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Research Paper

Characterization saprobic fungi on leaf litter of two species of trees in the Atlantic Forest, Brazil

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Abstract

We investigated the composition and structure of fungal communities associated with leaf litter generated by *Clusia nemorosa* and *Vismia guianensis* that belong to phylogenetically-related botanical families and exist together in a remnant of the Atlantic Forest in Bahia, Brazil. Samplings were conducted during wet (June2011) and dry (January2013) seasons in Serra da Jibóia. The fungi were isolated using particle filtration and the 1,832 isolates represented 92 taxa. The wet season yielded the largest number of isolates (1,141) and taxa (76) compared with the dry season (641 isolates and 37 taxa).The richness and diversity of fungal species associated with *C. nemorosa* (64 taxa, Simpson = 0.95) were higher compared with those of *V. guianensis* (59 taxa, Simpson = 0.90). Analysis of similarity (ANOSIM) revealed significant variations in the composition and community structure of fungi isolated from the two plants as a function of seasons. In contrast, nonmetric multidimensional scaling (NMDS) analysis show that the seasonality was an important influence on the distribution of fungal species. However, the populations of the saprobic fungal communities were dynamic, and several factors may influence such communities in the Atlantic Forest.

Key words: fungal communities, seasonality, rainforest, diversity, fungal ecology.

Introduction

The Atlantic Forest comprises a great diversity of plants, estimated at approximately 20,000 species, including 8,000 that are endemic (Myers *et al.*, 2000; SOS Mata Atlântica, 2013). This biome is home to one of the largest concentrations of species per square meter on the planet (Thomas *et al.*, 1998). Despite this robust biodiversity, the Atlantic Forest suffers major threats, and only 11.7% of its original area remains (Ribeiro *et al.*, 2009). Therefore, it is considered one of the 34 global hotspots (Morris, 2010).

As in other forest ecosystems, litter deposited on the soil of the Atlantic Forest comprises various plant debris, and leaves represent the most significant component (Werneck *et al.*, 2001). Fungi, among other microorganisms, contribute significantly to decomposition through their ability to degrade numerous compounds produced by plants, and the diversity of leaves in the litter allows the coexistence of various species (Promputtha *et al.*, 2010; Vorísková and Baldrian, 2013). This plurality is due, in part, to the recurrence of fungal species of certain host plants (Santana *et al.*, 2005; Paulus *et al.*, 2006b; Pasqualatti *et al.*, 2014) and particular stages of decomposition (Cornejo *et al.*, 1994; Yanna *et al.*, 2001; Santhi and Vittal, 2012).

Zhou and Hyde (2001) suggested the term "recurrence" to replace the *specific* terms and *preferences* commonly used to describe interactions of saprobic fungi that occur most often in association with a particular plant species compared with other species in the same environment. The reasons for the recurrence of fungi on different hosts may involve foliar structure and chemistry (Santana *et al.*, 2005; Paulus *et al.*, 2006b) or the initial decomposition of litter by the action of persistent endophytes present in the tissues of living leaves when they subsequently senesce and fall to the ground (Hyde *et al.*, 2007; Promputtha *et al.*, 2010; Unterseher *et al.*, 2013; Allegrucci *et al.*, 2014). Besides the recurrence of fungi harbored by particular plants,

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abiotic factors such as climate at collection sites and seasonality influence the composition and structure of fungal communities (Polishook *et al.*, 1996; Ormeño *et al.*, 2006; Paulus *et al.*, 2006b; Allegrucci *et al.*, 2014).

Numerous studies demonstrate the recurrence of saprobic fungi on substrates of decomposing plants, and fungi tend to recur at a higher rate according to the host's genus (Polishook *et al.*, 1996; Paulus *et al.*, 2006b; Kodsueb *et al.*, 2008; Cheewangkoon *et al.*, 2009; Magalhães *et al.*, 2011; Allegrucci *et al.*, 2014). For example, Paulus *et al.* (2006b) investigated the community of fungi associated with six plants and found low overlap among species, which may be accounted for by the phylogenetically unrelated families to which the host plants belong. Further, Paulus *et al.* (2006b) analyzed the influence of seasonality (wet and dry seasons) on the distribution of fungi and found that the taxonomy of the substrate was the most important variable. We are unaware of studies that determined whether saprobic fungi recur on a specific host or are generally present in the leaf litter of plants of phylogenetically related families.

