
Species composition, seasonal changes and community ordination of alkalotolerant micro fungal diversity in a natural scrub jungle ecosystem of Tamil Nadu, India

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One hundred and seven species of alkalotolerant fungi were isolated from different layers of litter of the Guindy Reserve Forest, Chennai, South India during a 2-year period. They comprised Zygomycota (7 species), Ascomycetes (4 species), hyphomycetes (86 species) and coelomycetes (10 species). The F1 litter layer, just beneath the recently fallen leaves, had the richest composition of fungi and the fungi were most abundant during the North East monsoons (September to November). Shannon's diversity index and Simpson diversity index λ indicate F1 layer to have the maximum species. The species distribution fell into the log series model and Fishers alpha was also highest for the F1 layer. Species richness indices computed also indicated that none of the species was more predominant. Values of species evenness computed hovered around 0.6 indicating a tilt towards even distribution of the species. The fungal community is a heterogenous assembly of species derived from a homogenous habitat with a log normal pattern of distribution formed due to the interplay of many independent factors governing the relative abundance of the species. Principal component ordination analysis reveals that the greatest variation in the species composition was due to the South West monsoon. Also, detrended correspondence data put the species abundance data for the four seasons in a linear arrangement.

Key words – Alkalotolerant fungi – Fungal diversity – Micro fungi – Plant Litter

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Introduction

One third of fungal diversity of the globe exists in India. Fungi play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling, as biofertilizers and many other ways (Manohara-chary et al. 2005). Fungi play an important role in plant litter decomposition in forest ecosystems through nutrient recycling and humus formation

in soil (Swift et al. 1979) because they attack the lignocellulose matrix in litter that other organisms are unable to assimilate (Kjoller & Struwe 1982, Cooke & Rayner 1984). Alkaline-adapted micro-organisms can be classified into two main groupings, alkaliphiles (also called alkalophiles) and alkalitolerants or alkalotolerant. The term alkaliphiles is generally restricted to those micro-organisms that actually require alkaline media for growth. The optimum growth rate of these micro-organisms

is observed in at least two pH units above neutrality. Organisms capable of growing at pH values more than 9 or 10, but with optimum growth rates at around neutrality or less, are referred to as alkalotolerant (Jones et al. 1994). Exploration of natural, alkalotolerant fungi for the production of industrially important alkaline active cellulose, protease, amylase, lipase, and mannose is gaining momentum. It is increasingly realized that fungi may be good sources of new compounds, beneficial to mankind. For example, in modern detergents there is a need for enzymes that actively work in alkaline conditions (Horikoshi 1999). Alkaline active enzymes are used to decompose the gelatin on film, degradation of paper in paper industry and in detergent additives. Alkaline enzymes have been also used in the hide-dehairing process. Given these extensive commercial application there is a need to build a germplasm collection of alkalotolerant fungi. The focus of this study is to enumerate the species diversity of alkalotolerant fungi inhabiting the different layers of the litter of a natural scrub jungle ecosystem and also to record the seasonal changes in species composition.

Materials and methods

Litter samples were collected from the Guindy Reserve Forest (GRF) is a 2.82 km² protected area of Tamil Nadu, located in Chennai, South India located at 13° 0' 20" N and 80° 13' 24" E. It is a dry evergreen scrub and thorn forest with over 350 species of plants including shrubs, climbers, herbs and grasses and over 24 varieties of trees, growth of colonies at alkaline pH in the laboratory.

Samples were taken from three layers of the litter namely: 1) L-layer which is the uppermost layer consisting of recently fallen senescent leaves lying loosely on the surface. The structure of the leaves is well preserved and they are yellowish to light brown in colour, dry and without any visible fungal growth; 2) F1-layer which occurs immediately below the L layer, has high moisture content and consists of dark brown leaves, usually compacted. The leaf structure is reasonably well preserved but slightly thinner and soft. The leaves show patches of visible fungal growth suggesting that they are under active decomposition; and 3) F2-

layer which is the lowermost layer lying between F1 layer and the soil surface below. The leaves are in advanced stages of decomposition, usually fragmentary, very thin and tightly compressed.

Litter samples were collected each month for 3 years. Each layer of litter samples were collected separately from different locations of GRF in sterile polythene zip cover for fungal analysis and the data was grouped to represent four seasons: i) South West monsoon (June-August); ii) North East monsoon (September-November); iii) Post monsoon period (December-February); and iv) Summer (March-May). The leaf samples were randomly selected from sealed polythene zip cover and cut into pieces approximately 1cm² with a pair of sterile scissors and washed in 100 ml of sterile water in a 250 ml Erlenmeyer flask. The flask was placed on a shaker for 30 minutes. From this initial suspension, serial dilutions were prepared. One ml of the required dilution (1/1000) was pipetted on the surface of already prepared replicate plates containing potato dextrose agar with antibiotic Streptomycin sulphate (300µg/ml). The medium was maintained to pH 8 using sodium carbonate (Horikoshi & Akiba 1982). The plates were incubated at room temperature in glass chambers under aseptic conditions for 4 days and then examined for fungal growth. All fungal colonies that developed were recorded, isolated and identified. The periodicity of occurrence of each species of fungus was calculated based on the number of samples in which they occurred against the total number of samples collected and the species were classified into four groups: 'most common' (76-100%), 'common' (51-75%), 'occasional' (26-50%) and 'sporadic' (1-25%).

The following diversity indices were calculated as a measure of α -diversity - Fisher's α , Shannon's diversity index (H'), Simpson's diversity index (λ), Margalef's richness index ($R1$), Menhinick's richness index ($R2$), and Evenness indices ($E1$, $E2$, $E3$, $E4$ and $E5$) using the software of Paleontological Statistics (PAST) Version 1.92. The Morisita-Horn index was calculated as a measure of between habitat diversity (β - diversity).

Table 1 List of fungal species isolated from three layers of litter of the Guindy Reserve forest and their periodicity of occurrence.

