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Higher turnover of endophytic fungal assemblages in the tissues of globose cactus *Melocactus ernestii* from Brazilian semi-arid biome

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Abstract
Endophytic fungi are extremely diverse in natural biomes, which display a unique plant-microbe association inside different living host tissues. Among the plants that shelter endophytes, the endophytic fungal assemblages of Cactaceae remain poorly understood. Our study characterized the taxonomy, diversity, and ecology of endophytic fungal assemblages living in different tissues of the cactus *Melocactus ernestii* present in the Brazilian Caatinga biome. A total of 222 endophytic fungi were obtained from roots, stems, and spines, which were identified in 99 operational taxonomic units (OTUs) of *Ascomycota* and *Basidiomycota* phyla. Most of the fungal taxa were recovered from root tissues, followed by stems and spines. The most abundant orders from *Ascomycota* were *Xylariales*, *Dothideomycetes*, and *Eurotiomycetes*. *Basidiomycota* is represented by *Cantharellales*, *Agaricales*, and *Geastrales*. Only *Nigrospora* sp. and *Preussia* sp. 1 were common among the three plant tissues, and 78.41% of the species were not shared among the populations and tissues. We detected similar richness patterns among the same tissue types using sample-based rarefaction and extrapolation curves. The multiple site dissimilarity across the plants and tissues showed greater disparities in species richness among *M. ernestii* fungal assemblages. These results highlight the compartmentalization of endophytic fungal species in the root tissue, and the endophytes sharing observed exclusively between spines and stems may reflect interactions of endophytic fungal assemblages with possible dependency on shared resources in cacti. Overall, our findings will an approach to understand changes in the diversity and the key roles of turnover of endophytic fungal assemblages in semi-arid environments.

Keywords Endophytes · Ecology · Fungi · Neotropical · Taxonomy · Caatinga

1 Introduction

Endophytes are microorganisms that form a unique plant-microbe association that is transiently symptomless, unobtrusive, and established entirely inside living host tissues such as bark, flowers, roots, stems, leaves, and seeds (Card et al. 2016). Endophytic communities may vary spatially in different tissues or organ types (Geisen et al. 2017). In addition, endophytic microorganism frequencies are influenced by climatic conditions (Unterseher et al. 2016), plant age (Liu et al. 2017), and other abiotic and biotic environmental factors, such as plant-soil and plant-plant interactions (Shymanovich and Faeth 2019). Among the endophytes, fungi represent one of the most diverse groups studied, which include mainly *Ascomycota* and *Basidiomycota* phyla taxa and their anamorphic forms (Rosa et al. 2010); these are represented by cosmopolitan generalist taxa, as well as singlets, which are considered rare and occur as minor taxa (eg. Silva-Hughes et al. 2015).

The Caatinga is a typical dryland ecosystem in Brazil and includes areas where rainfall is <65% of evaporative demand (Delgado-Baquerizo et al. 2013), which is among the most sensitive ecosystems to climate change and land degradation (Berdugo et al. 2020). Caatinga vegetation extends across approximately 735,000 km^2 of northeastern Brazil (Leal et al. 2005) and encompass the third center of diversity of the Cactaceae in East Brazil, which in turn, represents 40 different genera and 200 species, of which

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80% are endemic cactus (Taylor and Zappi 2004; Goettsch et al. 2015). Cacti exhibit strong ecological specialization and are sensitive to long-term climate change (Martorell et al. 2015; Hughes et al. 2016). Despite the importance of Cactaceae as a hotspot of endophytic fungi in drylands (Hubbard et al. 2014), there are only a handful of published studies on the diversity of these ecosystems. Previous research has reported that endophytic fungi associated with plants from drylands may contribute to the protection of their hosts against drought, salinity, herbivory, climate change, diseases caused by pathogenic microorganisms and improved plant growth by fungal volatile organic compounds (Redman et al. 2002; Hubbard et al. 2014; Camarena-Pozos et al. 2021).

Endophytes associated with Cactaceae have revealed fungal diversity estimates with new taxa associated included the new orders *Bezerromycetales* (Bezerra et al. 2017). However, only a limited number of studies on endophytic fungi associated with Cactaceae have focused on species from different ecosystems worldwide (Fisher et al. 1994; Suryanarayanan et al. 2005; Silva-Hughes et al. 2015; Fonseca-García et al. 2016; Camarena-Pozos et al. 2021; Gargouri et al. 2021).

Fisher et al. (1994) isolated 617 endophytic fungi (23 taxa, *Ascomycota*) from the 600-cladode fragments of *Opuntia stricta* in Australia. Suryanarayanan et al. (2005) screened 1050-cladode fragments 21 cactus species occurring in various localities within Arizona (USA) and isolated 900 endophytic fungi (22 taxa, *Ascomycota*) while Silva-Hughes et al. (2015) examined 540- cladode fragments from *Opuntia humifusa* in Missouri (USA) and identified 108 endophytic fungal (17 taxa, *Ascomycota* and *Basidiomycota*) by molecular biology techniques.

Fonseca-García et al. (2016) studied the microbial community composition of the two native and sympatric cacti species *Myrtillocactus geometrizans* and *Opuntia robusta* and detected 17 fungal classes principally composed for the *Ascomycota* orders *Pleosporales*, *Chaetothyriales*, *Capnodiales*, *Dothideales*, and *Hypocreales*, and of the *Basidiomycota* orders *Agaricales* and *Hymenochaetales*. Camarena-Pozos et al. (2021) detected that some fungal strains associated with agaves and cacti (*M. geometrizans* and *O. robusta*) produced volatile organic compounds (VOCs) capable of improving plant growth in *Arabidopsis thaliana* and host plants (*Agave tequilana* and *Agave salmiana*).

Gargouri et al. (2021) identified that fungal of root endosphere and rhizosphere samples of *Opuntia ficus-indica* from Tunisia belonged to the phylum *Ascomycota* (classes *Eurotiomycetes*, *Sordariomycetes*, *Dothiomycetes*, *Saccharomycetes*, and *Leotiomycetes*), followed by *Basidiomycota* (class *Agaricomycetes*), *Glomeromycota* (genus *Glomus*), and uncultured taxa. These author observed that increasing aridity correlates with an increase in connectivity of the root endosphere and rhizosphere fungal communities.

