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Agaricales (Basidiomycota, Fungi) in the Espinilho State Park, Rio Grande do Sul

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Agaricales (Basidiomycota, Fungi) in the Espinilho State Park, Rio Grande do Sul. A taxonomic study based on collections of macrofungi of the order Agaricales was conducted in the Espinilho State Park, located in Barra do Quaraí, southern Brazil, during three expeditions in April 2023, October 2023, and October 2024, encompassing both the rainy and dry seasons. During the samplings, 430 records were obtained (counted as one record per substrate patch, even when multiple basidiomata were present), distributed across 7 families and 11 genera. Abundance was higher during dry periods, decreasing markedly during the rainy season. We hypothesize that water accumulation in the soil of the sampled area, predominantly classified as a Haplic Gleysol (characterized by poor drainage and prolonged water saturation), causes stress to the mycelium, thereby hindering basidiomata development. This pattern contrasts with commonly reported findings in the literature, in which basidiomata production is typically associated with increased moisture and rainfall.

Keywords: Agaricomycetes, water accumulation, surveys, macrofungi, protected areas

Hongos Agaricales (Basidiomycota, Fungi) en el Parque Estatal Espinilho, Rio Grande do Sul. Se realizó un estudio taxonómico basado en colecciones de macrohongos del orden Agaricales en el Parque Estadual do Espinilho, ubicado en Barra do Quaraí, en el sur de Brasil, durante tres expediciones en abril de 2023, octubre de 2023 y octubre de 2024, abarcando tanto la temporada lluviosa como la seca. Durante los muestreos se obtuvieron 430 registros (contabilizados como un registro por parche de sustrato, incluso cuando estaban presentes múltiples basidiomas), distribuidos en 7 familias y 11 géneros. La abundancia fue mayor durante los períodos secos, disminuyendo notablemente durante la temporada lluviosa. Hipotetizamos que la acumulación de agua en el suelo del área muestreada, predominantemente clasificado como un Gleysol háplico (caracterizado por un drenaje deficiente y una saturación prolongada de agua), genera estrés en el micelio, dificultando así el desarrollo de los basidiomas. Este patrón contrasta con los hallazgos comúnmente reportados en la literatura, en los cuales la producción de basidiomas se asocia típicamente con un aumento de la humedad y la precipitación.

Palabras clave: Agaricomycetes, acumulación de agua, inventarios, macrohongos, áreas protegidas

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INTRODUCTION

These fungi play an important ecological role, acting predominantly as saprobes responsible for the decomposition of organic matter, thereby contributing to the biogeochemical nutrient cycle. In addition, some taxa act as symbionts by forming mycorrhizae with phanerogamic plants, enhancing nutrient uptake and plant resilience, or participate in the formation of basidiolichens (Dutra *et al.,* 2015; Vanni & Visconti, 2015; Sulzbacher, 2019). In Rio Grande do Sul, although several studies related to this order exist, there are few records of Agaricales in the Pampa biome, especially when compared to the number of studies conducted in Atlantic Forest from the state. *Maia et al.* (2015) report only 84 fungal species for the Pampa biome. Andrade *et al*. (2023), in the largest review of Pampa biodiversity conducted to date, mention the occurrence of 149 species of Basidiomycota for the biome, without, however, specifying the number of Agaricales. Furthermore, most conservation units in the state are located within the Atlantic Forest, which hinders surveys in the Pampa region (Brentano & Foleto, 2015; Rech & Canto-Silva, 2017).

