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Grazing exclusion-induced changes in soil fungal communities in a highly desertified Brazilian dryland

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ABSTRACT

Soil desertification poses a critical ecological challenge in arid and semiarid climates worldwide, leading to decreased soil productivity due to the disruption of essential microbial community processes. Fungi, as one of the most important soil microbial communities, play a crucial role in enhancing nutrient and water uptake by plants through mycorrhizal associations. However, the impact of overgrazing-induced desertification on fungal community structure, particularly in the Caatinga biome of semiarid regions, remains unclear. In this study, we assessed the changes in both the total fungal community and the arbuscular mycorrhizal fungal community (AMF) across 1. Natural vegetation (native), 2. Grazing exclusion (20 years) (restored), and 3. affected by overgrazing-induced degradation (degraded) scenarios. Our assessment, conducted during both the dry and rainy seasons in Irauçuba, Ceará, utilized Internal Transcribed Spacer (ITS) gene sequencing via Illumina® platform. Our findings highlighted the significant roles of the AMF families Glomeraceae (~71% of the total sequences) and Acaulosporaceae (~14% of the total sequences) as potential key taxa in mitigating climate change within dryland areas. Moreover, we identified the orders Pleosporales (~35% of the total sequences) and Capnodiales (~21% of the total sequences) as the most abundant soil fungal communities in the Caatinga biome. The structure of the total fungal community differed when comparing native and restored areas to degraded areas. Total fungal communities from native and restored areas clustered together, suggesting that grazing exclusion has the potential to improve soil properties and recover fungal community structure amid global climate change challenges.

1. Introduction

Drylands, encompassing about 40% of the Earth's surface, comprise both arid and semiarid habitats (Nickayin et al., 2022). Yet, certain regions within these arid and semiarid areas have undergone significant degradation due to a combination of natural ecosystem conditions and human activities (Araujo et al., 2022). This widespread degradation has led to desertification, impacting approximately 1.9 billion hectares and affecting 1.5 billion people worldwide (Albuquerque et al., 2020). Notably, the Brazilian semiarid region has witnessed desertification processes, primarily attributed to geological soil characteristics accelerated by improper land-use practices, impacting an area of 1.2 million km^2 (CGEE, 2016).

Improper land-use practices in the Brazilian semiarid region, specifically overgrazing of native vegetation (intensive grazing for extended periods without sufficient vegetation recovery), have been identified as

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significant contributors to the desertification process (Feltran-Barbieri and Féres, 2021). Overgrazing reduces the vegetation cover, leading to soil erosion, nutrient depletion, and a decrease in soil organic matter (SOM) (Oliveira Filho et al., 2019). Consequently, the desertification process caused by overgrazing has a detrimental impact on soil microbial communities (Pereira et al., 2021). Recent studies have underscored the effects of desertification through overgrazing on soil microbial communities, including bacteria archaea, and fungi, showing a reduction in in both abundance and diversity, richness, and ecological functions (Araujo et al., 2023; Pereira et al., 2021; Silva et al., 2022, 2024b).

While the impact of overgrazing on soil bacterial and archaeal communities has been extensively studied, there remains limited knowledge about how the soil fungal community responds to the process of desertification. The soil fungal community represents one of the most diverse assemblages of organisms on Earth, playing crucial ecological roles in soil processes such as organic matter decomposition and the transport of water and nutrients to plants (Dix and Webster, 2012). Taxonomically, the fungal community consists of nine phyla, including Opisthosporidia, Chytridiomycota, Neocallimastigomycota, Blastocladiomycota, Zoopagomycota, Mucoromycota, Glomeromycota, Basidiomycota, and Ascomycota (Naranjo-Ortiz and Gabaldón, 2019). Particularly, arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota, play essential functions in soils, including soil structuring (Genre et al., 2020) and enhancing plant growth through nutrient uptake, water absorption, and protection against pathogens (2019; 2011). In degraded lands, AMF has been recognized as a sensitive indicator (Vasar et al., 2021) and can also help mitigate the negative effect of degradation worldwide (Duarte and Maherali, 2022).

To mitigate further desertification, various restoration practices have been implemented in areas affected by overgrazing, among which grazing exclusion (GE) has been widely recognized as an effective approach for restoring degraded areas and promoting the restoration of ecosystem productivity (Zhang et al., 2021). Previous studies conducted in the Brazilian semiarid region have demonstrated that GE leads to notable improvements in soil properties (Oliveira Filho and Pereira, 2021). These improvements include increased levels of soil organic matter, soil enzymes (β-glucosidase, arylsulfatase, and phosphatase), and bacterial biomass (Oliveira Filho et al., 2019; Oliveira Filho and Pereira, 2021; Silva et al., 2024a). Moreover, there is an enhancement in the quality of metabolic capacities of plant growth-promoting bacteria and an increase in gene abundance associated with N and P nutrient cycles (Freitas Nunes Oliveira et al., 2021; Pereira et al., 2021; Silva et al., 2024b). Additionally, grazing exclusion has led to an increase in soil health after two decades of restoration, elevating it to levels comparable to native Caatinga vegetation (Lima et al., 2024). Furthermore, it has been shown to contribute to the recovery of bacterial diversity and richness (Pereira et al., 2021). Similar studies conducted worldwide have also indicated the potential of GE to restore soil properties (Liu et al., 2020; Xun et al., 2018; Zhang et al., 2021). However, there is currently a gap in research when it comes to assessing the fungal community in soils under desertification and restoration, specifically through overgrazing and GE, especially in the Brazilian semiarid region.

Unraveling the structure and composition of fungal communities in semiarid regions is essential, especially in highly degraded areas and potential restoration contexts. In this study, we hypothesized that the desertification process resulting from overgrazing alters the fungal community composition, while GE could effectively restore this community. To test this hypothesis, we conducted an analysis of the total fungal community, including AMF, through Internal Transcribed Spacer (ITS) sequencing via Illumina® platform. This analysis was carried out in soils under different conditions: desertification (due to overgrazing), restoration (through two decades of GE), and native vegetation (used as reference). Sampling was conducted in both dry and rainy seasons, within the Brazilian semiarid region.

2. Material and methods

2.1. Study site

The study was conducted in Irauçuba, Ceará, Brazil (3º44′46" S and 39º47′00" W) (Fig. 1). The average annual precipitation is ~454 mm, mainly concentrated between January and May (Fig. S1; FUNCEME, 2022). The mean temperature ranges from 26 ºC to 28 ºC (Alvares et al., 2013) and the soils are classified as Planosols (Oliveira Filho et al., 2019). In this region, the practice of overgrazing has been applied since 1960 and it has promoted high land degradation. In 2000, nine closed plots (2500 m^2) under GE were implemented to avoid animal grazing (Fig. 2). Then, these plots have been closed during the last two decades.

Soil samples were collected from three distinct areas at a depth of 0–10 cm depth: a degraded area (overgrazing), a restored area managed with grazing exclusion (GE), and a site with native Caatinga vegetation (reference). The soil sampling was done in both dry (October 2021) and rainy (April 2022) seasons. We collected soil samples in tree areas separated by approximately 2 km distance. To avoid pseudoreplication, each area has three different scenarios (degraded, restored, and native), each of which was replicated three times. At each replicated scenario, we sampled three points. At each point, we collected nine sub-samples, which were homogenized to create a composite sample. Thus, we analyzed 54 samples (3 areas × 3 scenarios× 3 replicates × 2 seasons). The experimental design is detailed extensively in Pereira et al. (2021) and Silva et al. (2022).

