Tropical Agricultural Research Vol. 13: 13-22 (2001)

Fungal Succession on *Michelia nilagirica* and *Semecarpus coriaceae* Leaf Litter

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ABSTRACT. *Michelia nilagirica and Semecarpus coriaceae are two common tree species, and are major litter contributors at the lower elevations of the Hakgala forest. Leaves of M. nilagirica decompose rapidly requiring 9-10 months to show an 88% decomposition. In contrast, S. coriaceae leaves require about two and half years for 38% decomposition. A comparative study of the fungal succession and its changes during the leaf litter decomposition of M. nilagirica and S. coriaceae was carried out as part of an attempt to determine the causes of the different rates of decomposition. Freshly fallen leaves of both species were collected into large nets. The litter bag technique was applied for Michelia leaves. The bags were sampled 3, 6 and 9 months after the placement on the forest floor. The analogous decomposition stages for Semecarpus leaf litter were selected from the forest floor and the dry weights were compared. Fungi were isolated from the leaf material of both species using the washing and plating method and identified using identification keys. Some fungal species were common to the analogous decomposition stages of both leaf species. Broomella acuta was the most common fungus isolated from each decomposition stage of both leaf species. Cladosporium cladosporioides, Trichoderma viride, Pseudobotrytis terrestris and Curvularia lunata were also isolated at high frequencies. However, the frequencies varied between decomposition stages and leaf species. Frequently isolated fungi from the four decomposition stages of both leaf species followed the general pattern of succession to some extent but some deviations observed is explained by studying the substrate utilizing capacities of the relevant fungal species.*

INTRODUCTION

In forest ecosystems the leaf litter input is the major source of nutrients through which, a major proportion of the net primary production is returned to the forest floor. The decomposition of these litter material is done mainly by soil inhabiting fungi (Kjøller and Struwe, 1992). It is well observed that no single fungal species is able to use all the components in leaf litter at once and therefore a succession of different groups of fungi will appear at different time intervals on different substrates (Kjøller and Struwe, 1980). Succession of both plants and fungi can be defined as a directional change in the composition, relative abundance and spatial pattern of species comprising communities (Frankland, 1998). The general scheme of fungal succession (Garrett, 1963) which Webster (1970) named the "nutritional hypothesis", discriminates between four stages beginning with the weak parasites invading the senescent tissue, secondly the primary sugar fungi utilizing the simple carbon compounds after the litter is deposited on the soil, thirdly the cellulose decomposers of the dead leaves and finally by the lignin decomposers. However,

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this pattern of fungal succession is not always observed. Reversed or deviated patterns of occurrence of individual fungal spp. have been indicated by a number of researchers (Frankland, 1966, 1969; Pugh *et al.*, 1972; Kjøller and Struwe, 1980, 1987,1989, 1992; Tillekeratna, 1989).

Fungal strains from soil and litter have been isolated and identified in large numbers and the purposes have varied from obtaining the basic knowledge on their ecological importance to industrial or other uses (Kjøller and Stuwe, 1992). These isolations have been carried out for temperate situations (Frankland, 1966, 1969; Visser and Parkinson, 1975; Flanagan, 1981; Gochenaur, 1984; Kjøller and Struwe, 1980, 1989, 1992), as well as from different tropical plant and tree litter (Aneja and Mehrotra, 1981; Bharat *et al.*, 1988). In Sri Lanka there are only a few recorded studies on isolation and identification of fungi from soil (Balasooriya and Deshappriya, 1996, 1997) and leaf litter (Kannangara *et al.*, 1997).

There are 97 tree species (over 15 cm GBH) of which 62 are endemic in the lower elevation of the Hakgala forest. Some of the common tree species found are *Syzygium revolutum* (Myrtaceae), *Psychotria bisulcata* (Rubiaceae), *Allophylus varians* (Sapindaceae), *Michelia nilagirica* (Magnoliaceae), *Memecylon parvifolium* (Melastomataceae), *Eugenia mabaeoids* (Myrtaceae), *Syzygium rotundifolium* (Myrtaceae), *Cinnamomum ovalifolium* (Lauraceae) and *Semecarpus coriaceae* (Anacardiaceae). Among these common tree species, the major leaf litter contributors are the two tree spp. *Michelia nilagirica* (*Wal sapu*) and *Semecarpus coriaceae* (*Kiri badulla*). *Michelia nilagirica* (Magnoliaceae) is very common in South India and in the montane forests above 1200-2350 m of Sri Lanka (Nooteboom, 1988). The tree has a large crown and the height is between 17-20 m. *Semecarpus coriaceae* (Anacardiaceae) is endemic to Sri Lanka and common in montane forests above 1200 m. The trees of this species are 16-20 m in height and have black drying juice in its inner bark which causes skin irritations (Meijer, 1983).

