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Occurrence of Yeast Species in Soils under Native and Modified Vegetation in an Iron Mining Area

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ABSTRACT: Soils of the *Quadrilátero Ferrífero* are rich in mineral resources, especially gold and iron. Soil management and mining activities greatly impact soil biology. However, studies addressed to the diversity of microorganisms and their ecological role in the recovery of these areas are scarce. This study aimed to assess the yeast occurrence in soils with natural vegetation (Atlantic Forest, neotropical savanna, and iron outcrop) and areas with anthropogenic modifications (*Eucalyptus* stand and rehabilitated area) in the *Quadrilátero Ferrífero* region. We isolated and identified a total of 68 yeast. Partial sequencing of the 26S ribosomal gene revealed the presence of six genera: *Saitozyma, Pseudozyma*, *Meyerozyma*, *Debaryomyces*, *Lipomyces*, and *Aureobasidium*. Overall, the yeast community was more diverse in the area with greater anthropic modification. Environmental variables, especially pH, soil organic matter, texture, and Al saturation index, explained 60 % of the variability. *Saitozyma podzolica* was the dominant species and was positively correlated with the presence of Al in soils. This study is the first report of the occurrence of yeast species in soils of the *Quadrilátero Ferrífero* region in the State of Minas Gerais and one of the few studies of yeast diversity in Brazilian soils.

Keywords: diversity, fungi, land use, mining.

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INTRODUCTION

The *Quadrilátero Ferrífero* region in Minas Gerais, Brazil, occupies an area of approximately 7,000 km^2 in the central-southeastern part of the state. It is internationally recognized as an important Precambrian terrain with significant mineral resources, especially gold and iron. This region is inserted in the Cerrado and Atlantic Forest biomes, which were extensively transformed by human activities such as urbanization and mining, generating impacts due to soil removal and subsequent changes in the vegetation cover (Azevedo et al., 2012).

Soils in this region are mainly ferruginous, with low water content, and low nutrient levels. High levels of heavy metals may occur in these soils and promote the selection of resistant plant species with physiological and/or morphological adaptations (Vincent and Meguro, 2008).

The flora in the *Quadrilátero Ferrífero* has been studied expensively, especially in iron outcrops, typical ecosystems associated with superficial iron crusts (Carmo and Jacobi, 2013). Studies report the diversity of the fauna (Salvador-Jr et al., 2011), rhizobial communities (Castro et al., 2017), nematodes (Caixeta et al., 2016), and mycorrhizal fungi (Teixeira et al., 2017; Vieira et al., 2017).

To know and evaluate the functions of microorganisms naturally inhabiting these environments is important, mainly because they play a crucial role in nutrient cycling and in improving nutrient availability; in addition, they are potential indicators of environmental changes mainly in the course of the recovery of degraded areas (Castro et al., 2017).

Soil yeast communities play an important ecological role in processes such as mineralization of organic matter via respiration or fermentation, P solubilization, transformation of N compounds and inorganic S, plant protection against pathogens, increasing plant root growth, and even act as food source for arthropods, bacteria, nematodes, and protists (Botha, 2011).

Yeasts occur in a broad range of different soil types from forest and cultivable land (Sláviková and Vadkertiová, 2000, 2003) to soils of extreme environments such as Antarctica (Connell et al., 2008). Factors such as temperature, humidity, chemical composition, and geographical location can influence the diversity of yeasts in these environments (Vishniac, 2006). However, our knowledge about yeasts living in soils is rather limited. A better understanding of species niches is crucial for analyses of diversity data and may hint to the discovery of unifying patterns of microbial species distribution as well as the influence of vegetation and chemical and physical soil properties.

We hypothesized that the yeast communities differ between soils with natural vegetation and soils with vegetation modified by mining activities. The aim of this study was to report the occurrence of yeasts present in soils under different types of vegetation and influenced by mining in *Quadrilátero Ferrífero* in Sabará, Minas Gerais, Brazil.

MATERIALS AND METHODS

Study sites and sampling

Soil samples were collected at the Center for Research and Conservation of Biodiversity in the *Quadrilátero Ferrífero* (CeBio) located in Sabará, Minas Gerais (Figure 1a), in September 2013. This study center was established by Vale S.A. in 2006 and is located in a deactivated mining area, comprising a total of 716.47 ha with different vegetation types, including natural and preserved areas (Atlantic Forest, neotropical savanna, and iron outcrops) and areas with anthropogenic modifications (*Eucalyptus* plantations and rehabilitated areas with grass) (Figure 1b).

These areas are rehabilitated with the use of non-native vegetation, i.e. *Eucalyptus* spp. and *Melinis minutiflora*. The grass has a special feature; i.e., it is formed by deposition of tailings, resulting from the practice of mining, and this area is in the primary stage of soil recovery.

Sampling was performed as described by Moreira et al. (2008) (Figure 1c). For soil sampling, the main point was georeferenced, and we sampled 12 points, forming two concentric circles around the sample point with a distance of 3 m; the outer circle had a radius of 6 m. Samples were taken at a layer of 0.00-0.20 m and homogenized to obtain a representative composite sample for each sampling point. Twenty composite soil samples were collected, four samples for each vegetation type: iron outcrop (IO), rehabilitated area with grass (RG), neotropical savanna (NS), *Eucalyptus* (E), and Atlantic Forest (AF). The samples were stored under refrigeration and transported to the Mycology Laboratory of the University of Brasilia (UNB).

Chemical and physical analyses were performed according to *Empresa Brasileira de Pesquisa Agropecuária* - Embrapa (Donagema et al., 2011), in the Soil Analysis Laboratory of the Department of Soil Science of the Federal University of Lavras, UFLA/MG. Phosphorus, K^+, Ca^{2+}, Mg^{2+}, and Al^{3+} contents, exchangeable acidity (H+Al), organic matter (OM), as well

