Unravelling the community of arbuscular mycorrhizal fungi associated with endemic plants from a neotropical dry forest

Antonio Marcos Miranda Silva (antoniomarcos@usp.br)
University of São Paulo
Henrique Petry Feiler
University of São Paulo
Gileno Vieira Lacerda-Júnior
Brazilian Agricultural Research Corporation
Paulo Ivan Fernandes-Júnior
Brazilian Agricultural Research Corporation
Saulo Tarso Aidar
Brazilian Agricultural Research Corporation
Victor Araújo Vieira Prudêncio Araújo
University of São Paulo
Filipe Pereira Matteoli
University of São Paulo
Arthur Prudêncio Araújo Pereira
Federal University of Ceará
Itamar Soares Melo
Brazilian Agricultural Research Corporation
Elke Jurandy Bran Nogueira Cardoso
University of São Paulo

Research Article

Keywords: environmental DNA sequencing, biodiversity-related knowledge, bromeliad, grass, Tripogon spicatus, mycorrhizal symbiosis

Posted Date: September 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2066211/v1

Abstract

Arbuscular mycorrhizal fungi form symbiotic associations with 80% of all known plants, allowing the fungi to acquire plant-synthesized carbon, and confer an increased capacity for nutrient uptake by plants, improving tolerance to abiotic and biotic stresses. We aimed to characterize the mycorrhizal community associated with *Neoglaziovia variegata* (so-called *caroa*) and *Tripogonella spicata* (so-called resurrection plant) using high-throughput sequencing of the partial 18S rRNA gene. Both endemic plants to neotropical dry forests and shrubland ecosystems were sampled in the Caatinga biome, located in northeastern Brazil. Illumina MiSeq sequencing of 37 rhizosphere samples (19 for *N. variegata* and 18 for *T. spicata*) revealed a distinct mycorrhizal community between the studied plants. There is a lack of information regarding the mycorrhizal composition of these plants, as revealed by our systematic review. According to alpha diversity analyses, *T. spicata* showed the highest richness and diversity based on the Observed ASVs and Shannon index, respectively. The four most abundant genera (higher than 10%) found were *Glomus*, *Gigaspora*, *Acaulospora*, and *Rhizophagus*, with *Glomus* being the most abundant in both plants. Nonetheless, *Gigaspora*, *Diversispora*, and *Ambispora* were specific for *N. variegata*, whilst *Rhizophagus*, *Paraglomus*, and *Archaeospora* were only associated with *T. spicata*. Therefore, the arbuscular mycorrhizal fungi community showed a genus-specific niche, and hence they may be differentially assisting the plants in the harsh environment of the Caatinga biome.

Introduction

Neotropical dry forests, also referred to as seasonally dry tropical forests (SDTFs), are one of the most threatened tropical forests in the world, with climate change being the main threat in the Americas, especially in Brazil, which comprises most of them (Miles et al. 2006; Santos et al. 2011; Siyum 2020). SDTFs cover extensive areas from Mexico (Central America) to Argentina (South America) and throughout the Caribbean (Dryflor et al. 2014).

The Brazilian Caatinga biome harbours the largest SDTFs, composed of a shrubland ecosystem that covers 844,453 km^2 and represents 10.1% of the Brazilian territory (IBGE 2019). According to Teixeira et al. (2021), only 1.3% of the Caatinga biome is fully protected. Therefore, conservation actions are urgently needed, as they have unique biodiversity patterns. The evolutionary history confined to this biome converged to its uniqueness, presenting plant species restricted to it (Pennington et al. 2009). Alongside, these endemic species co-evolved with a host microbiome that assisted them to survive so far (Bonfante and Genre 2008). Therefore, the Caatinga biome is a screening hotspot for microbes that may be used to mitigate abiotic stresses (Kavamura et al. 2013; Fernandes-Júnior et al. 2015; Taketani et al. 2017; Santana et al. 2020). However, little is known about the diversity and community composition of arbuscular mycorrhizal fungi (AMF) associated with plants that are endemic to dry forests.

The mycorrhizal symbiosis plays a key role in maintaining plant growth and, compared to other known symbioses (e.g., nitrogen-fixing bacteria and lichens), is the oldest of approximately 400 million years. AMF colonize over 80% of all plant species, and only very few plant families cannot be colonized by AMF, such as *Brassicaceae*, *Chenopodiaceae*, *Cyperaceae* and *Juncaceae* (Smith and Read 2010). Under SDTFs, some investigations have shown the AMF composition associated with *Bromeliaceae*, which is considered one of the most species-rich and ecologically important plant families in the neotropics (Butcher and Gouda 2020; Leroy et al. 2021). However, there are no studies on AMF communities associated with the bromeliad *Neoglaziovia variegata* (Arruda) Mez, endemic to the Caatinga biome, but there are studies showing its gastroprotective, antibacterial and acaricidal potential (Peixoto et al. 2016; Torres-Santos et al. 2021; de Lira et al. 2021).

Likewise, there are no studies investigating the AMF communities associated with *Tripogonella spicata* (Nees) P.M.Peterson & Romasch, belonging to the so-called resurrection plants. The term resurrection plant is due to its capacity to survive dehydration to an air-dried state for months, losing most of its cellular water, and quickly resume normal physiological activities after rehydration (Aidar et al. 2017; Oliver et al. 2020; Gechev et al. 2021). In addition, other plant species belonging to the families *Myrothamnaceae*, *Selaginellaceae*, *Velloziaceae*, and *Scrophulariaceae* are also known as resurrection plants (Alam et al. 2019). Indeed, the plant physiological mechanisms are decisive for rapid plant rehydration, but the associate rhizosphere microbiota may be the downstream agent that modulates the upstream response.

Thus, this investigation has pioneered in revealing the arbuscular mycorrhizal fungi composition using high-throughput sequencing of rhizosphere samples of *N. variegata* and *T. spicata*, two endemic plants from Caatinga. Therefore, our study may represent a step forward for bioprospecting programs aimed at finding potential AMF that can assist crop plants to tolerate abiotic stress, such as drought, in the soil.

Materials And Methods

Location site and characteristics

The investigation was conducted in the Caatinga biome in the State of Pernambuco located in northeastern Brazil (Fig. 1a and Fig. 1b). Sampling was performed in two nearby sampling areas of the Petrolina (9º 03' 58" S, 40 º 19' 14"W) and Lagoa Grande (8º 48' 11.6"S, 40º 14' 48.4"W) municipalities of the State of Pernambuco, Brazil (Fig. 1c). The climate is BSwh' according to the Köppen–Geiger classification, with an annual average temperature of 26.3°C and rainfall of 577 mm. The soil of both areas are classified as red–yellow Ultisol (Soil Survey Staff 2014), corresponding to Argissolo Vermelho-Amarelo in the Brazilian Soil Classification System (Embrapa 2018) (Fig. 1d). The physicochemical soil rhizosphere characterization associated with both plants is displayed in Table 1. More information about those sampling areas is present in da Silva et al. (2017), Fernandes-Júnior et al. (2015), and Santana et al. (2020). Detailed information regarding the phytogeographical patterns of the Caatinga biome is displayed in Moro et al. (2014) and Moro et al. (2016).

