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Succession and natural occurrence of saprobic fungi on leaves of *Berchemia floribunda* (climber) and their association with *Magnolia liliifera* (host)

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Abstract

Fungal succession on various plants from different regions of the world have been well-studied, however there has been no report comparing the fungi on leaves of a climber with those of the supportive plant. Fungi on leaves of *Berchemia floribunda*, a climber, were studied to fungal diversity and succession over a period of leaf decomposition. These fungi were compared with those on leaves of *Magnolia liliifera*, the supportive plant, using data from previous studies at the same site. Leaves of *B. floribunda* were placed with the upper or lower leaf surface adjacent to the forest floor, hung above the ground either under the host tree or other tree species, or placed on the forest floor under the host tree or under other trees to establish the effects of these treatments. These leaf bait trials did not affect the fungal diversity on the leaves. There was very little overlap between fungi on the climber leaves and those on the support tree. Only four saprobes from *B. floribunda* were also found on leaves of *M. liliifera*. We suspect that most of the fungi degrading leaves of *B. floribunda* were initially endophytes and became active saprobes once leaves started to decay.

Key words – Fungal diversity – Fungal ecology – Fungal succession – Leaf decomposition **–** Percentage occurrence

Introduction

Fungal succession has been observed in both terrestrial and aquatic ecosystems of tropical and temperate regions (Ho et al. 2002, Somrithipol et al. 2002, Suzuki et al. 2002, Yanna et al. 2002, Handa & Harada 2005, Tang et al. 2005, Paulus et al. 2006, Kodsueb et al. 2015, Matsuoka et al. 2018, Gao et al. 2019, Herzog et al. 2019). Most studies focused on the change in composition of fungal communities with changes of plant species, following different types of disturbances,

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such as animal, fire, land slide and deforestation (McMullan-Fisher et al. 2002, Suzuki et al. 2002, Zhang et al. 2017, Shi et al. 2019). There have been numerous studies of fungal succession on a variety of substrata, for instance cellulose film (Tribe 1957, 1961), other cellulosic substrates (Gorska 1982), plant litter (Promputtha et al. 2002, 2017, Tokumasu & Aoiki 2002, Yanna et al. 2002, Zhou & Hyde 2002, Handa & Harada 2005, Paulus et al. 2006, Voříšková & Baldrian 2013), and wool (Ghawana et al. 1997).

Previous studies have also investigated fungal succession on various plant species from different regions of the world (Frankland 1966, Richardson 2002, Sivichai et al. 2002, Somrithipol et al. 2002, Suzuki et al. 2002, Tokumasu & Aoiki 2002, Yanna et al. 2002, Zhou & Hyde 2002, Paulus et al. 2006, Voříšková & Baldrian 2013, Promputtha et al. 2017), but there has been no report comparing the fungi on leaves of a climber with those of the supportive plant. There have been few studies on fungal succession in Thailand, a tropical region (Kodsueb et al. 2015, Promputtha et al. 2017). Somrithipol et al. (2002) investigated succession of fungi on fruits and seeds of *Delonix regia* exposed on the forest floor. Sivichai et al. (2002) studied fungal colonization on two timbers, *Dipterocarpus alatus* and *Xylia dolabriformis*, exposed in a fresh water stream. Promputtha et al. (2002) investigated fungal succession on senescent leaves of *Manglietia garrettii* (later corrected to *Magnolia liliifera*) and found that the upper or lower leaf surface facing the forest floor had no effect on fungal communities developing on leaves.

In this study, leaves of *Berchemia floribunda* (a woody climber) in Doi Suthep-Pui National Park, Thailand were studied. The objectives were to evaluate fungal diversity, examine the succession of fungi over the period of leaf decomposition and determine the effect of various leaf placements on fungal diversity.

Materials & Methods

Two-hundred and fifty senescent leaves of *Berchemia floribunda* were collected from Doi Suthep-Pui National Park, Chiang Mai, Thailand in June (wet season). Selected senescent leaves were recently fallen and had yellow-green with fresh abscission scars. Ten of the senescent leaves were randomly selected to represent day 0. Bright-coloured wool was tied to the petioles of the other 240 senescent leaves to mark the samples. The leaves were randomly placed under *B. floribunda* and *M. liliifera* trees with one of the following treatments or control. In treatment 1, 50 leaves were hung under *B. floribunda*; in treatment 2, 50 leaves were placed on the forest floor under *B. floribunda*; in treatment 3, 50 leaves were hung under *M. liliifera* trees; and in treatment 4, 50 leaves were placed on the forest floor under *M. liliifera* trees. The control treatment comprised 40 senescent leaves tied with different coloured bright wool. These leaves were autoclaved twice at 121°C for 15 minutes and then returned to the forest. Ten of the sterilized leaves were hung under *B. floribunda*; 10 were placed on the forest floor under *B. floribunda*; 10 were hung under *M. liliifera* trees; and 10 were placed on the forest floor under *M. liliifera* trees (Fig. 1).

At each sampling time, five marked leaves from each treatment were collected. Marked leaves were collected at days 4, 8, 16, 24, 32, 40, 48, 56, 64 and 72. However at day 24, leaves were almost decayed and highly skeletonized comprising vascular tissue with attached remnants of inter-vein tissue. Sterilized leaves were collected at day 16, before complete decay (summarized experimental design of the trial is showed in Fig. 1). Samples were placed in separate plastic bags in the forest and taken back to the laboratory. Samples were incubated individually in plastic bags with the addition of sterile tissue paper moistened with sterile water. Samples collected at day 0 were examined for the presence of fungi on the day the experiment was set up. All other samples were examined using a microscope for the presence of fungi after one day of incubation. Sporulating fungi were mounted in water on glass slides and examined using differential interference contrast microscopy. Fungi were isolated by single spore isolation techniques (Chomnunti et al. 2014). Herbarium specimens of fungi were prepared and air-dried in an oven at 37°C for one week.