The Espinilho State Park, located within the Pampa, has soils classified as Solodic Carbonatic Melanic Gleysol, of hydromorphic character, medium texture, poor drainage, and prolonged water saturation, which lead to seasonal waterlogging. The mean annual temperature is around 20°C, with maximum values reaching 34°C in summer and average minimums of 8°C in winter. The mean annual precipitation is 1,100 mm, with January being the wettest month, averaging 120 mm, and August being the driest, with an average of 60 mm (Gardin *et al.,* 2016; Galvani, 2003; Watzlawick, 2014). The predominant vegetation, composed of espinilho [*Vachellia caven* (Molina) Seigler & Ebinger], inhanduvá [*Neltuma affinis* (Spreng.) C.E.Hughes & G.P.Lewis] and algarrobo [*Neltuma nigra* (Griseb.) C.E.Hughes & G.P.Lewis], is exclusive both within Rio Grande do Sul and in Brazil, making this conservation unit an important biodiversity refuge. In addition to this unique vegetation, the park also shelters rare fauna species, such as the pampas cat (*Leopardus munoai*) (Silveira *et al*., 2018), the pampas deer (*Ozotoceros bezoarticus*) (Bianchin, 2011) and the yellow cardinal (*Gubernatrix cristata*) (Seixas, 2012; Brasil, 2024). However, no efforts to inventory fungal diversity have been conducted in this Conservation Unit to date. Therefore, this study provides the first diversity data of Agaricales for the Espinilho State Park.

MATERIALS AND METHODS

Study area

Espinilho State Park is located in the far west of Rio Grande do Sul, in the Quaraí Chico stream microbasin, within the Uruguay River Hydrographic Basin. Situated in the municipality of Barra do Quaraí, the park covers an area of 1,617.14 hectares, with UTM coordinates 6654600N 446.500E and 6666400N 463.800E, at the tri-border region between Brazil, Argentina, and Uruguay. The park lies approximately 6 km from the urban center of Barra do Quaraí and 70 km from Uruguaiana (Brasil, 2024) (Figure 1).

According to Köppen's climatic classification, the region presents a Cfa climate type, characterized as subtropical, with average temperatures above 22 °C in the warmest month and ranging between -3 °C and 18 °C in the coldest month, resulting in an annual average of around 23.4 °C (Moreno, 1961). General precipitation is well distributed throughout the year, with an annual average between 1,300 mm and 1,500 mm (Almeida, 1996), ranging from an average of 120 mm in the wettest month (January) to 60 mm in the driest month (August) (Gardin *et al*., 2016).

Collections

Three sampling expeditions were carried out between 2023 and 2024, specifically in April

