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New Insights on Environmental Occurrence of Pathogenic Fungi Based on Metagenomic Data from Brazilian Cerrado Biome

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HIGHLIGHTS

- Investigation of pathogenic fungi sequences from the metagenomic data of Cerrado soils.
- The native vegetation samples show higher relative abundance of pathogenic fungi.
- Identification of 41 pathogenic fungal species associated with human and animal infections.

Abstract: Cerrado is the second largest biome in Brazil and majorly contributes to the country's grain production. Previous studies on soil metagenomics from the Cerrado revealed an outstanding microbial diversity. In this study, the abundance of pathogenic fungi was analyzed using metagenomic sequences of the Cerrado soils under native vegetation, and under agriculture with no-tillage and conventional tillage. In total, 128,627 sequences of fungi were identified, with 43,439 representing pathogenic fungi and were distributed as follows: native 17,301 (40%), no-tillage 13,780 (32%), and conventional tillage 12,358 (28%). We identified 41 pathogenic fungal species associated with human and animal infections. The data analysis revealed that the native soils had a higher relative abundance of fungal sequences, similar to pathogenic species sequences, in relation to the total eukaryotic sequences, than the conventional tillage and no-tillage treatments, which observed a reduction in fungal abundance because of anthropogenic activities.

Keywords: Pathogenic fungi; metagenomics; Cerrado biome.

INTRODUCTION

The Cerrado biome is a savannah-like region that belongs to the central part of Brazil, covering an area of approximately 2 million km^2 area [1]. It is the second largest Brazilian biome [2] and is considered one of the most biodiverse sites on the planet [3]. Currently, this biome contributes to the maximum production of grains in the country [4], which has consequently led to changes in native vegetation due to agricultural activities and deforestation [5]. Studies reporting the rich biodiversity of the Cerrado encompass the fauna [6], flora [5], and microorganisms [7-9].

Pathogenic fungi complete their life cycle in a host [10] and are causative agents of infections in humans, animals, and plants [11]. Human pathogenic fungi are responsible for approximately 1.5 million deaths per year [12], causing superficial, (sub)cutaneous, and systemic infections [11]. Most etiologic agents are reported in soil, vegetation, and decaying matter in humid environments, which colonize the host either by necessity or opportunity [11]. However, the routes of infection of pathogenic fungi remain unknown. Several studies have reported fungal spores dispersed in air are associated with pulmonary or disseminated infections [13], propagules present in soil and plant debris are related to cutaneous/subcutaneous mycosis in the warm-blooded host [14], and the hypothesis of infection via plants or by animals [15]. In addition, fungi colonize the skin, hair, and nails, which use keratin as a nutrient source [16].

Culture-independent methods such as metagenomics, have developed into a robust technique for understanding and comparing microbial diversity in the most distinct environments [1], especially to identifying microorganisms that are scarcely recovered from the environment using conventional methods [17]. In this context, this study aimed to investigate pathogenic fungal sequences using the metagenomic data of Cerrado soils, including non-disturbed soil covered with native vegetation, and agricultural soils under the no-tillage and conventional tillage systems.

MATERIAL AND METHODS

Analyzed dataset

The data sequences used in this study were obtained from a previous study on soil samples from the experimental station of Embrapa Cerrados in Planaltina, Federal District, Brazil (15°36′34”S and 47°44′36”W) [9]. The samples were classified by authors as native soil (undisturbed Cerrado *stricto sensu* with original soil conditions) and two cultivable soils. Cultivable soils were cropped for 23 years with soybean/maize under “no-till” (NT) and conventional tillage (CT) with breaks during the winter (dry season). The CT area was prepared annually by plowing and disking the soil before sowing, and to inclusion of weeds after harvest, whereas the NT area was managed without ploughing or disking [9].

The metagenomic sequences assessment were performed by an untargeted library (shotgun metagenomics) using the Ion Proton sequencer with mean read lengths of 58–288 bp. Low-quality reads (phred score < 15) and short reads (≤ 50 bp) were removed. High quality reads were to the MG-Rast server for first metagenomic analysis (https://www.mg-rast.org/) [18], using the previously defined taxonomic annotation parameters [9].

The taxonomy of the microbial community of Metagenomic analysis was processed by the standard pipelines of the MG-RAST server [18]. Basically, the hierarchical phylogenetic profile generated was compared by functional analyses of genes (16S, 18S, ITS, 28S, and 26S), in addition to the taxonomy linked to the functional genes. The reads were compared against the M5NR database [19] based on the "best hit classification" method using the following parameters: Max. E-value cutoff: $1e^{-5}$; Min.% Identity cutoff: 80%; Min. Alignment length cutoff of 50. Then all sequences were taxonomically analyzed, the data sequences of eukaryote organisms were downloaded from MG-Rast server [18].