In Brazil, there are only four studies of endophytic fungal assemblages associated with cacti *Opuntia ficus-indica*, *Cereus jamacaru*, and *Tacinga inamoena* sampled in the Caatinga biome (Bezerra et al. 2012; Bezerra et al. 2013; Freire et al. 2015; Bezerra et al. 2017); these studies displayed Cactaceae that support a rich and varied endophytic mycobiota. Bezerra et al. (2012) studied 45 cladode fragments *Opuntia ficus-indica* and isolated 44 endophytic fungi belonging to 13 táxons (Ascomycota). Bezerra et al. (2013) examined 1.215 cladode fragments of *Cereus jamacaru* and isolated 560 endophytic fungi belonging to 59 táxons distributed in 30 genus: *Ascomycota* (24), *Basidiomycota* (4), and traditional *Zygomycota* (2). Freire et al. (2015) studied the endophytic fungi composition of the hefht cladodes of *O. ficus-indica* and cladodes infested by an insect (Hemiptera), and observed major frequency of the genus in the healthy cladodes tissue belonging to nine families of seven orders. Bezerra et al. (2017) characterized by morphological and multigene phylogenetic analyses three new strains endophytic fungi associated with the cactus *Tacinga inamoena* from Caatinga. Those novel taxa were described two new genera *Bezerromyces* (*B. brasiliensis* and *B. pernambucoensis)* and *Xiliomyces* (*X. brasiliensis*), which were accommodate in a new family *Bezerromycetaceae* and a new order *Bezerromycetales*.

Among the cacti from Brazilian Caatinga, the *Melocactus ernestii* (Vaupel), known as the "Turk's cap" or "melon cactus" is a lithophytic cactus endemic to eastern Brazil, and ranks as the species of the genus with the widest geographical range and environmental exclusivity to rocky outcrops (Hughes et al. 2018). Additionally, *M. ernestii* is used in folk medicine for the treatment of influenza, pneumonia, colic, and bowel disease (Rocha and Agra 2002; Andrade et al. 2006; Taylor and Zappi 2004). According to Hughes et al. (2018), *M. ernestii* is a species that takes years to reach adulthood (ca. 30 years) and is subjected to longer colonization times and more favorable habitats for fungal establishment. Therefore, understanding whether the endophytic fungi richness is compartmentalized in the different tissues (i.e., compartmental hypothesis), or whether it is characterized by one unique assemblage, is crucial for improving our understanding of the biotic factors that drive species turnover. For the reasons described above, our study aimed to characterize the taxonomy, diversity, and ecology of endophytic fungal assemblages living in different tissues of *M. ernestii* present in the Brazilian Caatinga biome.

2 Materials and methods

2.1 Study site and plant sampling

Caatinga is characterized by long dry seasons with 240 to 1500 mm annual rainfall and with 5 to 6 months receiving <100 mm, and the potential evapotranspiration is high with 1500 and 2000 mm per year (Pennington et al. 2009; Moro et al. 2016). Tissues from two populations of *subsp. Melocactus ernestii* subsp. *ernestii* established on rocky outcrops were collected in 2010/2011 at (i) Pedra Azul (Mee-PA), Minas Gerais state (−15.991457° Lat, −41.308297° Long; 652 m a.s.l.) and (ii) Ipirá (Mee-Ip), Bahia state (−12.184444° Lat, −39.471306° Long; 248 m a.s.l.) in Seasonally Dry Tropical Forest (Fig. 1). Live tissues from 30 adult individuals (characterized by the presence of cephalium) of each population were divided into three classes (hereafter called niches) stems, roots, and spines (Fig. 1). Mee-PA and Mee-Ip populations have, respectively, establishment on kinzigitic geiss and granitoids outcrops, mean annual precipitation levels varying between 1005 and 684 mm, mean annual temperatures of 22.4 and 23 °C, and precipitation seasonality (coefficient of variation) of 70 and 25% according to the Worldclim 2 (~1 km resolution) (Fick and Hijmans 2017). Vouchers were deposited in the Herbarium of the Instituto de Ciências Biológicas of Universidade Federal de Minas Gerais (BHCB-UFMG 141490 and 154,338).

2.2 Isolation of fungal endophytes

The roots concentrated in the 0–2 cm substratum depth, stem pieces and spines located at one-quarters along stem length from the base of the plants were placed in disinfected individual plastic bags and stored for less than 24 h at 10 °C prior to the isolation of endophytic fungi. Fifteen fragments of each part of the plant were plated, totaling 45 fragments per plant and 2700 fragments in all. The 5 to 10-mm-long fragments were washed, and surface disinfected by immersion 70% ethanol (1 min), 2% sodium hypochlorite (3 min) and washed with sterile distilled water (2 min) (Silva-Hughes et al. 2015). Fragments were plating in potato dextrose agar (PDA, Himedia/India) and malt extract agar Base (MEA, Himedia/India), both media supplemented with chloramphenicol (200 μg/mL) (Sigma/USA). Plates were incubated for up to 60 days at 25 °C and the hyphal tip of each fungus growing out from the plant tissue was excised and transferred to a new PDA plate (Suppl. Fig. 1). To test the effectiveness of the surface disinfection, 100 μL of the last water rinse was plated on PDA and MEA, and incubated at 25 °C. The culture purity was assessed using colony morphology. Samples of the fungal colonies were stored in cryotubes with 20% sterile glycerol at −80 °C and maintained in glass flasks with sterile distilled water (Castellani 1967). The fungi were deposited in the Collection of Microorganisms and Cells of UFMG (UFMGCB).

2.3 Fungal identification

The protocol for DNA extraction followed Rosa et al. (2010). For the filamentous fungi, the internal transcribed spacer (ITS) region was amplified with the universal primers ITS1 and ITS4 (White et al. 1990). Amplification of the ITS region was performed as described by Rosa et al. (2010). The yeasts were grouped and identified according to protocols established by Kurtzman et al. (2011). Yeast molecular identities were confirmed by sequencing the D1-D2 variable domains of the large-subunit rRNA gene using the primers NL1 and NL4 as described by Lachance et al. (1999). Fungal isolates with query coverage and identity ≥99% were considered representing the same taxon. Representative consensus sequences of the fungal taxa were deposited into the GenBank database (Suppl.Table 1). To achieve species-rank identification based on ITS the consensus sequence was aligned with all sequences from related species retrieved from the NCBI GenBank database using BLAST (Altschul et al. 1997). Taxa that displayed query coverage and ≤ 98% identity or an inconclusive taxonomic position were subjected to phylogenetic ITS based analysis for comparison with sequences of ex type species deposited in the GenBank database. The information about fungal classification followed the databases of dictionary Kirk et al. (2008), and websites MycoBank (http://www.mycobank.org) and the Index Fungorum (http://www.indexfungorum.org).

