Article

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A relationship between fungi (Basidiomycota, Agaricomycetes, Agaricales) and nutrient content in riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) in southern Brazil

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ABSTRACT – (A relationship between fungi (Basidiomycota, Agaricomycetes, Agaricales) and nutrient content in riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) in southern Brazil). Due the tolerance in soil degraded, *Eucalyptus* is widely used in reforestation area. This study aims to evaluate the fungi that use *Eucalyptus grandis* W. Hill ex Maiden as substrate in reforestation area in southern Brazil. Fungi were identified and macronutrient and micronutrient contents were evaluated in order to understand the relationship between the fungi and the substrate. There were 200 specimens found, categorized into 25 species belonging to 10 families of Agaricales (Basidiomycota, Fungi). Substrates used by fungi were branches, roots, stems, humus, and soil. Macronutrients mean level found in fungi followed the order Ca>K>P>Mg, and micronutrients S>Fe>Mn>Cu/B>Zn. C:N ratio mean was 13:1, associated with substrate degradation potential, since the enzymatic production of fungi is affected by disposition of these nutrients. The data obtained in this study allowed a better understanding of fungi associated with the exotic arboreal substrate, and their nutritional significance in reforestation area.
Keywords: Agaricales, associations, Myrtaceae, nutrient, reforestation

RESUMO – (Uma relação entre fungos (Basidiomycota, Agaricomycetes, Agaricales) e teor de nutrientes em área ripária de reflorestamento com *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) no Sul do Brasil). Devido à tolerância em solos degradados, *Eucalyptus* é amplamente utilizado em áreas de reflorestamento. Este estudo teve como objetivo avaliar os fungos que utilizam *Eucalyptus grandis* W. Hill ex Maiden como substrato em uma área de reflorestamento no sul do Brasil. Para compreender a relação dos fungos com o substrato, os mesmos foram identificados e os teores de macronutrientes e micronutrientes foram avaliados. Foram encontrados 200 espécimes divididos entre 25 espécies pertencentes a 10 famílias de Agaricales (Basidiomycota, Fungi). Os substratos utilizados pelos fungos foram galhos, raízes, caules, húmus e solo. Os teores médios de macronutrientes encontrados nos fungos seguiram a ordem Ca>K>P>Mg, e os micronutrientes S>Fe>Mn>Cu/B>Zn. A relação C:N foi de 13:1, associada ao potencial de degradação do substrato, uma vez que a produção enzimática dos fungos é afetada pela disposição desses nutrientes. Os dados obtidos neste estudo auxiliam em uma melhor compreensão dos fungos associados ao substrato arbóreo exótico e sua importância nutricional na área de reflorestamento.
Palavras-chaves: Agaricales, associações, Myrtaceae, nutriente, reflorestamento

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Introduction

The *Eucalyptus* (Myrtaceae) is an arboreal genus that is frequently used in afforestation and reforestation operations because of its high tolerance in degraded soils (Njouonkou *et al*. 2021). In Rio Grande do Sul State this genus plays a significant role to economy, primarily in silviculture area (Echer *et al*. 2015). Soils that have been damaged by anthropic action, *Eucalyptus* is often used as a pioneer species for planting due their tolerance the acid pH and salinity (Taiz *et al*. 2018). However, it is an exotic species in Brazil, introduced in Rio Grande do Sul State in middle of XIX century (Trentim *et al*. 2014).

A limited number of studies reported ecological role of fungi in relation to substrates of exotic tree species, as well as their role in nutrients recirculation. According to Manzato *et al*. (2020), capable fungi to use exotic trees substrate are very important to biodegradation of this organic matter type. Fungi associated with these trees can break down and extract carbon compounds, including lignin, cellulose, and hemicellulose (de Araujo *et al*. 2018, Martins *et al*. 2018). The carbon/nitrogen ratio can provide insight into how native fungi diversity is associated with exotic tree species (Bononi *et al*. 2017, Njouonkou *et al*. 2021). Agaricales fungi have carbon-to-nitrogen ratio mean between 10:1 and 15:1 (Stoffella & Kahn 2001). A study conducted by D'Agostini *et al*. (2011) with fungi that decompose wood, the C:N ratio was noted as a factor in the time needed to breakdown organic matter in part due to the time required to produce decomposing enzymes, such as cellulase. The priming effect is attributed to the ability of fungi to decompose organic matter through the availability of fresh carbon (Fontaine *et al*. 2011). Nitrogen also interacts with decomposition system once the availability interferes with the diversity of fungi (Liao *et al*. 2020). Understanding the carbon-to-nitrogen ratio is essential since it is linked with type of organic matter and the decomposition process by fungi. In this context, size population, as well as the capacity of enzyme production and mineralization is related the fungi action in nutrient cycle, once the lignicolous species possess enzymatic apparatus which is designed to extract these compounds from wood, and terrestrial are involved in decomposition organic matter deposited in the soil (Bahram & Netherway 2022).

Macronutrients and micronutrients are acquired by fungi from substrate to which they grow or are attached in combination with the capacity to extract these nutrients (Silva-Neto *et al*. 2022). Therefore, the ability of fungi to absorb and retain certain nutrients is essential to comprehend how to occur nutritional cycle. In study with edible fungi, Malinowski *et al*. (2021) report that some species contained essential nutritional content. Edible fungi contain macronutrients and micronutrients suitable for human and animal consumption. They are functional foods because containing around of 20-35% proteins, 5-10% essential amino acids, vitamins and minerals, as well as low lipid levels (Altaf *et al*. 2020).