The data accessed are available online on the MG-RAST server with the following identifications for the datasets: mgp10523, mgp10541, and mgp10450. The metagenome dataset sequences were derived from three biological replicates of each of the three treatments: native soils (NATIVE 1, NATIVE 2 and NATIVE 3), cultivated under no-tillage (NT 1, NT 2 and NT 3), and conventional tillage (CT 1, CT 2 and CT 3) soil preparations [9].

Data mining

The abundance was determined from mining metagenomic data. In total, of 49,182,419 DNA sequences were evaluated. First, only eukaryotic sequences (406,972 sequences) were selected, followed by sequences related to the fungi kingdom (128,627 sequences) using in-house scripts in the Java programming language (http://www.java.com). A manual check was performed according to the literature for the screening of pathogenic fungi, totaling 43,439 sequences (Table1). The ggplot package [20] in R software (http://www.r-project.org/) was used for the figure.

Table 1. Summary of mining metagenomic data from surveys conducted with native vegetation of Cerrado (Native), and cropped with soybean/corn under no-tillage (NT) or conventional tillage (CT) systems.

Sample name	ID	Eukaryotic sequences	Fungi sequences	Pathogenic sequences
NATIVE 1	mgm4577669.3	46,172	18,669	6,215
NATIVE 2	mgm4578924.3	36,052	15,208	5,158
NATIVE 3	mgm4578925.3	40,824	17,944	5,928
NT 1	mgm4577671.3	50,849	15,321	5,194
NT 2	mgm4578714.3	45,729	13,077	4,381
NT 3	mgm4577672.3	50,210	12,023	4,205
CT 1	mgm4577670.3	49,117	12,739	4,201
CT 2	mgm4578926.3	51,228	13,803	4,710
CT 3	mgm4578927.3	36,791	9,843	3,447
Total	-	406,972	128,627	43,439

Relative abundance and Richness estimate

The relative abundance of each sample was calculated based on comparative parameters: 1) pathogenic sequences in relation to the community of Eukaryotic sequences; 2) fungi sequences in relation to the community of Eukaryotic sequences, and 3) pathogenic sequences in relation the community fungi sequences. The data are presented in percentage. Furthermore, Chao [21] was used to estimate the richness of the genera in each treatment based on the number of genera identified by data mining.

RESULTS

In total, 43,439 sequences representing pathogenic fungi were distributed as follows: native 17,301 (40%), no-tillage 13,780 (32%), and conventional tillage 12,358 (28%). Considering all the evaluated treatments, 4 phyla, 9 classes, 11 orders, 18 families, 28 genera, and 41 different species were classified taxonomically (Supplementary Table 1).

Overall, 28 genera were identified, of which 25 were observed in the native soil, 23 in the NT, and 23 in the CT. The most abundant genera were *Aspergillus* (38%), followed by *Fusarium* (13%), *Cryptococcus*

(10%), *Coccidioides* (7%), besides *Candida*, *Talaromyces* and *Yarrowia* (4%). Moreover, genera with 3% abundance included *Histoplasma* and *Schizophyllum* while *Blastomyces*, *Malassezia*, *Nakaseomyces*, *Paracoccidioides*, and *Trichophyton* displayed 2% of abundance. Less abundant genera, representing less than 1%, included *Clavispora*, *Debaryomyces*, *Encephalitozoon*, *Lodderomyces*, *Meyerozyma*, *Microsporum* and *Nannizzia*. The least abundant genera included *Basidiobolus*, *Conidiobolus*, *Epidermophyton*, *Exophiala*, *Millerozyma*, *Pneumocystis*, and *Rhinocladiella* (0.1% abundance) (Figure 1).

A comparison among the three treatments reveled that the genera *Aspergillus*, *Coccidioides*, *Talaromyces*, *Histoplasma*, *Blastomyces*, *Paracoccidioides*, *Trichophyton*, *Microsporum*, *Nannizzia*, *Pneumocystis*, *Basidiobolus* and *Rhinocladiella* displayed the highest number of sequences in the native soils. The NT soils featured higher abundances of *Fusarium*, *Cryptococcus*, *Schizophyllum*, *Malassezia*, *Meyerozyma*, *Encephalitozoon*, *Epidermophyton*, *Exophiala* and *Millerozyma* genera while CT soil were predominant by *Candida*, *Yarrowia*, *Clavispora*, *Lodderomyces*, *Conidiobolus* and *Debaryomyces* (Figure 1).

