Fungal succession on senescent leaves of *Manglietia garrettii* in Doi Suthep-Pui National Park, northern Thailand

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Twenty-two fungal taxa were identified on decaying senescent leaf baits of *Manglietia garrettii* during a 56 day study. Most of the taxa were the same as those occurring on naturally decaying leaves in the same forest area collected at the same time. Distinct fungal communities were observed to occur in sequence on the leaves, with the dominant species on the leaves being different at each succession stage. There was no noticeable effect on fungal communities whether the upper or lower leaf surface was in contact with the forest floor. The greatest fungal diversity occurred between day 4 and 40 (mature community stage), with most species being present on day 40. On day 56, leaves were found to be skeletonised, so the fungal communities had decreased in number.

Key words: biodiversity estimates, fungal distribution, fungal ecology, saprobic fungi.

Introduction

Succession has been defined as "the sequential occupation of the same site by thalli either of different fungi or different associations of fungi" (Rayner and Todd, 1979). Saprobic fungal succession can be classified as seral succession or substratum succession (Cooke, 1979; Frankland, 1992). Substratum succession refers to the succession of species that occur on any colonisable plant, animal or man-made material (Cooke, 1979; Frankland 1992), for example, the occurrence of different fungal taxa at different stages of decomposition on petioles of *Pteridium aquilinum* (Frankland, 1969, 1976). In this study, the sequential occurrence of fungi on senescent leaves of *Manglietia garrettii* in Thailand was studied.

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In succession, biotic communities change over time and a complete sequence of change can be observed (Yanna *et al.*, 2002). In general, fungal communities go through the following three succession stages (Dix and Webster, 1985).

Pioneer community

This community consists of pioneer species that have a low percentage occurrence (Dix and Webster, 1985). Pioneer species tend to be fast growing, short lived, and capable of rapid and wide dispersal (Luczkovich and Knowles, 2000). This community type has low species diversity and few species have high percentage occurrence (Dix and Webster, 1985).

Mature community

The species diversity in mature communities is high and has peaked. There is a number of species with low percentage occurrence. However, several species have a high level of occurrence. The dominant species have extremely high levels of occurrence. During the later stages of the mature community, the number of dominant species declines, but species diversity is still high (Dix and Webster, 1985).

Impoverished community

The species diversity and the number of species in an impoverished community, decline. The community is dominated by a few species with high levels of occurrence (Dix and Webster, 1985). These dominant species tend to be persistent and longer-lived species (Luczkovich and Knowles, 2000). However, there are still some species with low levels of occurrence (Dix and Webster, 1985).

In this study we examined the fungal succession on fallen leaves of *Manglietia garrettii*, a partly deciduous tree up to 25 m tall, which is locally common in Doi Suthep-Pui National Park. The leaves are relatively large (18-30 × 8-12 cm) and thick and were yellow-green at senescence.

The objectives of this study were to investigate the fungal communities occurring on senescent leaves of *Manglietia garrettii* during different stages of decay, over a 2 month period until complete decomposition, and to compare fungal communities from this succession study with those from naturally occurring samples (Promputtha *et al.*, in prep.).

Materials and methods

One-hundred and ten senescent leaves of *Manglietia garretii* were collected from Doi Suthep-Pui National Park, Chiang Mai, Thailand on 31 July 2001. Selected senescent leaves were fallen and yellow-green. Ten of the

senescent leaves were randomly selected to represent day zero. Bright-coloured wool was tied to the petiole of the other 100 senescent leaves to mark the samples. The leaves were randomly placed under *Manglietia garrettii* trees over an area of about 1 ha. Fifty leaves were arbitrarily placed under the trees with their lower surface touching the forest floor and the other 50 were placed with the upper surface touching the forest floor. At each sampling time, five marked senescent leaves with their lower surface touching the forest floor and five marked senescent leaves with their upper surface touching the forest floor were randomly collected. It was planned to collect marked senescent leaves at day 4, 8, 16, 24, 40, 56, 72 and 88. However at day 56, leaves were highly skeletonised comprising vascular tissue with attached remnants of non vascular tissue. Samples were placed in separate plastic bags in the forest and brought back to the laboratory. Samples were incubated individually in plastic bags with an addition of tissue paper moistened with sterilized water. Samples collected at day O were examined for the presence of microfungi on the same day of collection. All other samples were examined under a microscope for the presence of microfungi after one day of incubation and then periodically for up to two weeks. Squash mounts of sporulating fungi were made in water for examination with differential interference contrast microscopy. Fungi were isolated by single spore isolation (Choi *et al.*, 1999). Herbarium specimens of fungi were prepared and air-dried in an oven at 37 C, for one week.