To estimate the number of fungal species, it is important to determine whether fungi are recurrent or general, particularly in tropical regions (Zhou and Hyde, 2001; Hawksworth, 2001). The goals of the present study were as follows: (1) to investigate the composition and structure of the fungal community of plants in the same environment and (2) to evaluate the effects of plant species and seasonality in a diverse biological community. For this purpose, we identified the fungi associated with leaf litter of *Clusia nemorosa* G. Mey (Clusiaceae) and *Vismia guianensis* (Aubl) Choisy (Hypericaceae), which are locally abundant and belong to phylogenetically-related botanical families, during the wet and dry seasons in a remnant of Atlantic Forest.

Material and Methods

Study area and sampling

The Serra da Jibóia is located in South Reconcavo in the State of Bahia and consists of a complex of hills and mountains that is distributed over six municipalities (Santa Terezinha, Castro Alves, Elísio Medrado, Varzedo, São Miguel das Matas and Laje) (Figure 1). Expeditions were conducted in Morro da Pioneira (north of the Serra da Jibóia), in the municipality of Santa Terezinha (12°51' S and 39°28' W) (Figure1). The mean altitude of this area ranges between 750-840 m above sea level with a mean annual temperature and rainfall of 21 °C and 1,200 mm. Rainfall occurs most abundantly from April through July (Neves, 2005; Tomasoni MA, unpublished data). The remaining months are called dry months, because they are characterized by levels of precipitation < 100 mm. To evaluate the influence of seasonality on the community of fungi, we performed sampling during wet and dry seasons

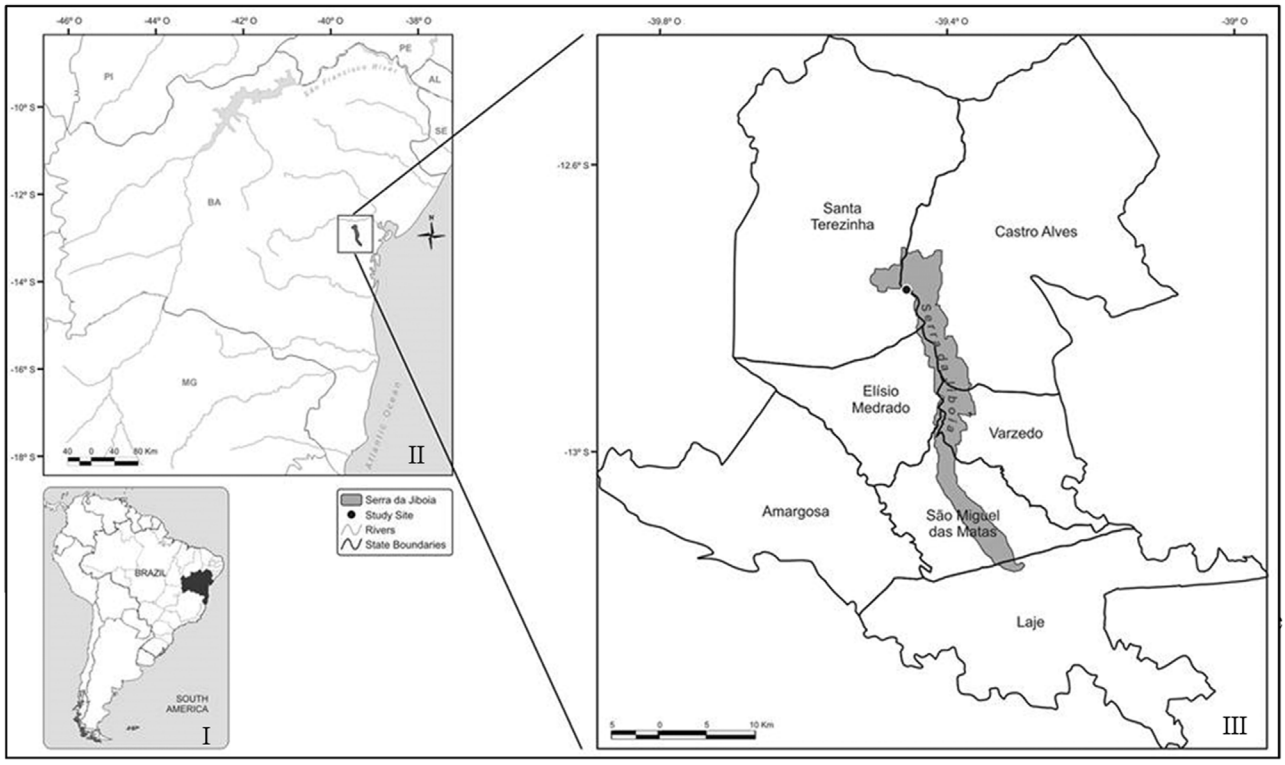

Figure 1 - Location of the Serra da Jibóia in the state of Bahia, Brazil (I, II); and collection site in the municipality of Santa Terezinha (Morro da Pioneira) indicated by the black point on the map (III).