S.No	Fungal Species	L-LAYER	F1-LAYER	F2-LAYER
	ZYGOMYCOTINA			
1	<i>Absidia corymbifera</i>	Occasional	Occasional	Occasional
2	<i>Cunninghamella echinulata</i>	Sporadic	Occasional	Sporadic
3	<i>C. elegans</i>		Sporadic	
4	<i>Helicostylum piriforme</i>		Sporadic	
5	<i>Mucor racemosus</i>	Common	Most common	Common
6	<i>Rhizopus stolonifer</i>	Common	Common	Common
7	<i>Syncephalastrum racemosum</i>	Occasional	Occasional	Occasional
	ASCOMYCOTINA			
8	<i>Anthostoma simplex</i>		Sporadic	
9	<i>Ascotricha chartarum</i>	Sporadic	Sporadic	
10	<i>Chaetomium longirostre</i>		Sporadic	Sporadic
11	<i>C.globosum</i>	Sporadic	Sporadic	Sporadic
	DEUTEROMYCOTINA: HYPHOMYCETES			
12	<i>Acremonium strictum</i>	Sporadic	Occasional	Sporadic
13	<i>Alternaria alternata</i>	Occasional	Common	Occasional
14	<i>A. longipes</i>	Occasional	Sporadic	
15	<i>Aspergillus candidus</i>	Sporadic	Sporadic	Sporadic
16	<i>A. flavipes</i>			Sporadic
17	<i>A. flavus</i>	Most common	Most common	Occasional
18	<i>A. fumigatus</i>	Common	Most common	Most common
19	<i>A. japonicus</i>	Common	Common	Occasional
20	<i>A. nidulans</i>	Occasional	Occasional	Sporadic
21	<i>A. niger</i>	Most Common	Most Common	Most common
22	<i>A. ochraceus</i>	Occasional	Most common	Occasional
23	<i>A. sydowii</i>			Sporadic
24	<i>A. tamarii</i>	Sporadic		Sporadic
25	<i>A. terreus</i>	Occasional	Occasional	Occasional
26	<i>A. versicolor</i>	Occasional	Occasional	Sporadic
27	<i>Aureobasidium pullulans</i>	Sporadic	Sporadic	
28	<i>Beltrania mangiferae</i>		Sporadic	
29	<i>B. rhombica</i>			Sporadic
30	<i>Beltraniella portoricensis</i>		Sporadic	
31	<i>Beltraniopsis asperisetifer</i>		Sporadic	
32	<i>Ceratosporium fuscescens</i>		Sporadic	
33	<i>Chrysosporium pannorum</i>	Sporadic		
34	<i>Chaetochara africana</i>		Sporadic	Sporadic
35	<i>Circinotrichum fertile</i>		Sporadic	
36	<i>Cladosporium cladosporioides</i>	Sporadic	Sporadic	Occasional
37	<i>C. oxysporum</i>	Occasional	Common	Occasional
38	<i>Curvularia lunata</i>	Occasional	Common	Occasional
39	<i>C. brachyspora</i>	Sporadic	Sporadic	Sporadic
40	<i>C. eragrostidis</i>	Occasional	Occasional	Occasional
41	<i>C. oryzae</i>	Occasional		
42	<i>C. pallescens</i>	Occasional		
43	<i>C. tuberculata</i>	Sporadic		Sporadic
44	<i>Cordana vasiformis</i>		Occasional	
45	<i>Dendryphon comosum</i>		Sporadic	
46	<i>Dictyosporium heptasporum</i>		Sporadic	
47	<i>Drechslera australiensis</i>	Sporadic	Occasional	Sporadic
48	<i>D. halodes</i>	Sporadic	Sporadic	Sporadic
49	<i>D. hawaiiensis</i>	Sporadic	Occasional	Sporadic
50	<i>D. rostrata</i>		Occasional	
51	<i>Fusarium moniliforme</i>	Sporadic		

S.No	Fungal Species	L-LAYER	F1-LAYER	F2-LAYER
52	<i>F. oxysporum</i>	Occasional	Common	Occasional
53	<i>F. solani</i>		Sporadic	
54	<i>Geotrichum candidum</i>	Sporadic		
55	<i>Gliocladium roseum</i>		Sporadic	Sporadic
56	<i>Gliomastix murorum</i>		Sporadic	
57	<i>Humicola grisea</i>		Sporadic	Sporadic
58	<i>Henicospora coronate</i>		Sporadic	
59	<i>Memmoniella echinata</i>	Sporadic	Sporadic	
60	<i>Monilia sitophila</i>	Sporadic		Sporadic
61	<i>Monodictys glauca</i>	Sporadic	Sporadic	Sporadic
62	<i>M. levis</i>	occasional	Sporadic	
63	<i>Myrothecium indicum</i>		Sporadic	
64	<i>M. gramineum</i>		Sporadic	
65	<i>M. roridum</i>	Sporadic	Sporadic	
66	<i>M. verrucaria</i>	Sporadic	Sporadic	Sporadic
67	<i>Nigrospora sphaerica</i>	Sporadic	Sporadic	Sporadic
68	<i>Paecilomyces carneus</i>	Sporadic	Occasional	Sporadic
69	<i>P. variotii</i>	Sporadic	Sporadic	
70	<i>Periconia clitoriae</i>	Sporadic		
71	<i>Penicillium citrinum</i>	Most common	Most common	Common
72	<i>P. chrysogenum</i>	Sporadic		Sporadic
73	<i>P. fellutanum</i>	Sporadic	Sporadic	Sporadic
74	<i>P. frequentans</i>	Sporadic		Sporadic
75	<i>P. funiculosum</i>	Sporadic		Sporadic
76	<i>P. islandicum</i>	Sporadic		
77	<i>P. oxalicum</i>	Sporadic		Occasional
78	<i>P. purpurescens</i>	Sporadic	Occasional	
79	<i>P. purpurogenum</i>	Sporadic	Sporadic	
80	<i>Pithomyces chartarum</i>		Sporadic	
81	<i>P. maydicus</i>	Sporadic	Sporadic	
82	<i>Selenosporella curvispora</i>		Sporadic	
83	<i>Scolecobasidium constrictum</i>	Sporadic	Occasional	Sporadic
84	<i>S. humicola</i>		Sporadic	
85	<i>Scopulariopsis brevicaulis</i>	Sporadic	Sporadic	
86	<i>Sporothrix</i> sp.		Sporadic	
87	<i>Stachybotrys atra</i>	Sporadic	Sporadic	Sporadic
88	<i>S. bisbyi</i>	Sporadic	Sporadic	Sporadic
89	<i>Thielaviopsis basicola</i>		Sporadic	
90	<i>T. paradoxa</i>		Sporadic	Sporadic
91	<i>Trichoderma atroviride</i>			Sporadic
92	<i>T. harzianum</i>	Occasional	Occasional	Occasional
93	<i>T. koningii</i>	Sporadic	Sporadic	
94	<i>T. polysporum</i>		Sporadic	
95	<i>T. pseudokoningii</i>		Sporadic	
96	<i>T. roseum</i>		Sporadic	Sporadic
97	<i>T. viride</i>	Occasional	Occasional	Sporadic
	COELOMYCETES			
98	<i>Bartalinia robillardoides</i>	Sporadic	Sporadic	Sporadic
99	<i>Colletotrichum dematium</i>		Sporadic	Sporadic
100	<i>Lasiodiplodia theobromae</i>	Sporadic	Sporadic	Sporadic
101	<i>Pestalotiopsis guepinii</i>	Sporadic	Sporadic	Sporadic
102	<i>Phoma betae</i>	Sporadic	Sporadic	
103	<i>P. capitulum</i>		Sporadic	
104	<i>P. exigua</i>		Sporadic	Sporadic
105	<i>P. lingam</i>	Sporadic	Sporadic	Sporadic
106	<i>P. palmicola</i>			Sporadic
107	<i>Phomopsis eugeniae</i>		Sporadic	

Species estimation through extrapolation

Extrapolation method was used to estimate the total number of species from empirical samples that make up the community, since complete inventories are practically impossible. The most widely used approach for estimating species richness is based on extrapolating from a known species – accumulation curve. This is obtained by plotting the number of species discovered, $S(n)$, within a plot, as a function of the effort expended (n) to find them. In this study, effort is the number of times samples were taken during the 3-year period of study. Following Colwell (2000), the asymptote of the species - accumulation curve, S_{max} , and a second parameter, B (number of season surveyed when species richness = $1/2 S_{max}$), were estimated using a transformation known as the Eadie-Hofstee equation, which assumes that $S(n)$ is a function of $S(n)/n$, the ratio of species to individuals in the sample. Based on this approach, Michaelis-Menten model and Coleman curve were fitted to the sampling data after randomizing them for 50 times. All these analyses were carried out using the EstimatesS-Win software Version 7.5.2 of Colwell (2000). Several estimators of diversity namely Chao 1 and Chao 2 and ACE, ICE and Jackknife 1 were calculated.

