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Effects of storage conditions and culture media on the saprobic fungi diversity in tropical leaf litter

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Abstract

The effects of methodology were observed in a community of fungi grown in culture associated with leaf litter of *Clusia nemorosa*, collected in the state of Bahia, northeast of Brazil. We examined the effects of variables as storage time (fresh leaf litter, 07, 14, 21 and 28 days) and temperature (room temperature and 4°C), and culture media (MYE and DRBC) on the diversity of fungi. Protocol of particle filtration was used to achieve the isolation of fungi. A total of 1.113 strains belonging to 67 taxa were isolated. The analysis of covariance (ANCOVA) indicated that richness and the number of isolates decreased significantly with storage time, but did not vary in relation to culture media. The differences between the communities related to the storage temperatures were not significant based on the t–test. The diversity analysis indicated that storage samples up to 14 days showed no significant differences in the communities when compared to fresh litter. Nonmetric multidimensional scaling (NMDS) shows a tendency of separation between the communities observed in the different temperatures and between the initial storage times and late. The results indicate that in the storage of leaves for up to 14 days no significant changes in the community were observed, however in longer storage periods there was a notable loss in both richness and quantity of fungi.

Key words – diversity – leaf litter – methodological aspects – storage litter – tropical fungi

Introduction

Fungi are hyperdiverse organisms and the global number of species is estimated to range from 1.5 to 3.3 million (Hawksworth 2012). Tropical rainforests are home to a wide diversity of species and leaf litter is one of the major reservoirs (Hawksworth & Rossman 1997). The leaf litter is the most significant part of the plant debris and its decomposition contributes to the maintenance and balance of the ecosystem in the forest through nutrient cycling (Xiaogai et al. 2013). The knowledge of factors that influence the composition and community structure of fungi is important for understanding the dynamics of the decomposition process since the diversity of fungi, associated with environmental conditions and the characteristics of the substrate, has significant effects on the rate of decay (Hättenschwiler et al. 2005, 2011).

There are several factors that influence the diversity of the saprobe fungi community. The most important ones seem to be related to the plant species (Paulus et al. 2006, Monkai et al. 2013), intrinsic factors to the study area, such as vegetation composition and climate (Polishook et al.

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1996, Yanna et al. 2001), and the seasonality (Kodsueb et al. 2008, Voříšková et al. 2014). Methodological aspects, used to access the saprobic fungi, also have significant influence on the diversity of the community. For example the sampling effort (Magurran 1988, Bills & Polishook 1994a), methods of pretreatment, storage of plant debris (storage time until processing) (Paulus et al. 2003a), methods for detection and isolation of culturable fungi (Collado et al. 2007, Unterseher & Schnittler 2009) as well as methods used for characterization and identification, are all factors to consider. (Bills & Polishook 1994a, Bills 1996).

The storage of the substrate for subsequent diversity analysis has been commonly performed in many studies, mainly due to the fact that collection areas are in remote sites (Talley et al. 2002) and because of the high volume of material to be processed simultaneously (Bills & Polishook 1994a, Polishook et al. 1996, Parungao et al. 2002). The effects of this procedure on diversity of fungi were assessed by Paulus et al. (2003a) in *Neolitsea dealbata* leaves in a rainforest in Australia. The authors observed that diversity was significantly affected since there was a decrease in richness and number of isolates with the increase in storage time.

The storage temperature may have a considerable influence on the conservation of fungi present in the samples. The storage of the litter at temperatures below 0°C (–15 to –20°C) did not affect the diversity of fungi (Kuter 1986), possibly, because the fungi are adapted to freezing for long periods in their natural harsh winters. In tropical regions, the effects of freezing on the diversity are unknown. In addition the refrigerated temperature (4°C) seems to preserve the diversity of the community, since Bills & Polishook (1994a) obtained high richness as well as quantity of fungal isolates in storage samples of 01 week in Costa Rica. However, the effects on the community were not assessed by the authors.

