Fungal Biology 123 (2019) 660–668

Contents lists available at ScienceDirect

Fungal Biology

journal homepage: www.elsevier.com/locate/funbio

Comparison of aquatic hyphomycetes communities between lotic and lentic environments in the Atlantic rain forest of Pernambuco, Northeast Brazil

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ARTICLE INFO

Article history:
Received 8 February 2018
Received in revised form
22 May 2019
Accepted 23 May 2019
Available online 31 May 2019

Corresponding Editor: Nik Money

Keywords:
Aquatic ecosystems
Asexual Ascomycota
Neotropics
Riparian vegetation

ABSTRACT

Riparian forests are important to aquatic ecosystems and produce large quantities of organic matter that are recycled by the microbial community that includes microscopic fungi. The aim of this study was to unveil and compare the diversity of aquatic hyphomycetes associated to submerged leaf litter of tropical lotic and lentic environments in the Atlantic Forest of Northeast Brazil. Six sampling events were carried out in six points of two study areas: Biological Reserve "Mata da Chuva" (MC) and Environmental Protection Area "Lagoa da Mata" (LM), in Pernambuco, Brazil. Twenty three taxa of hyphomycetes were identified resulting in 87 occurrences. In the lake LM, 13 taxa of hyphomycetes were identified with 34 occurrences and in the MC (stream), 20 taxa with 53 occurrences. Ten species were common to both areas. Diversity indices and fungal biomass (ergosterol) were mostly higher in the lotic system. The fungal community analysis did not show any structure regarding sampling periods or sampling points within an area, however the two areas are different. Although the turbulence of the water is considered important for the development of these aquatic fungi, it is possible to find a diverse community of hyphomycetes and considerable fungal biomass in the lentic environment.

1. Introduction

The Atlantic (rain) Forest is characterized for presenting luxuriant vegetation that holds a large associated biodiversity of macro- (fauna) and micro- (microbiota and mycota) organisms (Tabarelli et al., 2010). Although the importance of this ecosystem has been fully recognized with all its benefits, the total area of this forest in Brazil is as yet decreasing due to human activities. The forest that initially ranged around 1.3 million km^2 is presently reduced to about 29 % of its original area and only circa of 7 % is well preserved forest fragments with more than 100 ha (MMA, 2019).

Riparian vegetation can be found inserted in the forests. These consist of strips of vegetation surrounding water bodies that offer natural protection to rivers, streams and lakes. The riparian forests influence water quality, enhance regulation of the water regime, stabilize the river banks and reduce the silting of the river channel. However, they are influenced by floods, by the input of nutrients and by the aquatic ecosystems they surround (Naiman and Decamps, 1997).

Fungi are very diverse and found in different environments. One of the environments where fungi can develop and carry out their activities is water bodies, where they take part in the processes of decomposition of submerged dead organic matter alongside other organisms (Abelho, 2001), contributing to nutrient cycling and the maintenance of this ecosystem (Graça et al., 2005).

The fungi in freshwater environments also enhance substrate palatability due to increased fungal biomass that favors consumption by shredders (Canhoto and Graça, 2008; Bärlocher and Sridar, 2014). Freshwater fungi are expected to be mainly Ascomycota, however, a number of species develop only their asexual state and receive from specialists the general name hyphomycetes (Raja et al., 2018; Seifert et al., 2011) so they can be studied according to their ecological

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https://doi.org/10.1016/j.funbio.2019.05.013
1878-6146/

functions with no prejudice to their upper taxonomical position when molecular work, for example, places them in the correct Phylum. For the purpose of studying the process of decomposition in aquatic environments, the hyphomycetes were distributed in four groups, according to their morphology and biology: Ingoldian or aquatic, aeroaquatic, terrestrial-aquatic and submerged-aquatic (or facultative aquatic) hyphomycetes (Goh and Hyde, 1996). Recently, Raja et al. (2018) recognized three groups (aquatic [Ingoldian], aeroaquatic and miscellaneous mitosporic hyphomycetes). In general, Ingoldian fungi are found in clean, turbulent, well aerated waters, with their conidia dispersed in the foam or associated to organic substrates (Ingold, 1975; Webster and Descals, 1981). The other groups do not demand these exact characteristics (Raja et al., 2018).

The main habitats of these aquatic fungi are rivers, streams, rapids and waterfalls, where they occur in substrates like leaves, branches and twigs of trees (Shearer et al., 2004). However, they can also be found in aquatic environments of stagnate or slow moving water, like reservoirs and lakes (Schoenlein-Crusius et al., 2009, 2014; Raja et al., 2018).

Marques et al. (2008) reinforced the importance of documenting the biota of the Atlantic Forest Domain ecosystems considering that only a small fraction of these forests is protected. Also, Raja et al. (2018) stressed the importance of studying freshwater fungi as freshwater ecosystems are amongst the most threatened ecosystems of all and have about the highest rates of species losses. Although a number of efforts have been carried out to document the Atlantic Forest diversity, the aquatic hyphomycetes are as yet poorly studied in both lotic and lentic systems in Brazil. Thus, the aim of this study was to uncover and compare the diversity of aquatic hyphomycetes associated to submerged leaf litter in lotic and lentic environments in two areas of Atlantic Forest in the Northeast of Brazil.