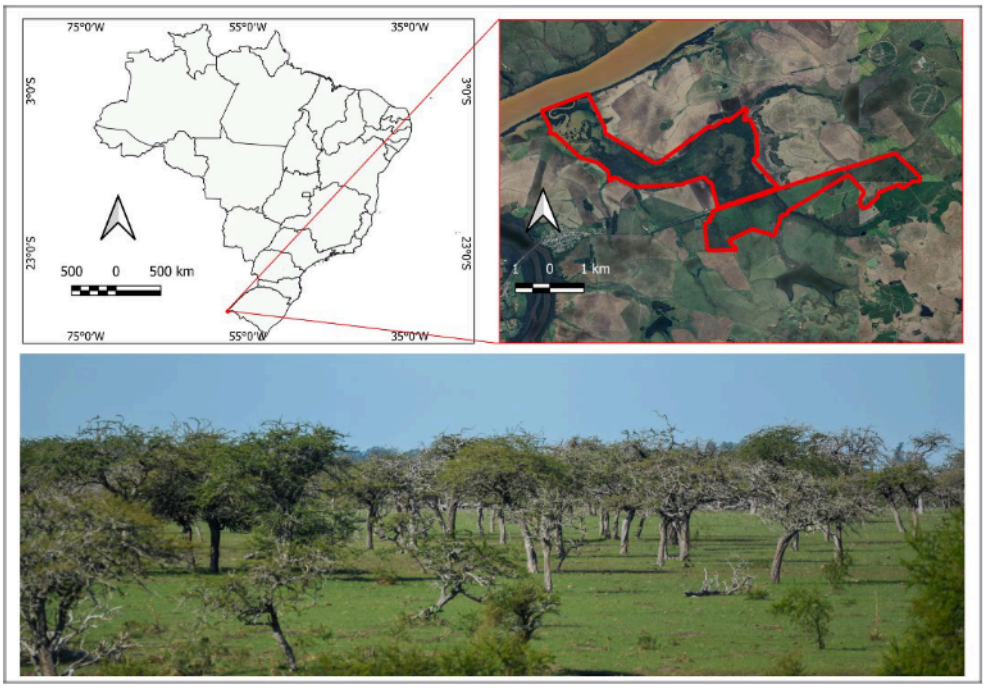


Figure 1. Map of Rio Grande do Sul, highlighting the area of Espinilho State Park, between UTM coordinates 6654600 (N) 446.500 (E) and 6666400 (N) 463.800 (E). Below, an illustrative photograph of the characteristic vegetation of the park

2023, October 2023, and October 2024. These months were strategically selected because the period from October to April concentrates most of the region's annual precipitation and erosive potential. In this study area, the highest rainfall volumes are historically recorded between October and January, with October exhibiting a historical average of 153 mm to 192 mm, and January averaging 142 mm, representing a significant contrast to the drier winter months (Wrege *et al*., 2012). During the surveys, mushrooms appeared more frequently and abundantly during soil drying phases after intense rainfall, as the characteristic waterlogging of the Gleysol temporarily limits mycelial fructification due to oxygen depletion. Material was collected using the walking survey method (Mueller *et al*., 2004), following pre-established trails throughout the conservation unit. After collection, the specimens were stored in individual plastic containers and taken to the Laboratory of Fungal Taxonomy (LATAF), where macro- and micromorphological analyses were conducted for species identification.

Identification

For measurements of macroscopic structures, a digital caliper was used to measure the number of lamellae per mm, pileus diameter, and stipe circumference and length. For microscopic measurements, an Olympus Corporation SZ2-ILST stereomicroscope and a Carl Zeiss Microscopy GmbH AXIO SCOPE.A1 microscope were used. With these microscopes, visualization and measurements of spores, pileus cortical layer, lamellar trama, cheilocystidia, pleurocystidia, and basidia were performed. Whenever possible, at least 50 structures of each type were observed.

RESULTS AND DISCUSSION

A total of 430 records were obtained, distributed among seven families: Agaricaceae (185),

Hymenochaetaceae (8), Irpicaceae (6), Bolbitieaceae (9), Strophariaceae (182), Pluteaceae (19), and Physalacriaceae (21), comprising 11 genera: *Pluteus* (19), *Agaricus* (64), *Phellinus* (8), *Leucoagaricus* (17), *Leucocoprinus* (66), *Hydnopolyporus* (6), *Panaeolus* (9), *Macrolepiota* (37), *Psilocybe* (182), *Cyptotrama* (21), and *Macrocybe* (1). Species abundance is presented in Figure 2.

Species richness was 15 species in the first site, 3 species in the second, and 2 species in the third, for a total richness of 18 species (Figure 3). The species richness of Basidiomycetes, analyzed through rarefaction, differed among the three phytophysiognomies, and the confidence intervals did not overlap, indicating significant differences based on the 95% confidence interval. In addition to interpolation (rarefaction), extrapolations were also performed, allowing estimates of species numbers if sampling effort were increased.

The Simpson index calculated for the three sites was 0.787. This relatively high value indicates low diversity, suggesting the presence of one or a few dominant species in the area, while the others occur in lower abundance. In other words, the macrofungal community of Espinilho State Park may be structured by the dominance of a small number of species, reflecting an uneven distribution of diversity.

The Shannon index value was 1.86, which suggests moderate diversity, indicating a relatively balanced composition of species present in the study area. Obtaining a value close to 2.0 generally indicates considerable diversity, although there is still room for greater richness or evenness within the community. These data highlight the importance of maintaining and monitoring this diversity, as it may be influenced by environmental factors, management, or human intervention. Comparing this index with similar studies may provide additional insights into the ecological status of the conservation unit and guide more effective conservation strategies.

