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Influence of different soil uses in the Pampa biome, southern Brazil, on fungal communities

Influência de diferentes usos de solo do bioma Pampa, sul do Brasil, nas comunidades fúngicas

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ABSTRACT

Anthropogenic activities in any natural ecosystem cause changes that affect the physical and biological environment, causing degradation. Likewise, these activities can cause significant changes in the chemical and physical factors of the soil, also impacting the microbiological community. The study aimed to evaluate the influence of soil biochemical and physicochemical characteristics on the development of fungal communities in three different soils of the Pampa biome. The soil types selected were: native forest, Eucalyptus plantation and pasture area, in two distinct periods: hot and cold. Microbiological, physicochemical and biochemical analyses of the soils were carried out. The following genera and species of fungi were identified: *Aspergillus* sp., *Aspergillus niger*, *Fusarium* sp., *Geotrichum* sp., *Isaria* sp., *Penicillium* sp., *Scedosporium* sp., *Trichoderma* sp., *Verticillium* sp. The results showed that the fungal community is being affected by soil composition characteristics, regardless of the sampled areas. *Aspergillus* sp. is associated with higher amounts of manganese, boron and the Ca+Mg/K ratio in the soil, while *A. niger* and *Trichoderma* sp. have a negative relationship with these variables. *Aspergillus* sp. showed a high ability to tolerate Mn. The most diverse area was pasture in the warm period, and the least diverse was Eucalyptus plantation in the cold period. The analyzed taxa had a reduction in the cold period compared to the warm one, except for *Aspergillus* sp. in the Eucalyptus area, which presented a greater amount in the cold period compared to the hot period. It can be seen that the use of soil in the Pampa biome for planting monocultures or for generating pastures interferes with its characteristics and distinctly affects the fungal community.

Keywords: soil management; physicochemical aspects of the soil; soil biochemistry; soil microorganisms.

RESUMO

As atividades antropogênicas em qualquer ecossistema natural causam modificações que afetam o ambiente físico e biológico, gerando degradação. Da mesma forma, essas atividades podem causar modificações significativas nos fatores químicos e físicos do solo, impactando também a comunidade microbiológica. O estudo objetivou avaliar a influência de características bioquímicas e físico-químicas do solo sobre o desenvolvimento de comunidades fúngicas em diferentes solos do bioma Pampa. Os tipos de solo selecionados foram: área de floresta nativa, área de plantação de eucalipto e área de pastagem, em dois períodos distintos: quente e frio. Foram realizadas análises microbiológicas, físico-químicas e bioquímicas dos solos. Identificaram-se os seguintes gêneros e espécie de fungos: *Aspergillus* sp., *Aspergillus niger*, *Fusarium* sp., *Geotrichum* sp., *Isaria* sp., *Penicillium* sp., *Scedosporium* sp., *Trichoderma* sp., *Verticillium* sp. Os resultados mostraram que a comunidade fúngica está sendo afetada pela composição das características do solo, independentemente das áreas amostradas. *Aspergillus* sp. está associado a maiores quantidades de manganês, boro e da relação Ca+Mg/K no solo, enquanto *A. niger* e *Trichoderma* sp. têm relação negativa com essas variáveis. *Aspergillus* sp. mostrou elevada capacidade de tolerância ao Mn. A área mais diversa foi a de pastagem no período quente, e a menos diversa foi a área de eucalipto no período frio. Os táxons analisados tiveram redução no período frio em relação ao período quente, com exceção de *Aspergillus* sp. na área de eucalipto, que apresentou maior quantidade em período frio em relação ao período quente. Pode-se constatar que a utilização do solo no bioma Pampa, para plantação de monoculturas ou para a geração de pastagens, interfere em suas características e afeta distintamente a comunidade fúngica.

Palavras-chave: manejo do solo; aspectos físico-químicos do solo; bioquímica do solo; microrganismos do solo.

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Introduction

The Pampa biome is located in South America and extends over an area of approximately 700,000 km^2, being present in Argentina, Uruguay and Brazil, where it exclusively occupies the state of Rio Grande do Sul. Its vegetation is quite characteristic, being composed of pastures, undergrowth, grasses and riparian forests. This biome is known for its rich diversity and has an abundant geological composition, formed by several microecosystems (Bolzan et al., 2016). The microbial biodiversity of the Pampa biome has not yet been sufficiently explored, and probably several species of microorganisms can still be found (Daniel Junior et al., 2018).

Despite its abundant diversity, the Pampa biome is still little known and studies about its communities are scarce. In recent years, the Pampa has been degraded to favor livestock and agricultural expansion, cattle breeding, and planting of soybean, corn and exotic tree species (Lupatini et al., 2013). This biome has one of the smallest networks of protected areas in the world and the smallest in Brazil (Lima et al., 2020).

Soil fungi play important roles for the ecosystem, as they are responsible for the decomposition of organic waste and xenobiotics, make biological control, establish symbiotic relationships, promote soil aggregation, mineralization of nutrients and cause diseases (Tortora et al., 2017). According to Moreira and Siqueira (2006), several factors affect soil organisms, so populations vary depending on the type of soil, vegetation and climatic conditions. Thus, large microbial variations can be found between distinct ecosystems in the same region or in different geographic regions.