2. Materials and methods

2.1. Study areas

The lake Lagoa da Mata (7º46′36.04″S and 34º51′52.73″W) is located in the Itamaracá Island, the metropolitan region of Recife and, comprising an area of 46,750 m^2, it is fed exclusively by rain water and has no drainage. The soil in the lake area is mostly Oxisol with low natural fertility. The island, and consequently the lake region, is inserted in a State Conservation Unity, namely the Wild Life Refuge Mata de Amparo that is part of a State Conservation and Sustainable Use Unity – Environmental Protection Area (in Portuguese Área de Proteção Ambiental de Santa Cruz, FADURPE–CPRH, 2010). Despite being located in a protected area, there is a large gap in the knowledge between the freshwater and marine environments in this island.

The Municipal Biological Reserve Mata da Chuva (08º32′20″S and 35º43′22″W) is within a region commonly known as Serra dos Macacos (Monkeys' Hills), in the municipality of Bonito. This is located in the micro-region called "brejo" that is altitude humid forests inserted in the semiarid region between the meridional "agreste" and the Atlantic Forest zone of Pernambuco State. The area accounts for approximately 175 ha at 750 m.a.s.l. (Santiago et al., 2004). In 2011, a municipal law nº 936 established the Municipal Biological Reserve (REBIOmu) Mata da Chuva within which one of the springs of the River Bonito is found. The water emerges naturally from the soil and forms a stream that runs through the forest and reaches approximately two meters wide at the sampling sites.

2.2. Sampling of submerged leaf litter

Six sampling events were carried out for decomposing submerged leaf litter around six points established in each study site. The sampling points were located at a minimum of 100 m and maximum of 200 m from each other, distributed linearly in the right margin of the stream and covering the entire perimeter (1077 m) of the lake. The sampling was carried out from Aug/2016 to Sep/2017 at 2 m intervals. Fully submerged samples were collected by hand, or with the aid of a 15 cm diameter plastic sieve attached to a 1 m pole, from the margin, reaching into the water body to a maximum of 1 m. Thus, 6 samples from each site were analyzed at each sampling event, totaling 72 samples composed of 8–10 leaves in this study. The leaves were taken into glass jars, with some water from the site, and transported to the laboratory where they were further processed according to Ingold (1975) and Schoenlein-Crusius and Milanez (1989).

2.3. Samples processing, slides preparation and identification of hyphomycetes from aquatic environments

In the laboratory, the leaves were gently rinsed under tap water to remove the excess of sediments and other detritus (Ingold, 1975) and then cut into fragments of approximately 1 cm^2. For each sample, 40 fragments were cut and placed in 4 Petri dishes with sterile distilled water, totaling 10 fragments per plate that were incubated at room temperature (approx. 26–28 °C) for 4–5 d (Schoenlein-Crusius and Milanez, 1989) prior to observation. In the following 30 d, after daily observation of leaf fragments, the water from the plates was delicately changed every other day.

The leaf fragments were mounted onto microscope slides with distilled water and examined under a light microscope with phase contrast for observation of fungal structures like hyphae, conidiophores and conidia, necessary for morphological identification of the specimens. For the identification of the fungi, the specialized literature (e.g. Ingold, 1975; Marvanová, 1997; Matsushima, 1971, 1975; Nilsson, 1964; Seifert et al., 2011) was consulted, using the observed morphological characteristics for comparison. After the confirmation of the presence of fungal reproductive structures in the leaf fragments, semi-permanent slides were made by exchanging the water for 90 % lactic acid and sealing with transparent nail varnish (Fiuza and Gusmão, 2013).

2.4. Analysis of climate (average rainfall and air temperature) and water variables

Water samples were collected into clean 500 ml plastic bottles and taken to the laboratory for pH and electrical conductivity measurements in 30 ml aliquots using a bench pHmeter and conductivity meter, respectively. The water temperature at each sampling point was registered *in situ* with a glass thermometer (mercury column) that was left in the river/lake for 3 min. The dissolved oxygen levels in the water was determined from water samples collected into amber BOD glass bottles, following the method of Winkler modified by Golterman (1969). Monthly averages of rainfall and air temperature were compiled from the website of the water and climate agency of Pernambuco (APAC) at <http://www.apac.pe.gov.br/>.

2.5. Estimates of fungal biomass

Ergosterol extraction was carried out according to Seitz et al. (1977) with a few adaptations to our samples. Using 2 g of leaf litter grounded in liquid N_2 with a mortar and pestle, the material was transferred to extraction tubes added of 10 ml HPLC grade ethanol plus pyrogallol (0.3 g/L). The tubes were agitated for 2 h in orbital agitator and, next, the content was transferred to centrifuge tubes and run at 12,000 g for 10 min at 10 °C. After centrifugation, the supernatant was transferred to a fresh tube where 1 ml of 60 % KOH was added, and placed in a water bath at 90 °C for 30 min. After incubation, a 1 ml distilled water was added plus 4 ml hexane to each tube, following vigorous agitation and resting for phase separation and the removal of the hexane phase to a new tube. The fractioning was repeated with 2 ml hexane. The tubes containing the hexane phase were placed open on a heating plate, in the fume hood for evaporation. When dry, each tube received 2 ml methanol following agitation for ~1 h. The extract was then transferred to freezer resistant tubes and stored at −20 °C until analysis.

The ergosterol in each sample was quantified by liquid chromatography (HPLC). Ten μl of each extract (Malosso et al., 2004) were injected in the system that used methanol as carrier at a flow of 1 ml per min, and was equipped with a C18 reverse phase column. Ergosterol was identified by an UV detector set to 282 nm. The quantity of ergosterol in each sample was calculated by applying the integrated area of the specific peak to the equation ($y = 3148.7x - 4081.8$; $R^2 = 0.9959$) derived from the ergosterol (Sigma−Aldrich) standard curve.