Relationship between relative abundance and species rank

The total abundance of the different species of fungi observed during the 3-year period was converted into a percentage and plotted against their ranks from the most abundant to least abundant species using PASTA software under the log series model.

In order to present the log normal model the data was rearranged by plotting the frequency of species (the ordinate) against the logarithm of abundance. The logarithms to the base 2 were used as per the conventional procedure in ecological studies so that each class or octave represents a doubling of the previous abundance class.

Community ordination technique

Principal Components Analysis was computed using the software Paleontological Statistics (Past) version 1.92 with the aim to

summarize the relationship between the samples. Sampling units (sites) were arranged in relation to multi coordinate axes such that their relative positions to the axes and to each other provided maximum information about their ecological similarities. The data on the species abundance was used to calculate the Eigen value based on which co-ordinate axes were represented so as to produce information on the similarity of the habitat types. To test for linearity, the data was further subjected to detrended correspondence analysis and the values of the first three axis obtained was used to plot the graph.

Results

Composition of mycoflora of the three litter layers

A total of 107 species belonging to 54 genera were isolated from the litter samples. These were made up of 7 species of Zygomycota, 4 species of Ascomycetes 86 species of hyphomycetes and 10 species of coelomycetes. The number of taxa recorded per sampling was higher in F1 layer (23-32) than F2 (14-22) or L (17-25) layers (Table -1). Seven species of Zygomycota were recorded in the F1 layer and 5 species in both the F2 and F3 layers. For Ascomycota, four species were recorded in F1 layer and two each in F2 and L layers. Seventy seven species of anamorphic fungi were recorded in the F1 layer, 61 species in the L layer and 54 species in the F2 layer. Five species of Zygomycota viz. *Absidia corymbifera*, *Cunninghamella echinulata*, *Mucor racemosus*, *Rhizopus stolonifer* and *Syncephalastrum racemosum* were isolated from all three layers of litter. In addition, *Cunninghamella elegans* and *Helicostylum piriforme* were isolated from F1 litter only. In the case of Ascomycetes, two species were isolated only from L and F2 litter and four species from F1 litter. Similarly, more hyphomycetes were isolated from F1 litter followed by F2 and L litter. For coelomycetes, most species were isolated from F2 litter followed by F1 and L litter. Forty one species were present in all three layers, 13 species were present only in L and F1 layers, 8 species were present in both F1 and F2 layers and 7 species were

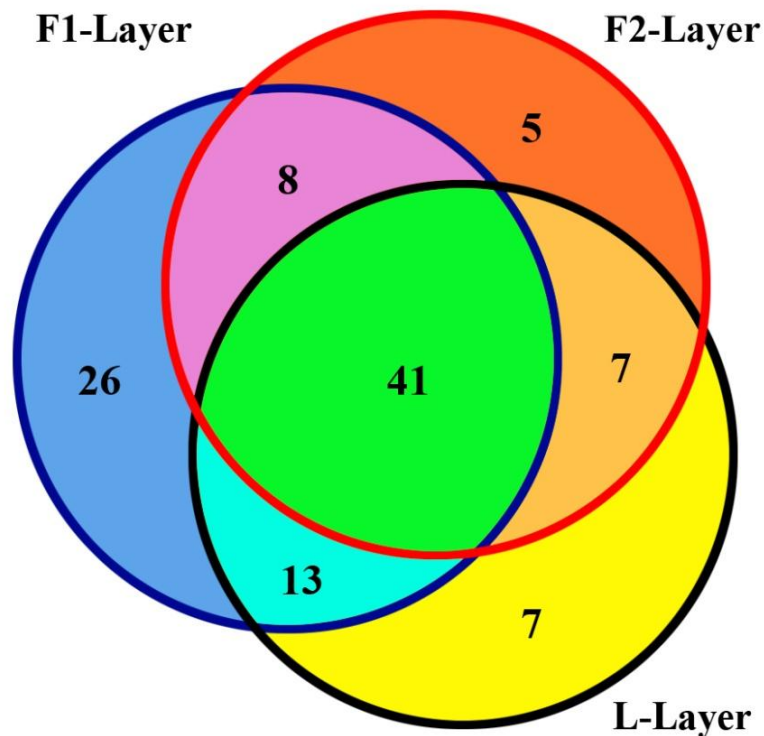


Fig. 1 – Composition of mycoflora among the three litter layers in the Guindy Reserve Forest – Number represents total number of species encountered

common to L and F2 layers. Twenty six species were specific to F1 layer; 7 and 5 species were specific to L and F2 layers, respectively. (Fig 1).

Although many species were recorded in all three layers, their periodicity of occurrence was not the same (Table 1). *Aspergillus niger* was the only species “most common” in all three layers of the litter. *Aspergillus flavus* and *Penicillium citrinum* were “most common” in L and F1 litter but had only the “occasional” and “common” status in the F2 litter. *Mucor racemosus* and *Aspergillus ochraceus* were most common in F1 litter but “common” and “occasional” in L and F2 litter, respectively. *Aspergillus fumigatus* was most common in F1 and F2 litter but “occasional” in the L litter sample.

Species diversity

Shannon index H is a diversity index taking into account the number of individuals as well as number of taxa. It varies from 0 for communities with only single taxa to high values for communities with more taxa each

with few individuals. The F1 layer had the maximum value of H. This estimator is biased because the total number of species in the community will be greater than the number of species observed in the sample. However if ‘n’ is large, this bias will be small. Simpson diversity index λ gives the probability that two individuals drawn at random from a population belong to the same species that the diversity of the community is low. Simpson mean λ values were higher for the F1 Layer than L and F2 layer. Fisher’s alpha is a useful index for log series models of distribution and the present data falls in this category. The diversity as indicated by Fishers alpha was highest for the F1 layer (Table 2).

Species richness

The total species richness S_{max} , which is a straight forward measure of species diversity, is given in Table 2. The maximum number of species was recorded in the F1 layer followed by the L layer and the lowest number of species was recorded in the F2 layer. A similar trend was observed for the data on the total number

Table 2 Diversity indices computed for the fungal species complex at different layers of the forest litter.

S.No	Diversity Indices	L-Layer	F1-Layer	F2-Layer
1	(S_{max}) Total Species	69	89	62
2	No. of Colony Forming Unit	3590	4418	2909
3	Dominance_D	0.03625	0.03279	0.0464
4	Berger-Parker	0.139	0.1213	0.1653
5	Margalef R1	8.307	10.48	7.648
6	Menhinick R2	1.152	1.339	1.15
7	Evenness_e ^{H/S}	0.6799	0.5989	0.6436
8	Equitability_J	0.9089	0.8858	0.8932
9	Simpson_1-D	0.9637	0.9672	0.9536
10	Shannon_H	3.848	3.976	3.686
11	Simpson mean λ	27.43	30.39	20.97
12	Fisher_alpha	12.12	15.79	11.13

of (colony forming unit) individuals wherein significantly fewer individuals were recorded in the deeper litter layer (F2 layer). Dominance value ranges from 0 when all taxa are equally present to 1 when one taxon dominates the community completely. The values observed here show a tendency to move towards zero. The Berger Parker dominance index expresses the proportion of the total share that is due to the dominant species. This value was also low for all three layers. Since the F2 layer had fewer species, the Berger-Parker dominance index was higher in the F2 layer than the L and F1 layer.

