Acta Botanica Brasilica - 34(4): 796-806. October-December 2020.
doi: 10.1590/0102-33062020abb0118

Communities of Mucorales (phylum Mucoromycota) in different ecosystems of the Atlantic Forest

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Received: March 25, 2020
Accepted: October 12, 2020

ABSTRACT

As primary decomposers of organic matter, mucoralean fungi have an important ecological role in edaphic systems in the Atlantic Forest. However, there is a knowledge gap regarding how communities of Mucorales are structured in soils of Atlantic Forest areas, and whether these communities are influenced by edaphic attributes in this domain. Thus, the current study aimed to understand the influence of edaphic attributes linked to species richness, abundance and composition of Mucorales in dense ombrophilous forest, '*tabuleiro*' forest, sandbank and mangrove ecosystems located in Pernambuco, Brazil. Altogether, twenty-three taxa, including seven new records, were reported from soil samples from the ecosystems. Species composition was similar among the ecosystems, except for mangrove, while species richness and diversity of Mucorales were highest in dense ombrophilous forest and '*tabuleiro*'. Together the soil variables were responsible for 35.5 % of the variation in species composition, with pH being responsible for 53.32 % and 47.24 % of the variation in richness and abundance of these communities, respectively. These data indicate that pH is the most important attribute in delimiting the structure of mucoralean communities in the study areas, with influence on the composition, richness, and abundance of these fungi.

Keywords: basal fungal order, diversity, ecology, Mucorales, Mucoromycota, Mucoromycotina, soil, taxonomy

Introduction

Mucorales, a basal fungal order that belongs to the subkingdom Mucoromyceta Doweld, comprises species morphologically characterized by the production of asexual structures, such as sporangia, sporangiola and merosporangia, and by the formation of a sexual spore, the zygospore, in a zygosporangium formed after the fusion of two gametangia (Spatafora *et al*. 2016; Tedersoo *et al*. 2018). These fungi have a worldwide distribution and have been commonly reported in animal dung, stored cereals, fruits, vegetables, and soil, although some species are facultative pathogens of plants, animals, and even other fungi (Hoffmann *et al*. 2013; Richardson & Rautemaa-Richardson 2020).

The mucoralean communities in soil play an important role in ecological processes as most of specimens are saprobes, that is, primary decomposers of organic matter and able to degrade mainly simple carbon sources, with some species capable of degrading pectin and hemicelluloses, as well as some lipids and proteins (Ferreira *et al*. 2013; Lima *et al*. 2016a). The biochemical properties of some Mucorales

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spp., such as the production of a large spectrum of enzymes, make these fungi essential for the recycling of nutrients (Richardson 2009; Ziaee *et al*. 2016).

Studies on how mucoralean communities are structured are extremely relevant since these fungi are pioneers in the ecological succession processes of several substrates (Richardson 2009; Spatafora *et al*. 2016). Despite this, ecological data regarding mucoralean communities in soil are still scarce, especially regarding the influence of edaphic attributes on these communities. There have only been three ecological studies focusing on soil mucoralean communities: two in semi-arid regions and one in the Atlantic Forest, all undertaken in Brazil, though none of them used this approach (Santiago *et al*. 2006; Santiago *et al*. 2013; Lima *et al*. 2016a; Lima *et al*. 2018a).

The Atlantic Forest domain is known for its large biodiversity and for containing a high number of endemic species of various taxonomic groups, comprising 8 % of global biodiversity (IBGE 2011; Rezende *et al*. 2018). This domain has been largely degraded due to the continuous cycle of extraction, agriculture and pasture practices that culminated in the deforestation and fragmentation of forest areas, which are therefore considered as higher priority hotspots for biodiversity conservation (Myers *et al*. 2000; Rezende *et al*. 2018). However, only 2 % of these areas are legally protected in conservation units such as Biological Reserves and Environmental Protection Areas (IBGE 2011).

Mucorales spp., like other fungi, contribute for the maintenance of ecosystems, including those of the Atlantic Forest, and in this process their communities are influenced by the physical and chemical factors of the soil (Lauber *et al*. 2008; Ziaee *et al*. 2016; Lima *et al*. 2018a). However, it is unknown whether soil chemical properties, including pH, as well as other environmental variables, such as vegetation, may influence the structure of the mucoralean communities. In this present study, we addressed the following questions: Do edaphic attributes influence the mucoralean communities? If so, what are the main soil parameters associated?

The current study aimed to understand how these chemical attributes influence the richness, abundance and species composition of these fungi in four ecosystems of Atlantic Forest, through ecological indices (diversity and evenness), quantitative and qualitative population data (frequency of occurrence and relative abundance), and similarity among these ecosystems. We also discuss how soil properties are linked to variation in these communities. Therefore, our research helps to provide an understanding of how local mucoralean communities are structured by edaphic attributes, mainly in tropical and subtropical forests.

Materials and methods

Study areas

This study was conducted in two areas: the Saltinho Biological Reserve (8°43'34.73"S – 35°10'37.26"W) and the Guadalupe Environmental Protection Area (8°46'11.52" S – 35°06'27.24" W), both located in the ecoregion of the Pernambuco coastal forests in the municipality of Tamandaré. The climate of both areas is characterized as humid (As'), according to Koppen, with an average annual temperature of 25 °C. Rainfall is evenly distributed throughout the year, with no truly dry season and higher precipitation between March and July, while the period of low rainfall occurs from October to December, with an annual precipitation of 1,500 to 2,000 mm. The vegetation in the Saltinho Biological Reserve is predominantly composed of dense ombrophilous forest. The phytogeographic domain that surrounds the Guadalupe Environmental Protection Area is diverse and comprises areas of '*tabuleiro*' forest, sandbanks and mangroves (IBAMA 2003; Rodrigues *et al*. 2010). All the above-mentioned ecosystems belong to the Brazilian Atlantic Forest floristic domain, which is inserted in the Tropical and Subtropical Moist Broadleaf Forest biome (http://ecoregions2017.appspot.com).