Soil degradation and lack of vegetation cover affects the growth of macro and microbial communities, since they cause changes in food availability, soil temperature and humidity. Species resistant to environments with low availability of nutrients are selected and cause reduction of carbon cycling, soil aeration and disintegration of organic matter, resulting in decreased microbial biomass (Granada et al., 2019).

Considering the hypothesis that soils used in different activities may present different physicochemical and biochemical conditions, possibly influencing the fungal community, this study aimed to evaluate the influence of biochemical and physicochemical soil characteristics on the development of fungal communities in different soils of the Pampa biome.

Material and Methods

Collection of the samples

Soil samples were collected in the city of Pantano Grande/RS, in the Pampa biome domain. Three different areas of land use were selected: native forest area, Eucalyptus plantation area and pasture area (Figure 1) — the native forest belonging to the Atlantic Forest biome. The Eucalyptus area has silvicultural practices of preparation and soil fertilization and was under cultivation for seven years. The pasture area was previously used in the plantation of monocultures and had not been interfered with in approximately five years. In each area, three collection points were defined, sampled in triplicate. The soil was collected with the aid of a modified Auger at a depth of 5 cm with 10 cm in diameter and sieved in buckets previously disinfected with 70 GL alcohol.

After that, the sample was placed in Stomacher hermetic bags and stored in a thermal box for temperature preservation. For the collection, at each point a 1 m^2 quadrant was defined, from which nine points were randomly selected to compose the final sample. The coordinates of each sampled site can be found in Chart 1. The collection was performed in two distinct periods, winter and summer. Samples from the cold period (mean temperature of 15°C) were collected in July 2017 and the hot period (mean temperature of 30°C) was collected in December 2019.

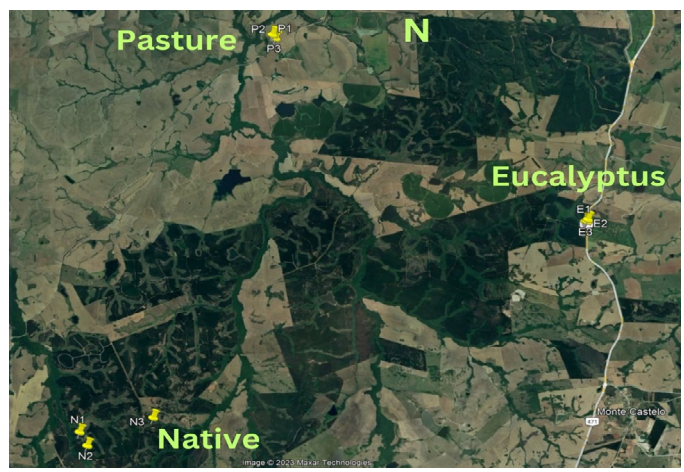

Figure 1 – Demarcation of the three collection points in the areas of native forest, Eucalyptus plantation and pasture selected for soil collection.
N: North. P1: Pasture 1; P2: Pasture 2; P3: Pasture 3; E1: Eucalyptus 1; E2: Eucalyptus 2; E3: Eucalyptus 3; N1: Native 1; N2: Native 2; N3: Native 3.
Source: Adapted from Google Earth.

Chart 1 – Coordinates of the sampled points for each land use area: native vegetation, Eucalyptus plantation and pasture*.

Collection points	Latitude	Longitude
Pasture 1	6655441.00 m S	360302.00 m E
Pasture 2	6655427.70 m S	360339.44 m E
Pasture 3	6651137.00 m S	356770.00 m E
Eucalyptus 1	6650246.00 m S	367850.00 m E
Eucalyptus 2	6650251.00 m S	367810.00 m E
Eucalyptus 3	6650306.83 m S	367871.16 m E
Native 1	6644258.02 m S	355770.85 m E
Native 2	6643880.08 m S	355960.58 m E
Native 3	6644681.81 m S	357534.26 m E

*Geographical coordinates of the three collection points of the different areas of land use analyzed in this study, native vegetation, pasture and Eucalyptus plantation.

Microbiological analyses

After soil collection, the samples were taken to the Microbiology Laboratory to be analyzed. For the extraction of fungi present in the soil, the decimal dilution technique was performed. 10 g of soil was weighed and 90 mL of saline peptone water was added. After one minute stirring in the homogenizer, decimal dilution was performed up to 10^{-5}. An aliquot of 0.1 mL was removed from each dilution and plated in a petri dish containing Sabouraud agar with chloramphenicol to prevent bacterial growth. The plates were incubated at 25°C for five days. After this period the count of Colony Forming Units (CFU)/ g of soil was performed. The colonies that showed growth after five days of incubation were isolated with the aid of a magnifying glass and inoculation loops in petri dishes with Sabouraud agar. The identification of genera was made by microculture. In a glass petri dish were placed two blades with two agar cubes with the fungus sown between them, a supporting blade and a cotton ball moistened. The microcultures were incubated at 25°C for seven days for growth of fungal structures. After this period, the slides were stained with lactophenol and KOH 20% and visualized under a microscope. The identification was based on the macro and micromorphological characteristics of the isolates. In the macroscopic study the reverse was observed of the colonies, such as in diameter, color of conidia and mycelium, texture, presence of exudates and soluble pigments. Microscopic structures such as conidiophores, conidiogenic cells and conidia were analyzed. For the identification of genera and species, the following literature was used: Domsch et al. (2007). The following isolates were identified: *Aspergillus* sp., *Aspergillus niger*, *Fusarium* sp., *Geotrichum* sp., *Isaria* sp., *Penicillium* sp., *Scedosporium* sp., *Trichoderma* sp., *Verticillium* sp. After being identified, the fungi were stored in test tubes with Sabouraud agar inclined to room temperature and in skim milk 10% in Eppendorf frozen at -20°C.