During the examination period, 50 naturally fallen decaying leaves of *B. floribunda* at various stages of decay were collected and examined immediately for comparison of fungal communities

with those from the succession study.

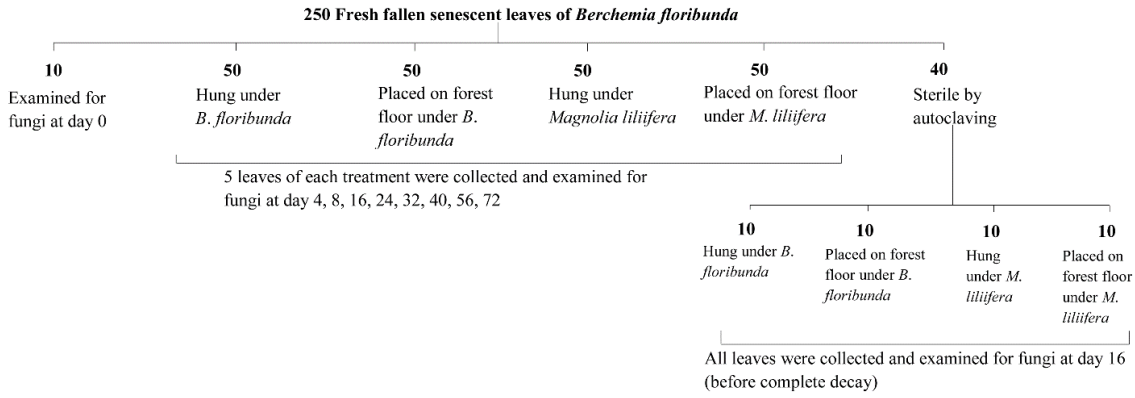

Figure 1 – Experimental design of leaf bait trials for fungal diversity investigation throughout decomposition period of *Berchemia floribunda* leaves. Statistical analyses and samples calculation are given in Promputtha et al. (2017).

Results

Fungi on naturally decaying leaves

Fungal taxa recorded on 50 naturally decaying leaves of *B. floribunda,* with their percentage occurrence, are listed in Table 1. Of the 40 fungal taxa identified, 32 species were found during the succession study and the eight different species were: *Berkleasmium phyllostachydis*, *Cladosporium oxysporum*, *Dictyosporium sacchari*, *Helicomyces* sp., *Lasiodiplodia theobromae*, *Nectria* sp. 2, *Periconia lateralis*, and *Verticillium* sp. 2. The dominant species were *Mycosphaerella* sp. 2 (100%), *Dictyochaeta tropicalis* (84%), *Beltrania rhombica* (74%), *Cylindrocladium floridanum* (64%), *Volutella* sp. 2 (64%), and an unidentified ascomycete 1 (58%).

Table 1 Percentage occurrence of fungi found on 50 naturally decaying leaves of *Berchemia floribunda*.

Fungus	Number of leaves	Percentage occurrence
Mycosphaerella sp. 2	50	100
Dictyochaeta tropicalis	42	84
Beltrania rhombica	37	74
Cylindrocladium floridanum	32	64
Volutella sp. 2	32	64
Unidentified ascomycete 1	29	58
Volutella sp. 3	26	52
Bacillispora sp.	24	48
Colletotrichum sp. 1	24	48
Dactylaria sp. 6	24	48
Ellisiopsis gallesiae	24	48
Colletotrichum sp. 2	23	46
Colletotrichum sp. 5	21	42
Beltraniopsis eusenbeckiae	20	40
Circinotrichum flexuosum	16	32
Colletotrichum sp. 3	15	30

Table 1 Continued.

Fungus	Number of leaves	Percentage occurrence
Periconia byssoides	15	30
Wiesneriomyces javanicus	14	28
Colletotrichum sp. 4	12	24
Cylindrocladium infestans	8	16
Dactylaria sp. 7	8	16
Idriella sp. 2	8	16
Dactylaria sp. 8	7	14
Acremonium sp.	6	12
Pithomyces chartarum	6	12
Cladosporium oxysporum	5	10
Verticillium sp. 1	5	10
Dictyochaeta cocophilum	4	8
Pestalotiopsis sp.	4	8
Diplodia theobromae	3	6
Stachybotrys sp.	3	6
Berkleasmium phyllostachydis	2	4
Cylindrocladium lucidum	2	4
Dictyosporium sacchari	2	4
Lasiodiplodia theobromae	2	4
Periconia lateralis	2	4
Helicomyces sp.	1	2
Memnoniella levispora	1	2
Nectria sp. 2	1	2
Verticillium sp. 2	1	2

Fungi on leaf bait treatments

Forty fungal taxa were identified on leaves of *Berchemia floribunda* throughout 24 days of the study (Table 2). These results are separated into those fungi on leaves either hung under *B. floribunda* plants or placed on the forest floor under *B. floribunda* plants, and those hung under *M. liliifera* trees or placed on the forest floor under *M. liliifera* trees. Thirty-two taxa were identified on leaves hung under *B. floribunda*, 33 on leaves placed on the forest floor under *B. floribunda*, 27 on leaves hung under *M. liliifera*, and 28 on leaves placed on the forest floor under *M. liliifera*. The overall dominant species were *Dictyochaeta tropicalis* (45%), *Mycosphaerella* sp. 2 (43%), *Colletotrichum* sp. 1 (34%), *Bacillispora* sp. (28%), and *Beltrania rhombica* (27%).