The dense ombrophilous forest (DOF) is a well-developed forest with a canopy of 20 to 30 m and species reaching 50 m in height. It includes species of Anacardiaceae, Euphorbiaceae, Lauraceae, Mimosaceae, Moraceae, Myrtaceae and Sapotaceae families (IBAMA 2003). '*Tabuleiro*' (TAB) is an ecosystem that develops along the edge of the Atlantic Forest, exhibiting plant species shared with the '*Cerrado*' floristic domain, which is part of the Tropical and Subtropical Savanna biome, in areas of sandy soil near the coast. The vegetation appears in open areas in coastal enclaves (Almeida *et al*. 2009). In addition, it can form a vegetative continuum with sandbank areas, sharing plant species with this ecosystem (Andrade-Lima 1970; Oliveira-Filho & Carvalho 1993). The vegetation of sandbanks (SAN) can vary in physiognomies, from herbaceous to arboreal types, in which plants like *Remirea maritima* Aubl. and *Canavalia rosea* (Sw.) DC. are found (Sampaio *et al*. 2005). Mangrove (MAN) ecosystems are exposed to conditions of extreme salinity, and are considered aquatic and terrestrial intermediary systems, with *Rhizophora mangle* (Rhizophoraceae), *Laguncularia racemosa* (Combretaceae) and *Avicennia* spp. (Acanthaceae) included in their composition (Castiglioni & Coelho 2011).

Sampling sites

Seven expeditions for soil collection were done monthly from June to December of 2014 at the Saltinho Biological Reserve and the Guadalupe Environmental Protection Area, specifically in areas of DOF (8°43'28.63" S – 35°10'45.89" W), TAB (8°45'08.65" S – 35°07'44.22" W), SAN (8°46'29.06" S – 35°06'35.21" W) and MAN (8°46'24.46" S – 35°06'27.22" W). At each of the above-mentioned areas, eight quadrants of 100 m^2 (10 × 10 m) were spatially dispersed and randomly distributed, with a minimal distance of 200 m among them. In each quadrant, using sterilized spatulas, eight soil sub-samples up to 5 cm deep were collected, placed in clean plastic bags and stored in styrofoam boxes with ice during transport to the Laboratory of the Universidade Federal de Pernambuco (UFPE). In the lab, the eight soil sub-samples collected in each quadrant were mixed to form one composite sample per quadrant, totaling eight composite samples in each area from each collection expedition. Considering the seven collection expeditions, 56 composite samples were analyzed from each area, for a total of 224 samples considering the four areas. The soil samples were stored in the laboratory (UFPE) for isolation of Mucorales and chemical analysis of the soil.

Isolation, purification and identification of Mucorales

Five milligrams of soil from each of 224 composite samples (672 Petri dishes) were sprinkled over Petri dishes containing wheat germ agar culture medium plus chloramphenicol (80 $mg.L^{-1}$) in triplicate (Benny 2008). Colony growth was monitored for 96 hours at 28°C. In order to purify the Mucorales, fragments of the colonies were transferred separately to MEA [malt extract agar, plus chloramphenicol (80 $mg.L^{-1}$)] (Benny 2008). Taxa were identified by observing their macroscopic (color, appearance and diameter of colonies) and microscopic (microstructures) characteristics, as described by Hesseltine & Ellis (1964), Benny & Benjamin (1975), Benny (1982), Schipper (1984; 1990), Zheng & Chen (2001), Domsch *et al.* (2007) and Zheng *et al.* (2007).

Soil analyses

Soil pH and other chemical soil analyses were performed from three analytical replicates per each composite soil sample from each quadrant of each area. The analyses were conducted at the 'Estação Experimental de Cana de Açúcar do Carpina' of the Universidade Federal Rural de Pernambuco, using standard methods to determine P, Ca, Mg, Na, K, Al^{3+}, H^+, S and cation exchange capacity (CEC) levels according to EMBRAPA (1998) and Jackson (2005).

Data Analysis

The frequency of occurrence (FO) of the species was estimated according to the following equation: FO = Ji/k x 100, in which: FO = frequency of occurrence of species i; Ji = the number of samples in which species i occurred, and k = the total number of soil samples. According to this formula, the species were classified as follows: very frequent (>10 %), frequent (5-10 %), infrequent (≧1-5< %), and rare (< 1 %) (Hyde & Sarma 2001). Species accumulation curves were also calculated per area, allowing the expected and observed richness to be calculated using the Chao 1 and Jackknife 1 estimators, respectively (Clarke & Gorley 2006). The Shannon Wiener diversity index and Pielou's evenness were estimated using R software (RStudio Team 2009). In addition, the richness and abundance (number of colony forming units) were calculated. The relative abundance of each species within the three studied areas was evaluated according to the following equation: RA = (Ni/N) x 100, where RA = relative abundance of the species i; Ni=number of CFU of the species i; N = total number of CFU of fungi in all samples in each area). According to this formula, each taxon can be classified as one of the following: RA < 0.5 % = rare; 0.5 ≤ RA < 1.5 % = occasional; 1.5 ≤ Ra < 3.0 % = common; or RA > 3.0 % = abundant. An analysis of indicator species was performed to assess the statistical significance of relationships among Mucorales species abundance in each area inventoried. The indicator value was calculated and the significance was obtained using the Monte Carlo test (500 random replications) according to Dufrêne & Legendre (1997), being considered indicator species when values were greater or equal to 25 % with $p<0.05$. The richness, abundance, Shannon diversity and Pielou's evenness values were submitted to analysis of variance (One-way ANOVA) using Past software and means were compared by the Tukey's pairwise test ($P \leq 0.05$) (Hammer *et al.* 2001). The soil chemical attributes were also submitted to analysis of variance and the means compared by the Tukey test ($p \leq 0.05$). NMDS (Non-Metric Multidimensional Scaling) was carried out to determine the composition of Mucorales species in the study areas. This analysis was based on a matrix of communities using Euclidean distance. In addition, Analysis of Similarity (ANOSIM) was used to detect significant differences among the groups identified in the NMDS. The influence of soil variables on the Mucorales community was evaluated using principal component analysis (PCA) and the existence of a relationship among Mucorales species richness, abundance and the soil variables was tested through multiple models. Before this test, multicollinearity among the ten soil variables was evaluated through the variance inflation factor (VIF), and only variables with a factor less than 5 were included in the linear model. To test the independence of the quadrants sampled within each area, we evaluated eight linear models, four to each variable (richness and abundance). Two standard linear models as follow: i) all soil predictor variables without quadrants as fixed effects and without random effects; ii) all soil predictor variables and quadrants as fixed effects without random effects; in addition to two mixed models; iii) all soil predictor variables as fixed effects and quadrants as random effects; iv) and a null model with only quadrants as random effects. These last two were adjusted by the Restricted Maximum Likelihood (REML) to avoid bias in the variance estimates of the models. The model selection was performed using the likelihood ratio test according to Zuur *et al.* (2009). All analyses were performed using R software and the RStudio interface with an alpha level of 0.05 (RStudio Team 2009).