2.4 Analysis of fungal assemblage structure

The recorded abundance of each fungal endophytic across sampling plants (i.e., 30 adult plants of each populations, divided into three tissues or partitions) was used to calculate the three integrated rarefaction/extrapolation curves, based on the first three Hill's numbers (Hill 1973; Chao et al. 2014): q = 0, an estimation of ${}^{0}\Delta$ or species richness; q = 1, ${}^{1}\Delta$ or Shannon index; q = 2, ${}^{2}\Delta$ or inverse Simpson index. Extrapolations, with values not greater than 2 times the real individual size, were made using the estimation of three Hill's numbers (q = 0, q = 1, q = 2; Colwell et al. 2012) to testing the sampling sufficiency in each tissue by population. We compared the estimator patterns among partitions using the Hill confidence interval as the mean of 999 replicate bootstrap runs (Chao et al. 2014; Budka et al. 2019). In this case, no overlap of 95% confidence intervals for Hill's numbers among partitions in each population are differed significantly at $p < 0.001$ (Colwell et al. 2012). Estimates were obtained using the "iNEXT" R package (Hsieh et al. 2019) in R 4.0 software (R Core Team 2020).

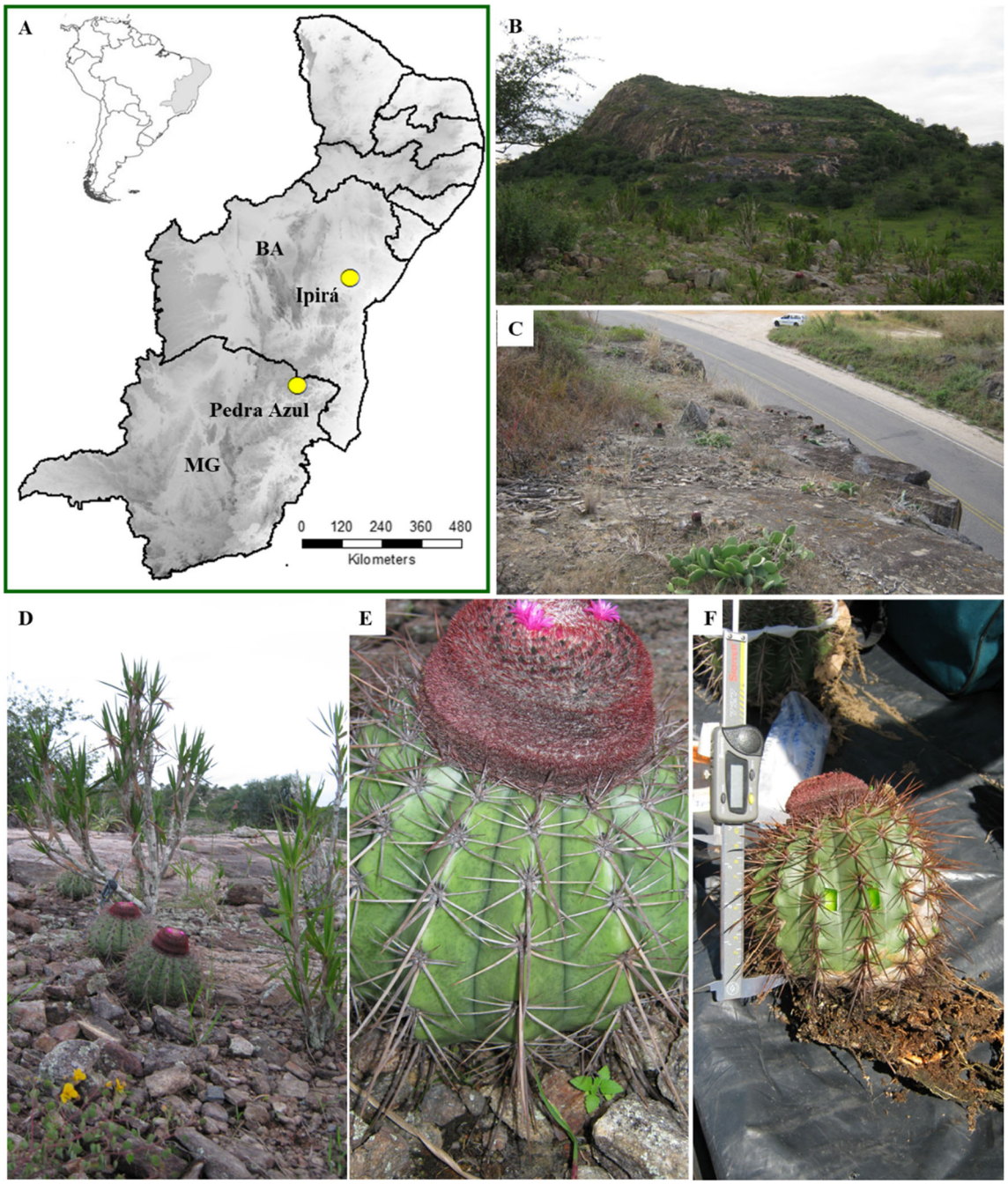

Fig. 1 *Melocactus ernestii* from two sites of Brazilian Caatinga: **A** Map of northwestern Brazil showing the sites (Ipirá and Pedra Azul) in which the plants were collected; **B** Ipirá site, Bahia State (BA); **C** Pedra Azul site, Minas Gerais State (MG); **D** Adult plants characterized by the presence of cephalium (Ipirá site); **E** *M. ernestii* in rocky outcrop; **F** Screened parts: stems, roots and spines

2.5 Abundance-based multiple-site dissimilarities

We explored the differences observed among fungal endophytes communities by additive partitioning of beta diversity among tissues and populations. The presence-absence (i.e., β_{sor} - incidence-based Sørensen index, a beta diversity metric ranges from 0 for no differentiation between assemblages, otherwise it measure to 1; Baselga 2010) and abundance matrices (i.e., $\beta_{\%Diff}$ - abundance-based extension of Sørensen index or percentage difference; Baselga 2013, 2017) were employed to calculate the dissimilarities between tissues and populations. These metrics can be understood with processes driving the species composition of communities. For incidence-based, these metrics were decomposed in two distinct components of dissimilarity: (i) turnover, or replacement of species between sites; (ii) nestedness, or species pool at a site being a strict subset of the species of another richer site (Baselga 2010). For abundance-based dissimilarity, two

complementary metrics were obtained: (i) $\beta_{BC.BAL}$ - balanced variation, or the abundance of a species declines from tissues and populations in the same magnitude than the abundance of other species increases from tissues and populations, and (ii) $\beta_{BC.GRA}$ - abundance gradient, or the abundance of all species equally declines or increases from one site (tissue or population) to another (Baselga 2013, 2017). Triangular plots were employed to explore similarities among endophytes assemblages present the in different tissues and populations of *M. ernestii*. Analyses were performed using R package 'betapart' in R 4.0 software (R Core Team 2020) with 10,000 permutations [R functions according to Baselga 2010, 2013, 2017].

