

Division - Soil Processes and Properties | Commission - Soil Biology

# Total fungi and yeast distribution in soils over native and modified vegetation in central Brazil

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**ABSTRACT:** Fungi are ubiquitous components of soil microbial communities, generally comprise the largest proportion of soil biomass. They can occur as filamentous forms or unicellular yeasts, in both, as free-living or symbionts. Next generation sequencing has allowed greater depth of the access to soil fungal diversity complementing culture-dependent results. In Brazil, the state of Minas Gerais is recognized for its mining activity, which modifies the vegetation cover and consequently the soil microbial communities. To describe the fungal community (total fungi and yeast) in a post-mining area, comparing natural and modified ecosystems, we used environmental metabarcoding of ITS2 region. We assessed four ecosystems, with different vegetation and levels of impact, ranging from none to high impact (Atlantic forest, Iron outcrops, Eucalyptus, and Grass). Sequence data were compared with culture data obtained from previous studies. The fungal communities (total fungi and yeast) were more similar between Eucalyptus and Atlantic Forest, while Grass and Iron outcrops ecosystems showed greater dissimilarity. Despite its modified state, Grass ecosystem presented the highest alpha diversity values. Yeasts represented a proportion of fungal communities ranging from 1.7 to 17 % of fungal sequences in soil. The Ascomycota:Basidiomycota ratio was higher for the total fungi analysis, while a greater proportion of Basidiomycota was observed with the yeast analysis. Grass ecosystem was the only exception, where a higher proportion of ascomycetous yeasts was detected. The yeast communities responded to the environmental stress caused by the mining activity, resulting in changes in the composition, mainly increasing the abundance of black yeasts. *Saitozyma podzolica* relative abundance obtained with ITS sequencing was coherent with the findings obtained with culture data. Despite greater diversity depth obtained by metabarcoding, sequence and culture data were complementary tools in describing the fungal soil community. This study contributes significantly to the inventory of yeast species in tropical and subtropical soils.

**Keywords:** tropical forest, post-mining, black yeast, *Saitozyma*, metabarcoding.

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## INTRODUCTION

Fungi perform important functions in soil, due to their role in both decomposition of organic matter and symbiosis with plants (Větrovský and Baldrian, 2013). Fungi have different forms of growth, ranging from unicellular organisms (yeasts) to large and complex mycelial networks (filamentous fungi) (Dunthorn et al., 2017). Overall, filamentous growth dominates the terrestrial environments and is more studied than soil yeasts, whose diversity is less explored. Most reports of soil yeasts are restricted to temperate and boreal climates (Yurkov, 2018).

Due to the close ecological interactions between fungi and plants, vegetation characteristics are one of the main determinants of fungal diversity and distribution, along with soil properties (Schappe et al., 2017). Seasonality is another important factor influencing the dynamics of fungal communities in the soil, as colder seasons may lead to reduced soil fungal activity (Zifčáková et al., 2016). Furthermore, anthropogenic activities of high economic impact, such as mining practices, influence the dynamics of the soil fungus community due to the removal of natural vegetation and subsequent reforestation. This practice of environmental recovery allows observing the dynamics of fungal communities during plant succession (Harantová et al., 2017). Studies on primary succession in areas degraded by mining activities highlight the importance of diversity in fungal communities for vegetation development on degraded lands (Harantová et al., 2017; Moreira and Vale, 2018).

Traditionally, fungal communities in soil were studied by isolation techniques and identified based on macroscopic and microscopic characteristics (Větrovský and Baldrian, 2013). Although the contribution of this classic method for the description of fungi diversity is undeniable, it is well known that it underestimates microbial diversity in soil. Emerging methodologies based on next generation sequencing are deepening the access to diversity and ecology of fungal communities (Lindahl et al., 2013; Schmidt et al., 2013; Tedersoo et al., 2015). For example, the metabarcoding of the ITS region made it possible to quickly observe changes in the fungal community to an increase in soil disturbance (Cho et al., 2017).

However, there are currently few published studies using metabarcoding to describe soil yeast communities (Dunthorn et al., 2017; Mašínová et al., 2017). To our knowledge, no study has been done on the influence of mining activities on yeast communities in tropical soils, using the metabarcoding approach. Based on cultivation techniques, our group described yeast communities in soils under different vegetation types at a post-mining site in Minas Gerais State, Brazil (Moreira and Vale, 2018), internationally recognized due to its mining industry and its rich mineral deposits (Azevedo et al., 2011).

Given the intrinsic conditions of our sample set and previous reports in the literature, we hypothesized that: a) fungal communities will differ in alpha and beta diversity among vegetation types; b) the yeast community will represent a small proportion of the total fungi from soil; c) yeast communities described with metabarcoding will differ in species composition from the communities described by culture-dependent methods. Therefore, this study aimed to apply the metabarcoding methodology in the study of fungal communities in soil, with an emphasis on the yeast community of a post-mining site.

## MATERIALS AND METHODS

### Study area and soil sampling

The study area is located at Centro de Pesquisa e Conservação da Biodiversidade do Quadrilátero Ferrífero (CeBio), in Sabará, Minas Gerais State, Brazil. CeBio is part of an area of VALE SA (Company) and constitutes an iron-mining site, known as *Córrego do Meio* mine area. Mining activities in the area ended in 2006. The entire area of the

Iron Quadrangle (*Quadrilátero Ferrífero*) is in an ecotone between the Atlantic Forest and Neotropical Savanna biomes, two Brazilian hotspots, the latter characterized by the occurrence of a mosaic of vegetation. The climate is semi-humid tropical, with two well-defined climatic seasons: humid summer and dry winter. According to Köppen's classification system, the climate is Cwa - humid temperate climate with dry winter and hot summer. The average annual temperature remains around 20 °C with annual rainfall ranging between 1300 to 2100 mm.

In the CeBio we studied different vegetation types, including unmodified ecosystems (Atlantic Forest and Iron outcrops), and ecosystems with vegetation modified by mining activity (eucalyptus and rehabilitated area with grass) (Table 1). Four points were sampled by the ecosystem. At each sampling point, 12 simple samples of the topsoil (0.00-0.20 m) were collected and homogenized to form a composite sample, according to the methodology described by Swift and Bignell (2001). The collected soil was packed in sterile plastic bags, sealed, and transported on ice to the laboratory for further analyses. The sample fractions were split into two: one was used to study yeast diversity by culture-dependent methods (Moreira and Vale, 2018), and the other fraction was used for fungal diversity studies based on ITS2 metabarcoding (present study).