2.2. Soil chemical, physical, and biological parameters

Total N, NH_4^+, and NO_3^- were determined by the Kjeldahl method (Nelson and Sommers, 1982; Vezzani et al., 2001; Freitas et al., 2013). Total organic carbon (TOC) was extracted using carbon oxidation in organic form with potassium dichromate ($K_2Cr_2O_7$) and determined by colorimetry. The content of Al, Ca, Mg, Na, and P were estimated according to EMBRAPA (2009). Electrical conductivity (EC) was measured using an electrical conductivity meter. The determination of cation exchange capacity (CEC) followed the methods described by Raij et al. (2001) for tropical soils. A pipette method was used to measure the clay fraction (Gee and Bauder, 1986), sieving was used to measure the sand fraction, and the silt fraction was determined by calculating the difference between the total mass of sand and clay fractions. A gravimetric method with oven drying was used to determine soil moisture, in which wet soil samples were weighed and then dried in an oven at 105 °C until they reached a constant mass. The samples were weighed again in the following step, and the mass difference represented the water mass.

The activities of the following enzymes, β-glucosidase (BG), urease (U), and acid phosphatase (AP), were determined according to publicly available methods. Briefly, the β-glucosidase (EC 3.2.1.21) activity was measured using ρ-nitrophenyl β-glucopyranoside as substrate under incubation (1 h, 37° C) in a modified buffer adjusted to pH 6.0. The ρ-nitrophenol formed was determined spectrophotometrically at 410 nm (Eivazi and Tabatabai, 1988). The activity of acid phosphatase (EC 3.1.3.2) was measured using disodium ρ-nitrophenyl phosphate as substrate under incubation (1 h, 37° C) in a modified universal buffer adjusted to pH 6.5. The amount of ρ-nitrophenol formed was measured spectrophotometrically at 420 nm (Tabatabai and Bremner, 1969). The urease (EC 3.5.1.5) activity was determined using the method of Kandeler and Gerber (1988) with urea as substrate under incubation (1 h, 37° C). The amount of ammonium (NH_4^+-N) produced was determined using the Kjeldahl method (Pereira et al., 2018). Glomalin was extracted following the Wu et al. (2014) and Wright and Upadhyaya (1998) procedures. Microbial biomass carbon (MBC) was determined by the chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). Soil respiration was estimated by quantification of CO_2-C released during 31 days of incubation at 28 ºC (Cardoso et al., 2013). The qCO_2 was calculated from basal respiration (CO_2-C.h^{-1}) per

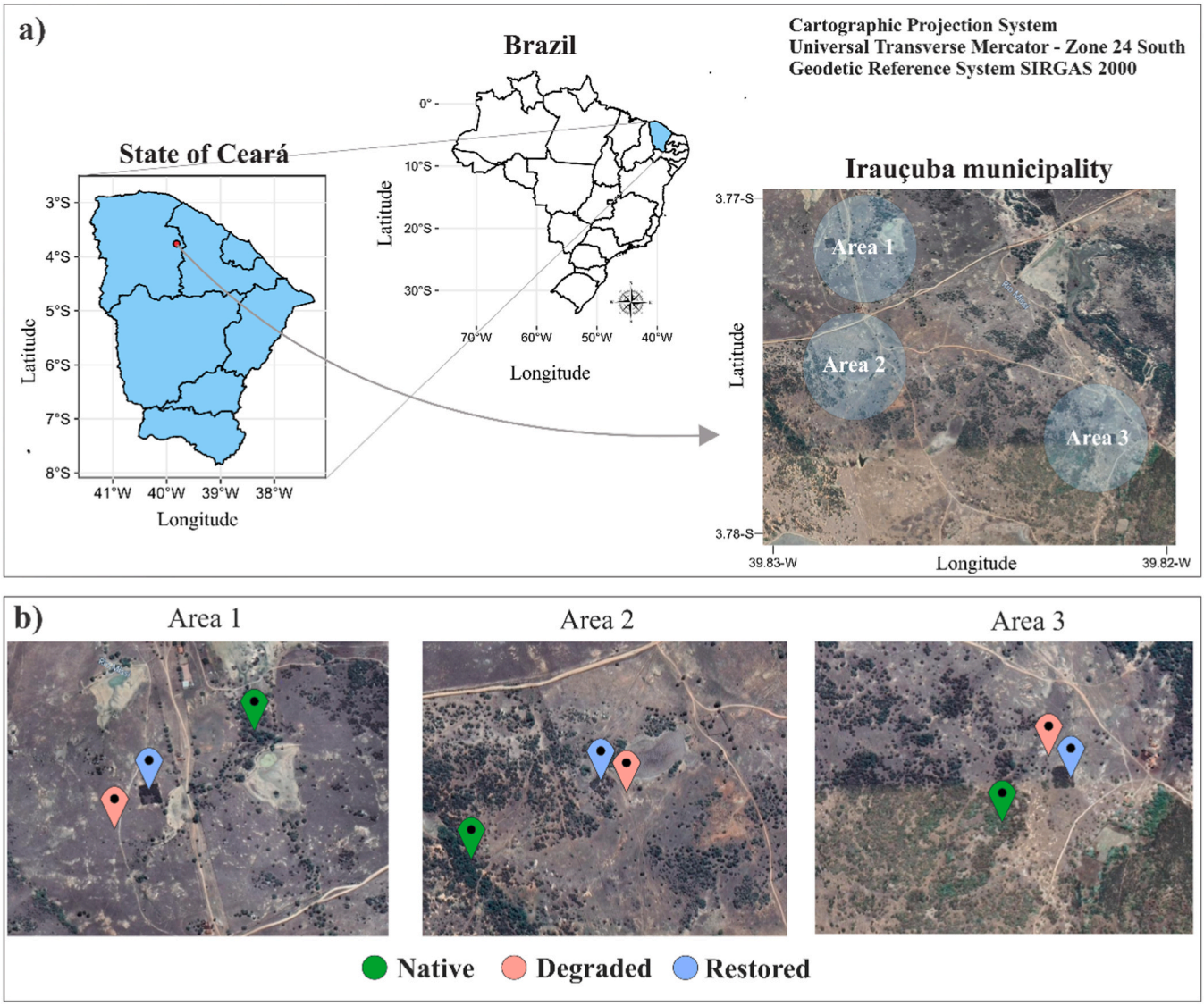

Fig. 1. Geographic location of Irauçuba Municipality, Ceará State, Brazil. Areas 1, 2, and 3 of soil sampling during dry and rainy seasons in Native Caatinga vegetation, Degraded areas by overgrazing and Restored soils by grazing-exclusion approach.

unit microbial biomass carbon (MBC). The values of soil parameters are shown in Table S1.

2.3. DNA extraction and Illumina sequencing

Genomic DNA was extracted from each sample using 0.25 g soil with the DNeasy PowerSoil Pro Kit (QIAGEN, DE, USA), following the manufacturer's protocols. The ITS amplicon libraries of the total fungal community were prepared following the ITS sequencing library protocol (Illumina®, San Diego, CA, USA). PCR was performed with DNA extracted from soil (~ 50 ng) with ITS1 (CTTGGTCATTTAGAGGAAGTAA) and reverse primer ITS2 (GCTGCGTTCTTCATCGATGC) (5.0 μL) (Smith and Peay, 2014). The KAPA 2x HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA) was used for the PCR reaction, and the AMPure XP kit was used for the purification reactions. Negative and positive controls will be introduced in all amplification procedures. The amplified products were sequenced by the MiSeq sequencing platform (Illumina®) using 250 bp paired-end reads following Illumina® sequencing protocols.

