Successional pattern of mycoflora associated with litter degradation in a *Cymbopogon caesius*-dominated tropical grassland

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Abstract

The pattern of fungal succession in *Cymbopogon caesius*-dominated tropical grassland in south India was studied by characterising litter mycoflora at different stages to understand the nature of litter degradation. The litter was dominated by numerous Hyphomycetes, and the fungal species of this substrate changed with progressive decomposition. The primary colonisers were mainly saprophytic fungi which survived well on undecomposed litter. The secondary colonisers were those capable of utilising high-molecular-weight compounds like lignin and cellulose. In the final stages of decomposition, *Rhizopus oryzae* and *Trichoderma viride* showed high survivability and the high percentage occurrence emphasised their role as climax species in *C. caesius* grasslands.

Introduction

Grassland is by far the largest single land use in the world (Peel 1986), and consequently its efficient utilisation and management are essential for sustainable food production and soil conservation (Tate *et al.* 1991a). In view of the rapid desertification of land resources in recent years, investigations are needed to effectively utilise and conserve grasslands. Soil micro-organisms are vital in the decomposition of organic matter to cycle C, N, P and S. Many ecological studies involve estimation of soil microbial biomass and activity (Kannan and Oblisami 1990a, 1990b; Tate *et al.* 1991b). The population and activity of microbes in soils are influenced by a variety of factors such as climate, soil fertility status and vegetation (Ross 1987; Sarathchandra *et al.* 1988; Okano *et al.* 1991). Our earlier investigation also indicated that the rate of litter degradation in a tropical grassland fluctuated depending on climatic factors (Senthilkumar *et al.* 1992). Relatively few reports describe the succession of micro-organisms during decomposition of the litter (Ponge 1991). Furthermore, these observations are limited to agricultural and forest soil ecosystems (Watson *et al.* 1974; Black and Dix 1977; Mitchell and Millar 1978).

This paper describes the fungal colonisation of decaying grassland litter in a tropical environment. The way species succeed each other has been studied by comparing mycofloras associated with different grades of decaying litter, as fungi are the primary decomposers of grassland litter (Coleman *et al.* 1980). Studies on the dynamics of mycoflora in this grassland ecosystem would provide information on the nature of litter degradation. The pattern of fungal succession in litter during various stages of decomposition was elucidated by analysis, over time, of mycoflora in the surface litter (L), the partially decomposed fermentation (F1), and the highly decomposed humus (F2) layers.

Materials and methods

Experimental site

The study was conducted in *Cymbopogon caesius*-dominated natural grasslands located in the foothills of Maruthamalai, Coimbatore, southern India. Soil physico-chemical characteristics and climatic features of the site were

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described by Senthilkumar *et al.* (1992). Briefly, the soil texture was sandy loam with a pH of 6.5. Total N and available P and K contents were 11, 0.6 and 39 mg/kg, respectively. The maximum temperature during the study period (October 1990–March 1991) ranged from 29–36 °C and the minimum temperature from 18–24 °C. Maximum rainfall of 134 mm was observed in October. Solar radiation was highest in February and March (239 and 221 calories/cm/day, respectively).

Collection of litter

C. caesius litter samples were collected at monthly intervals from the various layers with stainless steel forceps and spatula which were sterilised after each use with 70% ethanol. The samples were then placed in sterile Petri dishes and brought to the laboratory for immediate analysis. Three grades of litter were recognised for this study as described below:

Grade I litter: Hard, undecomposed, light brown to buff in colour, lying on the ground in a loose, uncompacted layer (L layer); it had high tensile strength and relatively low moisture content.

Grade II litter: Grey to black with softened tissues in the active phase of decomposition, low tensile strength and relatively high moisture content, lying in a more closely packed stratum of the fermentation layer or F1 layer.

Grade III litter: Greyish and fragmentary, relatively high moisture content, lying in a tightly compressed mat beneath the F1 layer (F2 layer); it was in the final stages of decomposition.