The physical-chemical soil properties were described in Moreira and Vale (2018). In general, the soils were characterized with high acidity, with low natural fertility (described by the low content of macronutrients), and high content of organic matter and Fe (iron). These are striking characteristics of the biomes and soil types present in the study area (Neotropical Savanna and Atlantic Forest). The exception was the Grass ecosystem, which presented low organic matter content and alkaline soils. This is due to the dominance of grass used in the environmental recovery of the area, and the sterile origin of the soil.

The dominant soil type in the studied ecosystems, according to the Brazilian Soil Classification System (SiBCS) (Santos et al., 2013) and World Reference Base for Soil Resources (IUSS Working Group WRB, 2015), were as follows: *Cambissolos Háplicos* (Cambisols), *Latossolo Vermelho* (Ferralsols), and *Neossolos Regolíticos Perféricos*

**Table 1.** Description of the ecosystems sampled in this study. The description of the soil properties can be found in Moreira and Vale (2018)

Ecosystems	ID	Latitude	Longitude	Impact level	Vegetation attributes <sup>(1)</sup>
Atlantic Forest	AF	19° 51' 41.41" S	43° 48' 7.90" O	None	Semi-deciduous seasonal forest region. Originally belonging to the Atlantic Forest Biome (BAF). Currently defined by secondary vegetation in different stages of natural regeneration.
Iron Outcrops	IO	19° 50' 2.06" S	43° 47' 7.40" O	None	Very preserved rock environment. Vegetation on ferruginous substrates where the dominance of Cactaceae and grasses represents the local physiognomy.
Eucalyptus	E	19° 51' 37.85" S	43° 48' 23.75" O	Medium	Reforestation environments. Originally belonging to the Atlantic Forest Biome (BAF). Homogeneous plantations of <i>Eucalyptus</i> spp. at different ages occupying extensive surfaces to remediate the impacts of the mining activity.
Grass	RG	19° 51' 38.59" S	43° 47' 47.36" O	High	Sterile/tailings piles and slopes. The ongoing environmental rehabilitation process, started in 2006, after the mining activity stopped. Currently, the soil is covered with grass, mostly <i>Melinis minutiflora</i> .

<sup>(1)</sup> Description provided by VALE SA Company (Filho, 2010).

(Regosols). The last type of soil is a proposed classification, exclusively for soils described in the Iron outcrops ecosystem (Coelho et al., 2017).

### Molecular analysis

Total environmental DNA (eDNA) was extracted from two replicates of composite samples. Total eDNA was extracted from 0.5 g of soil using the PowerLyzer® PowerSoil® DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA) according to the manufacturer's instructions. Fungal soil communities were characterized by sequencing the ITS2 region of rDNA using barcoded gITS7 and ITS4 primers (Ihrmark et al., 2012; Mašínová et al., 2017). PCR reactions contained 12.5 µL of GoTaq Master Mix 2X, 1 µL of each primer (10 µmol L<sup>-1</sup>), 0.3 µL of BSA (50 mg mL<sup>-1</sup>), and 1 µL of DNA template. Cycling conditions were 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Sequencing was performed on Illumina MiSeq, with the MiSeq Reagent Kit v2 (300 cycle) and paired-end sequencing libraries (2 × 150 bp), conducted by *BPI Bioinformática* (Botucatu, São Paulo, Brazil).

### Bioinformatic and statistical analysis

Sequencing delivered 555,769 reads, which were treated with Dada2 (Callahan et al., 2016) pipeline in R software (v 3.5.3). Dada2 was used for sequences filtering for quality [maxN = 0, maxEE = c(2,2), truncQ = 2] length (truncLen = 148), and to trim primer (trimLeft = 20). After quality evaluation, only forward sequences were used, which has been discussed before as a good option for taxonomy description of fungi (Pauvert et al., 2019). Forward sequences were dereplicated, amplicon sequence variants (ASV) were inferred and, finally, chimera checked and filtered out.

Both tables of ASV count per sample and ASV fasta sequences were exported out from R software to perform the taxonomic assignment in QIIME2 (Quantitative Insights Into Microbial Ecology) (Bolyen et al., 2019). The ASV count table was imported as biome table and converted to .qza artefact, as well as representative sequences of ASVs. Alpha and beta diversities were calculated in QIIME2. For alpha diversities, samples were rarefied to the smallest sample size (46,893 reads for all fungi, 953 for yeast only data), and Shannon diversity was calculated. Weighted Unifrac distances were calculated based on mafft aligned reads and rooted tree. Taxonomic assignment was performed with QIIME2 default pipeline classifier naïve Bayes against UNITE (Nilsson et al., 2019) dynamic database from February 2019. The Bray-Curtis dissimilarity was used in the construction of the UPGMA clustering. Nonmetric multidimensional scale (NMDS) was used to visualize the correlation between the soil physical-chemical properties [described in Moreira and Vale (2018)] and the distribution of the total fungi community. The Bray-Curtis dissimilarity was used in the construction of the NMDS, and the significant soil variables were chosen by direct selection with a test permutation. The NMDS was performed with the Vegan package (Oksanen et al., 2016) in the R software.

As our main objective was to compare total fungi and yeast's distribution in soils, and to compare with previous data obtained from yeasts isolation and culture, yeast species were filtered from the whole dataset. Several steps were performed to ensure that only yeast genera and/or species were considered, and that classification was up-to-date. A list of all described genera possessing yeast morphology was compiled from "The Yeasts: a taxonomic study" book (Kurtzman, 2011) and used in R software to filter from the total fungi taxonomic data. However, newly named species were not present in the list and were manually added, also based on previous culture results from our group (Moreira and Vale, 2018). The final list of genera was used to filter the biom table with QIIME2 (*qiime taxa filter-table*). The distance matrices from total fungi data and only yeast were used as input for a mantel correlation test using 999 permutations. Graphical representation was performed in R software using *ggplot2* package. Raw sequences were submitted to the NCBI database and can be found under the project PRJNA629830.