To optimize the sequencing of the mycorrhizal fungal communities, a nested reaction was performed to increase the sequencing effectiveness (Van Geel et al., 2014). The first PCR reaction was performed with primers NS31 (TTGGAGGGCAAGTCTGGTGCC) (Simon et al., 1992) and reverse AML2 (GAACCCAAACACTTTGGTTTCC) (Lee et al., 2008), a region-specific for this microbial classification within the small-subunit ribosomal region. For the second reaction, the primers used were AMV4.5NF (CGAAATTCAACTACGAGCTT) and AMDGR (ATGATTAATAGGGATAGTTGGG) (Sato et al., 2005). In the second set of primers, pre-adapters were inserted for subsequent ligation of the barcodes. The obtained material was submitted to standard sequencing on the MiSeq platform (Illumina®, San Diego, CA, EUA) with the V3 kit.

The FASTQ files for the 54 samples have been deposited in the NCBI database. The accession numbers for these files are available under BioProject ID PRJNA1083982 for ITS sequences and PRJNA1083924 for AMF sequences.

2.4. Data processing and statistical analyses

Data processing in this study was performed by a generalized Divisive Amplicon Denoising Algorithm 2 (DADA2) workflow written in snakemake (Callahan et al., 2016). Briefly, adapter trimming was performed with cutadapt, using the non-default settings of –max-n 0.

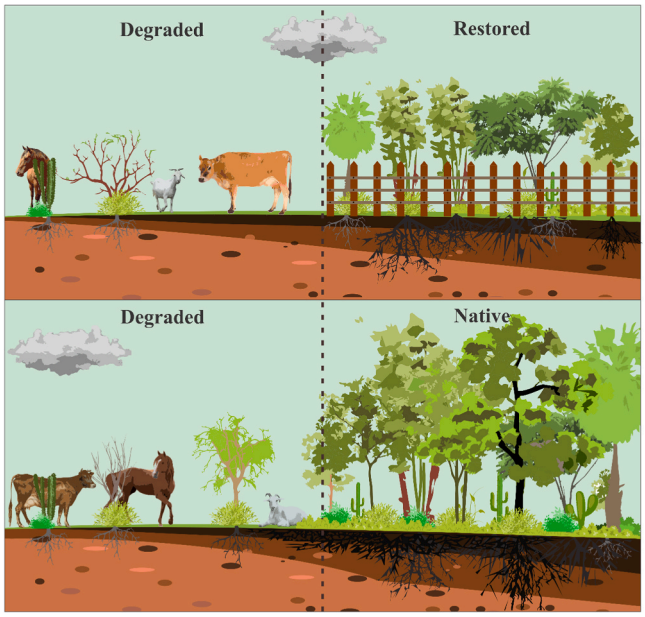

Fig. 2. Representation of native Caatinga vegetation (Native), soil degradation through overgrazing (Degraded) and 20 years of grazing exclusion by fencing (Restored).

Filtering of reads with DADA2 was performed with the non-default parameters of maxee=5 and truncq=5. The core of DADA2 and taxonomic assignment were run with default parameters and the UNITE fungal database for taxonomic assignment (Abarenkov et al., 2022).

We used the RStudio software for bioinformatic analyses. and proceed by filtering any ASV that did not have a class level taxonomic assignment or was not present in at least 5% of samples. Shannon's diversity index was calculated as an alpha diversity metric. With the VEGAN package, a principal coordinates analysis (PCoA) using the Bray-Curtis dissimilarity values was applied to evaluate differences among fungal communities in different treatments and seasons after 1000 permutations. A differential abundance analysis (DAA) using ANCOMBC was performed, and taxonomic groups from native and restored sequencing data were compared to degraded data ($p < 0.05$) as the rainy season was compared to the dry season (Lin and Peddada, 2020). Log2 changes were used in the DAA for order and family levels taxonomic ranks in the land variable treatment and seasons, respectively, i.e., log2 (native) - log2(degraded). Furthermore, we used redundancy analysis (RDA) to assess correlations between fungal communities' structure and soil attributes.

The variance homogeneity and normality were examined for our quantitative results using the Breusch–Pagan and Shapiro–Wilks tests. We then used nested ANOVA and compared groups of means with Tukey's test ($p < 0.05$).

3. Results

3.1. Soil chemical characterization

Soil pH ($CaCl_2$) ranged from 4 to 5, with degraded areas significantly more acidic than restored and native areas across dry and rainy seasons ($p < 0.05$). Soil organic carbon (SOC), Ca^{2+}, and EC were higher in native and restored areas regardless of season. During the dry season, restored areas exhibited higher available phosphorus (P) content compared to native and degraded areas, while natives had the highest P content during the rainy season. Total nitrogen (N) content was highest in native and restored areas and lowest in degraded areas, with restored and degraded areas showing seasonal differences, particularly higher N content in the dry season. NO_3^- contents did not vary significantly between areas during the rainy season, except for degraded areas showing the lowest values during the dry season. Ammonium (NH_4^+) levels were similar across all areas except for degraded areas, which had higher contents in the dry season. Native areas had lower Na^+ content during the dry season, with no significant differences between other areas or seasons. Moisture levels were generally higher during the rainy season across all treatments, with degraded areas having the lowest values. Exchangeable sodium percentage (ESP) increased more in degraded areas regardless of season. Al^{3+} contents showed no significant effect on soil management or season (Table S1).

Microbial biomass of C (MBC) was higher in native and restored areas, particularly during the rainy season, while degraded sites had the lowest MBC content, especially in the rainy season. β-glucosidase activity exhibited higher levels in native and restored compared to degraded soils. Additionally, it demonstrated elevated activity during the rainy season in degraded and restored. Urease activity was higher in native and restored soils and did not display seasonal variability. Phosphatase enzyme activity was notably higher in native and restored soils compared to degraded soils during the dry season. In the rainy season, phosphatase activity was higher in restored compared to degraded, while native exhibited intermediate values. Glomalin levels were higher in native areas compared to degraded areas. The restored areas exhibited intermediate results during the dry season, with no significant difference observed during the rainy season. The metabolic quotient (qCO_2) was higher in the dry season in all treatments; in this season, degraded areas were higher when compared to native, and restored showed intermediate results, while in the rainy season, native and restored areas were higher than degraded (Table S1).

3.2. Fungal community distance and diversity variation

The structure of the total fungal community differed when comparing native and restored areas to degraded areas (Fig. 3). The results indicated that the total fungal communities from native and restored areas clustered together. In contrast, the structure of the AMF community showed a significant distinction when comparing native, restored, and degraded lands (Fig. 3; $p < 0.05$). The PCoA indicated that the restored area clustered between the native and degraded areas, with a slight overlap observed between the AMF communities of restored and degraded areas ($p < 0.05$).

Both total fungal and AMF diversity varied according to sites and seasons (Fig. 4). In general, the alpha diversity of total fungal and AMF communities was higher in the rainy season. When comparing sites, native areas exhibited higher diversity of the total fungal community as compared to 20 years of grazing exclusion (restored). However, during the rainy season, the diversity of the AMF community was higher in restored and degraded areas than in the native area, with no observed variation during the dry season. Remarkably, the alpha diversity index of both total fungal and AMF communities showed no significant variation between native and degraded (Fig. 4).

