
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Aureobasidium pullulans
Pleosporales unidentified
Coniothyrium unidentified
Pyrenochaeta unidentified
Dictyosporiaceae unidentified
Dictyosporium heptasporum
Pseudocoleophoma bauhiniae
Didymellaceae unidentified
Neoascochyta unidentified
Didymosphaeriaceae unidentified
Paraphaeosphaeria unidentified
Spegazzinia unidentified
Spegazzinia parkeri
Keissleriella unidentified
Lophiostomataceae unidentified
Lophiotremataceae unidentified
Periconia unidentified
Phaeosphaeriaceae unidentified
Pleosporaceae unidentified
Alternaria alternata
Curvularia unidentified
Cryptocoryneum unidentified
Sporormiaceae unidentified
Preussia unidentified-1
Preussia unidentified-2
Westerdykella unidentified
Teichospora thailandica
Pleosporales unidentified
Dothideomycetes unidentified
Chaetothyriales unidentified
Herpotrichiellaceae unidentified
Penicillium unidentified-1
Penicillium unidentified-2
Sordariomycetes unidentified
Chaetosphaeriaceae unidentified
Chaetosphaeria unidentified
Dinemasporium unidentified
Chaetosphaeriaceae unidentified
Coniochaetales unidentified
Coniochaetaceae unidentified
Coniochaeta unidentified-1
Coniochaeta unidentified-2
Glomerellales unidentified
Hypocreales unidentified
Clonostachys unidentified
Clonostachys rosea
Trichoderma unidentified
Trichoderma harzianum
Nectriaceae unidentified-1
Fusarium unidentified-1
Fusarium unidentified-2
Nectriaceae unidentified-2
Sordariales unidentified
Chaetomiaceae unidentified-2
Chaetomiaceae unidentified-2
Lasiosphaeriaceae unidentified
Echria unidentified
Sordariaceae unidentified
Conlarium unidentified
Nigrospora unidentified
Nigrospora oryzae
Xylariaceae unidentified
Phialemoniopsis unidentified
Agaricomycetes unidentified-1
Clitopilus unidentified
Coprinellus unidentified
Auriculariales unidentified
Tulasnellaceae unidentified-1
Tulasnellaceae unidentified-2
Phallus rugulosus
Phallus unidentified
Vararia breviphysa
Sebacinales unidentified
Stereopsis radicans
Trechisporales unidentified-1
Trechisporales unidentified-2
Agaricomycetes unidentified-2
Dacrymycetaceae unidentified
Dacryopinax spathularia
Geminibasidium unidentified
Tremellomycetes unidentified
Naganishia unidentified
Solicoccozyma unidentified
Tremellales unidentified
Papiliotrema unidentified
Tremella unidentified
Cryptotrichosporon unidentified-1
Cryptotrichosporon unidentified-2
Mortierella unidentified-1
Mortierella unidentified-2
Mucoromycota unidentified
GS20 unidentified
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