2.6. Ecological analyses and diversity indices

Freshwater hyphomycetes were observed and identified on the leaf fragments and the presence of each species on each piece of substrate was accounted only once, even when a large number of conidiophores were detected for that particular species. The record of each leaf fragment was pooled together to account for one sample (composed of 40 fragments) per point per sampling expedition to facilitate comparison with other studies. Next, the ecological indices were calculated:

Shannon−Wiener's Diversity (H'): $H' = -\Sigma pi\ (ln\ pi)$ where $pi = ni/N$; N = total number of fungi sampled; ni = number of sampled fungi in a particular taxonomic group; ln = natural logarithm.

Pielou's Equitability (J'): $J = H'/H'_{max}$ where H'_{max} is the maximum possible diversity to be observed if all species present have equal abundance. $H'_{max} = \log S$ where S = total number of sampled species.

Berger−Parker's Dominance (d): $d = N_{max}/N_T$ where N_{max} is the number of individuals of the most abundant species, and N_T is the total number of individuals in the sample.

Species Richness: consists of the total number of species (S) in a sample unit.

Shannon−Wiener's Diversity (H'), Pielou's Equitability (J'), Berger−Parker's Dominance (d) and Dice/Sørensen similarity were calculated using the PAST 3 software (Hammer et al., 2001). PAST was also used to compare the ecological indices between the two areas by Diversity Permutation Test. To confront the observed richness against the estimated richness of the aquatic fungi species, the rarefaction curve was constructed based on the Bootstrap estimator (Sboot) and the associate confidence interval of 95 % calculated using EstimateS (Colwell, 2013). The bootstrap estimate is calculated with the following equation where p_k is the proportion of samples that contain species “k”, and m is the number of samples (Santos, 2003):

$$S_{boot} = S_{obs} + \sum_{k=1}^{Sobs} (1 - p_k)^m$$

The abiotic variables were analyzed using ANOVA and the significant averages were compared using the test of Tukey ($p < 0.05$). The abiotic data along with frequency of occurrence of hyphomycetes and the concentration of ergosterol were analyzed by principal components (Minitab 18, http://www.minitab.com/pt-br/) to verify whether there is any structuring in the fungal communities of the submerged leaf litter and what are the variables that contribute most to the observed structuring.

3. Results and discussion

3.1. Climate and water variables

The typical climate in the macro-region where the study sites are located presents wet winters (Apr to Sep) and dry summers (Oct to Mar). There was no rainfall registered in Nov 2016 in the municipality of Bonito (Fig. 1) and very little in Itamaracá Island while the higher levels of rainfall were registered for both locations in Jul 2017. According to the APAC records, in the dry months when sampling was carried out in Itamaracá Island (Lagoa da Mata), the levels of rainfall were higher than those in the municipality of Bonito (Mata da Chuva). This is certainly due to the fact that Itamaracá Island is in the Atlantic Ocean while the other site is located approx. 80 Km inland. The air temperature in the Reserve Mata da Chuva varied between 21 and 26.5 °C and in the lake Lagoa da Mata was from 24 to 27 °C (Fig. 1). Despite the differences in the numbers, they are not significant between sites ($p > 0.05$) for either rainfall or air temperature.

The water temperature did not differ significantly between the sampling points in either study site; however, it was significantly 9 °C warmer in the lake (Fig. 2). The amplitude of temperature variation along the seasons in the stream was only 2 °C while in the lake was 4 °C. This larger variation is probably due to thinner vegetation cover in the riparian zone of the lake combined with exposed water surface in the central part of the lake.

Significant pH variation between sampling points was detected only in the stream that was in general more acidic than the lake. The electrical conductivity (CE) and dissolved oxygen (DO) did not differ between sampling points in either site; however, the CE was significantly higher in the lake while the DO did not differ between areas (Fig. 2). Finding similar DO between these two aquatic systems could be explained by the amount of leaf litter deposited in the water bodies. In the stream, despite the movement of the water that input O_2 to the system, there is more substrate to be decomposed, and higher microbial activity consume more O_2 bringing its level down while the open surface of the lake facilitate phytoplankton photosynthesis, leveling these two systems with respect to O_2.

A recent study by Duarte et al. (2017) have shown that species richness was negatively affected by pH, conductivity and concentrations of nitrates and phosphorus in the water, however, a positive relationship was observed between species richness and water temperature. The heterogeneity in the community structure was influenced by environmental factors such as conductivity, nutrients concentrations and pH, thus, these authors concluded that the decomposer fungi in rivers exhibit biogeographic patterns that are selected by the environment making the environment responsible for the spatial variation in these fungal communities. However, their recommendation for caution is well placed since their data was collected mainly from temperate regions.

A tropical climate study in 15 Panamanian streams was published in 2010 in which some abiotic variables were analyzed (Bärlocher et al., 2010). The values for physical and chemical analysis of water reported there are in the same range of variation of those found in the present work (water temperature between 23.2 and 27.4 °C; pH between 6.6 and 7.5; O_2 between 4.3 and 8.2 mg L^{-1} and conductivity between 70 and 370 μS cm^{-1}) with no significant difference between stream groups (pristine, rural and urban). This might reflect in the similarities found for the fungal community.