Results

Overall, twenty-three species of Mucorales were isolated from the soils of Atlantic Forest ecosystems distributed among *Absidia*, *Backusella*, *Cunninghamella*, *Gongronella*, *Lichtheimia*, *Mucor*, *Rhizopus* and *Syncephalastrum*. The DOF and TAB areas had the greatest number of Mucorales colony-forming units per gram of soil (Tab. 1).

Gongronella butleri was very frequent (FO = 16.6 %) in DOF, followed by *G. brasiliensis* (FO = 7.14 %), *R. microsporus* (FO = 5.35 %) and *M. irregularis* (FO = 5.35 %). In TAB, four taxa were frequent: *G. butleri* (FO = 8.92 %), *C. elegans*, (FO = 5.95 %), *M. indicus* (FO = 5.95 %) and *S. racemosum* (FO = 5.95 %). *Rhizopus microsporus* (FO = 16.6 %) and *G. butleri* (FO = 5.35 %) were very frequent in SAN areas. According to the relative abundance of each taxon, most species of Mucorales were rare. *Gongronella butleri* was occasional in all ecosystems, except in MAN, while *R. microsporus* was occasional in SAN. Only two species were isolated from MAN: *R. stolonifer* (FO = 1.19 %, RA = 0.26 %) and *S. racemosum* (FO = 2.90 %, RA = 0.66 %), both of which were infrequent and rare (Tab. 2). Nine species were defined as indicators for specific ecosystems and could be described as characteristic for these areas: three for DOF and six for TAB (Tab. 2).

The average abundance (N), richness (S), Shannon Wiener index (H') and Pielou's evenness index (J') of the Mucorales communities were greatest in the soil of DOF and TAB areas, with no significant difference between these two areas (Fig. 1). SAN exhibited intermediate values while MAN exhibited the lowest values with fewer species and individuals than the other ecosystems with significant differences between extreme values (*e.g.* TAB *vs* MAN) (Fig. 1). The species accumulation curve indicated a sufficient sampling performance. The Chao 1 estimator indicated the same values for all areas (Fig. 2). However, Jackknife 1 revealed that richness may have been higher than what was observed in DOF (20.94 ± 1.7) (Fig. 2).

The highest similarity indices of the Mucorales community were observed between DOF and SAN areas (54.13 %), followed by SAN and TAB (50 %), and DOF and TAB (47.45 %) areas. The MAN community exhibited low similarities with all other areas (Tab. 3). This result was corroborated by the ANOSIM (Analysis of Similarity), which showed that MAN differs significantly from DOF, TAB and SAN areas. However, there were no significant differences in species composition among DOF, TAB and SAN areas (Fig. 3).

Principal component analysis (PCA) revealed low percentages of explanation, with the sum of the first two dimensions explaining 35.5 % of the variation (Fig. 4). Thus,

Table 1. Number of colony forming units of Mucorales per gram of soil (CFU.g-1) in the soil of dense ombrophilous forest (DOF), '*tabuleiro*' forest (TAB), sandbanks (SAN) and mangroves (MAN) from the Saltinho Biological Reserve and Guadalupe Environmental Protection Area. Values followed by the same lowercase letter do not differ in the Tukey test (p = 0.05).

Mucorales species	Areas			
	DOF	TAB	SAN	MAN
Absidia sp.	-	1.2×10^3	-	-
A. cylindrospora Hagem	0.8×10^3	-	-	-
A. pseudocylindrospora Hesselt. & J.J. Ellis	1.6×10^3	-	-	-
Backusella constricta D.X. Lima, de Souza & A.L. Santiago	0.4×10^3	-	-	-
Cunninghamella bertholletiae Stadel	0.8×10^3	-	-	-
C. blakesleeana Lendn.	-	2×10^3	1×10^3	-
C. echinulata Thaxt.	1.2×10^3	3×10^3	-	-
C. elegans Lendn.	0.8×10^3	6.2×10^3	-	-
C. phaeospora Boedijn	1.2×10^3	-	-	-
Gongronella brasiliensis C.A. de Souza, D.X. Lima & A.L. Santiago	10.6×10^3	-	1.4×10^3	-
G. butleri Peyronel & Dal Vesco	17.8×10^3	11.8×10^3	15.8×10^3	-
Lichtheimia brasiliensis A.L. Santiago, Lima & Oliveira	-	2.8×10^3	-	-
L. ramosa Vuill.	-	1.6×10^3	-	-
Mucor lusitanicus Bruderl.	0.8×10^3	-	-	-
M. hiemalis Wehmer	1.8×10^3	-	-	-
M. indicus Lendn.	1.4×10^3	5.8×10^3	-	-
M. irregularis Stchigel, Cano, Guarro & E. Álvarez	3.4×10^3	-	-	-
M. luteus Linnem. ex Wrzosek	-	3.2×10^3	-	-
M. simplex Tiegh.	0.8×10^3	1.4×10^3	-	-
Rhizopus arrhizus var. *arrhizus* A. Fisch	1.8×10^3	1.2×10^3	3.2×10^3	-
R. microsporus Tiegh	4×10^3	6.6×10^3	9.4×10^3	-
R. stolonifer (Ehrenb.) Vuill.	0.6×10^3	-	2.6×10^3	0.4×10^3
Syncephalastrum racemosum Cohn ex J. Schröt	5.6×10^3	4×10^3	0.6×10^3	1×10^3
Total	55.4×10^3a	50.8×10^3a	34×10^3b	1.4×10^3c