Leaves of *M. nilagiraca*, a fast decomposing species requires 9-10 months to show an 80% decomposition whereas leaves of *S. coriaceae*, a slow decomposing sp. requires about two and half years even for a 38% decomposition. Differences in the decomposition of different substrates are regulated by both abiotic (climate) and biotic (substrate quality, microbes and fauna) factors (Swift *et al.*, 1979). The present study was carried out to assess the fungal community structure and its changes (succession) during the leaf litter decomposition of *M. nilagirica* and *S. coriaceae* as a part of an extensive study to determine the causes of their different rates of decomposition.

MATERIALS AND METHODS

Location

The study site for the present investigation was located at the lower elevation (c. 1750 m) of the Hakgala strict natural reserve (SNR). The site slopes unevenly with slope angles varying from 5° to 35°. The climatic conditions of this forest during the period in which the present work was carried out were as follow:

The relative humidity during the day varied from 60.5-87.3% and in the night from 74.3-93%. The mean annual rain fall during the period of 1994-1999 was 1839.32 mm. The maximum average temperature in the forest site was the highest in March (22.7°C) and the lowest (18.2°C) was in July. The highest average minimum temperature (13.6°C) was in June and July and the lowest (10.5°C) was in December. The mean diurnal variation in temperature was 7.9°C.

Study plots

Five study plots (50×50 m) were located at the lower elevation of the Hakgala SNR. Four plots were set up in pairs with a gap of 100 m between the pairs. The fifth plot was set up 10 m below one of the pairs of plots. A random 1×1 m sub plot was marked on the forest floor in each of the five study plots for the placement of leaf litter bags.

Selection of decomposition stages

The following procedures were used to select each type of leaf litter of known ages following leaf fall. Freshly fallen leaves of both species were initially collected in large nets setup under the trees of each species at each of the five study sites.

M. nilagirica

The freshly fallen leaves were placed in nylon mesh bags (2 mm mesh size, 6 leaves per bag). The bags were then taken to the field and placed randomly in each of the five 1×1 m sub plots (20 bags in each sub plot). The samples were taken (2 bags from each sub plot) at 3, 6 and 9 months after placement in the field. At each sampling time, careful detailed visual observations were made of the decomposing leaves and the descriptions of colour, texture and condition were recorded. The four sampling times were; freshly fallen, leaves of 3 months (decomposition stage 1), 6 months (decomposition stage 2) and 9 months (decomposition stage 3) after leaf placement in the field. After completing all visual observations the leaf materials were used for the fungal isolation work.

S. coriaceae

Using the visual observations made for each decomposition stage of *Michelia* leaves, the analogous stages for *Semecarpus* leaf litter were selected from the forest floor of each of the five study plots for the subsequent isolation work. (The previously mentioned method cannot be used for *Semecarpus coriaceae* due to its very slow decomposition rate).

Isolation of fungi

Fungal isolations were carried out from the four decomposition stages (freshly fallen, decomposition stage 1, 2 and 3) of both leaf spp. using the following method. Samples were replicated five times.

Twelve leaf discs (5 mm diameter) were removed from each of the 5 replicate samples of leaves randomly using a sterilized cork borer. These discs were placed separately in 25 ml sterilized distilled water contained in 100 ml screw capped flasks and washed 20 times. This was the minimum number of washings required to eliminate the surface contaminants from leaf discs as estimated earlier (Kannangara *et al.*, 1996). Then 2 particles (1 mm) were removed aseptically from each of the 12 leaf discs. Thereafter a total of 120 litter particles (1 mm) from each of the four decomposition stages of both leaf types were placed on 2% malt extract agar supplemented with 0.01% streptomycin sulphate (one particle per petri dish). After 7-10 days of incubation, fungi that grew from each litter particle were separated into pure cultures. Subsequently slides containing fungal particles were prepared using the sticky tape method for identifications (Flegel, 1980) and were identified using keys (Barron, 1983; Domsch *et al.*, 1993). The percentage frequency of occurrence of fungi isolated in the leaf particles for each of the 5 replicate samples in each decomposition stage of both leaf spp. were calculated using the following equation:

$$\textit{Percentage frequency of occurrence} = \frac{\textit{Number of leaf pieces colonized by the fungus}}{\textit{Total no. of leaf particles plated}} \times 100$$