(considering the average rainfall of three months before collection, 114.5 mm and 53.16 mm, respectively).

The sampled plants are shrubs and the height reaches approximately 3 m. Samples of *C. nemorosa* (C1: S 12°51'19" and W 39°28'32"; C2: S 12°51'21" and W 39°28'31"; C3: S 12°51'18" and W 39°28'32") and *V. guianensis* (V1: S 12°50'57,3" and W 39°28'33,0"; V2: S 12°50'53,8" and W 39°28'31,4"; V3: S 12°50'51,7" and W 39°28'30") were chosen, taking into account the presence and amount of fallen leaves at their bases, and were marked for subsequent collection. One sample of each individual plant comprising 10 leaves were collected during each expedition, and 60 leaves of each plant species (30 each from wet and dry seasons) were processed no longer than 24 h after collection. A sample of *C. nemorosa* dry (C3d) was lost due to contamination and not considered in the analysis.

Isolation, characterization and preservation of fungi

The leaves were first washed with running water and then subjected to surface disinfection according to the method of Paulus *et al.* (2003a). Because the leaves of *V. guianensis* are smaller compared with those of *C. nemorosa*, leaf areas were standardized after disinfection. The leaves of *C. nemorosa* were divided into five areas and were removed by drawing a section equivalent to the leaf area of *V. guianensis* (18 cm^2). Particle filtration described by Bills and Polishook (1994), with modifications, was used to isolate fungi.

Each sample was homogenized in a blender for 1 min in 100 mL of sterile distilled water. The particulate material was washed with distilled water jets and filtered through a group of five stainless steel meshes with decreasing openings (1.0, 0.7, 0.5, 0.25, and 0.18 mm). The particles retained on the mesh of the smallest opening were transferred to centrifuge tubes and suspended in sterile distilled water (50 mL), vortexed for 1 min, and decanted. The supernatant was discarded, and the tube was filled with sterile distilled water to a volume of 50 mL. This procedure was performed four times, and the residual material was suspended in 20 mL of sterile distilled water. Aliquots (50 μL) of the suspension were transferred and inoculated in triplicate, using a Drigalsky stirring rod, into 90-mm diameter Petri dishes containing dichloran rose bengal chloramphenicol agar (DRBC, without the addition of dichloran) and malt yeast extract agar (MYE) supplemented with rose bengal (25 mg/L) and chloramphenicol (100 mg/L) (Paulus *et al.*, 2003a).

The plates were incubated at room temperature and light. Mycelial growth was observed daily from days 2-30, and after verification of hyphal growth, the particles were transferred to 60-mm diameter Petri dishes filled with culture medium containing corn-meal carrot agar (Castañeda-Ruiz, personal communication) and fragments of sterilized banana leaves (3 cm^2) to induce sporulation (Paulus *et al.*, 2003b).

Pure cultures were examined periodically using a stereomicroscope until reproductive structures were observed. These structures were transferred with the aid of a fine needle to slides with mounting medium containing PVL resin (polyvinyl alcohol lactic + alcohol + phenol) (Trappe and Schenk, 1982) and lactic acid. Fungi were identified morphologically, and the structures of taxonomic importance were compared with those described in the relevant basic and specific literature.

Isolates that did not sporulate were subsequently cultured in water agar (WA) and oatmeal agar (OA) with fragments of sterilized banana leaves. Sterile mycelia were grouped into morphotypes according to characteristics such as growth rate, margin shape, surface staining and reverse, lift and texture of the mycelia, and pigment production in the culture medium (Lacap *et al.*, 2003). Isolates of each identified species were preserved under mineral oil (Buell and Weston, 1947) and Castellani (1967) and deposited in the Coleção de Culturas de Microrganismos da Bahia (CCMB), Universidade Estadual de Feira de Santana.

Data analyses

Community diversity of saprobic fungi of both plant species was evaluated using Simpson's diversity index (Magurran, 1988). The number of taxa expected in the communities was calculated using the Chao1 estimator (Chao, 1984). The differences in richness among fungi isolated from *C. nemorosa* and *V. guianensis* were determined using rarefaction curves (Magurran, 1988). Similarities in species composition between the samples of leaf litter were verified by ordering the data using the NMDS method (*Nonmetric Multidimensional Scaling*) (Kruskal, 1964) from the Bray-Curtis dissimilarity matrix. The ANOSIM (*Analysis of Similarity one way*) permutation test was used to assess differences/dissimilarity between groups of samples (Clarke, 1993). SIMPER (*Similarity Percentages*) analysis was performed to identify the most influential species and those that contributed most to the dissimilarity between groups (Clarke, 1993). The analyses were conducted using Biodiversity Pro 2 (Mc Aleece, 1997), PAST v. 3.01 (Hammer *et al.*, 2013) and R 3.0.1 (R Core Team, 2013).