Physicochemical and biochemical analyses

In addition to the microbiological analyses, the following chemical analyses of clay, pH by the method SMP, potassium, phosphorus, percentage of organic matter, calcium, aluminum, magnesium, chlorine, copper, zinc, boron, sulfur and manganese were performed; and also physical analysis of sand, clay and silt. All these analyses were performed by an outsourced laboratory member of the Official Network of Soil Analysis Laboratories of the States of Rio Grande do Sul and Santa Catarina (ROLAS) and also a member of the Quality Control Program of Fertility Laboratories coordinated by the Embrapa System of Soil Analysis (PAQLF).

Physicochemical analyses of moisture, pH, granulometry and nitrogen were performed. To determine the humidity, a soil sample was placed in a Becker of known mass, weighed and left in the greenhouse for 24 hours. Then, the sample was placed in a desiccator and weighed again. The mass of the second weighing of the mass of the first weighing was discounted, thus obtaining the percentage of moisture present in the soil. The pH analysis was performed following the methodology of Embrapa (1997), adding 25 mL of distilled water in 10 g of soil and measuring with a pH meter after one hour rest. The granulometry aimed to characterize the amounts of silt, sand and clay present in the soil, as well as to determine its classification, and was performed according to the methodology proposed by Van Raij (2001). Nitrogen was quantified by protein tube digestion and distillation by the Kjeldahl method, following the Embrapa protocol (1997).

Biochemical analyses included microbial biomass, basal respiration and microbial quotient. The microbial biomass was determined by soil irradiation method and titration with ferrous ammoniacal sulfate 0.03 mol/L, according to the methodology described by Mendonça and Matos (2005). Basal respiration was performed following a protocol proposed by Stotzky (1965), where the soil rested for 40 days in hermetic pots with sodium hydroxide for analysis and was then titrated with hydrochloric acid PA. The microbial quotient (qMIC) was calculated following Anderson and Domsch (1993), using the values of microbial biomass and soil organic carbon.

Statistical analysis of the data

A restricted (canonical) ordering was performed through redundancy analysis based on distance — db-RDA (Legendre and Anderson, 1999). The distance was chosen according to the Bray-Curtis method. This analysis allowed evaluating the degree of association between the range of environmental variables and the taxonomic diversity of fungi found in the analyses in a multidimensional space. To conduct the db-RDA, the matrix of fungal taxa was standardized using the Hellinger method, while the matrix of environmental variables was standardized using the "standardize" method.

A multicollinearity analysis involving the environmental analyzes performed was developed in order to verify and exclude possible variables strongly correlated with the other predictor variables. This analysis was performed using the variance inflation factor (VIF) method through the *vifstep* function of the Uncertainty Analysis for Species Distribution Models — USDM package (Naimi et al., 2014). To evaluate if there was variation of fungal growth in hot and cold periods, two cluster analyses were made using spreadsheets (winter and summer) standardized by the Hellinger method, later transformed into Euclidean distance matrices. Afterwards, hierarchical groupings were conducted using the method of weighted peer groups with arithmetic mean (UPGMA). This procedure was visualized as grouped heat maps using the *pheatmap* function of the homonymous package (Kolde et al., 2012). All statistical analyses were performed in R programming language (Ihaka and Gentleman, 1996; R Core Team, 2018).

Results and Discussion

The results of the physicochemical and biochemical analyses of the three different areas of use of the studied soils, native vegetation,

pasture and Eucalyptus plantation, of the two periods analyzed (hot and cold), are presented in Table 1. The area with native vegetation showed higher humidity in the cold period than the other areas studied and periods analyzed. This same area also showed greater basal respiration, but in the hot period. Góes et al. (2021), in their study of the edaphic fauna of the Pampa biome, also observed that the soil with the highest moisture content was native vegetation. The higher values of humidity in areas of native vegetation can be justified by the greater coverage of the soil in relation to the other areas, because in field or crop, the soil covers are lower and deficient, leaving it exposed to the incidence of solar radiation, thus facilitating water loss by evaporation. Araújo et al. (2020b) demonstrated in their study that areas with native vegetation cover had greater biomass and carbon stock capacity compared to anthropic areas or areas in the process of anthropogenic change. In addition, as the vegetation regeneration stage advances, there is an increase in the amount of carbon and biomass. Basal respiration, higher in this area in the warm period compared to other areas, may also be related to greater soil cover, observed in places with native vegetation. According to Canei et al. (2018), basal respiration is one of the most used microbiological indicators to evaluate the activity of the soil heterotrophic microbiota and represents the CO2 produced by the microbial biomass during the processes of decomposition and mineralization of soil organic matter. The same study (Canei et al., 2018) also found higher values of basal respiration in native forest and araucaria forest than in pastures of the Pampa biome.