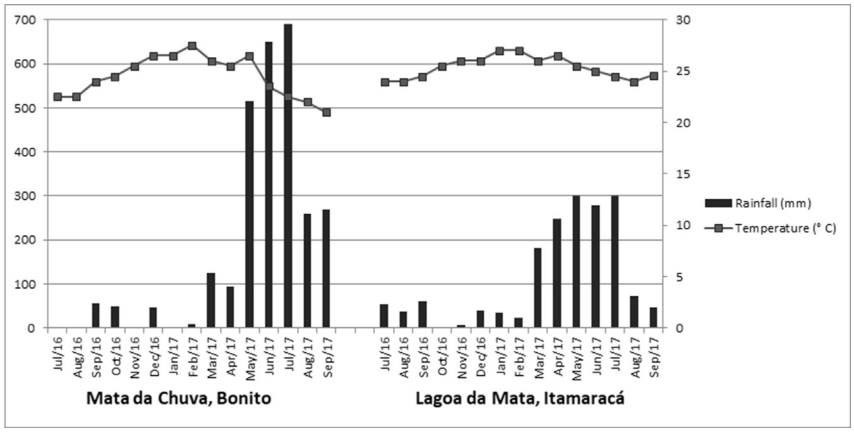

Fig. 1. Total monthly rainfall and air temperature in the municipalities of Bonito and Itamaracá Island, Pernambuco, from Jul 2016 to Sep 2017.

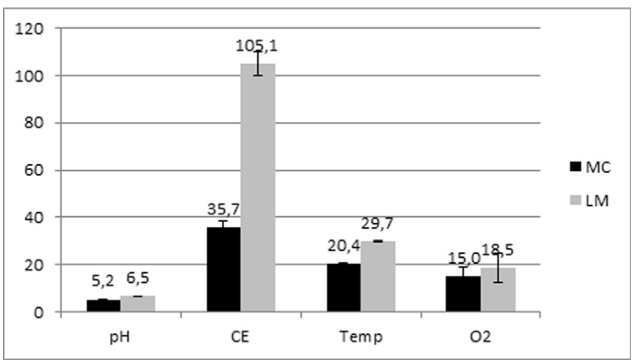

Fig. 2. Global average of the water abiotic variables in the stream at Mata da Chuva and the lake Lagoa da Mata, Pernambuco.

3.2. Species richness and occurrence of hyphomycetes in submerged leaf litter

In the six field trips were gathered 72 samples from the two areas. Twenty three taxa of hyphomycetes were identified totaling 87 occurrences. In the lake Lagoa da Mata, 13 taxa were identified with 34 occurrences, and 20 taxa with 53 occurrences were found in the stream of the Reserve Mata da Chuva.

Considering the biological groups in which the aquatic hyphomycetes *sensu lato* are divided, 11 taxa were identified as Ingoldian fungi, 7 taxa belong to the lignicolous terrestrial-aquatic hyphomycetes and 4 taxa to the aeroaquatic (Table 1).

In the stream of the Reserve Mata da Chuva, the taxa that occurred more frequently were *Triscelophorus monosporus* Ingold, *Triscelophorus acuminatus* Nawawi and *Colispora curvata* Nawawi & Kuthub. In the lake Lagoa da Mata, the most representative species were *Xylomyces acerosisporus* M.S. Oliveira, Malosso & R.F. Castañeda, *T. acuminatus* and *T. monosporus*.

These species seems to be very common and not novel for either the country or the Northeast Brazil since they have been reported before (Fiuza and Gusmão, 2013; Oliveira et al., 2015; Oliveira, 2016). Other species in Table 1 that are fully identified have also been reported. There are, however, 8 taxa that could not as yet be identified to species level and might be new, although we did not have success in culturing these fungi.

Fiuza et al. (2017) reported that there are 85 taxa of Ingoldian fungi known from Brazil from its different biomes. Considering the two Brazilian rain forests, 19 taxa are known from the Amazon and 53 from the Atlantic Forest. These lists of fungi contain species that are reported in our study, in Schoenlein-Crusius and Grandi (2003) review for South America and Bärlocher et al. (2010) study of the Panamanian streams.

3.3. Ecological aspects of the aquatic hyphomycetes

Comparing the six sampling events in the Reserve Mata da Chuva and those in the lake Lagoa da Mata, the period that presented the highest species richness for both areas was Sep/2017. The lower richness in the Mata da Chuva stream was accounted in Apr/2017, while in the lake was registered in Nov/2016. Except for Apr/2017, the lake Lagoa da Mata presented lower species richness compared to the stream in Mata da Chuva (Table 2).

In this study, the richness and diversity indices for the stream in Mata da Chuva were higher than those for the lake Lagoa da Mata. A comparative study of submerged leaf litter in the Guarapiranga reservoir (Schoenlein-Crusius et al., 2017), that is a lentic water body and the rivers Rio do Monjolinho and Rio Jacaré-Guaçú, that are lotic systems, the highest richness index and occurrence of taxa of hyphomycetes were registered in the lotic environment (Malosso, 1999). Eight taxa among those found were common between the two environments. This pattern of shared species between the two environment types was repeated in the present study. Studies following that of the late nineties in streams and lakes in the Municipal Park Ibirapuera (São Paulo city, Brazil) showed that the lakes presented higher richness than the streams (Schoenlein-Crusius et al., 2014). However, this is an urban park in the heart of the metropolitan region of São Paulo that has hundreds of visitors every day and it is as yet to be understood the impact of human recreational activities in the structure and dynamics of hyphomycetes communities in lakes and streams.

Among the taxa identified by Schoenlein-Crusius et al. (2014), *T. monosporus* Ingold and *T. acuminatus* Nawawi occurred more frequently and were also frequent in the present study. These results may be indicative that to some species the turbulence of the water is not relevant for their development and for conidia production (e.g. *Lunulospora curvula*), while for other species (e.g. *Xylomyces aquaticus*) the turbulence seems to be important. To some taxa such as *T. monosporus* and *T. acuminatus*, the turbulence factor may be of minor importance since they were abundantly found in both types of environments.