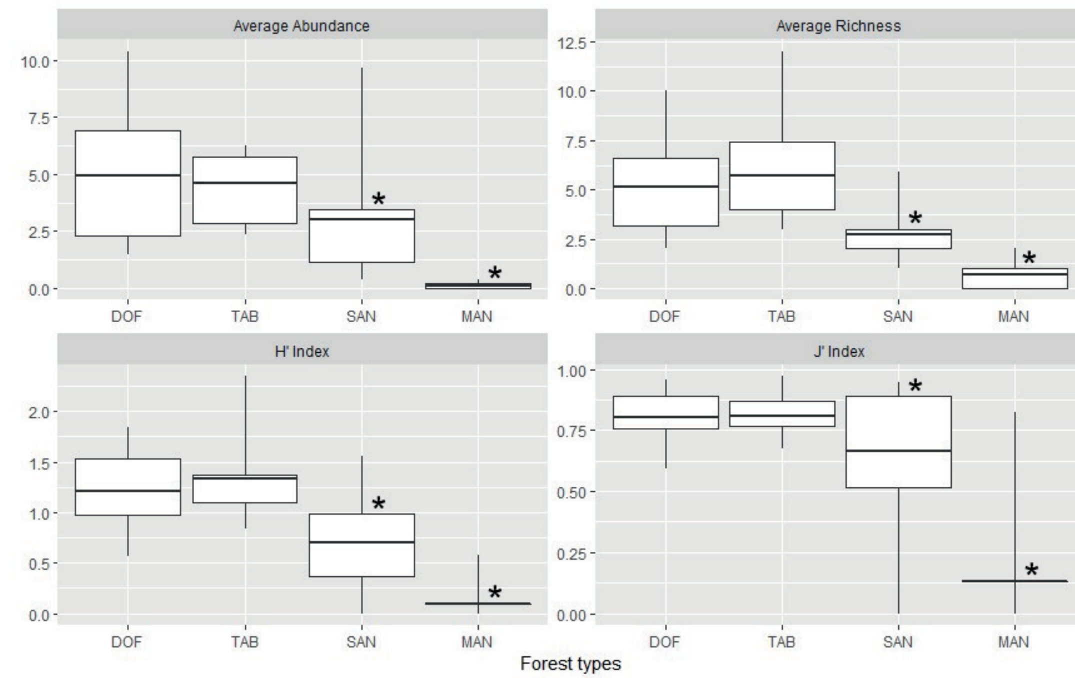

Figure 1. Average Richness (S), Abundance (N), Shannon Wiener index (H') and Pielou's Evenness index (J') of Mucorales communities from dense ombrophilous forest (DOF), '*tabuleiro*' forest (TAB), sandbanks (SAN) and mangroves (MAN) from the Saltinho Biological Reserve and the Guadalupe Environmental Protection Area. Asterisks (*) indicate significantly higher values of the evaluated attribute based on one-way ANOVA. Median (central dot), quartile (box), maximum and minimum (error bars) are shown.

Table 2. Frequency of occurrence (FO) and relative abundance (RA) of Mucorales in soils from dense ombrophilous forest (DOF), '*tabuleiro*' forest (TAB), sandbanks (SAN) and mangroves (MAN) from the Saltinho Biological Reserve and the Guadalupe Environmental Protection Area.

Species	Areas							
	DOF		TAB		SAN		MAN	
	FO	RA	FO	RA	FO	RA	FO	RA
Absidia sp.	-	-	2.97	0.17	-	-	-	-
A. cylindrospora	1.78	0.1	-	-	-	-	-	-
A. pseudocylindrospora	1.78	0.21	-	-	-	-	-	-
Backusella constricta	1.19	0.05	-	-	-	-	-	-
Cunninghamella bertholletiae	1.78	0.1	-	-	-	-	-	-
C. blakesleeana	-	-	3.57	0.29	1.78	0.2	-	-
C. echinulata.	1.19	0.16	4.76	0.44*	-	-	-	-
C. elegans	2.38	0.1	5.95	0.92*	-	-	-	-
C. phaeospora	3.57	0.16*	-	-	-	-	-	-
Gongronella brasiliensis	7.14	1.44*	-	-	1.78	0.28	-	-
G. butleri	16.66	2.4	8.92	1.76	5.35	1.56	-	-
Lichtheimia brasiliensis	-	-	2.97	0.41*	-	-	-	-
L. ramosa	-	-	4.16	0.23*	-	-	-	-
Mucor lusitanicus	1.78	0.1	-	-	-	-	-	-
M. hiemalis	1.73	0.24	-	-	-	-	-	-
M. indicus	1.19	0.18	5.95	0.86*	-	-	-	-
M. irregularis	5.35	0.46*	-	-	-	-	-	-
M. luteus	-	-	4.76	0.47*	-	-	-	-
M. simplex	1.78	0.1	1.78	0.2	-	-	-	-
Rhizopus arrhizus var. *arrhizus*	2.38	0.24	3.57	0.17	3.57	0.65	-	-
R. microsporus	5.35	0.54	4.16	0.98	16.60	1.93	-	-
R. stolonifer	1.19	0.08	-	-	2.97	0.53	1.19	0.26
Syncephalastrum racemosum	-	-	5.95	0.59	1.78	0.15	2.90	0.66

* Species with indicator value for a specific site (I value ≥ 25 % and $p < 0.05$; based on numbers of colony forming units; Dufrêene & Legendre 1997).