This study investigated fungi-plant-substrate association in a riparian reforestation area in southern Brazil with *Eucalyptus grandis* W. Hill ex Maiden monoculture. To understand this association, the study also analyzed the nutritional potential and chemical composition of Agaricales associated with this type of exotic substrate.

Material and methods

Study area – During the period 2021-2022 the collection was conducted at Fundação Estadual de Pesquisa Agropecuária (FEPAGRO), São Gabriel, Rio Grande do Sul State, Brazil (-30°20'13''S and -54°15'49''W). In the region, there is a weir of 37 m^2 protected by a reforestation area of 336 m^2 composed of *Eucalyptus grandis* W. Hill ex Maiden trees implemented in the year 2000. Previously, the area was planted with soy, and the same is still occurring in the farms in its surroundings. Temperatures oscillated from 3 to 35°C, with a mean of 10°C in winter, 18°C in autumn, 23°C in spring, and 30°C in summer. The average monthly precipitation ranges from 4 to 152 mm, with relative humidity from 19% to 70% (Embrapa 2022).

Sample – Fungi were collected under license SISBIO 79049-1 using the Rapid Survey method (Walter & Guarino 2006) with the modifications: perimeters of lines L1, L2, and L3 were subdivided according with limits of Brazilian riparian zones. Line 1 (active water channel) in the first 30 m from the water's edge. Line 2 (flood plain) for another 30 m from the end of the perimeter of L1. Line 3 (filter area) for another 30 m beyond the end of L2 (Brasil 1976). During the collection, a 5-minute interval was maintained, all specimens found were gathered up, and each line (L1, L2, and L3) there was one collector. The expeditions were seasonal with a total collection time of approximately 24 hours. To collect fungi from branches and stems of trees 1 cm diameter and 1 mm thick woody portion was removed near the basidiome. In the case of fungi close to the roots exposed on the soil surface, 1 cm in diameter and 1 mm thick were removed, and for this collection, the structure was followed up to the plant (de Araujo *et al*. 2018). For fungi found on soil, about 10 g were collected together with the basidiome. Samples were placed in paper bags and then dehydrated at 40°C for 48 hours.

Taxonomic identification – Fungal species were identified using the taxonomic key for Agaricales from Brazil (Putzke & Putzke 2017, 2019). Microscopy analyzes were conducted through cuts on the basidiomata lamellae and placing them on slides, which were then rehydrated with 3% KOH solution. The Olympus DP53 optical microscope was used to observe microstructures, such as spores, basidia, and hyphae.

Nutritional content – The dry mass of fungi collected was analyzed in relation to the nutritional levels. For species with pileus diameters smaller than 5 cm, duplicates were separated, while for species with larger diameters, only one unit was reserved. Analysis of macronutrients included carbon (C), nitrogen (N), calcium (Ca), magnesium (Mg), phosphorus (P), and potassium (K). The micronutrients analyzed were zinc (Zn), copper (Cu), sulfur (S), boron (B), iron (Fe), and manganese (Mn). Nutrient contents were analyzed following the determinations by Lutz (1985), and the C:N ratio was calculated using the results from chemical analysis, according to Mantovani *et al*. (2007). The analyzes were performed at Laboratório de Solos da Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul State, Brazil.

Statistics – Nutritional contents of fungi were computed and quantified using the BioEstat v.2.0 program in percentage terms (Ayres & Junior 2000). The means of nutritional content obtained with each species were submitted to Analysis of Variance (ANOVA) and compared by Tukey test at 5% level of error probability with ESTAT version 2 software (Estat 1994).

Results

The diversity of fungi found in the study area comprised 25 species associated with *Eucalyptus grandis* W. Hill ex Maiden, developing on branches (1), roots (1), stems (3), humus (7), and on the soil (13), totaling 200 specimens. In total, 10 families of Agaricales were found. Records of fungi included the occurrence of six species new to Rio Grande do Sul State, as well as 21 species associated with *E. grandis* for the first time (table 1 and figure 1).

Nutritional chemical analyzes of all mushrooms showed macronutrient content in following order Ca>K>P>Mg, and for micronutrients S>Fe>Mn >Cu/B/Zn. It was not possible to detect any differences in the concentration of Cu, B, or Zn. Terricolous fungi (soil and humus), when compared to lignicolous fungi (stem and branches), exhibited higher levels of macronutrients and micronutrients (table 2).

The carbon-to-nitrogen ratio (C: N) is summarized in Table 3. As result of this study, the C:N ratio of the species varied from 16.2 to 11.00 in C content, and from 1.58 to 0.66 in N content, with 13:1 mean. Considering total value of dry mass, C represented 93% and N 7%. Different terricolous fungi showed oscillations from 14.2 to 11.08 in C, and 1.43 to 0.81 in N. Oscillations between 14.70 and 11.08 in C, and 1.06 to 0.75 in N was found in humicolous fungi. In the case of lignicolous fungi, values on stems ranged from 12.90 to 10.90 in C, and 1.02 to 0.78 in N, while values on branches ranged from 12.98 to 9.79 in C, and 1.08 to 0.78 in N. Values associated with roots ranged between 15.1 and 12.20 in C, and 0.98 to 0.78 in N.

Table 1. Agaricales fungi found in the riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden, São Gabriel, Rio Grande do Sul State, Brazil.