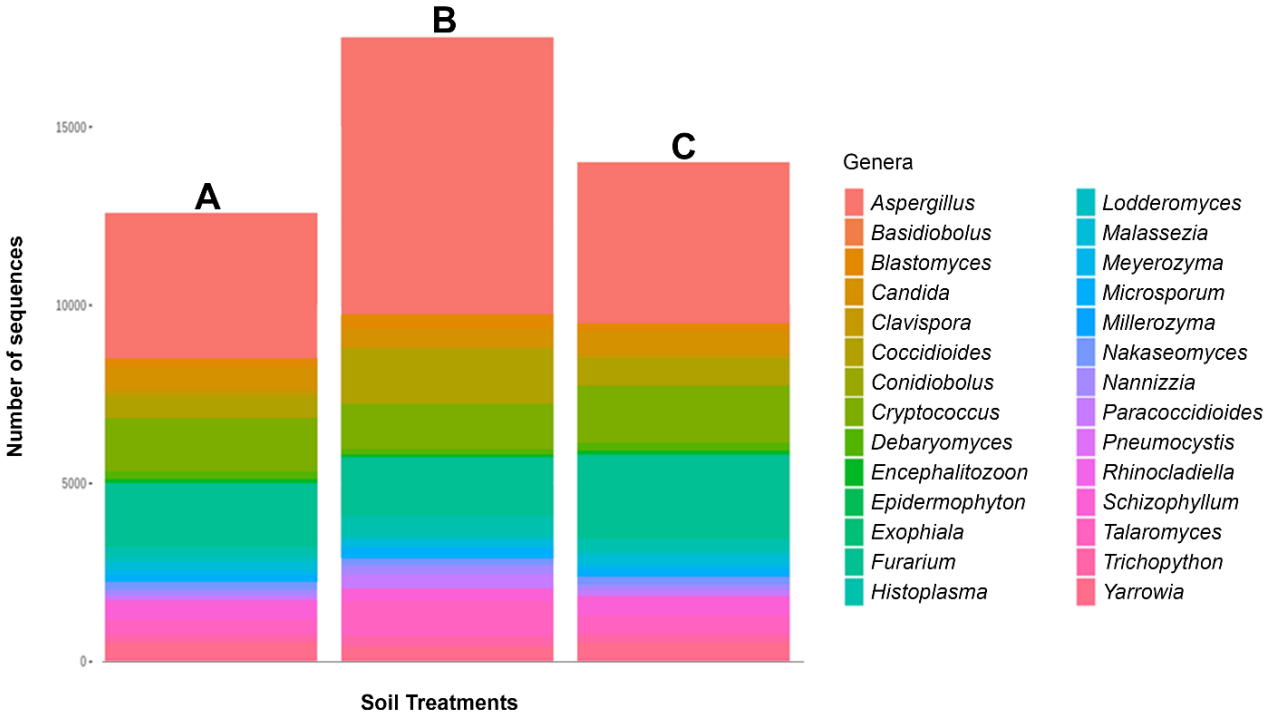

Figure 1. Abundance observed of the genera (associated with human and animal infection) based on the comparison of the number of sequences in each analyzed soil. In A: conventional tillage (CT), in B: undisturbed Cerrado soil (Native) and in C: no-tillage (NT). In the y-axis number of sequences and on the x-axis the treatments.

Altogether, among the soils evaluates, 41 species were reportedly identified as causal agents of diseases in humans and/or animals. With 31 species observed in all the three treatments (NT, CT and Native soils), whereas certain species such as *Exophiala pisciphila* and *E. dermatitidis* were discovered only in NT soils (Figure 2). The predominant specie was *Aspergillus fumigatus* (25.35%), mainly present in the native soils, followed by the *Fusarium solani* (13.35%) and *Cryptococcus neoformans* (9.78%), both of which were predominant in the cultivated soils (Figure 2).