RESULTS AND DISCUSSION

The visual observations made for the four decomposition stages of both leaf species are as follows:

Appearance of decomposition stages of both leaf species

Decomposition stage	*Michelia nilagirica*	*Semecarpus coriaceae*
Freshly fallen	Extremely smooth texture; Orange in colour; Lower surface lighter in colour than the upper surface; Cuticle not damaged; Fungal spores or hyphae rarely visible	Similar to freshly fallen leaves of *Michelia*; Orangish brown in colour
Decomposition stage 1	Rough texture; Dark brown in colour; Colour of lower surface darker than the upper surface; Black fungal spores common in the lower surface; Cuticle ruptured in some places	Similar to the leaves of same decomposition stage of *Michelia*.
Decomposition stage 2	Dark brown in colour; Lower surface darker than the upper surface. Damaged cuticle; One fourth of the area of leaf contained only the vascular system; Fungal colonization present on both surfaces	Similar to the leaves of same decomposition stage of *Michelia*; Some leaves contained whitish patches on both surfaces
Decomposition stage 3	Rough texture; Dark brown in colour; Cuticle absent in many places; Half of the leaf area contained only the vascular system.	Similar to the leaves of same decomposition stage of *Michelia*; Some leaves whitish in colour

Acremonium strictum and *Trichothecium roceum* were isolated at higher frequencies (21.67%) from freshly fallen *Michelia* leaf litter whereas from the freshly fallen leaves of *Semecarpus*, *Penicillium* variabile and *Broomella acuta* were isolated at frequencies of 17.5% and 16.67% (Table 1) respectively. *Broomella acuta* and the dark

Table 1. Percentage frequency of occurrence of most dominant fungi (occurring at frequencies higher than 4%) isolated from the four decomposition stages of both leaf species.

Fungal spp.	Frequency of occurrence (%)							
	Michelia				*Semecarpus*			
	F	1st	2nd	3rd	F	1st	2nd	3rd
Acremonium strictum	21.67	0	0	0	0	0	0	0
Acremonium kilience	13.30	0	0	0	0	0	0	0
Alternaria tenuissima	0	0	0	0	11.67	0	0	0
Amorphotheca resinae	0	0	0	0	0	5.00	0	0
Aspergillus sp. 1	0	0	0	0	0	0	0	50.83
Aureobasidium sp. 1	0	6.67	0	0	0	0	0	0
Basidiomycete sp. 1	5	0	0	0	0	0	0	0
Basidiomycete sp. 2	0	0	11.67	0	0	0	0	0
Broomella acuta	14.17	36.67	17.50	86.66	16.67	44.17	57.80	35.83
Chlorodium clamydosporis	0	0	0	0	0	10.00	0	0
Cladosporium cladosporioides	8.33	0	25.83	87.49	0	10.83	24.99	46.67
Cladosporium sphaerospermum	0	18.33	0	0	0	0	0	0
Cladosporium sp. 1	0	0	0	27.5	0	0	0	0
Cladosporium sp.2	0	0	0	10.83	0	0	0	0
Coelomycete sp. 1	0	13.3	0	0	0	0	0	0
Curvularia lunata	0	0	33.33	37.49	0	0	0	35.83
Cylindrocarpon didymum	0	11.62	0	41.67	0	0	0	0
Cylindrocarpon magnusianum	0	0	0	0	12.50	0	0	0
Cylindrocarpon sp. 1	0	5.83	0	0	0	0	0	0
Cylindrocarpon sp. 2	0	0	6.67	0	0	0	0	0
Cylindrocarpon sp. 3	0	0	5.00	0	0	0	0	0
Cylindrocladium parvum	0	0	0	0	0	10.00	0	0
Dark sterile sp. 1	4.17	0	0	0	5.83	0	0	0
Dark sterile sp. 2	0	0	0	0	8.33	0	0	0
Dark sterile sp. 3	0	0	0	0	10	0	0	0
Dark sterile sp. 4	0	11.67	0	0	0	0	0	0
Dark sterile sp. 5	0	0	0	0	0	6.67	0	0
Dark sterile sp. 6	0	0	0	0	0	10.00	0	0
Dark sterile sp. 7	0	0	28.33	0	0	0	0	0
Dark sterile sp. 8	0	0	39.17	0	0	0	0	0

Continued.....