The saprobic fungi are isolated and quantified using methods where selective culture media are widely used (Paulus et al. 2006, Collado et al. 2007). Such approaches have some advantages over other non-cultivable methods, based on molecular approaches, (Liew et al. 1998, Cuadros–Orellana et al. 2013), since it is possible to perform taxonomic, physiological, genetic and biotechnological studies with the fungal isolates (Morath et al. 2012, Gomes et al. 2013, Jeewon et al. 2013, Stadler et al. 2014). The use of a consortium of different culture media, favoring the detection of a greater diversity of fungi has been suggested by some authors (Cannon & Sutton 2004, Paulus et al. 2006).

Studies on the effects of methodological aspects on fungal communities become important because of the need to develop sampling methods efficient and consistent for conducting inventories and diversity studies, allowing comparisons between leaf litter fungal assemblages. In this paper, we evaluate the effects of storage time and temperature, along with the influence of culture media on the diversity of fungi recovered from decaying leaves in the rainforest.

Materials & Methods

Collection of leaves and assessment of the effects of storage conditions

Twenty fallen leaves of a plant of *Clusia nemorosa* G. Mey. were collected in a remnant of the Atlantic Forest in the state of Bahia, in northeastern Brazil (12° 51'S and 39° 28' W) in July/2011. The leaves were placed in paper bag, transported immediately to the laboratory and processed up to 12 h after the collection.

The leaves were divided into two treatment groups with 10 leaves each (A1 and A2). The treatments were stored at room temperature (A1) and 4°C (A2). Samples of each treatment were obtained after 07, 14, 21 and 28 days marked as T1, T2, T3 and T4, respectively to investigate the storage time. The samples in each time interval consisted of 10 pieces of 18 cm^2 (180 cm^2), each unit being obtained by leaf and treatment method. The leaves in each treatment were sampled continuously, in other words, after the removal of samples per time interval the remaining material was stored again in the appropriate treatment until the next sampling in the next time interval. Fresh material (leaf litter no storage) was named T0 and only seen in treatment A2. For comparisons among treatments, T0 from A2 was considered the control sample.

Processing of samples and particle filtration protocol

The leaves of *C. nemorosa* were initially washed in water to remove soil particles and other organisms that are attached to the leaf surface and subjected to a process for surface disinfection according to Paulus et al. (2003a). The samples were taken after this pretreatment procedure and, then, were processed using the method of particle filtration (Bills & Polishook 1994a).

Each sample was homogenized for 01 min in 100 mL of sterile distilled water in a household blender and the particulate material was washed with distilled water jets in a group of five stainless steel sieves with different mesh openings (1.0, 0.7, 0.5, 0.25, and 0.18 mm). The particles retained on the 0.18 mm sieve were transferred to a centrifuge tube and suspended in sterile distilled water (up to a volume of 50 mL), and then were stirred at Vortex for 01 min and allowed to settle. The supernatant was discarded and the tube was filled with sterile distilled water to a volume of 50 mL. This procedure was performed 04 times and the residual material was suspended in 20 mL of sterile distilled water. Aliquots (50µL) of the suspension were transferred and homogenized with the aid of a Drigalski handle in triplicate in Petri dishes of 90 mm diameter containing culture media.

Culture media

Two culture media were used for the isolation of the fungi: agar dichloran rose bengal chloramphenicol (DRBC) without dichloran and agar malt yeast extract (MYE) supplemented with rose bengal (25 mg/L) and chloramphenicol (100 mg/L) (Paulus et al. 2003a). The plates were incubated at room temperature with ambient light after the transfer of the particle suspension. The fungal growth was observed daily for 30 days, and once verified that hyphae was present in the particles, they were transferred to Petri dishes containing the culture media cornmeal and carrot agar (CCA) as described by Castañeda–Ruiz (personal communication), together with sterilized fragments of banana leaves (Paulus et al. 2003b). This procedure was performed to induce the reproductive stage of fungi isolated and/or for the characterization of morphotypes (Lacap et al. 2003).