During the experimental period, 90 naturally fallen decaying leaves of *Manglietia garrettii* at various stages of decay were also collected for comparison of fungal communities with those from the succession study. These results are to be published by Promputtha *et al.* (in prep.) and are used for comparison here.

Statistical analysis and sample calculation

The fungi found in this study are presented in terms of percentage occurrence. Fungal taxa with an overall percentage occurrence equal to or higher than ten are regarded as dominant species. These fungal taxa are plotted to illustrate changes in the dominant species throughout the experimental period.

$$\text{Percentage occurrence} = \frac{\text{Number of leaves on which fungus was detected}}{\text{Total number of leaf samples examined}} \times 100$$

Similarity index = 2c / a+b
a: the number of species in habitat A
b: the number of species in habitat B
c: the number of species in common in habitats A and B

A 3-dimensional correspondence analysis was performed to examine the differences in fungal communities at different times of decay, and the effect of which surface of the leaf touched the forest floor. Several new species names are introduced in this paper followed by the ending sp. nov. These are not yet formally described taxa, but the publications describing them are *in prep.*

Results

Percentage occurrence

Twenty-two taxa were identified on senescent leaves of *Manglietia garrettii* during the succession process and their percentage occurrences are listed in Table 1. These results are separated into those fungi on leaves with their upper surface touching the forest floor and those fungi on leaves with their lower surface touching the forest floor. The overall most common species were *Hyponectria manglietiae* sp. nov. (62.9%), *Volutella* sp. (55.7%), *Gliocladium* sp. 3 (38.6%), *Sporidesmium crassisporum* (34.3%) and *Cylindrocladium floridanum* (32.9%) (Table 1).

Effect of upper or lower leaf surface touching the forest floor on fungal communities

Twenty taxa were identified on leaves with their upper surface touching the forest floor during the succession process. This comprised 10 ascomycetes and 10 anamorphic taxa (9 hyphomycetes and 1 coelomycete). The dominant species were *Hyponectria manglietiae* sp. nov. (60%), *Volutella* sp. (60%), *Gliocladium* sp. 3 (37%), *Sporidesmium crassisporum* (34.3%), *Bionectria ochroleuca* (25.7%), *Cylindrocladium floridanum* (22.8%), *Phaeosphaeria* sp. (17%), *Dactylaria longidentata* (11.4%) and *Lasiosphaeria* sp. (11.4%) (Table 1).

Seventeen fungi were found on leaves with their lower surface touching the forest floor throughout the decay process. This comprised 7 ascomycetes and 10 anamorphic taxa (9 hyphomycetes and 1 coelomycete). The dominant species were *Hyponectria manglietiae* (65.7%), *Volutella* sp. (51.4%), *Cylindrocladium floridanum* (42.8%), *Gliocladium* sp. 3 (40%), *Sporidesmium crassisporum* (34.3%), *Bionectria ochroleuca* (28.5%), *Lasiosphaeria* sp. (17%) and *Dactylaria longidentata* (14.3%) (Table 1).

Three-dimensional correspondence analysis of fungal communities on senescent leaves of *Manglietia garrettii* showed that the surface of leaves touching the forest floor had no effect on the fungal communities (Fig. 1).

Table 1. Number of senescent leaves of *Manglietia garrettii* on which fungi occurred during the succession process.