Isolation and identification of fungi

The mycoflora of the litter samples was analysed by plating them on 2% malt extract agar containing 185 $\mu g/cm^2$ streptomycin. Twenty litter (stem with attached leaf) samples each measuring 2.5 cm in length were randomly collected under each grade. Each sample was cut into 10 inocula and plated in a Petri dish containing medium. Development of fungal colonies was noticed from the third day of incubation (22 ± 1 °C) and recorded. Fungal hyphae, spores and fruiting structures were mounted and stained with 0.05% solution of cotton blue or acid fuchsin in lactophenol. Fungal colonies were identified by their morphological characteristics (Thom and Church 1926; Thom 1930; Gilman 1957; Barron 1968; Booth 1971; Ellis 1971; Subramanian 1971; Domsch *et al.* 1980; Udaiyan and Hosagouder 1991).

The qualitative aspects of fungal flora associated with different stages of litter degradation were further examined by a moist-chamber incubation technique where sterilised Petri dishes with moistened filter papers served as moist chambers. This also ensured the identification of species whose isolation was not favoured by the plating technique.

Frequency of occurrence

Percentage occurrence of various fungi was calculated to assess their relative density and distribution in the litter during each sampling month.

$$\text{Percentage occurrence} = \frac{\text{Number of litter samples in which a particular fungus was observed}}{\text{Total number of samples examined}} \times 100$$

The term 'percentage frequency' was used to assess the establishment and survivability of fungi in the litter (Udaiyan and Manian 1990; 1991). On the basis of frequency, fungi were classified into 5 frequency classes as follows: rare (1–20% occurrence), occasional (21–40%), frequent (41–60%), common (61–80%) and dominant (81–100%).

$$\text{Percentage frequency} = \frac{\text{Number of sampling months in which a particular fungus was recorded}}{\text{Total number of sampling months}} \times 100$$

Results

Surface litter

The percentage occurrence and frequency of all the species of fungi isolated from the surface litter over a period of 5 months are shown in Table 1. The species with highest mean percentage occurrence in descending order were: *Curvularia lunata*, *Mucor racemosus*, *Rhizopus*

Table 1. Percentage occurrence[1] and frequency of mycoflora associated with different grades of *Cymbopogon caesius* litter in south India.

Fungi isolated	Surface litter			F1 litter			F2 litter		
	% occurrence	% frequency	Freq. class	% occurrence	% frequency	Freq. class	% occurrence	% frequency	Freq. class
Acremonium sp.	8	40	O[2]	—[3]	—	—	—	—	—
Alternaria alternata	9	80	C	—	—	—	—	—	—
A. dianthicola	—	—	—	9	60	F	6	20	R
A. tenuissima	7	80	C	—	—	—	—	—	—
Ascochyta vulgaris	—	—	—	—	—	—	8	80	C
Aspergillus carneus	8	100	D	—	—	—	—	—	—
A. flavus	—	—	—	—	—	—	7	80	C
A. nidulans	5	80	C	—	—	—	—	—	—
A. niger	9	100	D	14	100	D	10	100	D
A. terreus	—	—	—	5	80	C	11	100	D
Aureobasidium pullulans	—	—	—	—	—	—	4	80	C
Chaetomium lunasporium	3	80	C	10	60	F	—	—	—
C. spirale	—	—	—	6	80	C	—	—	—
Colletotrichum dematium	—	—	—	3	80	C	—	—	—
Curvularia lunata	17	100	D	21	100	D	—	—	—
C. senegalensis	4	20	R	—	—	—	—	—	—
Drechslera halodes	1	40	O	—	—	—	—	—	—
Emericella nidulans	—	—	—	4	80	C	—	—	—
Fusarium solani	6	60	F	—	—	—	—	—	—
Melanospora sp.	4	100	D	10	100	D	—	—	—
Mucor racemosus	16	100	D	7	40	O	—	—	—
Nigrospora spherica	6	20	R	—	—	—	—	—	—
Phoma herbarum	—	—	—	—	—	—	4	40	O
Pithomyces chartarum	—	—	—	—	—	—	6	60	F
Rhizopus nodosus	15	100	D	—	—	—	—	—	—
R. oryzae	—	—	—	—	—	—	23	100	D
R. stolonifer	—	—	—	27	100	D	—	—	—
Scytalidium lignicola	—	—	—	—	—	—	4	80	C
Spegazzinia lobulata	—	—	—	—	—	—	2	60	F
S. tessarthra	—	—	—	—	—	—	7	40	O
Taeniolella exilis	—	—	—	—	—	—	2	60	F
Thielavia terricola	—	—	—	—	—	—	2	20	F
Trichoderma koningii	—	—	—	—	—	—	12	100	D
T. viride	—	—	—	—	—	—	19	100	D