3.3. Relationship between fungal community, soil parameters, and land uses across different seasons

The redundancy analysis conducted between both the total fungal and AMF communities and soil properties revealed a closer relationship between restored and native areas (Fig. 5). In both restored and native areas, microbial biomass C, enzymatic activity, glomalin, OM content, and soil moisture showed positive correlations with the total fungal community (Fig. 5a, b). The orders Eurotiales and Hypocreales were strongly correlated with the native area, while Venturiales and Capnodiales correlated with restored areas. In the degraded area, Mycosphaerellales and Botryosphaeriales showed a high correlation in the dry season, while Mycosphaerellales and Chaetothyriales correlated with the degraded area in the rainy season. Regarding the AMF community, the families Acaulosporaceae, Claroideoglomeraceae, and

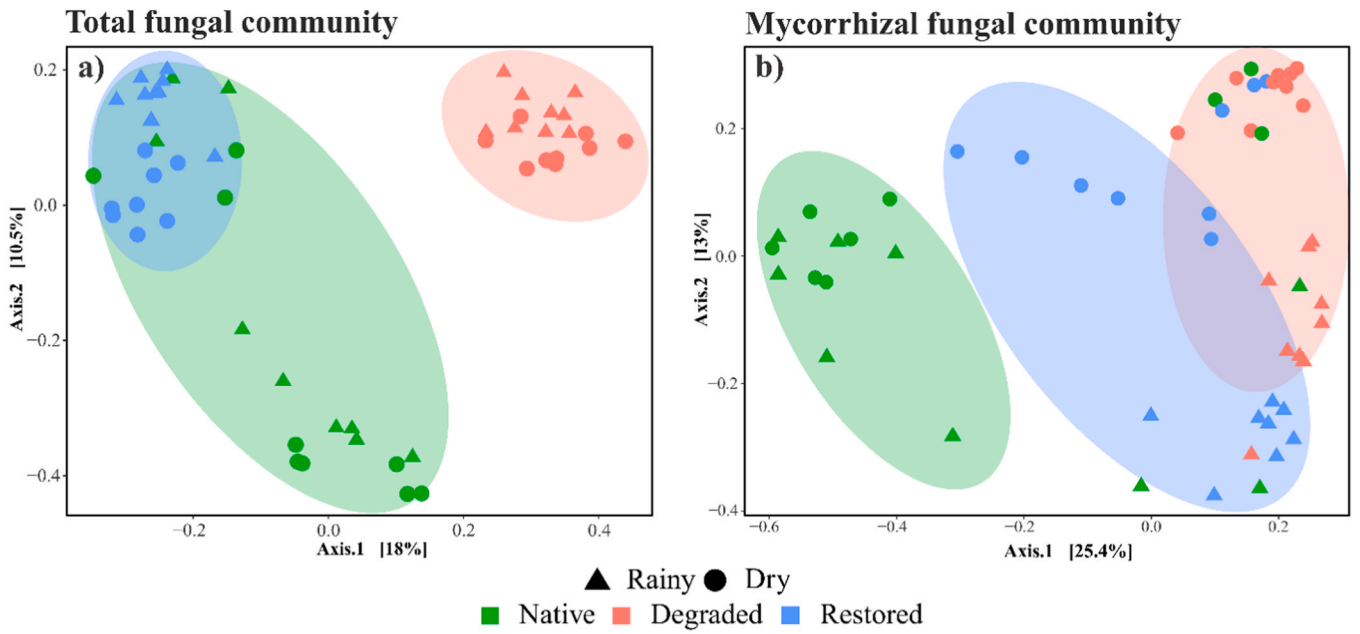

Fig. 3. Principle Coordinate Analysis (PCoA) based on bray-Curtis distance of total fungal community (a) and Mycorrhizal fungal communities (b) under Native Caatinga vegetation (green), Degraded system by overgrazing (pink) and Restored area by grazing-exclusion management (blue) during dry (circle) and rainy (triangle) seasons.

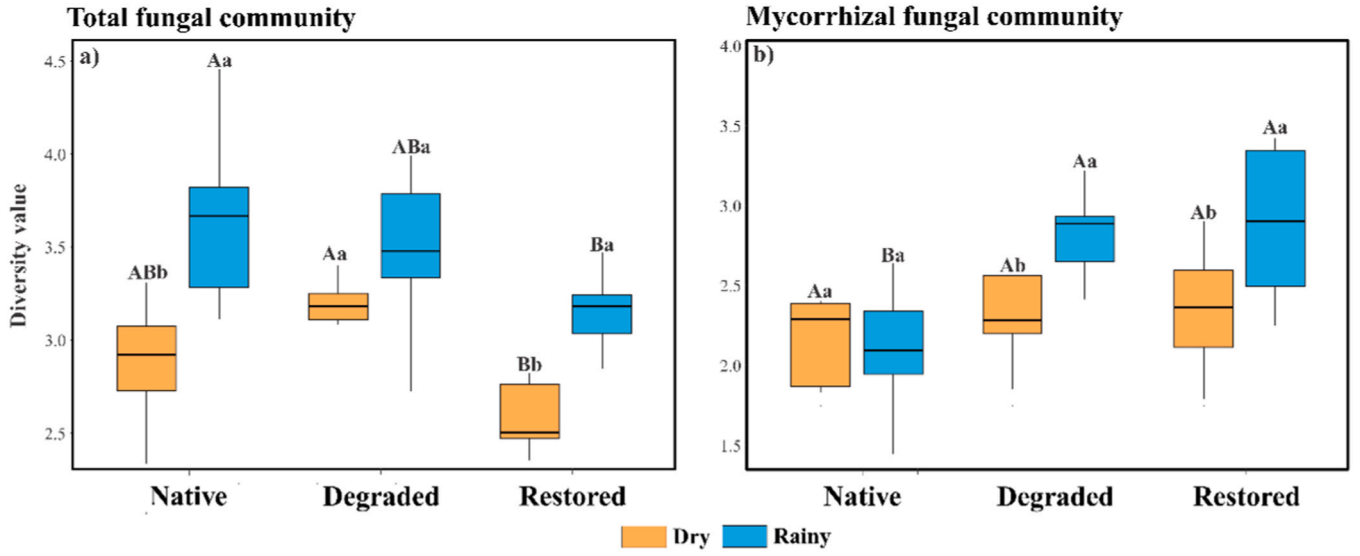

Fig. 4. Alpha diversity, based on Shannon's index of total fungal community (a) and mycorrhizal fungal community (b) in soil under native Caatinga vegetation, degraded by grazed systems and restored by grazing exclusion. Capital letters compared soil management within each season, and lowercase letters compared seasons within each soil management.

Gigasporaceae were strongly correlated to the degraded area in the dry season, while in the rainy season, only Glomeraceae presented correlation (Fig. 5c, d). The family Diversiporaceae correlated to the native area, while Archeosporaceae showed a correlation with the restored area.

Soil organic matter content had a positive correlation with fungal abundance, mainly Capnodiales and Eurotiales. Conversely, clay content correlated with a decreased abundance of fungal orders Pleosporales and Hypocreales. Additionally, nitrogen, phosphorus, and potassium contents, exerted an influence on the fungal community. Specifically, elevated phosphorus levels were linked to a reduction in the abundance of orders Sordariales and Agaricales (Fig. 5a, b).