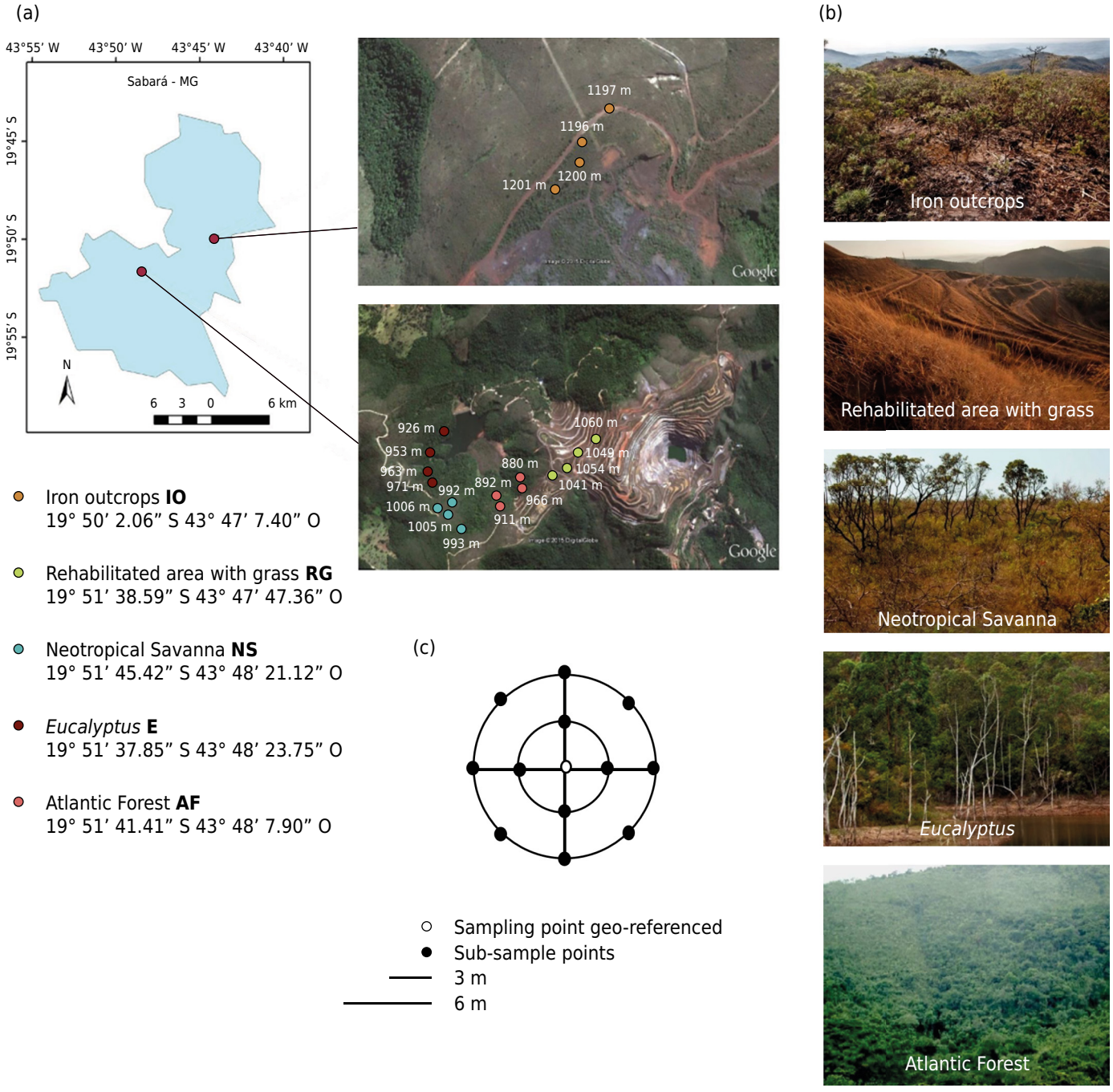

Figure 1. Geographical location of studied areas, environments, and sampling strategy. Geographical location of the *Quadrilátero Ferrífero* and the Center for Research and Conservation of Biodiversity (a); land use types sampled (b); and sampling strategy (c).

as sand, silt, and clay contents were evaluated. These results were used to calculate other properties, such as the sum of exchangeable bases (SB), cation exchange capacity at pH 7 (T), capacity of effective cation exchange (t), aluminum saturation index (m), and base saturation index (V). The chemical and physical properties of the soils samples from the different study areas are shown in table 1.

Isolation of yeasts

From each sample, 10 g of soil were homogenized in 90 mL of 0.1 % peptone water. The suspension was vortexed using a shaker for 1 h at 200 rpm. All soil samples were analyzed in three replicates, and each of the replicates was used to produce three dilutions (10^{-1}, 10^{-2}, and 10^{-3}). Subsequently, the dilutions were plated on MYGP medium (0.3 % malt extract, 0.3 % yeast extract, 0.5 % peptone, 1.0 % glucose, 2.0 % agar) with a pH of 5.6 (Masoud et al., 2004). To the culture medium, we added chloramphenicol (34 mg mL^{-1}) to inhibit bacterial growth. The Petri dishes were incubated at room temperature for 5 days.

The obtained yeast colonies were divided into morphological types, and each colony type was isolated in pure culture, which was morphologically characterized as described in Dias and Schwan (2010), observing macro-morphological characteristics such as color (white, beige, orange, pink, etc.), size (small, medium, or large), appearance of the colony (pasty or creamy), shape of the edge (smooth or filamentous), profile (flat, convex, high), colony surface (grainy, concentric grooves), and growth (slow or fast).

Subsequently, the colonies were stored in 15 % glycerol at -80 °C and deposited in the Yeast Culture Collection of the Department of Plant Pathology at the University of Brasília.

Table 1. Mean values of the chemical and physical properties of the soils from the Atlantic Forest, the *Eucalyptus* stand, the neotropical savanna, the iron outcrop, and the rehabilitated area with grass at the Center for Research and Conservation of Biodiversity in the *Quadrilátero Ferrífero* (CeBio), Sabará, Minas Gerais

Property[(1)]	Atlantic Forest	Neotropical savanna	Iron outcrop	Eucalyptus	Rehabilitated area with grass
pH(H_2O)	4.65	4.95	4.50	4.72	5.95
OM (g kg^{-1})	47.7	34.6	95.7	50.9	13.2
K (mg dm^{-3})	68.50	48.00	56.00	38.00	32.50
P (mg dm^{-3})	1.93	1.49	3.81	1.93	6.65
Ca^{2+} ($cmol_c$ dm^{-3})	0.57	0.50	0.77	0.12	1.15
Mg^{2+} ($cmol_c$ dm^{-3})	0.42	0.15	0.15	0.15	0.42
Al^{3+} ($cmol_c$ dm^{-3})	1.75	1.35	1.55	2.60	0.10
Zn (mg dm^{-3})	7.56	0.88	1.80	0.76	0.61
Fe (mg dm^{-3})	94.54	102.73	236.71	122.66	43.32
Mn (mg dm^{-3})	67.83	22.46	17.09	18.26	41.90
Cu (mg dm^{-3})	3.02	2.10	0.69	2.76	0.73
H+Al ($cmol_c$ dm^{-3})	9.86	6.56	22.54	12.17	1.49
SB ($cmol_c$ dm^{-3})	1.17	0.77	1.07	0.37	1.65
t ($cmol_c$ dm^{-3})	2.92	2.12	2.62	2.97	1.75
T ($cmol_c$ dm^{-3})	11.03	7.33	23.61	12.54	3.15
V (%)	10.54	10.99	5.29	2.95	48.93
m (%)	60.69	63.47	57.86	87.51	7.42
Clay (g kg^{-1})	30.50	27.50	20.50	35.75	20.50
Silt (g kg^{-1})	42.25	36.75	17.50	34.00	32.75
Sand (g kg^{-1})	27.25	35.75	62.00	30.25	46.75