Table 1. Cont'd .....

Fungal spp.	Frequency of occurrence (%)							
	Michelia				*Semecarpus*			
	F	1st	2nd	3rd	F	1st	2nd	3rd
Dark sterile sp. 9	0	0	18.33	0	0	0	0	0
Dark sterile sp. 10	0	0	13.33	0	0	0	0	0
Dark sterile sp. 11	0	0	5.83	0	0	0	0	0
Dark sterile sp. 12	0	0	24.17	0	0	0	0	0
Dark sterile sp. 13	0	0	9.17	0	0	0	0	0
Dark sterile sp. 14	0	0	0	0	0	0	9.17	0
Dark sterile sp. 15	0	0	0	0	0	0	25.00	0
Dark sterile sp. 16	0	0	0	64.17	0	0	0	0
Dark sterile sp. 17	0	0	0	0	0	0	0	45.83
Dark sterile sp. 18	0	0	0	0	0	0	0	38.33
Fusarium sporotrichioides	0	0	0	0	0	5.00	0	0
Fusarium sp. 1	0	0	0	0	0	12.50	0	0
Fusarium sp. 2	0	0	0	16.67	0	0	0	0
Geotrichum candidum	0	0	0	0	0	5.83	6.67	0
Gliomastix sp. 1	0	0	0	0	0	0	9.17	0
Glomerella cingulata	0	11.67	0	0	8.33	0	0	0
Hyaline sterile sp. 1	0	0	0	0	7.50	0	0	0
Hyaline sterile sp. 2	0	0	0	0	5.00	0	0	0
Hyaline sterile sp. 3	0	16.66	0	0	0	0	0	0
Hyaline sterile sp. 4	0	0	0	0	0	15.00	0	0
Hyaline sterile sp. 5	0	0	0	14.18	0	0	0	0
Hyaline sterile sp. 6	0	0	0	0	0	0	0	60.83
Hyaline sterile sp. 7	0	0	0	0	0	0	0	24.09
Mortierella vinacea	0	0	0	0	0	16.67	25.00	0
Nectria coccinea	19.17	12.50	10.00	0	0	0	5.83	0
Nodulosporium sp.1	0	0	0	0	0	10.83	0	0
Penicillium variabile	0	0	0	0	17.50	10.00	0	0
Penicillium sp. 1	0	0	5.83	0	5.00	0	0	0
Penicillium sp. 2	0	0	0	0	5.80	0	0	0
Pithomyces sp. 1	0	0	0	14.17	0	0	0	0
Pseudobotrytis terrestris	0	0	0	0	0	0	21.66	42.49
Rhizopus sp. 1	0	0	0	0	0	0	0	57.50
Trichoderma hamatum	0	5.00	0	0	0	0	10.00	0
Trichoderma harzianum	0	0	27.50	0	0	0	0	0
Trichoderma piluliferum	0	0	6.67	87.50	0	0	19.17	13..33
Trichoderma pseudokoningii	0	17.50	5.83	0	0	0	0	0
Trichoderma viride	0	25.83	55.87	35.83	0	8.33	35.80	65.00
Trichoderma sp. 1	0	0	0	0	0	6.67	0	0
Trichoderma sp. 2	0	0	0	0	0	0	0	49.17
Trichothecium roseum	21.67	0	0	0	0	0	0	0

F - Freshly fallen leaves 1st - Decomposition stage 1 2nd - Decomposition stage 2;
0 - Not isolated 3rd - Decomposition stage 3

sterile sp. 1 were the only common fungi (with different frequencies of occurrence) the freshly fallen leaves of both species. The fungi isolated from the senescent leaves of both leaf spp. are considered to be weak parasites or saprophytic fungi that exist on sugars and simple carbon compounds present in the phylloplane. Fungal communities at this stage are different from those observed in aspen poplar leaves (Visser and Parkinson, 1975), red alder leaves (Kjoller and Struwe, 1980) and both senescent pine and spruce needles (Tillekeratna, 1989). However, most of the reported experiments have been recorded for temperate situations. The difference in the fungal community structure of the present study as compared to the above mentioned studies may be due to the different climatic factors (temperature, rain fall and relative humidity) as well as the difference in substrate quality of different types of leaf litter.