The pasture area showed higher pH (in the cold period) and higher microbial biomass (in the hot period) than the other areas. Eucalyptus plantation showed higher average microbial quotient (*q*MIC) in the hot period. The high pH in the soil indicates alkalization and can be a result of the addition of basic components such as limestone (liming), used in the preparation of the soil for pasture (Bernardi et al., 2018). According to Novak et al. (2022), soil biomass is an important nutrient cycling parameter in ecosystems and represents the fraction of organic matter that is most rapidly decomposed. In contrast to the present study, this author reported a greater amount of biomass in soil with native vegetation than in soil with agricultural cultivation. This is probably because the forest is not stable, it is still under stress.

The chemical analysis of the soil composition of the three types of studied areas showed that the pasture presents a higher mean in the cold period compared to the hot period (Table 2). Phosphorus is found in inorganic, organic and microbial forms in soils and its dynamics can be controlled by a combination of chemical and biological properties. Phosphorus is highly limited in tropical soils, thus the renewal of organic P and the rapid recycling of P from litter are the main processes to provide this macronutrient to plants in natural ecosystems (Soltangheisi et al., 2019). Groppo et al. (2015) also reported higher amounts of phosphorus in agricultural soils, such as pastures, in relation to the amounts found in native vegetation soils. The cold period showed even more phosphorus and potassium than the hot period. This occurs due to the higher fertilization that occurs in this period, as a strategy to improve the quantity and quality of the pasture, affected by low temperatures and frost (Pazeto et al., 2015). In relation to potassium, the highest levels in the soil under pasture may occur due to the immobilization of this micronutrient in the biomass of the trees and the export through crops, mainly grain. In the pasture, the non-renewal of the soil and the addition of residues (vegetables and animals) provide higher concentration of K in the surface layer of the soil. The animals influence the redistribution of nutrients by consumption, through defoliation of pasture, and their return to the soil via excretion (Assis et al., 2019).

The analysis of variance, Anova 1 criterion, showed no statistical difference when comparing the physicochemical, chemical and biochemical variables of the soil of the three areas studied (native vegetation, Eucalyptus plantation and pasture) in both periods, hot and cold.

Table 1 – Mean of the physicochemical and biochemical variables of the three collection points in the areas of Eucalyptus, native vegetation and pasture in the hot and cold periods*.

Variables	Eucalyptus		Native		Pasture	Cold
	Hot	Cold	Hot	Cold	Hot	
Humidity (%)	12.41	16.20	11.60	24.10	12.53	18.20
pH	5.44	5.60	5.78	5.41	6.59	7.35
Silt (%)	9.99	8.90	10.74	7.16	11.65	10.5
Clay (%)	39.24	25.71	24.43	24.25	35.54	18.68
Sand (%)	50.76	65.38	64.82	69.67	52.79	70.95
Nitrogen (g/Kg)	10.23	12.36	15.76	12.83	13.23	12.83
Microbial biomass (µg/g of C in the soil)	456.81	404.85	379.6	348.69	924.86	343.16
Basal respiration (µg CO2/g soil/hour)	0.63	0.40	0.74	0.48	0.60	0.72
Microbial quotient (%)	2.13	2.34	1.26	1.62	1.60	1.86

*Arithmetic mean of the analyses of the physicochemical and biochemical variables of the three soil collection points in the areas of Eucalyptus, pasture and native vegetation in the hot and cold periods of the Pampa biome analyzed in this study.

Table 2 – Mean of chemical variables from soil analyses of the three areas studied in each type of plant formation, Eucalyptus, native and pasture of the Pampa biome*.

Variables		Eucalyptus		Native		Pasture	Cold
		Hot	Cold	Hot	Cold	Hot	
pH SMP		6.03	5.37	6.17	5.07	6.40	6.57
Phosphorus (mg/L)		3.53	6.93	4.33	18.30	54.70	64.97
Potassium (mg/L)		94.33	63.00	88.33	57.67	128.67	359.67
Organic matter (%)		3.40	3.10	4.77	3.23	3.27	3.27
Aluminum (cmolcL^{-1})		0.60	0.50	0.17	1.47	0.53	0.00
Calcium (cmolcL^{-1})		3.20	5.03	2.63	1.37	4.07	7.27
Magnesium (cmolcL^{-1})		0.90	1.40	1.03	0.50	1.67	2.63
H+Al (cmolcL^{-1})		4.47	9.13	3.87	13.40	1.67	2.33
CTC (cmolcL^{-1})	Effective	4.90	7.07	4.07	3.50	8.13	10,80
	pH 7.0	8.83	15.67	7.73	15.37	9.80	13.13
% Saturation CTC	Al	15.67	7.30	4.27	44.50	0.00	0.00
	Bases	48.87	41.87	49.73	12.77	83.13	82.00
	K	2.73	1.00	2.80	1.00	3.90	6.97
Ca/Mg Ratio		3.37	3.77	2.57	2.70	2.50	2.77
Ca/K Ratio		12.53	31.50	14.30	8.67	14.57	8.00
Mg/K Ratio		3.63	8.53	5.33	3.10	5.83	2.87
(Ca+Mg)/K Ratio		16.17	40.03	19.63	11.77	20.40	10.87
Sum of bases (cmolcL^{-1})		4.30	6.53	3.90	2.03	8.13	10.80
Sulfur (mg/L)		6.43	18.87	6.83	19.57	6.57	9.47
Zinc (mg/L)		2.33	9.23	3.03	55.97	6.90	24.53
Copper (mg/L)		1.17	1.10	1.40	2.00	2.17	2.97
Boron (mg/L)		0.50	0.70	0.59	0.93	0.60	1.12
Manganese (mg/L)		22.67	68.00	11.00	35.67	2.67	22.33
Iron (%)		0.00	0.16	0.00	0.20	0.00	0.29
Sodium (mg/L)		0.00	7.00	0.00	5.33	0.00	15.67