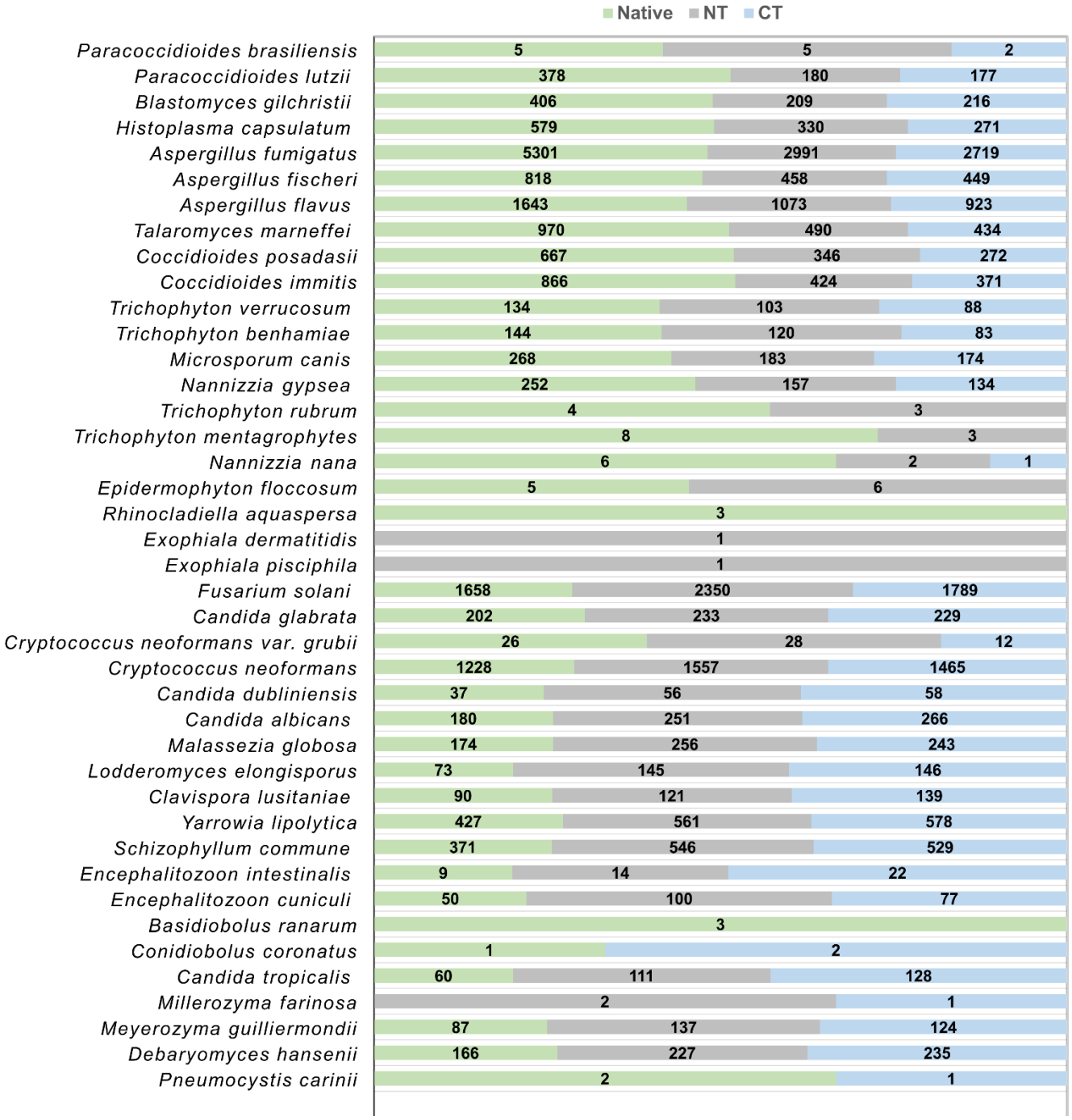

Figure 2. Distribution of species reported as causative agents of diseases in humans and/or animals on No-tillage (NT), undisturbed Cerrado (Native), and conventional tillage (CT) soils.

Furthermore, the relative abundance analyses in relation to the eukaryotic community revealed that the native soils have a notable fungal diversity, including pathogenic species (Table 2).

In addition, the genera richness analysis estimated the values of 53.90, 39.33, and 41.75 for native soils, NT, and CT, respectively, revealing that native soils are 35% richer than the others. However, comparing the presence of pathogenic species sequences to the dataset sequences of the fungal community, we observed a similar relative abundance of pathogenic fungi in the three different soils (Table 2). Nevertheless, certain species from the order Onygenales predominated in the native soils, validating the relative abundance and richness data observed for this soil, which were 35% richer than the others (Figure 2).

Table 2. Fungal Relative abundance in Brazilian Cerrado soils

	Pathogenic sequences / Eukaryotic sequences	Fungi sequences / Eukaryotic sequences	Pathogenic sequences / fungi sequences
Native 1	13,46053885	40,43359612	33,290481547
Native 2	14,30711195	42,1835127	33,916359811
Native 3	14,52087008	43,95453655	33,036112350
NT 1	10,21455683	30,13038604	33,901181385
NT 2	9,580353824	28,59673293	33,501567638
NT 3	8,374825732	23,9454292	34,974631955
CT 1	8,553046807	25,9360303	32,977470759
CT 2	9,194190677	26,94424924	34,123016735
CT 3	9,369139192	26,75382566	35,019811033

DISCUSSION

The metagenomic analysis of soils belonging to the Cerrado biome from three different treatments (undisturbed Cerrado (Native), no-tillage (NT) and conventional tillage (CT) soils) revealed the presence of saprobe fungi, and opportunistic and real pathogens. In this study, we identified sequences belonging to pathogenic fungi, and the results highlight that native soil displays higher richness and relative abundance of fungal sequences and pathogenic species sequences corresponding to the number of eukaryotic sequences, than in soils subjected to agricultural practices (Table 2). This indicates a reduction in fungal biodiversity owing to anthropogenic activity, which was also observed in previous studies on the Cerrado biome [1,9,22].

In recent times, global epidemiological data have shown a significant increase in the incidence of invasive fungal diseases in humans [10, 15, 23] and in animals [24]. Among the species identified, *Aspergillus fumigatus* (25.35%) exhibited the highest relative abundance (Figure 2), which is an important allergen that causes aspergillosis and is a major cause of human morbidity and mortality worldwide [25]. In Brazil, epidemiological data are rather scarce because of the difficulty in correct diagnosis [26], and studies with environmental isolates of *A. fumigatus* and *A. flavus* demonstrated a 20%–25% rate of the itraconazole resistance [27]. Furthermore, often present in soil and air samples [28], they have been abundantly identified as soils natives. It is suggested that soil management using certain approach seems to alter the frequency of the fungal occurrence in the environment.