Code	Taxa	Upper surface touching the forest floor									Lower surface touching the forest floor									Overall percentage occurrence
		Day							No.	Percentage occurrence	Day							No.	Percentage occurrence	
		0	4	8	16	24	40	56			0	4	8	16	24	40	56			
MS067	*Anthostomella tenacis*						2		2	5.7										2.9
MS065	*Albonectria rigidiuscula*				1				1	2.9										1.4
MS072	*Bionectria ochroleuca*			2	2	2	3		9	25.7				2	2	5	1	10	28.6	27.1
MS026	*Colletotrichum gloeosporioides*						1	1	2	5.7						1	2	3	8.6	7.1
MS029	*Cylindrocladium floridanum*		3	1	1	2	1		8	22.9		3	4	4	2	2		15	42.9	32.9
MS051	*Dactylaria longidentata*				1	3			4	11.4				2	3			5	14.3	12.9
MS071	*Fusarium* sp. 1												1					1	2.9	1.4
MS063	*Fusarium* sp. 2		1						1	2.9										1.4
MS068	*Gliocladium* sp. 1		1						1	2.9		2						2	5.7	4.3
MS042	*Gliocladium* sp. 2		2						2	5.7		3						3	8.6	7.1
MS046	*Gliocladium* sp. 3			3	4	5	1		13	37.1			5	4	5			14	40	38.6
MS054	*Hyponectria manglietiae* sp. nov.		2	2	3	4	5	5	21	60		1	4	4	4	5	5	23	65.7	62.9
MS024	*Hyponectria manglietiagarrettii* sp. nov.					1			1	2.9										1.4
MS028	*Hyponectria suthepensis* sp. nov.		1						1	2.9		1						1	2.9	2.9
MS050	*Hypoxylon* sp.						2	1	3	8.5										4.3
MS019	*Ijuhya parilis*			1					1	2.9										1.4
MS056	*Lasiosphaeria* sp.	1	1	2					4	11.4	1	4	1					6	17.1	14.3
MS036	*Phaeosphaeria* sp.			1	1	1	3		6	17.1			2					2	5.7	11.4
MS061	*Phomopsis* sp.	1	1						2	5.7	1	1						2	5.7	5.7
MS075	*Pseudohalonectria suthepensis* sp. nov.	1							1	2.9						1	1	2	5.7	4.3
MS002	*Sporidesmium crassisporum*			1	2	2	2	5	12	34.3			1	2	2	2	5	12	34.3	34.3
MS060	*Volutella* sp.	2	2	5	5	5	2		21	60	1	3	3	4	5	2		18	51.4	55.7

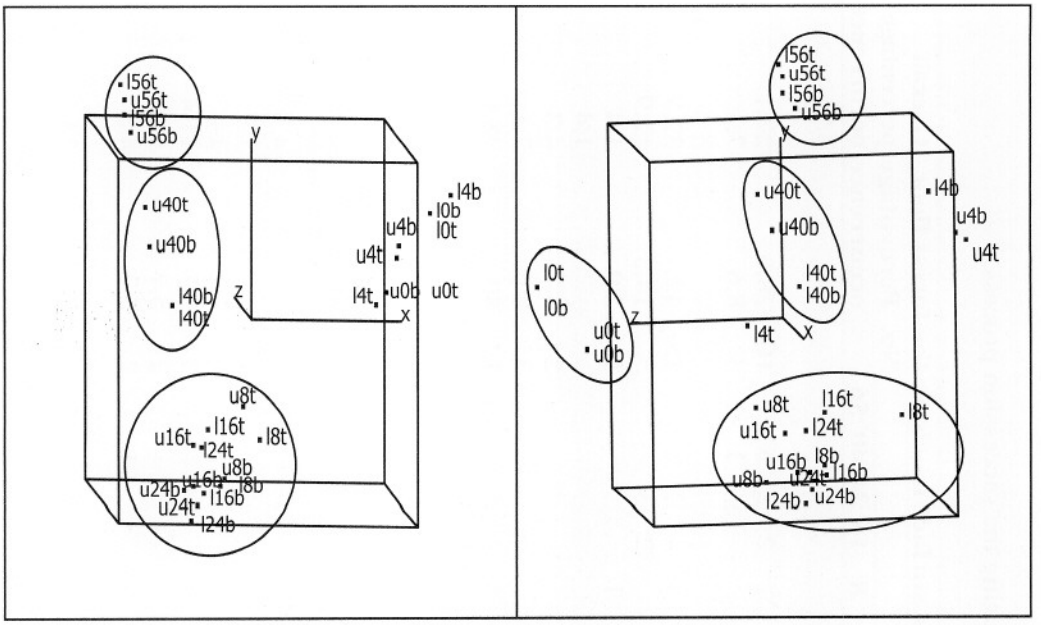

Fig. 1. Three-dimensional correspondence analysis of fungal on samples of *Manglietia garrettii*. U: upper surface touching the forest floor. L: lower surface touching the forest floor. 0, 4, 8, 16, 24, 40, 56: sampling times and stages of succession (day). T: top side of leaf. B: bottom side of leaf.