Family	Species	Substrate	N°	FR	Occurrence	Reference
Agaricaceae	*Agaricus rufoaurantiacus* Heinem, 1961	Soil	8	*	PE, RS, and SP	Pegler 1997, Costa *et al.* 2022
Entolomataceae	*Clitopilus scyphoides* Singer, 1946	Soil	8	*	PR and RS	Singer 1961, de Meijer 2006
Entolomataceae	*Entoloma* sp. (Fr.) P. Kumm,1871	Soil	2	-	WD	Putzke & Putzke 2017
Hydnangiaceae	*Laccaria fraterna* (Sacc.) Pegler, 1965	Soil	48	*	PR and RS	Putzke 1999, Meijer 2008
Hymenogastraceae	*Galerina* sp. Earle, 1909	Humus	2	-	WD	Putzke & Putzke 2019
Hymenogastraceae	*Gymnopilus earlei* Murrill, 1913	Humus	2	*	MT, PR, and RS	Araujo 1984, Sobestiansky 2005, Bononi *et al*. 2017
Hymenogastraceae	**Gymnopilus junonius* (Fr.) PD Orton, 1960	Stem	2	*	AM	Silva 2015
Hymenogastraceae	*Gymnopilus pampeanus* (Speg.) Singer, 1951	Stem	13	*	RS and SP	Singer 1953, Pleigler 1997, Sobestiansky 2005
Hymenogastraceae	**Gymnopilus paraenses* (Berk.) Pegler, 1988	Humus	2	*	PA	Araujo 1984

Table 1. Cont.

Family	Species	Substrate	N°	FR	Occurrence	Reference
Hymenogastraceae	*Gymnopilus subtropicus* Hesler, 1969	Root	5	-	PR, PB, and RS	Sobestiansky 2005, Meijer 2008, Magnago *et al.* 2015
Hymenogastraceae	**Gymnopilus zenkeri* (Henn.) Singer, 1951	Humus	6	*	RJ	Albuquerque 2006
Lycoperdaceae	**Apioperdon pyriforme* (Schaeff.) Vizzini, 2017	Soil	11	*	SP	Baseia 2005
Lycoperdaceae	*Lycoperdon perlatum* Pers, 1796	Soil	6	*	PE, RS, and SP	Baseia 2005, Cortez *et al.* 2013
Mycenaceae	*Mycena galericulata* (Scop.) Gray, 1821	Soil	12	*	RS	Rick 1961
Pluteaceae	*Pluteus cervinus* (Schaeff.) P. Kumm, 1871	Humus	16	*	PR and RS	Singer 1956, Cortez & Coelho 2005
Physalacriaceae	*Oudemansiella canarii* (Jungh.) Höhn, 1909	Branches	4	*	MG, RS, and SC	Putzke & Pereira 1988, Rosa & Capelari 2009
Psathyrellaceae	*Coprinellus domesticus* (Bolton) Vilgalys, Hopple & Jacq. Johnson, 2001	Humus	2	*	RS	Cortez & Coelho 2005, Meijer 2006
Psathyrellaceae	*Coprinus lagopus* (Fr.) Fr. 1838	Soil	8	*	PR and RS	Pleigler 1997, Meijer 2008
Psathyrellaceae	**Parasola lactea* (AH Sm.) Redhead, Vilgalys & Hopple, 2001	Soil	3	*	PR	Meijer 2006
Psathyrellaceae	*Psathyrella argillospora* Singer, 1973	Soil	1	*	RS	Singer 1953, Cortez & Coelho 2005, Meijer 2006
Psathyrellaceae	*Psathyrella hypertropicalis* Guzmán, Bandala & Montoya, 1988	Humus	1	*	RS	Singer 1953, Cortez & Coelho 2005
Psathyrellaceae	**Psathyrella murrillii* AH Sm. 1972	Soil	4	*	MT and SP	Pleigler 1997, Bononi *et al.* 2008
Strophariaceae	*Pholiota* sp. (Fr.) P.Kumm, 1871	Stem	18	-	WD	Putzke & Putzke 2019
Strophariaceae	*Stropharia rugosoannulata* Farl. ex Murrill, 1922	Soil	1	*	RS	Pleigler 1997
Tricholomataceae	*Lepista nuda* (Bull.) Cooke, 1871	Soil	15	*	RS	Guerrero & Homrich 1999

Note: Abundance of individuals (N°). First record in *Eucalyptus grandis* W. Hill ex Maiden (FR = *), and data shortage (-). Observation of first occurrence in Rio Grande do Sul State (*) next to the species name. States of occurrence in Brazil: Amazonas (AM), Minas Gerais (MG), Mato Grasso (MT), Pará (PA), Pernambuco (PE), Paraná (PR), Rio Grande do Sul (RS), Santa Catarina (SC), São Paulo (SP). Genus widely distributed in Brazil (WD). Source: Authors (2022).