The second major relative abundance was *Fusarium solani*, was predominant in cultivated soils, which was higher in NT than in CT (Figure 2). Recognized as a phytopathogen that causes crop loss, this fungus causes opportunistic infections in humans [29]. Furthermore, studies of invasive fusariosis in Brazil have shown that *Fusarium* spp. are associated with agricultural activities [30]. Their greater abundance in cultivated soils may be related to the fact that the soils evaluated have been cropped with corn and soybean, and the incidence of fusariosis has been extensively reported in these plants [31,32].

In our analyses, the third most abundant fungus was *Cryptococcus neoformans* (9.78%) distributed in the three treatments, but more frequently observed in no-tillage (Figure 2). This fungus is opportunistic due to its ability to grow at body temperature, produce melanin and polysaccharide capsules, causing cryptococcosis in immunocompetent and immunocompromised individuals [33,34]. Epidemiological data showed that the mortality in Brazil reached up to 60% in HIV-infected patients [26].

Fungi of the order Onygenales identified in this study was significantly abundance in native soils, dominant with dimorphic fungi such as *Paracoccidioides lutzii* (1.69%) and *P. brasiliensis* (0.03%), which causes paracoccidioidomycosis, an endemic disease in the Brazilian Cerrado region and restricted to Latin America [35]. It is the chief systemic mycosis affecting the Brazilian population, and the eighth largest cause of mycoses-associated mortality [36,37], which can also infect animals [38,39]. Moreover, *Histoplasma capsulatum* has been recognized as an endemic agent in Brazil, particularly in the Midwest [40]. Followed by *Coccidioides immitis* (3.82%) and *C. posadasii* (2.96%). The chemical properties of soils previously described [9] may represent a selection factor for these agents. For example, the amount of organic matter observed in the native soils (3,666) was greater than that in NT (3,209) and CT (2,751) (Supplementary Table 2).

Likewise, the native soils are more acidic (pH 4.687) than the NT (pH 5.670) and CT (pH 5.647), which may influence the selection of these agents, which are epidemiologically reported in soils with a high content of organic matter (Supplementary Table 2).

The most abundant opportunistic species was *C. albicans* (1.60%), observed as a prevalent causal agent of onychomycoses in northeast of Brazil [41]. In addition, *Yarrowia lipolytica* anamorph of *C. lipolytica* (3.61%) causing blood infections [42], and *Malassezia globosa* (1.55%) are considered relevant agents of superficial mycoses in humans and animals [43]. Yeasts grow in a wider pH range (between 5 and 6) [44,45], which could explain their abundance in NT and CT soils. Herpotrichilaceous fungi have been identified in low relative abundance (Figure 2), which include *Rhinocladiella aquaspersa,* a rare agent of chromoblastomycosis [46]; *Exophiala pisciphila,* associated with infection in cold-blooded animals [47], although in isolated cases, it can infect humans [48]; and *E. dermatitidis* an opportunistic pathogen that causes peritonitis [49], cystic fibrosis, phaeohyphomycosis, and chromoblastomycosis in humans [50].

Moreover, the fungus *Talaromyces marneffei* (4.36%) and *Blastomyces gilchristii* (1.91%) were more predominant in native soil, but at low frequencies (Figure 2). This fact may justify the rare cases in Brazil and Latin America [51, 52].

Although low in relative abundance, the zygomycetes *Conidiobolus coronatus* and *Basidiobolus ranarum* are clinically important because they cause conidiobolomycosis and basidiobolomycosis, respectively [53]. Furthermore, *Schizophyllum commune* was identified, which is a Basidiomycetes and an occasional human pathogenic agent of respiratory infections [54]. With respect to animal pathogenic fungi, *Pneumocystis carinii*, which is responsible for lung infections in rats [55] were identified. In addition, to *Encephalitozoon cuniculi* and *E. intestinalis* were observed, which cause microsporidiosis in rats, and several other infections in mammals [56-58] (Figure 2).

CONCLUSION

Fungi are ubiquitous organisms found associated with soil, plants, rock animals, and water sources in the environment, wherein human and animals are frequently exposed to these fungi. However, relatively few fungal species are capable of infecting human and animal hosts, and their environmental isolation is rarely correlated with the epidemiological data, which could be attributed to the limitations of isolation methods and/or frequency of the species in highly specific niches. In this scenario, metagenomic assays can be a relevant tool to overcome this shortcoming.