Succession of fungi

Three-dimensional correspondence analysis of fungal communities on senescent leaves of *Manglietia garrettii* showed that there were at least three succession communities, the pioneer community (day 0-3), the mature community (day 4-40) and the impoverished community (day 41-56). The overall number of fungi found at different stages of leaf decay are given in Fig. 2. and the overall percentage occurrence of dominant species at different stages of decay are shown in Fig. 3. The fungal community composition was distinct at each stage of succession. In the pioneer community stage, fungal communities were low in number and had a low percentage occurrence. The dominant species at this stage was *Volutella* sp. The highest species diversity was present during the mature community stage and the dominant species (upper and lower leaf combined) were *Bionectria ochroleuca* (27.1%), *Cylindrocladium floridanum* (32.9%), *Dactylaria longidentata* (12.9%), *Gliocladium* sp. 3 (38.6%), *Hyponectria manglietiae* sp. nov. (62.9%), and *Lasiosphaeria* sp. (14.3%). In the impoverished community stage, the species diversity and number of species declined. The community was dominated by a few species with relatively high percentage occurrence. Dominant species were *Hyponectria manglietiae* (62.9%) and *Sporidesmium crassisporum* (34.3%).

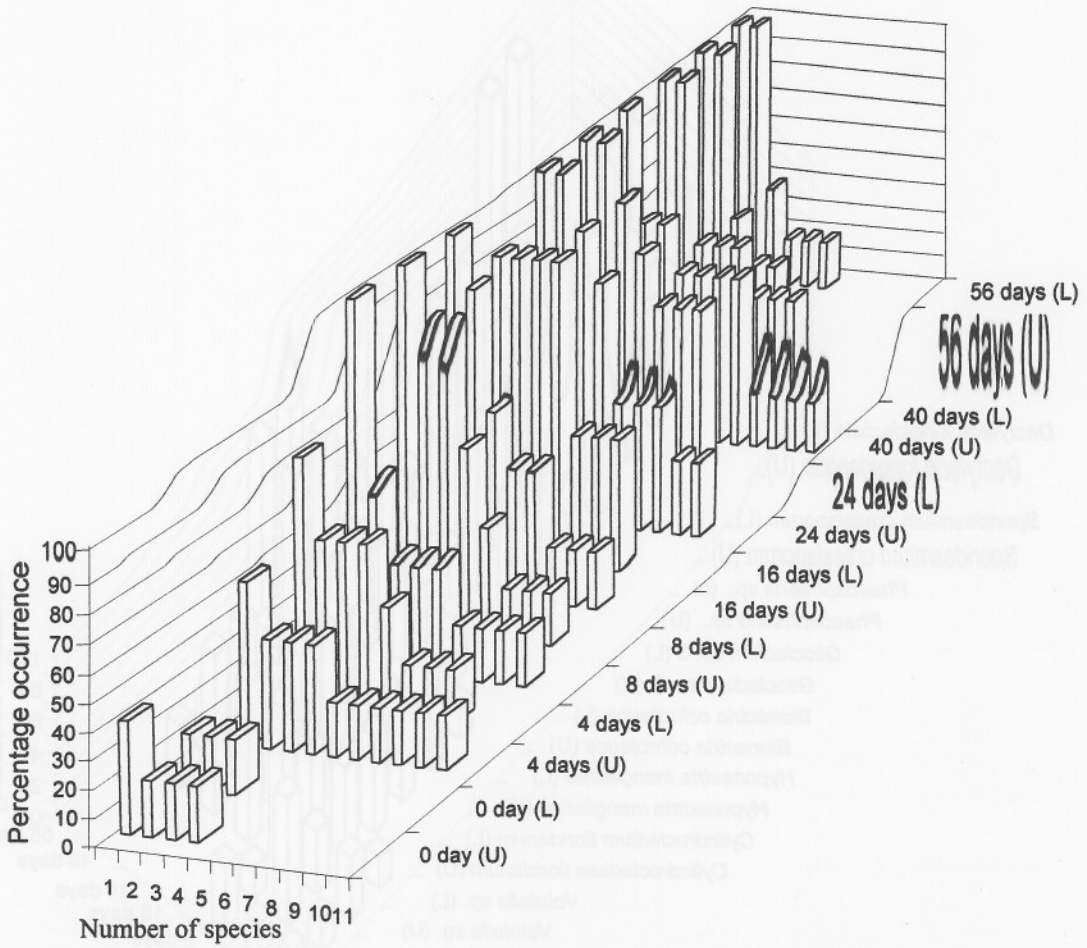

Fig. 2. Fungal species occurrence distribution at different sampling times. Species in each sampling time are ordered with most abundant on the left to the least abundant on the right.