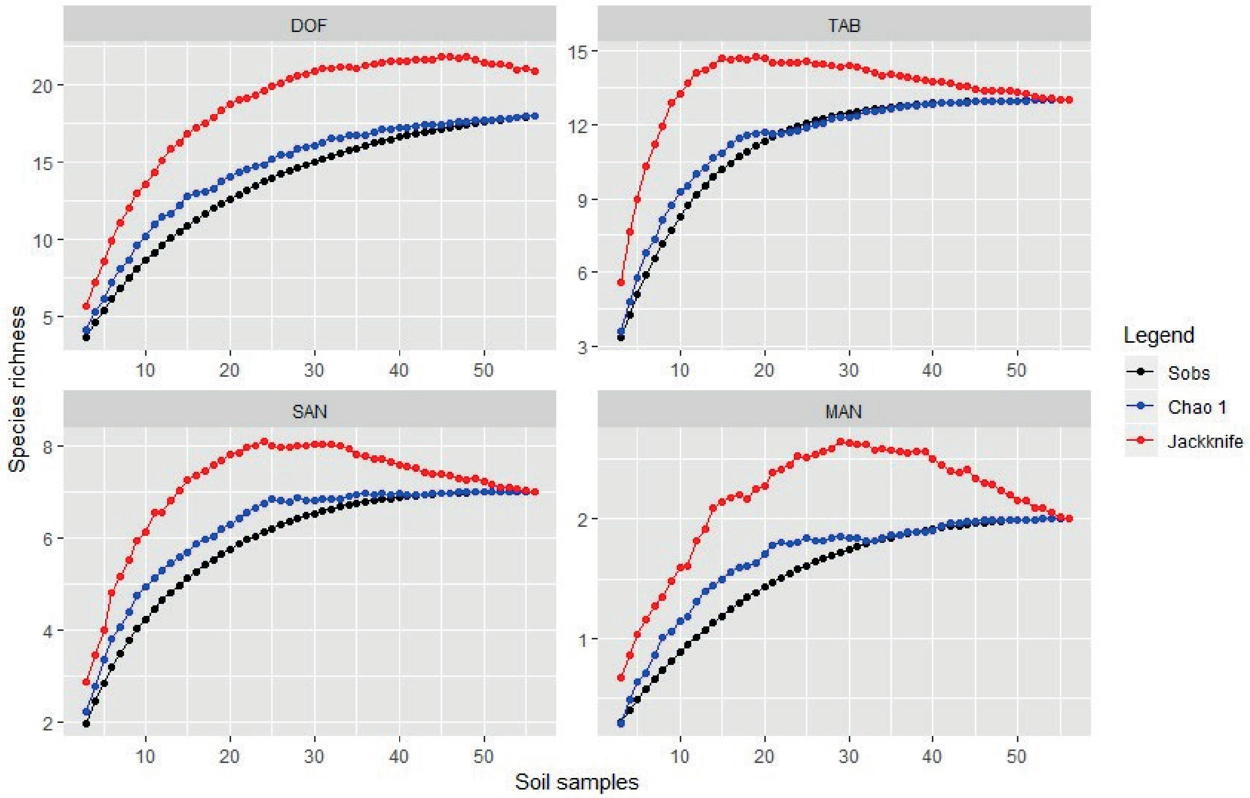

Figure 2. Chao 1 and Jackknife 1 Richness estimators of Mucorales from dense ombrophilous forest (DOF), '*tabuleiro*' forest (TAB), sandbanks (SAN) and mangroves (MAN) from Saltinho Biological Reserve and Guadalupe Environmental Protection Area.

the contribution of chemical-physical attributes to the composition of soil varied between 7 % and 8 % (Fig. 4). In general, the concentration of edaphic attributes analyzed differed among areas, with some exceptions between TAB-SAN (Mg, Na, K and S) and MAN-SAN (P and Al^{3+} + H^+) (Tab. 4). At DOF, there were high concentration of phosphorus, magnesium, potassium, soil acidity (Al^{3+} + H^+) and cation exchange capacity, while at MAN, high levels of sodium and sulfur were found. Soil data revealed three groups of communities: (i) DOF; (ii) TAB + SAN; (iii) MAN. After colinearity tests, only P, pH, Ca and Mg were selected for multiple models. Comparing the models, we did not obtain significant differences among them, which indicates that the dependence among the quadrant samples does not significantly affect these variables (Tab. 5). Thus, we selected the simplest model (model i) to test the influence of soil variables on the abundance and richness of Mucorales. According to these regressions, only pH significantly affected the richness and abundance of the Mucorales communities (Multiple regression - Richness; $F(4.23)=8.71$, $p < 0.01$; Multiple regression - Abundance; $F(4.22)=6.82$, $p < 0.01$), being responsible for 53.32 % and 47.24 % of the variation in richness and abundance found in the Mucorales communities, respectively (Fig. 5).

Table 3. Similarity of Mucorales composition between dense ombrophilous forest (DOF), '*tabuleiro*' forest (TAB), sandbanks (SAN) and mangroves (MAN) from the Saltinho Biological Reserve and the Guadalupe Environmental Protection Area.