This exploratory metagenomic study of soils from the Brazilian Cerrado region identified the presence of forty-one fungal species considered pathogenic to human and animal hosts. The data analysis revealed that the native soils contained a higher relative abundance of fungal sequences and pathogenic sequences in relation to the number of eukaryotic sequences based on the richness, compared with the conventional tillage and no-tillage soils, corroborating with previous studies that observed a reduction in fungal biodiversity because of anthropogenic activities.

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REFERENCES

1. De Castro AP, Quirino BF, Pappas G, Kurokawa AS, Neto EL, Krüger RH. Diversity of soil fungal communities of Cerrado and its closely surrounding agriculture fields. Arch Microbiol. 2008;190(2):129–39.
2. Silva JMC, Bates JM. Biogeographic Patterns and Conservation in the South American Cerrado: A Tropical Savanna Hotspot. Bioscience. 2002;52(3):225.
3. Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. Nature [Internet]. 2000;403(February):853–8.
4. Jepson W. A disappearing biome? Reconsidering land-cover change in the Brazilian savanna. Geogr J. 2005;171(2):99–111.
5. Sano EE, Rodrigues AA, Martins ES, Bettiol GM, Bustamante MMC, Bezerra AS, et al. Cerrado ecoregions: A spatial framework to assess and prioritize Brazilian savanna environmental diversity for conservation. J Environ Manage [Internet]. 2019;232(July 2018):818–28. Available from: https://doi.org/10.1016/j.jenvman.2018.11.108.

6. Costa WJEM. Three new species of the killifish genus *Melanorivulus* from the Rio Paraná Basin, central Brazilian Cerrado (cyprinodontiformes, aplocheilidae). Zoosystematics Evol. 2017;94(1):17–27.
7. Araujo ASF, Bezerra WM, dos Santos VM, Nunes LAPL, de Lyra M do CCP, do Vale Barreto Figueiredo M, et al. Fungal diversity in soils across a gradient of preserved Brazilian Cerrado. J Microbiol. 2017;55(4):273–9.
8. Noriler SA, Savi DC, Aluizio R, Palácio-Cortes AM, Possiede YM, Glienke C. Bioprospecting and structure of fungal endophyte communities found in the Brazilian biomes, pantanal, and Cerrado. Front Microbiol. 2018;9(JUL):1–14.
9. Souza RC, Mendes IC, Reis-Junior FB, Carvalho FM, Nogueira MA, Vasconcelos ATR, et al. Shifts in taxonomic and functional microbial diversity with agriculture: How fragile is the Brazilian Cerrado? BMC Microbiol [Internet]. 2016;16(1):42. Available from: http://www.biomedcentral.com/1471-2180/16/42
10. De Hoog GS, Ahmed SA, Danesi P, Guillot J, Gräser Y. Distribution of pathogens and outbreak fungi in the fungal kingdom. Emerg Epizoot Fungal Infect Anim. 2018;3–16.
11. Ginter-hanselmayer G, Nenoff P. Clinically Relevant Mycoses. Clinically Relevant Mycoses. Springer. 2019.
12. Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC. Hidden killers: Human fungal infections. Sci Transl Med. 2012;4(165):1–10.
13. Sephton-Clark PCS, Muñoz JF, Ballou ER, Cuomo CA, Voelz K. Pathways of Pathogenicity: Transcriptional Stages of Germination in the Fatal Fungal Pathogen Rhizopus delemar . mSphere. 2018;3(5):1–16.
14. Sanchotene KO, Madrid IM, Klafke GB, Bergamashi M, Terra PP Della, Rodrigues AM, et al. *Sporothrix brasiliensis* outbreaks and the rapid emergence of feline sporotrichosis. Mycoses. 2015;58(11):652–8.