Table 1
Chemical characterization of rhizosphere soil associated with Caatinga plants

RhizospherePlant	pH	SOM	P	S	K	Ca	Mg	B	Cu	Fe	Mn	Zn	Na	Al	H + Al	SB	CEC	Sand
	$CaCl_2$	g kg^{-1}	—mg kg^{-1}—		——mmol kg^{-1}——			————mg kg^{-1}————						————mmol kg^{-1}————				———
N. variegata	5.1	11.0	< 3.0	< 5.0	1.2	11.0	3.0	0.28	0.5	19.0	10.5	1.3	9.0	< 0.02	15.0	15.2	30.2	726.0
T. spicata	5.4	10.0	5.0	6.0	2.5	6.0	3.0	0.3	0.3	30.0	4.3	0.7	15.0	< 0.02	12.0	11.5	23.5	

pH: active acidity, SOM: soil organic matter, P: phosphorus, S: sulfur, K: potassium, Ca: calcium, Mg: magnesium, B: boron, Cu: copper, Fe: iron, Mn: manganes sodium, Al: Aluminum, H + Al: potential acidity, SB: sum of bases (Ca, Mg and K), CEC: cation exchange capacity

Sampling of the plant rhizosphere

Forty-eight native plants were studied, among which 24 rhizosphere samples were from *N. variegata* (Fig. 1e) and the other 24 rhizosphere samples from *T. spicata* (Fig. 1f) rhizosphere. Sampling was carried out in October 2018 in the late dry season, and the rhizosphere soil was sampled according to Batista et al. (2020). Briefly, plants were removed from the soil using a shovel, followed by manual agitation and considering the aggregates adhered to the roots as rhizosphere soil. The samples were stored at the Brazilian Agricultural Research Corporation [Embrapa Semi-arid] (9º 03' 58" S, 40º 19' 14" W) until shipment to the University of Sao Paulo, in the municipality of Piracicaba in the State of Sao Paulo (22º 42' 35" S, 47º 38' 05" W) where they were stored at − 80°C prior to molecular analysis.

Soil rhizosphere DNA extraction

Samples of freeze-dried soil (400 mg) were used for DNA extraction with the PowerSoil DNA Isolation kit (QIAGEN Inc., Valencia, CA, USA), according to the manufacturer. DNA concentrations were determined using the Qubit quantification platform with Quant-iT dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA). DNA quality was verified by electrophoresis in 1% agarose gel using tris-acetate-EDTA buffer (1x TAE), 5µl extracted DNA and 1µl GelRed™ stained (0.5 µg mL^{-1}), followed by visualization on a UV transilluminator (DNR – Bio Imaging Systems/MiniBis Pro).

Arbuscular mycorrhizal fungi sequencing and data analyses

Only 19 samples of *N.variegata* and 18 samples of *T. spicata* presented enough DNA concentration and quality for sequencing. Sequencing was carried out using MiSeq platform (250 bp paired-end) provided by the NGS Soluções Genômicas Facility (Piracicaba, São Paulo, Brazil), and libraries built using a 500-cycle V2 Sequencing kit. A nested PCR (polymerase chain reaction) was used to amplify 850 bp (base pair) fragment covering part of the 18S rRNA, a small subunit (SSU) ribosomal RNA gene (van Geel et al. 2014). For the first amplification step, the forward primer NS31 (5'-TTGGAGGGCAAGTCTGGTGCC-3') (Simon et al. 1992) and the reverse primer AML2 (5'-GAACCCAAACACTTTGGTTTCC-3') (Lee et al. 2008) were used, generating a fragment size of 550 bp. For the second amplification step, the forward primer AMV4. 5NF (5'- AAGCTCGTAGTTGAATTTCG – 3') and the reverse primer AMDGR (5'-CCCAACTATCCCTATTAATCAT – 3') (Sato et al. 2005), were used, generating a fragment size of 300 bp. Sequencing data were processed using QIIME2 (Bolyen et al. 2019) classify-sklearn command using MaarjAM database (Öpik et al. 2010). The workflow used in our investigation is depicted in Fig. S1. Briefly, raw reads were demultiplexed, quality-filtered, joined, and grouped within amplicon sequence variants (ASVs) using DADA2 (Callahan et al. 2016). Subsequently, the taxonomic, diversity, and abundance analysis were performed. Sequences were submitted to the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database with the BioProject PRJNA861682.

Alpha and beta diversity analyses were performed following Silva et al. (2021). The alpha diversity metrics used here were Shannon index (ASV diversity), Observed ASVs, Chao1 (ASV richness), and Faith's phylogenetic diversity (Faith PD). Differences were detected by Wilcoxon signed rank test, a non-parametric test (Krzywinski and Altman 2014). Changes in beta diversity between the sampled plants were tested using principal coordinate analysis (PCoA) with Bray-Curtis distances coupled with a permutational analysis of variance (PERMANOVA, 999 permutations), considering *p*-adjusted by Bonferroni correction. We used a network analysis based on the Newman-Girvan algorithm for determining edge betweenness to detect the different mycorrhizal communities within each sampled plant. For this method, high-betweenness edges are removed sequentially (recalculating at each step) and the best partitioning of the network is selected (Girvan and Newman 2002). We used RNAseq pipeline edgeR (Robinson et al. 2010) and limma voom (Ritchie et al. 2015), available from the Bioconductor project (Huber et al. 2015), to investigate differential abundances, and therefore, reveal which mycorrhizal taxonomic groups were enriched or depleted between sampled plants based on the log-fold changes (logFC). For such analyses, we considered the Benjamini–Hochberg false discovery rate correction (Gaiero et al. 2021).

For clarity, we defining relative and differential abundances as they are used quite often in this paper. For relative abundance, we considered the fraction of the taxa observed in the feature table relative to the sum of all taxa in the sample, and therefore varying between 0 and 100%. Differential abundance was considered as being the differentially abundant taxa between two or more environments, in our case between plants, based on the log-fold changes (Lin and Peddada 2020).

Mini-review on investigations with *N. variegate* and *T. spicata*

We performed a mini-review to obtain studies that reported the plant species evaluated in our study, i.e., *Neoglaziovia variegata* (Arruda) Mez and *Tripogonella spicata* (Nees) P.M.Peterson & Romasch. We used Web of Science as our primary database and ScienceDirect to search full texts published in scientific

journals and used the search string "Neoglaziovia variegata" OR "caroa" for N. variegata plants, and the search string "Tripogonella spicata" OR "Tripogon spicatus" for *T. spicata* plants. We investigated the search terms in title, abstract, and keywords. We did not use "resurrection plant" as a search string, although resurrection plants are a relatively small group, they exhibit a wide taxonomic diversity, comprising several families of plants as reviewed by Gechev et al. (2021). In addition, we used "Tripogon spicatus" as a search string due to the old classification for this species (Royal Botanic Garden 2021).

Results

Mini-review on investigations with *N. variegate* and *T. spicata*

A total of 20 articles published in the last 27 years (from 1995 to 2022) were found for *N. variegata*, and most of them revealed chemical molecules acting as acaricidal and antibacterial agents. In addition, we observed several studies showing the seed morphometric and physiological characterization of *N. variegata* (Table S1). Whilst for *T. spicata* a total of 7 articles published in the last 10 years (from 2012 to 2022) were found, part of them related to the characterization and use of the rhizosphere bacterial community as plant growth-promoting agents. We also observed other studies relating botanical aspects of *T. spicata* (Table S2).

Overview of amplicon sequencing

A total of 527,246 high quality mycorrhizal sequences were generated by Illumina Miseq sequencing, with an average of 14,249.89 sequences per sample (Table S3). The rarefaction curves showed an adequate sequencing depth, allowing the access of the majority of microbial diversity (Fig. 2a). Mycorrhizal sequences were grouped into 175 ASV. *Neoglaziovia variegata* (Arruda) Mez and *Tripogonella spicata* (Nees) P.M.Peterson & Romasch shared only 4% of mycorrhizal ASVs (Fig. 2b).

Differential abundance analyses between mycorrhizal communities

Relative and differential taxonomic abundance results were presented at the order and genus level due to the wide use of the scientific community that investigates microbial communities with amplicon sequencing (Straub et al. 2020). Regardless of the taxa level, the mycorrhizal composition and the specific taxa abundance shifted between the two plants (*N. variegata* and *T. spicata*).