In the dry season, P levels correlated positively with the presence of Acaulosporaceae and Claroideoglomeraceae, while both families showed a negative correlation with soil EC. Additionally, Diversiporaceae and Glomeraceae exhibited a positive relationship with soil glomalin levels, alongside negative correlations with soil clay content and Na^{+}. Conversely, Archeosporaceae had a positive correlation with soil clay content, exchangeable ESP, and soil moisture, while showing a negative correlation with soil N contents. Gigasporaceae showed a positive correlation with β-glucosidase enzyme activity and a negative correlation with soil Al^{3+} content (Fig. 5c). In the rainy season, the associations between AMF families and soil parameters were distinct. Glomeraceae and Diversiporaceae were positively linked with SOC content, alkaline phosphatase enzyme activity, and soil moisture, while exhibiting negative correlations with soil Na^{+} content. Gigasporaceae,

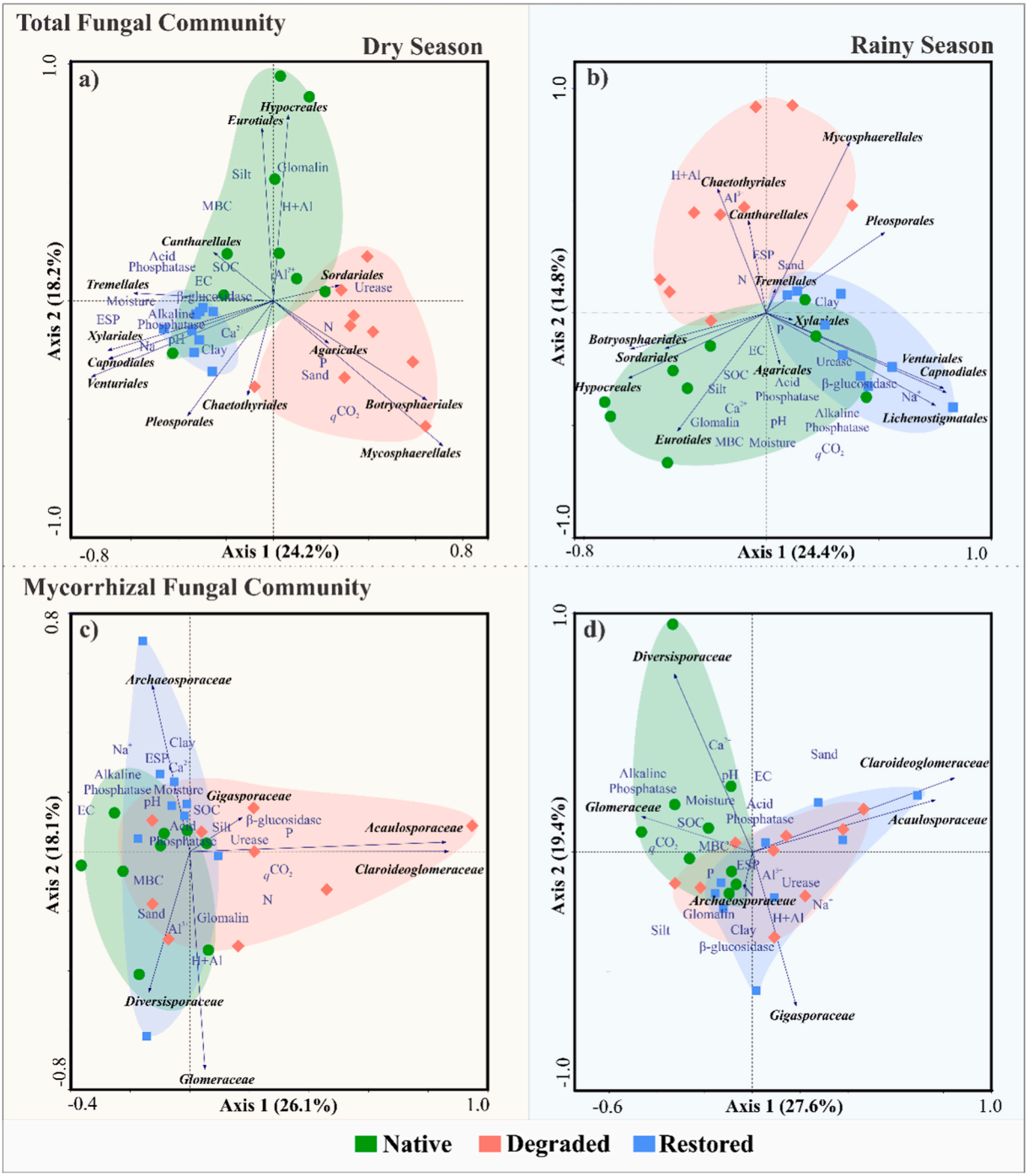

Fig. 5. Redundancy Analysis (RDA) of soil chemical, physical, and biological attributes against fungal communities structure. Total fungal community in the dry season (a) and the rainy season (b). Mycorrhizal fungal community in the dry season (c) and the rainy season (d). SOC: Soil organic carbon, EC: Soil Electrical Conductivity, ESP: Exchangeable Sodium Percentage, MBC: Microbial biomass C,.

as in the dry season, displayed a positive correlation with β-glucosidase enzyme activity. Claroideoglomeraceae exhibited a positive correlation with higher soil sand content and a negative correlation with soil silt content. Lastly, Archeosporaceae demonstrated a positive correlation with soil glomalin content and a negative correlation with soil electrical conductivity (Fig. 5d).

3.4. Relative abundance of total fungal and AMF groups

Regarding taxonomical composition, there was a variation in the relative abundance of total fungal orders according to seasons and areas (Fig. 6). Pleosporales (~35% of the total sequences) were dominant for all areas regardless of the season. In the dry season, the native area showed a higher relative abundance of Hypocreales (~21% of the total sequences), followed by Pleosporales (~20%), and Capnodiales (~19%); while the degraded area showed a higher abundance of Mycosphaerellales (~20%), Botryosphaeriales (~5%), and Agarlicales (~3%). The restored area revealed a higher abundance of Capnodiales (~15%), Venturiales (~7%), Xylariales (~4%), and Mycosphaerellales (~3%). In the rainy season, the degraded area showed higher abundance of Chaetothyriales (~9%), Sordariales (~7%), and Hypocreales (5%), while restored areas presented higher abundance of Capnodiales