[(1)] pH in water at a ratio of 1:2.5; Ca^{2+}, Mg^{2+} and Al^{3+} extracted by KCl 1 mol L^{-1}; H+Al: extractor SMP; SB: sum of bases; T: cation exchange capacity at pH 7.0; t: effective cation exchange capacity; V: bases saturation; m: Al saturation; OM: organic matter, oxidation $Na_2Cr_2O_7$ 2 mol L^{-1} + H_2SO_4 5 mol L^{-1}; P, K, Fe, Zn, Mn, Cu: Mehlich-1 extractor; S: extractor monocalcium phosphate acetic acid; B: hot water extractor.

Identification of yeasts

The DNA was extracted from the cell precipitate, obtained by centrifugation of the yeast culture grown in liquid MYGP medium over 48 h at 28 °C under stirring. Cells were lysed using extraction buffer (200 mmol L^{-1} Tris-HCl, 250 mmol L^{-1} NaCl, 25 mmol L^{-1} EDTA pH 8, 0.5 % SDS). Proteins were precipitated with chloroform: isoamyl alcohol (24:1) and subsequently centrifuged for 10 min at 16,000 *g*. The DNA was precipitated with isopropanol for 1 h at -20 °C. After this, the DNA samples were centrifuged and washed with 70 % ethanol. The DNA was then suspended in 30 µL of milli-Q water and stored at -20 °C.

The D1/D2 domains of the LSU rRNA gene were amplified by PCR, using the following universal primers: NL1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG-3') and NL4 (5'-GGT CCG TGT TTC AAG ACG G-3') to identify ascomycetes and basidiomycetes, as reported by Kurtzman and Robnett (1998) and Fell et al. (2000). The PCR conditions were as follows: initial denaturation at 94 °C for 3 min, followed by 33 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 30 s, and extension at 72 °C for a 1 min, and final extension at 72 °C for 6 min.

The PCR products were treated with the Exo-SAP enzyme (Affymetrix, Inc. Cleveland, Ohio, USA) and sent to sequencing in the Catholic University of Brasilia (UCB, Brasília, Brazil), using the sequencer ABI 3130xl Applied Biosystems, according to the Sanger methodology (Sanger et al., 1977). All isolates found were sequenced in both directions.

For species identification, the obtained nucleotide sequences were compared with sequences deposited in the NCBI GenBank (National Center for Biotechnology Information), using the BLASTn algorithm (Altschul et al., 1990).

Analysis

The physical-chemical soil properties were submitted to analysis of variance; means were compared by the Scott-Knott test at 5 % probability using the Assistat 7.7 statistical program (Silva and Azevedo, 2016).

The Past software (version 3.13) was used for the construction of the phenetic similarity dendrogram, the calculation of the diversity indices, principal components analysis (PCA), and canonical correspondence analysis (CCA). Phenetics analysis and morphological grouping of colonies were performed by building a binary matrix of presence (1) or absence (0) of the morphological characteristic observed for each colony, and a phenetics similarity dendrogram was built by paired group algorithm, using Jaccard's similarity coefficient for calculation. To identify similarities among the samples of the four areas evaluated, we used principal components analysis (PCA), based on a correlation matrix with soil chemical properties. To correlate the distribution of yeasts with the abiotic variables of the soil and the environment, we applied canonical correspondence analysis (CCA) (Hammer et al., 2001).

RESULTS

Soil analysis

Soil chemical characterization (Table 1) was performed according to the recommendations of the Soil Fertility Commission of the State of Minas Gerais (Alvarez V et al., 1999). Overall, the studied soils are characterized by high acidity, total acidity, and Al^{3+} saturation, with the exception of the soil of the rehabilitated area. The soils presented a low natural fertility with extremely low Ca^{2+}, Mg^{2+}, Al^{3+}, base sum (SB), base saturation (V), and effective cation exchange capacity (t).

Exchangeable acidity (H+Al) was low in the rehabilitated area, very high in the iron outcrop, and good in the Atlantic Forest, the neotropical savanna, and the *Eucalyptus* site. This property promotes a high potential of cation exchange capacity, indicating that

the soil has the ability to retain more nutrients, despite the low pH in these areas. This ability to retain more nutrients can be seen in the amount of organic matter, which was higher in the natural areas and lower in the rehabilitated area. Soils of all site types had high Fe content, which is a result of the soil and rock types in this region.

In the PCA, including all results, the principal components accounted for 60 % of the total variance. The first component accounted for 37 % and the second component for 23 % of the total variability (Figure 2).

The generated scatterplot revealed relationships between the areas studied. There was a discernible pattern in the distribution of soil samples, forming three distinct groups as follows: one containing the site RG, one containing the site IO, and one containing the sites E, AF, and NS (Figure 2).

The sites RG and IO were mainly influenced by pH and OM, respectively. Soils of RG were the poorest in OM due by the dominance *Melinis minutiflora*. The removed subterranean soil was accumulated on the surface, forming piles of soil that are currently under recovery in the course of vegetation rehabilitation.

The texture of the sampled soils was mostly medium; sandy in some points of the sites IO and RG and clayey in the sites AF and E. The levels of Al^{3+} were high in *Eucalyptus* soils, where we obtained the highest isolation of yeasts (Tables 2 and 3). The Al^{3+} saturation index was high in all sites, except in the rehabilitated site.

Considering the parameters V and SB, which relate to soil fertility, the soil samples of the site RG showed the highest fertility, indicating successful recovery of the vegetation layer.