## RESULTS

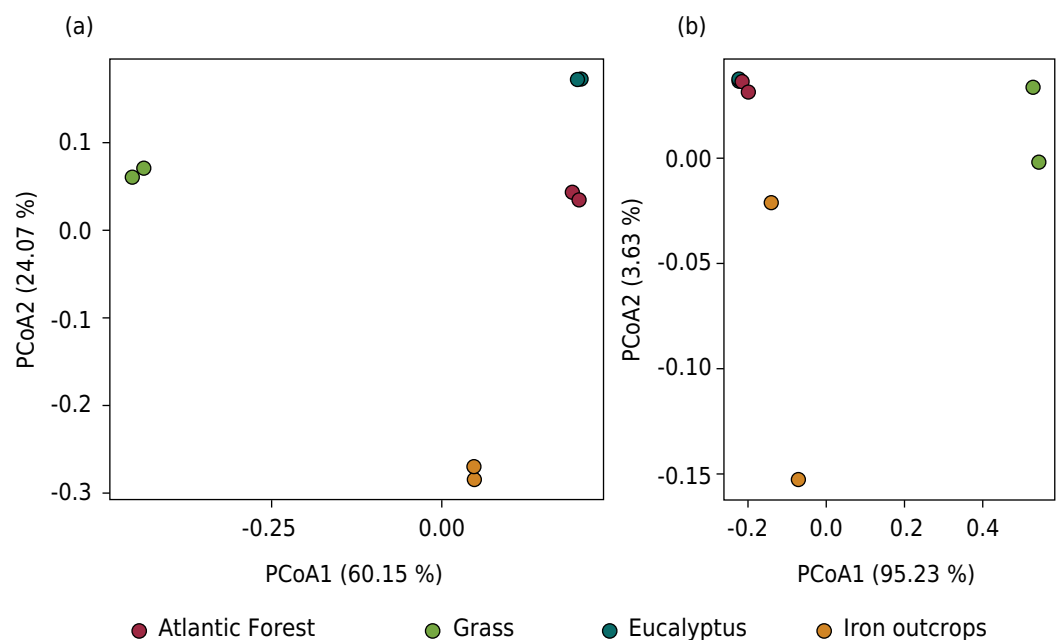
### Total fungi and yeast diversity

Quality filtering leads to a total of 506,233 reads, with an average of 63,279 reads per sample ( $\pm 9,597$ ), which composed a total of 3,309 ASVs, belonging to 173 fungi taxa (at the family level). About 50 % of the sequences were identified only up to the phylum level, being characterized as unclassified or unidentified. A total of 37,689 reads have been identified as yeast or yeast-like fungi in all eight samples, which composed a total of 57 ASVs, belonging to 38 yeast taxa (at genus level).

The principal coordinates analysis (PCoA) was used to represent the similarities between fungal communities present in each ecosystem, based on the weighted Unifrac metric that uses relative abundance and phylogenetic distance (Figure 1). Samples ordered closer together have a greater similarity than those more distant. The PCoA showed great reproducibility between the two replicates by the ecosystem. The first three axes explained 98.25 % of the data. For total fungi (Figure 1a), the first axis explained most of the data variability (60.15 %), separating samples from Iron outcrops from other ecosystems, while the second axis explained 24.08 % of the variability and separates the grass from other ecosystems. Samples were significantly distinct from each other (PERMANOVA;  $p$ -value = 0.012).

When considering only yeast data, the first axis only explained 95.23 %, corresponding to the greatest difference of grass samples against all others (Figure 1b). A Mantel test was performed between the weighted Unifrac distance matrices obtained with all fungi data against only yeast data. The spearman correlation with 999 permutations showed correlated beta-diversity distribution of both data ( $Rho = 0.9$ ;  $p$ -value = 0.001). We could see a greater similarity between Eucalyptus and the Atlantic Forest total fungi community diversity, which was more evident when analyzing the yeast community. Fungal communities (total fungi and yeast) of the Grass and Iron outcrops ecosystems showed greater dissimilarity among themselves, and in comparison to other ecosystems (Figure 1).

Grass samples presented the highest alpha diversity values for the total fungal and yeast communities (Figure 2). Considering the total fungi ASV abundance, no significant



**Figure 1.** Weighted Unifrac distances of total fungi (a) and only yeast diversity (b). Colors correspond to each sampled ecosystem. Grass represents the “Rehabilitated area with grass”.

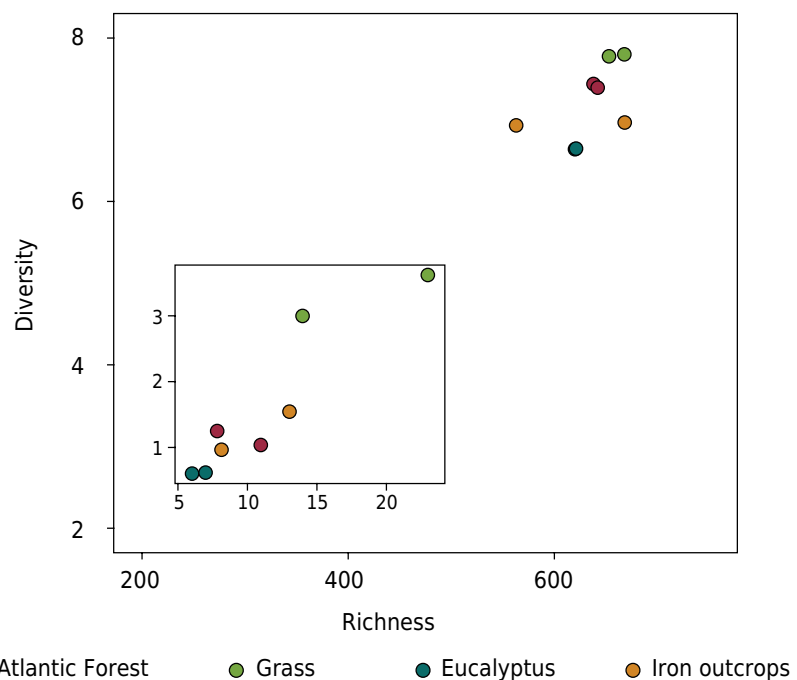
difference was observed for the observed ASVs (richness). On the other hand, the ecosystems differed regarding Shannon diversity, lowest in Eucalyptus, and increasing to Iron outcrops, Atlantic Forest, and Grass, respectively. When only yeasts were considered, Shannon diversity was only significantly increased in Grass samples.

The analysis of NMDS corroborates the distribution of fungi communities demonstrated by PCoA (Figure 3). The micronutrients P, Al, Fe, Cu, the texture (silt and sand), organic matter (OM) and pH, and the soil properties V (base saturation), t (effective cation exchange capacity), m (saturation index of aluminum), and H+Al (total acidity) were the variables with the most significant influence on the total fungi community structure (permutation test <0.05). Atlantic forest and Eucalyptus ecosystems were positively correlated with the aluminum content in the soils (m and Al). While Iron outcrops were correlated with total acidity (H+Al) and content of organic matter (OM) and iron (Fe), and the Grass ecosystem was positively correlated with pH and base saturation (V) (Figure 3).

### Fungi taxonomic composition per site

The majority of fungal sequences were assigned to the Ascomycota (78.7 %) and Basidiomycota (15.4 %) phylum. Mucoromycota was represented by 1.5 % of all sequences, and the other phyla represented less than 1 % of all sequences. Fungi from the Ascomycota phylum dominated in all ecosystems. In Eucalyptus and Atlantic Forest, only the Basidiomycota was slightly more abundant, representing 26 and 18 % of the sequences, respectively. For yeast communities, the majority of sequences were assigned to the Basidiomycota (93.8 %). However, yeast sequence from the Ascomycota dominated in Grass ecosystems (Figure 4).