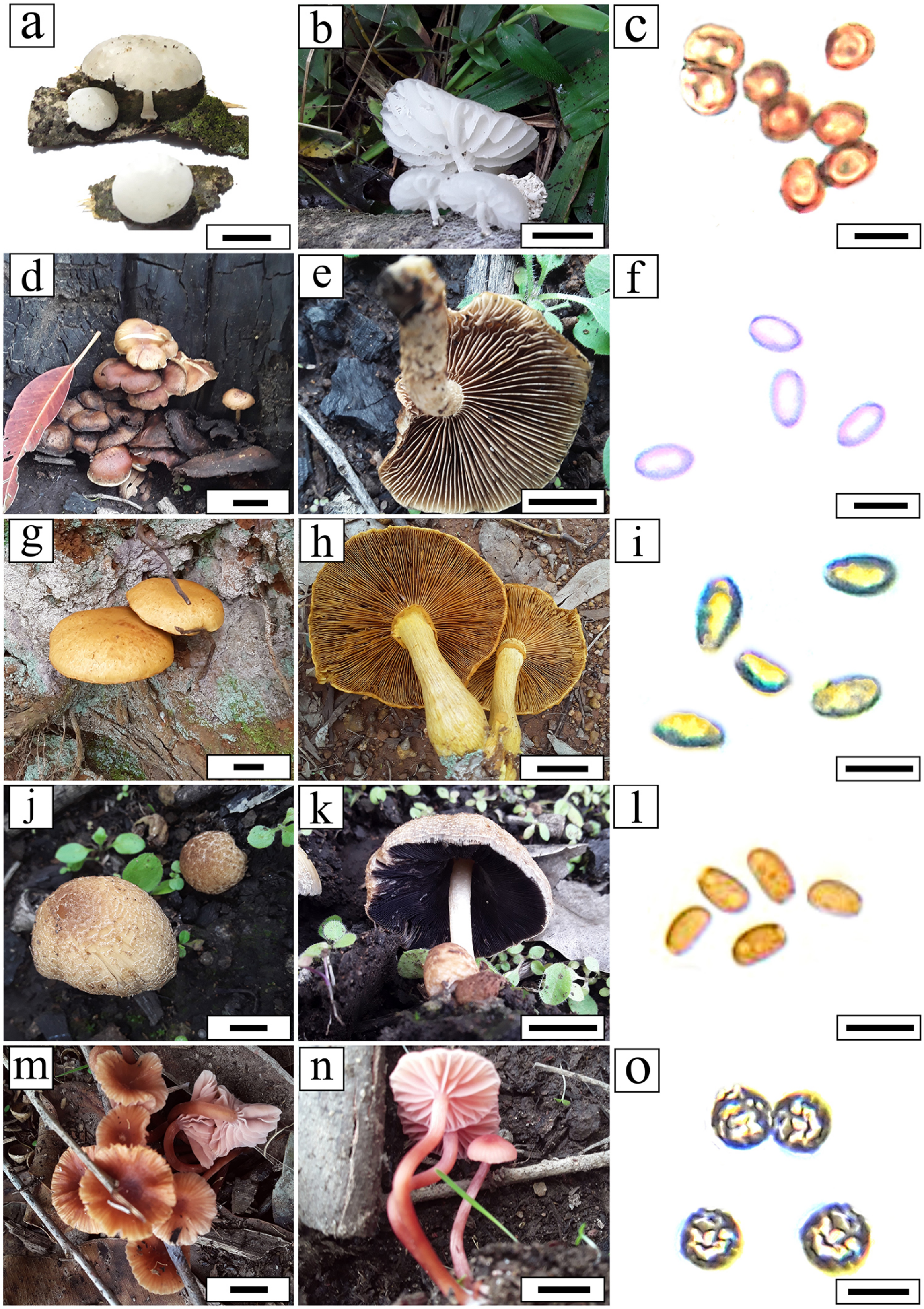

Figure 1. Macrostructures and microstructures of some fungi found in association with *Eucalyptus grandis* W. Hill ex Maiden, São Gabriel, Rio Grande do Sul State, Brazil. *Oudemansiella canarii* (Jungh.) Höhn on branches. a-c. *Pholiota* sp. (Fr.) P.Kumm on stem. d-f. *Gymnopilus junonius* (Fr.) PD Orton on roots. g-i. *Coprinellus domesticus* (Bolton) Vilgalys, Hopple & Jacq. Johnson on humus. j-l. *Laccaria fratera* (Sacc.) Pegler on soil. m-o. Pileus top view (a, b, c, g, and m), bottom view (b, e, h, k, and n), and spores (c, f, i, l, and o). Note: Scales (a, b, d, e, g, h, j, k, m, and n) 25 mm. Scales (c, f, i, l, and o) 10 µm. Source: Authors (2022).

Table 2. Macronutrient and micronutrient contents of fungi with occurrence in riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden, São Gabriel, Rio Grande do Sul State, Brazil.