Since S_{max} is dependent on the sample size it is limited as a comparative index. Hence two well known indices to measure species richness that are independent of sample size namely Margalef and Menhinick were computed. The values of both these indices were higher in the F1 layer than the L and F2 layers. However, in order to accept these indices according to their face value, it is assumed that a functional relationship exists between the total number of species and total number of individuals observed in the community.

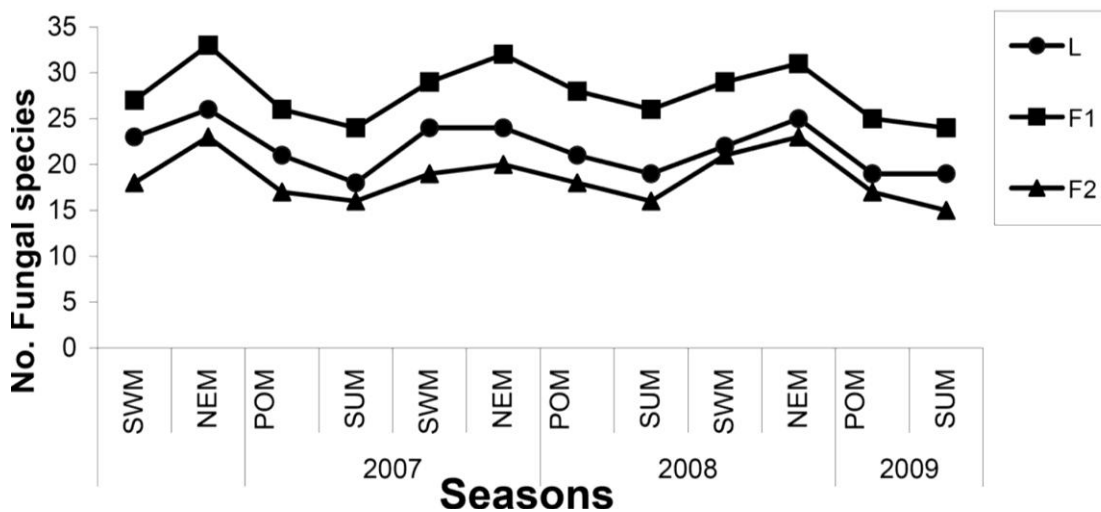
Species evenness

In analyzing species diversity it is important to also evaluate the equitability or evenness, which indicates the pattern of distribution of individuals between the species.

Evenness refers to how the species abundances are distributed among the species. When all species in a sample are equally abundant, the evenness index would have a maximum value of one and decrease towards zero as the relative abundances of the species moves away from evenness. Evenness values calculated hovered around 0.6 indicating a tilt towards even distribution of the species. A similar trend was observed for the Equitability J values. Simpson index (1-D) measures evenness of the community from zero to one. The value observed here approximated towards one (Table 2).

Seasonal changes in the fungal species composition

Monthly data on the number of species of fungi inhabiting the forest litter sample was grouped into four periods representing four seasons namely (Fig. 2). Throughout the period of study, the F1 Layer of the forest litter was the most species rich followed by the L layer and F2 layer. The trend indicates that the habitat was highest in fungal species during the NE Monsoon (Sep-Nov). A significant positive correlation was recorded between the fungal species of the three layers and the mean monthly rainfall. RH also appeared to have an influence on the number of fungal species especially that of the F1 layer, while no correlation was observed with temperature (Table 3).



SWM - Southwest monsoon, NEM –Northeast monsoon, POM - Post monsoon SUM – Summer

Fig. 2 – Comparison of number of species recorded in different seasons in the three layers of the forest litter.

Extrapolation of forest litter fungal species diversity

A few species of fungi are represented by large number of colony forming units and a large number of species by few colony forming units. These relative abundances represent the basic pattern of niche utilization in the community. The characteristic distribution of the various fungal species forming the community becomes evident when relative abundance of each species in the community is plotted in the order of its rank from the most abundant to the least abundant (Fig. 3). This clearly indicates a log normal pattern of distribution pointing towards interplay of many independent factors governing the relative abundance of the species.

Sample rarefaction and non-parametric estimators

The species (Sample rarefaction) accumulation curve obtained by plotting the cumulative number of species collected against the sampling effort (months) is provided (Fig. 4). In all three layers, the curve assumed asymptote at 12 sampling efforts. The order in which samples are taken alters the shape of the curve. The data basically being a time series

one indicate more species of fungi were collected during September to November months which coincided with the rainy season (Fig. 2). Therefore, to eliminate the influence caused by temporal variation, the sample order was randomized 100 times. Extrapolation to estimate the total species complement, S_{max} is only possible if the species accumulation curve is derived from a reasonably homogenous community. Therefore, heterogeneity was tested by rarefaction approach which generates the curve for samples by selection without replacement. If the expected curve rises more steeply from the origin, community is heterogeneous. The curve in this study arose slowly and reached asymptote indicating that the sampling effort was sufficient to predict the species richness. Several non-parametric estimators were calculated for extrapolating the species accumulation curve (Table 4). Values of the abundance based coverage estimator (ACE) of equaled the observed values of the total species fungi in each of the three layers of the litter. Similarly, the incidence based coverage estimator (ICE) also approximated the observed total number of species. Value of Chao 1, Chao 2 and Jack 1 also added support to the observed values of total species recorded in each layer of the litter.

Table 3 Correlation coefficient computed between the number of fungal species and the prevailing weather parameters.

Litter layer	Average Temperature (°C)	Monthly mean Relative Humidity	Mean monthly Rainfall (mm)
L-Layer	-0.105	0.575*	0.882**
F1-Layer	-0.226	0.711**	0.918**
F2-Layer	-0.21	0.672*	0.879**

*Significant at P=0.05, ** Significant at P=0.01

Change in diversity along the litter gradient (β diversity)

The changes in species diversity along a transect running through the layers of the forest litter was computed by using data on the total number of individuals of each species recorded during the 12 sampling periods (Table 5).

β -Diversity measures two attributes namely the number of distinct habitats within the region and the replacement of species by another between disjoint parts of the same habitat. The most effective approach used to

compare samples along a gradient is the similarity indices. The best index for presence-absence data is the Jaccard and for quantitative data is the Morista-Horn.

Values of the Jaccard classic as well as the Sorensen classic indicate that the F1 and F2 layers (samples 2 and 3) were more closely similar than either L and F2 (samples 1 and 3). The Morisita-Horn and Bray-Curtis indices based on quantitative data also indicate similar results.