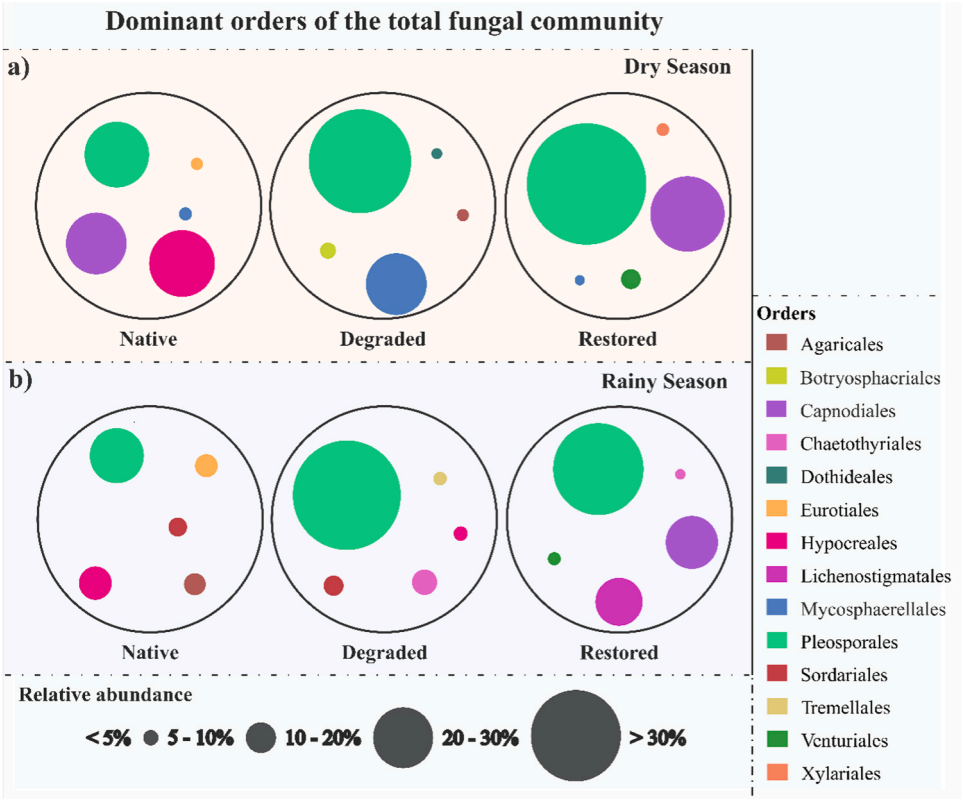

Fig. 6. Five dominant orders of total fungal community within each treatment (Native, Degraded, and Restored) in the dry season (a) and the rainy season (b).

(~19%), Lichenostigmatales (~18%), and Venturiales.

Regarding the AMF community, the family Glomeraceae (~71%) presented the highest abundance in all areas and seasons (Fig. 7). However, each area presented other abundant AMF families. Thus, Archaeosporaceae (5%) was the second most abundant in restored areas, followed by Claroideoglomeraceae, in the dry season. In the rainy

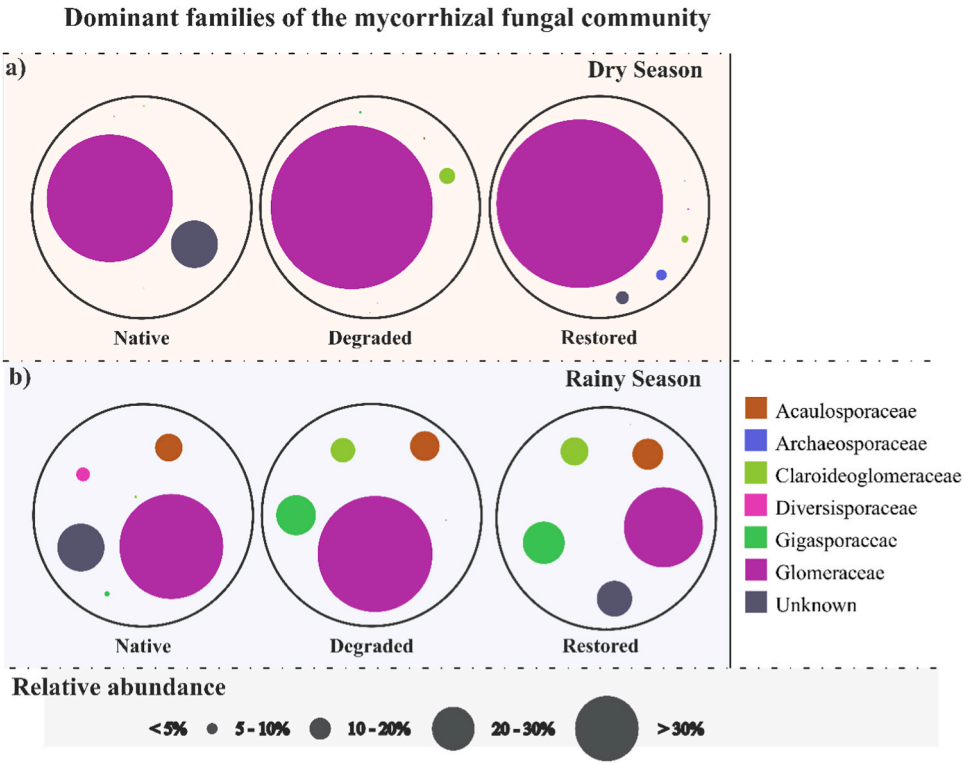

Fig. 7. Five dominant families of mycorrhizal fungal community within each treatment (Native, Degraded, and Restored) in the dry season (a) and the rainy season (b).

season, Gigasporaceae (~19%), Acaulosporaceae (~13%), and Claroideoglomeraceae (~11%) were abundant in the restored area. In the native area during the dry season, Claroideoglomeraceae and Diversisporaceae were abundant, while in the rainy season, Acaulosporaceae (~13%), Diversisporaceae (~12%), and Gigasporaceae (~2.5%) were abundant. In the degraded area, Claroideoglomeraceae (~7%), Gigasporaceae (~3%) were abundant in the dry season, while Gigasporaceae (~20%), Acaulosporaceae (~14%), and Claroideoglomeraceae (~13%), were abundant in the rainy season.

3.5. Differential abundance of total fungal and AMF groups

The differential abundance analysis based on log2 changes at the order level showed that 14 orders in the total fungal community differed between areas and seasons (Fig. 8a, b). Regarding the AMF community, four families differed between areas (Fig. 8c), and three varied between seasons (Fig. 8d). In the total fungal community, Eurotiales, Mortierellales, and Trichosporonales were abundant in the native area, while Lichenostigmatales, Orbiliales, and Xylariales were abundant in the restored as compared to the degraded area. The orders Capnodiales, Helotiales, and Trichosporonales were abundant in both native and restored areas as compared to the degraded area. In contrast,