Species	Substrate	Macronutrients $cmol_c L^{-1}$					Micronutrientes $mg L^{-1}$				
		P	K	Ca	Mg	S	Cu	Zn	Fe	Mn	B
Agaricus rufoaurantiacus	Soil	0.48	2.41	5.76	0.04	2.02	0.02	0.01	0.33	0.04	0.03
Clitopilus scyphoides	Soil	0.47	1.98	4.98	0.05	1.99	0.01	0.01	0.47	0.02	0.01
Entoloma sp.	Soil	0.41	2.02	5.44	0.04	2.03	0.01	0.01	0.35	0.04	0.02
Laccaria fraterna	Soil	0.43	2.23	4.68	0.05	2.02	0.02	0.02	0.43	0.05	0.01
Galerina sp.	Humus	0.31	2.07	3.43	0.03	2.01	0.02	0.01	0.37	0.05	0.01
Gymnopilus earlei	Humus	0.35	2.00	3.37	0.03	2.00	0.02	0.01	0.35	0.05	0.01
Gymnopilus junonius	Stem	0.40	1.87	3.86	0.02	1.99	0.02	0.02	0.32	0.03	0.03
Gymnopilus pampeanus	Stem	0.41	1.79	2.98	0.02	1.98	0.02	0.02	0.33	0.03	0.03
Gymnopilus paraenses	Humus	0.32	2.05	3.40	0.03	2.02	0.02	0.01	0.34	0.05	0.01
Gymnopilus subtropicus	Root	0.45	2.04	3.88	0.02	2.03	0.02	0.02	0.32	0.03	0.03
Gymnopilus zenkeri	Humus	0.31	2.00	3.38	0.03	2.01	0.02	0.01	0.36	0.05	0.01
Apioperdon pyriforme	Soil	0.44	2.01	5.02	0.05	2.10	0.01	0.02	0.35	0.06	0.02
Lycoperdon perlatum	Soil	0.48	1.99	4.34	0.05	2.32	0.01	0.01	0.41	0.06	0.01
Mycena galericulata	Soil	0.45	2.03	5.03	0.04	1.98	0.02	0.02	0.44	0.05	0.03
Pluteus cervinus	Humus	0.30	1.97	3.33	0.03	2.09	0.02	0.01	0.38	0.05	0.01
Oudemansiella canarii	Branches	0.41	1.86	3.45	0.02	2.01	0.02	0.02	0.36	0.03	0.03
Coprinellus domesticus	Humus	0.31	1.99	3.34	0.03	2.00	0.02	0.01	0.37	0.05	0.01
Coprinus lagopus	Soil	0.44	2.05	5.00	0.05	2.12	0.02	0.02	0.33	0.05	0.03
Parasola lactea	Soil	0.45	2.09	4.33	0.04	2.00	0.02	0.01	0.43	0.04	0.01
Psathyrella argillospora	Soil	0.42	1.90	4.99	0.05	2.01	0.01	0.02	0.40	0.05	0.03
Psathyrella hypertropicalis	Humus	0.33	2.00	3.35	0.03	2.02	0.02	0.01	0.34	0.05	0.01
Psathyrella murrillii	Soil	0.46	1.99	5.03	0.04	1.98	0.01	0.02	0.34	0.05	0.02
Pholiota sp.	Stem	0.42	1.78	2.95	0.02	2.00	0.01	0.01	0.30	0.03	0.03
Stropharia rugosoannulata	Soil	0.43	2.00	4.90	0.06	1.98	0.01	0.02	0.39	0.04	0.02
Lepista nuda	Soil	0.44	2.07	4.98	0.04	2.02	0.02	0.02	0.40	0.03	0.02

Macronutrients $cmol_c L^{-1}$

Fungi/Substrate	P	K	Ca	Mg
Soil	0.44 ± 0.02 cA	2.02 ± 0.13 bA	4.99 ± 0.38 aA	0.05 ± 0.00 dA
Humus	0.31 ± 0.01 cB	2.00 ± 0.03 bA	3.36 ± 0.03 aB	0.03 ± 0.00 dB
Stem	0.41 ± 0.01 cA	1.79 ± 0.04 bB	2.98 ± 0.51 aC	0.02 ± 0.00 dB
Branches	0.41 ± 0.00 cA	1.86 ± 0.00 bB	3.45 ± 0.00 aB	0.02 ± 0.00 dB

Micronutrientes $mg L^{-1}$

	S	Cu	Zn	Fe	Mn	B
Soil	2.02±0.09aA	0.01±0.00dB	0.02±0.00dA	0.40±0.04bA	0.05±0.01cA	0.02±0.00dB
Humus	2.01±0.03aB	0.02±0.00dA	0.01±0.00dB	0.36±0.01bB	0.05±0.00cA	0.01±0.00dB
Stem	1.99±0.01aC	0.02±0.00dA	0.02±0.00dA	0.32±0.01bC	0.03±0.00cB	0.03±0.00dA
Branches	2.01±0.00aB	0.02±0.00dA	0.02±0.00dA	0.36±0.00bB	0.03±0.00cB	0.03±0.00dA

Note: Means ± standard deviation followed in the same lowercase letters in columns and uppercase letters between substrates do not differ significantly by Tukey test at 5% level of error probability. Source: Authors (2022).

Table 3. Carbon-to-nitrogen ratio (C: N) of fungi with occurrence in riparian area of reforestation with *Eucalyptus grandis* W. Hill ex Maiden, São Gabriel, Rio Grande do Sul State, Brazil.