The highest occurrence of fungi in the stream of Mata da Chuva was registered in Jan/2017, while in the lake Lagoa da Mata was in Sep/2017. The lowest occurrence for both study areas was in Nov/2017, coinciding with the lowest rainfall. Only in the fourth and sixth samplings (Apr/2017 and Sep/2017) the lake Lagoa da Mata

Table 1
Occurrence of aquatic and facultative aquatic hyphomycetes from six sampling points observed during six sampling trips to two areas (Itamaracá Island – Lake Lagoa da Mata and Bonito Municipality – Reserve Mata da Chuva) in Pernambuco, Northeast Brazil.

Taxa/Location	Lagoa da Mata - Itamaracá Island							Reserve Mata da Chuva - Bonito							
	L1	L2	L3	L4	L5	L6	Total	M1	M2	M3	M4	M5	M6	Total	G.T.[a]
Aquatic (Ingoldian) hyphomycetes															
Anguillospora sp.							0		X	X		X	X	4	4
Articulospora tetracladia Ingold				X			1							0	1
Blodgettia indica Subram.							0		X	X				2	2
Colispora curvata Nawawi & Kuthub.	X		X			X	3	X	X	X		X	X	5	8
Flagellospora sp.		X		X	X		3		X	X				2	5
Ingoldiella hamata D.E. Shaw							0		X					1	1
Lunulospora curvula Ingold						X	1							0	1
Pyramidospora sp.	X			X	X		3		X					1	4
Triscelophorus acuminatus Nawawi	X	X		X	X	X	5	X	X	X	X	X	X	6	11
Triscelophorus magnificus R.H. Petersen						X	1	X						1	2
Triscelophorus monosporus Ingold	X	X	X	X	X		5	X	X	X	X	X	X	6	11
Terrestrial-aquatic (lignicolous) hyphomycetes															
Beltrania rhombica Penz.				X		X	2	X	X	X	X		X	5	7
Beltrania sp.							0				X			1	1
Codinea sp.							0					X		1	1
Cryptophiale kakombensis Piroz.							0		X					1	1
Cryptophiale udagawae Piroz. & Ichinoe							0		X					1	1
Endophragmiella sp.							0	X	X			X	X	4	4
Sporidesmiella sp.							0				X			1	1
Verticicladus amazonensis Matsush.						X	1							0	1
Aeroaquatic hyphomycetes															0
Xylomyces acerosisporus M.S. Oliveira, Malosso & R.F. Castañeda	X	X	X	X	X		5	X	X	X	X			4	9
Xylomyces aquaticus (Dudka) KD Hyde & Goh							0	X	X	X	X		X	5	5
Xylomyces giganteus Goh, WH Ho, KD Hyde & KM Tsui			X				1					X		1	2
Xylomyces sp.	X		X		X		3	X						1	4
Occurrence							34							53	87

[a] G.T.: Grand Total.

Table 2
Species richness, occurrence, Shannon–Wiener's diversity (H′), Pielou's equitability (J) and Berger–Parker's dominance (d) indices for aquatic hyphomycetes communities in each sampling event in the Reserve Mata da Chuva (MC) and lake Lagoa da Mata (LM), Pernambuco.

Ecological Indices	Aug/2016		Nov/2016		Jan/2017		Apr/2017		Jul/2017		Sep/2017	
	MC	LM	MC	LM	MC	LM	MC	LM	MC	LM	MC	LM
Richness (S)	8	5	5	2	6	5	2	5	10	6	11	8
Occurrence	81	34	23	11	115	37	25	35	98	22	94	119
Diversity (H′)	1.05	1.42	1.13	0.3	1.48	1.11	0.69	1.13	2.05	1.45	1.78	1.41
Equitability (J)	0.5	0.88	0.7	0.44	0.82	0.69	1	0.7	0.89	0.81	0.74	0.68
Dominance (d)	0.64	0.35	0.43	0.91	0.31	0.62	0.52	0.48	0.2	0.36	0.34	0.51

presented higher occurrence of fungi than the stream of Mata da Chuva (Table 2). The reason for this inverted behavior is yet to be found but it does not seem to be related to temperature or rainfall since these variables did not differ significantly between sites.

Regarding the diversity index in the studied areas, Jul/2017 is highlighted due to higher values for both the stream and the lake. The lowest diversity index in the Lagoa da Mata was in Nov/2016, coinciding with lowest rainfall and contrasting with the Reserve Mata da Chuva that presented lowest diversity in Apr/2017, that was when the rainy season was already in its second month (Table 2).

The equitability index was higher in the stream in all sampling events except for Aug/2016. The maximum (value equals to 1) was observed in Apr/2017 for the stream, although most of the values were considered high. Therefore, the distribution of individuals among the detected species was well balanced for most of the studied period. The lowest values of equitability were detected in Nov/2016 for the lake and in Aug/2016 for the stream. Consequently, it was in these moments with lowest equitability that the higher values of dominance were found (Table 2).

In general, the areas presented significant difference in the indices for richness and diversity that was higher for the stream in the Reserve Mata da Chuva. However, for equitability and dominance there was no significant difference between the studied areas (Table 3).

