Original article

A higher altitude enclave of humid forest in the semi-arid of Brazil holds a diversity of conidial fungi on leaf-litter

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ABSTRACT

Brejos de Altitude are enclaves of higher altitude humid forests in the semiarid lowlands of the North-eastern of Brazil. They present unique characteristics in terms of soil and air humidity, temperature, vegetation cover, and biodiversity. Due to these conditions, many cattle ranchers and farmers develop activities that have caused habitat loss and fragmentation of biodiversity. In this study, we aimed to describe the diversity of conidial fungi that occur in the leaf litter of the riparian vegetation in a *Brejo de Altitude* in Pernambuco, Brazil. Decomposing leaf material was collected from the forest floor in the dry and rainy periods of 2019, incubated in moist chambers and observed daily for fungal structures, for up to 45 days, under dissecting microscope and light microscope. Eighty-four taxa of fungi were identified, totaling 335 occurrences. The air and soil temperature, and precipitation showed an influence on the fungal community. Species richness was greater in the dry period and abundance was greater in the rainy period. The multivariate analyses revealed differences in the conidial fungi community between the dry and rainy periods. A high richness of leaf litter conidial fungal was uncovered in this area of humid forest surrounded by the semiarid vegetation of Caatinga.

Keywords: Asexual *Ascomycota*, Atlantic Forest, taxonomy, diversity, ecology.

Introduction

Tropical rainforests are found in Africa, Asia, and Central and South America. In Brazil, these forests are divided between the Amazon rainforest, considered the largest tropical forest in the world covering 40 % of South America (Müller 2020), and the Atlantic Forest that is the second-largest tropical forest in South America (Marques *et al.* 2021).

The Atlantic Forest includes varied ecosystems such as ombrophilous forests (dense, mixed, and open), seasonal semi-deciduous and seasonal deciduous forests, mangroves, coastal tablelands, associated altitude fields, and humid altitude forests called "*Brejos de Altitude*". The latter are Atlantic Forest enclaves or islands surrounded by the semiarid vegetation of the Caatinga (Serviço Florestal Brasileiro 2010).

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The *Brejos* present significant heterogeneity in the physical environment (Svenning 2001) [dry and wet, flat or hilly, wind-driven or wind-protected, hot and cold, sunny and shaded sites (Valencia *et al*. 2004; Russo *et al*. 2005)]. Therefore, a very diverse community of plants and animals has evolved in this area. Due to the privileged environmental conditions of the *Brejos*, many ranchers and farmers have been attracted to these regions for cattle breeding and cultivation of banana, coffee, manioc and other vegetables, for example. These activities have caused habitat fragmentation and biodiversity loss (Silva & Tabarelli 2000; de Medeiros & Cestaro 2020).

The importance of these islands of humid forest surrounded by semiarid vegetation is not limited to their biological richness and endemism, but it also includes what they can offer in terms of food, water, and other natural resources. Some *Brejos* regions have river springs and small streams that contribute to the maintenance of the humid forest characteristics and favor biological diversity. Where springs and streams are found, riparian forests connect ecological processes, performing extremely important functions in maintaining water quality and stability of margin soils. The riparian forest also regulates the exchange processes between terrestrial and aquatic systems, and allows circulation of animals and gene flow of species, besides being considered a preferred habitat for many species. However, riparian forests have been degraded in many areas, mainly due to the advanced agricultural practices along streams (Rodrigues & Nave 2001; Castro 2012).

Despite the poor fertility of the soil (Kaspari *et al*. 2008), the microbial community decomposing leaf litter is responsible for nutrient cycling and facilitating nutrients availability from the leaf litter which is the main source of organic matter (Costa *et al*. 2010; Stahl *et al*. 2013; Albuquerque *et al*. 2018). Fungi, especially conidial fungi, are capable of decomposing leaf litter. These fungi produce spores of asexual origin, called conidia, whose main function is dispersion that guarantees the survival of the species and colonization of fresh substrates (Seifert *et al*. 2011).

In addition, conidial fungi have an important role in promoting nutrient cycling in different terrestrial and aquatic habitats (Mueller *et al*. 2004; Cavalcanti & Milanez 2007; Seifert *et al*. 2011). When leaves fall to the ground, they are colonized by several species simultaneously or successively, which contribute to the degradation of various substrates and release of substances, thus enriching the soil (Castro *et al*. 2011; Kirk *et al*. 2013). Despite their importance, taxonomic studies on conidial fungi in leaf litter in Brazil are as yet insufficient, as very few research groups are dedicated to this group of fungi (Grandi & Silva 2006; Magalhães *et al*. 2011; Santa-Izabel *et al*. 2011; Monteiro *et al*. 2019) and only limited studies have been carried out in *Brejos* (Costa *et al*. 2016a; b; c; Santa-Izabel & Gusmão 2018; da Silva *et al*. 2019).

Therefore, the study of leaf litter colonizers in the *Brejos* is much needed and can aid the understanding of fungal diversity and community structure in the tropical forests during the dry and rainy periods, and obtaining a deeper knowledge on biotic and abiotic factors affecting community assemblage.

In this context, we addressed the following questions: (i) Are there differences in the composition and structure of the conidial fungal community on leaf litter between the dry and rainy periods in *Brejos*? (ii) What variables most influence changes in the fungal community in *Brejos*? The main aim of this study was to describe the diversity of conidial fungi associated with the leaf litter decomposition in the riparian vegetation of an Atlantic Forest formation known as *Brejo de Altitude,* Brazil. Consequently, we will contribute to the knowledge of these fungi in the semiarid region of Northeast Brazil and in the Neotropics.

Materials and methods

Study area

The Professor João Vasconcelos Sobrinho Municipal Natural Park (PJVS), 08°21'20.92"S and 36°1'42.98"W (entrance), is located in a region known as Serra dos Cavalos, an Integral Conservation Unit that includes two municipalities (Caruaru and Altinho) in the Agreste region of Pernambuco. With an area of 359 ha, it shelters an exuberant and diversified forest. Most of the park is covered by Dense Ombrophilous Montane Forest (Tavares *et al*. 2000) containing large trees, besides lianas, epiphytes and ferns distributed on the windward slope (Barros & Fonseca 1996).

Due to its rugged topography, with contour lines that vary from 800 to 950 m, a reasonably well defined drainage occurs, with two main water courses: the Chuchu and Capoeirão creeks (Braga *et al*. 2002). Within the park there are three large dams, Serra dos Cavalos, Guilherme de Azevedo and Jaime Nejaim, which serve as a strategic reserve for the region's public supply system, serving 387,000 inhabitants (Braga *et al*. 2002). The region of the park is located on the geological layer of the crystalline basement, with a small thickness of soils formed by processes of weathering (Braga *et al*. 2002; EMBRAPA 2006). In the park one can find variations of soil-Yellow Argissolos, and associated Red-Yellow Argissolos and Neossolos Litholithos, and Red-Yellow Argissolos (EMBRAPA 2001; 2006).

High temperatures in most of the year with intense heat strokes favor evaporation (Pinheiro-Filho 2019). Air and soil temperature were measured at the collection points with a digital thermometer, while accumulated precipitation data were provided by the Pernambuco water agency APAC (http://www.sirh.srh.pe.gov.br/apac/).