*Arithmetic mean of the chemical variables of the three soil collection points in the areas of Eucalyptus, pasture and native vegetation in the hot and cold periods of the Pampa biome analyzed in this study.

Regarding the fungi found in the three areas studied, in the hot and cold periods (Table 3), it can be observed that 265 fungi were sampled, distributed in eight genera and one species. The hot period obtained a total of 206 fungi and the cold period a total of 59, that is, there was a reduction of approximately 71.35% (or 147 individuals) of the fungal community sampled in the cold period. In relation to the total of specimens sampled in each area, the pasture area (n = 134) showed to be richer in fungi, followed by native vegetation (n = 79) and Eucalyptus plantation (n = 52). When analyzing the species richness in relation to hot and cold periods, the highest richness was found in the pasture area in the warm period (n = 101), followed by the area with native vegetation in the warm period (n = 63) and the pasture area in the cold period (n = 33). The Eucalyptus plantation area in the hot period showed an n = 42, and the area with native vegetation in the cold period showed an n = 16. The area with Eucalyptus plantation in the cold period was the one with the lowest richness, with an n = 10.

Regarding fungal diversity, the pasture area presented eight taxa in total, one species and nine fungal genera. Of these, eight were found in the hot period and four in the cold period, demonstrating a 50% reduction in diversity in the cold period. The native vegetation area presented seven taxa in total, seven in the hot period and three in the cold period, showing a reduction of 58% in the cold period compared to the hot one. The area with Eucalyptus plantation showed six taxa in the hot period and three in the cold period, with a reduction of 50% of diversity in the cold period. The Shannon-Weaver diversity index ranged from 1.15 in the area with native vegetation in the cold period to 1.54 in the area of Eucalyptus plantation in the warm period.

The pasture area obtained an index of 1.34 and the native vegetation area of 1.17 (both for the hot period). The area with Eucalyptus plantation and the pasture area of the cold period obtained indexes of 1.49 and 1.29, respectively. The Shannon-Weaver diversity index considers equal weight between rare and abundant species and the lower the index value, the lower the diversity of the sample (Furtado and Vieira, 2020); thus, the most diverse area was pasture in the hot period and the least diverse was the Eucalyptus area in the cold period.

The mean of taxa found in the three studied areas ranged from 3.2 in the Eucalyptus plantation area to 8.8 in the pasture area, the mean of the native vegetation area being 8.0. The standard deviation was 4.28 in the Eucalyptus area, 15.85 in the area with native vegetation and 16.51 in the pasture area.

The three most abundant taxa found in the total of the three areas analyzed in this study were *Aspergillus* sp., *Aspergillus niger* e *Fusarium* sp. The genus *Aspergillus* stands out as an excellent producer of secondary metabolites of industrial and environmental interest.

Table 3 – Quantities of fungi found in areas of native vegetation, Eucalyptus plantation and pasture, in the hot and cold periods, of the Pampa biome*.

Areas	Fungi	Hot period	Cold period	Total area
Eucalyptus	*Aspergillus* sp.	4	8	52
	Aspergillus niger	24	1	
	Fusarium sp.	3	1	
	Isaria sp.	1	0	
	Scedosporium sp.	1	0	
	Trichoderma sp.	9	0	
Native	*Aspergillus* sp.	39	12	79
	Aspergillus niger	13	3	
	Fusarium sp.	4	1	
	Trichoderma sp.	4	0	
	Geotrichum sp.	1	0	
	Penicillium sp.	1	0	
	Scedosporium sp.	1	0	
Pasture	*Aspergillus* sp.	34	20	134
	Aspergillus niger	29	8	
	Fusarium sp.	15	3	
	Geotrichum sp.	1	2	
	Isaria sp.	1	0	
	Penicillium sp.	3	0	
	Trichoderma sp.	17	0	
	Verticillium sp.	1	0	
Total (hot and cold):		206	59	

*Species or genera of fungi, with their respective quantities, found in the three areas analyzed: pasture, native and Eucalyptus plantation, in the two periods: hot and cold in the Pampa biome.