The communities of aquatic hyphomycetes in the two studied areas presented 60 % similarity according to the index of Sørensen. Regarding the similarities of the mycota between the six sampling points in the stream of Mata da Chuva, M2 and M3 presented the highest similarity (approximately 80 %), followed by M1 and M6

Table 3
Comparison of species richness, Shannon–Wiener's diversity (H′), Pielou's equitability (J′) and Berger–Parker's dominance (d) for aquatic hyphomycetes communities between the stream in the Reserve Mata da Chuva and the lake Lagoa da Mata, Pernambuco.

Ecological Indices	Mata da Chuva	Lagoa da Mata	*Perm p(eq)*[a]
Richness (S)	20	13	0.06
Diversity (H′)	2.07	1.85	0.01
Equitability (J′)	0.69	0.72	0.52
Dominance (d)	0.33	0.32	0.80

[a] *Perm p(eq)*: probability of having equal diversities. If *p(eq)* is greater than 0.05, there is no significant difference.

with 75 % similarity between them (Fig. 3, cophenetic correlation coefficient 0.77). M5 was the least similar, sharing 50 % of the taxa with the other sampling points. In the lake Lagoa da Mata, the higher similarity was found between L1 and L5 (~82 %), followed by L2 and L4 (~73 %) and the least similar was L6 that shared only 30 % of the taxa with the other sampling points. L6 is very close (less than 100 m) to the area where the public accesses the lake for swimming and barbecuing, which generates a lot of extra impact that the other sampling points do not have.

Although the studied areas present distinct characteristics between them, there was elevated similarity between the communities of hyphomycetes when the two areas were compared. This may be directly related to the affinity of the fungal species in the community of these areas with the characteristics of the Rain Forest. Considering the sampling points of each area there was similarity between the mycota even with different turbulence levels between the sites, in agreement with the report of Schoenlein-Crusius et al. (2014) and the discussion of Duarte et al. (2017) about geographic distance between water bodies.

The species accumulation curve for the stream in the Mata da Chuva estimated the maximum species richness of hyphomycetes in 23, and in this study 20 species have been found (Fig. 4A). That accounts for 83 % of the estimated taxa for that area. In the lake Lagoa da Mata, the maximum richness was estimated in 15 while 13 have been found in the study, accounting for 87 % of estimated taxa (Fig. 4B). In this analysis we used the bootstrap estimator that was initially developed by Efron (1979) as a method related to Jackknife, however, it was later treated as a more widely applicable and trustworthy than Jackknife (Smith and van Belle, 1984). Bootstrap differs from the other non-parametric estimators as it is not restricted to the rare species but uses data of all collected species to estimate total richness (Colwell and Coddington, 1994; Santos, 2003).

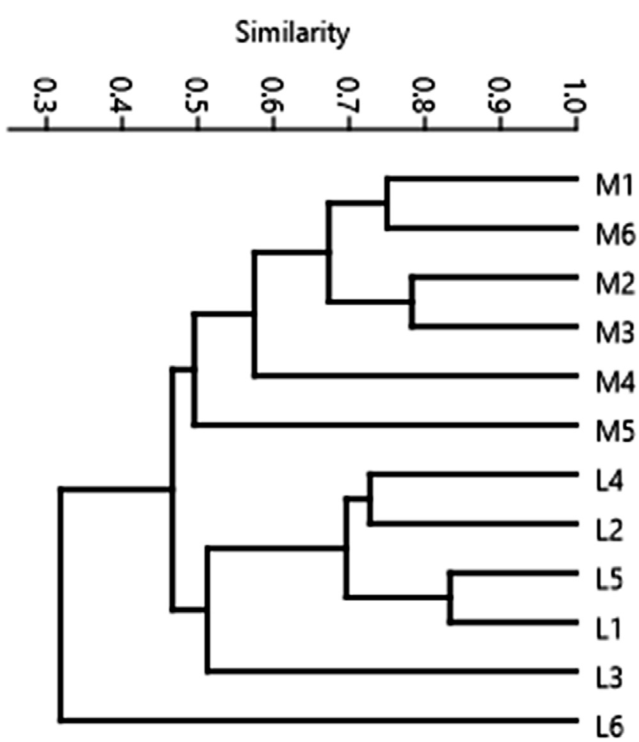

Fig. 3. Sörensen similarity dendrogram obtained by comparisons of the mycota between sampling points of the stream in the Reserve Mata da Chuva, Bonito (M) and the lake Lagoa da Mata, Itamaracá island (L), Pernambuco.

Accumulation curves for the studied areas, and the associated statistics, express the diversity of species expected and what was actually found. In this study of hyphomycetes in submerged leaf litter, the high percentage of uncovered species indicates that a good sampling effort was employed, although the species richness is not high in these sites. Shearer et al. (2015) registered higher diversity of taxa with smaller sampling effort than the employed in this work showing that the fungal communities in those areas are richer and more complex than in our study. For the lake Lagoa da Mata, the lower richness may be related to the fact that the water body does not have any tributary or outlets (no water flow) which concentrates leaves of the plant species in the immediate neighborhood of the lake, limiting the diversity of available substrates. However, the data (this study and the literature) are as yet insufficient to confirm this.

3.4. Fungal biomass determination based on ergosterol

Ergosterol concentrations are presented (Fig. 5) as average for samples of each area in different sampling periods and showed small differences between sampling events in both areas. In the stream of Mata da Chuva, the submerged leaf litter presented higher average levels of ergosterol in Aug/2016 (1.7 mg/g) and Nov/2016 (1.5 mg/g) while in the samplings of 2017 and Sep/2017, the samples from the lake Lagoa da Mata presented higher concentrations (1.3 and 1.2 mg/g). The lowest average of ergosterol was registered in Jan/2017 (0.6 mg/g) in the lake Lagoa da Mata.