Sample characterization

Four collection expeditions of decomposing leaf material on the forest floor were conducted from May 2019 to November 2019 in PJVS. The six sampling points (Table 1) are located along the Chuchu stream, before it flows into the Guilherme de Azevedo reservoir, 4.5 m from the watercourse bank and about 100 m apart from each other. At each point, a frame (25 cm^2) was randomly cast three times and the framed leaves were collected into plastic bags, taken to the laboratory and placed into three perforated plastic containers inside a tray, positioned at a 45° angle below the faucet and the running water falling in the tray but not directly on the leaves. This gentle washing for 30 minutes is to eliminate debris and nematodes (Castañeda-Ruiz *et al.* 2016). The leaves were next air dried on newspaper or paper towel for about 10 minutes and were cut with scissors into fragments of approximately 7 cm^2 to be placed in Petri dishes lined with a filter paper moist with sterile distilled water (moist chamber) for taxonomic analysis of fungi. Each moist chamber held 3 leaf fragments and six chambers were mounted for each sampling point. These plates were incubated in a styrofoam box that contained a 1 cm layer of water at the bottom, at room temperature. A few drops of glycerin were added to break the surface tension of the water. After 72 hours, the incubated material was observed with a stereomicroscope and light microscope, and the analysis continued for 45 days. Fungal structures were mounted on semi-permanent slides with 90 % lactic acid and permanent slides with polyvinyl alcohol in lactoglycerol (PVLG) for identification of the conidial fungi according to the literature such as Ellis (1971; 1976), Matsushima (1971; 1975; 1985; 1993), Seifert *et al.* (2011). The new species records were deposited in the Herbarium Pe. Camille Torrend – URM (University of Recife Mycology). Fungal structures were documented using a Nikon Eclipse Ni-U microscope with DIC optics and a Nikon DS-Fi2 digital camera.

A total of 144 plates (moist chambers) were analyzed, with three fragments per plate, six plates per collection point, six collection points in the area, and four expeditions, resulting in 432 leaf fragments.

Ecological indices for analyses of the fungal community

The frequency of occurrence of conidial fungi (F) was calculated according to the formula: F = n × 100/N, where n = number of samples in which a species was recorded, and N = total number of samples (144 moist chambers). The following frequency classes were established: F ≤ 10% = sporadic; 10 < F ≤ 30% = infrequent; 30 < F ≤ 70% = frequent; and F > 70% = very frequent (Dajoz, 1983).

For the constancy of the detected species, the following formula was applied: C= p.100/P, where: p = number of expeditions in which a fungal species was found and P = total number of expeditions (4). The taxa were divided according to the following constancy categories: C ≤ 25% accidental, 25% < C ≤ 50% accessory and C > 50% = constant (Dajoz 1983).

For Shannon-Wiener's diversity index (H'), we used H' = - $\sum$ *pi* (*ln pi*), where pi = ni/N; N = total number of individuals sampled; ni = number of individuals sampled from the taxonomic group; ln = neperian logarithm. Pielou's equitability (J) was calculated using the formula: J = H'/H'$_{max}$, where H'$_{max}$ is the maximum possible diversity that can be observed if all species of conidial fungi have equal abundance. H'$_{max}$ = log S, where S = total number of species of conidial fungi sampled. Berger-Parker's dominance was calculated using the formula: d = N$_{max}$ / N$_t$ where N$_{max}$ is the number of occurrences of the most abundant species and Nt is the total number of occurrences in the sample. Shannon-Wiener's Diversity (H'), Pielou's equitability (J) and Berger-Parker's dominance indices were calculated using the program PAST 3.18c (Hammer *et al.* 2013). The maximum species richness of conidial fungi in the sampling area was calculated using the *Chao* 1 (1st order) estimator (Santos 2003).

Statistical analyses

Pearson's correlation analysis was performed using R (R Development Core Team 2018) to identify whether there was correlation between the occurrence of fungi and the abiotic variables (precipitation, air and soil temperature) regarding the wet and dry season.

Table 1. Geographic position of the sampling points at the Parque Natural Municipal Professor João Vasconcelos Sobrinho (Serra dos Cavalos), Pernambuco, Brazil.

Points (P)	Latitude (N)	Longitude (W)	Altitude (m)
P1	8° 21' 46"	36° 1' 53"	843.06
P2	8° 21' 48"	36° 1' 51"	817.96
P3	8° 21' 48"	36° 1' 43"	852.56
P4	8° 21' 49"	36° 1' 46"	851.84
P5	8° 21' 47"	36° 1' 48"	828.77
P6	8° 21' 51"	36° 1' 38"	830.21

The classification of the resulting r values was: r= 0.1-0.3 (weak correlation), r= 0.4-0.6 (moderate correlation) and r= 0.7-1 (strong correlation) (Dancey & Reidy 2006).

The conidial fungal species composition of the community was compared between the dry and rainy periods using the multivariate statistical method NMDS (Nonmetric Multidimensional Scaling), based on the Bray-Curtis dissimilarity matrix (Kruskal 1964). The one way ANOSIM test was used to verify dissimilarity of the groups formed in the NMDS (Clarke 1993).

The similarity index between sampling periods was calculated from binary data, using the Morisita-Horn's coefficient and the dendrogram was constructed using the UPGMA grouping method and the SPSS Statistics software (version 22.0, International Business Machines Corp., Armonk, NY).

Principal Component Analysis – PCA (Turk & Pentland 1991) was applied to test whether the composition of the leaf litter conidial fungi community differs between sampling periods and if any of the abiotic variables interfere with this change using PAST 3.18c (Hammer *et al*. 2013).

Results

Air and soil temperature and precipitation

Four variables were used to test whether there were differences between the wet and dry seasons. The Shapiro-Wilk's test confirmed the normal distribution of the data: occurrence of taxa (p = 0.429), pluviometric precipitation (p = 0.273), air temperature (p = 0.478) and soil temperature (p = 0.899). Therefore, parametric statistics were applied.

According to the results of the univariate analysis, the number of taxa was not influenced by environmental factors such as soil and air temperature or pluviometric precipitation (Figure 1). The temperature range for growth of most fungi is between 20 °C and 30 °C. During the sampling months, the soil temperature varied from 20 °C to 24.5 °C and the air temperature from 21 °C to 28 °C (Figure 1). Regarding the pluviometric precipitation, it was observed that the greater occurrence of fungi coincided with the greater precipitation (148 mm) in July, 2019. When the precipitation was lower (14.2 mm) in November, 2019, the occurrence of fungi decreased compared to the previous collection period (September, 2019), but it was not the lowest occurrence (Figure 1).

It was observed that sampling points 1, 2 and 3, closer to the dam, had denser vegetation and larger amounts of leaf litter, and the area was, in general, more humid. From sampling point 4 onwards, there was a decrease in the leaf litter on the ground, and the vegetation was drier in points 5 and 6 (closer to the local unpaved road).

Occurrence and richness of taxa

Eighty-four species were identified in 432 leaf fragments. These species belong to 68 genera of which 33 were *incertae sedis*, 32 families and six classes (*Sordariomycetes*, *Dothideomycetes*, *Leotiomycetes*, *Eurotiomycetes*, *Orbiliomycetes* and *Tritirachiomycetes*). Among these taxa, two are new species; three are new records to the Neotropics and two to South America (Table 2).