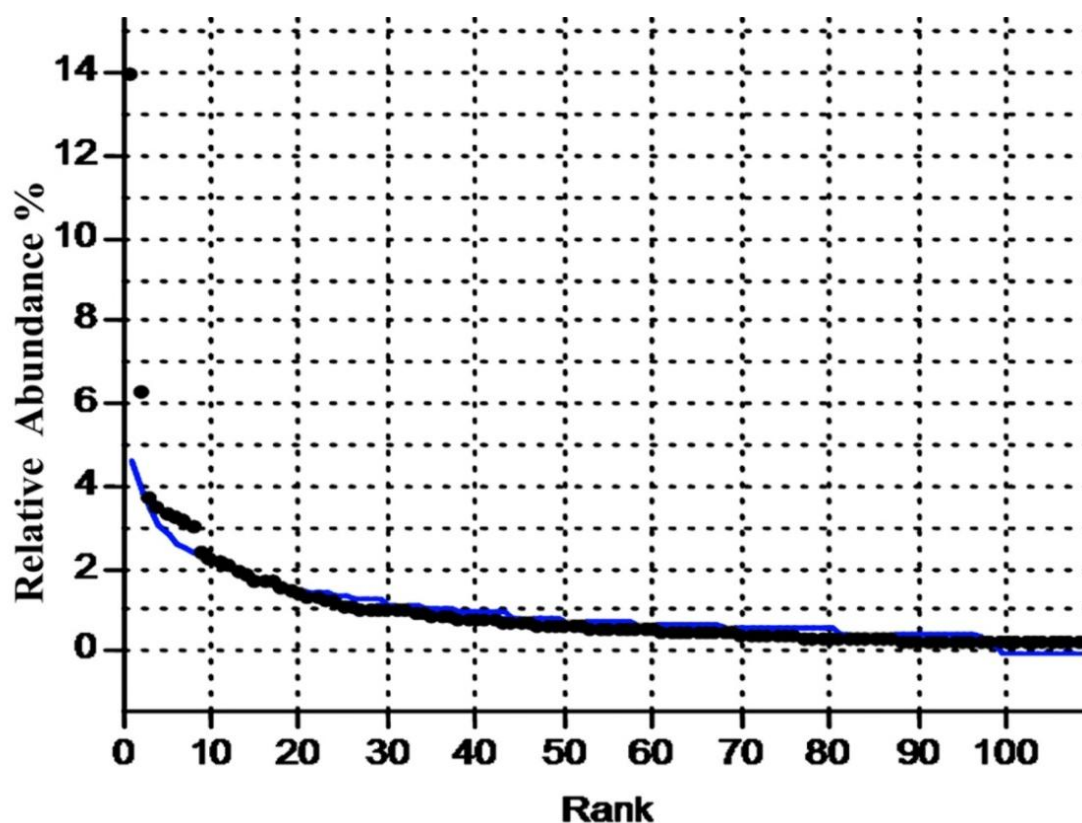


Fig. 3 – Relationship between relative abundance of fungal species and their rank in the forest litter community.

Table 4 Non parametric estimators of the species accumulation curve.

Layers	ACE Mean	ICE Mean	Chao 1 Mean	Chao 2 Mean	Jack 1 Mean	Jack 1 SD (analytical)	Bootstrap Mean	MM Means (1 run)
L-Layer	69	71.85	69	69.63	74.5	2.53	74.06	88.84
F1-Layer	88	89.54	88	88.07	90.75	1.44	94.16	116.05
F2-Layer	61	61	61	61	61	0	64.49	83.53

ACE= Abundance based coverage Estimator, MM mean = Michaelis Menton mean ICE= Incidence based Coverage Estimator

Community classification and ordination

Community classification plays a fundamental role in understanding the natural system that has led to the observed grouping of the species. Each species in the group has a number of common characteristics that set them apart from each other group. An attempt has been made here to classify the species in the community using a polythetic, hierarchical, agglomerative techniques namely cluster analysis.

Fig. 5 classifies the litter fungal community into clusters based on the Jaccard similarity indices. The L and F2 layers of litter formed a cluster based on a 60% similarity resembled one another and formed a group that was distinct from *Curvularia oryzae*. The remaining fungal species formed two distinct

between them and was separated from the F1 layers by a difference of about 8% only.

The data recorded on the abundance of individual species of fungi over the 12 season of sampling was used to group the various species reflecting their overall resemblance to one another. Dendrograms of the clustering of the species using Euclidean distance is provided (Fig. 6). *Aspergillus niger* formed a separate group in the community. Similarly the non-sporulating fungi group also differed from the other species of fungi. *Aspergillus ochraceus*, *A. flavus*, *A. fumigatus*, *Mucor racemosus*, *Rhizopus stolonifer* and *Penicillium citrinum* groups. Therefore, arbitrarily six distinct groups are identifiable based on the abundance of the fungal species.

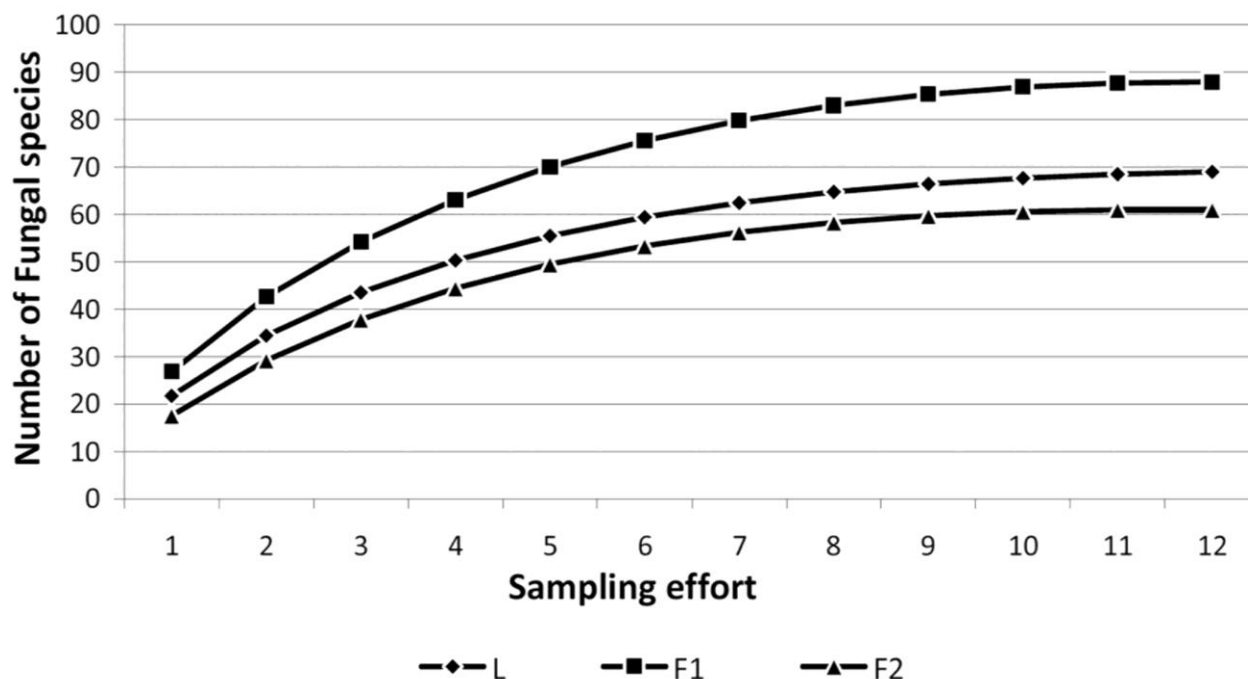
**Fig. 4** – Species accumulation curve

Table 5 Comparison for fungal species diversity among the three layers of the forest litter.