Effect of leaf bait treatments on fungal communities

Thirty-two taxa were found on leaves hung under *B. floribunda* plants. The dominant species were *Mycosphaerella* sp. 2 (64%), *Colletotrichum* sp. 1 (40%), *Dictyochaeta tropicalis* (32%), *Bacillispora* sp. (28%), and *Colletotrichum* sp. 5 (28%) (Fig. 3). Thirty-three fungal taxa were found on leaves placed on the forest floor under *B. floribunda* plants. The dominant species were *Dictyochaeta tropicalis* (60%), *Mycosphaerella* sp. 2 (48%), *Colletotrichum* sp. 1 (44%), *Colletotrichum* sp. 2 (32%), *Beltrania rhombica* (32%), and *Beltraniopsis esenbeckiae* (32%) (Fig. 4).

Twenty-seven fungal taxa were found on leaves hung under *M. liliifera* trees. The dominant species were *Dictyochaeta tropicalis* (32%), *Colletotrichum* sp. 2 (28%), *Bacillispora* sp. (28%), *Colletotrichum* sp. 1 (24%), *Mycosphaerella* sp. 2 (24%), and *Beltrania rhombica* (24%) (Fig. 5). Twenty-eight fungal taxa were found on leaves placed on the forest floor under *M. liliifera* trees.

The dominant species were *Dictyochaeta tropicalis* (56%), *Mycosphaerella* sp. 2 (36%), *Beltrania rhombica* (36%), *Bacillispora* sp. (32%), and *Ellisiopsis gallesiae* (32%) (Fig. 6).

Three-dimensional correspondence analysis of fungal communities on decaying leaves of *B. floribunda* showed that the bait treatment of leaves had no effect on colonization of saprobic fungi on *B. floribunda* (Fig. 2). The fungal communities on leaves hung or placed on the forest floor under *B. floribunda* were similar to those hung or placed on the forest floor under *M. liliifera* in both species diversity and occurrence.

Table 2 Number of leaves of *Berchemia floribunda* on which fungus occurred during the succession process.

Fungus	HB						FB						HM						FM						Overall %
	Day					%	Day					%	Day					%	Day					%	
	0	4	8	16	24		0	4	8	16	24		0	4	8	16	24		0	4	8	16	24		
Colletotrichum sp. 1	1	3	5	1		40	1	5	5			44	1	2	3			24	1	1	4	1		28	34
Colletotrichum sp. 2	1	1	2			16	1	3	4			32	2	1	3	1		28	1	1	3			16	23
Mycosphaerella sp. 2	1	4	5	5	1	64	1	3	5	1	2	48	2	1	0	1	2	24	1	3	1	2	2	36	43
Bacillispora sp.		1	0	5	1	28			2	2	2	24		1	1	3	2	28		2	2	3	1	32	28
Beltrania rhombica		1	1	1	1	16		1	3	1	3	32		1	3	2		24		1	2	2	4	36	27
Beltraniopsis esenbeckiae		1	1	2	1	20		1	3	1	3	32		1	1	2	1	20		1	2	2	2	28	25
Colletotrichum sp. 3		1	2			12		3	4			28		1	1			8		3	2	1		24	16
Colletotrichum sp. 4		1	1			8		1	2			12		1				4		1	2			12	9
Colletotrichum sp. 5		1	1	4	1	28		1	5			24			1	3	1	20		1	2	2	1	24	24
Cylindrocladium floridanum		1	3	1		20		2	1			12		2	2	1		20		1	2	1		16	17
Cylindrocladium infestans						0		1	1			8			1			4		1	1			8	5
Cylindrocladium lucidum						0		1				4						0						0	1
Dactylaria sp. 6		1	0	4		20		1	2	3		24		1	1			8		1	2	1		16	17
Dictyochaeta tropicalis		3	4	0	1	32		1	5	5	4	60		2	2	3	1	32		2	4	5	3	56	45
Ellisiopsis gallesiae		1	1	1		12		1	3	2		24		1	1	2		16		1	4	2	1	32	21
Idriella sp. 2		1	0	3	1	20			1			4			1			4						0	7
Memnoniella levispora		1				4						0						0						0	1
Periconia byssoides		1	0	4		20		1	3	1		20		1	2	1		16			1	1		8	16

Table 2 Continued.

Fungus	HB						FB						HM						FM						Overall %
	Day					%	Day					%	Day					%	Day					%	
	0	4	8	16	24		0	4	8	16	24		0	4	8	16	24		0	4	8	16	24		
Pithomyces chartarum		1	0	4		20						0			1			4						0	6
Stachybotrys sp.		1				4						0						0						0	1
Stachybotrys cf. *albipes*		1				4						0						0						0	1
Unidentified ascomycete 1		1	1	1		12		1	3	1	1	24		1	2	1	1	20		1	2	3	1	28	21
Unidentified hyphomycete 1		1	0	0	1	8						0						0						0	2
Verticillium sp. 1		1				4		1				4						0		1				4	3
Volutella sp. 2		1	1	1	1	16		2	0	1		12		1	1	1		12		1	2	1		16	14
Volutella sp. 3		1	1	1	1	16		2	1	2	2	28		1	1	0	1	12		1	2	1	1	20	19
Acremonium sp.			1	1		8			1	0	1	8			1			4			1	1		8	7
Circinotrichum flexuosum			1	2	1	16			1			4			1	1		8			1	1		8	9
Cladosporium sp. 1						0			1			4						0						0	1
Dactylaria sp. 7			1	2		12			1	1		8				1		4				1		4	7
Periconia cookei						0			1			4						0						0	1
Unidentified hyphomycete 2						0			1			4						0						0	1
Cladosporium oxysporum				1		4				1	1	8						0				1		4	4
Dactylaria sp. 8				4		16				2		8						0				2		8	8
Spiropsis sp.				1		4						0						0						0	1
Wiesneriomyces javanicus				2		8				1	1	8				1	1	8				1	1	8	8
Dactylaria sp. 9					1	4						0						0						0	1
Dictyochaeta cocophilum						0					1	4					1	4					1	4	3
Diplodia theobromae						0					2	8					1	4					1	4	4
Pestalotiopsis sp.						0					2	8					1	4					2	8	5