In four sampling expeditions, 335 occurrences were recorded. *Repetophragma fasciatum*, was the highest detected taxa (27 times), followed by *Wiesneriomyces laurinus* (26 times) and *Beltrania rhombica* (23 times). Regarding the class of frequency, there was a predominance of sporadic (95.23 %) and infrequent (4.76 %) taxa (Figure 2). Relating to the constancy of taxa, 68 % were accidental, 19 % accessory and 13 % constant (Figure 2).

Pearson's correlation analysis between the occurrence of conidial fungi, precipitation, air, and soil temperature resulted in moderate correlation between occurrence and precipitation, and strong negative correlation between occurrence and air or soil temperatures. As expected, air and soil temperatures had a strong positive correlation (Table 3).

Diversity indices for the leaf litter conidial fungi community

Based on the data of species richness of conidial fungi identified in the PJVS, a rarefaction curve was constructed using the *Chao* 1 index (1st order), representing the estimate of maximum local richness of conidial fungi. The rarefaction curve of the observed species did not reach the asymptote, showing that there is still a tendency to increase the detected species richness if samplings of leaf litter conidial fungi in the region were to be continued. The rarefaction curve in this study estimated that the maximum species richness to be found is 101, and 84 species were found, therefore 83.1 % of the estimated taxa was uncovered (Figure 3).

The distribution of species detected in the sampling periods revealed greater richness in the 3rd sampling (dry period), while abundance was greater in the 2nd sampling (wet period) (Table 4). The diversity index was higher in the 3rd sampling. The equitability was high in all sampling periods, with variation from 0.89 to 0.95 between the 1st (wet period) and 3rd sampling (dry period). The dominance was higher in the wet season (Table 4). In general, significant differences were found between the wet and dry seasons for the indices of diversity, equitability, and dominance; however, for richness there was no significant difference (Table 4).
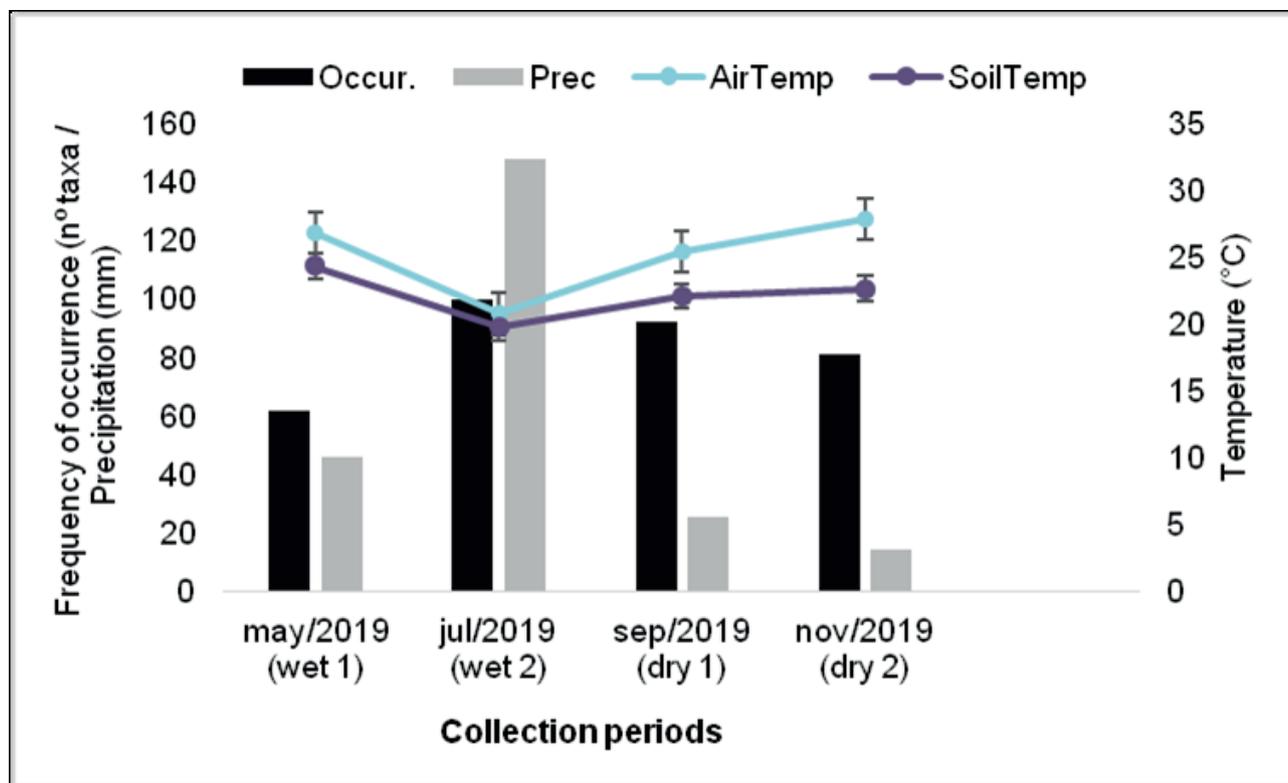
Figure 1. Distribution of precipitation, temperature variation (air and soil) and occurrence of taxa between sampling periods in the Parque Natural Municipal Professor João Vasconcelos Sobrinho - Serra dos Cavalos, PE-Brazil.
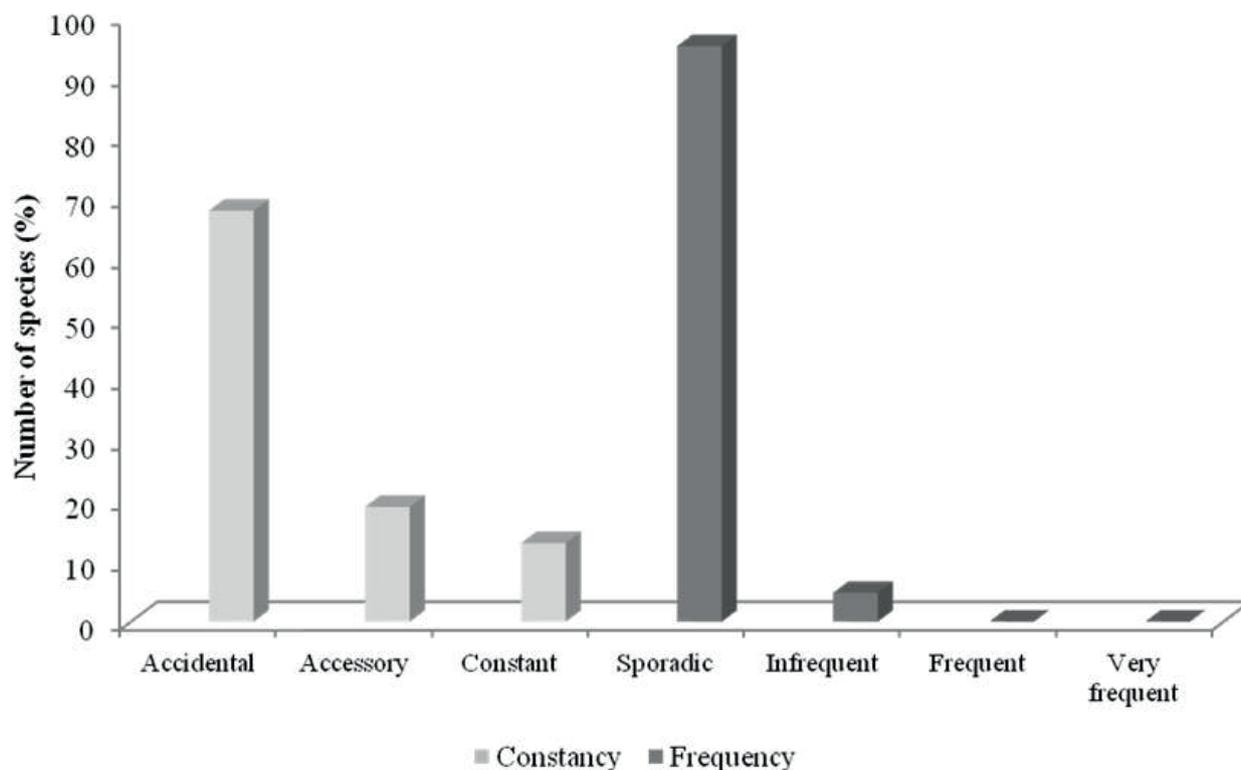

Figure 2. Constancy and frequency of occurrence of taxa of conidial fungi in leaf litter undergoing decomposition in the Parque Natural Municipal Professor João Vasconcelos Sobrinho-Serra dos Cavalos, PE-Brazil.