First Sample	1	1	2
Second Sample	2	3	3
Sobs First Sample	69	69	89
Sobs Second Sample	89.00	62.00	62.00
Shared Species Observed	55	49	50
ACE First Sample	69	69	89
ACE Second Sample	89	62	62
Jaccard Classic	0.533	0.597	0.495
Sorensen Classic	0.699	0.748	0.662
Chao-Jaccard-Raw Abundance-based	0.759	0.758	0.747
Chao-Jaccard-Est Abundance-based	0.759	0.758	0.747
Chao-Sorensen-Raw Abundance-based	0.863	0.862	0.855
Chao-Sorensen-Est Abundance-based	0.863	0.862	0.855
Chao-Sorensen Est Abundance- based	0.863	0.862	0.855
Morisita-Horn	0.94	0.932	0.923
Bray-Curtis	0.734	0.737	0.683

Sample 1=L Layer, Sample 2=F1 Layer, Sample 3= F2 Layer

Principal component ordination

A community ordination technique was performed in which the seasons of sampling are arranged in relation to the coordinate axes such that their relative position to the axes and to each other provides maximum information F1 layer is provided (Fig.7).

PCA helps in breaking down or partitioning a resemblance matrix into a set of perpendicular axes or component. Each PCA axis or component correspond to an eigenvalue of the matrix. The eigenvalue is the variance accounted for by the axes. The eigenvalues are extracted in the descending order of magnitude

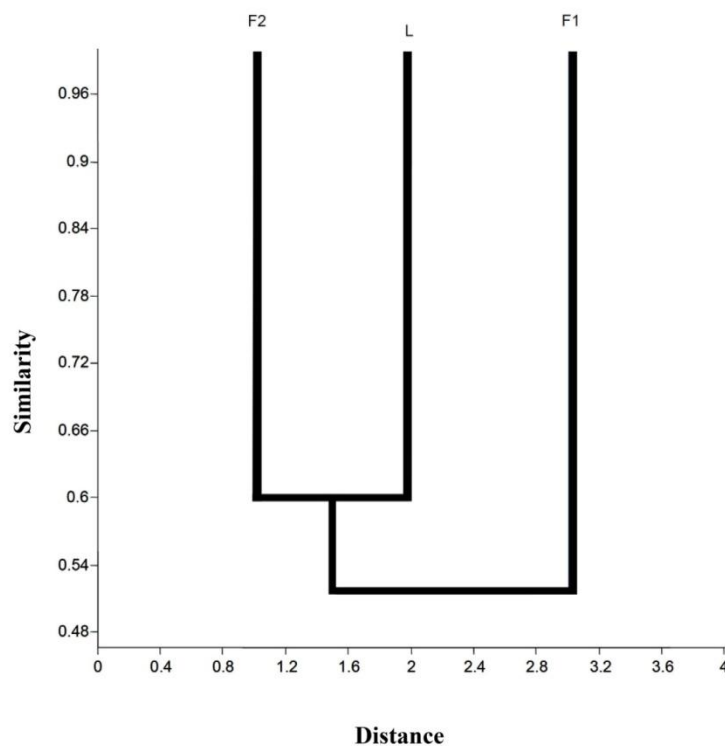


Fig. 5 – Dendrogram for the cluster analysis classifying the three layers of the forest litter based on the Jaccard index of similarity.

Table 6 Principal components analysis for four seasons based on the abundance of 23 fungal species of the F1 Layer: Coordinate of the first four axis.

Seasons	Axis 1	Axis 2	Axis 3	Axis 4
SWM	-0.32	0.10	-1.46	-2.73
NEM	-1.28	-0.11	0.78	-2.17
PM	0.75	1.23	0.43	1.09
SUM	0.85	-1.21	0.25	4.20

such that the correspondingly PCA components represent correspondingly greater to lesser amount of variation in the matrix. Hence, the first few PCA axes upon which species (season) is positioned will represent the largest percentage of the total variation. Values of the first 4 components are given (Table 6). The PC ordination plotting Component 1 vs Component 2 indicate that the data of the fungal species abundance during the North East monsoon and

South West monsoon were positioned towards the -ve side of the axis while the post monsoon and summer were towards the +ve side. Also the greatest variation was accounted to be due to the South West monsoon (Fig.7).

The component 3 would account for lesser variation in the matrix than 1 and 2. Plotting of component 3 vs component 1, also indicates the data during the North East monsoon and South West monsoon to fall on

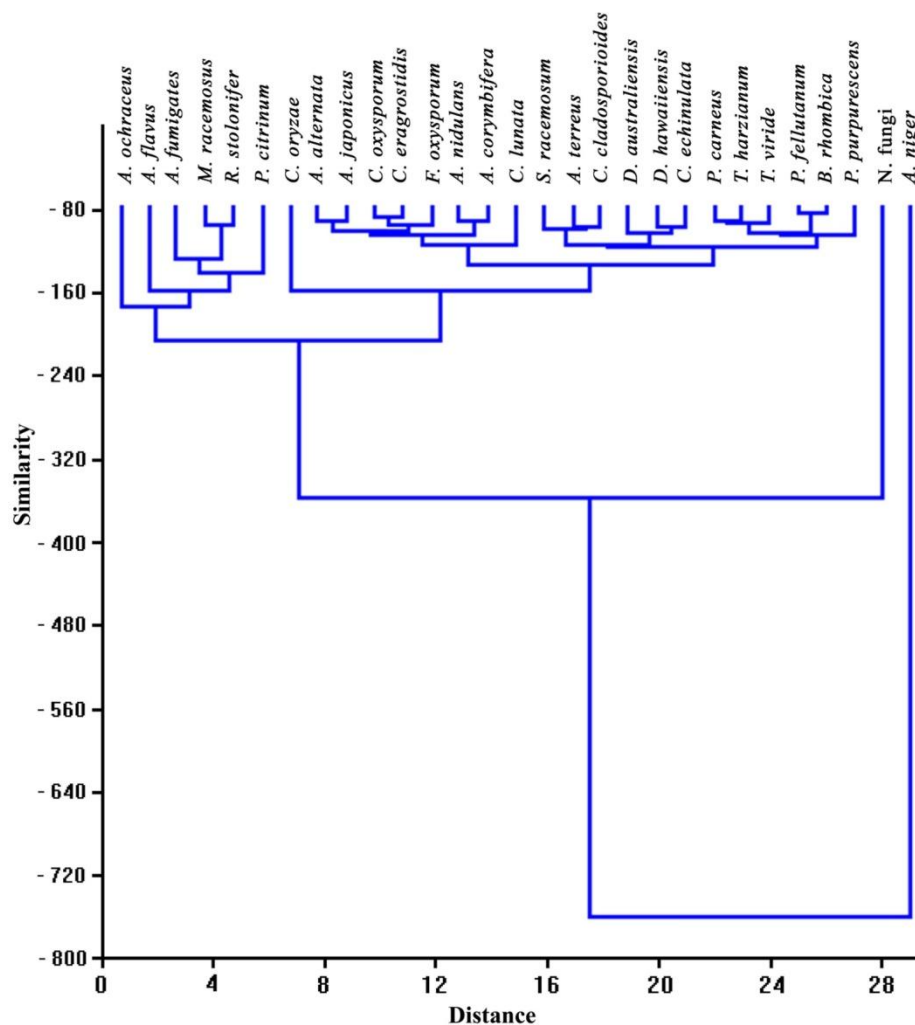


Fig. 6 – Dendrogram clustering the 29 abundant fungal species of the forest litter using euclidean distance.

Table 7 Detrended correspondence analysis value: Coordinates of the first three axis.