Regarding relative abundances at the order level for *N. variegata*, the mycorrhizal composition was summarized by the predominance of Glomerales (71.0%), Diversisporales (21.0%), and Archaeosporales (8.0%), whilst for *T. spicata* the predominance was based on Glomerales (76.0%), Diversisporales (18.0%), and Paraglomerales (6.0%) (Fig. 3a). At the genus level for *N. variegata*, the mycorrhizal composition was composed of *Glomus* (68.0%), *Gigaspora* (11.0%), *Ambispora* (8.0%), *Diversispora* (7.0%), *Claroideoglomus* (3.0%), *Acaulospora* (2.0%), and *Scutellospora* (1.0%), while for *T. spicata* the mycorrhizal composition was based on the presence of *Glomus* (62.0%), *Acaulospora* (16.0%), *Rhizophagus* (11.0%), *Paraglomus* (6.0%), *Claroideoglomus* (3.0%), *Scutellospora* (1.5%), and *Diversispora* (0.5%) (Fig. 3b).

Differential abundance results followed the same pattern observed for relative abundance at order level, but this did not occur at the genus level. Overall, at the order level for *N. variegata* there was an enrichment of Glomerales, Diversisporales, and Archaeosporales, while for *T. spicata* there was an enrichment of Paraglomerales (Fig. 3c). At the genus level, substantial enrichments were found in *N. variegata* for the genera *Glomus*, *Claroideoglomus*, *Gigaspora*, and *Ambispora*. Equally, substantial enrichments were observed in *T. spicata* for the genera *Acaulospora*, *Rhizophagus, Paraglomus*, and *Archaeospora*. There was depletion of *Diversispora* in *T. spicata*, whereas in *N. variegata* it was enriched (Fig. 3d).

Alpha and beta diversity, and community detection

The alpha-diversity of the mycorrhizal community varied significantly according to the plants, with the highest Shannon diversity ($p < 0.05$), observed ASV ($p < 0.01$), Chao1 ($p < 0.01$), and Faith PD ($p < 0.05$) being found in *T. spicata* (Fig. 4). Likewise, beta-diversity showed higher dissimilarities of the mycorrhizal community between the plants based on Bray-Curtis distance, which was confirmed by the PERMANOVA ($p < 0.001$) (Fig. 5a, Table S4).

According to the algorithm to perform community detection based on edge betweenness (Newman-Girvan), we noticed a different pattern within the same plant, with the most pronounced differentiation found for *N. variegata*. Overall, four different mycorrhizal communities were detected within *N. variegata*, in which each one was composed of at least three samples. Higher modularity was found for *N. variegata* (0.240331), reflecting dense connections within communities and sparse connections across communities (Fig. 5b). While for *T. spicata* we observed a prevalence of only one community and lower modularity (0.005478) (Fig. 5c).

Discussion

Unravelling the composition of the mycorrhizal community associated with endemic plants of hostile environments can be a step forward to develop cutting-edge projects aimed at the bioprospection of microbes that may help plants to cope with abiotic stresses (Sangwan and Prasanna 2022). The role of arbuscular mycorrhizal fungi (AMF) in providing key ecosystem services is well known (Hannula and Morriën 2022). However, we can find restricted approaches that disbelieve the necessity of considering the mycorrhizal community for the plant health under harsh environments or when managing crops in agriculture (Lugo et al. 2015; Ryan and Graham 2018). On the other hand, we firmly advocate that these ancient symbiotic groups are crucial for the soil-plant sustainability. We go further and argue that there are keystone taxa of AMF, which, combined with the physiological plant traits, are essential to help plants to overcome drought events. Notwithstanding, it is reassured that AMF is among the most ubiquitous plant mutualists that improve plant growth and yield by facilitating the uptake of phosphorus and water (Kaur and Suseela 2020).

In our investigation, two plants were sampled in the Caatinga biome, which present a xeric shrubland vegetation. The first plant was *Neoglaziovia variegata* (Arruda) Mez, an endemic bromeliad popularly known as "caroa" (in Portuguese), which presents medicinal, ornamental and fibre production potential (de Lira et al. 2021; Farias and Dantas 2022). The second plant was *Tripogonella spicata* (Nees) P.M.Peterson & Romasch, a grass distributed in the tropics and subtropics of America, popularly known as resurrection plant due to its rapid rehydration capacity (Fernandes-Júnior et al. 2015; Denchev and Denchev 2018). The lack of information about these plants was detected by our mini-review, especially when they are considered as a host of microbes that can help crop plants to tolerate shortages of water in the soil. Therefore, as far as we are concerned, our investigation is the first study to describe the mycorrhizal community associated with the rhizosphere soil of *N. variegate* and *T. spicata*, consequently encouraging further investigations.

Here, the AMF community found in the rhizosphere of *N. variegata* differs from the rhizosphere of *T. spicata*, and this may be related to the phylogenetic host specificity. In other words, the two plants studied can have their own niche since they are not phylogenetically closely related. Thus, these plant species can be harbouring a different AMF community and, therefore, they exploit their resources in the soil in different ways (Terradas et al. 2009; Veresoglou and Rillig 2014; dos Passos et al. 2021). Although the AMF community differed between the plants, more than 90% of the mycorrhizal community for both plants was composed of the order Glomerales and Diversisporales. Likewise, Leroy et al. (2021), investigating the taxonomic and functional diversity of root-associated fungi in bromeliads (none of them being *N. variegata*), found the order Glomerales to be dominant, and *Rhizophagus*, *Funneliformis* and *Glomus* to be the main genera, while here for our bromeliad, the main genera found were *Glomus*, *Gigaspora*, *Ambispora* and *Diversispora*. These taxonomic differences are expected, since life forms and nutritional modes drive the root fungal community structure in bromeliads.

On the other hand, we can also find similar results with a distinct host, although it is known that the partner specificity in mycorrhizal symbiosis occurs at the level of ecological groups, rather than at the species level (Öpik et al. 2009). For example, dos Passos et al. (2021), evaluating the composition of the AMF community of soil samples from the rhizosphere of *Mimosa tenuiflora* (legume), found the order Glomerales to be dominant and argued that some taxa of this order are recognised for colonising plants first, allowing their establishment in diverse environments. Several studies using native plants of the Caatinga have shown similar results (Goto et al. 2010; Pagano et al. 2013; Marinho et al. 2019; Maia et al. 2020; dos Passos et al. 2021; Sousa et al. 2022).

We observed that *T. spicata*, besides the highest diversity indices, presented a well-structured AMF community according to the algorithm for community detection, which may result in benefits for the plant. We couple this evidence with the observed enriched taxa (*Acaulospora*, *Rhizophagus, Paraglomus*, and *Archaeospora*), and argue that these genera may play a crucial role in the desiccation tolerance trait of *T. spicata*. Indeed, it has been gathered evidences that *Rhizophagus* sp. and *Acaulospora* sp. have a pronounced effect on the response of plants to water shortage in the soil, especially due to their intrinsic character of stress-tolerance and widespread geographical distribution, adapting to adverse environmental conditions (Chagnon et al. 2013; Savary et al. 2018). For example, Ortiz et al. (2015) showed that inoculation of *Rhizophagus intraradices* significantly enhanced the relative water content in *Trifolium repens*, particularly when associated with bacteria. Also, Chitarra et al. (2016) working with *Solanum lycopersicum* 'San Marzano nano' demonstrated the efficiency of *R. intraradices* to minimize drought stress-imposed effects, showing that the enhancement of water transport combined with an increase of plant osmolites, stomatal density and gene expression related to plant hormones are the main altered mechanisms. Furthermore, there are investigations that show the positive effect of *Acaulospora* sp. on promoting the plant-growth response under water stress in soil (Yooyongwech et al. 2016; Porto et al. 2020). Little is known about how *Paraglomus* sp. and *Archaeospora* sp. can overcome the negative effects of drought on plants.