Table 2. Occurrence of conidial fungi, frequency and category of constancy in decomposing leaf litter detected in four sampling expeditions to the Parque Natural Municipal Professor João Vasconcelos Sobrinho-Serra dos Cavalos, PE-Brazil. *Ingoldian fungi; ** Aeroaquatic hyphomycetes; *** Coelomycetes; ● Sexual ascomycetes; 0 = No occurrence; □ New species; Δ First record to the Neotropics; ◊ First record to South America. W1-W2 = collection wet periods and D1-D2 = collection dry periods

FAMILY	TAXA	W1	W2	D1	D2	Freq.	Class of Freq.	Constancy %
Incertae sedis	*Acremonium* sp	0	0	0	1	0.7	sporadic	accidental
Incertae sedis	*Acumispora verruculosa* Heredia, R.F. Castañeda & R.M. Arias	0	0	1	0	0.7	sporadic	accidental
Cordycipitaceae	*Akanthomyces lecanii* (Zimm.) Spatafora, Kepler & B. Shrestha	0	0	0	2	1.4	sporadic	accidental
Pleosporaceae	*Alternaria alternata* (Fr.) Keissl	0	2	0	0	1.4	sporadic	accidental
Venturiaceae	*Anungitopsis speciosa* R.F. Castañeda & W.B. Kendr.	0	0	1	0	0.7	sporadic	accidental
Incertae sedis	*Bactridium flavum* Kunze	0	1	2	0	2.1	sporadic	accidental
Beltraniaceae	*Beltrania querna* Harkn.	0	4	4	4	8.3	sporadic	constant
Beltraniaceae	*Beltrania rhombica* Penz	13	5	0	5	16.0	infrequent	constant
Beltraniaceae	*Beltraniella portoricensis* (F. Stevens) Piroz. & S.D. Patil	4	3	0	7	9.7	sporadic	constant
Beltranieaceae	*Beltraniopsis esenbeckiae* Bat. & J.L. Bezerra	0	2	2	2	4.2	sporadic	constant
Nectriaceae	*Calonectria ovata* D. Victor & Crous	0	0	0	1	0.7	sporadic	accidental
Cancellidiaceae	**Cancellidium applanatum* Tubaki	0	0	2	0	1.4	sporadic	accidental
Castanediellaceae	*Castanediella cagnizarii* (R.F. Castañeda & W.B. Kendr.) Crous, Hern.-Restr. & M.J. Wingf.	1	0	0	0	0.7	sporadic	accidental
Microascaceae	*Cephalotrichum microsporum* (Sacc.) P.M. Kirk	1	0	0	0	0.7	sporadic	accidental
Chaetomiaceae	*Chaetomium* sp	1	0	0	0	0.7	sporadic	accidental
Hamatocanthoscyphaceae	*Chalara aurea* (Corda) S. Hughes	2	0	0	0	1.4	sporadic	accidental
Xylariaceae	*Circinotrichum flexuosum* K.G. Karand., S.M. Kulk. & Patw	0	0	0	2	1.4	sporadic	accidental
Xylariaceae	*Circinotrichum maculiforme* Nees	0	0	0	3	2.1	sporadic	accidental
Xylariaceae	*Circinotrichum olivaceum* (Speg.) Piroz.	0	0	0	2	1.4	sporadic	accidental
Incertae sedis	*Cladosporiella cercosporicola* Deighton	0	0	0	2	1.4	sporadic	accidental
Cladosporiaceae	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries	0	0	0	1	0.7	sporadic	accidental
Glomerellaceae	****Colletotrichum* sp	0	0	0	1	0.7	sporadic	accidental
Cordycipitaceae	*Cordyceps* sp	1	0	0	0	0.7	sporadic	accidental
Chaetosphaeriaceae	*Cryptophiale kakombensis* Piroz.	0	1	0	2	2.1	sporadic	accessory
Nectriaceae	*Cylindrocladium ellipticum* Alfieri, C.P. Seym. & Sobers	0	0	2	0	1.4	sporadic	accidental
Orbiliaceae	*Dactylella candida* (Nees) de Hoog	0	0	1	0	0.7	sporadic	accidental
Torulaceae	*Dendryphion comosum* Wallr.	1	0	2	0	2.1	sporadic	accessory
Torulaceae	*Dendryphion vinosum* (Berk. & M.A. Curtis) S. Hughes	0	1	0	0	0.7	sporadic	accidental
Chaetosphaeriaceae	*Dictyochaeta assamica* (Agnihothr.) Aramb., Cabello & Mengasc.	3	0	5	0	5.6	sporadic	accessory
Chaetosphaeriaceae	*Dictyochaeta fertilis* (S. Hughes & W.B. Kendr.) Hol.-Jech.	0	1	0	0	0.7	sporadic	accidental
Chaetosphaeriaceae	*Dictyochaeta triseptata* (Matsush.) R.F. Castañeda	0	0	1	0	0.7	sporadic	accidental
Chaetosphaeriaceae	****Dinemasporium lanatum* Nag Raj & R.F. Castañeda	2	0	0	0	1.4	sporadic	accidental
Incertae sedis	*Ellisembia bawanglingensis* S.C. Ren & X.G. Zhang	1	0	1	0	1.4	sporadic	accessory
Nectriaceae	*Fusarium* sp	0	0	1	0	0.7	sporadic	accidental
Incertae sedis	*Guarroa acutispora* (Matsush.) Heredia & R.F. Castañeda	0	0	1	0	0.7	sporadic	accidental
Incertae sedis	*Gyrothrix verticiclada* (Goid.) S. Hughes & Piroz.	0	3	0	0	2.1	sporadic	accidental
Xylariaceae	*Hansfordia pulvinata* (Berk. & M.A. Curtis) S. Hughes	0	0	0	4	2.8	sporadic	accidental
Chaetosphaeriaceae	Δ *Menisporopsis multisetulata* C.K.M. Tsui, Goh, K.D. Hyde & Hodgkiss (**URM 94402**)	0	6	0	0	4.2	sporadic	accidental
Chaetosphaeriaceae	*Menisporopsis theobromae* S. Hughes	3	9	4	0	11.1	infrequent	constant
Chaetosphaeriaceae	*Menisporopsis pirozynskii* Varghese & V.G. Rao	0	0	3	0	2.1	sporadic	accidental
Magnaporthaceae	*Mycoleptodiscus terrestris* (Gerd.) Ostaz.	0	0	4	2	4.2	sporadic	accessory
Stachybotryaceae	*Myrothecium inundatum* Tode	0	5	0	0	3.5	sporadic	accidental

Table 2. Cont.