Areas	DOF	TAB	SAN
TAB	47.45 %	-	-
SAN	54.13 %	50 %	-
MAN	4.92 %	3.83 %	5.64 %

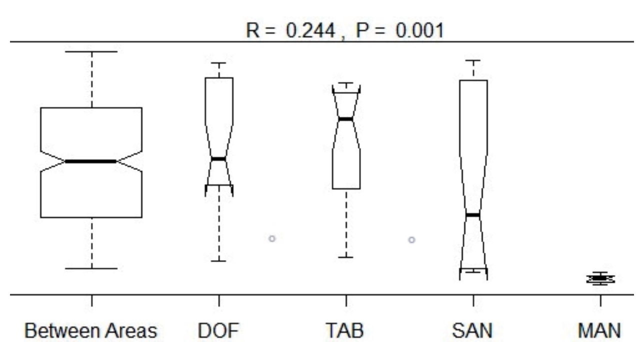

Figure 3. Analysis of Similarity (ANOSIM) results among communities of Mucorales of dense ombrophilous forest (DOF), '*tabuleiro*' forest (TAB), sandbanks (SAN) and mangroves (MAN) from the Saltinho Biological Reserve and the Guadalupe Environmental Protection area. Average dissimilarity calculated using Bray-Curtis distance.

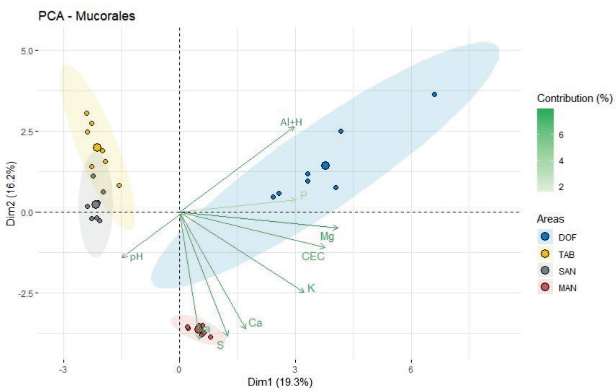

Figure 4. Two-dimensional Projection of Principal Component Analysis (PCA) of seven soil samples in each different vegetational type at the Saltinho Biological Reserve and the Guadalupe Environmental Protection Area. The colored dots represent soil samples and larger are the samples centroids. Green arrows show evaluated soil nutrients and their contribution to the variation of principal components. Legend: "Al" Aluminum, "Ca" calcium, "CEC" cation exchange capacity, "H" hydrogen, "K" potassium, "H" hydrogen, "Mg" magnesium, "Na" sodium, "P" phosphorus, "S" sulfur.

Discussion

This manuscript reports ecological data regarding the communities of Mucorales in DOF, TAB, SAN and MAN ecosystems located in Pernambuco, Brazil. Most of the mucoralean species isolated in the present study have already been reported in other inventories of Atlantic Forest soil (Eicker 1969; Varghese 1972; Ogbonna & Pugh 1982; Rambelli *et al*. 1984; Bettuci & Roquebert 1995; Schoenlein-Crusius *et al*. 1996; Schoenlein-Crusius & Milanez 1997; Schoenlein-Crusius & Milanez 1998; Schoenlein-Crusius *et al*. 2006; Maia *et al*. 2006; Lima *et al*. 2018a). However, seven new records for this domain are reported herein: *Absidia* sp., *Cunninghamella bertholletiae*, *C. elegans*, *Gongronella brasiliensis*, *Lichtheimia ramosa*, *Mucor indicus*, and *M. irregularis*, which represent an addition of almost 10 % of the currently known species in the Brazilian Atlantic Forest for which 66 species of Mucorales are currently recorded (Flora do Brasil 2020). Although DOF, TAB, SAN and MAN are part of the same domain, the Mucorales communities in these ecosystems varied in relation to richness and abundance of species.

Some genera were found predominantly or exclusively in DOF, such as *Absidia*, *Backusella* and *Mucor*. *Absidia* species are described as common in tropical forests soils, whereas *B. constricta* is the only species of this genus reported in DOF (Pfenning & Abreu 2006; Lima *et al*. 2016b; Lima *et al*. 2018a). Although several *Mucor* species have been previously reported in the Atlantic Forest (Flora do Brasil 2020), *M. irregularis* isolated during this survey was recorded for the first time in South America (Lima *et al*. 2018b). *Cunninghamella* species have been commonly found in the soil of the Atlantic Forest and soils of subtropical regions (Domsch *et al*. 2007; Lima *et al*. 2018a). Although *S. racemosum* is a common soil species, this is the second report of it in Brazilian Atlantic Forest soils (Domsch *et al*. 2007; Lima *et al*. 2018a). *Rhizopus* and *Lichtheimia* are ubiquitous, but they are more common in soils from the Brazilian semi-arid areas than in Brazilian Atlantic Forest soils (Santiago *et al*. 2013; Lima *et al* 2016a; Lima *et al*. 2018a). Among the isolated species, some are indicators or characteristic of these ecosystems, such as *C. phaeospora*, *G. brasiliensis* and *M. irregularis*, found in DOF, and *C. echinulata*, *C. elegans*, *L. brasiliensis*, *L. ramosa*, *M. indicus* and *M. luteus* observed in TAB (Dufrêne & Legendre 1997). These indicator species might be useful to define the habitat preference of these species (Bouffaud *et al*. 2016).

Table 4. Soil chemical attributes at areas of dense ombrophilous forest (DOF), '*tabuleiro*' forest (TAB), sandbanks (SAN) and mangroves (MAN) from the Saltinho Biological Reserve and the Guadalupe Environmental Protection Area.