15. Queiroz-Telles F, Buccheri R, Benard G. Sporotrichosis in immunocompromised hosts. J Fungi. 2019;5(1):1–23.
16. De Hoog GS, Dukik K, Monod M, Packeu A, Stubbe D, Hendrickx M, et al. Toward a Novel Multilocus Phylogenetic Taxonomy for the Dermatophytes. Mycopathologia. 2017;182(1–2):5–31.
17. Pylro V, Roesch L. The Brazilian Microbiome. Current Status and Perspectives. Springer. 2017.
18. Glass EM, Meyer F. The Metagenomics RAST Server: A Public Resource for the Automatic Phylogenetic and Functional Analysis of Metagenomes. Handb Mol Microb Ecol I Metagenomics Complement Approaches. 2011;I:325–31.
19. Wilke A, Harrison T, Wilkening J, Field D, Glass EM, Kyrpides N, et al. The M5nr: a novel non-redundant database containing protein sequences and annotations from multiple sources and associated tools. BMC Bioinformatics [Internet]. 2012;13(1):141. Available from: http://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-13-141
20. Wickham, H. ggplot2: Elegant Graphics for Data Analysis. New York: Springe. 2009.
21. Anne Chao. Nonparametric Estimation of the Number of Classes in a Population. Scand J Stat. 1984;11(4):265–70.
22. Bresolin JD, Bustamante MMC, Krüger RH, Silva MRSS, Perez KS. Structure and composition of bacterial and fungal community in soil under soybean monoculture in the Brazilian cerrado. Brazilian J Microbiol. 2010;41(2):391–403.
23. Guinea J, Zaragoza Ó, Escribano P, Martín-Mazuelos E, Pemán J, Sánchez-Reus F, et al. Molecular identification and antifungal susceptibility of yeast isolates causing fungemia collected in a population-based study in Spain in 2010 and 2011. Antimicrob Agents Chemother. 2014;58(3):1529–37.
24. Seyedmousavi S, Netea MG, Mouton JW, Melchers WJG, Verweij PE, de Hoog GS. Black yeasts and their filamentous relatives: Principles of pathogenesis and host defense. Clin Microbiol Rev. 2014;27(3):527–42.
25. Lamoth F. *Aspergillus fumigatus*-related species in clinical practice. Front Microbiol. 2016;7(MAY):1–8.
26. Costa MC, Pereira de Sá N, Johann S, Santos DA. Social, environmental and microbiologic aspects of endemic mycoses in Brazil. New Microbes New Infect [Internet]. 2019;29:100496. Available from: https://doi.org/10.1016/j.nmni.2018.11.004
27. Bedin Denardi L, Hoch Dalla-Lana B, Pantella Kunz de Jesus F, Bittencourt Severo C, Morais Santurio J, Zanette RA, et al. *In vitro* antifungal susceptibility of clinical and environmental isolates of *Aspergillus fumigatus* and *Aspergillus flavus* in Brazil. Brazilian J Infect Dis [Internet]. 2018;22(1):30–6. Available from: http://dx.doi.org/10.1016/j.bjid.2017.10.005
28. Pringle A, Baker DM, Platt JL, Wares JP, Latgé JP, Taylor JW. Cryptic speciation in the cosmopolitan and clonal human pathogenic fungus *Aspergillus fumigatus*. Evolution (N Y). 2005;59(9):1886–99.
29. Zhang N, O'Donnell K, Sutton DA, Nalim FA, Summerbell RC, Padhye AA, et al. Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. J Clin Microbiol. 2006;44(6):2185–90.
30. Nucci M, Varon AG, Garnica M, Akiti T, Barreiros G, Trope BM, et al. Increased incidence of invasive fusariosis with cutaneous portal of entry, Brazil. Emerg Infect Dis. 2013;19(10):1567–72.