[1] Values represent the mean of 5 sampling months.
[2] The "dominant", "common", "frequent", "occasional" and "rare" forms are indicated by "D", "C", "F", "O" and "R", respectively.
[3] Not isolated.

nodosus, *Aspergillus niger* and *Alternaria alternata*. The monthly results of 4 dominant species which recorded high percentage occurrence are shown in Figure 1. Of the 15 species isolated, 6 were dominant, 4 were common, one was frequent, 2 were occasional and 2 were rare. The dominant species in surface litter were: *Aspergillus carneus*, *A. niger*, *Curvularia lunata*, *Melanospora* sp., *Mucor racemosus* and *Rhizopus nodosus*.

F1 litter

The species with highest relative density in the F1 litter (i.e. percentage occurrence) (Table 1) during various sampling months in descending order were: *Rhizopus stolonifer*, *Curvularia lunata*, *Aspergillus niger*, *Melanospora* sp. and *Chaetomium lunasporium*. The monthly pattern of percentage occurrence of the former 4 species is shown in Figure 1. *Aspergillus niger*, *Curvularia lunata*, *Melanospora* sp. and *Rhizopus*

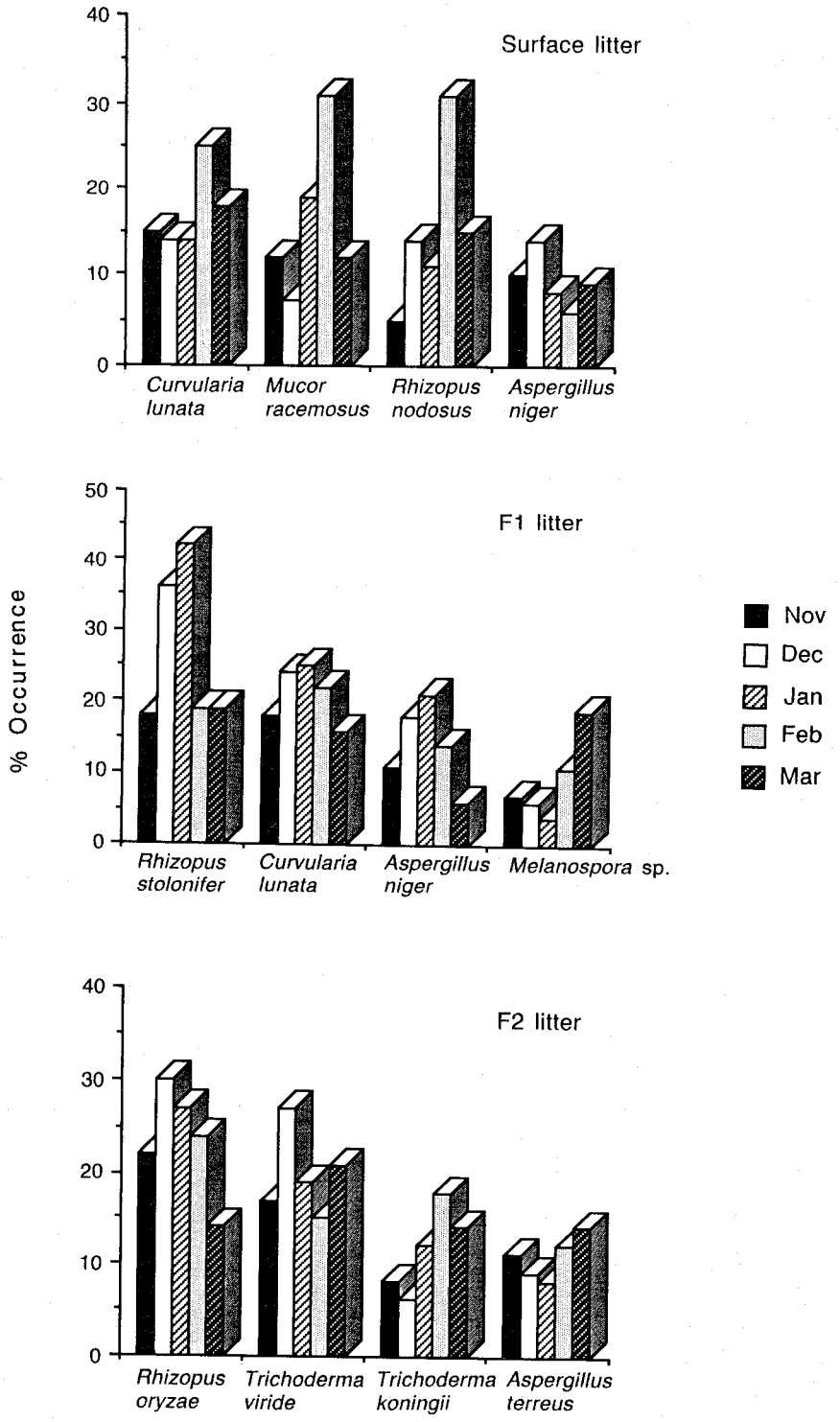

Figure 1. Percentage occurrence of the predominant fungi from surface, F1 and F2 litter during different sampling months from November–March.