Data analyses

Caculations were made using the t-test in order to compare the number of fungi and richness between treatments A1 and A2. The evaluation of the effects of storage time and culture media in relation to the number of isolates and richness of fungi obtained from each treatment was performed by the analysis of covariance (ANCOVA, Quinn & Keough 2002). The significance level of 5% was established for both analyzes, t-test and ANCOVA.

The diversity of fungal communities in each investigative time was evaluated by Shannon and inverse Simpson indexes, the last one represented as *1–D* (Magurran 1988). The confidence interval of 95% in both indexes was calculated using the bootstrap method (Grünwald et al. 2003). To search for general differences in composition among fungal treatments, the date was analyzed using a multivariate method, specifically the nonmetric multidimensional scaling (NMDS, Kruskal 1964), from the Bray-Curtis dissimilarity matrix. The permutation test ANOSIM (*Analysis of Similarity*) was used to assess the significance of the pattern indicated by the NMDS (Clarke 1993).

The statistical analyses were conducted using softwares: PAST v. 3.01 (Harmer et al. 2013) and R 3.01 (R Core Team 2013) using the vegan package (Okasen et al. 2013).

Results

A total of 1.113 throughout specimens, comprising 67 taxa, were isolated from the leaf litter of *C. nemorosa*. The number of fungi and species richness decreased in relation to storage time in both treatments. In treatment A1, the number of isolates ranged from 40 to 235, and in relation to richness, ranged from 14 to 43 taxa. In A2, the number of isolates and richness varied from 62 to 235 and from 25 to 43 taxa, respectively. The culture medium DRBC provide the growth of 595 fungi, belonging to 57 taxa, while in MYE 518 specimens represented by 51 fungal taxa were grown (Table 1).

Table 1 Numbers of isolated and richness of saprobic fungi on litter of *Clusia nemorosa* at different storage time and temperature, and culture media

Storage time/ culture media	Number of isolates		Total	Richness		Total
	A1 (room temperature)	A2 (4°C)		A1 (room temperature)	A2 (4°C)	
T0[a] (fresh material)	235	235	235	43	43	**43**
T1 (07 days)	152	207	359	35	35	**49**
T2 (14 days)	113	143	256	28	31	**36**
T3 (21 days)	60	101	161	20	23	**27**
T4 (28 days)	40	62	102	14	25	**28**
Total	**600**	**748**	**1.113**	**57**	**60**	**67**
DRBC	304	408	595	47	49	**57**
MYE	296	340	518	42	48	**51**
Total	**600**	**748**	**1.113**	**57**	**60**	**67**

[a]The number of isolates and richness obtained at T0 were repeated in A1 and A2, but the total amount was quantified only once.

The t-test revealed no differences between treatments (isolated, *t=0.24, df=16, P=0.81*; richness, *t=0.14, df=16, P=0.90*). The ANCOVA revealed a negative correlation between the number of isolates and richness of fungi with regards to storage time. The use of both culture media as well as the interaction between the culture media and storage time had no effect on the community of fungi (Table 2).

Table 2 Analysis of covariance (ANCOVA) of numbers of isolate and richness of saprobic fungi on litter of *Clusia nemorosa* at different storage time and temperature, and culture media

Treatment	Number of isolates			Richness		
	df	***F***	***P***	***df***	***F***	***P***
A1 (room temperature)						
Culture media	1	0.017	0.901	1	5.691	0.054
Storage time	1	29.847	0.002	1	172.035	0.012
Culture media * storage time	1	0.126	0.735	1	0.844	0.394
Residual	6			6		
A2 (4°C)						
Culture media	1	3.560	0.108	1	0.043	0.842
Storage time	1	78.246	<0.001	1	36.186	<0.001
Culture media * storage time	1	0.004	0.950	1	0.232	0.647
Residual	6			6		

The Shannon diversity index obtained its maximum at T0 (*H'=3.16*) and decreased in relation to time of storage in both A1 and A2 (*H'=2.22* and *H'=2.68*, respectively). In A1 the index values were higher compared to the values in A2, except on T4A2 (Table 3). Comparisons of the confidence intervals between T0 and each storage time showed significant differences at T3 and T4. It was considered significant because there was no overlap in values between the confidence intervals (Table 3).