FAMILY	TAXA	W1	W2	D1	D2	Freq.	Class of Freq.	Constancy %
Stachybotryaceae	*Myrothecium verrucaria* (Alb. & Schwein.) Ditmar	0	0	0	3	2.1	sporadic	accidental
Ophioceraceae	● *Ophioceras leptosporum* (S.H. Iqbal) J. Walker	1	3	0	1	3.5	sporadic	constant
Incertae sedis	*Paraceratocladium polysetosum* R.F. Castañeda	1	0	0	0	0.7	sporadic	accidental
Stachybotryaceae	*Paramyrothecium roridum* (Tode) L. Lombard & Crous	1	3	4	0	5.6	sporadic	constant
Aspergillaceae	*Penicillium* sp	0	1	2	0	2.1	sporadic	accessory
Periconiaceae	*Periconia byssoides* Pers	0	0	3	0	2.1	sporadic	accidental
Mycosphaerellaceae	*Periconiella ilicis* P.M. Kirk	1	0	0	0	0.7	sporadic	accidental
Pestalotiopsidaceae	****Pestalotiopsis trachycarpicola* Yan M. Zhang & K.D. Hyde	0	0	0	2	1.4	sporadic	accidental
Incertae sedis	*Phaeodactylium biseptatum* R.F. Castañeda, Iturr. & Rob. Fernández	0	0	0	2	1.4	sporadic	accidental
Incertae sedis	*Physalidiella elegans* (Luppi Mosca) Rulamort	0	0	1	2	2.1	sporadic	accessory
Incertae sedis	□ *Pleurotheciopsis* sp	1	0	1	2	2.8	sporadic	constant
Incertae sedis	*Polyscytalum ciliatum* J.A. Cooper	0	1	3	0	2.8	sporadic	accessory
Nectriaceae	*Pseudonectria buxi* (DC.) Seifert, Gräfenhan & Schroers	2	0	1	0	2.1	sporadic	accessory
Didymellaceae	*Pseudopithomyces chartarum* (Berk. & M.A. Curtis) Jun F. Li, Ariyaw. & K.D. Hyde	0	0	1	0	0.7	sporadic	accidental
Incertae sedis	*Repetophragma fasciatum* (R.F. Castañeda) R.F. Castañeda, Gusmão & Saikawa	4	8	8	7	18.8	infrequent	constant
Incertae sedis	*Repetophragma filiferum* (Piroz.) R.F. Castañeda, Gusmão & Heredia	2	0	0	0	1.4	sporadic	accidental
Herpotrichiellaceae	*Rhinocladiella cristaspora* Matsush.	2	0	2	0	2.8	sporadic	accessory
Cochleáromycetaceae	◊****Satchmopsis sacciformis* R.F. Castañeda **(URM 94403)**	1	5	4	0	6.9	sporadic	constant
Sympoventuriaceae	*Scolecobasidium humicola* G.L. Barron & L.V. Busch	1	0	0	0	0.7	sporadic	accidental
Incertae sedis	*Solosympodiella clavata* Matsush.	0	0	0	1	0.7	sporadic	accidental
Wiesneriomycetaceae	*Speiropsis pedatospora* Tubaki	0	1	0	0	0.7	sporadic	accidental
Wiesneriomycetaceae	*Speiropsis scopiformis* Kuthub. & Nawawi	2	0	0	0	1.4	sporadic	accidental
Incertae sedis	◊● *Stellosetifera malaysiana* Matsush.**(URM 94405)**	0	1	3	0	2.8	sporadic	accessory
Pleosporaceae	*Stemphylium botryosum* Wallr.	0	0	3	0	2.1	sporadic	accidental
Incertae sedis	*Subulispora procurvata* Tubaki	0	1	0	0	0.7	sporadic	accidental
Tetraplosphaeriaceae	*Tetraploa aristata* Berk. & Broome	0	2	0	0	1.4	sporadic	accidental
Chaetosphaeriaceae	*Thozetella cristata* Piroz. & Hodges	0	0	2	5	4.9	sporadic	accessory
Chaetosphaeriaceae	*Thozetella pinicola* S.Y. Yeung, Jeewon & K.D. Hyde	0	6	1	0	4.9	sporadic	accessory
Chaetosphaeriaceae	Δ *Thozetella serrata* Whitton, K.D. Hyde & McKenzie **(URM 94404)**	4	0	0	0	2.8	sporadic	accidental
Chaetosphaeriaceae	*Thozetella submersa* F.R. Barbosa & Gusmão	0	0	2	0	1.4	sporadic	accidental
Chaetosphaeriaceae	□ *Thozetella* sp	0	0	2	0	1.4	sporadic	accidental
Torulaceae	*Torula herbarum* (Pers.) Link	1	0	0	0	0.7	sporadic	accidental
Hypocreaceae	*Trichoderma* sp	0	0	3	0	2.1	sporadic	accidental
Incertae sedis	**Triscelophorus deficiens* (Matsush.) Matsush.	0	0	0	1	0.7	sporadic	accidental
Tritirachiaceae	*Tritirachium oryzae* (Vincens) de Hoog	0	1	0	0	0.7	sporadic	accidental
Vermiculariopsiellaceae	*Vermiculariopsiella immersa* (Desm.) Bender	0	3	0	0	2.1	sporadic	accessory
Plectosphaerellaceae	*Verticillium* sp	1	2	0	0	2.1	sporadic	accessory
Cainiaceae	*Vesiculozygosporium echinosporum* Bunting & E.W. Mason	0	0	0	3	2.1	sporadic	accidental
Nectriaceae	*Volutella ciliata* (Alb. & Schwein.) Fr.	0	0	4	5	6.3	sporadic	accessory
Wiesneriomycetaceae	*Wiesneriomyces laurinus* (Tassi) P.M. Kirk	3	14	5	4	18.1	infrequent	constant
Incertae sedis	Δ *Yuccamyces purpureus* Gour, Dyko & B. Sutton **(URM 94401)**	1	5	0	0	4.2	sporadic	accidental
Zygosporiaceae	*Zygosporium oscheoides* Mont.	0	0	0	2	1.4	sporadic	accidental
	OCCURRENCE	62	100	92	81	335		
	RICHNESS	29	29	37	30			

Table 3. Pairwise Pearson's correlation between fungal occurrence, precipitation, air, and soil temperatures in the Parque Natural Municipal Professor João Vasconcelos Sobrinho-Serra dos Cavalos, PE-Brazil, according to the sampling periods.