Discussion

Biodiversity

Twenty-two fungi were identified on the baited leaves at various stages of succession and most have been shown to occur on naturally occurring decaying leaves in the same vicinity (Promputtha *et al.*, pers. obs.). This number is moderately high following direct examination. Parungao *et al.* (2002) examined 10 leaves from different tree types in rainforests in northern Queensland, Australia and found between 0-14 taxa on each leaf type. Photita *et al.* (2001) examined the large leaves of *Musa* sp. 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.* (pers. obs.) recorded between 17 and 27 taxa on *Musa* 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

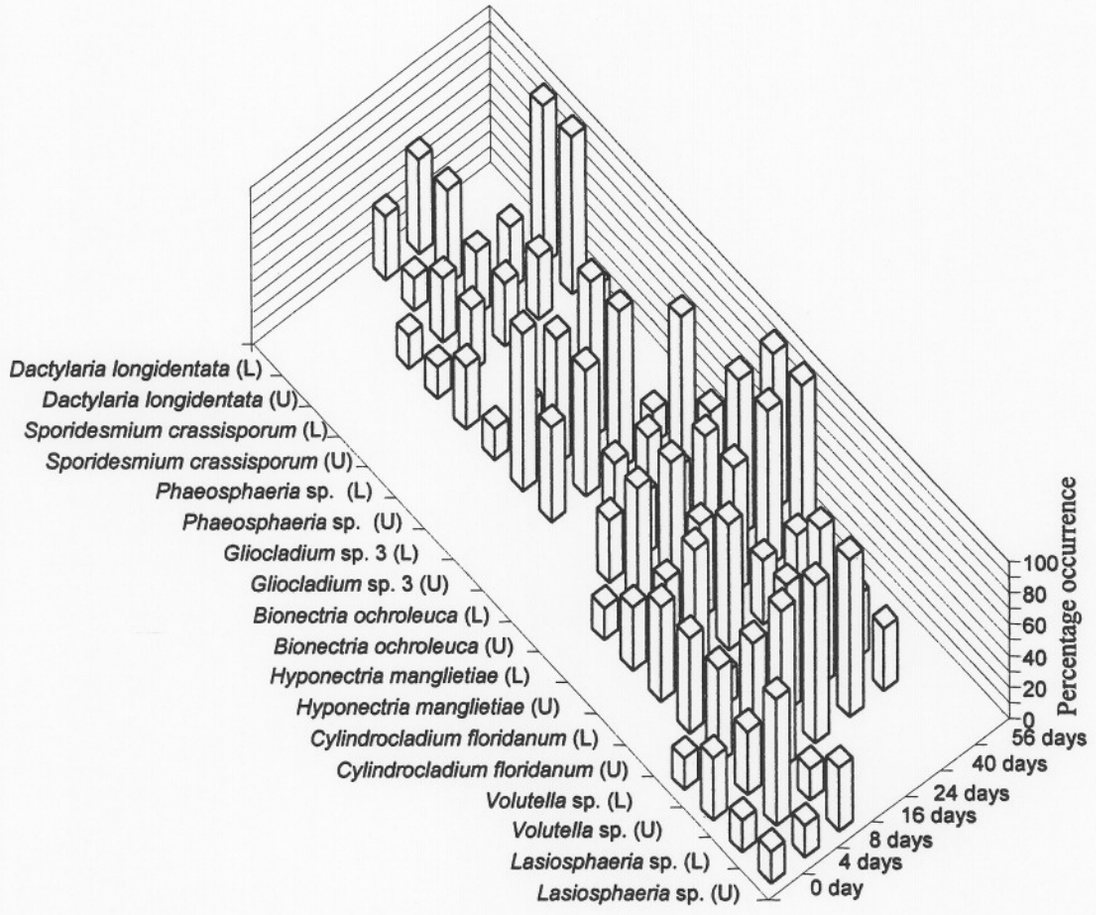

Fig. 3. Percentage occurrence of dominant fungi on leaves throughout the experimental period.

leaves of *Guarea guidonia* in Puerto Rico. 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.*, 2002), however, this method will also detect dormant spores and may therefore not be a good reflection of the fungi involved in decaying leaves.