stolonifer were the dominant fungi in terms of frequency while *Aspergillus terreus, Chaetomium spirale, Colletotrichum dematium* and *Emericella nidulans* were common.

F2 litter

The species with the highest mean percentage occurrence in F2 litter in descending order were as follows: *Rhizopus oryzae, Trichoderma viride, T. koningii, Aspergillus terreus* and *A. niger* (Table 1). Species with high mean relative densities in the litter during all sampling months are presented in Figure 1. All 5 categories of fungal colonisers were found in the F2 litter. Among 16 different species, *Aspergillus niger, A. terreus, Rhizopus oryzae, Trichoderma koningii* and *T. viride* were dominant. The commonly occurring ones were *Ascochyta vulgaris, Aspergillus flavus, Aureobasidium pullulans* and *Scytalidium lignicola.*

Discussion

It is evident from these results that tropical litter is colonised by numerous Hyphomycetes and the type of fungal species occurring changes as decomposition progresses. Although fungi capable of infecting the living leaf (e.g. *Alternaria alternata, A. tenuissima*) or colonising the phylloplane (e.g. *Aspergillus* spp., *Chaetomium lunasporium, Curvularia lunata, Rhizopus nodosus*) appear first, they give way to other colonists with greater ability to degrade the dead leaf (e.g. *Ascochyta vulgaris, Scytalidium lignicola, Taeniolella exilis, Trichoderma* spp.) which is a component of litter.