Results

We isolated 1,832 species of fungi representing 92 taxa from the leaf litter of *C. nemorosa* and *V. guianensis*. The greatest number of fungi (1,141) and the richness of taxa (76) were observed during the wet season (Table 1). The Simpson's index (1-D) for communities of fungi demonstrated higher diversity of that one isolated from *C. nemorosa* (0.95) compared with *V. guianensis* (0.90). The confidence intervals indicated that the difference between the indices, although small, was statistically significant, because there was no overlap between values. Considering the same sampling effort, the estimated richness obtained

using Chao1 was 70 taxa for *C. nemorosa* and 66 taxa for *V. guianensis* (Table 1).

Fewer fungi (869) with the highest richness of taxa (64) were isolated from *C. nemorosa*. During the wet season, the leaves of *C. nemorosa* harbored more taxa (54), and the leaves of *V. guianensis* harbored a greater richness of species (31) during the dry season (Table 1 and Figure 2). Rarefaction curves showed no stability; however, the slopes of the curves of samples collected during the dry season were shallower (Figure 2). Given the smaller sample size of 291 isolates used to perform a random resampling, the expected values of richness displayed by the rarefaction curves were 45, 38, 28, and 20 for samples Cw, Vw, Cd, and Vd, respectively (Figure 2). However, the differences observed between Cw and Vw were not significant, because there was overlap between confidence intervals (Cw: 41-49; Vw: 34-42).

Among the sporulating isolates, 68 taxa were asexual (13 coelomycetes and 55 hyphomycetes), and nine produced sexual reproductive structures in culture. Four ascomycetes formed connections with their asexual forms as follows: *Calonectria gracilipes*/*Cylindrocladium graciloideum*, *Glomerella cingulata*/*Colletotrichum gloeosporioides*, *Guignardia* sp./*Phyllosticta* sp. and *Pseudomassaria carolinensis*/*Beltraniella portoricensis*. Taxa *Pestalotiopsis* spp. (14.2%), *P. carolinensis* (12.5%), *Chaetosphaeria* sp. (6.6%), *G. cingulata* (6.6%), *B. rhombica* (6.3%), sterile mycelium sp.7 (6.1%), *Penicillium minioluteum* (3.7%), and *Phomopsis* sp.2 (3.2%) were the most abundant and common in both substrates, and the first four taxa were most prevalent on *V. guianensis* (Table 2).

Clusia nemorosa harbored 33 unique taxa and *V. guianensis* yielded 28 taxa. Thirty-one taxa were shared between the two plants (34%), and 66% did not overlap. Among taxa shared between *C. nemorosa* and *V. guianensis*, the most abundant were *B. rhombica*, *Chaetosphaeria* sp., *G. cingulata*, sterile mycelium sp.6, *P.*

Table 1 - Richness, number of isolates, Simpson's diversity, and Chao1 of the fungal communities from leaves of *C. nemorosa* and *V. guianensis* during the wet (w) and dry (d) seasons in the Serra da Jibóia, Bahia, Brazil.

Substrates	N° isolates		Total	Richnes		Total	Simpson[a]	Chao1[a, b]
	w	d		w	d			
C. nemorosa	578	291	869	54	20	64	0.95 (0.94-0.96)	70 (65-88)
V. guianensis	563	400	963	45	31	59	0.90 (0.89-0.91)	66 (57-101)
Total	1141	691	1832	76	37	92	0.94 (0.93-0.95)	98 (94-114)

[a]95% confidence intervals.
[b]Analysis performed with 5 samples for each plant.