Comparing the plants, we noticed a different mycorrhizal enrichment for *N. variegata* for the genera *Claroideoglomus* (order Glomerales), *Gigaspora* (order Diversisporales), and *Ambispora* (order Archaeosporales). Among these genera, *Claroideoglomus* sp. has been the most studied in the Caatinga and has been shown to be promising, because of its rapid establishment and symbiotic interactions with a plant host (Chagnon et al. 2013; Pedone-Bonfim et al. 2018). These results fit well with the C-R-S framework for AMF proposed by Chagnon et al. (2013), who classified AMF species into three functional groups, namely, competitor (C), ruderal (R), and stress tolerating (S). They argued that species belonging to the genus *Gigaspora* sp. have competitive traits (higher soil hyphae density and stronger carbon-sink strength), and *Claroideoglomus* sp. have ruderal traits (higher growth rate and more efficient hyphae healing). *Ambispora* sp. appears to exhibit stress tolerating traits, such as low growth rate and long-lived mycelium (Antunes et al. 2011; Chagnon et al. 2013). Therefore, our research indicates a complementarity of functional enrichment strategies for *N. variegata*, relating this to our result of the different mycorrhizal communities detected within *N. variegata* based on the community detection algorithm.

In addition, we also shed light on some limitations and future perspectives. First, we must consider that the entire AMF community may not have been accessed due to limitations when using the molecular approach, i.e., bias from DNA extraction to low accuracy of the DNA reference databases (Zinger et al. 2020; Leroy et al. 2021). Second, it is important to consider that precipitation and temperature regulate the composition and diversity of the AMF community (Pedone-Bonfim et al. 2018; Teixeira-Rios et al. 2018; Sousa et al. 2022). Therefore, considering that our sampling was executed at the end of the dry season, we could have different results for the rainy season. Ultimately, we believe that AMF and bacteria consortia from the studied plants may potentiate drought protection on crops, making of *N. variegata* and *T. spicata* a hotspot for bioprospection projects in the near future.

Conclusions

We concluded that, although arbuscular mycorrhizal communities are differentially associated with *N. variegata* and *T. spicata*, they have *Glomus* sp. as the most abundant genus. Furthermore, we argue that the differential enrichment of the genera *Gigaspora*, *Diversispora*, *Ambispora*, *Rhizophagus*, *Paraglomus*, and *Archaeospora* play a key role in the desiccation tolerance traits in both plants. As the first investigation to describe the mycorrhizal community associated with these plants, we believe that more studies are needed that use this knowledge to define cutting-edge projects aimed at screening microbes to mitigate drought stress in crop plants in the near future.

Declarations

Author contributions

AMMS, ISM, and EJBNC conceived and designed the study. AMMS, HPF, GVLJ, IFJ, and STA collected the data. AMMS and FPM performed bioinformatic analyses. AMMS, HPF, VAVPA, and APAP analysed the data. AMMS wrote the first draft of the manuscript, and all co-authors contributed to revisions.

ACKNOWLEDGMENTS

We thank Luís Fernando Baldesin and Denise Mescolotti for their collaboration as technicians of the Microbiology Laboratory of the Department of Soil Science at ESALQ/USP, Brazil. Likewise, we are grateful to the whole staff of Embrapa Semiárido for laboratory supervision during our sampling excursion in the Caatinga. Finally, we would like to thank Samuel E. Jones for critically reading the text and for all his contributions to improve its improvement.

FUNDING DECLARATION

This study was funded by the São Paulo Research Foundation (FAPESP) (#2019/13436-8, #2019/27682-0, #2017/24785-8, and #2016/18944-3) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

References

1. Aidar SDT, Chaves ARDM, Fernandes Júnior PI, et al (2017) Vegetative desiccation tolerance of *Tripogon spicatus* (Poaceae) from the tropical semiarid region of northeastern Brazil. Funct Plant Biol 44:1124–1133. https://doi.org/10.1071/FP17066
2. Alam A, Dwivedi A, Emmanuel I (2019) Resurrection plants: Imperative resources in developing strategies to drought and desiccation pressure. Plant Sci Today 6:333–341. https://doi.org/10.14719/pst.2019.6.3.542
3. Antunes PM, Koch AM, Morton JB, et al (2011) Evidence for functional divergence in arbuscular mycorrhizal fungi from contrasting climatic origins. New Phytol 189:507–514. https://doi.org/10.1111/j.1469-8137.2010.03480.x
4. Batista AM, Libardi PL, Giarola NFB (2020) Evaluation of the soil aggregation induced by the plant roots in an Oxisol by turbidimetry and water percolation. Rhizosphere 16:100265. https://doi.org/10.1016/j.rhisph.2020.100265
5. Bolyen E, Rideout JR, Dillon MR, et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0209-9
6. Bonfante P, Genre A (2008) Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. Trends Plant Sci 13:492–498. https://doi.org/10.1016/j.tplants.2008.07.001
7. Butcher D Gouda E (2020) The new bromeliad taxon list. Utrecht, the Netherlands: University Botanic Gardens. http://bromeliad.nl/taxonlist. Accessed 01 December 2021
8. Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869
9. Chagnon PL, Bradley RL, Maherali H, Klironomos JN (2013) A trait-based framework to understand life history of mycorrhizal fungi. Trends Plant Sci 18:484–491. https://doi.org/10.1016/j.tplants.2013.05.001
10. Chitarra W, Pagliarani C, Maserti B, et al (2016) Insights on the impact of arbuscular mycorrhizal symbiosis on tomato tolerance to water stress. Plant Physiol 171:1009–1023. https://doi.org/10.1104/pp.16.00307
11. da Silva AF, de Freitas ADS, Costa TL, et al (2017) Biological nitrogen fixation in tropical dry forests with different legume diversity and abundance. Nutr Cycl Agroecosystems 107:321–334. https://doi.org/10.1007/s10705-017-9834-1
12. de Lira KL, Machado FDF, Viana AFSC, et al (2021) Gastroprotective activity of *Neoglaziovia variegata* (Arruda) Mez.(*Bromeliaceae*) in rats and mice. Journal of Medicinal Food, 24:1113-1123. https://doi.org/10.1089/jmf.2020.0182
13. Denchev TT, Denchev CM (2018) *Tilletia tripogonellae* (*Tilletiaceae*), A new smut fungus on *Tripogonella spicata* (*Poaceae*) from Argentina. Ann Bot Fenn 55:273–277. https://doi.org/10.5735/085.055.0407
14. dos Passos JH, Maia LC, de Assis DMA, et al (2021) Arbuscular mycorrhizal fungal community structure in the rhizosphere of three plant species of crystalline and sedimentary areas in the Brazilian dry forest. Microb Ecol 82:104–121. https://doi.org/10.1007/s00248-020-01557-y
15. Dryflor, Banda R, Delgado A, et al (2014) Plant diversity patterns in neotropical dry forests and their conservation implications. Science 353:1–125.
16. Embrapa (2018) Brazilian system of soil classification, 5th ed. Embrapa, Rio Janeiro, Brazil, 356 p.
17. Farias LAA de C, Dantas BF (2022) Morphometric characterization and functional traits of fruits and seeds of *Neoglaziovia variegata* (Arruda) Mez. J Seed Sci 44:e202244021. http://dx.doi.org/10.1590/2317-1545v44250044
18. Fernandes-Júnior PI, Aidar S de T, Morgante CV, et al (2015) The resurrection plant *Tripogon spicatus* (*Poaceae*) harbors a diversity of plant growth promoting bacteria in northeastern Brazilian caatinga. Rev Bras Cienc do Solo 39:993–1002. https://doi.org/10.1590/01000683rbcs20140646
19. Gaiero JR, Tosi M, Bent E, et al (2021) Soil microbial communities influencing organic phosphorus mineralization in a coastal dune chronosequence in New Zealand. FEMS Microbiol Ecol 97:1–16. https://doi.org/10.1093/femsec/fiab034