Broomella acuta (36.67%) and *Trichoderma viride* (25.83%) were isolated in higher frequencies from the 1st decomposition stage of *Michelia* leaf litter. *Broomella acuta* and *Mortierella vinaceae* were isolated at higher frequencies from the 1st decomposition stage of *Semecarpus* leaf litter with percentage frequencies of 44.17 and 16.67% respectively. *Broomella acuta* and *Trichoderma viride* were common to this decomposition stage of both leaf species, but in different frequencies. In the forest floor of the Hakgala natural forest, the leaf material belonging to the 1st decomposition stage of both leaf spp. were observed in the L layer of the forest floor material. The fungi (1st saprophytes) isolated from the 1st decomposition stage of both leaf litter are thought to exist on simple carbon compounds such as sugars, starch and amino acids.

Decomposition stages 2 and 3 of both leaf species were found in the F1 and F2 layers of the forest floor material respectively in the Hakgala natural forest. *Trichoderma viride* (55.87%) and the dark sterile sp. 8 (39.17%) were the fungi isolated in higher frequencies from the 2nd decomposition stage of *Michelia* litter meanwhile *Broomella acuta* (57.8%) and *Trichoderma viride* (35.8%) were isolated from the *Semecarpus* leaf litter at higher frequencies. *Trichoderma viride*, *Cladosporium cladosporioides*, *Broomella acuta* and *Nectria coccinea* were common to the leaf litter of both species at different frequencies of occurrence. The role of fungi in the 2nd stage is probably that of cellulose and lignin decomposition.

Cellulose and lignin decomposition with associated secondary saprophytic activities are probably the main functional features of the fungi isolated from the 3rd decomposition stage of both leaf species which are found in the F2 layer of the forest floor. *Trichoderma piluliferum* (87.5%), *Cladosporium cladosporioides* (87.49%) and *Broomella acuta* (86.66%) were isolated in higher frequencies from *Michelia* leaf litter. *Trichoderma viride*, hyaline sterile sp. 6 and the *Rhizopus* sp. were the most dominant fungi isolated with the frequencies of 65, 60.8 and 57.5% respectively from *Semecarpus* leaf litter of the 3rd decomposition stage. *Trichoderma piluliferum*, *Cladosporium cladosporioides*, *Broomella acuta*, *Curvularia lunata* and *Trichoderma viride* were commonly isolated with different frequencies of occurrence from both leaf species.

After the fall of leaves they are rapidly colonized by the litter mycoflora. At this time a considerable competitive interaction between the fungi inhabiting the leaves before leaf fall and the natural microflora and fauna of the forest floor could occur. Some of the phylloplane fungi inhabiting leaves before fall will persist after the leaves have reached the forest floor.

Fig. 1 and 2, separately show the pattern of the persistence of some dominant fungi isolated from various decomposition stages during the decomposition process. The frequency of occurrence of *Broomella acuta* in both leaf material increased from freshly fallen stage to the 1st decomposition stage. This may be due to the heavy sporulation and the greater competitive ability of *Broomella acuta* to compete with the other members of the litter mycoflora (Domsch *et al.*, 1993).