HB: Leaves hung under *B. floribunda* tree

FB: Leaves placed on forest floor under *B. floribunda*
HM: Leaves hung under *M. liliifera*
FM: Leaves placed on forest floor under *M. liliifera*

Fungal colonization on sterilized leaves

Fourteen fungal taxa were found on 40 sterilized leaves of *B. floribunda* (control group), collected at day 16 of decomposition (Table 3). All of these fungi were the same species as those found on non-sterilized leaves and colonized as dominant species. Dominant species on non-sterilized leaves, which were absent from sterilized leaves were *Colletotrichum* spp. 1, 2, 3, 5, *Bacillispora* sp., *Dactylaria* sp. 6, and unidentified ascomycete 1.

Table 3 Percentage occurrence of fungi found on sterilized leaves (control group) of *Berchemia floribunda*, ten leaves in each group.

Fungal taxa	Percentage occurrence				Overall percentage occurrence
	HB	FB	HM	FM	
Beltrania rhombica	100	100	80	100	95
Beltraniopsis esenbeckiae	60	50	70	70	62.5
Cylindrocladium floridanum	90	100	70	100	90
Cylindrocladium infestans	40	30	50	40	40
Dactylaria sp. 7	10				2.5
Dictyochaeta tropicalis	100	100	100	100	100
Diplodia theobromae	20	10		10	10
Ellisiopsis gallesiae	100	100	100	100	100
Mycosphaerella sp. 2	10				2.5
Periconia byssoides			10		2.5
Pithomyces chartarum	20	10			7.5
Pestalotiopsis sp.	40	20	30	40	32.5
Volutella sp. 3			10		2.5
Volutella sp. 2		10			2.5

HB: Leaves hung under *B. floribunda*
FB: Leaves placed on forest floor under *B. floribunda*
HM: Leaves hung under *Magnolia liliifera*
FM: Leaves placed on forest floor under *M. liliifera*

Succession patterns of fungi on senescence leaves of *Berchemia floribunda*

There were three distinct succession communities on decaying leaves of *Berchemia floribunda* over 24 days of decomposition; the pioneer community (day 0–4), the mature community (day 8–16), and the impoverished community (day 24 onwards) (Fig. 3). The fungal community composition was distinct at each stage of succession. During the pioneer community stage, fungal communities were low in number of species and the species also had a low percentage occurrence. The dominant species at this stage were *Colletotrichum* sp. 1, *Colletotrichum* sp. 2, and *Mycosphaerella* sp. 2. The highest species diversity was present during the mature community stage and they also had high percentage occurrence. The dominant species were *Bacillispora* sp., *Beltrania rhombica*, *Colletotrichum* sp. 3, *Colletotrichum* sp. 5, *Cylindrocladium floridanum*, *Dactylaria* sp. 6, *Dictyochaeta tropicalis*, *Ellisiopsis gallesiae*, *Periconia byssoides*, unidentified ascomycete 1, *Volutella* sp. 2, and *Volutella* sp. 3. In the impoverished community stage, the species diversity and number of species decreased. Species from earlier stages of decomposition persisted through this stage, but at low frequency.