Identification of yeasts, species composition, and diversity analysis

A total of 68 yeast isolates were recovered from the five sampled areas: 24 isolates from the *Eucalyptus* stand, 19 from the Atlantic Forest, nine from the neotropical savanna, eight from the rehabilitated area, and eight from the iron outcrop. Due to the low number of yeast colonies per plate (<15), we were not able to count the colony-forming units (CFUs). The morphological characterization of the colonies grouped the isolates in 19

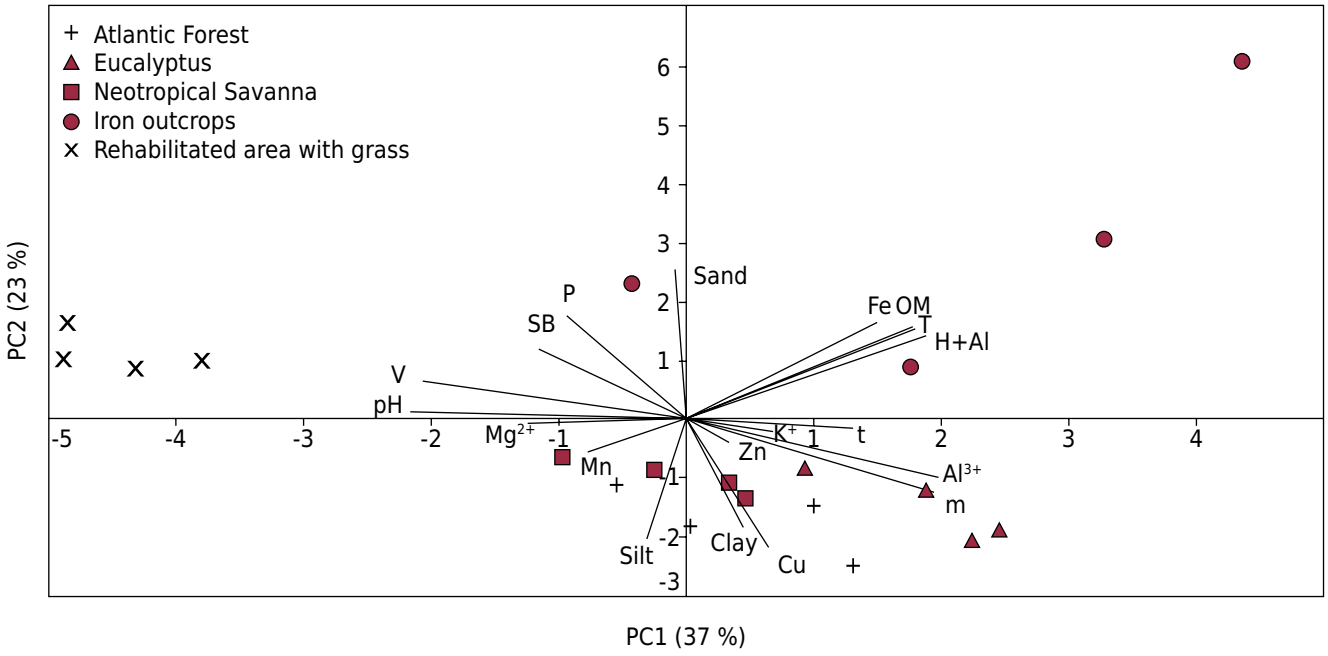

Figure 2. Principal components analysis (PCA) of chemical and physical properties of four soil samples per area of the *Quadrilátero Ferrífero* region. H+Al = exchangeable acidity; OM = organic matter; SB = sum of Ca^{2+}, Mg^{2+}, and K^{+}; t = effective cation exchange capacity; T = cation exchange capacity at pH 7.0; V = base saturation of CTC at pH 7.0; m = Al^{3+} saturation.

different morphotypes, and the majority of the colonies presented a white to beige color. The morphotypes mainly differed in terms of border type and colony profile. According to the morphological characterization, isolates of the same area presented more similar characteristics, grouping in the same clade (Figure 3).

Based on analyzes of 26S ribosomal RNA gene sequences, the 68 yeasts recovered from the soil belonged to six genera, namely: *Saitozyma*, *Pseudozyma*, *Meyerozyma*, *Debaryomyces*, *Lipomyces*, and *Aureobasidium*.

Of these isolates, only 58 were identified to the species level, using the 26S ribosomal RNA gene sequences. For the 10 isolates belonging to the genera *Meyerozyma*, *Aureobasidium*, *Debaryomyces*, and *Pseudozyma*, it was necessary to sequence the Internal Transcribed Spacer (ITS) region to identify them to the species level. The sequences of all yeasts isolated in this study were deposited in GenBank (Table 2).

Among the various genera identified, the relative abundance of the Basidiomycota and Ascomycota phyla was 82 and 18 %, respectively. In the phylum Basidiomycota, the species *Saitozyma podzolica* was the most abundant (81 %) and present in all areas, except in the rehabilitated site. In contrast, the phylum Ascomycota was less abundant but more diverse, with four genera, of which *Meyerozyma* was most abundant (8 %).

The species composition of the yeast community was homogeneous in areas with similar vegetation, which highlights the dominance of the genus *Saitozyma* and the similarity of the yeast community between samples of the Atlantic Forest and the *Eucalyptus* site and between the neotropical savanna and the iron outcrop. Samples of the rehabilitated area were the most diverged regarding the composition of yeast communities (Figure 4).

Species richness, dominance, evenness, and the Shannon diversity index and Simpson's index for the different sites are presented in table 3. For the calculation of the diversity indices, only the isolates identified to the genus level were considered as a collection of closely related samples that represented a single unnamed phylogenetic species.

The highest species richness was measured in the rehabilitated area. Dominance (D) ranged from values near zero in the rehabilitated area, indicating that all taxa were present in equal amounts, to values close to 1 in all the other sites, indicating that only one taxon dominated the community.

Simpson's index (1-D), which measures the community evenness comparing the proportion of different species, indicated that the structure of yeast communities was the same for similar vegetation types, such as the Atlantic Forest and the *Eucalyptus* stand.

The Shannon index (H) was higher in the rehabilitated area, indicating a high diversity in this vegetation type, in contrast to the Atlantic Forest and the *Eucalyptus* stand. The latter site obtained the highest number of individuals (Table 2), but the indices showed a low diversity because of the dominance of a single taxon, the species *S. podzolica*.

The results of the canonical correspondence analysis (CCA) (Figure 5) of the yeast species isolated from the five sites showed that *S. podzolica*, *L. kononenkoae*, *A. leucospermi*, *Meyerozyma* sp., and *Debaryomyces* sp. are mainly associated with high values of Al^{3+}, K^{+}, t, m, OM, H+Al, and clay content. *Aureobasidium* sp. was associated with SB, Mg^{2+}, and P, while *Pseudozyma* sp. was associated with pH, V, silt, and sand. These two species were isolated only from the rehabilitated area. *Debaryomyces polymorphus* was only isolated from Atlantic Forest soil and it is not associated with any specific soil properties.