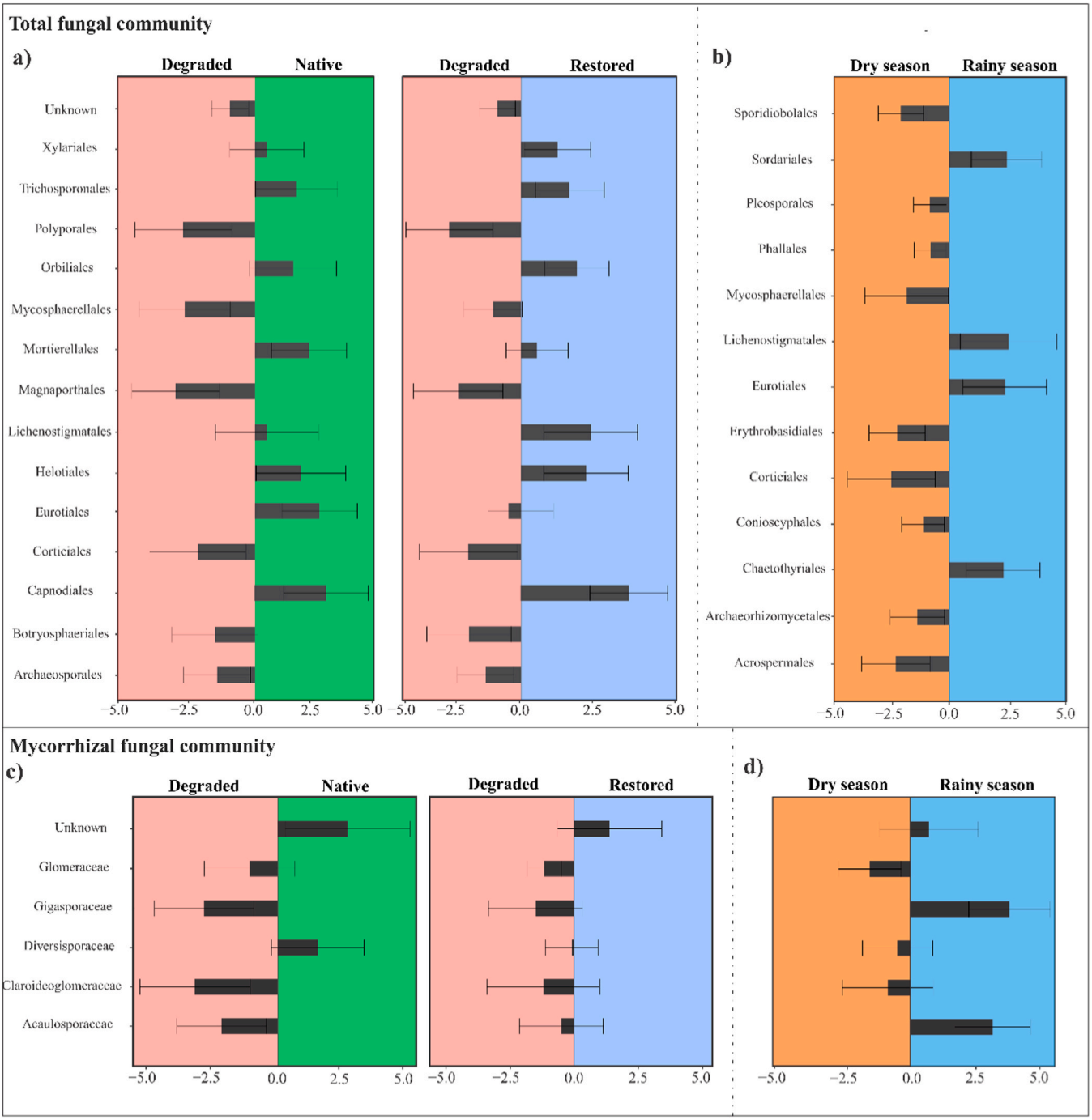

Fig. 8. Differential analysis based on log2 changes of Order level taxonomic ranks in the land variable treatment (Native and Restored) and season (Rainy) in reference to the degraded and dry season, respectively, for total fungal community and Mycorrhizal fungal community.

Archaeosporales, Botryosphaeriales, Corticiales, Magnaporthales, and Polyporales were abundant in degraded areas as compared to native and restored areas. Regarding the AMF community, the abundance of Acaulosporaceae, Claroideoglomeraceae, and Gigasporaceae was higher in the degraded area as compared to the native area, and there was no difference regarding to restored area. The families Acaulosporaceae and Gigasporaceae were higher in the rainy season, and Glomeraceae showed a higher abundance in the dry season.

4. Discussion

Soil microbial communities play essential functions within the soil ecosystem (Mendes et al., 2017), including the cycling of nutrients (such as C, N, P, and S) and litter decomposition (Teste et al., 2017; Cotta et al., 2019). This is particularly important for the maintenance of arid and semiarid regions. In the Brazilian semiarid region, studies comparing microbial communities in degraded and under restoration lands have primarily focused on bacterial and archaeal communities, which perform multiple soil functions (Araujo et al., 2023; Pereira et al., 2021, 2022). However, it is noteworthy that fungal communities also play crucial roles in soil ecosystems, significantly contributing to soil structuring and enhancing root surfaces. Recognizing this research gap, our study aims to assess both total fungal and AMF communities in soils affected by desertification due to overgrazing and undergoing restoration through grazing exclusion.

In general, our results confirm our overall hypothesis, demonstrating that the desertification process driven by overgrazing has a significant impact on the fungal community, including AMF. Conversely, grazing exclusion appears to hold potential for restoring the fungal community and improving soil properties. It is noteworthy that the AMF community in degraded areas was originally native, and over time, the most adaptable groups have thrived, becoming more prevalent in this environment condition. The overlap of samples between degraded and restored areas serves as a clear illustration of this process. While the variability observed in native samples aligns with expectations, restored areas display an intermediate state, with a subtle overlap between degraded and restored (Fig. 3b). This gradual transition towards the restoration of native communities in restored areas suggests a positive result, albeit with lingering influences from the degraded environment. Additionally, the levels of C-, N-, and P-acquiring enzymes were higher in both native and restored soils (see Fig. S2), which could be attributed to the continuous presence of vegetation in the native area (e.g., herbaceous plants, stunted trees, and bushes) and the restored area (grazing exclusion as secondary vegetation) (Oliveira-Filho, 2019). These differences observed between native and restored areas compared to the degraded area are indicative of a shift in the overall structure of the total fungal community.

Specifically, our findings reveal a decrease in the abundance of fungal groups such as Capnodiales and Eurotiales in degraded lands, possibly linked to vegetation losses duo toovergrazing, as these fungal orders are typically associated with plants (Crous et al., 2009; Barbosa et al., 2020). In contrast, we observed an increase in Mycosphaerellales, a dominant order that encompasses a wide variety of fungal species with different host preferences and distinct environmental conditions (Dai et al., 2022). Furthermore, desertification is associated with an elevated abundance of arbuscular mycorrhizal fungi families, including Glomeraceae and Acaulosporaceae, which aligns with previous studies reporting a high abundance of AMF in degraded lands in the Brazilian semiarid region (Silva et al., 2022; Sousa et al., 2014). Notably, during the rainy season, the native soil exhibited the highest fungal community diversity, as indicated by Shannon's index, while the restored soil displayed intermediate diversity levels ($p < 0.05$). These results are consistent with prior research on soil bacterial communities conducted by Pereira et al. in 2021.

Furthermore, Pleosporales emerged as the most abundant order across all areas in both the dry and rainy seasons. Within the class Dothideomycetes (Ascomycota), Pleosporales is recognized as one of the largest orders and includes species with a global distribution, encompassing semiarid grasslands (Knapp et al., 2019, 2015; Pintye and Knapp, 2021). Fungal species from this order primarily act as saprotrophs, but can also colonize plant residues (Knapp et al., 2012; Phookamsak et al., 2014). Notably, certain subsets within this taxonomic group include plant endophytes, particularly dark septate endophytes, which play pivotal roles in ecosystem dynamics (Knapp et al., 2015).

During the dry season, the order Capnodiales exhibited the second-highest dominance in the restored areas and ranked third in the native areas, in contrast to its lower prevalence in degraded areas. Concurrently, we observed a scarcity of competitive decomposer taxa, such as Basidiomycetes. Capnodiales typically thrive on leaf surfaces, especially in environments where plant-sucking insects produce honeydew (Chomnunti et al., 2014). Moreover, this order has been identified as a dominant fungal group on rock surfaces and other highly exposed ecosystems (Ruibal et al., 2005). Some species within Capnodiales are commonly referred to as "black sooty molds" due to their melanized spores and hyphae (Coleine et al., 2022). The melanization of spores confers resistance against microbial attacks and abiotic degradation induced by UV radiation, thereby enhancing their viability during long-range dispersal, as elucidated by Wyatt et al. (2013). The prevalence of Capnodiales in the soils of a tropical dry forest like the Caatinga may be attributed to the resilience conferred by melanized spores, enabling them to withstand the challenging environmental conditions of this ecosystem. Factors such as water stress and elevated soil salt levels can be considered stressors for certain microbial groups, potentially explaining why Capnodiales dominates in such conditions (Carbone et al., 2021; Ji et al., 2023).