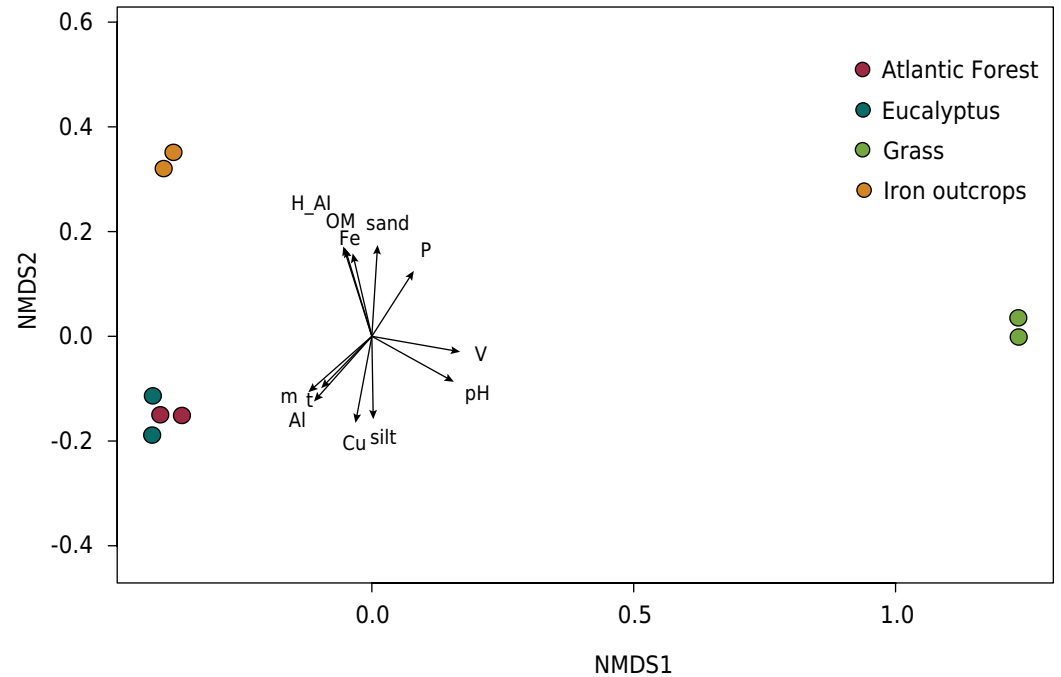
The taxonomic composition of the fungal community (at family level) was different among ecosystems, mainly due to differences in the abundance of the main taxa, which were Aspergillaceae, Trimorphomycetaceae, Herpotrichiellaceae, and Trichocomaceae (Figure 5). It is possible to observe that, as demonstrated at PCoA, Eucalyptus and Atlantic Forest ecosystems showed greater similarity in terms of taxonomic composition.



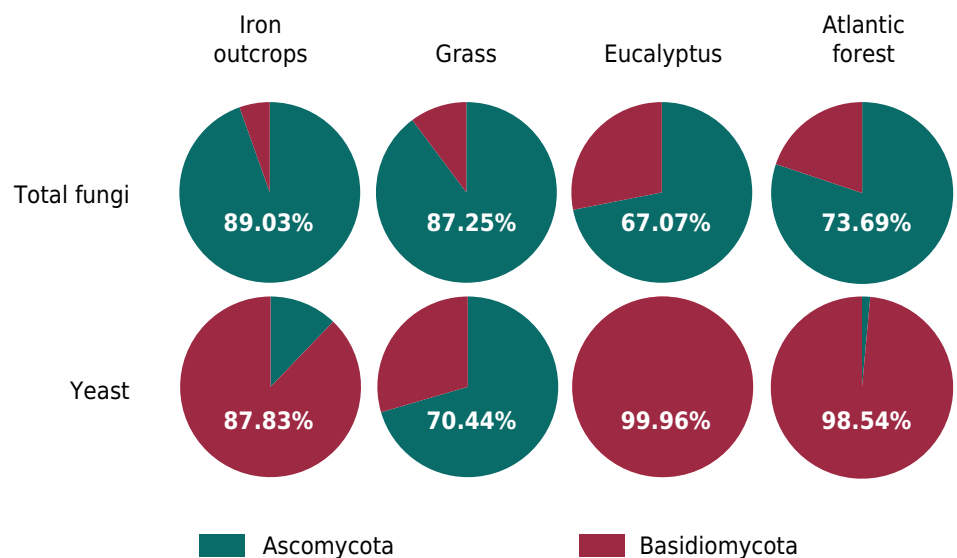
**Figure 2.** The  $\alpha$ -diversity - observed ASVs for richness and Shannon diversity - of total fungi (bigger graph) and only yeast (inside graph).

On the other hand, the Grass and Iron outcrops ecosystem showed greater Bray-Curtis dissimilarity regarding the other ecosystems. This seems to be mainly due to the greater abundance of Aspergillaceae in Iron outcrops, representing more than 50 % of the relative abundance, and of Herpotrichiellaceae in Grass. In contrast, Eucalyptus and Atlantic Forest had a greater abundance of the Trimorphomycetaceae taxa, belonging to the Basidiomycota (Figure 5).

In the Aspergillaceae, the most abundant fungal genera were *Penicillium* (26 % of ascomycetous sequences) and *Aspergillus* (2.5 %), especially in Iron outcrops. Trichocomaceae, abundant mainly in Eucalyptus, had a greater abundance of the



**Figure 3.** Nonmetric multidimensional scaling (NMDS) plot tracking community similarity and soil variables correlation. Soil variables (permutation test <math><0.05</math>). P: phosphorus; Al: aluminum; Fe: iron; Cu: copper; OM: organic matter; H+Al: total acidity; m: saturation index of aluminum; t: effective cation exchange capacity; V: saturation index of bases; texture (silt, sand); and pH.