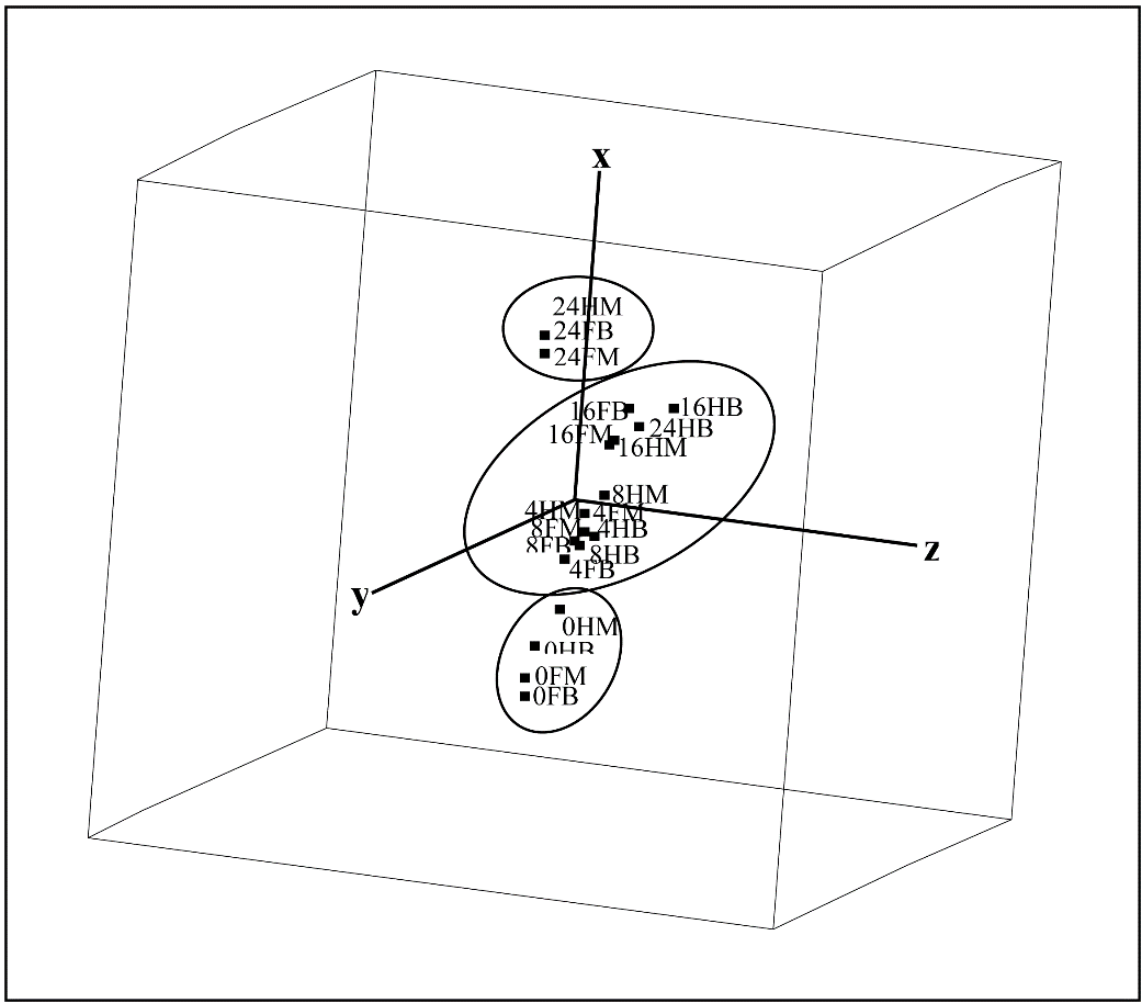

Figure 2 – Three-dimensional correspondence analysis of fungal communities on leaves of *B. floribunda* either hung under *B. floribunda* trees (HB) or placed on forest floor under *B. floribunda* trees (FB), and those hung under *M. liliifera* trees (HM) or placed on forest floor under *M. liliifera* trees (FM) over a 24 day period. 0, 4, 8, 16, 24 sampling times (days).

Discussion

Fungal succession

There have been several studies of the fungal succession on various materials, including submerged wood in fresh water (Ho et al. 2002, Kane et al. 2002, Sivichai et al. 2002, Newman et al. 2015, Matsuoka et al. 2018), leaf litter (Osono 2002, Promputtha et al. 2002, 2017, Yanna et al. 2002), pods of *Dolenix regia* (Somrithipol et al. 2002) and dung (Richardson 2001, 2002). Numerous studies have established similar patterns in fungal occurrence with time during fungal succession, there being early, intermediate and late colonizers (Jones & Hyde 2002). Changes of species composition throughout the decay process have been observed and classified by several authors (Dix & Webster 1985, Promputtha et al. 2002, 2017, Yanna et al. 2002, Zhou & Hyde 2002). In the present study, the fungal communities grouped into three succession stages, the pioneer stage, mature stage and impoverished stage (Dix & Webster 1985, Promputtha et al. 2002, 2017, Yanna et al. 2002, Voříšková & Baldrian 2013). Leung (1998) divided fungi from bamboo into two groups, early colonizers and regular inhabitants, while Zhou & Hyde (2002) divided fungi identified on bamboo into five groups, early colonizers, middle stage colonizers, later colonizers, regular inhabitants and sporadic inhabitants.

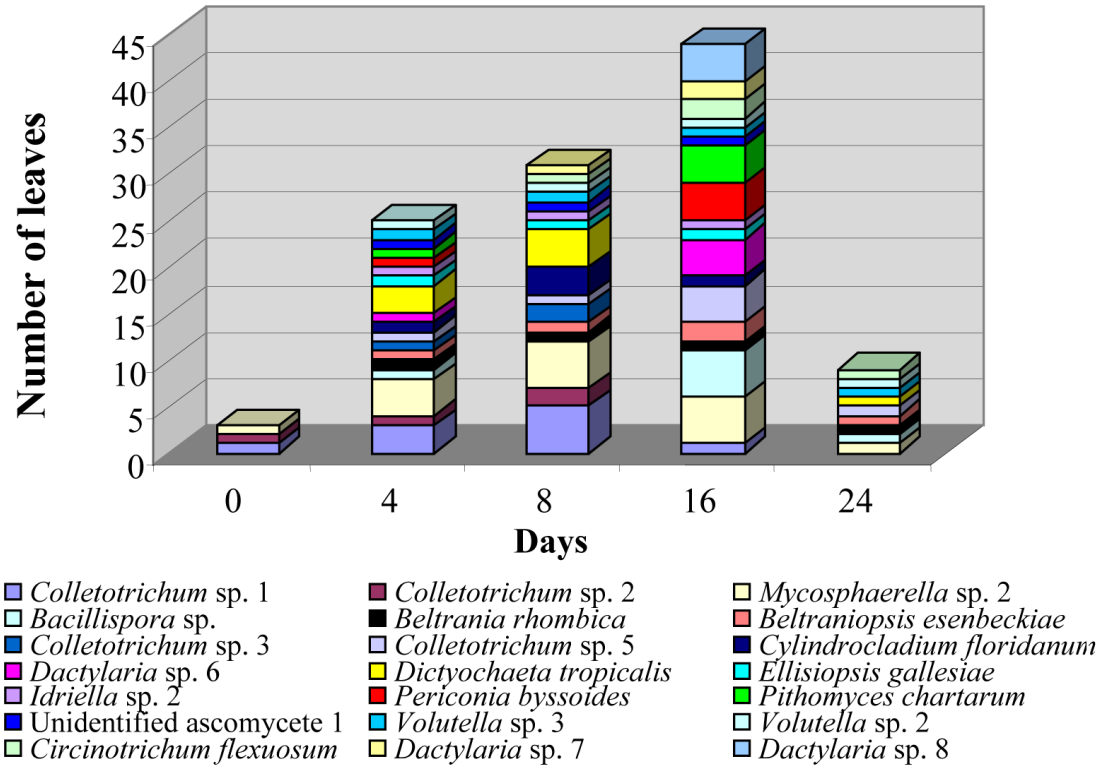

Figure 3 – Succession pattern of dominant fungi on leaves of *Berchemia floribunda* hung under *B. floribunda* plants during a 24 day period.