This genus is part of a group of non-pathogenic microorganisms capable of producing enzymes and has been widely researched. They are filamentous fungi that stand out for their ease of cultivation and secretion of enzymes directly in the production medium (Nascimento et al., 2014). Correa et al. (2023), in their study with fungi, showed that most isolates of the genera *Penicillium*, *Aspergillus* and *Trichoderma* used the herbicide glyphosate as a source of nutrients, degrading it and decreasing its concentration in the environment.

A. niger is a species of *Aspergillus* sp. used in the development of an inoculant that promotes plant growth. This microorganism has the ability to solubilize mineral phosphates and is considered safe in food production (Araújo et al., 2020a). According to Nascimento et al. (2021), *A. niger* grows rapidly and sporulates well in common culture media. In addition, this microorganism does not present specificity for host plants, thus being advantageous in relation to symbiotic microbes, for which the production of inoculants can be complex. For Daniel Junior et al. (2018), the genus *Fusarium*, the third most abundant taxon in this study, is one of the most cited genera in studies related toproduction of bioherbicides. This genus has herbicidal activity against different species of grasses.

This study analyzed 35 environmental variables, but the variance inflation factor (VIF) showed which were correlated with each other. After model selection, only eight variables were maintained in the final db-RDA model, as shown in Table 4. The proportion of inertia (i.e. mean quadratic contingency coefficient) explained that the variability in the composition of fungi as a function of their relationship with the environmental variables of the soil was 63%, while 37% of the variability was not explained. The first two axes explained 51% (1º = 41.1%; 2º = 10.7%). The quality of the db-RDA model was relevant (R2 = 0.70) and significant (Anova, F[8.9] = 1.90, p-value = 0.02).

Table 4 – Variance inflation factor analysis displaying 12 variables retained and fit for the following narrow-order analyses#.

Environmental variables (soil)	VIF
1. Silt	1.69
2. Clay*	2.02
3. (Ca + Mg)/ K Ratio*	2.40
4. Basal respiration*	2.74
5. Colony forming units (CFU)	2.79
6. Microbial quotient*	2.98
7. Total nitrogen*	3.33
8. Manganese*	3.34
9. Copper*	3.74
10. pH	3.80
11. Sulfur	3.82
12. Boron*	4.75

#Variance inflation factor, of the 12 variables retained and suitable for restricted ordering analyses; *variables retained in the final db-RDA model.

When comparing the values of soil variables of the three areas studied, it can be seen that although the three areas analyzed, native vegetation, pasture and Eucalyptus plantation did not differ among themselves in the composition of the community of fungi, Table 4 shows the variation in soil components for each of them. Thus, *q*MIC, clay percentage, (Ca + Mg)/K ratio, manganese and copper seem to be higher in Eucalyptus plantation areas (Table 5). According to De Morais et al. (2021), the substitution of natural vegetation in the local biome for the production of forest crops can cause changes in quantitative and qualitative carbon stocks, in the microbial biomass of the soil and, consequently, in soil quality.

The higher *q*MIC is related to the lower carbon accumulation by microorganisms in this location, often due to the lower availability of substrate. With the addition of quality organic matter or when there are no stress situations, there is an increase in microbial biomass and, consequently, an increase in *q*MIC (Gonçalves et al., 2019). For Oliveira et al. (2022), in their study with Eucalyptus crops, the prolonged cultivation time of this plant caused increases in litter accumulation, organic matter and soil organic carbon.

The soils with pasture showed a mean rate of basal respiration and boron content above the other two areas studied. The values of the basal respiration rate are directly related to the increase or reduction of C available in the soil. A higher basal respiration rate may mean the release of nutrients in the soil, since the decomposition of plant material may make nutrients available to plants (Quirino et al., 2020). Alves et al. (2022), in their study, also obtained higher mean basal respiration in pastures. According to these authors, these areas promote a high availability of organic matter and have dense root mass, favoring the formation of microbial biomass in the rhizosphere. Basal soil respiration is considered the main measure to evaluate microbial activity and consists of the total sum of all metabolic functions, of which CO2 is produced.

Table 5 – Mean values of the soil variables kept in the final model of the db-RDA restricted ordering analysis by type of vegetation depending on the sampled area*.

Vegetation	Eucalyptus	Native	Pasture
Clay	32.4 (09.1)	24.3 (14.4)	27.1 (11.1)
(Ca+Mg)/ K Ratio	28.1 (14.5)	15.7 (08.3)	15.6 (05.5)
Basal respiration	0.51 (0.17)	0.61 (0.21)	0.66 (0.18)
Microbial quotient	2.23 (1.29)	1.44 (0.99)	1.73 (0.98)
Total nitrogen	11.3 (4.0)	14.3 (5.7)	13.0 (2.9)
Manganese	45.3 (34.4)	23.3 (25.6)	12.5 (11.6)
Copper	1.13 (0.22)	1.70 (0.51)	2.56 (0.85)
Boron	0.60 (0.16)	0.75 (0.29)	0.86 (0.32)

*Mean values with their respective standard deviations (between parentheses) of the soil variables kept in the final model of the analysis restricted db-RDA ordering by type of vegetation depending on the sampled area.