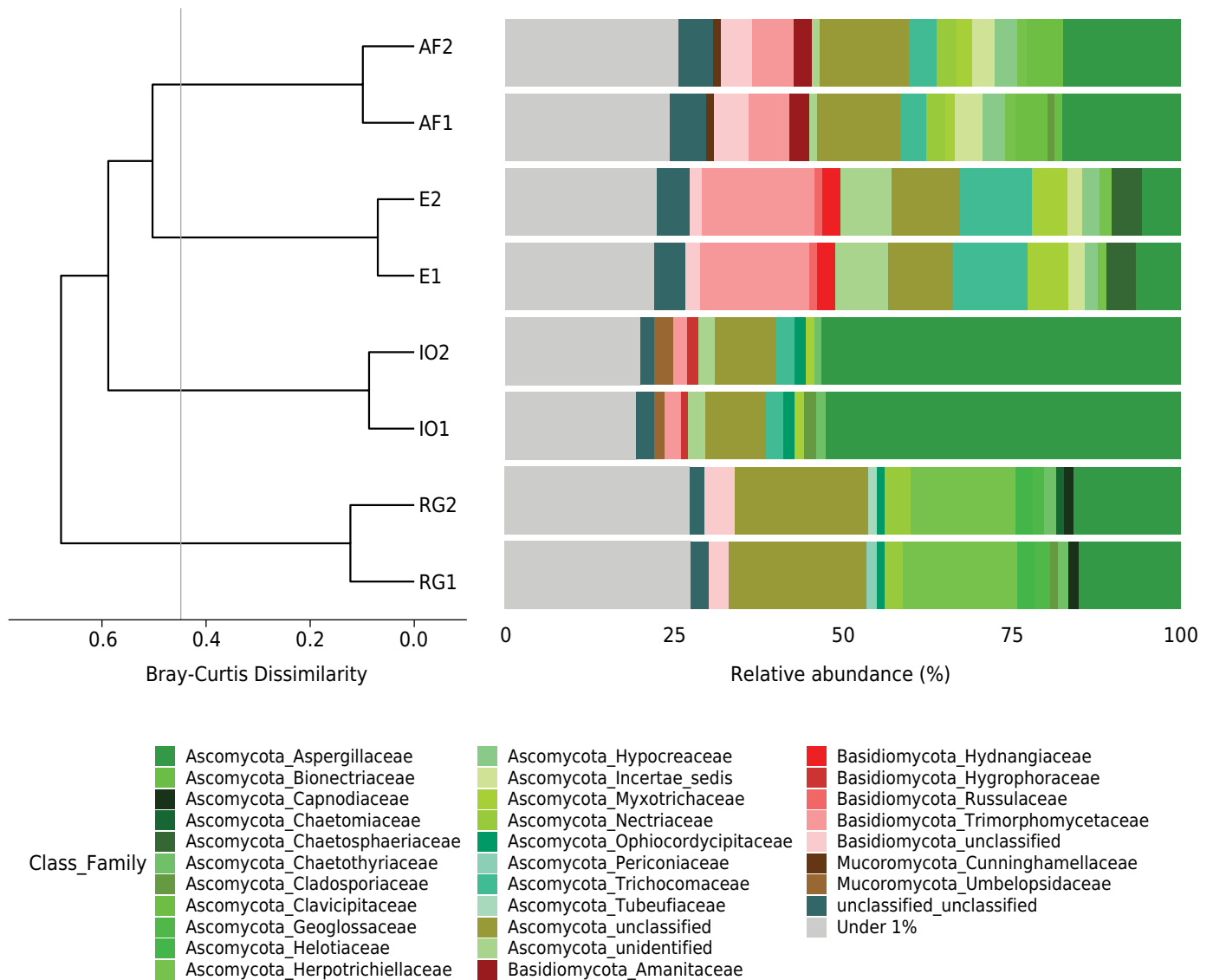
**Figure 4.** Ascomycota:Basidiomycota ratio for all sequences distributed among ecosystems, in both total fungi and yeasts. Percentages correspond to the dominant phylum in each ecosystem.

*Talaromyces* and *Sagenomella* genus, with 3 and 2 % of ascomycetous sequences, respectively. For Grass, the Herpotrichiellaceae had a greater abundance of the *Coniosporium* and *Exophiala* genera, with less than 1 % of the ascomycetous sequences. Trimorphomycetaceae, the most abundant fungal family in Eucalyptus and Atlantic Forest, highlighted the *Saitozyma* genus (44 % of basidiomycetous sequences).

### Yeast abundance and comparison with culture data

To study the distribution of yeast genera and species across the four ecosystems, we have filtered the taxa that are known as yeasts or yeast-like fungi. From the total fungal data, samples presented varied proportions of yeasts from 1.7 to 17 % depending on the sample (Table 2). Grass ecosystems presented the lowest and Eucalyptus the highest proportions.

We identified 38 yeast taxa from sequence data. Unlike the pattern presented by the total fungi, in the yeast community, Basidiomycota predominated over the Ascomycota. The exception was for Grass, where a higher proportion of ascomycetous yeasts was detected (Figure 4). *Saitozyma podzolica* was the species with the highest relative abundance. There is a negative correlation of its relative abundance regarding the level of impact on the soil, being relatively smaller in Grass. On the other hand, *Knufia tsunedae*, the



**Figure 5.** Fungal taxonomic community composition (family level) in all four vegetation types. UPGMA clustering formed four groups with greater than 45 % dissimilarity. Under 1 % represents all families with <1 % relative abundance. AF: Atlantic Forest; E: eucalyptus; IO: Iron outcrops; RG: rehabilitated area with grass.



**Table 2.** Average relative abundance (%) of yeast species and genera by site: comparison between sequence data/culture data. Relative abundance of each taxon by the total fungi for sequence data is expressed within parenthesis