The Simpson index was higher at T0 and T1 having the same values (*1–D=0.94*) and, similar to the Shannon index, decreased in relation to time of storage in both A1 and A2 (*1–D=0.85* and *1–D=0.89*, respectively) (Table 3). Significant differences between the confidence intervals of indexes were found at T3A2 and T4A1 (Table 3).

Table 3 Diversity index Shannon and Simpson for saprobic fungi on litter of *Clusia nemorosa* at different storage time and temperature

Samples[a]	Shannon index (*H'*)	Simpson index (*1–D*)
T0	3.16 (3.03–3.27)	0.94 (0.92–0.95)
T1A1	3.15 (3.03–3.24)	0. 94 (0.93–0.95)
T1A2	3.11 (2.99–3.19)	0.94 (0.93–0.95)
T2A1	2.97 (2.83–3.07)	0.93 (0.91–0.94)
T2A2	2.96 (2.83–3.08)	0.93 (0.91–0.94)
T3A1	2.73 (2.51–2.82)	0.92 (0.89–0.93)
T3A2	2.5 (2.43–2.75)	0.87 (0.84–0.91)
T4A1	2.22 (2.15–2.46)	0.85 (0.83–0.90)
T4A2	2.68 (2.56–2.93)	0.89 (0.85–0.93)

[a]T0 (fresh material), T1 (07), T2 (14), T3 (21), T4 (28) days of storage; A1 (room temperature) and A2 (4°C).

Among all samples, only 25 shared taxa were found, between T0 and A1, 31 taxa, between T0 and A2, 30 taxa, and between temperature treatments 32 taxa were observed. It was found that 08 taxa were exclusive to T0, 07 to A1 and 10 to A2 (Table 4). The frequency of the most common taxa decreased with time of storage from 44% in T0 to 24% and 28% in the treatments A1 and A2, respectively (Table 4).

Asexual fungi (hyphomycetes 62.7% + coelomycetes 12% = 74.7%) were isolated more frequently than the sexual stage (9%). Sterile mycelia were observed at a percentage of 16.4%. Three connections of sexual-assexual stages were recorded: *Pseudomassaria carolinensis* (*Beltraniella portoricensis*), *Glomerella cingulata* (*Colletotrichum gloeosporioides* complex) and *Calonectria gracilipes* (*Cylindrocladium graciloideum*). Rare taxa, considering the *singletons* and *doubletons*, accounted for 32.8% of the fungi community that were obtained mostly (81.8%) during the first time intervals of storage (T0 and T1, in both treatments). The fungi more abundant were *Dactylaria belliana* (11%), *P. carolinensis* (7.5%), *Beltrania rhombica* (7%) and *Volutella minima* (6.2%) (Table 4).

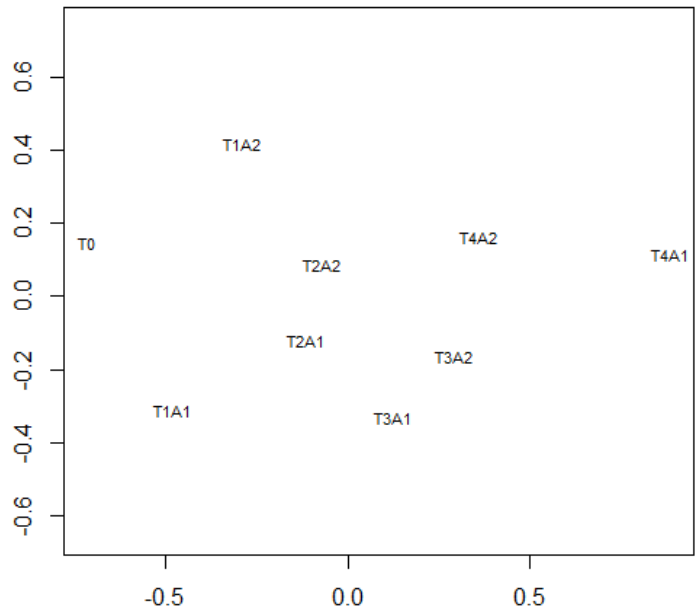
Table 4 Taxa and number of saprobic fungi on leaf litter of *Clusia nemorosa* at different storage time and temperature[a]