The order Hypocreales, highly prevalent in native area, encompasses numerous genera of significant agronomic importance. Notably, the genus *Trichoderma*, renowned for its well-studied biological control capabilities, stands out as a prime example (Gangaraj et al., 2023). Conversely, the order also includes the genus *Fusarium*, which houses several plant pathogenic species (Nikitin et al., 2023). Several other orders, including Botryosphaeriales, Eurotiales, Lichenostigmatales, Mycosphaerellales, and Xylariales, exhibited increased abundance with notable differences among treatments. It is important to note that these orders contain pathogens with economic significance. However, it is crucial to emphasize that the presence of these pathogens in the soil does not necessarily equate to plant disease occurrence. This relationship is linked to soil suppressiveness or conduciveness (Schlatter et al., 2017), an area that warrants further investigation through microbial ecology and plant microbiome studies within a soil desertification gradient.

Our study underscores the beneficial impact of grazing exclusion on the restoration of semiarid soils facing desertification. Notably, AMF plays a crucial role in enhancing plant resilience against abiotic stresses induced by climate change, thereby reducing plant losses and improving adaptability, dispersal, and survival rates (Asmelash et al., 2016; Israel et al., 2022; Silva et al., 2022). This emphasizes the pivotal role of AMF in maintaining soil ecosystem health, as highlighted by Ferlian et al. (2021) and Genre et al. (2020). One of the key functions associated with AMF is the production and secretion of glomalin, a glycoprotein known to significantly contribute to soil aggregation and the stabilization of soil aggregates (Asmelash et al., 2016; Silva et al., 2022). Our findings reveal higher glomalin content in native areas, particularly during the rainy season, likely attributable to the protective influence of vegetation against various forms of soil erosion. Moreover, our results showed that, although overgrazing has led to soil degradation, AMF spore abundance remains unaffected by degradation, as reported by Silva et al. (2022). Additionally, it is worth noting that fungal spores constitute the most abundant fungal infectious propagules in the soil (Bueno and Moora, 2019).Differences in the composition of fungal families, such as Glomeraceae, Gigasporaceae, Acaulosporaceae, and Claroideoglomeraceae, were clearly observed across different areas and seasons. In particular, Glomeraceae emerged as the most prevalent family across all areas and

seasons, corroborating the findings of Carrillo-Saucedo et al. (2018), who identified Glomeraceae as dominant in a tropical dry forest in Jalisco, Mexico. Previously, Silva et al. (2022) assessed the morphological characteristics of fungal spores and found Glomeraceae to be the most abundant family in both native and restored areas in the Brazilian semiarid region. Notably, certain species within the Glomeraceae and Acaulosporaceae families have been identified in disturbed environments, such as those contaminated with metals, due to their robust sporulation capacity and plant colonization capabilities (Moreira et al., 2015).

The classification of AMF via spore quantification and DNA sequencing has provided comprehensive insights into AMF community dynamics across various ecological settings. Silva et al. (2022) using taxonomical characterization of spores revealed that restored soils exhibited significantly higher Shannon's diversity index compared to native soils. Additionally, the spore analysis identified a higher abundance of AMF spores in restored soils, with an average of 450 spores per gram of soil compared to 300 spores per gram in native soils. Dominant families and species of AMF, such as Gigasporaceae, Acaulosporaceae, Glomeraceae, and Ambisporaceae, were quantified in the spore analysis, with Gigasporaceae comprising of total spores, Acaulosporaceae, Glomeraceae, and Ambisporaceae. Similarly, the sequencing data quantified various AMF families correlated with specific land types and seasons, including Acaulosporaceae, Claroideoglomeraceae, Gigasporaceae, Glomeraceae, Diversiporaceae, and Archeosporaceae.

Importantly, our previous investigation, devoid of sequencing data, provided pivotal groundwork in discerning AMF distribution and abundance based on morphological characteristics and taxonomic classification. Thus, the current sequencing data offer molecular insights into AMF species classification across distinct habitats and seasons. Additionally, the analysis unveiled correlations between specific AMF families and environmental parameters, with restored soils exhibiting significantly higher concentrations of organic matter compared to native soils, contributing to the observed differences in AMF community structure. Seasonal fluctuations in AMF composition underscore the dynamic nature of these fungal communities. The PCoA delineated spatial segregation of AMF communities, with restored areas exhibiting an intermediate clustering between native and degraded sites, indicative of transitional community states.

Our study unequivocally demonstrates that desertification in the Caatinga biome has a profound impact on both the diversity and composition of soil fungal communities. Conversely, our findings highlight the potential of grazing exclusion in restoring fungal communities and improving soil properties, as evidenced by the increased abundance of beneficial AMF families like Glomeraceae and Acaulosporaceae in restored soils. Moreover, our study underscores the importance of seasonal variations in shaping fungal community dynamics, with native soils exhibiting higher diversity during the rainy season compared to restored soils. Through the integration of spore quantification and DNA sequencing data, we have provided a comprehensive understanding of AMF community dynamics across different land types and seasons. Our study not only enhances our knowledge of fungal ecology in semiarid ecosystems but also emphasizes the importance of microbial conservation efforts in mitigating desertification and promoting ecosystem resilience. Moving forward, further research is warranted to elucidate the functional roles of specific fungal taxa in ecosystem processes and to develop targeted strategies for sustainable soil management in semiarid regions.

5. Conclusions

This study has demonstrated that soil desertification resulting from overgrazing significantly alters the status of fungal communities. Moreover, both the total fungal communities and AMF exhibited sensitivity to both desertification and restoration processes. Notably, the Glomeraceae and Acaulospora families emerge as crucial groups warranting further investigation under these soil conditions in semiarid regions. The desertification process, driven by overgrazing, contributes to the degradation of soil chemical, physical, and biological properties, resulting in alterations in the structure, diversity, and composition of fungal communities. In contrast, grazing exclusion, serving as an ecological practice for the restoration of semiarid soils, holds the potential to ameliorate soil properties and positively influence fungal communities in the face of ongoing global climate changes. Consequently, long-term grazing exclusion can be considered a promising strategy for the restoration of degraded soils in the Brazilian semiarid region.

CRediT authorship contribution statement

Arthur Prudêncio de Araujo Pereira: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing. **Vania Maria Marciel Melo:** Conceptualization, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Elke Jurandy Bran Nogueira Cardoso:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization, Data curation, Funding acquisition. **Danilo Ferreira Silva:** Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jorge L. Mazza Rodrigues:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Victor Lucas Vieira Prudêncio Araujo:** Conceptualization, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Filipe Pereira Matteoli:** Conceptualization, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Lucas William Mendes:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Ademir Sérgio Ferreira Araujo:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Laibin Huang:** Data curation, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Christian Erikson:** Investigation, Formal analysis, Data curation, Methodology, Software, Validation, Visualization, Writing – review & editing. **Antonio Marcos Miranda Silva:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

The accession numbers for these files are available under BioProject/NCBI ID PRJNA1083982 for ITS sequences and PRJNA1083924 for AMF sequences

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2024.127763.

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