Seasons	Axis1	Axis2	Axis3
SWM	0.14	0.15	0.15
NEM	0	0	0
PM	0.39	0.37	0.39
SUM	0.6	0.32	0.35

the left hand side while the post monsoon and summer on the right hand side. The biplot depicting the positioning of the various species about the centre is also presented. Fig. 7 depicts the plotting of component 3 with the component 2. The 23 species of fungi appears to be dispersed around the central axis indicating no specific orientation.

Detrended correspondence analysis

In studies where the sampling units occur at wide environmental gradients, the species abundance patterns are typically non-linear and this can cause difficulties in interpretation. To overcome this, detrended correspondence analysis was attempted. The values of the first three axis obtained by computing the detrended correspondence analysis is provided to in (Table 7). Detrended correspondence analysis plotting axis 1 and axis 2 (Fig. 8) and axis 2 with axis 3 indicate a linear arrangement of the four seasons. Species abundance contributing to the data of the North East monsoon and South West monsoon was closer to the central axis than the post monsoon and summer indicating the ecological importance of environment on the species distribution.

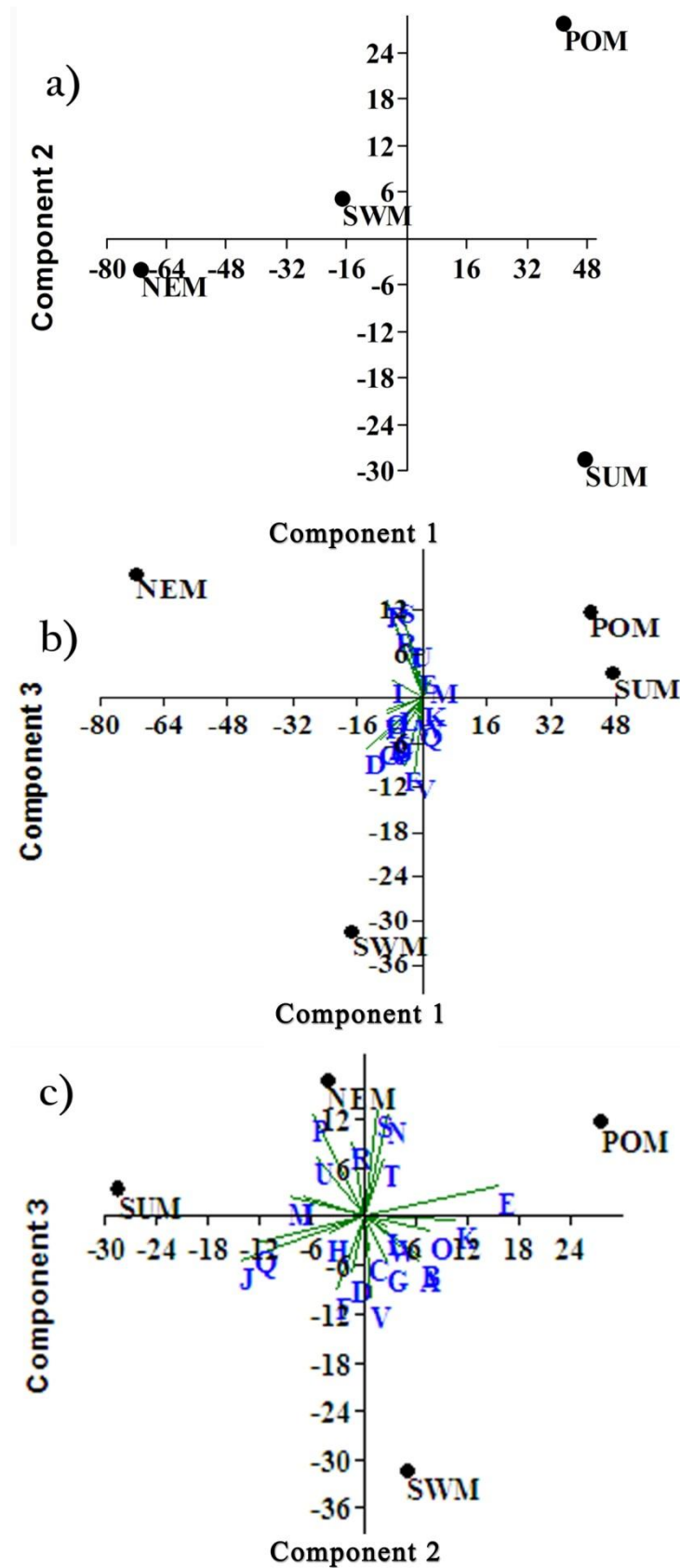
Discussion

Investigations of tropical forest soil fungi have focused largely on those associated with leaf litter (Paulus et al. 2006, Duong et al. 2008) and most assemblages of micro fungi sporulating on decomposing leaves have been identified following laboratory incubation (Parungao et al. 2002, Rambelli et al. 2004, Thongkantha et al. 2008). However, investigation of total soil fungal assemblages in tropical forests have been more limited and have relied on dilution plating approaches (Mohanty & Panda 1994, Persiani et al. 1998).

There has been no specific study on the alkalotolerant species inhabiting the leaf litter and in this sense the present study appears unique. A total of 107 species were identified from this forest ecosystem and these species were capable of growing in alkaline pH. There was a distinct pattern of occurrence of fungal species in the different layers of the litter, indicating the specific functional role they play in the process of litter degradation. The exclusive occurrence of fungal species in particular layers was also reported by Watson et al. (1974) for hard wood and pine litter. That the fungal diversity varies depending on the needle type has been emphasized by Tokumasu et al. (1997) in studies on microfungal communities in pine leaf litter in Thailand. Such selective distribution of fungal species at different strata of the habitat might relate to the specific functional role they play in the community and also signifies the specific niche requirements of the species.

Periodicity of occurrence and percentage frequency

In the present study a somewhat arbitrary grouping of fungi has been made on the basis of their periodicity of occurrence i.e., the number of samplings in which each fungus was found to occur as against the total number of samplings. Four groups were recognized; species showing 76-100% periodicity of occurrence were regarded as “most common”, 51-75% as “common”; 26-50 % as “occasional” 1-25% “sporadic”. Not that this is a perfect classification but yet it was felt that this would give some idea on the periodicity of occurrence and facilitate description. Although several fungal species were common components of the mycoflora of all the three layers of the Guindy Forest litter, it was noticed that each layer had its own characteristic flora with regards to their periodicity of occurrence. *Aspergillus niger*



SWM – Southwest monsoon NEM –Northeast monsoon POM - Post monsoon SUM – Summer

Fig. 7 – Principal component ordination: Position of four seasons based on fungal species abundance **a)** Principal Component 1 Vs 2 **b)** Component 1 Vs Component 3 and **c)** Component 2 Vs Component 3.

showed the highest average percentage occurrence in all the three layers of litter. *Penicillium citrinum* was among the 'most common' category in L layer of the litter. *Mucor racemosus*, *A. flavus*, *A. fumigatus*, *A. ochraceus*, and *Pencillium citrinum* were 'most common' in the F1 litter. *Aspergillus fumigatus* was among the 'most common' category in F2 litter. Such difference among different categories of litter were reported by Vittal (1973), Sudha (1978), and Dorai & Vittal (1988). In general, many fungi appeared sporadically with low frequency. Only a few species were constant throughout the decomposition process. A similar observation was made by Watson et al. (1974) and Heredia (1993).