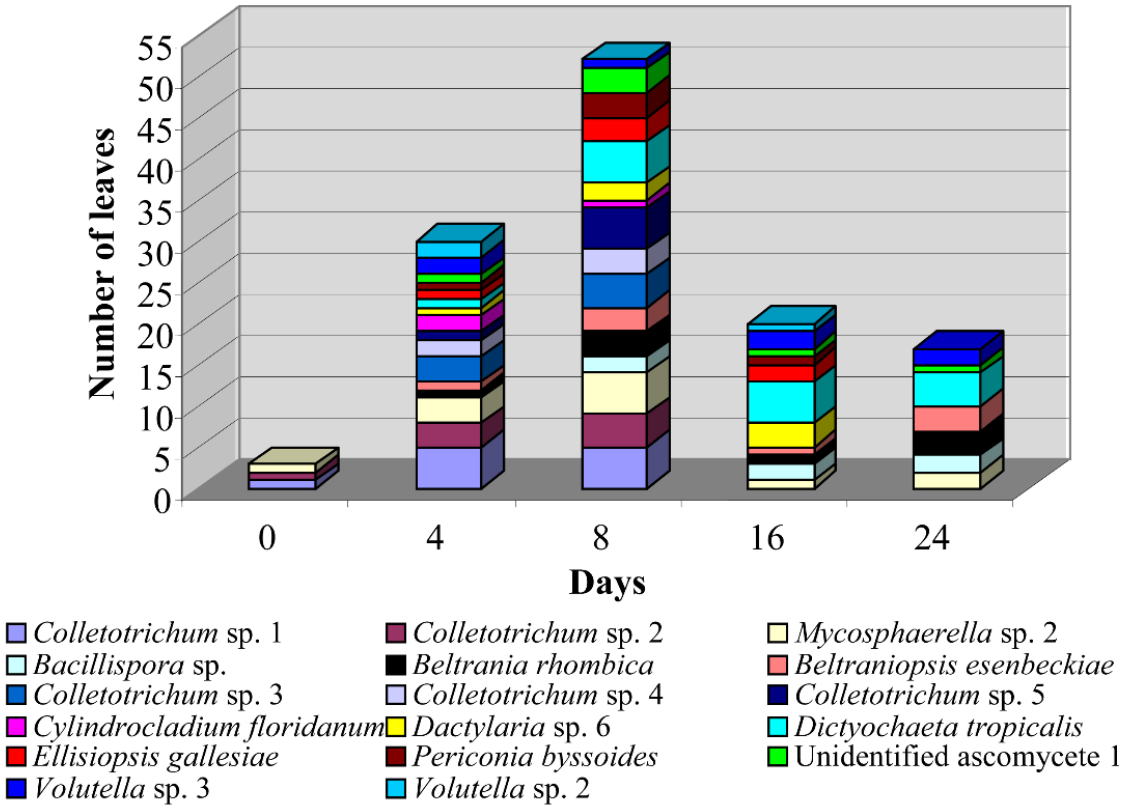

Figure 4 – Succession pattern of dominant fungi on leaves of *Berchemia floribunda* placed on forest floor under *B. floribunda* plants during a 24 day period.

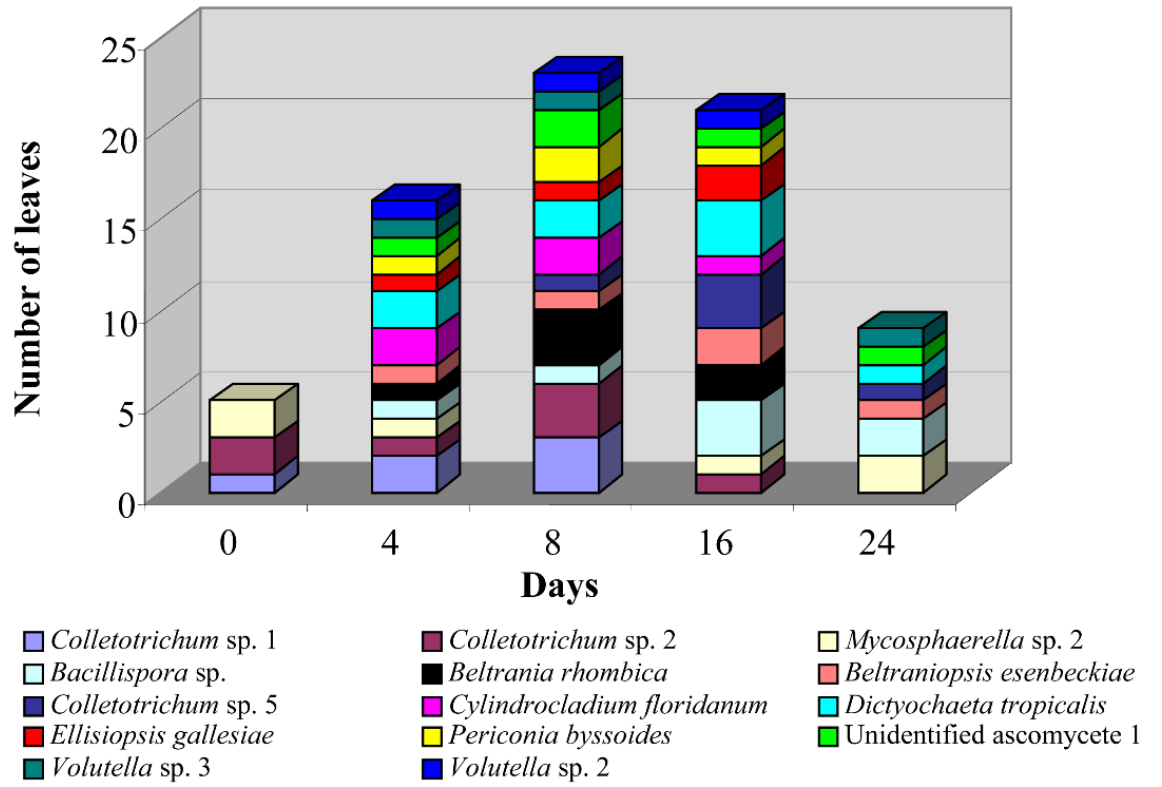

Figure 5 – Succession pattern of dominant fungi on leaves of *Berchemia floribunda* hung under *Magnolia liliifera* tree during a 24 day period.