Species	Substrate	Mean	Minimum	Maximum	Mean	Minimum	Maximum
			μg C g^{-1}			μg N g^{-1}	
Agaricus rufoaurantiacus	Soil	14.35	13.6	15.1	1.08	0.73	1.43
Clitopilus scyphoides	Soil	14.20	12.2	16.2	1.15	0.80	1.50
Entoloma sp.	Soil	12.15	10.1	14.2	1.08	0.73	1.43
Laccaria fraterna	Soil	11.95	10.9	13.0	1.11	0.81	1.41
Galerina sp.	Humus	12.90	14.1	11.7	0.83	0.68	0.98
Gymnopilus earlei	Humus	12.5	14.0	11.0	0.81	0.66	0.96
Gymnopilus junonius	Stem	11.90	10.9	12.9	0.92	0.82	1.02
Gymnopilus pampeanus	Stem	11.25	10.4	12.1	0.83	0.73	0.93
Gymnopilus paraenses	Humus	12.40	10.1	14.7	0.86	0.66	1.06
Gymnopilus subtropicus	Root	13.65	12.2	15.1	0.88	0.78	0.98
Gymnopilus zenkeri	Humus	13.05	11.9	14.2	0.92	0.77	1.07
Apioperdon pyriforme	Soil	13.30	12.5	14.1	1.23	0.88	1.58
Lycoperdon perlatum	Soil	14.65	13.5	15.8	1.13	0.93	1.33
Mycena galericulata	Soil	12.95	11.9	14.0	1.05	0.80	1.30
Pluteus cervinus	Humus	12.90	10.9	14.9	0.90	0.75	1.05
Oudemansiella canarii	Branches	11.38	9.78	12.98	0.93	0.78	1.08
Coprinellus domesticus	Humus	12.90	10.9	14.9	1.12	0.77	1.47
Coprinus lagopus	Soil	12.50	11.0	14.0	1.12	0.87	1.37
Parasola lactea	Soil	11.75	10.4	13.1	1.23	0.98	1.48
Psathyrella argillospora	Soil	13.75	12.5	15.0	1.19	0.84	1.54
Psathyrella hypertropicalis	Humus	13.50	11.8	15.2	0.91	0.76	1.06
Psathyrella murrillii	Soil	12.65	10.9	14.4	0.91	0.76	1.06
Pholiota sp.	Stem	12.70	11.2	14.2	0.93	0.78	1.08
Stropharia rugosoannulata	Soil	13.30	10.9	15.7	1.15	0.80	1.50
Lepista nuda	Soil	13.00	11.8	14.2	1.09	0.89	1.29

Source: Authors (2022).

Discussion

A large number of species were found on soil, compared to other types of substrates. According to Putzke & Putzke (2017) *Agaricus rufoaurantiacus* occupies the interior of forests as its natural habitat. This is the first report of association with *E. grandis*. *Apioperdon pyriforme* and *Lycoperdon perlatum* also grow on soil (Alves & Cortez 2014, Xu *et al*. 2019), and *L. perlatum* has already been associated with *Eucalyptus* spp. in India (Natarajan & Purushothama 1987). This is the first occurrence of *A. pyriforme* in Rio Grande do Sul, as well as association with *E. grandis*.

Clitopilus scyphoides has been observed growing alongside *Eucalyptus globulus* Labill. and *Eucalyptus macarthur* H. Deane & Maiden in Spain (Aragón 2002). Due to the fact that *Clitopilus austroprunulus* Morgado, GM Gates & Noordel, 2012 was registered under *Eucalyptus regnans* F. Muell, *Clitopilus* sp. with *Eucalyptus cladocalyx* F. Muell and *Eucalyptus baxteri* (Benth.) Maiden & Blakely ex J.M.Black; it appears that the genus has an affinity for exotic tree substrates, all records occurred in Australia (Crous *et al*. 2012). Also, *C. austroprunulus* has been associated with *E. regnans* in Africa (Decock 2012).

In Russia, *Entoloma* sp. has been recorded along with *Eucalyptus brassiana* S.T. Blake (Crous *et al*. 2018), and in Australia, the genus has already been identified with *E. baxteri* and *Eucalyptus dunnii* Maiden. Also, *Entolama nipponicum* Kasuya, Nabe, Noordel & Dima, 2019 was cataloged with *E. grandis* in same region (Catcheside 2006, Bahram & Netherway 2022). There is a wide distribution of occurrence of genus in Brazilian biomes (Putzke & Putzke 2017).

Laccaria fraterna is an ectomycorrhiza with record of association involving *Eucalyptus diversicolor* F. Muell., *Eucalyptus globulus* (Labill.), and *Eucalyptus tereticornis* Sm., respectively in India, Scotland, and Australia (Reddy & Natarajan 1995, Dunstan *et al*. 1998, Mason *et al*. 2000). This is the first report of *E. grandis* associated with this fungi.

In this study, *Mycena galericulata* was found on soil next to *E. grandis* and this is report first of association. Species has already an association record in Spain with *E. globulus* (Lorenzo *et al*. 2009). Associated with *Eucalyptus* spp. other species of the same genus, such as *Mycena pseudoinclinata* AH Sm. 1947 in Italy (La Rosa *et al*. 2009), *Mycena neerimensis* Grgur, 1998 in Australia (Grgurinovic 1998), and *Mycena filopes* (Bull.) P. Kumm, 1871 in Brazil (Sobestiansky 2005) have already been found.

This is record first of *Coprinus lagopus* associated with *E. grandis*. However, the fungi already have cataloged with *E. globulus* in Peru (Ordóñez & Rabanal 2019). Furthermore, *Coprinus* sp. has an occurrence record with *Eucalyptus* sp. in Brazil (Manzato *et al*. 2020).

In Paraná a record of *Parasola lactea* was described (Meijer 2006), for Rio Grande do Sul and the association with *E. grandis* this is report first. However, only *Parasola conopilea* (Fr.) Örstadius & Larss, 2008 is cited in association with *Eucalyptus gomphocephala* DC. in Australia (Bougher & Cook 1983).

Psathyrella argillospora, *Psathyrella hypertropicalis,* and *Psathyrella murrillii* do not have known associations with *Eucalyptus*. Additionally, this is the first report of *P. murrillii* occurrence in Rio Grande do Sul State. Only in Costa Rica *Psathyrella ovispora* Deschuyteneer, Heykoop & Moreno, 2019 was associated with *Eucalyptus* spp. (Crous *et al*. 2019).