The values of ergosterol detected in this investigation are within the range of values reported in Brazil and in tropical regions (Gonçalves et al., 2006, 2007; Lopes et al., 2015; Martins et al., 2017). The alternating values (high and low) of ergosterol detected in different leaf litter samples were expected since the leaves present different chemical composition and fungal colonization depend on the available nutrients, inhibitory substances, climate and humidity, besides the physiological status of the fungi (Barajas-Aceves et al., 2002; Gessner and Chauvet, 1994; Gessner et al., 2007). During the fungal development, changes that occur in the synthesis and organization of ergosterol in the cells can be due to simple changes in the environment but also to a change in the phase of the fungal life cycle thus interfering in the result of the analysis and data interpretation (Newell, 1992; Behalová et al., 1994; Gessner and Chauvet, 1994). The first time ergosterol was used to analyze aquatic hyphomycetes biomass in Brazil was in 1999 by Malosso (Schoenlein-Crusius et al., 2017). This was followed by another graduation works that also applied this technique (Moreira, 2006). After those, the works by Gonçalves et al. (2006, 2007), Lopes et al. (2015) and Martins et al. (2017) were published, however, they reported their data as μg of ergosterol per g of ash free dry mass (AFDM), therefore, their data are not directly comparable to ours. To date, there is as yet a scarcity of ergosterol data from submerged leaf litter in Brazil, especially in the Northeast where, to the best of our knowledge, this is the first report. Despite the samples have been taken from Atlantic Forest sites, the climate conditions can be very different between our study area and the Southeast (São Paulo and Minas Gerais states) where the other studies were carried out.

Pearson's correlation analysis was carried out to verify whether there is any correlation between the occurrence of fungi and the concentration of ergosterol in the decomposing submerged leaf litter. It has been detected that for the lake Lagoa da Mata there was no correlation between the two variables since the observed value (0.04) is close to zero. For the stream in the reserve Mata da Chuva, the correlation was moderate and negative (−0.43). This inverse behavior (increased ergosterol when detected diversity decreases) can be interpreted as few species are dominating in the

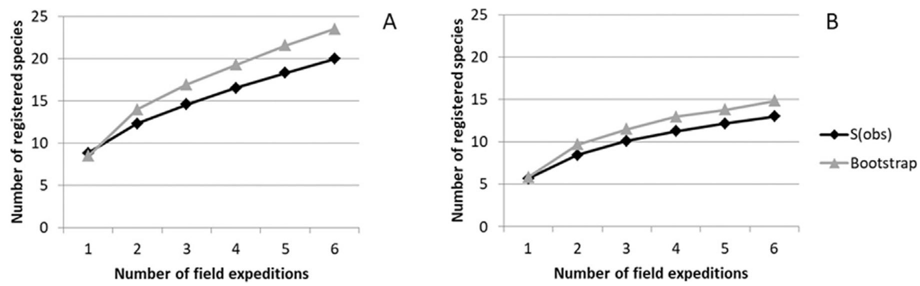

Fig. 4. Accumulation curves for taxa of hyphomycetes in submerged leaf litter and nonparametric Bootstrap estimate for the stream of the Reserve Mata da Chuva, Bonito (A) and the lake Lagoa da Mata, Itamaracá Island (B), Pernambuco.

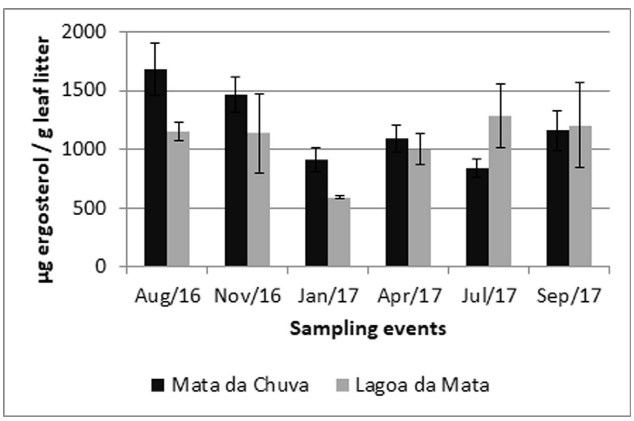

Fig. 5. Average concentration of ergosterol in submerged leaf litter in the stream of the Reserve Mata da Chuva and the lake Lagoa da Mata in different sampling events.

environment during certain periods or under certain conditions. Duarte et al. (2006), in their experiment on the potential effect of species richness on overall fungal production (ergosterol buildup), detected a significant difference in the levels of ergosterol in leaf litter when the four species of fungi were inoculated instead of the sum of the biomasses of individual inoculation and also that whenever one particular species was present, the amount of ergosterol was higher.

3.5. *Aquatic hyphomycetes community structure analysis*

A principal components analysis (PCA) including frequency of occurrence of taxa, ergosterol concentration in the substrate, water pH, electric conductivity, dissolved oxygen and temperature (Fig. 6), showed that the first two components explained 70 % of variance, separating the two study areas in the direction of the first component (X axis), and CE was one of the variables that contributed to this separation, along with pH and temperature, with equivalent vector loadings.

Based on the variables include in this research, no systematic structure was found for the communities in these two areas regarding either sampling points or sampling periods. Although samples from each site were separated along the X axis, forming 2 clusters.