20. Gechev T, Lyall R, Petrov V, Bartels D (2021) Systems biology of resurrection plants. Cell Mol Life Sci 78:6365–6394. https://doi.org/10.1007/s00018-021-03913-8
21. Girvan M, Newman MEJ (2002) Community structure in social and biological networks. Proc Natl Acad Sci USA 99:7821–7826. https://doi.org/10.1073/pnas.122653799
22. Goto BT, Silva GADA, Yano-Melo AM, Maia LC (2010) Checklist of the arbuscular mycorrhizal fungi (Glomeromycota) in the Brazilian semiarid. Mycotaxon 113:251–254. https://doi.org/10.5248/113.251
23. Hannula SE, Morriën E (2022) Will fungi solve the carbon dilemma? Geoderma 413: 115767. https://doi.org/10.1016/j.geoderma.2022.115767
24. Huber W, Carey VJ, Gentleman R, et al (2015) Orchestrating high-throughput genomic analysis with Bioconductor. Nat Methods 12:115–121. https://doi.org/10.1038/nmeth.3252
25. IBGE (2019) Biomas e sistema costeiro-marinho do Brasil : compatível com a escala 1:250 000 /Coordenação de Recursos Naturais e Estudos Ambientais, Rio de Janeiro, Brasil. 168 p.
26. Kaur S, Suseela V (2020) Unraveling arbuscular mycorrhiza-induced changes in plant primary and secondary metabolome. Metabolites 10:1–30. https://doi.org/10.3390/metabo10080335
27. Kavamura VN, Santos SN, Silva JL da, et al (2013) Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. Microbiol Res 168:183–191. https://doi.org/10.1016/j.micres.2012.12.002
28. Krzywinski M, Altman N (2014) Non-parametric tests. Nat Methods 11:467–468. https://doi.org/10.1017/cbo9781139164832.008
29. Lee J, Lee S, Young JPW (2008) Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 65:339–349. https://doi.org/10.1111/j.1574-6941.2008.00531.x
30. Leroy C, Maes AQM, Louisanna E, et al (2021) Taxonomic, phylogenetic and functional diversity of root-associated fungi in bromeliads: effects of host identity, life forms and nutritional modes. New Phytol 231:1195–1209. https://doi.org/10.1111/nph.17288
31. Lin H, Peddada S Das (2020) Analysis of microbial compositions: a review of normalization and differential abundance analysis. npj Biofilms Microbiomes 6:. https://doi.org/10.1038/s41522-020-00160-w
32. Lugo MA, Reinhart KO, Menoyo E, et al (2015) Plant functional traits and phylogenetic relatedness explain variation in associations with root fungal endophytes in an extreme arid environment. Mycorrhiza 25:85–95. https://doi.org/10.1007/s00572-014-0592-5
33. Maia LC, Passos JH, Silva JA, et al (2020) Species diversity of Glomeromycota in Brazilian biomes. Sydowia 72:181–205. https://doi.org/10.12905/0380.sydowia72-2020-0181
34. Marinho F, Oehl F, da Silva IR, et al (2019) High diversity of arbuscular mycorrhizal fungi in natural and anthropized sites of a Brazilian tropical dry forest (Caatinga). Fungal Ecol 40:82–91. https://doi.org/10.1016/j.funeco.2018.11.014
35. Miles L, Newton AC, DeFries RS, et al (2006) A global overview of the conservation status of tropical dry forests. J Biogeogr 33:491–505. https://doi.org/10.1111/j.1365-2699.2005.01424.x
36. Moro MF, Lughadha EN, Filer DL, et al (2014) A catalogue of the vascular plants of the Caatinga Phytogeographical Domain: A synthesis of floristic and phytosociological surveys. Monograph. https://doi.org/10.11646/phytotaxa.160.1.1
37. Moro MF, Nic Lughadha E, de Araújo FS, Martins FR (2016) A phytogeographical metaanalysis of the semiarid Caatinga domain in Brazil. Bot Rev 82:91–148. https://doi.org/10.1007/s12229-016-9164-z
38. Oliver MJ, Farrant JM, Hilhorst HWM, et al (2020) Desiccation tolerance: avoiding cellular damage during drying and Rehydration. Annu Rev Plant Biol 71:435–460. https://doi.org/10.1146/annurev-arplant-071219-105542
39. Öpik M, Metsis M, Daniell TJ, et al (2009) Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New Phytol 184:424–437. https://doi.org/10.1111/j.1469-8137.2009.02920.x
40. Öpik M, Vanatoa A, Vanatoa E, et al (2010) The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188:223–241. https://doi.org/10.1111/j.1469-8137.2010.03334.x
41. Ortiz N, Armada E, Duque E, et al (2015) Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: Effectiveness of autochthonous or allochthonous strains. J Plant Physiol 174:87–96. https://doi.org/10.1016/j.jplph.2014.08.019
42. Pagano MC, Zandavalli RB, Araújo FS (2013) Biodiversity of arbuscular mycorrhizas in three vegetational types from the semiarid of Ceará State, Brazil. Appl Soil Ecol 67:37–46. https://doi.org/10.1016/j.apsoil.2013.02.007
43. Pedone-Bonfim MVL, da Silva DKA, Maia LC, Yano-Melo AM (2018) Mycorrhizal benefits on native plants of the Caatinga, a Brazilian dry tropical forest. Symbiosis 74:79–88. https://doi.org/10.1007/s13199-017-0510-7
44. Peixoto RDM, Silva WELE, Almeida JRGS, et al (2016) Antibacterial potential of native plants from the caatinga biome against *Staphylococcus* spp. isolates from small ruminants with mastitis. Rev Caatinga 29:758–763. https://doi.org/10.1590/1983-21252016v29n328rc
45. Pennington RT, Lavin M, Oliveira-Filho A (2009) Woody plant diversity, evolution, and ecology in the tropics: Perspectives from seasonally dry tropical forests. Annu Rev Ecol Evol Syst 40:437–457. https://doi.org/10.1146/annurev.ecolsys.110308.120327
46. Porto DL, Arauco AM de S, Boechat CL, et al (2020) Arbuscular mycorrhizal fungi on the initial growth and nutrition of *Parkia platycephala* benth. Under water stress. Cerne 26:66–74. https://doi.org/10.1590/01047760202026012671
47. Ritchie ME, Phipson B, Wu D, et al (2015) Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43:e47. https://doi.org/10.1093/nar/gkv007

48. Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140. https://doi.org/10.1093/bioinformatics/btp616
49. Royal Botanic Gardens, Kew (2021) The World Checklist of Vascular Plants (WCVP). https://doi.org/10.15468/6h8ucr. Accessed 13 November 2021
50. Ryan MH, Graham JH (2018) Little evidence that farmers should consider abundance or diversity of arbuscular mycorrhizal fungi when managing crops. New Phytol 220:1092–1107. https://doi.org/10.1111/nph.15308
51. Sangwan S, Prasanna R (2021) Mycorrhizae helper bacteria: Unlocking their potential as bioenhancers of plant–arbuscular mycorrhizal fungal associations. Microb Ecol 1–10. https://doi.org/10.1007/s00248-021-01831-7
52. Santana SRA, Voltolini TV, Antunes G dos R, et al (2020) Inoculation of plant growth-promoting bacteria attenuates the negative effects of drought on sorghum. Arch Microbiol 202:1015–1024. https://doi.org/10.1007/s00203-020-01810-5
53. Santos JC, Leal IR, Almeida-Cortez JS, et al (2011) Caatinga: The scientific negligence experienced by a dry tropical forest. Trop Conserv Sci 4:276–286. https://doi.org/10.1177/194008291100400306
54. Sato K, Suyama Y, Saito M, Sugawara K (2005) A new primer for discrimination of arbuscular mycorrhizal fungi with polymerase chain reaction-denature gradient gel electrophoresis. Grassl Sci 51:179–181. https://doi.org/10.1111/j.1744-697x.2005.00023.x
55. Savary R, Masclaux FG, Wyss T, et al (2018) A population genomics approach shows widespread geographical distribution of cryptic genomic forms of the symbiotic fungus *Rhizophagus irregularis*. ISME J 12:17–30. https://doi.org/10.1038/ismej.2017.153
56. Silva AMM, Estrada-Bonilla GA, Lopes CM, et al (2021) Does organomineral fertilizer combined with phosphate-solubilizing bacteria in sugarcane modulate soil microbial community and functions? Microb Ecol. 84:539–555. https://doi.org/10.1007/s00248-021-01855-z
57. Simon L, Lalonde M, Bruns TD (1992) Specific amplification of 18S fungal ribosomal genes from vesicular- arbuscular endomycorrhizal fungi colonizing roots. Appl Environ Microbiol 58:291–295. https://doi.org/10.1128/aem.58.1.291-295.1992
58. Siyum ZG (2020) Tropical dry forest dynamics in the context of climate change: syntheses of drivers, gaps, and management perspectives. Ecol Process 9:25. https://doi.org/10.1186/s13717-020-00229-6
59. Smith SE, Read DJ (2010) Mycorrhizal symbiosis. Academic press.
60. Soil Survey Staff (2014) Soil Survey | NRCS Soils. http://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/survey/. Accessed 13 November 2021
61. Sousa NMF, Roy J, Hempel S, et al (2022) Precipitation and temperature shape the biogeography of arbuscular mycorrhizal fungi across the Brazilian Caatinga. J Biogeogr 49:1137–1150. https://doi.org/10.1111/jbi.14376
62. Straub D, Blackwell N, Langarica-Fuentes A, et al (2020) Interpretations of environmental microbial community studies are biased by the selected 16S rRNA (Gene) amplicon sequencing pipeline. Front Microbiol 11:1–18. https://doi.org/10.3389/fmicb.2020.550420
63. Taketani RG, Kavamura VN, dos Santos SN (2017) Diversity and technological aspects of microorganisms from semiarid environments. In: de Azevedo J, Quecine M (eds.) Diversity and benefits of microorganisms from the tropics. Springer, Cham, pp 3-19 https://doi.org/10.1007/978-3-319-55804-2_1
64. Teixeira LP, Lughadha EN, Silva MVC da, Moro MF (2021) How much of the Caatinga is legally protected? An analysis of temporal and geographical coverage of protected areas in the Brazilian semiarid region. Acta Bot Brasilica 35:473–485. https://doi.org/10.1590/0102-33062020abb0492
65. Teixeira-Rios T, da Silva DKA, Goto BT, Yano-Melo AM (2018) Seasonal differences in arbuscular mycorrhizal fungal communities in two woody species dominating semiarid caatinga forests. Folia Geobot 53:191–200. https://doi.org/10.1007/s12224-018-9314-7
66. Terradas J, Peñuelas J, Lloret F (2009) The Fluctuation Niche in Plants. Int J Ecol 959702. https://doi.org/10.1155/2009/959702
67. Torres-Santos PT, Farias IF, Almeida MD, et al (2021) Acaricidal efficacy and chemical study of hexane extracts of the leaves of *Neoglaziovia variegata* (*Bromeliaceae*) against the tick *Rhipicephalus microplus*. Exp Appl Acarol 84:263–270. https://doi.org/10.1007/s10493-021-00611-9
68. Van Geel M, Busschaert P, Honnay O, Lievens B (2014) Evaluation of six primer pairs targeting the nuclear rRNA operon for characterization of arbuscular mycorrhizal fungal (AMF) communities using 454 pyrosequencing. J Microbiol Methods 106:93–100. https://doi.org/10.1016/j.mimet.2014.08.006
69. Veresoglou SD, Rillig MC (2014) Do closely related plants host similar arbuscular mycorrhizal fungal communities? A meta-analysis. Plant Soil 377:395–406. https://doi.org/10.1007/s11104-013-2008-2
70. Yooyongwech S, Samphumphuang T, Tisarum R, et al (2016) Arbuscular mycorrhizal fungi (AMF) improved water deficit tolerance in two different sweet potato genotypes involves osmotic adjustments via soluble sugar and free proline. Sci Hortic (Amsterdam) 198:107–117. https://doi.org/10.1016/j.scienta.2015.11.002
71. Zinger L, Donald J, Brosse S, et al (2020) Advances and prospects of environmental DNA in neotropical rainforests. Adv Ecol Res 62:331–373. https://doi.org/10.1016/bs.aecr.2020.01.001

Figures

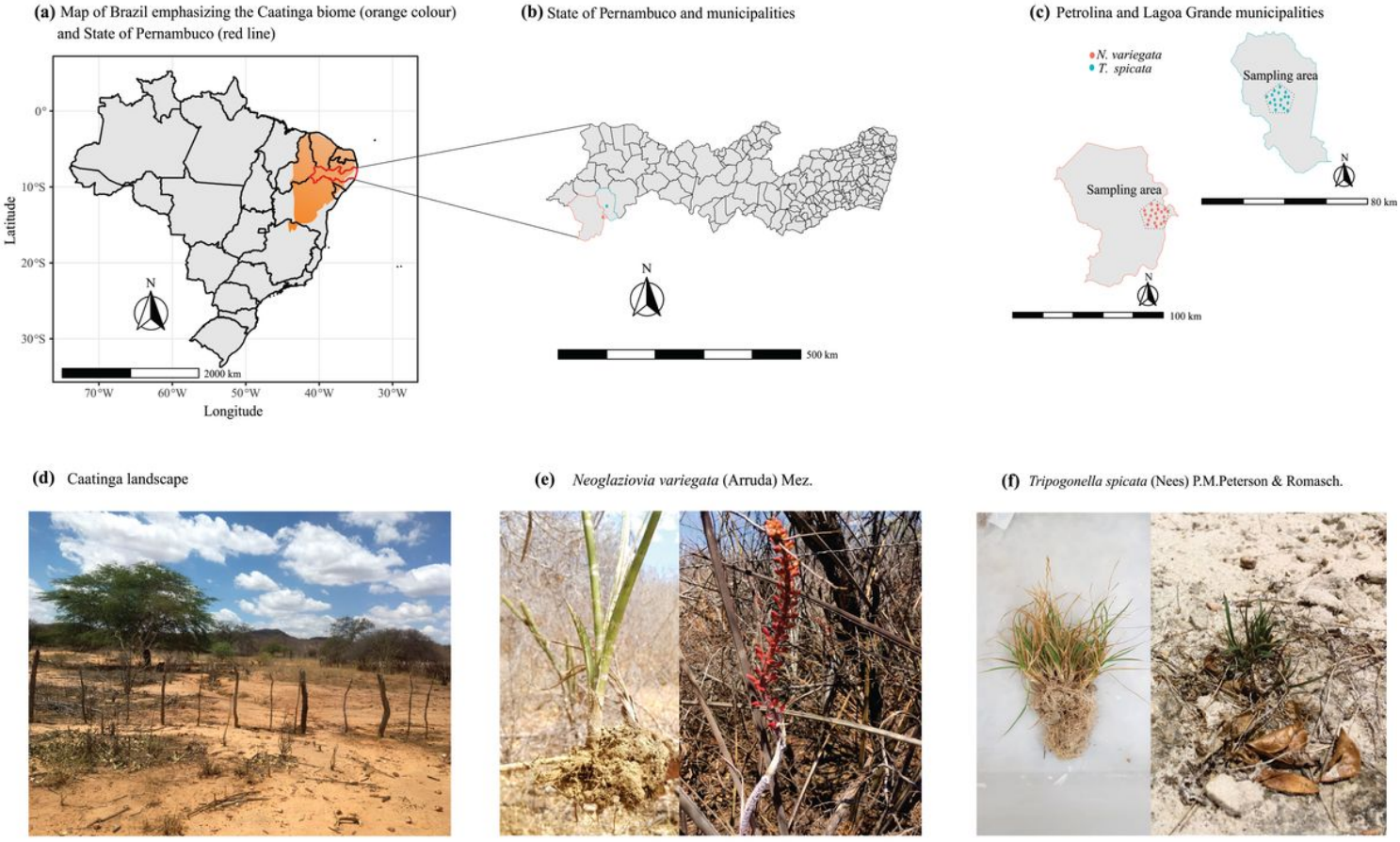

Figure 1

Location of the sampling area and distribution of the Caatinga biome in Brazil, **a** map of the State of Pernambuco in Brazil, showing its municipalities, highlighting the municipalities where the sampling was carried out, **b** map of Petrolina and Lagoa Grande municipalities and sampling points, **c** a common landscape of the Caatinga biome during late dry season showing some *Mimosa tenuiflora* trees, **d**sampled plant *Neoglaziovia variegata*(Arruda) Mez., a bromeliad so-called “caroa”, **e** sampled plant *Tripogonella spicata* (Nees) P.M. Peterson & Romasch., a grass so-called the resurrection plant, **f**