3 Results

3.1 Taxonomic composition

A total of 222 endophytic fungi isolates were obtained from 2700 fragments of roots, stems, and spines of *M. ernestii*. A total of 136 fungal isolates were recovered from *M. ernestii* at the Pedra Azul (Mee-PA), as well as 161 from the Ipirá (Mee-Ip) site, which represents 99 operational taxonomic units (OTUs) of *Ascomycota* and *Basidiomycota* using molecular biology methods (Suppl. Table 1). *Ascomycota* was represented by two subphyla, *Pezizomycotina* and *Saccharomycotina*. *Pezizomycotina* was represented by *Sordariomycetes* (39%), *Dothideomycetes* (30%), *Eurotiomycetes* (14%), and *Pezizomycetes* (1%). *Xylariales* (*Sordariomycetes*) was the most abundant, followed by *Dothideomycetes* (*Pleosporales*) and *Eurotiomycetes* (*Eurotiales*). *Basidiomycota* was represented by only the *Agaricomycetes* class, distributed in the following order: *Cantharellales*, *Agaricales*, and *Geastrales* (Fig. 2).

The majority of the fungal taxa were recovered from root tissues, followed by the stem and spine (Fig. 3 and Suppl. Table 2). In general, 78.41% of the species were not shared among the *M. ernestii* populations and tissues. Only *Nigrospora* sp. and *Preussia* sp. 1 were common among the three plant tissues. *Preussia* sp. 2 and *Preussia minimoides* occurred in the stem and spine, and *Aspergillus calidoustus*, *Penicillium* cf. *griseofulvum*, and *Sphaerobolaceae* sp. in the root and spine. Endophytic root and stem assemblages shared *Fusarium oxysporum*, *Hypocreaceae* sp. 1, and *Phoma* sp. Only the yeasts *Aureobasidium pullulans* and *Candida parapsilosis* were isolated from *M. ernestii* stem.

3.2 Fungal assemblage composition

Among the standardized sample size of 30 plants of *M. ernestii*, the root tissues exhibited the highest values of endophyte diversity: A total of 41 (Ro_BA) and 36 (Ro_MG) endophytes were detected (°Δ), the Shannon index was 29.99 ° and 26.13 ° (^{1}Δ), and the inverse Simpson diversity index was 22.46 and 19.53% (^{2}Δ) (Fig. 4 and Suppl. Table 2). We detected similar richness patterns among the same tissue types using sample-based rarefaction and extrapolation curve; however, the variations among the tissues was mainly due to the low number of species observed in the spines of BA (Sp_BA) and stems of BA and MG (St_BA - St_MG) locations, which did not overlap with St_MG, Ro_BA, and Ro_MG according to rarefaction.

Rarefaction curves indicated that only root tissues showed close to sufficient sampling (Fig. 4). Tissue types revealed different patterns in the rarefaction/extrapolation curves, mainly between roots and spines-stems (Fig. 4), and the confidence intervals did not overlap. In addition, the curve order was similar for every tissue type, with decreasing values of species richness, Shannon diversity, and inverse Simpson index (exception observed in Sp_BA with a small initial sample). These data represent the effects of frequent species numbers. The species richness curve for the tissues continuously increased with increasing sample size, whereas the curves representing the Shannon and inverse Simpson indices grew considerably only in the initial part.

Comparing tissue types, the highest sample completeness was detected for root tissues, with an initial increase in the °Δ curve and tendency to stationarity. The other tissue types also exhibit an increase after the initial phase, with highlight to

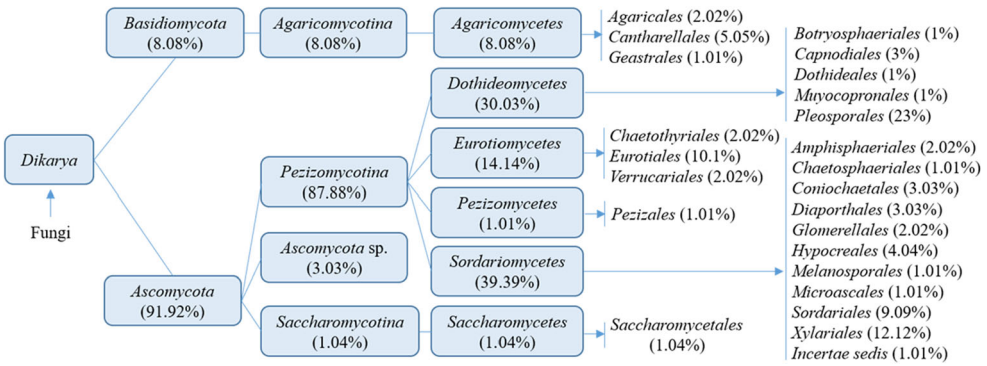

Fig. 2 Schematic representation of phylogenetic placement of endophytes fungal taxa isolated from *Melocactus ernestii* tissues from different locations of Brazilian Caatinga. Percentages indicate the total number of isolates obtained out of a total of 222 isolates for the indicated group