Yeast taxa	Sequence data/ culture data (%)			
	Iron outcrop	Grass	Eucalyptus	Atlantic Forest
Ascomycota				
Arthoniomycetes	<i>Phaeococcomyces nigricans</i>	0.2699 (0.007)/0	3.133 (0.061)/0	
	<i>Phaeococcomyces</i> unidentified		4.739 (0.107)/0	
Dothideomycetes	<i>Aureobasidium</i> unclassified		0.752 (0.019)/2.941	
	<i>Aureobasidium melanogenum</i>	2.770 (0.077)/0	7.635 (0.156)/0	
	<i>Aureobasidium thailandense</i>			1.382 (0.094)/0
Eurotiomycetes	<i>Exophiala</i> unclassified			0.036 (0.006)/0
	<i>Exophiala bergeri</i>	0.347 (0.010)/0		
	<i>Exophiala pisciphila</i>		15.563 (0.319)/0	
	<i>Knufia</i> unclassified	5.378 (0.149)/0	8.889 (0.188)/0	
	<i>Knufia tsunedae</i>		30.109 (0.619)/0	
Saccharomycetes	<i>Knufia</i> unidentified	1.157 (0.032)/0		
Saccharomycetes	<i>Diutina catenulata</i>	0.193 (0.005)/0		
Sordariomycetes	<i>Sporothrix</i> unclassified	0.121 (0.003)/0		
	<i>Sporothrix</i> unidentified	3.238 (0.090)/0		
Basidiomycota				
Cystobasidiomycetes	<i>Occultifur externus</i>		0.188 (0.005)/0	
	<i>Cyrenella elegans</i>			0.733 (0.048)/0
Exobasidiomycetes	Unclassified		2.167 (0.046)/0	
	<i>Meira nashicola</i>			0.091 (0.015)/0 0.112 (0.007)/0
Microbotryomycetes	<i>Sakaguchia</i> unclassified			0.009 (0.001)/0
	<i>Rhodotorula mucilaginosa</i>	0.887 (0.025)/0		
Tremellomycetes	<i>Rhodotorula taiwanensis</i>	1.178 (0.032)/0	0.408 (0.010)/0	0.040 (0.007)/0 0.345 (0.022)/0
	<i>Goffeauzyma</i> unclassified	0.154 (0.004)/0		
	<i>Heterocephalacria arrabidensis</i>		0.596 (0.015)/0	
	<i>Solicoccozyma</i> unclassified		0.920 (0.022)/0	
	<i>Genolevuria</i> unclassified		0.564 (0.014)/0	
	<i>Vishniacozyma victoriae</i>			0.109 (0.018)/0
	<i>Papiliotrema perniciosus</i>		0.752 (0.019)/0	
	<i>Papiliotrema</i> unidentified		0.282 (0.007)/0	
	<i>Cryptococcus</i> unidentified	2.084 (0.058)/ 0	0.262 (0.004)/0	
	<i>Tremella</i> unclassified	0.097 (0.003)/ 0	0.251 (0.006)/0	
	<i>Tremella</i> unidentified		0.533 (0.013)/0	
	<i>Saitozyma</i> unclassified		0.846 (0.021)/0	
	<i>Saitozyma podzolica</i>	82.030 (2.278)/ 10.294	21.411 (0.431)/0	99.454 (16.43)/33.823 92.337 (6.058)/25
	<i>Cryptotrichosporon</i> unclassified	0.097 (0.003)/ 0		
	<i>Apiotrichum</i> unclassified			0.449 (0.031)/0
<i>Apiotrichum laibachii</i>			1.621 (0.106)/0	
<i>Apiotrichum sporotrichoides</i>			0.262 (0.043)/0 2.390 (0.155)/0	
<i>Cutaneotrichosporon smithiae</i>			0.631 (0.043)/0	

second most abundant species, followed by *Exophiala pisciphila*, both ascomycetous, were detected only in Grass. The presence of the *Aureobasidium* in this ecosystem is also notable, with a relative abundance of 8.4 % against 2.77 and 1.38 % in Iron outcrops and Atlantic Forest, respectively.

*Saitozyma podzolica* and *Aureobasidium* sp. were species detected by the two approaches (sequence and culture data) (Table 2). In both studies, *Saitozyma podzolica* had the highest relative abundance. The only exception was for culture data, where this species was not detected in the Grass samples.

## DISCUSSION

### Vegetation and soil variables effect

Soil fungal communities differed among the studied ecosystems, both in structure and taxonomic composition which supported our first hypothesis. These findings are consistent with the literature that reports the importance and influence of vegetation type on soil fungal communities (Urbanová et al., 2015; Schappe et al., 2017). In this study, we point out two interesting results regarding vegetation, in both total fungi and yeast communities. First, the great similarity between the fungal communities of the Atlantic Forest and Eucalyptus; and second, the low similarity of the communities in Iron outcrops and Grass, among themselves, and with the other ecosystems. It is already known that plant species, especially dominant species, are the main determinants of fungi composition and distribution in soils, mainly in litter and topsoil (Urbanová et al., 2015).

Originally, the present Eucalyptus ecosystem was an area of the Brazilian Atlantic Forest, which is now undergoing a revegetation process to mitigate the effects of mining. The comparison between reference systems with natural vegetation and areas under environmental recovery allows evaluating the efficiency of the restoration (Derhé et al., 2016). Eucalyptus and Atlantic Forest showed similarities in soil properties, especially in terms of Al content, as viewed by NMDS. The similarity of soil fungal and yeast communities between Eucalyptus and the Atlantic Forest suggests that revegetation can restore this ecosystem as close as possible to the natural conditions.

On the other hand, Grass and Iron outcrops ecosystems had the most different fungal communities. We highlight the origin of the soil from sterile waste (anthropogenic soil) and rocky outcrops (iron-rich), for Grass and Iron outcrops, respectively. The origin of the soil is evident when we observe the soil properties of these samples. Grass had a very low organic matter content ( $13.2 \text{ g kg}^{-1}$ ) when compared to Iron outcrops ( $95.7 \text{ g kg}^{-1}$ ). On the other hand, Iron outcrops had a high iron content when compared to Grass,  $236.71$  and  $43.32 \text{ mg dm}^{-3}$ , respectively (Moreira and Vale, 2018). The NMDS analysis corroborates these data, where Iron outcrops ecosystem was positively correlated with the content of organic matter and Iron, while Grass ecosystem was correlated with pH and base saturation index (V). The base saturation index (V) is a good parameter of soil fertility conditions, and its positive correlation with Grass ecosystem can be indicative of the recovery of that soil.

Approaches to recover degraded areas include sowing a mixture of plant species that have rapid growth and are useful for incorporating biomass into the system and accelerating the formation of dense vegetation coverings, such as grass (Figueiredo et al., 2018; Gastauer et al., 2018). Thus, the Grass ecosystem is still in the initial stages of recovery, configuring itself as an environment in natural succession. Usually, environments in natural succession begin with microbial communities containing only a few species, followed by changes in the community that increase diversity and complexity (Harantová et al., 2017). This is not coherent with our data, since grass was the most diverse within the

four sites, which can indicate a disturbed site with community not yet established. Our findings are consistent with Rodrigues et al. (2013), in which the local taxonomic diversity of soil bacteria increases after the conversion of the Amazon rainforest to agriculture, but communities become more similar across space.

We believe that the difference in the fungal community at Iron outcrops is related to the type of soil, typically known as ferruginous soil. Since this soil determines the plant species able to grow in this ecosystem (Jacobi et al., 2007; Skirycz et al., 2014), it can also influence the fungal community, either directly or indirectly. The soil and vegetation developed in the Iron outcrops areas reveal many peculiarities that completely differentiate them from the surrounding environments and other Brazilian biomes. In addition, these ecosystems are among the most threatened and least studied in the state of Minas Gerais, where this study is one of the only reports on yeast diversity.