During the surveys, mushrooms appeared more frequently and abundantly during dry periods. This pattern contrasts with traditional mycological observations where macrofungal richness and abundance typically peak during the rainy season, as documented in tropical and subtropical ecosystems (Andrew *et al*., 2013; Cavalcante *et al*., 2024). While mushroom deve-

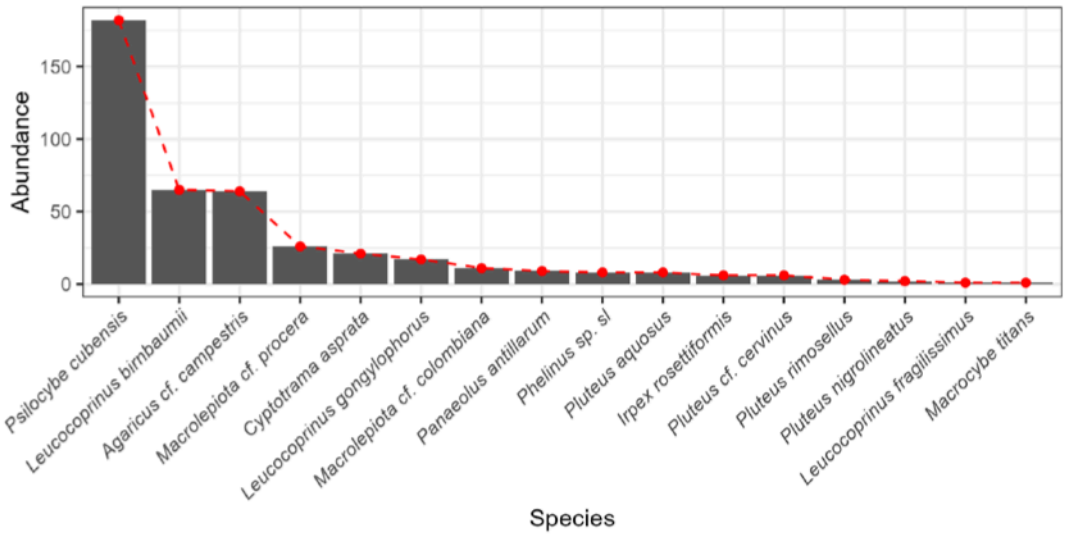


Figure 2. Species abundance in Espinilho State Park, Pampa biome, Rio Grande do Sul, Brazil