Caumo et al. (2021), in their study with plant communities, found that the areas with pastures presented higher species richness and that the presence of dry material in the soil is higher in non-pastured areas. The presence of herbivores is relevant in the recycling of vegetative material, changing the dynamics of nutrient cycling by accelerating the process.

Finally, total nitrogen seems to be, on average, more represented in soils with native vegetation (Table 5). According to Dalal et al. (2021), in the last 70 years, there has been an increase in the deposition of various forms of nitrogen in the soil, even in soil with native vegetation.

According to redundancy analysis, it can be observed that the fungal community is affected distinctly by the composition of the soil characteristics (Table 6), regardless of the areas sampled, as previously shown by the Permanova.

The genus *Aspergillus* sp. is more associated with higher amounts of manganese, boron and Ca+Mg/K ratio in soil (Figure 2 on the right), while taxa such as *A. niger* and *Trichoderma* sp. have a negative relationship with these variables. Thus, the latter will be absent or less frequent when these characteristics are predominant in the environment (Figure 2 on the left). According to Mohanty et al. (2017), in their study on fungus-assisted manganese bioleaching, *Aspergillus* sp. showed a high ability of tolerance to this micronutrient under various optimization conditions. Vera et al. (2021) found that the dose of B and the type of soil management have a significant effect on the structure of the fungal community, observing higher abundances of *Aspergillus* sp. *Penicillium* sp. and *Conlarium* sp. in soils with traditional agriculture and high levels of boron.

It should be noted that other species of the genus *Aspergillus* may have a behavior quite different from *A. niger*, considering that both taxonomic entities were diametrically opposed to each other and thus responding to soil components differently. In contrast, both *A. niger* and *Trichoderma* sp. appear to occur in greater abundance in soil types where there is a higher concentration of clay and where basal respiration is more intense (Figure 2 left).

Table 6 – Scores for restricted variables with correlations between pattern of fungal composition and soil composition*.

Environmental variables (soil)	CAP1	CAP2
Clay	-0.57	0.18
(Ca + Mg)/ K Ratio	0.40	0.06
Basal respiration	-0.44	0.22
Microbial quotient	-0.01	0.12
Total nitrogen	-0.04	-0.15
Manganese	0.75	0.24
Copper	0.03	-0.57
Boron	0.45	-0.21

*Scores for the restricted variables according to the db-RDA model showing the correlations between the fungal composition pattern and the soil composition pattern.

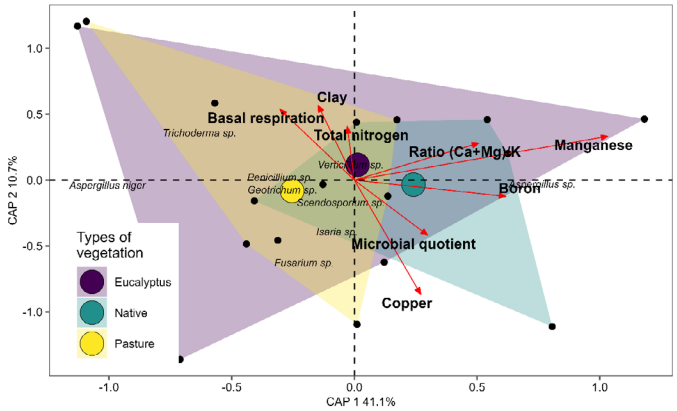

Figure 2 – Distance-based redundancy analysis (Bray-Curtis) showing the correlation between the fungal community and soil characteristics (gradients) of three areas with distinct vegetation in the Pampa biome. (i) Each fungal taxon is represented by a centroid (weighted mean) where it is most likely to occur or is more abundant, surrounded by the most representative sampling points (black) of its occurrence; (ii) The arrows (in red) point in the direction where the taxa were more abundant; (iii) The origin, direction, and size of the arrows indicate, respectively, the sign and correlation with the axes of db-RDA (importance for ordination), thus displaying their association with the variability in fungal community composition and how each organism responds (positive or negative relationship) to soil environmental variables; (iv) The polygons encompass the outermost sampling points of each area with native vegetation, Eucalyptus, or pasture, while (v) The larger colored points within them show the centroid of each group. Genera such as *Penicillium* sp., *Scedosporium* sp., and *Isaria* sp. are located closer to the center of the graph, overlapping with the environmental variables of microbial quotient and total nitrogen.

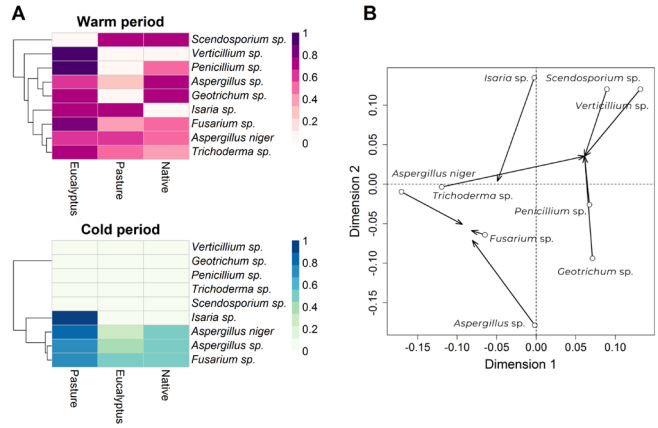

Figure 3 – (A) Heatmaps grouped based on matrices standardized by the Hellinger method of fungal diversity sampled in three areas of the Pampa biome during a warm (upper) and a cold (lower) period. (B) Procrustes analysis showing the agreement between the two heatmaps grouped in A*. *Cophenetic correlation coefficients: 0.81 (hot) and 0.99 (cold). In B, the empty points indicate the position (proximity or distance) of fungal taxa according to the similarity (grouping) observed during the warm period. Now, the arrowheads indicate the corresponding change (displacement) when the cold period is analyzed. The length of the residues indicated by the straight lines shows how concordant (in this case, the absence thereof) are both configurations (clusters) after the ideal Procrustes fit.