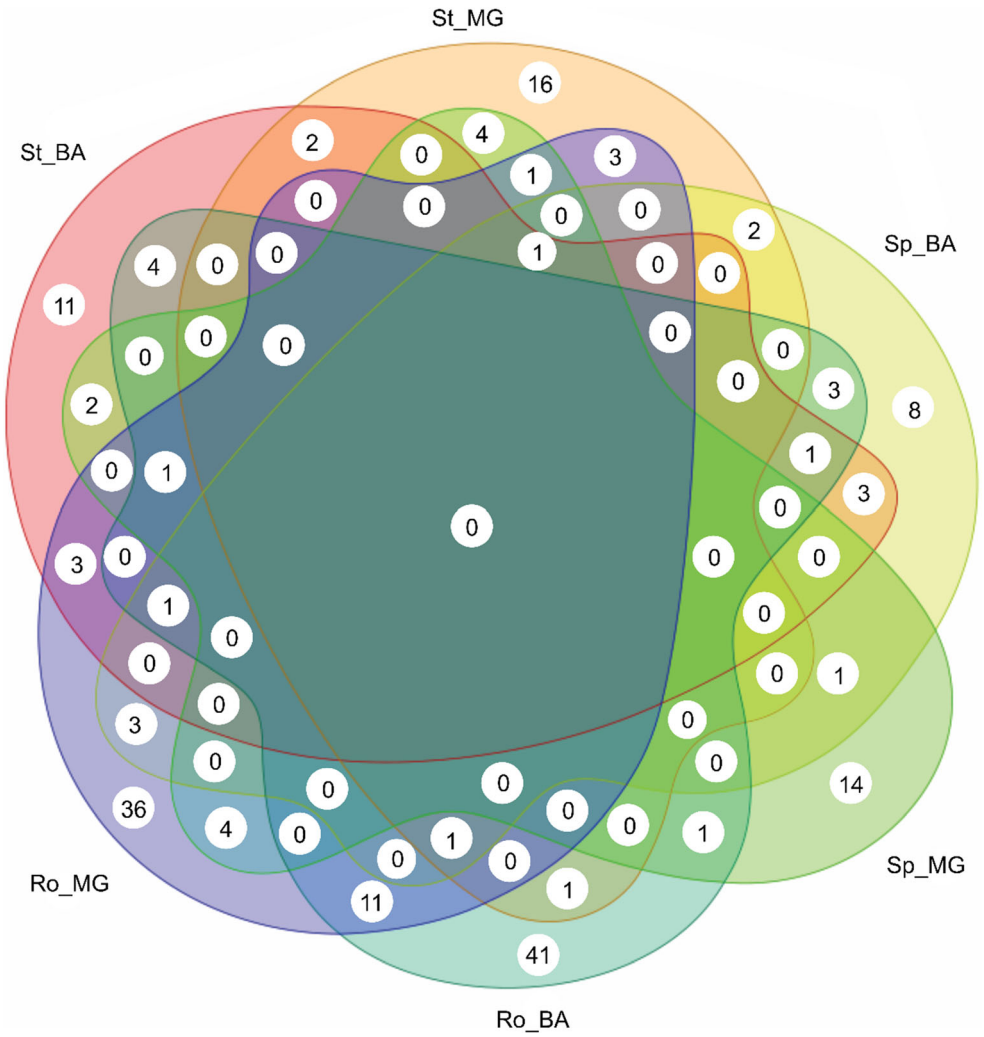

Fig. 3 Venn diagrams showing the fungal taxa distribution among the different *Melocactus ernestii* tissues (Ro, roots; Sp, spines; St, stems) and two populations (BA, Bahia; MG, Minas Gerais)

St_MG tissue; in this sense, new species are detected with additional survey plants. The extrapolation curves (dotted lines) suggest an increase in species with continued sampling. This evidence was confirmed by extrapolation of the total species number, where the prediction of complete richness in St_MG of 37 species. A total of 21 fungal taxa were expected to be detected by extensive surveying. In contrast, the observed richness of Sp_BA (eight species) requires only an extrapolation of four additional species to be sampled (Suppl. Table 2).

Intersection of root tissue curves and intersection of spines and stems curves was observed (°Δ species richness; Fig. 4), which suggests that the species richness is significantly different among these tissues. In this case, root tissues were the richest in species, and the spines and stem tissues remained the poorest in species. An intermediary pattern of richness was observed in Minas Gerais (St_MG). A similar pattern was observed for diversity metrics related to the number of frequent species ($^1\Delta$, Shannon index) and highly frequent species ($^2\Delta$, inverse Simpson index) (Fig. 4). In both cases, significant differences were observed between roots and other tissues, except for St_MG, which had an intermediary pattern. Our inferences were made at a significance level of $P < 0.001$.

The coverage-based sample curves for the tissue types of *M. ernestii* were variable (Suppl. Fig. 2). The coverage for root tissues was estimated to be ~85% for the reference sample size of 100 (Ro_MG) and 109 (Ro_BA) individuals. For any sample size less than 100, the curve shows that the sample completeness for Ro_MG is estimated to be similar to that of Ro_BA treatment, as evidenced by overlapping confidence intervals. From the sample completeness curve, when the sample size in the Ro_MG treatment was doubled from 100 to 200 individuals, the sample coverage increased from 85% to 96%. A similar coverage pattern was observed for Ro_BA. In general, the other coverage curves for spines and stems show that for any sample size of less than 37 individuals, the estimate is similar. In addition, when the sample size is doubled, the sample coverage increases from St_BA 80% to 86%, from St_MG 60% to 67%, from Sp_BA 60% to 88%, and from Sp_MG 79% to 92%.