Lepista nuda was associated to *Eucalyptus fasciculosa* F. Muell in Australia (Catcheside 2006), and *Eucalyptus camaldulensis* Dehnh in Italy (Venturella & La Rocca 2001). Equal to *Stropharia rugosoannulata* is known to occur in agricultural soils (Stamets 2005), but this is record first of both in association with *E. grandis*.

Galerina includes humicolous, terrestrial and lignicolous species (Spahr 2018). In Brazil, the genus is widely distributed and recorded occurrence in Rio Grande do Sul State (Singer 1953). Putzke & Putzke (2019) mention 17 species in Brazil and only *Galerina montivaga* Singer, 1969 it is humicolous. The genus also occurs on branches and litter of *Eucalyptus* spp. (Manzato *et al*. 2020).

Only *Pholiota conissans* (Fr.) MM Moser, 1953; *Pholiota highlandensis* (Peck) Singer, 1952; *Pholiota limonella* (Peck) Sacc. 1887; *Pholiota nameko* (T. Itô) S. Ito & S. Imai 1933; *Pholiota spumosa* (Fr.) Singer, 1948; and *Pholiota squarrosoides* (Peck) Sacc. 1887 has occur register in Brazil. In terms of associations with tree species, *P. nameko* has a record with *Eucalyptus saligna* Sm. in Paraná (Paccola *et al*. 2001), as well as *P. highlandensis* and *Pholiota communus* (Cleland & Cheel) Grgur, 1997 with *Eucalyptus marginata* Donn ex Sm. in Australia (Robinson & Williams 2011).

This study found *Gymnopilus* species directly associated with the stem and root. In roots *Gymnopilus subtropicus* was found. *Gymnopilus earlei* and *Gymnopilus zenkeri* were found in humus mainly close to the roots. *G. zenkeri* has already been associated with *Eucalyptus* sp. in France (Njouonkou *et al*. 2021), *Gymnopilus junonius* with *Eucalyptus* spp. in Uruguay (Barneche *et al*. 2017), *Gymnopilus spectabilis* (Weinm.) AH Sm. 1949 and *G. pampeanus* in India (Kaur & Rather 2015), *G. pampeanus* in Argentina (Colavolpe & Albertó 2014), as well as *Gymnopilus corticophilus* Rees, 1999; *Gymnopilus tomentulosus* Rees, 1999; *Gymnopilus* tasmanicus Rees, 1999; *Gymnopilus eucalyptorum* (Cleland) Singer, 1947; *Gymnopilus tyallus* Grgur, 1997; and *Gymnopilus moabus* Grgur, 1997 in Australia (Rees *et al*. 1999). It is the first occurrence record associated with *E. grandis* and in Rio Grande do Sul State for *G. junonius*, *G. paraensis,* and *G. zenkeri*.

Pluteus cervinus grows on wood humus (Putzke & Putzke 2019). However, this is the first record of association with *E. grandis*. In relation the others species, *Pluteus ludwigii* Ferisin, Justo & Dovana, 2019 has been recorded growing on *E. grandis* humus in Russia (Ordóñez & Rabanal 2019), *Pluteus albotomentosus* Malysheva & Malysheva, 2014 and *Pluteus extremiorientalis* Malysheva & Malysheva, 2014 with *Eucalyptus urophylla* S.T.Blake in Indonesia (Crous *et al*. 2014).

Coprinellus domesticus integrates another new association record with the tree species of this study. Using *Eucalyptus* spp. humus as substrate, *Coprinellus* sp. was cataloged in Australia (Tyagi *et al*. 2019).

Lignicolous *Oudemansiella canarii* grows in stem and branches. It is described growing in the stem of *Eucalyptus* sp. in Argentina (Alberti *et al*. 2021), this is the first record of association with *E. grandis*.

Macronutrient and micronutrient contents analyzed in this study can provide a basis for further analyzes regarding application nutritional of these fungi. Although the fungi studied exhibit associations with *E. grandis*, there are not techniques or standards for cultivating them in exotic tree. *A. rufoaurantiacus, L. fraterna, G. pampeanus, G. paraensis, G. zenkeri, A. pyriforme, L. perlatum, O. canarii, C. lagopus,* and *L. nuda* are classified as non-toxic and potentially edible (Crosier *et al*. 1949, Ruegger *et al*. 2001, Ndong *et al*. 2011, Akatin 2013, Putzke & Putzke 2017, 2018, Sridhar & Karun 2019, Altaf *et al*. 2020). This group not contemplate the fungi cultivated in Brazil for edibility purposes, so a more robust analysis of their potential for consumption is required. Moreover, *G. earlei, G. subtropicus, M. galericulata, P. lactea, P. argillospora, P. hypertropicalis,* and *P. murrillii* do not present sufficient data regarding their edibility or toxicity. There are not data on the edibility of *C. scyphoides, G. junonius, P. cervinus, C. domesticus,* and *S. rugosoannulata*, but levels of toxicity have already reported in studies involving these species (Gabriel *et al*. 1997, Novikova 2001, Hartley *et al*. 2009, Wu *et al*. 2011, Lee *et al.* 2020), and therefore their consumption should not be considered.