	Occur. of fungi	Precipitation	Air temperature	Soil temperature
Occur. of fungi	1			
Precipitation	0.52	1		
Air temperature	-0.73	-0.94	1	
Soil temperature	-0.94	-0.75	0.87	1

If r = 0, there is no relationship between the two variables. Values closer to 1 or -1 indicate a perfect correlation between the two variables.
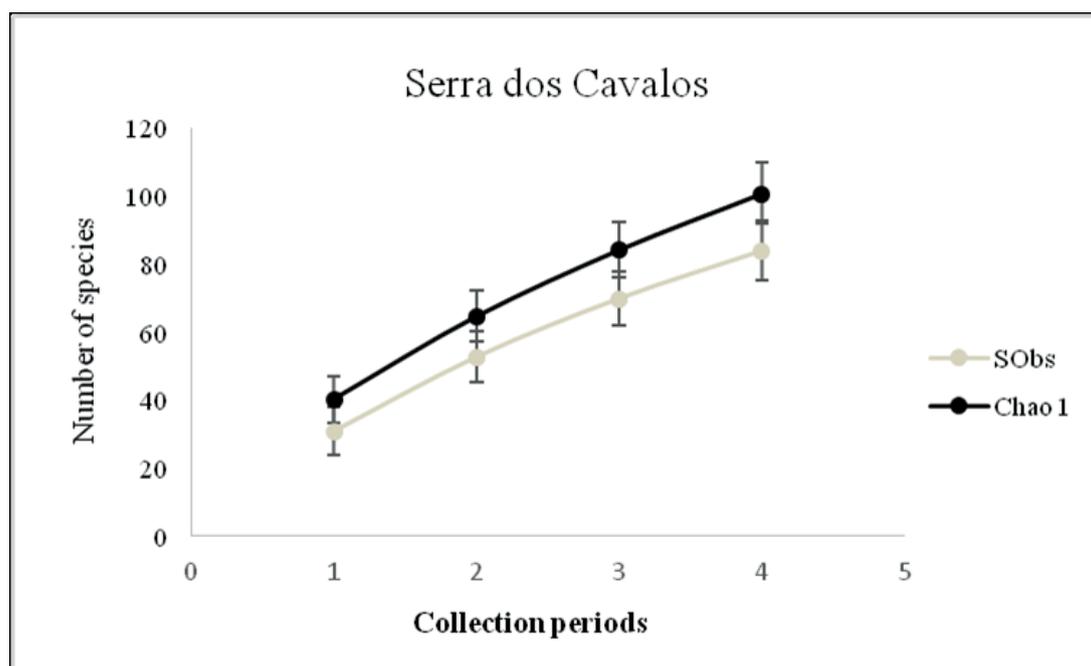
Figure 3. Rarefaction curve of conidial fungi in decomposing leaf litter in the Parque Natural Municipal Professor João Vasconcelos Sobrinho-Serra dos Cavalos, PE-Brazil, and richness estimates using the nonparametric estimator Chao 1 (black line), SObs: observed richness (grey line).

Table 4. Ecological indices regarding the species of conidial fungi collected in the Parque Natural Municipal Professor João Vasconcelos Sobrinho-Serra dos Cavalos, PE-Brazil.

ECOLOGICAL INDICES	WET SEASON**		DRY SEASON**		Perm p (eq)
	W1 (May/2019)	W2 (Jul/2019)	D1 (Sep/2019)	D2 (Nov/2019)	
Richness (R)	29	29	37	30	0,09
Occurrence (O)	62	100	92	81	0*
Shannon-Wiener (H')	3.02	3.05	3.43	3.22	0.002*
Equitability (J')	0.89	0.90	0.95	0.94	0.003*
Berger-Parker's Dominance	0.07	0.06	0.03	0.04	0.002*

Perm p(eq): probability of having equal diversities. If p(eq) is greater than 0.05, there is no significant difference. *Significant difference. **D and W = collection periods.

Changes in the structure of the leaf litter conidial fungi community between seasonal periods

Multidimensional scaling ordination of samples based on occurrence of conidial fungi species separated them by both dry and wet periods. This trend is confirmed when observing the result obtained by ANOSIM analysis, which revealed a significant difference between the communities in the dry and wet periods (R= 0.4954, p = 0.0053) (Figure 4). One cluster was formed between the communities detected in 1st and 2nd sampling (wet period), showing structuring according to sampling events and good separation between the dry and wet periods. General similarity between wet and dry seasons was $\cong$ 45 %; however, the community in 4th sampling (dry period, D2) was less similar ($\cong$ 35 %) (Figure 5). The principal component analysis (PCA), including frequency of occurrence of fungi, air and soil temperature, and rainfall, is shown in Figure 6. PC1 and PC2 explained 83.6 % of variance, separating the six points in the dry period (D1-D6) from the same six points in the wet period (W1-W6). Air and soil temperatures were the variables that most contributed to this separation.
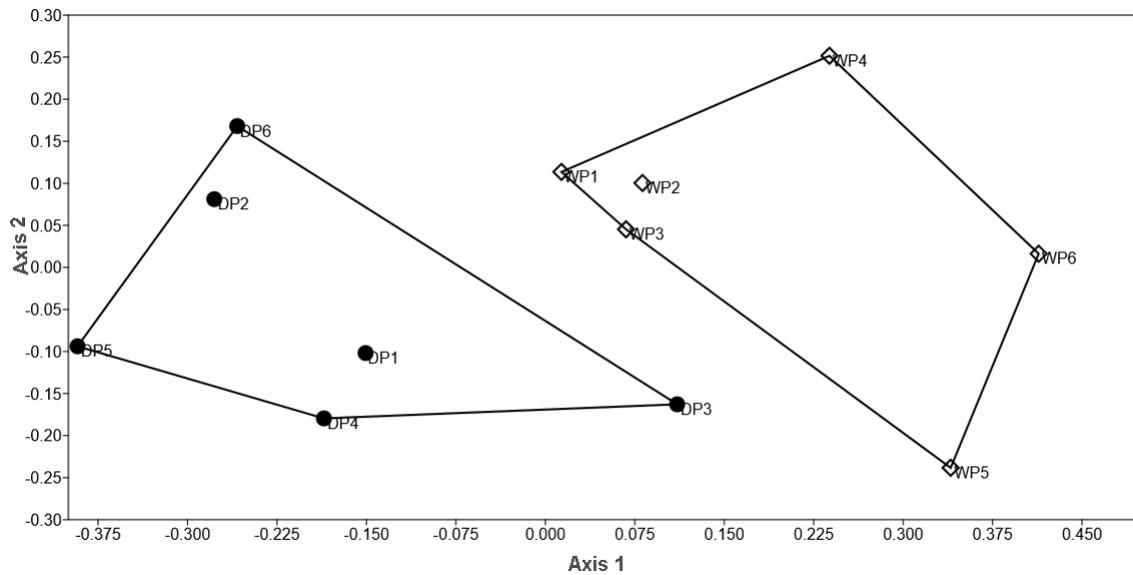
Figure 4. Non-metric multidimensional scaling (NMDS) ordination plot illustrating the conidial fungi communities of decomposing leaf litter in six sampling points in the wet (WP1-WP6) and dry (DP1-DP6) periods in the Parque Natural Municipal Professor João Vasconcelos Sobrinho-Serra dos Cavalos, PE-Brazil. Stress = 0.18.
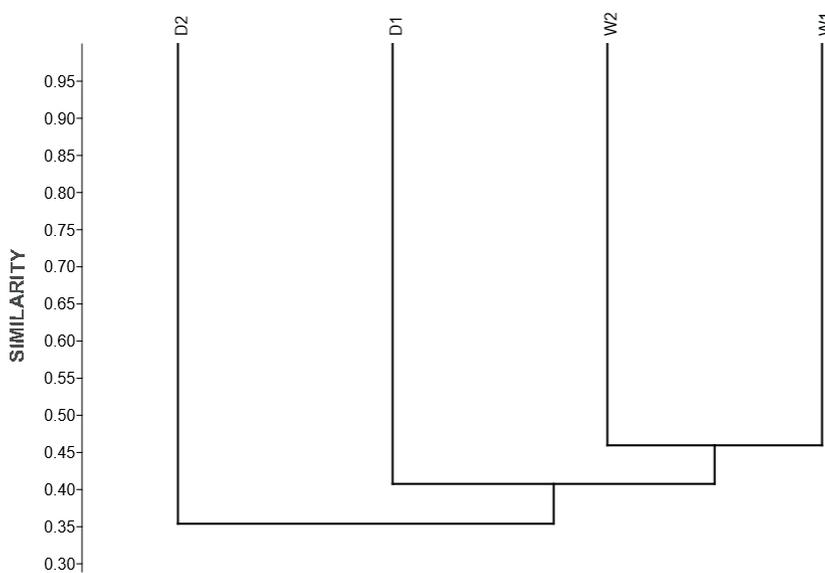