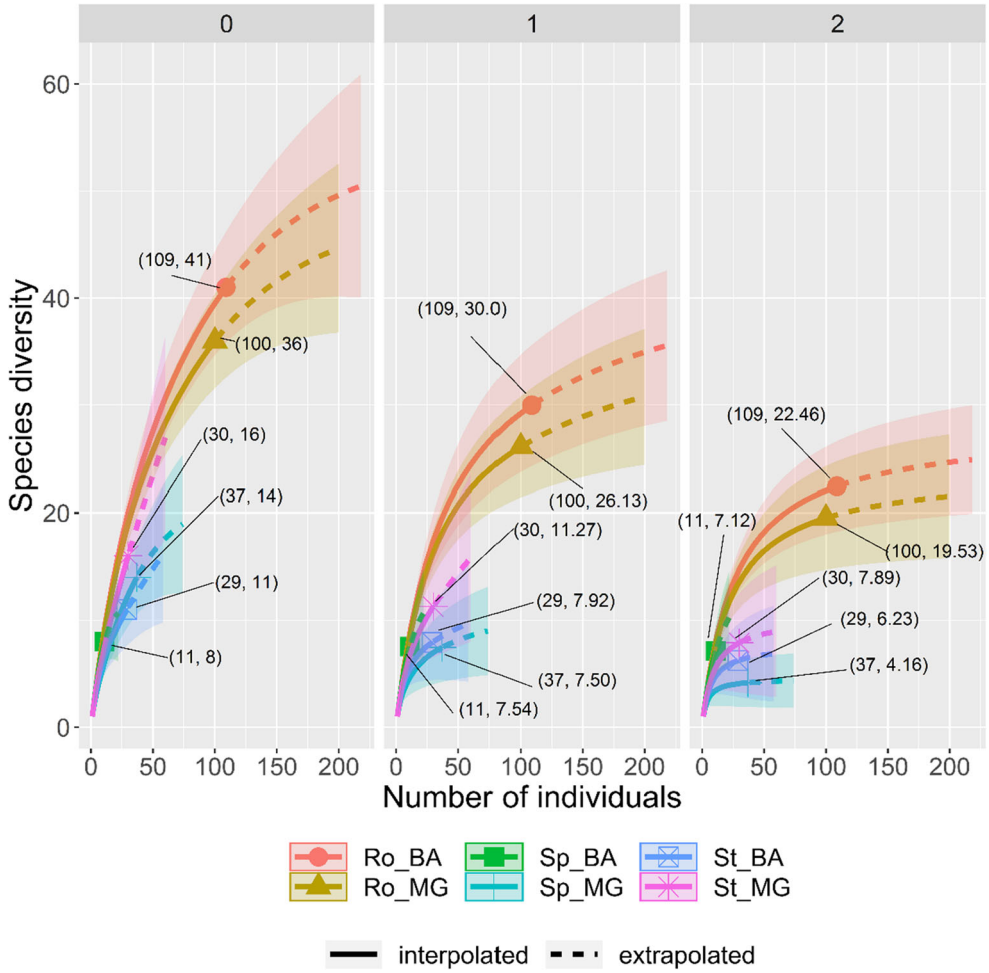

Fig. 4 Comparison of sample-size-based rarefaction (solid line) and extrapolation (dashed line, double the size of the reference sample) of tissues fungal endophytes diversity (Ro, roots; Sp, spines; St, stems) on two populations (BA, Bahia; MG, Minas Gerais) of *Melocactus ernestii* based on Hill's numbers ${}^{q}\Delta$ with q = 0 (species richness), q = 1 (Shannon index) and q = 2 (inverse Simpson index). The shaded area represents 99% confidence intervals obtained using the bootstrap method on the basis of 999 repetitions. The numbers in parentheses are the sample size and the observed Hill numbers for each reference sample

We compared the coverage-based diversities of the tissue samples for q = 0 (left panel), q = 1 (middle panel), and q = 2 (right panel), employing our maximum coverage (i.e., 85%, Ro_MG) for the reference sample (Suppl. Fig. 3). Since the confidence envelope overlapped in the root tissues, the richness of endophytes was similar. However, root tissues were significantly higher than in the spine and stem tissues for standardized sample coverage between 10% and 85%. In addition, the richness of St_MG was significantly higher than that of any other, above 55%. For the Shannon diversity (${}^{1}\Delta$), the root tissue is more diverse than the spines and stem tissues. The difference between root compositions was observed, but the confidence envelopes overlapped. For Shannon diversity (${}^{2}\Delta$) when coverage is less than 60%, Sp_BA and St_MG have almost the same diversity but are more diverse than St_BA and Sp_MG.

4 Abundance-based multiple-site dissimilarities

The multiple site dissimilarity across the total plants and tissues showed higher differences in species richness of endophytic fungal assemblages of *M. ernestii* (β_{Sor} mean ± SD: 0.82 ± 0.08), with similar values to abundance balanced variation ($\beta_{\%Diff}$ 0.85 ± 0.09). Changes in endophyte richness among the six assemblages were mostly due to species replacement through turnover, which accounted for 92.2% (i.e., 0.83 out of 0.90) of the total beta diversity at tissues/population locations. By complementarity, nestedness (species loss or gain) accounted for only 7.8% (0.07) of these changes. In accordance with the aforementioned data, differences in endophyte species were mostly due to individual substitution from tissue to tissue, which accounted for 92.1% (0.84) of total beta diversity. The abundance gradient accounted for the remaining 7.9% (0.07).

Pairwise incidence-based dissimilarities in total beta diversity among tissues ranged from 0.68 (Stem-Spines of Bahia) to 0.91 (BA - MG, Spines; mean of all pairwise comparisons = 0.82; Fig. 5). Turnover values (72.4% ± 0.09) were always higher than the nestedness values (8.3. ± 0.09). Higher turnover values were observed between pairwise spines and populations (0.875, BA and MG), followed by pairwise stems from BA and MG (0.818) and stems and roots from MG (0.813). Abundance-based pairwise dissimilarities among

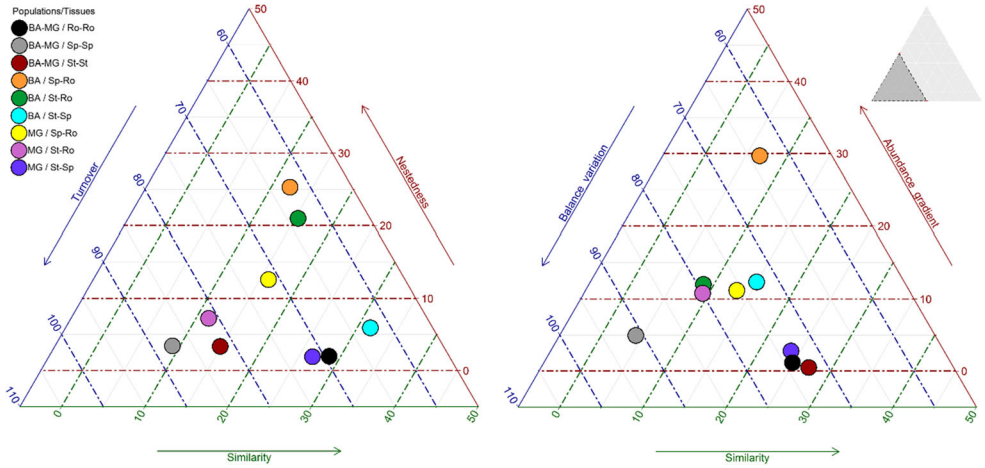

Fig. 5 Triangular plots representing the beta diversity components for pairwise comparisons among tissues and populations. Left panel, incidence-based metrics of species richness (turnover, nestedness); and right panel, abundance-based metrics (balanced variation, abundance gradient). Similarity was estimated as 1-dissimilarity. Two-letter codes identify each tissue as follows: Ro - roots; St - stems; Sp - spines; and two populations: BA = Ipirá (Mee-Ip), Bahia State; MG = Pedra Azul (Mee-PA), Minas Gerais State

tissues ranged between 0.72 (BA - MG, Stems) and 0.96 (BA - MG, Spines; mean = 0.85 ± 0.09). The balance of variation explained dissimilarities in all pairwise comparisons, in contrast to abundance gradient comparisons.

5 Discussion

In this present study, we examined the composition, diversity partitioning, and compartmentalization of endophyte fungi in tissues of *M. ernestii*, a cacti endemic to rock outcrops in hot drylands in eastern Brazil. Most of the fungal genera obtained as endophytes of *M. ernestii* have been previously described as endophytes. Fewer fungi within the *Basidiomycota* are isolated as endophytes (Vieira et al. 2012), and only nine species are associated with cacti: *Rhodotorula foliorum*, *R. minuta*, *R. mucilaginosa*, *R. pilati*, *R. sonckii*, *Sporobolomyces salmonicolor*, *Sterigmatomyces elviae, Tritirachium dependens* recovered from the stem of *Cereus jamacaru* (Bezerra et al. 2013), *Cryptococcus flavescens* (*Tremellomycetes*) recovered from *Opuntia humifusa* (Silva-Hughes et al. 2015), and eight OTUs which belong to *Agaricales*, *Cantharellales*, *Corticiales*, *Filobasidiales*, and *Tremellales* orders from the root (endosphere and rhizosphere) of *Opuntia ficus-indica* (Gargouri et al. 2021).