Schoenlein-Crusius et al. (2009) sampled submerged leaf litter in an urban park during 30 m and found that the occurrence of aquatic hyphomycetes was mainly influenced by the trophic level of the aquatic environments. In their analysis of abiotic data with the number of fungal occurrences revealed that the first two axes accounted for 81.7 % of data variability while separated samples of the eutrophic sites from the others regardless of seasons. In a posterior study (Schoenlein-Crusius et al., 2014) in another park of São Paulo city, the authors reported that ANOSIM pointed out a significant difference between samples taken from the same sites within different seasons while they were not significantly different when taken from different sites within one season. These findings indicate a lack of pattern for hyphomycetes behavior in urban waters of São Paulo city.

The sensitivity of the aquatic hyphomycetes to decreased oxygen levels in the water was a probable reason to regard them as bioindicators of water quality, however, their ability to metabolize dissolved organic matter and to survive using inorganic nutrients (Gessner, 1997) did not fully support this idea, according to Schoenlein-Crusius et al. (2009).

The pH, especially when it is low, can also be a physiological stressor to the aquatic community. Sales et al. (2015) believe that acidic water could affect the rates of fungal metabolism but could not be considered the sole factor accounting for low species numbers and spore concentrations for aquatic hyphomycetes in a Cerrado stream since other factors like low concentrations of dissolved oxygen could also reflect low diversity of aquatic hyphomycetes (Medeiros et al., 2009), with reports of less than half the number of species of aquatic hyphomycetes in a tropical stream (Chamier, 1987) when dissolved oxygen dropped from about 6.0 mg/L to about 3.8 mg/L.

Sales et al. (2015) found that ergosterol concentration (fungal biomass) was correlated with leaf mass loss and reported that the sporulation rates of aquatic hyphomycetes peaked at the beginning of the rainy season, when fewer hyphomycetes species were present. In the tropical stream of their study, where the physical and chemical characteristics of the water varied little during the year, their hypothesis that changes related to leaf chemical composition could be the cause of changes in the abundance and composition of the fungal assemblages was not confirmed since no significant differences were found in the analyses of lignin, cellulose, fiber, phosphorous, and polyphenols. Therefore, it is as yet advisable to agree with their conclusion that future studies are necessary to understand the patterns of richness and the successional process of these fungi in tropical streams.

4. Conclusions

The studied areas of the Atlantic Forest present a diverse community of fungi associated to the submerged decomposing leaf litter similar to other sites in tropical regions of Brazil. The significant differences between the water bodies seem to impact the fungal communities less than the expected, especially regarding the turbulence of the water that, although it is considered an important factor for the development of aquatic fungi, it is possible to find a rich community of hyphomycetes in lentic environments.

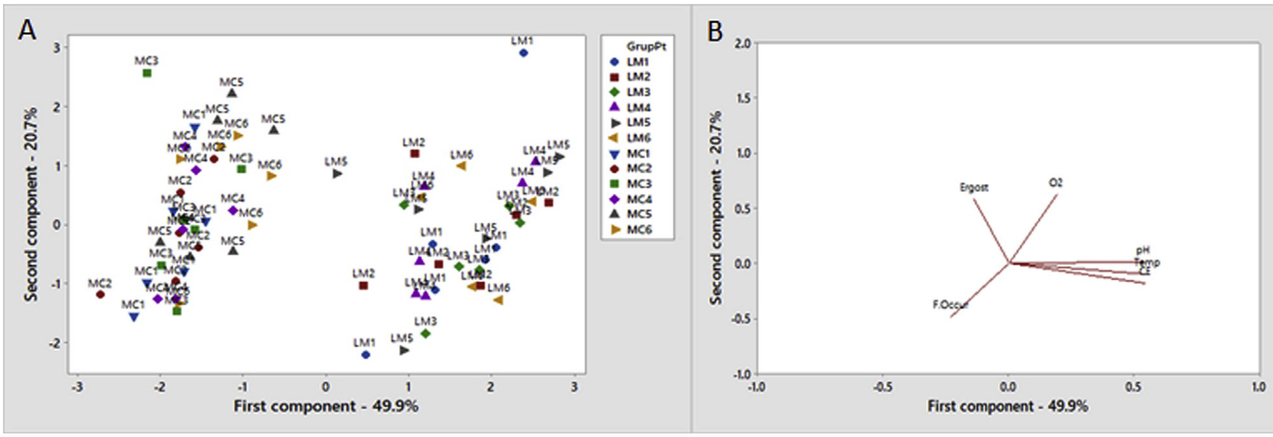

Fig. 6. Principal component analysis comparing abiotic variables, occurrence of hyphomycetes and levels of ergosterol in submerged leaf litter samples of the lake Lagoa da Mata and the stream in the Mata da Chuva. A – Scores of first and second components; B – Vector loading. The graph explains 70 % of data variance.

There is a large variation in the levels of ergosterol detected in the substrate between different studies therefore comparison between them are not always direct, however, when comparison is possible, the levels of fungal biomass in the study sites are within reported range. The climatic conditions of the studied sites are very similar therefore; the only structure found in the hyphomycetes community was related to the lotic and lentic sites. The data in this study contribute to increase the knowledge about the distribution and ecological aspects of aquatic hyphomycetes in Brazil and in poorly studied areas in the State of Pernambuco, consequently increasing the knowledge for tropical regions.

Acknowledgements

The authors are thankful to the Post-Graduation Program in Biology of Fungi and the Department of Mycology of the Universidade Federal de Pernambuco for the facilities; to Prof. Antônio Fernando Morais de Oliveira for helping with ergosterol analysis; to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the scholarship to the first author; and to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for financial support (grant Nº 88881.062172/2014-01) to EM and RFCR.

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