Table 2. Identification of yeast strains isolated from the soil samples based on 26S rRNA gene sequencing

Isolate ID	Origin	Phylum	Species or genus	GenBank accession number	ID	Best blast match [GenBank]
					%	
CLUnB 440	Neotropical Savanna	Basidiomycota	*Saitozyma podzolica*	KX902984	100	JQ672617
CLUnB 441	Neotropical Savanna	Basidiomycota	*Saitozyma podzolica*	KX902985	99.7	FJ743620
CLUnB 442	Neotropical Savanna	Basidiomycota	*Saitozyma podzolica*	KX902986	99.3	FJ743621
CLUnB 443	Neotropical Savanna	Basidiomycota	*Saitozyma podzolica*	KX902987	99.6	JQ672617
CLUnB 444	Neotropical Savanna	Basidiomycota	*Saitozyma podzolica*	KX902988	99.6	JQ672617
CLUnB 445	Neotropical Savanna	Ascomycota	*Meyerozyma sp.*	KX902989	99	AB773382; JQ398673
CLUnB 446	Neotropical Savanna	Basidiomycota	*Saitozyma podzolica*	KX902990	99.5	JQ672617
CLUnB 447	Neotropical Savanna	Basidiomycota	*Saitozyma podzolica*	KX902991	99.6	JQ672617
CLUnB 448	Neotropical Savanna	Basidiomycota	*Saitozyma podzolica*	KX902992	100	JQ672617
CLUnB 449	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX902993	99.7	JQ672617
CLUnB 450	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX902994	99.7	JQ672617
CLUnB 451	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX902995	99.8	JQ672617
CLUnB 452	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX902996	99.6	JQ672617
CLUnB 453	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX902997	99.8	JQ672617
CLUnB 454	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX902998	99.5	JQ672617
CLUnB 455	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX902999	99.6	JQ672617
CLUnB 456	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX903000	99.8	JQ672617
CLUnB 457	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX903001	99.8	JQ672617
CLUnB 458	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX903002	99.4	JQ672617
CLUnB 459	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX903003	99.6	JQ672617
CLUnB 460	Atlantic Forest	Ascomycota	*Debaryomyces polymorphus*	KX903004	99	FN545820
CLUnB 461	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX903005	99.6	JQ672617
CLUnB 462	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX903006	99	JQ672617
CLUnB 463	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX903007	99.5	JQ672617
CLUnB 464	Atlantic Forest	Ascomycota	*Lipomyces kononenkoae*	KX903008	100	JN940881
CLUnB 465	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX903009	99.3	JQ672617
CLUnB 466	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX903010	100	JQ672617
CLUnB 467	Atlantic Forest	Basidiomycota	*Saitozyma podzolica*	KX903011	99.5	JQ672617
CLUnB 468	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903012	99.5	JQ672617
CLUnB 469	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903013	99.8	JQ672617
CLUnB 470	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903014	99.5	JQ672617
CLUnB 471	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903015	99.8	JQ672617
CLUnB 472	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903016	99.8	FJ797568
CLUnB 473	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903017	100	JQ672617
CLUnB 474	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903018	99.6	JQ672617
CLUnB 475	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903019	100	JQ672617
CLUnB 476	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903020	99.6	JQ672617
CLUnB 477	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903021	99.8	JQ672617
CLUnB 478	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903022	99.6	JQ672617
CLUnB 479	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903023	99.5	JQ672617
CLUnB 480	Eucalyptus	Ascomycota	*Debaryomyces sp.*	KX903024	100	KF830186; KC006846
CLUnB 481	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903025	99.8	JQ672617
CLUnB 482	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903026	100	JQ672617
CLUnB 483	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903027	100	JQ672617
CLUnB 484	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903028	99.8	JQ672617
CLUnB 485	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903029	99.6	JQ672617
CLUnB 486	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903030	98.7	JQ672617
CLUnB 487	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903031	99.3	JQ672617
CLUnB 488	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903032	99.5	JQ672617
CLUnB 489	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903033	99.3	JQ672617
CLUnB 490	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903034	99.3	JQ672617
CLUnB 491	Eucalyptus	Basidiomycota	*Saitozyma podzolica*	KX903035	99.8	JQ672617
CLUnB 492	Rehabilitated area with grass	Ascomycota	*Meyerozyma sp.*	KX903036	98	AB831021; AB916575
CLUnB 493	Rehabilitated area with grass	Ascomycota	*Aureobasidium leucospermi*	KX903037	100	KJ159030
CLUnB 494	Rehabilitated area with grass	Ascomycota	*Meyerozyma sp.*	KX903038	99	AB772556; JQ686905
CLUnB 495	Rehabilitated area with grass	Ascomycota	*Meyerozyma sp.*	KX903039	100	AB772556; KJ794711
CLUnB 496	Rehabilitated area with grass	Ascomycota	*Meyerozyma sp.*	KX903040	100	AB831044; KF359926
CLUnB 497	Rehabilitated area with grass	Basidiomycota	*Pseudozyma sp.*	KX903041	97	AB617892; AB548951
CLUnB 498	Rehabilitated area with grass	Ascomycota	*Aureobasidium sp.*	KX903042	99	KJ159028; JN712559
CLUnB 499	Rehabilitated area with grass	Ascomycota	*Aureobasidium sp.*	KX903043	99	KJ159028; JN712559
CLUnB 500	Iron outcrops	Basidiomycota	*Saitozyma podzolica*	KX903044	99	JQ672617
CLUnB 501	Iron outcrops	Basidiomycota	*Saitozyma podzolica*	KX903045	99	JQ672617
CLUnB 502	Iron outcrops	Ascomycota	*Meyerozyma sp.*	KX903046	100	AB773382; JQ398673
CLUnB 503	Iron outcrops	Basidiomycota	*Saitozyma podzolica*	KX903047	100	JQ672617
CLUnB 504	Iron outcrops	Basidiomycota	*Saitozyma podzolica*	KX903048	100	JQ672617
CLUnB 505	Iron outcrops	Basidiomycota	*Saitozyma podzolica*	KX903049	100	JQ672617
CLUnB 506	Iron outcrops	Basidiomycota	*Saitozyma podzolica*	KX903050	100	JQ672617
CLUnB 507	Iron outcrops	Basidiomycota	*Saitozyma podzolica*	KX903051	100	JQ672617

Table 3. Diversity indices of yeast communities from the Atlantic Forest, the *Eucalyptus* stand, the neotropical savanna, the iron outcrop, and the rehabilitated area with grass