In our study, we recovered *the Agaricomycetes* class, *Cantharellales* order (*Ceratobasidium* sp., *Ceratobasidiaceae* sp. 1, *Ceratobasidiaceae* sp. 2, and *Ceratobasidiaceae* sp. 3), *Agaricales* order (*Agaricomycetidae* sp. and *Tricholomataceae* sp.) isolated from roots, and *Geastrales* order (*Sphaerobolaceae* sp.) recovered from roots and spines of *M. ernestii*. These results were similar to previous reports, which obtained *Cantharellales* and *Agaricales* orders from roots of *Opuntia robusta and O. ficus-indica* (Fonseca-García et al. 2016; Gargouri et al. 2021).

Fungi from the *Ceratobasidiaceae* family play an important ecological role as pathogens, saprotrophs, mycorrhizal symbionts, and endophytes (Veldre et al. 2013). González and Tello (2011) detected two endophytic species of *Ceratobasidium* from *Vitis vinifera*, and Decruse et al. (2018) studied the seed germination and seedling growth promoted by a *Ceratobasidiaceae* sp. in *Vanda thwaitesii*, an endangered orchid species endemic to South Western Ghats, India, and Sri Lanka. *Agaricomycetidae* and *Tricholomataceae* mainly include saprotrophic taxa, but they can be isolated as mycorrhizal and endophytes (Schulz and Boyle 2006; Tejesvi et al. 2010). Fonseca-García et al. (2016) revealed the presence of *Agaricales* (mainly *Henningsomyces* species) in the stem endosphere community of the cacti *Myrtillocactus geometrizans*. Toju et al. (2013) detected endophytic fungi of the genus *Cortinarius* (*Agaricomycetidae*) associated with *Quercus serrata*, whereas Lana et al. (2011) isolated the endophytic *Moniliophthora perniciosa* (*Tricholomataceae*) associated with *Theobroma cacao*. The *Sphaerobolaceae* family represents mainly hummus species with few lignicolous, termites, and endophytic fungi, as the isolates from its family are associated with *Bouteloua gracilis* (Herrera et al. 2010).

The *Ascomycota* genera *Alternaria*, *Aspergillus/Neosartorya*, *Aureobasidium, Candida*, *Chaetomium*, *Cladosporium*, *Collariella*, *Curvularia*, *Diaporthe*, *Cochliobolus/Curvularia*, *Epicoccum*, *Fusarium*, *Nigrospora*, *Penicillium*, *Pestalotiopsis*, *Phoma*, and *Xylaria* were found to be associated with cacti. However, among the fungi identified as endophytes of *M. ernestii*, the genera *Acrocalymma*, *Astrocystis*, *Bartalinia*, *Ceratobasidium*, *Colletotrichum*, *Daldinia*, *Didymella*, *Gelasinospora*,

Neurospora, *Lecythophora*, *Microsphaeropsis*, *Muyocopron*, *Neoscytalidium*, *Preussia*, *Sclerostagonospora*, *Setophoma*, and *Thozetella* were reported for the first time as endophytic of cacti. The high number of new registers can be explained by the fact that most fungal endophyte studies focus only on stem or root tissues. In this study, fungi were isolated from root (70.83%) and spine (16.6%) tissues. In addition, other studies on endophytic fungi from cacti may have underestimated species diversity by using only morphological techniques and by not identifying several sterile fungi.

Aureobasidium pullulans and *Mycoleptodiscus indicus* associated with *M. ernestii* stems were isolated from dominant species. Suryanarayanan et al. (2005) and Silva-Hughes et al. (2015) showed results similar to those of *Aureobasidium pullulans* as the dominant species in cacti from USA. However, the *Cladosporium* genus was the most abundant species in the cladodes of *Opuntia stricta*, *O. ficus-indica*, and one of the most abundant species in *Cereus jamacaru* (Fisher et al. 1994; Bezerra et al. 2012; Bezerra et al. 2013).

Aureobasidium pullulans, a dimorphic species, are often considered transient inhabitants of leaf and fruit surfaces, including the cactus (Ganter et al. 2017). It is one of the most widespread saprophytes and endophytic fungi. Previous studies have reported the ability of *A. pullulans* to promote plant growth and suppress several fungal plant pathogens, such as *Botrytis cinerea, Fusarium culmorum, Penicillium expansum, Rhizopus stolonifer,* and *Aspergillus niger* (Ippolito et al. 2000; Castoria et al. 2001; Wachowska and Głowacka 2014; Sun et al. 2019). However, *A. pullulans* has been reported as a causal agent of disease in grapes (Morgan and Michailides 2004) and in the stem and fruit of the cactus Pitaya (*Hylocereus undatus* and *H. polyrhizus*) in China (Liu et al. 2017).

Mycoleptodiscus indicus is a dematiaceous hyphomycete fungus found as saprotrophs leaflet decomposers of *Paubrasilia echinata*, which in turn, are endophytes associated with *Echinacea purpurea*, *Borreria verticillata*, and *Opuntia ficus-indica*; phytopathogens that can cause diseases in economically important plants such as *Vanilla fragans* (Bezerra and Ram 1986; Grandi and Silva 2006; Dewar and Sigler 2010; Bezerra et al. 2012; Rosa et al. 2012; Andrioli et al. 2014; Maboni et al. 2019). *Preussia* sp. 1 and *Sphaerobolaceae* sp. were the most abundant species of spines. Members of the *Preussia* taxa are predominantly coprophilous, but they are isolated from soil, wood, or plant debris and as endophytes (Gonzalez-Menendez et al. 2017). *Preussia* species were the most frequent genera in plants collected in arid areas, such as the Arizona desert and Sonoran Desert (Massimo et al. 2015; González-Menéndez et al. 2018).