Area	P	pH	Ca	Mg	Na	K	$Al^{3+} + H^{+}$	S	CEC
DOF	8.4a	5.5c	1.3b	1.4a	0.1b	0.3a	8.8a	2.7b	12.4a
TAB	3.00c	5.25d	0.65c	0.64c	0.06c	0.04c	4.55b	1.40c	6.30c
SAN	4.71b	6.07a	0.56d	0.64c	0.05c	0.05c	2.37c	1.35c	3.39d
MAN	4.57b	5.74b	2.79a	1.04b	4.23a	0.2b	2.29c	8.33a	10.70b

Values followed by the same lowercase letter do not differ in the Tukey test (p = 0.05).

Table 5. Likelihood ratio test for eight linear models performed to evaluate the influence of soil variables on the richness and abundance of Mucorales in four forest types from the Saltinho Biological Reserve and the Guadalupe Environmental Protection Area. Legend: DF - Degrees of Freedom; AIC - Akaike information criterion.

Compared models	X^2	DF	p-value	1º AIC	2º AIC	Variable
Model i) - Model iii)	0.0	1	1	118.24	120.24	Richness
Model ii) - Model iii)	7.65	5	0.17	122.58	120.24	Richness
Model iii) - Null Model	7.96	4	0.06	120.24	122.20	Richness
Model i) - Model iii)	0.0	1	1	137.32	139.32	Abundance
Model ii) - Model iii)	8.24	5	0.14	141.08	139.32	Abundance
Model iii) - Null Model	9.41	4	0.06	139.32	140.74	Abundance

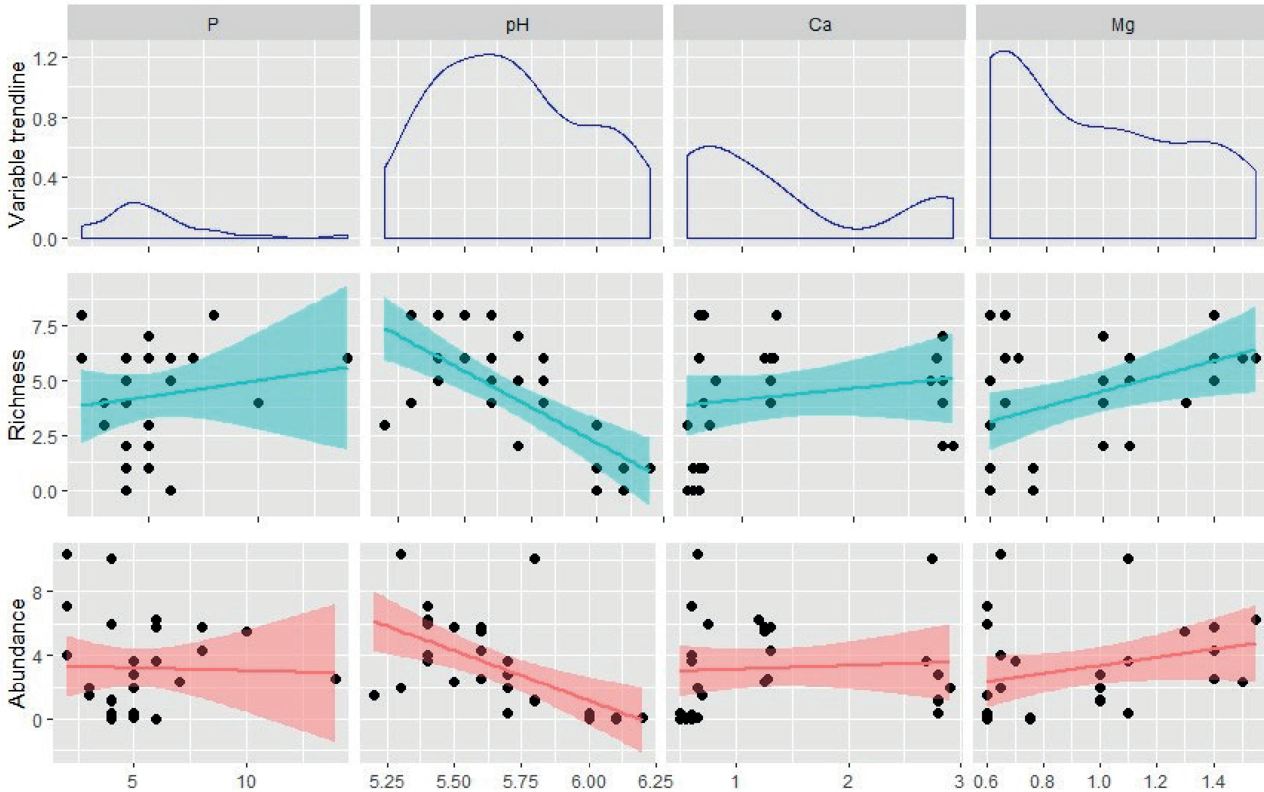

Figure 5. Multiple regression with P, pH, Ca and Mg showing a negative effect of pH on the richness and abundance of Mucorales communities in the Saltinho Biological Reserve and the Guadalupe Environmental Protection Area.

Although Mucorales species were present in the soil of all the ecosystems studied, the frequency of occurrence and the relative abundance of the majority of species were low. Other studies have also shown that the majority of mucoralean species in soil is infrequent and abundance is relatively low (Lima *et al*. 2016a; Oliveira *et al*. 2013). However, *G. butleri* was occasional, very frequent or frequent in most ecosystems with the exception of MAN. This species was reported as frequent in tropical forests and with a greater distribution in Atlantic Forest ecosystems (Lima *et al*. 2018a). It is considered generalist in terms of habitat use due to its broad geographic distribution (Persiani *et al*. 1998). This result was expected, as species of Mucorales commonly have few taxa with a high frequency of occurrence and a clear dominance over other species (Richardson 2009; Lima *et al*. 2016a; Lima *et al*. 2018a).