Diversity index	Land use type				
	Atlantic Forest	Neotropical savanna	Iron outcrop	*Eucalyptus*	Rehabilitated area with grass
Richness (S)	3	2	2	2	4
Individuals	19	9	8	24	8
Dominance (D)	0.8061	0.8025	0.7813	0.9201	0.3438
Simpson (1 - D)	0.1939	0.1975	0.2188	0.07986	0.6563
Shannon (H)	0.4095	0.3488	0.3768	0.1732	1.213
Equitability (J)	0.3727	0.5033	0.5436	0.2499	0.875

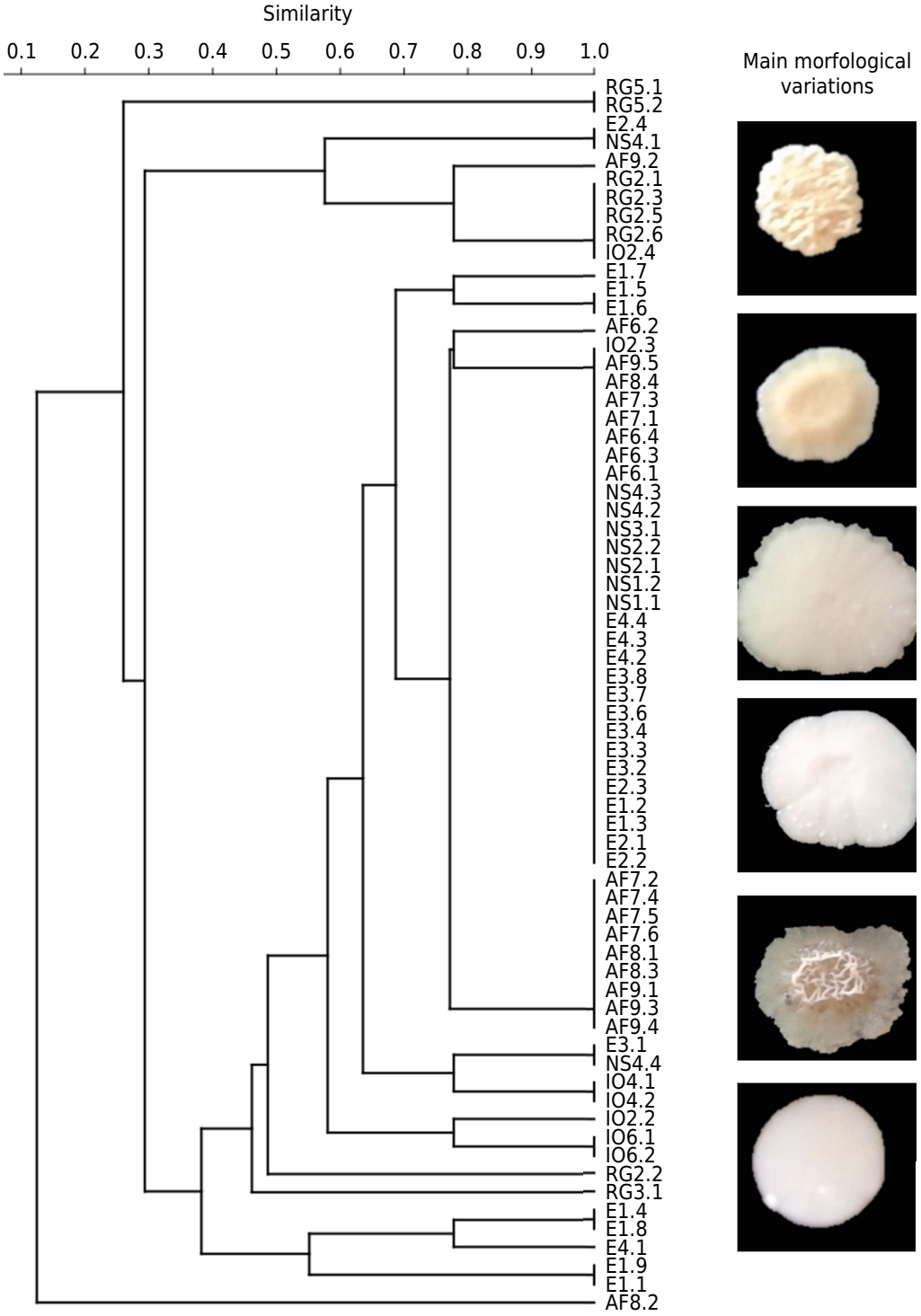

Figure 3. Dendrogram of phenetic similarity and main morphological variations.