Seasonal changes in species composition

The GRF was highest in fungal species during the North East monsoon. In the southern Indian plains, the ideal season for fungal growth has been observed to be the monsoon period (Maria & Sridhar 2004) which is in agreement with the present study. Zhou & Hyde (2002) also suggested that seasonality has an effect on fungal composition in the habitat, as more fungi were present during the wet season. They concluded that rainfall positively impacted on fungal occurrence, but temperature and relative humidity appeared to have little influence. Many authors have reported that seasonality affects fungal diversity. Tokumasu (1998) observed that the temperature at the surface of decaying needle litter of *Pinus densiflora* was a major factor contributing to seasonal changes in interior fungal communities. Pandey & Dwivedi (1984) also observed that *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, and *F. psidii* on the leaves of *Psidium guajava* were recorded more frequently in the rainy season. Similarly, in the present study, *Periconia clitoriae*, *Lasiodiplodia theobromae*, *Pestalotiopsis guipenii*, and *Beltraniopsis asperisetifer* were isolated only during the North East monsoon. Leung (1998) also suggested that seasonal factors, especially air temperature and rainfall affected the development of the fungal communities on bamboo litter. Rainfall is therefore, an important factor that has the effect of increasing fungal diversity. A significant positive correlation was recorded between the

fungal species of the three layers of the GRF litter and the mean monthly rainfall. Relative humidity (RH) also appeared to have an influence on the number of fungal species especially that of the F1 layer, while no correlation was observed with temperature.

The log normal distribution of fungal community observed in the present study indicates that the niche of a species is dependent on a multitude of different factors and that the amount of resources used by each species is normally distributed. This distribution pattern also indicates that many independent factors govern the relative abundances of the species and the fungal community at GRF habitat appears to be a heterogeneous assemblage although the samples were drawn from a homogenous habitat. Detrended correspondence analysis plotting axis 1 with axis 2 and axis 2 with axis 3 indicate a linear arrangement of the four seasons for the data. Species abundance contributing to the data of the north east monsoon and south west monsoon was closer to the central axis than the post monsoon and summer indicating the ecological importance of environment on the species distribution.

Senthilkumar et al. (1993) reported that *Acremonium* sp., *Alternaria alternata*, *Aspergillus flavus*, *A. nidulans*, *A. niger*, *A. terreus*, *Aureobasidium pullulans*, *Chaetomium lunasporium*, *Colletotrichum dematium*, *Curvularia lunata*, *Drechslera halodes*, *Emericella nidulans*, *Fusarium solani*, *Mucor racemosus*, *Nigrospora sphaerica*, *Pithomyces chartarum*, *Rhizopus stolonifer*, *Trichoderma koningii*, and *T. viride*, were major mycoflora associated with litter degradation in tropical grasslands of south India. Similar species were also encountered in litter samples of GRF. Some interesting conclusions emerged from results of this investigation. Firstly, even though the freshly fallen leaves are rich in nutrients, they are in less utilizable form; the fungal communities require a helping hand from shredders (snails and other macro-invertebrates). Whereas, the middle layer (F1 layer) is usually rich in coarse particulate organic matter (Garrett 1963, Kirk et al. 1980) which can be solely and easily handled by the fungal community. Hence, the explosion of diversity was observed in F1 layer.

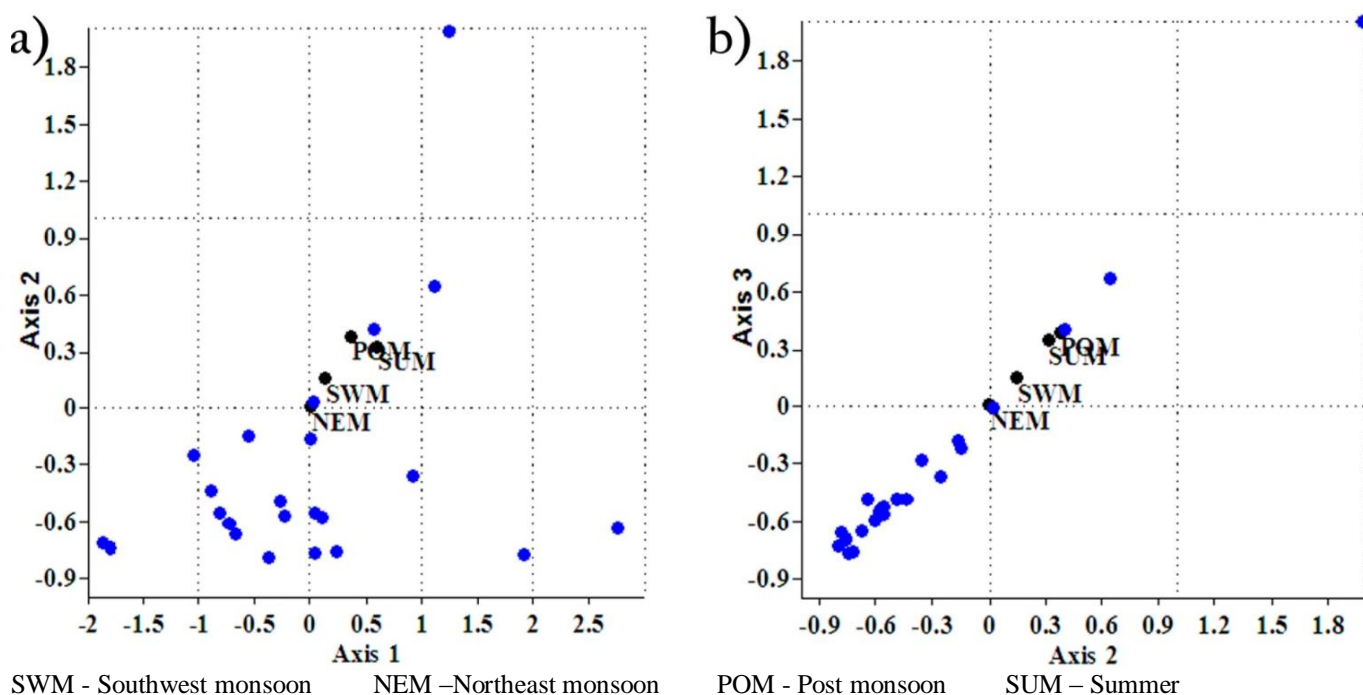


Fig. 8 – Detrended correspondence analysis for four seasons based on the abundance of 23 fungal species of the F1 Layer **a)** axis 1 vs axis 2 **b)** axis 2 vs axis 3.

Secondly, the specific association of fungal species in different layers is due to their niche specialization. Senthilkumar et al. (1993) reported that most of the fungal flora occurring in the later stages of decomposition (F1 litter layer) were efficient degraders of cellulose and lignin. In the present study more fungal species were recorded in F1 litter layer compared to L and F2 layer.

During the later stages of decomposition the initial colonizers gradually disappeared and were replaced by new colonizers. This could be due to the availability of different kinds of nutrients from the substrate at various stages of decomposition. This phenomenon probably plays a vital role in specific association of fungal species in different layer in the litter. Few of the fungi occurring in surface litter are well known plant pathogens, an observation also reported by Stakman & Harrar (1957). This supports the hypothesis that fungi capable of infecting living leaf could be the initiators of litter degradation.

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