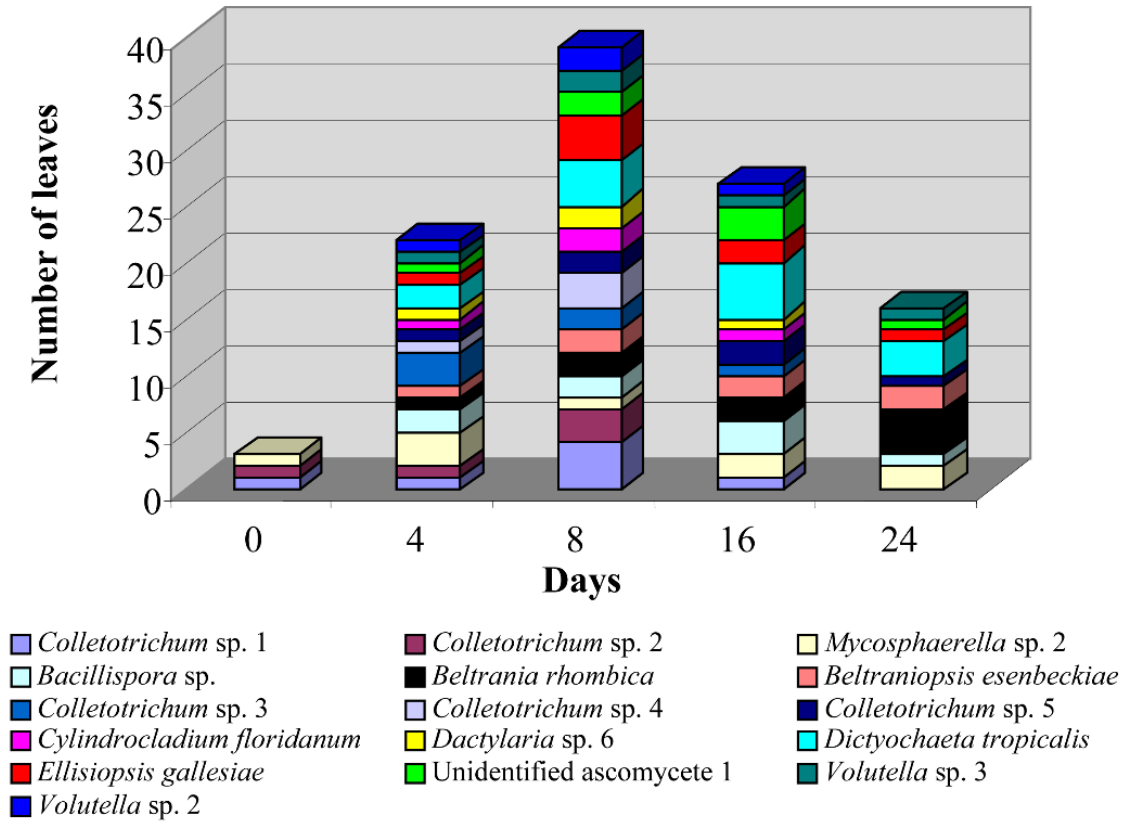

Figure 6 – Succession pattern of dominant fungi on leaves of *Berchemia floribunda* placed on forest floor under *Magnolia liliifera* tree during a 24 day period.

Fungal diversity

The fungal communities on leaves of *Berchemia floribunda* during the succession period grouped into three stages, (1) pioneer communities, those fungi occurring during day 0–4 and disappearing thereafter, (2) mature communities, those fungi occurring during an intermediate period of decay and disappearing thereafter, and (3) impoverished communities, those fungi occurring in the latter stages of decay and present until the end of the study. We identified 40 taxa from leaves of *Berchemia*, a climber growing on *Magnolia liliifera* trees. In a similar study at the same site, Promputtha et al. (2017) identified 23 taxa from *Magnolia liliifera*. Only four species (10%), *Beltrania rhombica*, *Colletotrichum gloeosporioides*, *Cylindrocladium floridanum*, and *Phyllosticta capitaliensis*, colonizing leaves of *Berchemia floribunda*, overlapped with the fungi on leaves of *Magnolia liliifera*.

In previous leaf litter fungal succession studies, Parungao et al. (2002) examined ten leaves from different tree species in rainforests in northern Queensland, Australia and found between 0–14 taxa on each leaf species. Photita et al. (2001) examined the large leaves of *Musa acuminata* in Hong Kong and found 20 taxa on the leaves at Nim Shue Wan and 18 taxa on the leaves in Fung Yuen. In Thailand, Photita et al. (2003) recorded between 17 and 27 taxa on *Musa acuminata* leaves at various sites. Polishook et al. (1996) identified between 8 and 15 species from leaves of *Manilkara bidentata* and 9 and 11 species from leaves of *Guarea guidonia* in Puerto Rico. Nine taxa occurred on decomposing leaves, two species on freshly fallen leaves and four species on senescent leaves of *Fagus crenata* (Osono 2002). Promputtha et al. (2002, 2017) recorded 22 taxa on *Manglietia garrettii* and 23 taxa on *Magnolia liliifera* leaves. Forty-one taxa were found on leaves of the palm *Nypa fruticans* (Hyde & Alias 2000). Appreciably greater numbers of fungi have been identified using indirect methods (pulverization-washing-plating of particle suspensions) to detect fungal diversity in litter (e.g. Polishook et al. 1996, Paulus et al. 2003).