Our results are in agreement with Harantová et al. (2017), who evaluated the development of microbial communities in areas degraded by mining activity, and demonstrated that vegetation was the strongest determinant for shaping communities of fungi and bacteria. These authors suggested that the initial composition of the microbial community will determine the future vegetation to develop in that place. The dissimilarity found in fungal communities between sites reflects the different composition of plant species between the sites evaluated, as previously revealed on a global scale (Prober et al., 2015).

### **Total fungi and yeast diversity**

Our analyses were based on the concept of ASVs (Amplicon Sequence Variants) instead of OTU (Operational Taxonomic Unit). We chose this approach because ASVs provide finer resolution consistent with biological significance based on sequence variation, without imposing arbitrary limits on dissimilarity that define molecular OTUs (Callahan et al., 2017). The ITS region was chosen because they are conserved in large groups of organisms, contain enough variation to be informative at the phylogenetic level, and because it is the universal DNA barcode marker for fungi (Schoch et al., 2012; Lindahl et al., 2013). In the ITS region, both ITS1 and ITS2 have been used to describe the fungi community in the soil and produce similar results when used as metabarcodes (Blaalid et al., 2013). We opted for the ITS2 region to facilitate comparison with the first reports of metabarcoding for the yeast community (Mašíňová et al., 2017).

For fungi total communities, at the phylum level, 78.7 % of the fungal sequences were assigned to Ascomycota. The highest proportion found for Ascomycota is in agreement with other studies that used the same approach for soil fungus communities (Urbanová et al., 2015; Harantová et al., 2017; Chen et al., 2017; Schappe et al., 2017). According to the global diversity pattern of fungi in the soil, the Ascomycota: Basidiomycota ratio tends to be higher in ecosystems with sparse vegetation (e.g. grasslands) and decreasing as vegetation density increases (e.g. temperate deciduous forests) (Tedersoo et al., 2014). The increase in the relative abundance of Basidiomycota in forest ecosystems (Atlantic Forest and Eucalyptus) may be related to the rate of decomposition in this ecosystem favored by dense and closed vegetation, which generates a greater input of material to be degraded (Peršoh, 2015), and because many saprophyte fungi in forest ecosystems are basidiomycetes (Sterkenburg et al., 2015). It is believed that a succession might be observed regarding a decrease in Ascomycota relative abundance and increased Basidiomycota as the decomposition process intensifies (Vorisková and Baldrian, 2013).

The fungal taxa per site once again support our first hypothesis, showing that communities differ in terms of composition between ecosystems. These findings are in agreement with Mueller et al. (2014), who evaluating the effect of deforestation on the Amazon rainforest described that fungal communities in primary forests (as Eucalyptus) were more similar to those found in secondary forests (as Atlantic Forest), and both differed

from the communities found in deforested pastures (as Grass). The study suggests that the recovery of the fungal community is closely linked to that of plants.

The majority of taxa uncovered in this study appear to be soil associated at some level. *Penicillium* and *Aspergillus* were detected in all ecosystems, but with higher relative abundance in Iron outcrops. These are genera with ubiquitous distribution and typical soil inhabitants (Gams, 2007) and have been reported in other studies based on high-throughput sequencing (Vorisková and Baldrian, 2013; Vorisková et al., 2014; Urbanová et al., 2015). It is noteworthy that most of the references available for comparison are for temperate soils, whereas in tropical soils are still scarce. However, even in tropical forests, many taxa are similar to those found in temperate latitudes, with only variations in species abundance and richness, mainly for cosmopolitan taxa (Gams, 2007).

*Coniosporium* and *Exophiala*, the most abundant fungal genera in Grass, are recognized as black microcolony fungi and black yeasts inhabitants of extreme environments (Marzban et al., 2013) and have been associated to urban environment (Sterflinger and Prillinger, 2001; Isola et al., 2016; Antonelli et al., 2020). The abundance of these genera in the Grass ecosystem may be related to their biological characteristics, similar to high-stress tolerance and ability to survive severe and hostile conditions (Marzban et al., 2013), as the limitation of nutrients expressed by the low content of organic matter in Grass. The most abundant fungal genera in Eucalyptus and Atlantic Forest were *Saitozyma*, *Talaromyces*, and *Sagenomella*. These genera have been described as indigenous inhabitants of the soil and other habitats (de Beeck et al., 2015; Zhai et al., 2016; Peterson and Jurjević, 2017; Yurkov, 2018).

From the 37,689 sequences of the fungal ITS2 region that represented 57 ASVs for yeast, we have shown the small proportion that the yeast community represented regarding total fungi. This supported our second hypothesis and is in accordance with previous reports for soil yeast communities accessed by metabarcoding (Mašíňová et al., 2017). At the phylum level, 59.5 % of the yeast sequences were assigned to Basidiomycota, 36.5 % were assigned to Ascomycota. Unlike the total fungi analysis, for the soil yeast communities, there is a predominance of Basidiomycota, agreeing with what is expected for soil yeasts (Yurkov, 2018). However, the intensity of land management (Yurkov et al., 2012), agricultural practices (Stringini et al., 2008), and soil pollution (Tepeeva et al., 2018) can change the ratio between Basidiomycota and Ascomycota. This is observed for the Grass ecosystem, where a greater relative abundance of ascomycetous yeasts was found.

*Saitozyma podzolica* was the most abundant species in all ecosystems. This is an indigenous soil yeast and has been the most common and most frequently reported species (Buée et al., 2009; Yarwood et al., 2010; Mašíňová et al., 2017; Harantová et al., 2017). The relative abundance of *S. podzolica* decreased as the impact on the soil increased. This finding is in agreement with the literature, where the modification of the vegetation generates the reduction of this species in the soil (Yurkov et al., 2012; Boonmak et al., 2019). Harantová et al. (2017) describe the increase in the relative abundance of *S. podzolica* in soils as the reestablishment of vegetation before the impact of mining activity. Based on previous reports, and on our findings by both sequence data and culture data, we suggest that this species has great potential to be used as an indicator of quality in soils impacted by anthropic activities.

Grass samples differed from the others in terms of taxa composition and showed high values for alpha diversity. This difference in grass is significant because of the greater richness of yeast species found and especially because of the species *Knufia tsunedae*, which is more abundant than *Saitozyma podzolica* in these samples compared to the others. It is interesting to observe this balance between the yeast genera when comparing Grass to environments with medium or none impact. Microorganisms respond quickly

to environmental stress caused by soil management, resulting in rapid changes in their diversity, abundance, and activity (Vadkertiová et al., 2017).