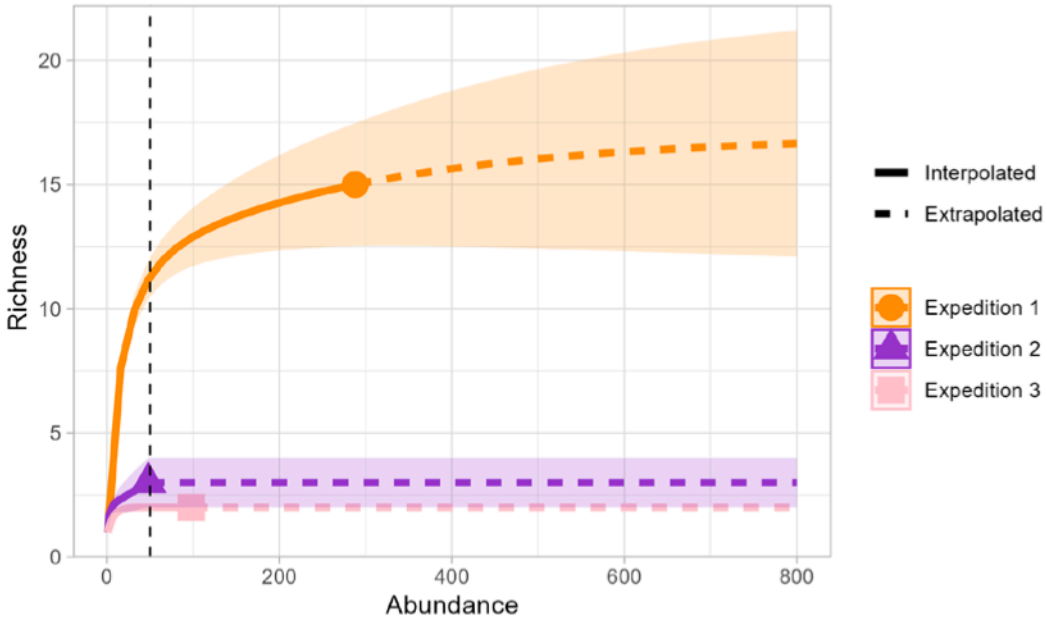


Figure 3. Rarefaction and extrapolation curves based on abundance for Agaricales fungi collected in Espinilho State Park, Rio Grande do Sul. The X axis represents abundance (number of occurrences) and the Y axis represents richness (number of species). Solid lines represent interpolated (observed) richness, and dashed lines represent extrapolated (estimated) richness. Shaded areas indicate the 95% confidence interval. The vertical dashed line indicates the standardized comparison point (lowest sampling effort)

lopment universally requires adequate substrate, temperature, and moisture (Teixeira *et al*., 2023), our findings indicate that the optimal humidity threshold for basidiome emergence in this ecosystem is met only when waterlogged conditions recede. At the sampling sites, the typical drainage constraints of the Haplic Gleysol lead to prolonged water accumulation during rainy periods. Rather than favoring development, this excessive saturation likely hinders basidiome formation by causing severe physiological or hypoxic stress to the mycelium, meaning that the specific moisture requirements for fruiting are only satisfied during the better-drained, drier intervals.

Marchiori *et al*. (2013) reported that the soil at Espinilho State Park is composed of fine, dry sand, which becomes saturated when in contact with rain, potentially leaving the area completely waterlogged. This saturation can compact the sand layer, creating a barrier that retains water on the surface. Thus, fungal hyphae may experience stress during these periods, hindering reproduction.

Moreover, as shown in Table 1, the distribution of species among the different substrates also supports this interpretation. It was observed that records of mushrooms on soil (SOL) and leaf litter (SER) were proportionally lower compared to the other substrates, especially during rainy periods. These substrates are directly exposed to water accumulation and to soil compaction, as previously described, which may limit oxygen availability and hinder the development of hyphae and the formation of basidiomata. On the other hand, mushrooms found on living trees (AVV) and dead trees (AMT) occurred more frequently, possibly because these substrates remain more stable and are less prone to water saturation. Thus, the variation in abundance among substrates suggests that excessive moisture may be

Table 1. Distribution of mushroom species by substrate in each of the phytophysiognomies studied in Espinilho State Park. AVV = Live tree, AMT = Dead tree, SOL = Soil, SER = Leaf litter, EST = Manure

	SOL	AVV	AMT	EST	SER
Macrolepiota cf. *colombiana*	11	0	0	0	0
Phelinus sp.	0	8	0	0	0
Leucocoprinus gongylophorus	17	0	0	0	0
Irpex rosettiformis	0	0	6	0	0
Panaeolus antillarum	9	0	0	0	0
Agaricus cf. *campestris*	64	0	0	0	0
Psilocybe cubensis	0	0	0	182	0
Pluteus cf. *cervinus*	0	0	0	0	6
Pluteus aquosus	0	0	0	0	8
Pluteus nigrolineatus	0	0	2	0	0
Pluteus rimosellus	0	0	3	0	0
Leucocoprinus fragilissimus	1	0	0	0	0
Leucocoprinus birnbaumii	64	1	0	0	0
Cyptotrama asprata	21	0	0	0	0
Macrolepiota cf. *procera*	26	0	0	0	0
Macrocybe titans	1	0	0	0	0

acting as a limiting factor, explaining the higher occurrence of mushrooms during dry periods.

Between April and October, a drastic decline in abundance and richness was observed (Figure 4). This decrease could be linked to a reduction in average temperature during that period. It has been documented that basidiomata formation requires specific optimal temperatures, and temperatures falling below these thresholds can significantly limit mushroom development, as observed in cultivation trials of subtropical species (Colavolpe & Albertó, 2014).