Time of substrate decomposition on high diversity

Fungal diversity was high on leaves of *Berchemia floribunda* between day 4–16, with most species present on day 4 (Table 2). The time for fungal communities to reach a peak of species diversity, or fungal activity therefore varied between different studies. For example, complete decomposition of sugarcane bagasse needed 20 weeks and maximum colony counts were recorded during weeks 6–13 (Sandhu & Sidhu 1980). In a 2-year study of the decomposition of leaf and root litter of pineapple, the number of species, the number of viable propagules associated with the litter and the rate of weight loss were maximum during the middle phase of the litter decomposition (Tiwari et al. 1994). During one year of complete decomposition process of fronds of *Phoenix hanceana* (120 days for leaves, 150 days for rachis tips and 200 days for mid-rachides and rachis-bases), species diversity also peaked at the middle stage of decomposition (Yanna et al. 2002). The time taken for decomposition of leaf litter varies enormously, for example, 10 years to full decomposition of pine needles and one year for leaves in ash and sycamore woods (Tokumasu & Aoiki 2002); a few weeks for tropical forest leaves (Hudson 1980); 14 months for leaves of sugarcane (Hudson 1962); 19 months for stems of couch grass (Hudson & Webster 1958); two years for litter of pineapple (Tiwari et al. 1994); one year for leaves and rachis-tips of *Phoenix hanceana* and 18 months for mid-rachides and rachis-bases of *Phoenix hanceana* (Yanna et al. 2001).

Fungal diversity on sterile leaves

The saprobic fungi detected on sterilized leaves of *B. floribunda* were similar to those fungi found on non-sterilized leaves, although the numbers of fungal species on sterilized leaves was lower. Fryar et al. (2001) investigated the effect of autoclaving on the colonization pattern of fungi and found that the number of fungal species colonizing non-autoclaved wood was higher than on autoclaved wood. The overlap of fungi occurring on non-sterilized and sterilized leaves may result from the fungi being host-specific (Zhou & Hyde 2001). The chemical content of leaves may effect germination or growth of specific fungal species resulting on re-occurrence of specific fungal taxa

on the sterilized leaves. The reasons as to why saprobic fungi may occur recurrently on certain hosts is unclear, but may be related to the presence of these fungi as endophytes (Photita 2003, Promputtha et al. 2007, 2010, Jeewon et al. 2013, 2017, 2018), although the fact that similar fungi occur on the sterilized leaves places doubt on this.

Effect of leaf bait condition on the diversity of fungi

We also wanted to verify whether a leaf suspended above the ground had a different fungal community developing on it as compared to a leaf placed on the ground, either under the host tree or under another tree species in close proximity. This would possibly indicate whether the leaf litter fungi originate from the soil, colonize the leaves through aerial dispersal or are present in the leaves at senescence. In the latter case, they most likely are endophytes that become saprobes. Fungal communities on all baited groups were similar; the dominant species and the times of their occurrence were also similar. The results indicate that baiting leaves in different conditions had no effect on the diversity of fungi. The fact that leaves placed on the forest floor are similar to those hung in the tree canopy would suggest that the saprobes do not colonize through soil contact. The fact the leaves hung under different trees are similar also suggest that saprobes are not aerially dispersed, unless chemical components within the leaf control which saprobes can grow on an individual leaf. The results therefore indicate that saprobes may have been derived from endophytes within the leaves as suggested by Wong & Hyde (2001), Ghimire & Hyde (2004), Hyde et al. (2007) and Promputtha et al. (2007, 2010).

Fungal communities on baits versus those on naturally decaying samples

Examination of fungi on naturally occurring samples is likely to result in finding fungi that only occur during certain stages of decay. For instance, in this study fungal communities on naturally occurring decaying leaves were similar to those found on mature community stage of leaf baits (Tables 1, 2). Fungal communities which change rapidly, or taxa that are present for a relatively short time, may easily be missed on collections of naturally occurring samples, especially those that appear only during the pioneer or impoverished stages. For instance, *Gliocladium* sp. 2 on leaves of *Magnolia liliifera* was dominant (occurrence up to 60%) for a short period only during the early succession study. It was not dominant on the naturally occurring sample. In addition, *Dokmaia monthadangii* on *Magnolia liliifera* was dominant (occurrence up to 11%) on naturally occurring leaves, but was not found on the leaf baits (Table 2). The percentage similarities between fungi on naturally occurring samples and on the succession baits of *Magnolia liliifera, Meliosma simplicifolia* and *Berchemia floribunda* were high at 77%, 88.9% and 66.7%, respectively. This study has demonstrated that examination of naturally occurring samples and leaf baits from initial senescence to complete decomposition are essential to obtain a full understanding of the fungal diversity on leaves of *Magnolia liliifera, Meliosma simplicifolia* and *Berchemia floribunda.*

Factors supporting higher diversity

The reasons for higher diversity of fungi in this study compared to some previous studies are unclear. Large leaves, with a greater surface area may provide more substrata for fungal growth and thus support a larger number of species (Promputtha et al. 2002). Photita et al. (2001) reported that the large leaves of *Musa acuminata* supported a higher diversity than the smaller leaves of other tree species. The three-tree species studied had different sized leaves. In the case of *Berchemia floribunda*, the decaying period is only 24 days, but still more species were obtained than from some longer decaying periods (Polishook et al. 1996, Osono 2002).

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