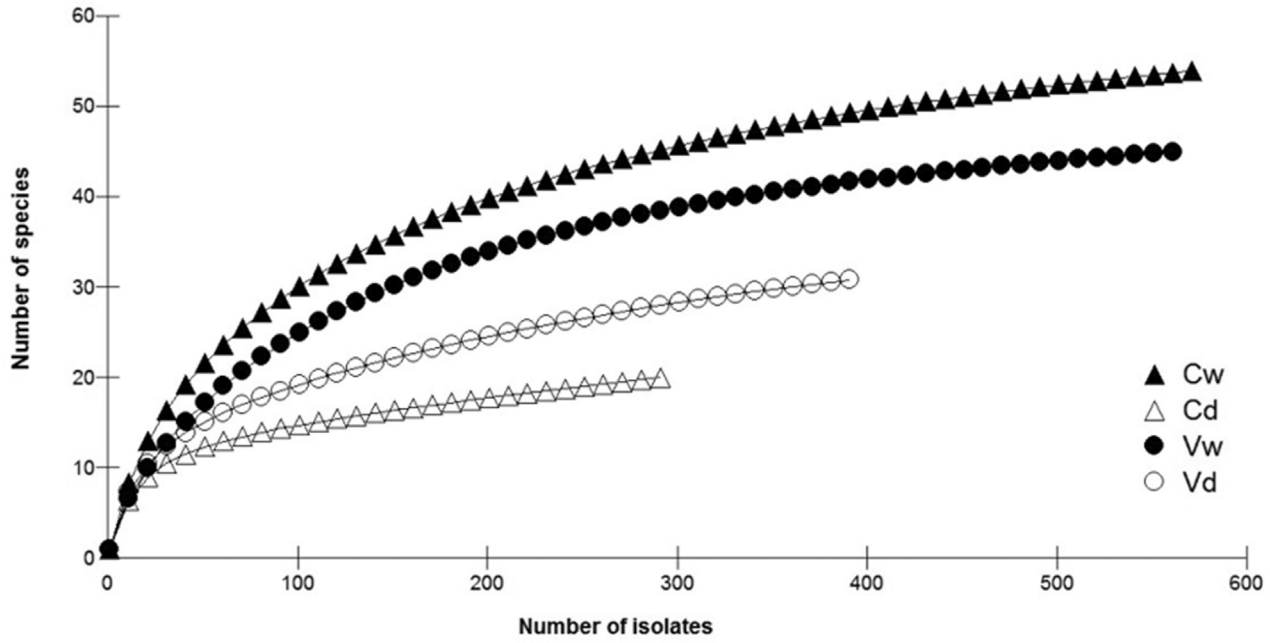

Figure 2 - Rarefaction curves of fungi isolated from leaves of *C. nemorosa* (C) and *V. guianensis* (V) during the wet (w) and dry (d) seasons in the Serra da Jibóia, Bahia, Brazil.

Table 2 - Number of fungi associated with leaf litter of *C. nemorosa* and *V. guianensis* collected during the wet (w) and dry (d) seasons in the Serra da Jibóia, Bahia, Brazil.

Taxa	*C. nemorosa*		*V. guianensis*		Total
	w	d	w	d	
Pestalotiopsis spp.	86	0	174	0	260
Pseudomassaria carolinensis M.E. Barr & Hodges	62	1	100	67	230
Chaetosphaeria sp.	24	1	65	31	121
Glomerella cingulata (Stoneman) Spauld. & H. Schrenk	36	0	25	60	121
Beltrania rhombica Penz.	52	0	18	46	116
Sterile mycelium sp.6	5	91	14	2	112
Penicillium minioluteum Dierckx	0	31	0	37	68
Phomopsis sp.2	4	31	3	20	58
Gliocladiopsis sp.	49	0	0	0	49
Phomopsis sp.4	22	24	0	0	46
Dactylaria belliana B.C. Paulus, Gadek & K.D. Hyde	41	0	0	0	41
Satchmopsis brasiliensis B. Sutton & Hodges	0	0	2	38	40
Guignardia sp.	0	28	0	7	35
Penicillium brevicompactum Dierckx	16	0	18	0	34
Sterile mycelium sp.7	2	10	6	14	32
Phomopsis sp.1	0	18	5	8	31
Rare taxa[a]	179	56	133	70	438
Total	578	291	563	400	1 832

a Fungal isolates present at a frequency of < 1.5% were as follows: *Acremonium* spp.1 and 2, *Ardhachandra cristaspora,* ascomycetes spp.1-5, *Aspergillus ochraceus, Atrosetaphiale fragelliformis, Bartalinia* cf. *robillardoides, Beltraniella* cf. *botryospora, Calonectria gracilipes*, coelomycetes sp., cf. *Chaetosphaeronema* sp.1, *Chalara alabamensis, Chalara* cf. *paramontellica*, *Cladosporium-like* sp.1, *Coleophoma* sp., *Cryptophialoidea fasciculata*, *Curvularia geniculata*, *Cylindrocladium candelabrum*, *Cylindrocladium floridanum*, *Cylindocladium gracile*, *Cylindrocladium pauciramosum*, *Dactylaria* cf. *acerosa*, *Dactylaria* cf. *biseptata*, *Dictyochaeta simplex*, *Dinemasporium* sp., *Fusarium lateritium*, *Fusarium solani*, *Gyrotrix* cf. *pediculata*, hifomiceto sp.1-3, *Idriella* cf. *cubensis*, *Idriella lunata*, *Idriella ramosa*, *Idriella* cf. *variabilis*, *Idriella* spp.1 and 2, *Lasiodiplodia theobromae*, *Menisporopsis theobromae, Metarhizium anisopliae,* sterile mycelia spp. 1-5 and 8-15, *Ochroconis variabilis*, *Ochroconis* spp.1 and 2, *Paliphora intermedia*, *Parasympodiella laxa*, *Phomopsis* sp.3, *Pyrenochaeta* sp., *Sclerostagonospora* sp., *Scolecobasidiella* cf. *tropicalis*, *Speiropsis scopiformis*, *Stachybotrys chartarum*, *Subulispora longirostrata*, *Thozetella cristata*, *Thozetella gigantea*, *Vermiculariopsiella immersa*, *Verticillium* sp., *Volutella minima*, *Wiesneriomyces laurinus*, *Zygosporium mansonii.*