*Knufia tsunedae* was the second most abundant species, followed by *Exophiala pisciphila*, which are two ascomycetous found only in Grass. The presence of the genus *Aureobasidium* in this ecosystem is also notable (8.4 % of relative abundance). *Knufia*, *Aureobasidium*, and *Exophiala* are part of a group known as the black yeast. These are pigmented fungi known for their ability to grow in oligotrophic environments and with multiple stress factors similar to temperature changes, UV radiation, osmotic stress (Botha, 2011). Black yeast has been associated with anthropogenic and polluted environments (Isola et al., 2016; Babic et al., 2017). We suggest that the oligotrophic conditions (low organic matter content) and stressful characteristics of an ecosystem formed from piles of sterile tailings from mining may favor the abundance of taxa known to be tolerant and with better adaptive capacity to survive in these conditions.

Consistent with our results, Harantová et al. (2017) found a greater abundance of the genus *Knufia* in the initial stages of succession in a post-mining area, followed by a decrease in *Knufia* and an increase in the relative abundance of *Saitozyma* in the final stages of vegetation succession. This balance of genera that can possibly be used as indicators suggest that the grass ecosystems has not yet reached stability, being in recovery, or in the process of ecological succession. In addition, the relative abundance of Ascomycota significantly decreases over time during succession, where the abundance of Basidiomycota increases (Harantová et al., 2017). This finding can be seen for Eucalyptus, a revegetated area, where Basidiomycota practically represented 100 % of the yeast sequences. It will probably also be found in the Grass ecosystem if it continues to be monitored during the years of the succession of vegetation.

It is known that the healthy functioning of the soil depends on the abundance and diversity of microorganisms, and that the increase in biodiversity also increases the functional capacities of the ecosystem (Grządziel, 2017). The results obtained on abundance and species richness in total fungus and yeast communities point to an important concept in microbial ecology, functional redundancy. Thus, the richness and diversity of species found in the grass area give stability to this ecosystem in its initial stages of environmental recovery, since the loss of one or more species does not dramatically affect the functioning of the ecosystem (Grządziel, 2017; Escalas et al., 2019). Furthermore, under changed environmental conditions, high biodiversity and species richness can be beneficial in providing a set of species with the relevant traits to thrive in the harsh environment (Fetzer et al., 2015).

### **Yeast diversity: metabarcoding versus culture**

Comparing sequence and culture data, only *Saitozyma podzolica* and *Aureobasidium* sp., were common in both studies, confirming our third hypothesis. Different approaches will result in different results, although the dominance of *Saitozyma podzolica* in both studies revealed a congruence between them. On the other hand, many environmental microorganisms have a low growth rate under laboratory conditions, which often leads to them not being detected through cultivation (Yurkov and Pozo, 2017).

High-throughput sequencing has increased the detection of yeasts as an important component of microbial communities in neotropical (Dunthorn et al., 2017; Vaz et al., 2017) and temperate soils (Mašínová et al., 2017). In Neotropical forests in South and Central America, the environmental metabarcoding revealed that yeasts dominate fungal communities in terms of the number of sequencing reads and OTUs (Dunthorn et al., 2017). The authors attribute this dominance of yeasts to predominantly watery conditions in the soils due to frequent, sometimes daily, flooded soil conditions. Nevertheless, the main species detected by sequence data have commonly been isolated using cultivation

techniques (Stringini et al., 2008; Yurkov et al., 2012; Mestre et al., 2014; Glushakova et al., 2017; Boonmak et al., 2019).

Recent and still few studies, addressing culture and sequence data, have revealed that soil yeasts represent a group of well cultivable microorganisms (Mašínová et al., 2017, 2018). That study showed a good overlap between cultivated and detected species, confirming that soil yeasts can be reasonably well cultivated. However, when comparing our data, we see some important differences between culture and sequence data. The *Debaryomyces*, *Lipomyces*, *Meyerozyma*, and *Pseudozyma* genera were detected in the culture data, but not in the sequence data. All four genera were present in the database used to perform the taxonomic assignment. The first three genera are ascomycetes and the last basidiomycetes. Ascomycetous yeasts have a rapid growth that can be further favored by the nutritional conditions of the culture media used (Yurkov and Pozo, 2017). We suggest that this characteristic can often mask the predominance of basidiomycetous, and facilitate its detection by culture methods, even if it is in smaller proportions in the samples. On the other hand, due to the bias embedded in the metabarcoding methodology, mainly due to the markers used, may have led to the non-detection of these taxa. The choice of primers affects the results of amplicon-based metabarcoding studies. Among the limitations of the marker used, we can mention the variation in primer coverage at different taxonomic levels due to differences in degeneration and the location of primer sets, generation of chimeric and incompatible readings, as well as great variation in the abundance of species (Li et al., 2020).

For culture data, we have two considerations that may have limited access to diversity. First, in the isolation stage, several yeast colonies grow on the same plate, and many with similar morphology, although they may represent different species. The common choice of a colony among several on the same plate, but representing different species of yeast, creates bias in later analyses. Second, we use only one generic culture medium for the isolation and growth of yeasts, the YM agar. Although all known cultivable yeast species can grow in this medium, it is possible that fast-growing species have been favored, masking the diversity of those of slow growth.

Our results are consistent with those found by Mašínová et al. (2017), where there are also no reports of the genera *Debaryomyces*, *Pseudozyma*, *Meyerozyma*, and *Lipomyces*. The first three, although frequently reported in soil, are genera associated with the phylloplane (Limtong and Kaewwichian, 2015; Nasanit et al., 2015). *Lipomyces*, on the other hand, is an indigenous genus in soils most easily detected from the mineral soil horizons, as there is an increase in the abundance of this genus with the depth of the soil (Glushakova et al., 2017). Here, we evaluate only the topsoil, where the abundance of this genera tends to be lower.





This is the first study to our knowledge to directly compare assessments of yeast diversity in tropical soils in South America, using conventional cultivation techniques and environmental DNA sequencing. The combination of a culture-independent metagenomic with a culture-based approach allows for a more realistic understanding of the diversity of yeast species.





## CONCLUSION



Soil fungal communities respond to the predominant vegetation type. Eucalyptus, a revegetated ecosystem, showed high similarity with the Atlantic Forest, the equivalent pristine ecosystem before the disturbance. Grass, an ecosystem in rehabilitation, and Iron outcrops, an endemic ecosystem in Minas Gerais State, presented different communities. Grass presented the greatest species richness. The yeast community represented a significant proportion of the total fungi community, depending on the site. The proportion of Basidiomycota and Ascomycota in the yeast community may be indicative of soil quality



in post-mining areas. *Saitozyma podzolica* showed the highest relative abundance in both approaches, sequence, and culture data. Both approaches were complementary in describing the fungal soil communities. This study contributes significantly to the inventory of yeast species in tropical and subtropical soils, a region still insufficiently sampled.

## AUTHOR CONTRIBUTIONS



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