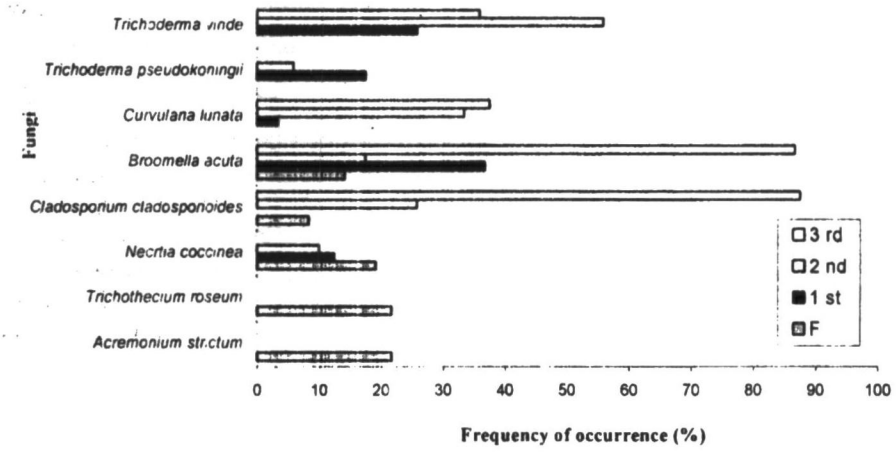

Fig. 1. Percentage frequency of occurrence of some dominant fungi isolated from the freshly fallen leaves to the decomposition stage 3 leaf litter of *Michelia*.

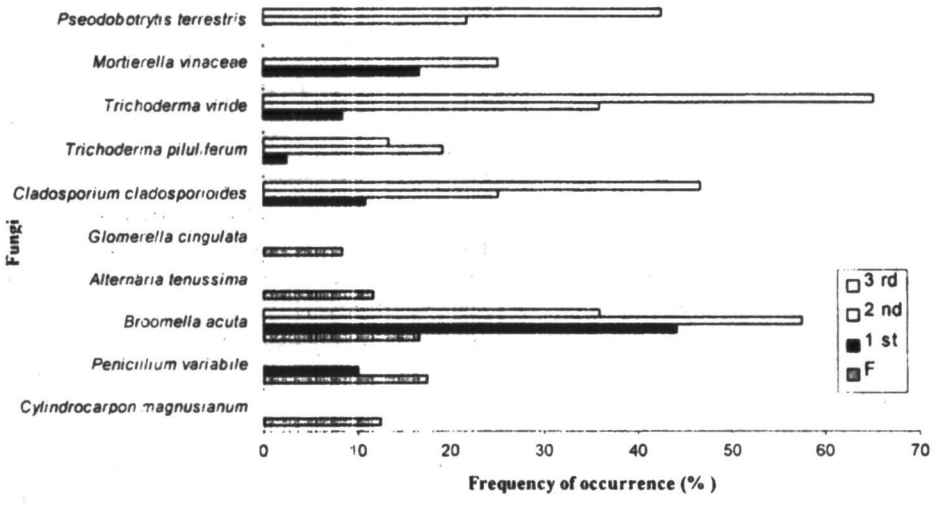

Fig. 2. Percentage frequency of occurrence of some dominant fungi isolated from the freshly fallen leaves to the decomposition stage 3 leaf litter of *Semecarpus*.

In *Michelia* leaf litter, the frequency of occurrence of *Broomella acuta* decreased by 19% from decomposition stage 1 to decomposition stage 2 and again increased by 69% in the decomposition stage 3. In *Semecarpus* leaf litter, frequency of the occurrence of the *Broomella acuta* increased by 14% again from the decomposition stage 1 to decomposition stage 2 from where it decreased by 22% to decomposition stage 3. These decreases in frequency of occurrence may be due to some environmental or biological factors such as interactions with the other organisms.

Some of the fungal spp. isolated from the freshly fallen leaves of both leaf types did not persist after some time. (*Acremonium strictum*, *Trichothecium roseum* in *Michelia* leaves and *Cyllindrocarpon magnusianum*, *Alternaria tennusima* and *Glomerella cingulata* in *Semecarpus* leaf litter.) This is mainly due to the competitive inhibition of their growth by the litter colonizing fungal spp. The invasion of the litter material at the 1st decomposition stage by *Trichoderma viride* and its persistence to the 3rd decomposition stage was observed in both leaf species.

CONCLUSIONS

The present study on the fungal succession on *Michelia* and *Semecarpus* leaf litter showed the presence of common as well as different fungal spp. in the analogous decomposition stages of both leaf species. Therefore, the difference of the decomposition rates between the two leaf types does not appear to be solely due to the occurrence of completely different fungal communities. However further observations on their physiological roles in utilizing the organic compounds (substrate utilization abilities) present in leaf litter indicated that stepwise fungal utilization of the storage and structural components of plants is not always found.

The fungal spp. isolated from the freshly fallen and decomposition stages 1, 2 and 3 of both leaf species appeared to represent the 4 categories of fungi (weak parasites, primary sugar fungi, cellulose decomposers and lignin decomposers).

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