Figure 5. Similarity of the conidial fungi community associated with leaf litter in decomposition on the fores floor, over the dry and wet seasons in the Parque Natural Municipal Professor João Vasconcelos Sobrinho - Serra dos Cavalos. The dendrogram is an UPGMA Morisita-Horn analysis. Cophenetic correlation coefficient = 0.80. Sampling periods are indicated as D1-D2 (dry) e W1-W2 (wet).
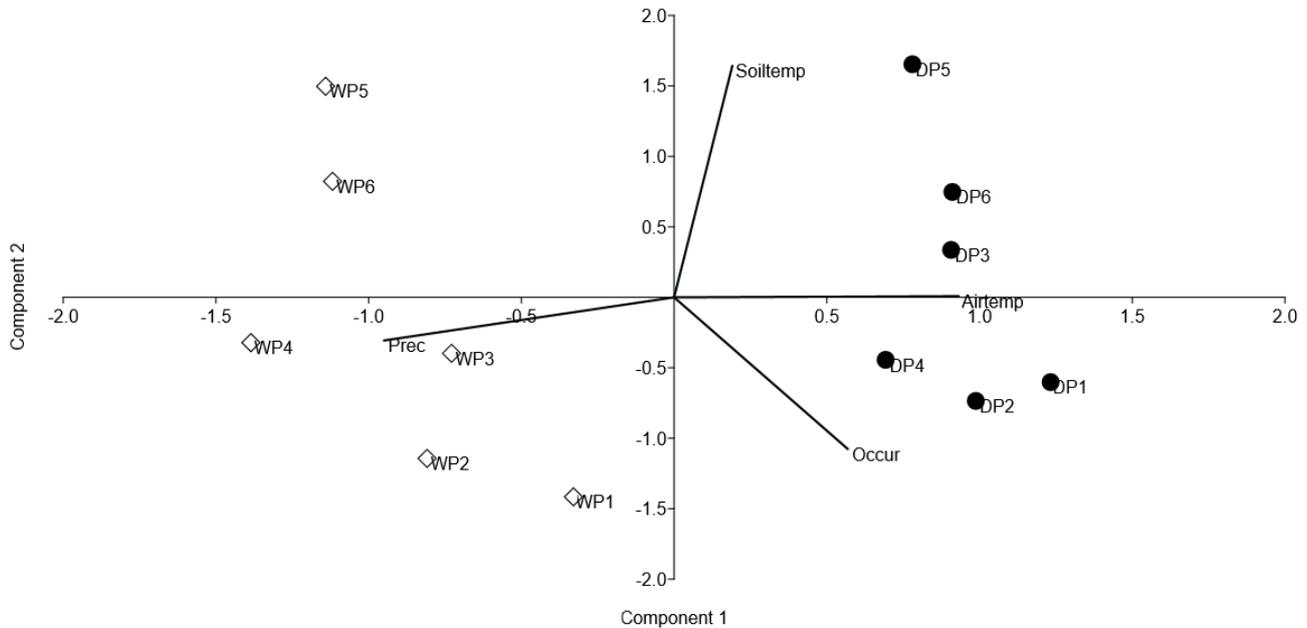
Figure 6. Principal Components Analysis of the fungi community in six sampling points in the Parque Natural Municipal Professor João Vasconcelos Sobrinho-Serra dos Cavalos, PE-Brazil over the dry (DP1-DP6) and wet seasons (WP1-WP6).

Discussion

In this study, we observed that soil and air temperatures are within optimal levels for the development of fungi (Maia 1983) (Figure 1). In these ecosystems, temperatures do not vary greatly, and the water regime plays a key role in soil microbial dynamics (Lodge *et al*. 1994). Therefore, rainfall has apparently influenced the increase in the number of fungi in the decomposing leaves. Rainfall has an influence on leaf litter production because it can induce the fall of senescent leaves (Liu 2012). In addition, non-senescent leaves can also be shed due to heavy rainfall at some times of the year (Scheer 2009) and, consequently there will be an increase in colonization by the fungi and boost fungal richness (Swaty *et al*. 1998). The largest deposits of leaf litter in humid forests occur in the dry season, and that increases the intensity of leaf litter decomposition in the wet season (Delitti 1984; Barbosa & Faria 2006).

In this study, the Class *Sordariomycetes* (42) showed the greatest richness, followed by *Dothideomycetes* (12) (Table 2). *Sordariomycetes* is the second-largest class of the *Ascomycota* phylum (Kirk *et al*. 2008; Hyde *et al*. 2013) and has a cosmopolitan distribution (Zhang *et al*. 2006). These representatives are found in a variety of habitats, mostly terrestrial taxa and several freshwater taxa (Cai *et al*. 2002; Jones *et al*. 2015). Some species are pathogens and endophytes of various plants, others cause diseases in arthropods and mammals (Maharachchikumbura *et al*. 2015; Hyde *et al*. 2016). The majority of species are saprobes

and are linked in nutrient cycling, and some species are fungicolous (Zhang *et al*. 2006; PeiGui *et al*. 2000).

Beltrania rhombica (Table 2) is considered cosmopolitan and commonly found associated with leaf litter in taxonomic studies, but also in diversity and succession studies of fungi in Brazil (Maia 1983; Castro *et al*. 2012; Magalhães *et al*. 2013), Japan (Milagres *et al*. 2018), India (Pirozynski & Patil 1970), Cuba (Delgado-Rodriguez *et al*. 2002), and United States (Heredia-Abarca 1994). Likewise, *Wiesneriomyces laurinus* has a cosmopolitan distribution and the taxon is reported in studies in Brazil (Gusmão & Grandi 1997; Silva & Grandi 2008), Australia (Paulus *et al*. 2007), Myanmar (Thaung 2008), and Canada (Pratibha *et al*. 2015), among others. Although *Wiesneriomyces laurinus* is collected in terrestrial environment, it also has a wide distribution in aquatic environment (Sridhar & Kaveriappa 1989; Rajashekhar & Kaveriappa 2000; 2003). Since *W. laurinus* produces sporodochium with pointed setae, these structures can become attached to submerged plant substrates (Goh & Hyde 1996); moreover, many terrestrial conidial fungi called immigrant fungi, can be found in association with submerged plant substrates (Park 1972), which may explain the wide occurrence of this fungus in association with aquatic environment as well. *Repetophragma fasciatum* has distribution restricted to neotropical region, mainly Central and South America, and was the taxon with the highest occurrence in this study. This species was found in studies of fungal diversity on decaying leaves of an unidentified plant (Castañeda-Ruiz *et al*. 2006; Santos *et al*. 2018).