The present study also indicated that *A. niger* was dominant in all stages of litter decomposition. This indicates the high survivability of this species in tropical grassland vegetation. *Curvularia lunata, Chaetomium lunasporium* and *Melanospora* sp. were present in surface and F1 litter and their intensity of occurrence increased with the progress of degradation. However, they were eliminated from the final stages (i.e. F2 litter) of litter degradation. *Alternaria* spp., *Drechslera halodes* and *Fusarium solani* were observed only in surface litter, suggesting that most of the primary colonisers were weak saprophytes exhibiting short survivability. It can also be inferred that few of the fungi occurring in surface litter are well-known plant pathogens (Stakman and Harrar 1957). This supports the hypothesis that fungi capable of infecting living leaf could be the initiators of litter degradation. During the later stages of decomposition the initial colonisers gradually disappeared, and were replaced by new colonisers. 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 determining the succession of mycoflora in the litter. The initial colonisers are mainly utilisers of simple organic compounds whereas the later ones are capable of exploiting complex organic molecules such as cellulose and lignin (Garrett 1963). In our study, most of the fungal flora occurring in the later stages of decomposition were efficient degraders of cellulose and lignin. *Aspergillus* spp., *Chaetomium* sp. and *Trichoderma* spp. are capable of using cellulose as a sole source of carbon for their growth and multiplication. Similarly, *Scytalidium lignicola* can utilise ligno-cellulosics as an energy source (Kirk *et al.* 1980).

Fungal succession during foliage senescence and decomposition has been studied extensively by plant and soil mycologists, especially on pine litter in forest ecosystems (Ponge 1991). However, there are no such investigations in tropical grassland ecosystems (Coleman *et al.* 1980), which makes it impossible to compare the present results with other similar studies. Among the investigations on fungal successions in monocotyledons, Meredith (1962) reported on the fungal colonisation of collapsed and decaying leaves of banana (*Musa* sp.) in Jamaica. He showed that the primary colonisers in litter decomposition when inoculated on the healthy epidermal cells were able to penetrate host cells by means of appressoria and this ability was lacking for the late colonisers. These primary colonisers are prolific producers of conidia and thereby exhaust readily available nutrients from the substrate. Hudson (1968) studied the succession of microfungi on aging leaves of *Saccharum officinarum*. The primary colonisers of monocot leaves as reported by Hudson (1968) and Meredith (1962) were similar to those observed in this study. However, a number of different genera/spp. as well as the varying successional patterns observed, indicate that the colonisation

and succession vary with plant species plus climatic and edaphic factors.

The influence of different sampling months on mycofloral successions can be seen in Figure 1. Although no precise relationship was observed, it is clear that the variation in the percentage occurrence during different sampling months highlights the influence of climate. Our previous report also supported the contention that variation in the decomposition rates of litter is a function of climatic conditions (Senthilkumar *et al.* 1992). It should also be noted that, as decomposition progresses, even the true litter fungi (primary and secondary colonisers) are suppressed and the most active species belong to the fungi inhabiting soil. Thus, generally the succession will be of phylloplane mycoflora followed by litter mycoflora and then by soil mycoflora. However, this varies across ecosystems and depends on a variety of factors including the type of vegetation. An investigation of the mycoflora associated with soils at the study site revealed that the inoculum for litter-degrading fungi originated mainly from the soil (Senthilkumar *et al.* 1992). It should be mentioned that, besides the species isolated in this study, a number of others would be expected with different isolation techniques.

Our study indicates that the Hyphomycetes play a predominant role in grassland litter degradation in the tropics, and there is a successional pattern of fungal flora. Primary colonisers on grassland litter were mostly saprophytes, few being well known for their ability to cause disease on crop plants. The secondary colonisers were those fungi with the ability to utilise lignin and cellulose for growth. Notably, in *Cymbopogon caesius*-dominated grasslands, the role of *Rhizopus oryzae* and *Trichoderma viride* is considered to be indispensable in litter degradation due to their high level of occurrence and greater survivability during the final stages of decomposition. To our knowledge, this is one of the first reports dealing with the mycoflora of tropical grasslands. Further studies on the role of other factors such as nutrients, climate and vegetation, would elucidate their importance in litter decomposition in grasslands.

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(Received for publication January 6, 1993; accepted June 15, 1993)