31. Zheng N, Zhang LP, Ge FY, Huang Wk, Kong LA, Peng DL, et al. Conidia of one *Fusarium solani* isolate from a soybean-production field enable to be virulent to soybean and make soybean seedlings wilted. J Integr Agric [Internet]. 2018;17(9):2042–53. Available from: http://dx.doi.org/10.1016/S2095-3119(17)61891-4
32. Ranzi C, Camera JN, Deuner CC. Influence of continuous cropping on corn and soybean pathogens. Summa Phytopathol. 2017;43(1):14–9.
33. May RC, Stone NRH, Wiesner DL, Bicanic T, Nielsen K. *Cryptococcus*: From environmental saprophyte to global pathogen. Nat Rev Microbiol [Internet]. 2016;14(2):106–17. Available from: http://dx.doi.org/10.1038/nrmicro.2015.6
34. Huang W, Liao G, Baker GM, Wang Y, Lau R, Paderu P, et al. Lipid flippase subunit Cdc50 mediates drug resistance and virulence in *Cryptococcus neoformans*. MBio. 2016;7(3):1–13.
35. Teixeira MM, Theodoro RC, De Oliveira FFM, MacHado GC, Hahn RC, Bagagli E, et al. *Paracoccidioides lutzii* sp. nov.: Biological and clinical implications. Med Mycol. 2014;52(1):19–28.
36. Coutinho ZF, Wanke B, Travassos C, Oliveira RM, Xavier DR, Coimbra CEA. Hospital morbidity due to paracoccidioidomycosis in Brazil (1998-2006). Trop Med Int Heal. 2015;20(5):673–80.
37. Shikanai-Yasuda MA, Mendes RP, Colombo AL, de Queiroz-Telles F, Kono ASG, Paniago AMM, et al. Brazilian guidelines for the clinical management of paracoccidioidomycosis. Rev Soc Bras Med Trop. 2017;50(5):715–40.
38. Bagagli E, Franco M, Bosco SDMG, Hebeler-Barbosa F, Trinca LA, Montenegro MR. High frequency of *Paracoccidioides brasiliensis* infection in armadillos (*Dasypus novemcinctus*): An ecological study. Med Mycol. 2003;41(3):217–23.
39. Richini-Pereira VB, Bosco SDMG, Griese J, Theodoro RC, Macoris SADG, Da Silva RJ, et al. Molecular detection of *Paracoccidioides brasiliensis* in road-killed wild animals. Med Mycol. 2008;46(1):35–40.
40. Almeida M de A, Almeida-Silva F, Guimarães AJ, Almeida-Paes R, Zancopé-Oliveira RM. The occurrence of histoplasmosis in Brazil: A systematic review. Int J Infect Dis [Internet]. 2019;86:147–56. Available from: https://doi.org/10.1016/j.ijid.2019.07.
41. Silva-Rocha WP, de Azevedo MF, Chaves GM. Épidémiologie et distribution des espèces fongiques des mycoses superficielles dans le Nord-est du Brésil. J Mycol Med [Internet]. 2017;27(1):57–64. Available from: http://dx.doi.org/10.1016/j.mycmed.2016.08.009
42. Trabelsi H, Chtara K, Khemakhem N, Néji S, Cheikhrouhou F, Sellami H, et al. Fungemia Caused by *Yarrowia lipolytica*. Mycopathologia. 2015;179(5–6):437–45.
43. Velegraki A, Cafarchia C, Gaitanis G, Iatta R, Boekhout T. *Malassezia* Infections in Humans and Animals: Pathophysiology, Detection, and Treatment. PLoS Pathog. 2015;11(1):1–6.
44. Vacca I. Fungal physiology: Acidic pH interferes with *Candida* persistence. Nat Rev Microbiol. 2017;15(7):382.
45. Zhang T, Wang NF, Liu HY, Zhang YQ, Yu LY. Soil pH is a key determinant of soil fungal community composition in the Ny-Ålesund Region, Svalbard (High Arctic). Front Microbiol. 2016;7(FEB):1–10.
46. González GM, Rojas OC, González JG, Kang Y, De Hoog GS. Chromoblastomycosis caused by *Rhinocladiella aquaspersa*. Med Mycol Case Rep [Internet]. 2013;2(1):148–51. Available from: http://dx.doi.org/10.1016/j.mmcr.2013.08.001
47. De Hoog GS, Vicente VA, Najafzadeh MJ, Harrak MJ, Badali H, Seyedmousavi S. Waterborne *Exophiala* species causing disease in cold-blooded animals. Persoonia Mol Phylogeny Evol Fungi. 2011;27:46–72.
48. Kebbe J, Mador MJ. *Exophiala pisciphila*: a novel cause of allergic bronchopulmonary mycosis. J Thorac Dis. 2016;8(7):E538–41.
49. Pinheiro RL, Cognialli RCR, Barros RC, de A. Pinto T, Cunha MFM, Tahan TT, et al. Peritonitis by *Exophiala dermatitidis* in a pediatric patient. Med Mycol Case Rep [Internet]. 2019;24(January):18–22. Available from: https://doi.org/10.1016/j.mmcr.2019.02.001
50. Kirchhoff L, Olsowski M, Rath PM, Steinmann J. *Exophiala dermatitidis*: Key issues of an opportunistic fungal pathogen. Virulence [Internet]. 2019;10(1):984–98. Available from: https://doi.org/10.1080/21505594.2019.1596504
51. Chan JFW, Lau SKP, Yuen KY, Woo PCY. *Talaromyces* (*Penicillium*) *marneffei* infection in non-HIV-infected patients. Emerg Microbes Infect [Internet]. 2016;5(3):e19-9. Available from: http://dx.doi.org/10.1038/emi.2016.18
52. Dalcin D, Rothstein A, Spinato J, Escott N, Kus J V. *Blastomyces gilchristii* as cause of fatal acute respiratory distress syndrome. Emerg Infect Dis. 2016;22(2):306–8.
53. Vilela R, Mendoza L. Human Pathogenic Entomophthorales. Clin Microbiol Rev. 2018;31(4):1–40.
54. Chowdhary A, Randhawa HS, Gaur SN, Agarwal K, Kathuria S, Roy P, et al. *Schizophyllum commune* as an emerging fungal pathogen: A review and report of two cases. Mycoses. 2013;56(1):1–10.
55. Weisbroth SH. *Pneumocystis*: Newer knowledge about the biology of this group of organisms in laboratory rats and mice. Lab Anim (NY). 2006;35(9):55–61.

56. Goodwin D, Gennari SM, Howe DK, Dubey JP, Zajac AM, Lindsay DS. Prevalence of antibodies to *Encephalitozoon cuniculi* in horses from Brazil. Vet Parasitol. 2006;142(3–4):380–2.
57. Lindsay DS, Goodwin DG, Zajac AM, Cortés-Vecino JA, Gennari SM, Rosypal AC, et al. Serological Survey for Antibodies to *Encephalitozoon cuniculi* in Ownerless Dogs From Urban Areas of Brazil and Colombia. J Parasitol. 2009;95(3):760–3.
58. Malčeková B, Halánová M, Sulínová Z, Molnár L, Ravaszová P, Adam J, et al. Seroprevalence of antibodies to *Encephalitozoon cuniculi* and *Encephalitozoon intestinalis* in humans and animals. Res Vet Sci. 2010;89(3):358–61.