Fungi may be sensitive to changes in the vegetation type (Heinemeyer *et al*. 2004), but the distribution of a species in a given soil is also influenced by abiotic factors such as temperature, pH, salinity, amount of organic matter and nutrients (Cruz *et al*. 2017). The soil variables analyzed together in the present study were responsible for 35.5 % of variation in the species composition of the Mucorales community. Of all test variables, Na and S exerted greater influence on the species composition of communities in MAN, whereas $Al^{3+} + H^{+}$ exerted greater influence on the species composition in DOF. Additionally, differently from what has been reported by Lauber *et al*. (2008) and Rousk *et al*. (2010) in other fungal communities, we also found a direct influence of pH on the composition of Mucorales in our soil samples, mainly in TAB and SAN.

Some surveys have indicated that the structure of soil fungal communities is more influenced by nutrients than pH (Lauber *et al*. 2008; Rousk *et al*. 2010). However, we found that pH was responsible for 53.32 % and 47.24 % of the variation in richness and abundance found in the Mucorales communities, respectively. This became clear after observing that the richness and abundance of Mucorales decreased when pH increased (Fig. 5). SAN, for example, seemed more affected by pH variation, since it had a higher pH than the other areas, and the means of richness and abundance were lower than DOF and TAB (Tab. 4, Fig. 1). According to Glassman *et al*. (2017), the pH is a strong factor of shaping fungal communities due to the fact that it affects the availability of all soil nutrients, and an acidic pH is most favorable to the development of Mucorales (Richardson & Rautemaa-Richardson 2020). On the other hand, the variation of other edaphic factors of ecosystems, climatic conditions, as well as fungal physiology and competition for organic matter resulting from niche overlap may also alter the microbial structure in the rhizosphere (Bills *et al*. 2004; Pandey & Palni 2007).

The community of Mucorales in DOF showed a high number of $CFU.g^{-1}$ of soil. This fact may be explained by the low average soil pH value of this area, in addition to the high pool nutrient availability and potential acidity (H^+ + Al^{3+}) in these soils (Tabarelli *et al*. 2005; Cruz *et al*. 2013), determining factors for the increased abundance and diversity of fungi in an ecosystem (Heijden *et al*. 2008; Lauber *et al*. 2008). The richness and diversity of Mucorales in DOF were also high, as well as in TAB. However, according to Jackknife 1, the estimated richness was attained for all study areas, except for DOF, which indicates that the richness obtained was lower than expected.

Although the mucoralean diversity in TAB was greater than in SAN, both ecosystems are similar in Mucorales species composition. This result was expected, since TAB and SAN naturally form a vegetation continuum that is difficult to define, sharing various plant species (Tavares 1964; Andrade-Lima 1970), moreover, both ecosystems share soil characteristics, such as Mg, Na, K and S. In addition, both TAB and SAN exhibit sandy soils, with open vegetation and intense solar radiation (Tavares 1964; Andrade-Lima 1970), which may explain the higher occurrence of the thermotolerant genera *Lichtheimia* and *Rhizopus* in these areas, since the high incidence of solar radiation favors the sporulation and germination of these fungal sporangiospores (Abdullah & Al-Bader 1990; Lima *et al*. 2016a).

Although MAN exhibits a soil pH favorable to the growth of Mucorales, it is considered an extreme ecosystem, with low soil oxygenation and high concentration of salinity and sulfides (Hossain & Nuruddin 2016; Doi *et al*. 2018), which was corroborated by our soil analysis (Tab. 4, Fig. 4) and seemed determinant for the lower Mucorales richness, abundance and diversity in this area. The lower species richness found in MAN soils explains the low similarity in species composition among MAN and the other areas (Tab. 3). However, species composition among DOF, TAB and SAN areas were quite similar, which suggests homogeneous communities among these areas. Due to the peculiar characteristics of MAN soil, such as high salinity, poor aeration and high temperatures, this ecosystem is a receptive habitat for thermophilic/thermo-tolerant and halophilic/halotolerant fungi (Doi *et al*. 2018; Jaitly & Rai 1982). Only *R. stolonifer* and *S. racemosum* were isolated from MAN soils in this study and both are hereby reported for the first time in mangrove sediments in Brazil. Species of these genera have been reported in mangrove ecosystems in India (Senthilkumaran *et al*. 2016) and on substrates with high saline concentration, such as marine algae in the Red Sea, Egypt (Abdel-Gawad *et al*. 2014). According Abdel-Gawad *et al*. (2014), the abundance of Mucorales increases with increased temperature and pH, and decreases with increased salinity. Therefore, the presence of these species in MAN may indicate that they are halotolerant.

The present study highlights the ecology of Mucorales in the Brazilian Atlantic Forest, increasing knowledge of the diversity, richness, frequency of occurrence and relative abundance of Mucorales in the soil of this domain. *Gongronella butleri* is common in Atlantic Forest ecosystems, except in mangrove ecosystems, which are not suitable for the establishment of most Mucorales species. The present study is a pioneering survey on Mucorales communities in ecosystems of '*tabuleiro*' forest and Brazilian mangrove. Although the chemical variables analyzed in the soil, such as Al^{3+} + H^+, Na and S, influence Mucorales composition, pH was the key edaphic attribute that influenced the composition, richness and abundance of mucoralean communities. Future research may identify the influence of other variables, such as the availability of organic matter and soil temperature, on the structure of these communities.

Acknowledgements

This study was financed by the project 'URM Herbarium: characterization and availability of the collection as a source of microbiological resources' (FACEPE – APQ – 0143-2.12/15) and 'Diversity of Mucoromycotina in the different ecosystems of the Atlantic Forest of Pernambuco' (FACEPE – First Projects Program PPP/FACEPE/CNPq – APQ – 0842-2.12/14). We thank the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco for the scholarships awarded to Diogo Xavier Lima. We also thank Roger Fagner Ribeiro Melo for his revision of the manuscript.

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