Taxa		A1				A2			
	T0	T1	T2	T3	T4	T1	T2	T3	T4
Acremonium sp.1	4	0	0	4	0	0	0	0	0
Acremonium sp.2	0	0	0	0	0	2	0	0	0
Ardhachandra cristaspora	3	0	0	1	0	2	1	0	0
Ardhachandra selenoides	0	0	0	0	0	7	2	0	1
ascomycete sp.	0	0	0	0	0	7	0	0	0
Atrosetaphiale fragelliformis	0	0	0	0	0	0	0	0	1
Beltrania rhombica	29	15	3	1	0	20	8	1	1
Calonectria gracilipes	2	7	1	3	0	3	8	1	1
Chalara cf. *paramontellica*	0	0	0	0	0	2	0	0	0
Dactylaria cf. *biseptata*	4	1	0	0	2	0	1	1	3
Gliocladiopsis cf. *elgholli*	21	11	2	1	0	0	2	7	1
Gliocladiopsis cf. *mexicana*	0	10	0	0	0	0	0	0	0
Idriella cf. *cubensis*	2	0	0	0	0	0	0	0	0
Idriella cf. *variabilis*	3	2	2	6	1	2	2	2	3
Neofusicoccum sp.	0	0	0	0	0	1	0	0	0
Scolecobasidiella cf. *tropicalis*	0	1	0	0	0	0	0	0	0
Chaetosphaeria sp.	10	1	1	0	0	6	1	0	0
Cladosporium–like sp.	1	1	0	0	0	0	0	0	0