carolinensis, *P. minioluteum*, *Pestalotiopsis* spp., and *Phomopsis* sp.2. Some of these fungi such as *B. rhombica*, *P. minioluteum* and *Phomopsis* sp.2 were isolated almost with the same frequency, while others such as *Chaetosphaeria* sp., *G. cingulata,* sterile mycelium sp.6, and *P. carolinensis* were more frequent in the leaves of one plant. Some fungi were unique, such as *Gliocladiopsis* sp., *Phomopsis* sp.4, and *Dactylaria belliana* isolated from *C. nemorosa*, and *Satchmopsis brasiliensis*, *Beltraniella* cf. *botryospora*, and sterile mycelium sp.13 isolated from *V. guianensis*. About climate seasons existed differences in community composition with the fungi *Chaetosphaeria* sp., *P. brevicompactum*, *P. carolinensis*, and *Pestalotiopsis* spp. associated with the wet season exclusively or most often, while *Guignardia* sp., *P. minioluteum*, *Phomopsis* sp.2, and sterile mycelium sp.7 were associated predominantly or solely with the dry season (Table 2).

ANOSIM analysis indicated a significant difference in community compositions of fungi associated with the leaves of *C. nemorosa* or *V. guianensis* and between seasons (R = 0.8, P = 0.0004). NMDS analysis revealed a strong separation between samples collected during the wet (left) and dry (right) seasons as well as between *C. nemorosa* (top) and *V. guianensis* (lower) (Figure 3).

SIMPER analysis indicated greater dissimilarity between communities of *C. nemorosa* during the wet and dry seasons, and the taxa that contributed most were *Pestalotiopsis* spp. and sterile mycelium sp.6. The lowest dissimilarity was observed between the communities of *C. nemorosa* and *V. guianensis* during the wet season, and the taxon *Pestalotiopsis* spp. made an important contribution (Table 3).

Discussion

Using the technique of particle filtration, we report here the isolation of a large number of fungi from the leaf litter of *C. nemorosa* and *V. guianensis* in from the Atlantic Forest of Brazil during wet and dry seasons. This approach favors the isolation of fungi that grow slowly by reducing

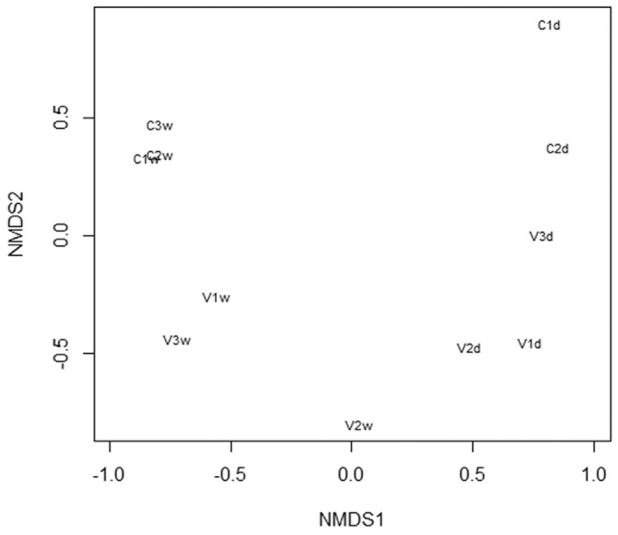

Figure 3 - Two-dimensional ordering using NMDS of communities of fungi associated with leaf litter of *C. nemorosa* (C) and *V. guianensis* (V) during the wet (w) and dry (d) seasons in the Serra da Jibóia, Bahia, Brazil. Stress = 0.06.

the plant substrate to particles with lengths of a few micrometers that theoretically harbor one fungus per particle, thereby decreasing competition between fungi and increasing the contact surface of the substrate with the culture medium (Kirby *et al.*, 1990; Bills and Polishook, 1994).