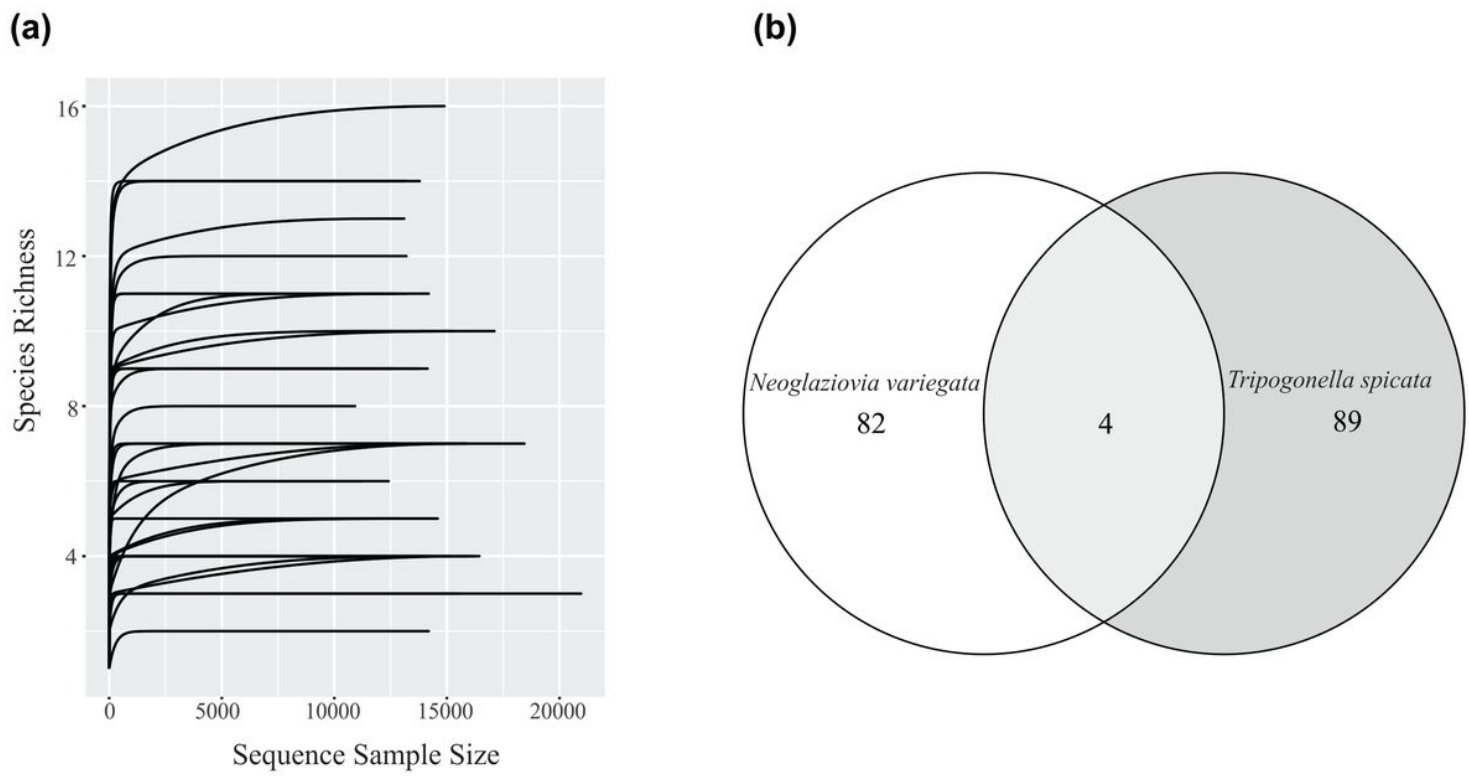

Figure 2

Sequencing rarefaction curves indicating the sampling depth per sample for mycorrhizal community measured by NS31/AML2 primers targeted to ITS and 18S rRNA amplicon sequencing, **a** and Venn diagrams of the overall amplicon sequence variants (ASVs) distribution across the sampled plants for mycorrhizal community, **b**

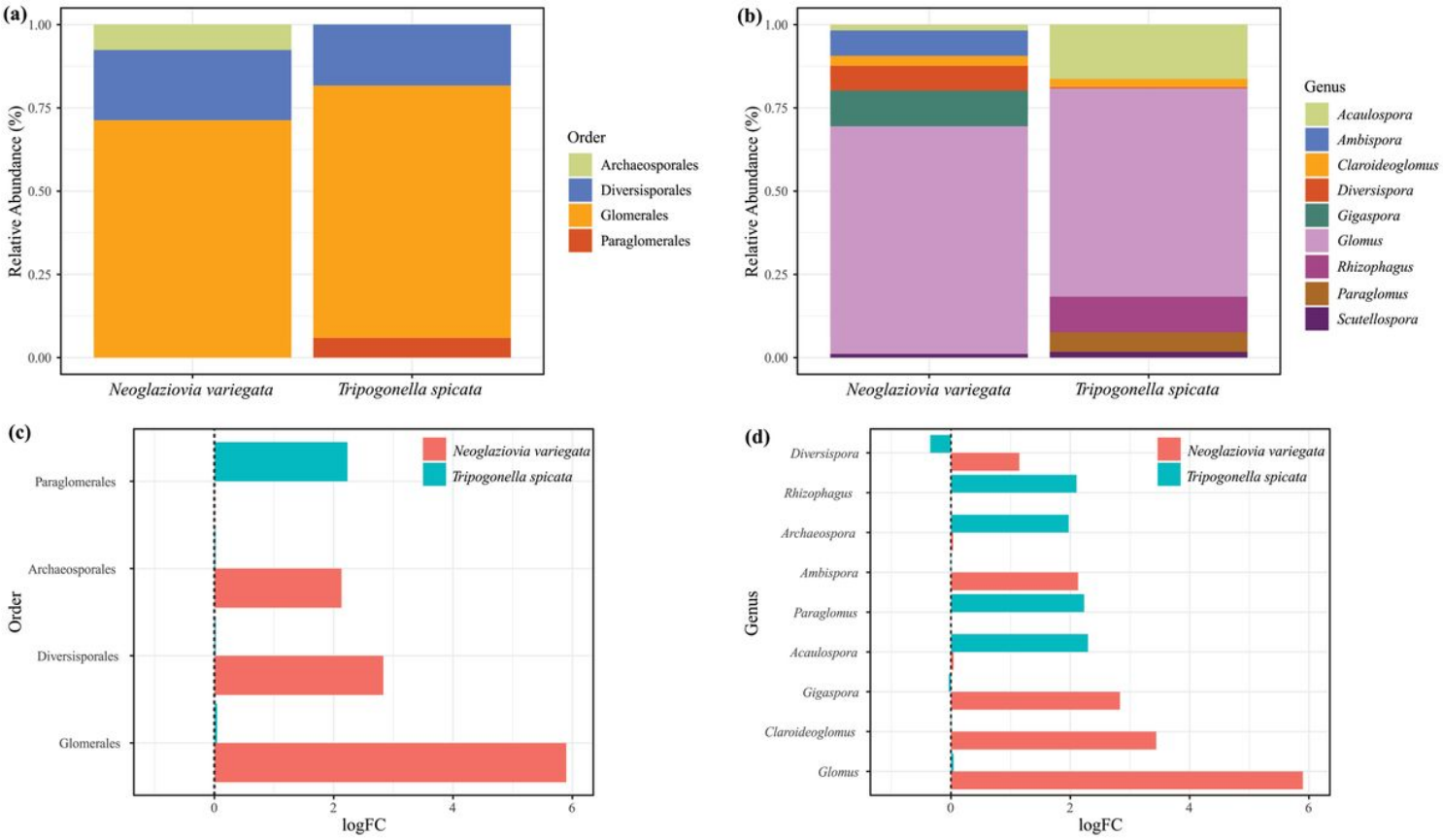

Figure 3

Community composition of arbuscular mycorrhizal fungi (AMF) in two plants sampled in the Caatinga biome based on the relative abundance of order, **a** and genus, **b** taxa. Differential abundance analysis considering order, **c** and genus, **d** taxa of the AMF community for the plants with the results expressed by log-fold changes (logFC)