The data on the frequency of occurrence of fungi (Figure 2) are in accordance with other studies carried out in Brazil, in which most of the fungi is sporadic and infrequent (Magalhães *et al*. 2011; Monteiro *et al*. 2019). The predominance of accidental species (Figure 2) was also verified in other studies of richness of conidial fungi that decompose leaf litter in the Atlantic Forest (Marques *et al*. 2008; Barbosa *et al*. 2009; Santana *et al*. 2017), and *Brejos* (Santa-Izabel & Gusmão 2018). The data in these studies show that the predominance of accidental taxa of conidial fungi leaf litter can be influenced by environmental factors such as humidity and temperature, and nutritional factors during decomposition. The constant taxa suffer less influence from these conditions (Table 3). In this study, small numbers of constant and frequent taxa were observed; a fact that reinforces the analyses of the authors mentioned above in tropical forests. The small number of constant species can be the result of several factors, such as large number of tree species per hectare making available numerous substrates in different periods (Santana *et al*. 2017). Also, the high percentage of sporadic species found in this study matches results from Atlantic Forest areas (Santana *et al*. 2017). Similarly, climate changes (Suseela & Tharayil 2018), the stage in which the substrate decomposes and trichomes (Parungao *et al*. 2002) in the leaves are related to the presence of sporadic species.

In tropical forests, where environmental conditions such as humidity and temperature are optimal for the development of microfungi (Ferreira & Chauvet 2011; Geraldes *et al*. 2012), changes in temperature can inhibit fungi activity, consequently, it promotes drastic changes in leaf litter decomposition (Bärlocher *et al*. 2013; Gonçalves *et al*. 2013). Moreover, other conditions such as the preference for a specific vegetal substrate is related to nutritional factors and secondary metabolites offered by the substrates (Rambelli *et al*. 2004).

The data in this study show that the rarefaction curve continues to rise (Figure 3), indicating that the number of samples may not have been sufficient to adequately characterize the hyphomycetes community in the leaf litter of the investigated area. Due to the increasing number of fungi at each sampling, scientists have been encouraged to use various approaches to estimate species richness, such as accumulation curves, parametric and non-parametric estimators to describe mycological communities (Unterseher *et al*. 2005; Lindner *et al*. 2006). Studies of microfungi in humid forests have achieved lower (Costa & Gusmão 2015) or similar (Unterseher *et al*. 2008) coverages than the found in this study using non-parametric richness estimators. Although the data showed that the curve did not reach the asymptote, it is considered a good sampling effort. It is very difficult to observe the stabilization of the curve, especially in tropical regions, due to microbiota instability revealed as new species are added to the list every time a new sample is collected (Colwell & Coddington 1994; Costa & Gusmão 2015; Costa *et al*. 2010; Ferreira *et al*. 2020).

The analysis of the diversity indices between the wet and dry periods showed significant variation (Table 4); however, species richness did not differ significantly. The availability of water or nutrients, besides plant heterogeneity and other factors may have influenced this result, so that seasonal changes have not affected the number of species occurring in the leaf litter. Plant heterogeneity is greatest in tropical forests (Wright 2002). A higher diversity of plant species found at a site may be a consequence of considerable environmental heterogeneity, as observed in this study and by Heredia-Abarca (1994).

There was no significant variation of diversity among sampling points, although occurrence in point 1 was significantly higher. This fact can be explained by some peculiar features of each point: points 1, 2 and 3 had larger amount of leaf litter and the area was more humid. From point 4 onwards, there was a decrease in the leaf litter, and the vegetation was drier in points 5 and 6. Other factors such as plant composition may also have contributed to the taxa variations between sampling points. Environment heterogeneity aggregates a series of parameters such as physical and chemical features, humidity, temperature, seasonality, decomposition stages of the leaf litter, wind, luminosity, and others that may have influenced the richness of taxa (Takyu *et al*. 2002).

The number of taxa in the dry period was higher than in the wet period in this study, in agreement with the results presented by Kodsueb & Lumyong (2019). Few studies suggest seasonal changes as a factor that influences the conidial fungi community in tropical forests (Lodge *et al*. 2004; Nikolcheva & Bärlocher 2005; Costa & Gusmão 2015). In contrast, many studies consider the seasonal factor as active in shaping the community of conidial fungi in temperate regions, as there are more significant changes in temperature, humidity, and rainfall (Gessner 1977; Kuter 1986; Thongkantha *et al*. 2008). Despite these reports, it is not possible to state that seasonality can affect the community (Kodsueb *et al*. 2008) because other abiotic factors (humidity, nutrient availability) can also influence the composition and structure of conidial fungi communities (Polishook *et al*. 1996; Ormeño *et al*. 2006; Paulus *et al*. 2007; Allegrucci *et al*. 2014).

The nMDS and ANOSIM analyses showed separation of the fungal communities between dry and wet periods in the 1-2 axis (Figure 4). Similar results were also found by Costa & Gusmão (2015). Their ANOSIM analysis indicated a significant difference in community of fungi between seasons (R = 0.8, P = 0.0004), and the nMDS analysis revealed a strong separation between samples collected during the wet and dry periods.

The hierarchical clustering analysis using UPGMA showed a low similarity (Figure 5) between the collection periods (wet and dry) of conidial fungi.

The conidial fungi that colonize mixed leaf litter or several substrates (leaves, branches, bark, and petioles) can present low similarity between sampling points of the same collection area. This is due to differences in the leaf litter constituents such as cellulose, lignin, secondary metabolites and other components (Voříšková & Baldrian 2013) that are related to different plant species and environment characteristics.

For the variables analyzed in this study, there is a structure in the fungal community in the two (seasonal) sampling periods (Figure 6). The rainfall variable separated the samples from the wet period and the air and soil temperature variables separated the samples corresponding to the dry period along the x axis. Prihatini *et al.* (2015) found no correlation between fungal communities and temperature using a PCA; however, these authors consider that rainfall is a significant environmental variable influencing fungal community. Bärlocher (1992) reported that in some forests the leaves accumulate in the soil until the beginning of the rainy season, ensuring the development of fungi. This fact may also explain the unpredictable behavior of the fungal community in tropical forests and, in addition, clarify the difference in the species composition for these fungi. The structural complexity in forests may create more microhabitats and microclimates for fungi, providing more resources and surfaces to be exploited (Lodge & Cantrell 1995).

This study of the conidial fungi community in an area that corresponds to about 1 % of the preserved area (359 ha) in the park revealed high richness of taxa. We contributed to the knowledge of the diversity of conidial fungi associated with leaf litter in the riparian zone of a humid forest formation inserted in a semi-arid region, adding new registers to the Northeast Brazil, South America and the Neotropics. The data indicates that there is still a great diversity to be uncovered in this park, and one can predict that the same is true to other areas in the semi-arid of the Northeast.

We confirmed the differences between dry and wet periods, with temperature variation and rainfall favoring substrate colonization by conidial fungi in a tropical forest, and rainfall influencing frequency of occurrence of the conidial fungi. However, even though univariate and multivariate analysis showed separation of the community between dry and wet seasons, it is not possible to affirm that the abiotic factors analyzed in this work are the only ones to have influence over this fungi community. Therefore, it is important to increase the number of variables analyzed in the studies to better understand how these influence the community of conidial fungi in humid forests and other ecosystems.

Acknowledgements

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