The species of fungi isolated from the leaves of *C. nemorosa* was richer and more diverse, although fewer samples were analyzed (05 samples), compared with those isolated from *V. guianensis* (06 samples). The characteristics of the leaves of both plants are distinct. For example, the leaves of *C. nemorosa* are thicker, and those of *V. guianensis* are thinner and brittle. A positive correlation between the richness of fungi and leaf thickness was demonstrated by Paulus *et al.* (2006b), who found a greater number of species of fungi associated with the leaves of *Cryptocarya mackinnoniana* that are thicker compared with the other leaves analyzed such as *Darlingia ferruginea* and *Elaeocarpus angustifolius*. This correlation may be attributed to the larger volume of available substrate for mycelial growth. Other features that were not evaluated in the present study, such as foliar area and chemistry, contribute alone or together to the growth of fungi in leaf litter (Santana *et al.*, 2005; Paulus *et al.*, 2006b). The lower Simpson's diversity observed for community isolated from *V. guianensis* was associated with higher frequencies of taxa such as *P. carolinensis* and *Pestalotipsis* spp, which are present most frequently in the leaves of many trees in tropical forests (Bills and Polishook, 1994; Paulus *et al.*, 2006).

We isolated more taxa during the wet season compared with the dry season (76 and 37, respectively). Comparing the communities of fungi according to season, those of wet season showed greater richness in *C. nemorosa* (54) and those of dry season showed greater richness in *V. guianensis* (31). Precipitation is considered a key environmental factor for the spread of fungi and the decomposition

Table 3 - Analysis of the percentage similarity (SIMPER) of fungal communities isolated from leaf litter of *C. nemorosa* (C) and *V. guianensis* (V) during the wet (w) and dry (d) seasons in the Serra da Jibóia, Bahia, Brazil.

Samples	Average dissimilarity (SIMPER in %)	Promoting species dissimilarity	Contribution (%)
Cw x Cd	91	*Pestalotiopsis* spp.	10.87
		sterile mycelium sp.6	10.87
		P. carolinensis	7.7
Cw x Vw	50.4	*Pestalotiopsis* spp.	15.3
		Gliocladiopsis sp.	8.5
		D. belliana	7.1
Cw x Vd	62.1	*Pestalotiopsis* spp.	14.1
		Gliocladiopsis sp.	8.1
		D. belliana	6.7
Cd x Vw	90.4	*Pestalotiopsis* spp.	22.5
		P. carolinensis	12.8
		sterile mycelium sp.6	10
Cd x Vd	74.8	sterile mycelium sp.6	17.2
		P. carolinensis	12.7
		G. cingulata	11.6
Vw x Vd	64.3	*Pestalotiopsis* spp.	28.1
		P. minioluteum	6
		S. brasiliensis	5.8

of organic matter (Cannon and Sutton, 2004). For example, under high humidity, the decaying plant debris are more densely colonized by fungi (Paulus *et al.*, 2006b), particularly because the leaves represent the most significant part of the litter and contribute greater biomass and nutrients compared with other plant substrates (Vorísková and Baldrian, 2013).

The species composition of communities was influenced by the seasons. For example, *Pestalotiopsis* spp. and *P. brevicompactum* were present only in the wet season in the leaf litter from both plants, and *P. minioluteum* and *Guignardia* sp. were associated only with the dry season. The characteristics of the types of sporulation (dry or wet spores) and the morphologies of conidia may be related to the dispersion medium or adhesion to the substrate (Jones, 2006). *Pestalotiopsis* species produce asexual spores with terminal appendages and are retained in a damp mass. Both features enhance the adhesion of spores to the substrate surface so that sporulation and dispersion depend on high humidity (Nag Raj, 1993; Jones, 2006). Rainfall influences the distribution of *Guignardia* ascospores, because they are transported by air currents over long distances and are therefore influenced by rainfall. In the present study, the majority of specimens were in their asexual stage, known as *Phyllosticta*, whose spores have a mucilage, which protects against desiccation in an adverse environment (Kriel *et al.*, 2000). In contrast, *Penicillium* comprises species that produce dry spores that are efficiently dispersed by wind (Cannon and Sutton, 2004).

Rarefaction curves did not reach stability due to the large number of fungi isolated by the particle filtracion. The slope of the rarefaction curve for samples acquired during the dry season was shallower, demonstrating that the collection effort was satisfactory. However, the data for samples collected during the wet season indicate that new collections may increase the number of taxa. Therefore, regardless of the representation of plant species, the community present during the wet season always comprised greater numbers of isolates and species richness (Figure 2). Considering the overall communities of fungi, the richness estimated using Chao1 approached the number of taxa observed, indicating that the techniques used for sampling and isolating fungi were efficient.