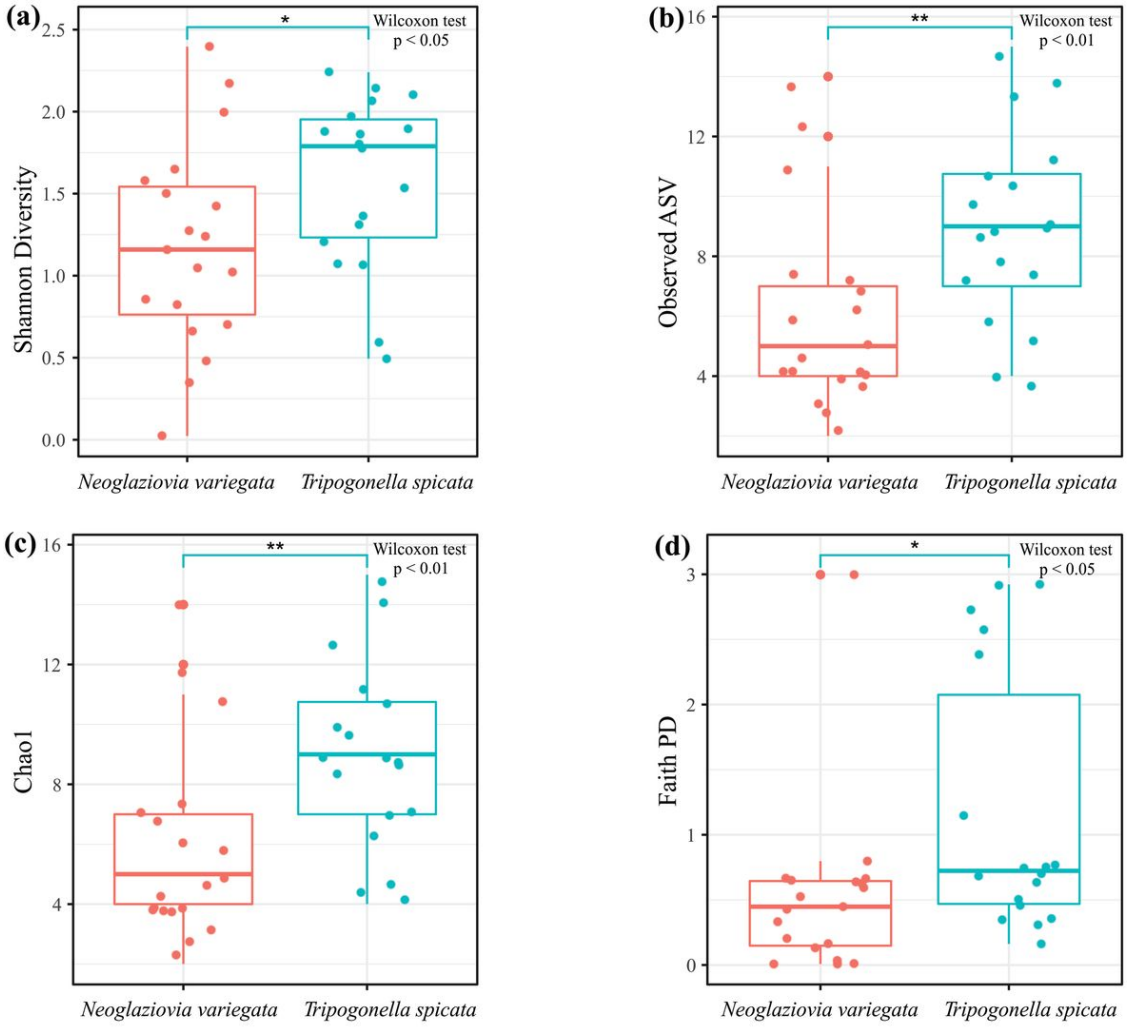

Figure 4

Alpha diversity indices considering the two plants sampled in the Caatinga biome, expressed by Shannon diversity, **a** observed ASV [amplicon sequence variant], **b** Chao1, **c** and Faith's phylogenetic diversity, **d**. Statistical differences are denoted as *($p < 0.05$) and **($p < 0.01$) by the Wilcoxon test

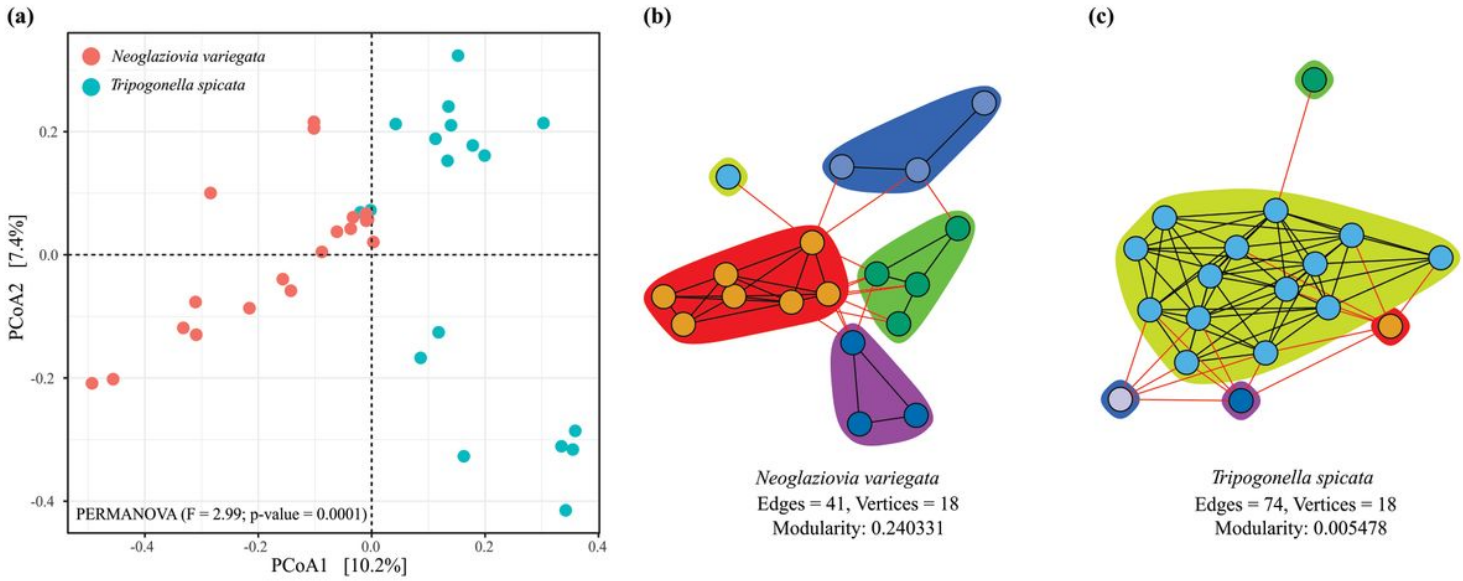

Figure 5

Beta diversity expressed by principal coordinate analysis (PCoA) using Bray-Curtis distances, depicting mycorrhizal data from two plants sampled in the Caatinga biome, **a**. Network analysis for mycorrhizal community detection within *N. variegata*, **b** and *T. spicata*, **c** based on edge betweenness (Newman-Girvan). Each node represents the samples and colours represent the different mycorrhizal communities detected

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterials.docx
- FigureS1.jpg