According to Branco *et al*. (2022), fungi possess homeostatic mechanisms to defend themselves against environmental variations. However, extreme physical conditions can override these cellular defenses. Griffin (1963) documented that water-saturated soils induce severe physical stress on the mycelium, preventing hyphal growth due to oxygen depletion. Following this reasoning, the prolonged waterlogging observed in the Haplic Gleysol of the Espinilho State Park after intense rainfall events likely imposes a similar hypoxic and physical stress on the macrofungal mycelium, restricting mushroom emergence during peak rainy periods.

Furthermore, seasonal thermal extremes also play a critical role in limiting community development in the study area, where summer temperatures regularly reach up to 34 °C. This field observation aligns with empirical laboratory models; for instance, Zhang *et al*. (2016) demonstrated that filamentous fungi exposed to heat stress (such as 33 °C) exhibit significantly inhibited mycelial growth and reduced hyphal branching due to severe physiological heat shock. Therefore, the drastic declines in richness and abundance observed in the park are modulated by a dual constraint system: severe hypoxic stress during waterlogged rainy periods and destructive thermal stress during the high-temperature summer months.

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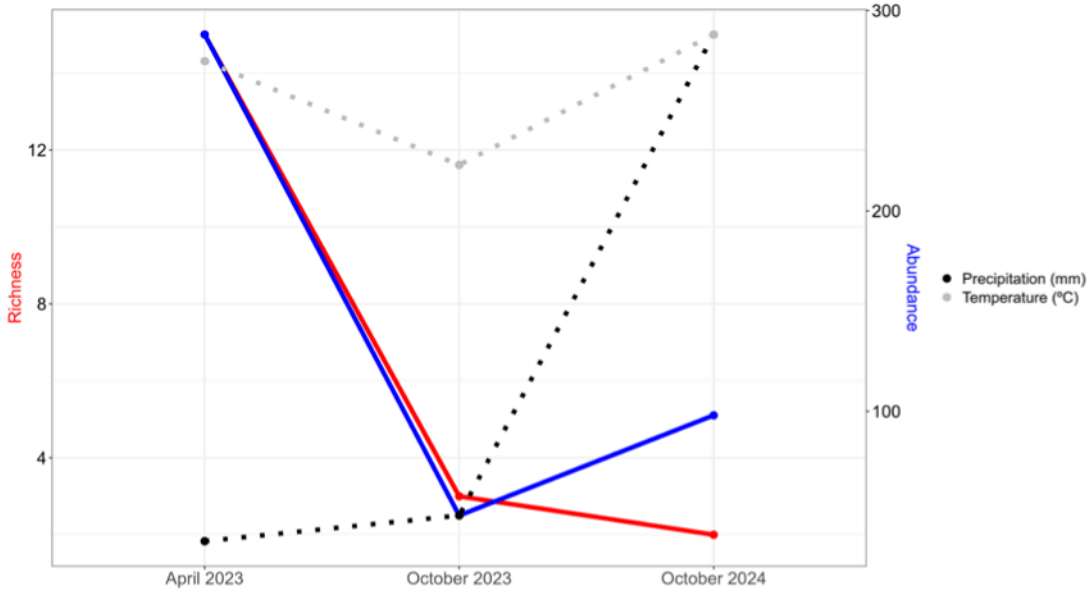


Figure 4. Rainfall graph with species abundance and richness

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AUTHORS CONTRIBUTIONS

M.A. Velloso: Conceptualization, Methodology, Fieldwork, Microscopic analysis, Statistical analysis, Literature review, Writing – original draft. J.R. Velloso: Fieldwork, Microscopic analysis, Statistical analysis, Literature review, Data curation, Visualization. L.L. Vilazante: Fieldwork. F. Machado-Teixeira: Fieldwork. J.P. Souto Dias: Fieldwork. A.L. Klotz Neves: Fieldwork. F. Aires Sousa: Fieldwork. J. Putzke: Conceptualization, Supervision, Methodology, Resources, Writing – review & editing.

CONFLICTS OF 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.

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