Polishook *et al.* (1996) observed similar percentages of similarity and complementarity (26-32% and 68-74%, respectively) among the communities of saprobic fungi of *Manilkara bidentata* and *Guarea guidonia* in a tropical rainforest in Puerto Rico to those reported here for the Atlantic Forest. Santana *et al.* (2005) found distinct communities among five plant species, with a complementary level of 68% for the 10 most frequently isolated fungi. Monkai *et al.* (2013) reported very low levels of similarity between the communities of *Magnolia liliifera* and *Cinnamomum iners*, reaching values of 1.92% to 8.51%, considering species and genus, respectively. Pasqualetti *et al.* (2014) investigated the fungi of 17 species of trees in Mediterranean areas and observed communities with variable percentages of overlapping species (6-47%). However, when the community of fungi between species of the same genus was investigated, the similarity was greater as observed between *C. nemorosa* and *Clusia melchiorii* (60%) (Barbosa *et al.*, 2009).

This pattern is commonly observed in studies of fungi with a broad host range, in contrast to others that are restricted to one plant species, indicating a level of recurrence in the colonization of decaying plant debris (Parungao *et al.*, 2002; Rambelli *et al.*, 2004; Santana *et al.*, 2005; Paulus *et al.*, 2006b; Hyde *et al.*, 2007; Monkai *et al.*, 2013). There are significant differences among the constituents of leaf litter of different plant species, such as lignin, cellulose and secondary compounds as well as other components. Some of these compounds may inhibit fungal growth, whereas others require a variety of enzymes for their degradation (Vorísková and Baldrian, 2013). These differences in the components of leaves may contribute to differences in the composition and frequency of fungi that have been consistently identified among plant species in temperate (Ormeño *et al.*, 2006; Allegrucci *et al.*, 2014; Pasqualetti *et al.*, 2014) and tropical forests (Paulus *et al.*, 2003a; Rambelli *et al.*, 2004; Paulus *et al.*, 2006b; Magalhães *et al.*, 2011; Monkay *et al.*, 2013).

The spatial and temporal heterogeneity of the community is best viewed using NMDS, which reveals the tendency of the separation between the communities of the plant species (C and V), particularly between the wet (w) and dry (d) seasons (Figure 3). ANOSIM analysis reveals that the composition and richness of fungal communities were significantly different between plant species and seasons, and SIMPER analysis indicates that *Pestalotiopsis* spp., *P. carolinensis*, and sterile mycelium sp.6 were the taxa that contributed most to the dissimilarity between communities.

The NMDS data indicate that seasonality may serve as an important factor for the distribution of fungi when compared to the nature of the plant substrate. *Clusia nemorosa* and *V. guianensis* belong to different botanical families, Clusiaceae and Hypericaceae, respectively, but because they are phylogenetically related (APG III, 2009), this may favor the differences, similarities, or both observed among fungal communities. A different result was reported by Paulus *et al.* (2006b) who investigated the distribution of fungi from four phylogenetically unrelated botanical families (Elaeocarpaceae, Lauraceae, Moraceae, and Proteaceae) in a rainforest in Australia. These researchers found that the fungal community was more closely related to the plant species as a function of richness and community composition compared with seasonality, which is indicated by the low percentage of overlapping species (13-22%). Allegrucci *et al.* (2014) obtained the same re-

sults for fungi associated with *Scutia buxifolia* and *Celtis tala* in a xeric forest in Argentina.

In summary, the data presented here indicate that leaf litter in the Atlantic Forest contains a robust richness of fungi that differs depending on plant species and seasonality. Major differences in the fungal communities were observed between the wet and dry seasons when compared with the plant species of origin, because a significant number of fungi were shared between the two plants, likely because the plants belong to phylogenetically related botanical families and share certain biochemical characteristics such as latex production. According to Paulus *et al.* (2006b), adaptation of saprobic organisms to hostile environments such as tissues containing latex or phenolic compounds plays an important role in the development of recurrence in certain saprobic fungi. When fungal communities of phylogenetically distant plants were investigated, the plant species of origin was the most important factor influencing the compositions of communities (Paulus *et al.*, 2006b). Although a core group of fungi was common to both plants, the majority of taxa were confined exclusively to one plant. Our results show a high recurrence of fungi to closely related plants that coexist in the same environment and show further that the saprobic fungal communities in rainforests are dynamic. Moreover, the factors that regulate such communities in the Atlantic Forest are complex.

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