Taxa		A1				A2			
	T0	T1	T2	T3	T4	T1	T2	T3	T4
coelomycete sp.1	0	0	0	0	0	1	0	0	0
coelomycete sp.2	2	0	0	0	0	0	0	0	0
Cylindocladium gracile	5	6	5	8	0	1	7	8	3
Dactylaria belliana	30	9	1	8	7	25	20	5	16
Dictyochaeta novae-guineensis	0	0	0	0	1	0	0	0	0
Dictyochaeta simplex	0	1	0	0	0	1	0	0	0
Fusarium decemcellulare	0	0	0	0	0	2	0	0	0
Fusarium lateridium	1	13	0	0	0	0	0	0	0
Fusarium solani	1	0	0	0	1	2	0	8	4
Glomerella cingulate	10	1	2	0	0	3	11	0	0
hyphomycete sp.	1	0	1	0	0	0	0	1	0
Idriella lunata	5	4	2	0	0	1	0	0	1
Idriella ramosa	1	0	1	0	0	0	0	0	0
Idriella sp.1	3	0	0	0	0	0	1	0	0
Idriella sp.2	0	1	0	0	0	0	0	0	0
Idriella sp.3	3	0	0	0	0	0	0	0	0
Lasiodiplodia theobromae	2	0	0	0	0	0	0	0	0
Metarhizium anisopliae	3	0	0	0	0	0	0	1	0
Mycoleptodiscus terrestris	0	0	0	0	0	0	1	0	0
Nigrospora sphaerica	0	1	0	0	0	0	0	0	0
Ochroconis sp.	2	0	0	0	0	0	0	0	0
Ochroconis variabilis	1	0	0	0	0	0	0	0	0
Parasympodiella laxa	1	0	0	0	0	2	0	0	0
Penicillium brevicompactum	11	7	10	1	0	5	1	0	0
Penicillium minioluteum	12	0	4	5	0	8	3	6	0
Pestalotiopsis spp.	4	13	7	1	6	8	3	2	2
Phomopsis sp.1	6	0	0	0	0	0	0	0	0
Phomopsis sp.2	2	0	0	0	0	3	1	0	0
Pseudomassaria carolinensis	24	8	7	5	0	14	22	2	1
Satchmopsis brasiliensis	0	1	0	0	0	0	0	0	0
Selenodriella fertilis	0	0	0	0	0	1	0	0	0
Speiropsis scopiformis	7	1	0	3	1	5	1	2	1
sterile mycelium sp.1	0	1	1	0	0	0	6	1	1
sterile mycelium sp.2	1	1	18	2	3	14	0	0	1
sterile mycelium sp.3	0	4	1	1	3	1	0	1	0
sterile mycelium sp.4	0	3	4	3	0	3	3	4	1
sterile mycelium sp.5	2	4	3	0	0	10	3	1	2
sterile mycelium sp.6	2	0	1	0	0	3	2	0	1
sterile mycelium sp.7	1	3	6	0	1	0	7	0	0
sterile mycelium sp.8	1	0	0	0	0	0	0	0	0
sterile mycelium sp.9	0	1	5	1	1	4	2	1	1
sterile mycelium sp.10	0	2	10	2	1	0	5	1	0
sterile mycelium sp.11	1	9	2	0	0	24	5	2	1
Thozetella cristata	3	2	0	0	0	0	0	0	0
Trichoderma sp.	0	0	8	0	1	0	2	0	1
Verticillium sp.	1	3	4	1	0	10	1	13	8
Volutella minima	1	3	1	3	11	4	10	30	6
Wiesneriomyces laurinus	6	0	0	0	0	0	1	0	0
Xylariaceae sp.	0	1	0	0	0	0	0	0	0
TOTAL	**235**	**152**	**113**	**60**	**40**	**207**	**143**	**101**	**62**

[a]T0 (fresh material), T1 (07), T2 (14), T3 (21), T4 (28) days of storage; A1 (room temperature) and A2 (4°C).

The distribution pattern of fungi community among the samples obtained by the ordination method NMDS is shown in Figure 1. The graph shows a tendency of separation between the treatments (A1, bellow, and A2, above) and between the initial storage times (T1 and T2, left), along with T0, and late (T3 and T4, right). The result of ANOSIM indicated that the differences between the communities were not significant (*R= – 0.064*, *P=0.635*).



T0 (fresh material)
T1 (07 days)
T2 (14 days)
T3 (21 days)
T4 (28 days)
A1 (room temperature)
A2 (4°C)

Fig. 1 – Nonmetric multidimensional scaling (NMDS) ordination of saprobic fungi comunity on leaf litter of *Clusia nemorosa* at different storage time and temperature. Stress: 0.09

Discussion

Although the particle filtration protocol proved to be efficient in the isolation of a significant amount of fungi associated with leaf litter, the richness obtained in this study was lower when compared to other studies in tropical forests using the same technique. Bills & Polishook (1994b) isolated 1.676 fungi and grouped it in 122 morphotypes, from four samples of senescent leaves of *Heliconia mariae*. Polishook et al. (1996) isolated 101 and 173 taxa from a sample of five grams of leaf litter of *Guarea guidonea* and *Manilkara bidentata*, respectively. Paulus et al. (2003a) investigated the fungi in leaf litter of *Neolitsea dealbata* using four samples of five grams, and managed to isolate 736 fungi distributed among 112 taxa.