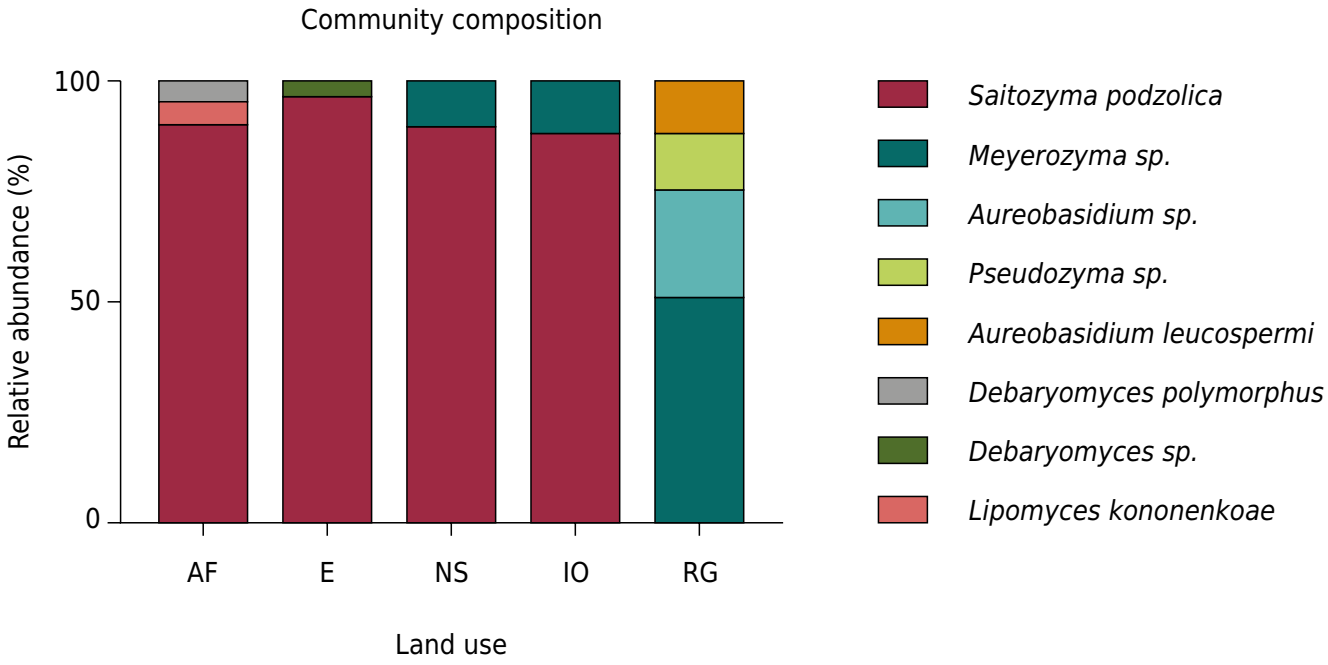

Figure 4. Composition of yeast communities in the Atlantic Forest (AF), the *Eucalyptus* stand (E), the neotropical savanna (NS), the iron outcrop (IO), and the rehabilitated area with grass (RG).

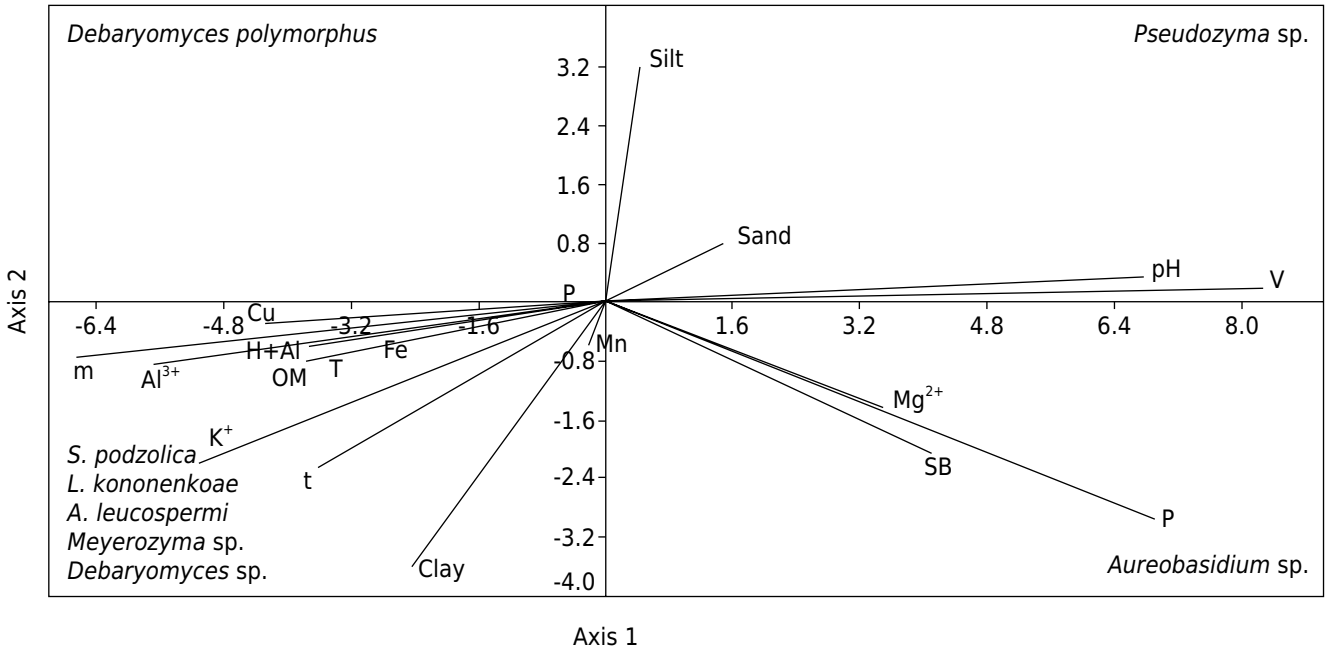

Figure 5. Canonical correspondence analysis (CCA) of soil yeast species of the Atlantic Forest, the *Eucalyptus* stand, the neotropical savanna, the iron outcrop, and the rehabilitated area with grass.

DISCUSSION

We isolated 68 yeasts from five different land use types located in a deactivated mining area with natural vegetation and revegetated areas. Species belonged to both Ascomycota and Basidiomycota, although the latter was dominant. The samples were collected during the dry season; therefore, there is a greater relation between the occurrence and distribution of yeasts and the soil properties compared to the vegetation type.

For example, the soils of the *Eucalyptus* stand and the Atlantic Forest contained clay and had a medium texture, and we isolated high numbers of yeasts, probably because of the

high water retention capacity of these soils. This was shown in the results of the CCA, where a higher soil clay content was positively correlated with most of the isolated species.

The importance of water for yeast growth has been described in a sterile sandy loam soil (Vishniac, 1995) and subsequently confirmed by Vreulink et al. (2007), determining that lower soil moisture levels may have a negative effect on the size of soil yeast populations. In forests soils, the yeast population sizes undergo seasonal changes, which can partly be ascribed to changes in soil moisture content (Sláviková and Vadkertiová, 2000). However, in this study, it was not possible to determine the influence of water content on the soil yeast community, as this variable was not measured.

Saitozyma was the dominant genus in all areas, and several studies on the diversity of yeasts in soils have reported the occurrence and dominance of this genus, both in soils with natural vegetation and in cultivated soils (Vital et al., 2002; Sláviková and Vadkertiová, 2003; Wuczkowski and Prillinger, 2004; Vishniac, 2006; Connell et al., 2008; Mestre et al., 2011; Yurkov et al., 2012).

Saitozyma podzolica was the main detected species (81 % of isolates), present in all areas studied, except in the rehabilitated area. This species was dominant in the *Eucalyptus* stand, the neotropical savanna, and the Atlantic Forest. According to the results of the CCA, the occurrence this species was strongly associated with a high Al^{3+} content in these areas. This was, however, not the case in the rehabilitated area, where the Al^{3+} content was low (Table 1) and this species was not detected. *Saitozyma podzolica* has first been described in a soil with the accumulation of OM, Fe and Al oxides, and with sandy texture and acids (Fell and Statzell-Tallman, 1998), similar to soils of the *Eucalyptus* stand and the Atlantic Forest, where *S. podzolica* was dominant.