The genus *Trichoderma* has a phytostimulating effect on plants, with direct and indirect impacts, improving its growth and productivity. This genus has also shown potential in the growth and productivity of various crops (Sani et al., 2020). Utomo et al. (2016) demonstrated an association between *Trichoderma* sp. and higher levels of organic carbon and soil organic matter. Organic matter and organic carbon may be associated with increased basal respiration of the soil, explaining the correlation observed in the present study.

The genus *Fusarium* showed a correlation with higher amounts of copper in the soil as opposed to the remaining community of fungi (Figure 2). Ragasa et al. (2021) also reported the presence of species of the genus *Fusarium* in soil with high concentrations of copper. Copper is known to be an essential micronutrient. However, high concentrations associated with agricultural waste are highly toxic to most organisms.

Through redundancy analysis, it is possible to infer that the occurrence of organisms such as *Geotrichum* sp., *Verticillium* sp., *Penicillium* sp., *Scedosporium* sp. and *Isaria* sp., when positioned near the origin (x and y axes equal to 0.0), is poorly explained by any of the soil variables.

Regarding the periods studied, according to the clusters generated, there is a change in the pattern formed in the hot period when compared to the cold period (Figure 3A, higher). For example, while *A. Niger*, *Fusarium* sp., *Trichoderma* sp. and *Isaria* sp. are grouped next to each other, suggesting a change of individuals comparable along the areas sampled (Eucalyptus, native and pasture), *Aspergillus* sp. aligns with *Geotrichum* sp., indicating a pattern of modification of individuals more analogous to each other than to the taxa previously mentioned (Figure 3A, top). In the cold period, *A. Niger*, *Fusarium* sp. and *Isaria* sp. have reduced abundance of individuals, but maintain the same pattern of changes along the areas along with *Aspergillus* sp., while *Trichoderma* sp. modifies considerably, not presenting any individual in this period (Figure 3A, bottom). It should also be noted that all taxa analyzed in this study had a reduction in the cold period compared to the hot period, except for *Aspergillus* sp. in the area of Eucalyptus, with greater quantity in the cold period compared to the hot period. This is probably because this genus of fungus has numerous biological characteristics that allow its survival in regions that have extreme conditions (Verweij et al., 2016).

The Procrustes analysis showed that there was no agreement between the order generated between one period and another, suggesting that the pattern of diversity found in the hot period (point) will not be the same as the cold period, as indicated by the head of the arrow in Figure 3B (M2 = 0.82, correlation = 0.41, p-value = 0.52). The size of the residues (length of the arrows) in the Procrustes analysis indicates great modifications from one period to another, with the exception of *Fusarium* sp., which has a very corresponding proportion of change between each of the areas in both periods, as indicated by the smaller size of its residue in Figure 3B.

Conclusion

The three most abundant taxa found in the analyzed areas were *Aspergillus* sp., *Aspergillus niger* and *Fusarium* sp. Shannon's diversity index demonstrated that the area with the greatest diversity of fungi is pasture in the hot period, and the least diverse is Eucalyptus in the cold period. Of 35 environmental variables, eight showed correlation with the fungal composition in the studied areas. This study showed that the fungal community did not differ in the areas studied, native vegetation, pasture and Eucalyptus plantation, but was affected by the soil composition characteristics in all areas analyzed. The genus *Aspergillus* sp. is more associated with higher amounts of manganese, boron and Ca+Mg/K ratio in soil, while *A. niger* and *Trichoderma* sp. have a negative relationship with these variables. Regarding the periods studied, all taxa had a reduction in the cold period compared to the hot period, except for *Aspergillus* sp., which demonstrated the opposite for the Eucalyptus plantation. It can be seen that the use of soil in the Pampa biome for planting monocultures of the Eucalyptus type or for the generation of pastures interferes with its characteristics and distinctly affects the fungal community.

Contribution of authors:

STROHER, A. L.: Conceptualization; Investigation; Visualization; Writing – original draft. MÜLLER, T.: Conceptualization; Data curation; Visualization; Writing – original draft; Writing – review & editing. HEIDRICH, D.: Conceptualization; Data curation; Formal Analysis; Investigation; Methodology; Supervision; Validation. MACIEL, M. J.: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – review & editing. REMPEL, C.: Conceptualization; Data curation; Methodology; Project administration; Resources; Supervision; Validation; Writing – review & editing. SILVA, G. L.: Conceptualization; Data curation; Formal Analysis; Software; Supervision; Validation.

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