The larger robust leaves (e.g. *Musa* sp.) appear to support a higher diversity than the smaller delicate leaves (Photita *et al.*, 2001). Wong and Hyde (2001) have shown that grasses with a more durable, strongly sclerenchymatic structure also support a higher fungal diversity than those with softer herbaceous tissues. The reason(s) for the higher diversity is unclear. The larger surface area offered by larger leaves may provide more substrata for fungal growth and thus support a larger number of species. The leaves may also decay

more slowly, thus providing substrata over a longer period allowing the development of more fungal species. Other factors (e.g. pH, lignocellulose content) may also account for the higher species diversity.

The highest fungal diversity occurred between day 4 and 40 with the most species being present on day 40 (Fig. 3). On day 56, leaves were found to be mostly skeletonised, so the fungal communities had decreased in number. The time for fungal communities to reach a peak of species diversity or fungal activity has varied in different studies. For example, complete decomposition of sugarcane bagasse needed 20 weeks and maximum colony counts were recorded during weeks 6-13 (Sandhu and 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 (Tawari *et al.*, 1994).

Fungal succession

There have been several studies of fungal succession in temperate regions (e.g. Dickinson, 1967; Frankland, 1969, 1992). In each of these studies a sequential occurrence of fungi was observed. In general, fungal communities were classified into three succession stages (Dix and Webster, 1985), the pioneer stage, the mature stage and the impoverished stage. Other authors have used the terms pioneer, early and later successional groups, which may be considered the same. Banana leaves, for instance, were first colonised by pioneer colonisers, such as *Deightoniella torulosa*, *Nigrospora* spp. and *Verticillium theobromae*, and were later replaced by species of *Alternaria*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Paecilomyces* and *Penicillium* with time (Meredith, 1962). Changes in species composition throughout the decay process were observed in the present study and communities can be grouped into the three successional stages used in the above studies.

The time of fungal decomposition of leaf litter varies enormously. For instance, in cool temperate pine forest, it may take ten years to fully decompose pine needles, within one year for decomposition of leaves in ash and sycamore woods, and only a few weeks in a tropical forest (Hudson, 1980). The time for decomposition of monocotyledons in topical regions has been shown to be relatively short, e.g. 14 months for leaves of sugarcane (Hudson, 1962), 19 months for stems of couch grass (Hudson and Webster, 1958), 2 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). In contrast the

decomposition of senescent leaves of *Manglietia garrettii* in this study was rapid, leaves were completely decayed within two months.

In this study, baits were periodically retrieved from the forest floor, followed by direct visual examination after incubation in a moist chamber. This method is most widely used in succession studies of plant litter (Webster, 1957; Srivastava *et al.*, 1983; Hyde, 1991). Other methods include culture plating (Pugh, 1958; Srivastava *et al.*, 1983) and leaf disk washing (Sandhu and Sidhu, 1980). Leaf disk washing would exclude fungi that grow slowly or cannot grow at all on agar plates. It would also encourage the growth of fast growing ubiquitous species, such as *Penicillium*, and the results would probably be unrepresentative (Lee and Hyde, 2002). Employing different methods might give a more accurate result (Yanna *et al.*, 2001).

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 fungal communities on naturally occurring decaying leaves in this study were similar to those found on mature stage leaf baits (Promputtha *et al.*, pers. obs.). Fungal communities which change rapidly, or taxa that are present for relatively short time periods may easily be missed on collections of naturally occurring samples, especially those that appeared during the pioneer and impoverished stages. For instance, *Gliocladium* sp. 2 was moderately dominant (occurrence up to 60%) for a short period of time only during the early succession study (Promputtha *et al.*, pers. obs.). It was not dominant on the naturally occurring sample. In addition, *Dokmaiensis monthadangii* gen. and sp. nov. was dominant (occurrence up to 11%) on naturally occurring leaves, but was not found on senescent leaf baits. The percentage similarity between naturally occurring samples and succession baits was high at 77% (Promputtha *et al.*, pers. obs.). This study has demonstrated that examination of naturally occurring samples and leaf baits from its initial senescence to complete decomposition are essential to obtain a full understanding of the fungal diversity on leaves of *Manglietia garrettii*.

Effect of leaf surface touching the forest floor

We also wanted to establish whether the surface of the leaf that touched the forest floor had any effect on the fungal communities that developed on the leaves. Fungal communities on the leaves with their upper surface touching the forest floor were similar to those with their lower surface touching the forest floor. The dominant species and time of their occurrence were similar (percentage similarity 77%). *Cylindrocladium floridanum*, *Hyponectria*

manglietiae sp. nov., *Sporidesmium crassisporum*, and *Volutella* sp. were common on both leaves with their upper or lower surface touching the forest floor.

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