The genus *Pseudozyma*, also belonging to the phylum Basidiomycota, was found in this study, although species of this genus are usually inhabitants of the phyllosphere (Renker et al., 2005). This species was only isolated from the rehabilitated area and mainly associated with the pH. In addition, species of the genus *Pseudozyma* may represent anamorphs of some teleomorph plant pathogens (Kurtzman et al., 2015) and may be associated with the predominant vegetation in this area.

The phylum Ascomycota was not dominant among the samples, with only 12 isolates (18 %), but was more diverse, including the genera *Meyerozyma*, *Debaryomyces*, *Lipomyces*, and *Aureobasidium*. Of these, *Aureobasidium* and *Debaryomyces* have adaptive advantages that ensure their long-term survival in the soil (Botha, 2006). Species of *Aureobasidium* are dimorphic and have an intrinsic ability to adapt to environmental stress due to rapid changes between the yeast and mycelial forms (Gostinčar et al., 2014). *Debaryomyces* spp. are osmotolerant and can grow in medium containing NaCl concentrations above 4 mol L^{-1} (Breuer and Harms, 2006); they can therefore grow in environments with low water activity, such as the soil studied here, which was collected in the dry season.

Species that were found in the greatest numbers, such as *S. podzolica*, are considered autochthonous because they grow and persist within the habitat and originate within the system (Botha, 2011). In contrast, the species isolated in smaller quantities are considered allochthones and may originate from outside the system (Botha, 2011), such as *D. polymorphus* or *Pseudozyma* sp., for example. However, it is reasonable to assume that the soil yeast populations reported in this study may represent only a portion of the total yeast communities in the soil, mainly due to the limitations of culture-based isolation techniques.

In this study, the highest isolation rates were detected in the *Eucalyptus* stand (35 %) and the Atlantic Forest (28 %), which might be explained by the dense vegetation and the greater nutrient availability in these sites (Mendes et al., 2012).

Nutrient-rich soil supports a wider diversity of yeast species than nutrient-poor soil, and yeasts may survive by using strategies such as exopolymeric capsule and

biofilm formation to sequester moisture and nutrients (Spencer and Spencer, 1997; Botha, 2006).

Principal components analysis (PCA) showed that pH, SB, P, Al^{3+}, Fe, OM, H+Al, Al^{3+} saturation index (m), and texture were decisive for the separation of the sites. The *Quadrilátero Ferrífero* region has its natural vegetation cover shared between the neotropical savanna and the Atlantic Forest. The soil is characterized by high levels of Fe, Al^{3+}, and some heavy metals as well as an acid pH (Vincent and Meguro, 2008; Azevedo et al., 2012), which corroborates the grouping of soil samples generated by the PCA, where samples of the same vegetation type tended to be grouped based on soil properties.

Furthermore, the soils of the *Quadrilátero Ferrífero* are mostly derived from itabirite (metamorphosed iron-formation composed of Fe oxides, silica, and quartz) (Jacobi et al., 2007), which explains the high Fe content in all environments evaluated.

The CCA showed that pH, texture, Al^{3+}, and P content as well as total acidity were the main variables that determined the distribution of yeasts in the soils. Carvalho et al. (2013) associate the isolation of various species of yeasts of the neotropical savanna to the soil physicochemical properties, where approximately 90 % of *Candida* species were closely related to high levels of Al^{3+} present in the sampled soils.

It has already been demonstrated that the environmental variable that correlated best with soil yeast richness is pH (Vreulink et al., 2007). In our sites, pH levels were rather low, which is not surprising, since a low pH favors fungal growth.

Despite the strong influence of the soil physicochemical properties, the presence of a particular yeast species in the soil results from the sum of its adaptive capacities and interactions with the soil microbial community (Botha, 2011).

We evaluated soils under different vegetation types and noted impacts of the vegetation on the soil microbial community. The Atlantic Forest site and the *Eucalyptus* stand had similar soil yeast communities, while the communities of the Neotropical Savanna and the iron outcrop resembled each other. However, the yeast community of the rehabilitated areas differed from those of the other sites.

The conversion of forests into open vegetation results in changes in the structure of the soil microbial communities. We observed an increased proportion of ascomycetous yeasts in the rehabilitated site compared to sites with natural vegetation. Yurkov et al. (2012) studied yeast diversity in soils under different management regimes and reported that the yeast community composition differed considerably between different vegetation types. In addition, basidiomycetes yeasts were prevalent in forests, while ascomycetes yeasts were prevalent in grassland.

Factors such as the quality of OM might have generated differences in nutrient availability, with higher levels in the rehabilitated site, favoring the predominance of Ascomycetes. Ascomycetous yeasts exhibit rapid growth rates in the presence of mono-, di, and trisaccharides, since they lack enzymes to break down complex compounds (Fonseca and Inácio, 2006). Land management changes the soil nutrient balance and provides easy-to-use carbon sources favoring the growth of ascomycetous yeasts (Yurkov et al., 2012).

Furthermore, the recent introduction of grasses to the rehabilitated site may explain the high diversity index values (Shannon and Simpson) found in this area, which currently recovers from environmental stressors and is subject to colonization by microorganisms. The similarity between the yeast communities of the *Eucalyptus* stand and the Atlantic Forest indicates that rehabilitation in this area was successful.

Different phytophysiognomies under natural vegetation are the major determinant of biological soil functioning (Mendes et al., 2012). The structure of the microbial community

of soils covered by different communities of plants or undergoing different management practices differs greatly (Quirino et al., 2009).

Previous studies have reported that yeast communities in different locations share few species, and some taxa were restricted to a single sampling site, such as *Pseudozyma* sp., which, in this study, was found only in the rehabilitated site. This may be due to a sampling bias, where factors associated with environmental heterogeneity, such as vegetation type, soil type, and climatic conditions at the time of sampling can influence the results (Sláviková and Vadkertiová, 2000; Maksimova and Chernov, 2004; Vishniac, 2006; Yurkov et al., 2012).

CONCLUSIONS

The occurrence of yeast species in soils under different vegetation types in the *Quadrilátero Ferrífero* in Minas Gerais was influenced by chemical and physical soil properties, such as pH, phosphorus, aluminum, index aluminum saturation (m), exchangeable bases, the sum of Ca^{2+}, Mg^{2+}, and K^{+} (SB), clay, as well as the vegetation type.

Molecular identification of the isolates showed the dominance of *Saitozyma podzolica* species in four areas and its close relationship with the presence of aluminum.

Highest yeast diversity was found in the rehabilitated site, indicating successful revegetation.

Our paper highlights the preliminary results of the occurrence of yeast species in the *Quadrilátero Ferrífero* region, showing the need for future studies to evaluate the yeast diversity in the region with improved cultivation techniques.

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