Cochliobolus eragrostidis, *Fusarium oxysporum*, and *F. solani* were the dominant species in the roots of *M. ernestii*. The other OTUs showed low abundance and may represent rare species, included the genera *Aspergillus/Neosartorya, Chaetomium* and *Collariella* that were reported as endophytic from the root endosphere and rhizosphere associated with *O. ficus-indica* in aridity gradient (Gargouri et al. 2021). Species of *Cochliobolus*/*Curvularia* are commonly described as endophytes in cacti (Bezerra et al. 2012, 2013; Freire et al. 2015; Gargouri et al. 2021). Bezerra et al. (2013) reported *Cochliobolus* species as endophytes in the stems of *Cereus jamacaru* and *Opuntia humifusa*, and roots of *O. ficus-indica* cactus (Bezerra et al. 2013; Silva-Hughes et al. 2015; Gargouri et al. 2021). In addition to cacti, species of the genus *Cochliobolus* cause diseases in banana (*Musa* sp.), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*), and maize (*Zea mays*) (Moraes et al. 2006; Völz et al. 2020).

Members of the *Fusarium* genus have been isolated from plants and soils as pathogens, saprobes, and endophytes, including *Cereus jamacaru*, *Opuntia stricta*, and *Opuntia ficus-indica* cactus (Fisher et al. 1994; Souza et al. 2010; Bezerra et al. 2013; Bonfim et al. 2013; Freire et al. 2015; Gargouri et al. 2021). Usually, *Fusarium oxysporum* infects the host by the root, obstructing the vascular system and reducing the flow of water from the roots to the top of the plant, consequently producing withering (Leslie and Summerell 2006). However, *F. oxysporum* has been observed to be asymptomatic in three healthy tissues of *E. purpurea* and was able to produce extracts with antifungal activity, suggesting a protective effect on the plant (Carvalho et al. 2016).

Root-associated fungal communities are important components of ecosystem processes that affect plant growth and vigor by influencing the quality, direction, and flow of nutrients and water among plants and fungi (Trivedi et al. 2020). In our study, we also recovered the pigmented fungi *Alternaria*, *Aureobasidium*, *Aspergillus*, *Cladosporium*, *Cochliobolus*/*Curvularia, Epicoccum*, *Nigrospora*, and *Phoma*. Pigmented endophytic fungi have been associated with tolerance to UV radiation and desiccation, establish and survive in arid sites, or to confer abiotic and biotic tolerance to their plant hosts (Redman et al. 2002; Mandyam and Junpponem 2005; Sterflinger et al. 2012; Khidir et al. 2010; Ali et al. 2018). For example, melanized dark septate fungi associated with roots could facilitate the uptake of water and nutrients such as N, P, K, and Mg in arid ecosystems (Barrow and Osuna 2002; Barrow 2003; Mandyam and Junpponem 2005; Barrow et al. 2008; Vergara et al. 2018; Gargouri et al. 2021). Morsy et al. (2010) suggested that the endophytic-host relationships might be influenced by the involvement of the melanin pigment and by thermophilic proteins, as observed in cultures of *Curvularia protuberata*. *Melocactus ernestii* grows in rock outcrop microhabitats (cavities and crevices) with low substrate amounts, which can make water more ephemeral (Hughes et al. 2018). The joint effect of succulence and crassulacean acid metabolism (CAM) (Lüttge 2004), in

association with dark septate fungi, can promote the water storage and water-use efficiency of *M. ernestii* (Fonseca-García et al. 2016).

We have shown that the species richness of endophytes in *M. ernestii* varies across different tissue types. Based on a double sample size, the roots showed the highest species richness (°Δ) among the two populations, followed by stems and spines, which were poor in species richness. The same pattern was observed for all diversity metrics (species richness, Shannon ($^1\Delta$), and inverse Simpson ($^2\Delta$) indices). The multiple comparative approaches employed here (Chao et al. 2014) demonstrated the relationship between sample size and sample completeness in host tissues. More sampling effort is necessary to achieve a stationary level (or completeness) of species richness in spines (>7%) and stems (≈25%). The sterilization protocols and growth conditions, standardized and applied to all samples (minimizing biases in comparative analyses communities), can influence the discrepancy observed in the richness capture in stem and spine tissues. In this case, the rarefaction and extrapolation approach enabled the precise comparison of endophytic diversity in different tissues and improved the precision of diversity estimates (Chao et al. 2014).

The diversity patterns among tissue types in *M. ernestii* support the hypothesis of compartmentalization into modules (ecological networks), that is, the biotic factors represent the main driver of selection (Toju et al. 2014; Coleman-Derr et al. 2016; Fonseca-García et al. 2016; Trivedi et al. 2020). The difference in the number of isolates from the stem, spine, and root assemblages might be due to variations in the characteristics of each plant tissue sampled, such as the thick and waxy cuticle and the low frequency of stomata in cacti; these adaptations to reduce evaporative water loss may represent additional barriers that inhibit infection by parasitic fungi, as well as the colonization of endophyte species, resulting in less diversity when compared to other plant species (Zimmermann and Granata 2002; Suryanarayanan et al. 2005; Luz et al. 2006; Silva et al. 2006; Fonseca-García et al. 2016).

In addition, there are other characteristics of plant tissue, such as biomass and secondary substances, which may justify the low colonization of stems and spines in relation to the roots (Mauseth 2004; Shade et al. 2017). Finally, belowground conditions (low UV radiation and atmospheric aridity) are co-determinants of the differences observed among tissues (roots, stems, leaves, and spines; Arnold and Lutzoni 2007; U'Ren et al. 2012).

The endophyte sharing observed exclusively between spines and stems may reflect interactions of endophytic fungal species and/or communication between the two tissues in *M. ernestii*. According to Schill and Barthlott (1973), chemiluminescence experiments suggest that spines of *Discocactus horstii* can act as capillaries and provide an increase in water absorption by the plant. *Discocactus* and *Melocactus* are the closest in a strongly supported clade (Ritz et al. 2007). In this case, it is not surprising to suppose that the globose genera *Melocactus* and *Discocactus*, the closest relatives to each other, present common mechanisms of water supplementation (Schill and Barthlott 1973; Mauseth 2006) and influence the sharing and interactions of endophytic fungal species. In additional, Gargouri et al. (2021) suggested that the interactions among ecological networks could mean that microbes have a higher dependency on shared resources in arid sites.

6 Conclusion

We show that fungal endophytic assemblages across tissues differ with respect to species richness and abundance. The beta diversity among tissues was mainly attributed to the replacement of endophytic species or individuals rather than nestedness. In this context, we suggest that species replacement among tissue compartments is driven by biotic factors. In comparison to complementarities, nestedness and abundance gradient contributed relatively little towards the differences among tissues, suggesting interactions of endophytic fungal species with possible sharing of resources in tissues. Our results highlight the key roles of turnover of endophytic fungal assemblages and summarize the increasing body of evidence. Overall, this work could contribute to estimates of fungal diversity in small scale; to describe novel taxa, which indicates that fungal phylogenetic diversity and function in semi-arid environments.

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