In terms of macronutrient, P, K, Ca, and Mg remained within the range already described for edible Agaricales species, such as *Agaricus bisporus* (JE Lange) Imbach, 1946; *Lentinula edodes* (Berk.) Pegler, 1976; and *Pleurotus* spp. (Silva-Neto *et al*. 2022). The contents of these macronutrients were higher in terricolous fungi than in other fungi. Nutritional retention of fungi is related to their availability in the substrate. Neina (2019) demonstrates that Ca is widely distributed in soil, predominantly in Ca^{2+} form, but specific mechanisms in each plant permit Ca to enter in permeable channels. In all species analyzed, Ca was the most abundant macronutrient. This result supports Agrahar-Murugkar & Subbulakshmi (2005) findings in *Calvatia gigantean* (Batsch ex Pers.) Lloyd, *Cantharellus cibarius* Pe. 1821, *Russula integra* (L.) Fr. 1838, *Gomphus floccosus* (Schwein.) Singer, 1945, and *Lactarius quieticolor* Romagn, 1958, which oscillated between 5 and 9 $cmol_c\ L^{-1}$.

The values in K content were consistent with described in literature. Different species of mushrooms evaluated in Poland had similar K contents with this study (Rudawska & Leski 2005). However, Malinowski *et al*. (2021) infer higher level with ranging between 5-20 $cmol_c\ L^{-1}$in *Boletus edulis* Bull. 1782, *Imleria badia* (Fr.) Vizzini, 2014, and *Leccinum scabrum* (Bull.) Gray, 1821.

The P content was similar to described for *A. bisporus* (Beyer 2003). Rudawska & Leski (2005) report Mg content in different mushroom species analyzed did not differ, as well as in the species of fungi analyzed in this study.

Micronutrients S, Fe, Mn, Cu, B, and Zn showed values consistent with previously described in literature (Beyer 2003, Agrahar-Murugkar & Subbulakshmi 2005, Rudawska & Leski 2005, Fidanza *et al*. 2010, Malinowski *et al*. 2021). Rudawska & Leski (2005) in analysis of S content with mushrooms found higher level, as in our results. Nevertheless, high concentrations of S can be toxic to plants (Taiz *et al*. 2018). Minor fluctuation of values of S among the species this study occurred, it is possible to infer that fungi are capable of accumulating this micronutrient.

Mn, Cu, and Zn contents did not show expressive oscillations, with exception of Fe, which obtained the highest value among metals. Fidanza *et al*. (2010) report that metallic micronutrient levels generally remain stable in edible Agaricales. Similarly, the Cu and Zn contents were also less expressive and very small in comparison to other micronutrients analyzed (Fidanza *et al*. 2010). Rudawska & Leski (2005) who analyzed the micronutrient content of mushrooms, presented a similar order of contents found in our data. According to Rudawska & Leski (2005), metallic micronutrients in mushrooms are primarily related to acidic pH of soil. It is important to emphasize that *Eucalyptus* are tolerant to acidic soils (Pinto & de Negreiros 2021). In this study there was not conducted analysis soil, but due to the presence of metals with higher levels in terrestrial fungi, we can infer that the fungi presented these values influenced by the availability of these micronutrients in substrate. Additionally, B content which is considered esterified was found in small concentrations, similar the date by Rudawska & Leski (2005).

Carbon-to-nitrogen ratio (C: N) of analyzed fungi oscillated of 10:1 to 15:1, compatible with values in literature for edible Agaricales species (Stoffella & Kahn 2001). However, none of species found contained C:N ratio values described, thus the values presented are unpublished. Carbon and nitrogen levels in fungi biomass are directly related to the rate of decomposition and mineralization. In study by D'Agostini *et al*. (2011) with *L. edodes*, *Pleurotus ostreatus* (Jacq.) P. Kumm, 1871, and *Agaricus blazei* Murrill, 1945, when analyzing the C:N ratio, discovered that mycelial growth was proportional to this ratio. In this premise, mycelial growth was reduced in proportions greater than 15:1 or less than 10:1 (D'Agostini *et al*. 2011).

Organic matter is main source of C for fungi, but the assimilation of N depends not only of substrate also of fixation by microorganisms (Liao *et al*. 2021). According to Martins *et al*. (2018) *P. ostreatus* obtained rapid degradation of basidiomata when the C:N ratio was lower than 10:1, and generated basidiomata of higher fresh biomass value when the C:N ratio was in the expected range (15:1 to 10:1). In contrast, increasing these proportions for *A. blezei* cultivation was beneficial. Kopytowski (2002) found that the C:N ratio yielded higher quality and productivity in the 20:1 ratio, higher than those found in this study. However, in edible species the C:N ratio does not take into account substrate type, but rather biomass profitability.

Conclusions

Association of fungi with *Eucalyptus grandis* W. Hill ex Maiden substrate it is paramount importance in nutritional cycling at reforestation area. Moreover, our analyzes showed levels of primary and secondary essential nutrients in species found, and these values can be used as a basis for new studies of their nutritional potential. The C:N ratio help in understanding ability of fungi to absorb and recycle nutrients from organic matter, since the enzymatic production of fungi is affected by its disposition. Fungi in this study showed adapted to the exotic tree substrate, their ecological interaction may have played a significant role in restructuring the reforestation area studied.

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Conflicts of interest

There is no conflict of interest.

Author contributions

Alice Lemos Costa: Contribution to sampling; data analysis and interpretation; manuscript preparation; critical revision and adding intellectual content.

Cassiane Furlan-Lopes: Contribution to sampling and manuscript preparation.

Fernando Augusto Bertazzo-Silva: Contribution to sampling and manuscript preparation.

Ana Luiza Klotz-Neves: Contribution to sampling and manuscript preparation.

Kamille Rodrigues Ferraz: Contribution to sampling and manuscript preparation.

Ana Flavia Zorzi: Contribution to sampling and manuscript preparation.

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