Despite using the same technique mentioned above, a lower richness was obtained (67 taxa), probably, due to the differences in sampling methods and the characteristics of the substrates of the plants. The samples were determined by leaf area because the weight parameter varies depending of the environmental conditions including the temperature, an extremely important factor. Another fact to be taken into consideration are that the leaves of *C. nemorosa* have a thick cuticle (Fernandes 2007) that acts as a physical barrier that may hinder penetration and colonization of the leaf tissue by some fungi (Canhoto & Graça 1999).

In addition to the sampling method, other methodological factors could contribute to the differences in composition and community structure of culturable fungi. Among the parameters evaluated in the study, the use of distinct culture medium (DRBC and MYE) did not differ in the isolation of fungi. This observation was not supported by Cannon & Sutton (2004), which reported that the use of selective media favored the detection of a large number of fungi in leaf litter, while the spectrum of selectivity of these culture media are not well known. The culture media used in this study were complex and it is possible that the similarity of some chemical compounds and the final pH favored the growth of a variety of quite similar fungi.

Although the temperature is an important factor that regulates the metabolic activity of fungi (Pietikäinen et al. 2005; van der Wal et al. 2013), its effects on the composition of communities are not well understood and little information is available. The leaf litter stored at room temperature (A1), lost more humidity and was observed to be more dry and brittle during the experiment when compared to the litter stored at 4° C (A2). Although A2 showed a higher number of isolates and an increase in richness there were no significant differences between treatments. These observations indicate that for the storage time evaluated, the storage temperatures showed no different to obtain a higher number fungi.

The parameter that strongly influenced the fungal community was the time of storage of the substrate for further processing (Paulus et al. 2003a). In both treatments A1 and A2, the storage time of 28 days yielded a decrease in both number and richness of fungal isolates. This result corroborated the findings of Paulus et al. (2003a) whose study of fallen leaves of the *Neolitsea dealbata* used storage periods of 01, 07, 21 e 28 days and found that the number of isolates differed significantly in relation to storage time. However the richness was not affected for up to 21 days of storage. Considering that the same leaves were continuously processed, the storage time interval (fresh material to 28 days) may also have contributed to the lower richness found in this study.

The Shannon and Simpson's diversity indices indicated that the diversity of fungi decreases with storage time. However, the Shannon index was more sensitive, indicating that leaves stored up to 14 days had no significant effect on the community. As mentioned above, Paulus et al. (2003a) found no differences in the richness up to 21 days of storage, although the authors did not assess storage leaves for 14 days. The Shannon index values are considered high, because for many studies the index usually ranges between 1.5 (low diversity) and 3.5 (high diversity) (Margalef 1972).The lowest values of Simpson's index (0.85 to 0.89) indicate a community with a greater dominance of taxa.

The result of the NMDS indicated that the observed differences in the number of isolates and taxa richness between the storage time intervals (early and late) and treatments (A1 and A2) could separate communities of fungi, wherein storage initial periods showed higher values compared to later periods. However, this pattern was not confirmed by the ANOSIM test indicating that more samples could be analyzed.

Given the observed community, some species of fungi were detected early in the process (initial storage) (ex. *Fusarium lateridium*, *Lasiodiplodia theobromae* and *Metarhizium anisopliae*), while others were found during the later periods of storage (ex. *Ardachandra selenoides*, *Trichoderma* sp. e *Volutella minima*). The dynamics of the species composition and structure of communities associated with litter indicate that some fungi may be more resistant to water stress, which would be present in a dormant or less physiologically active state, giving considerable longevity to the fungus under conditions of lower humidity (Dix 1985, Paulus et al. 2003a).

This study indicates that the storage time had a significant effect on the diversity of fungi in leaf litter while the storage temperature and the use of different selective media showed no significant differences in isolation. The diversity of stored leaves for up to 14 days, was substantially similar to the diversity of material no storage, however longer storage periods showed a significant loss pertaining to both richness and abundance of fungi saprobic. Based on these data is important to assess whether changes in fungal diversity are acceptable for the proposed research or if just presence/absence data are needed. If it is important to get a more diverse community, then the isolation of fungi should be performed within 14 days after the collection of fallen leaves.

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