Fungi on decaying leaves of *Magnolia liliifera* and *Cinnamomum iners* show litter fungi to be hyperdiverse

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Monkai J, Promputtha I, Kodsueb R, Chukeatirote E, McKenzie EHC, Hyde KD 2013 – Fungi on decaying leaves of *Magnolia liliifera* and *Cinnamomum iners* show litter fungi to be hyperdiverse. Mycosphere 4(2), 292–301, Doi 10.5943/mycosphere/4/2/12

Diversity of fungi on decaying leaves of *Magnolia liliifera* and *Cinnamomum iners* collected during the dry season at Doi Suthep-Pui forest, Chiang Mai, Thailand were studied and compared. Thirty-five taxa were identified from *Magnolia liliifera* comprising 8 sexual (ascomycetes) and 27 asexual taxa. The most abundant species found were *Sporidesmium* sp., *Colletotrichum fructicola* and *Stachybotrys parvispora*. Seventeen taxa were identified from *Cinnamomum iners* comprising 2 ascomycetes and 15 asexual taxa. Anamorph of *Eutypa* sp. 2 and *Pleurophragmium* sp. were the most abundant species on *Cinnamomum iners*. There is very little overlap between the fungi occurring on the two host species. Distinct fungal communities were found between the two hosts at each stage of decomposition. Decaying leaves of both hosts collected in the early stage of decomposition supported a greater fungal diversity than those collected at the later stage of decomposition. Saprobic fungi on the two plants are shown to be hyperdiverse.

Key words – biodiversity estimates – fungal ecology – saprobic fungi

Article Information
Received 11 March 2013
Accepted 14 April 2013
Published online 20 April 2013
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Introduction

Saprobic fungi function as decomposers of organic materials and recycle nutrients to other organisms (Cooke & Rayner 1984). The number of fungi worldwide was estimated at 1.5 million species by Hawksworth (1991, 2001b). However, only 70,000 species are presently described leaving the remaining 1.43 million (or 95%) as being undescribed. It is therefore important to search for these undescribed fungi which occur in unexplored habitats, hosts or poorly studied countries especially in tropical regions (Hawksworth & Rossman 1997). Forests of the northern part of Thailand have great plant diversity (Gardner et al. 2000) and several studies on their fungal diversity have been carried out in recent years (Duong et al. 2008, Thongkantha et al. 2008,

Wang et al. 2008, Osono et al. 2009, Bhilabutra et al. 2010, Sysouphanthong et al. 2010, Zhao et al. 2010, Vasilyeva et al. 2012). This has resulted in taxonomic advances (see Fournier et al. 2010, Boonmee et al. 2011, 2012, Liu et al. 2011) and a better understanding of Thailand's fungi. However, only a few published studies have established the diversity of fungal communities and overlapping fungi on litter types in the tropics (Yanna et al. 2001, Ananda & Sridhar 2004, Paulus et al. 2006, Yule & Gomez 2008). There are still many unidentified taxa which need to be discovered (Duong et al. 2004).

Magnolia liliifera (L.) Baill. (*Magnoliaceae*) is an evergreen tree and in northern Thailand, is commonly located in Doi Suthep-Pui National Park (Gardner et al. 2000). *M. liliifera* leaves are large and thick and this host is a good source for saprobic fungi (Promputtha et al. 2004). Promputtha et al. (2002, 2004) studied saprobic fungi on *M. liliifera* leaves in the forest of Doi Suthep-Pui National Park during the wet season of 2001. They found many diverse fungal communities and undescribed species (e.g. *Anthostomella monthadoia*, *Dokmaia monthadangii*, *Hyponectria manglietiae*, *Pseudohalonectria suthepensis*). Saprobic fungi on decaying woody litter of *M. liliifera* in the forest of Doi Suthep-Pui National Park during the wet and dry seasons were reported by Kodsueb et al. (2008). The results showed that samples collected in the dry season provided greater species richness than samples collected in the wet season.

Cinnamomum iners Reinw. ex Blume (*Lauraceae*) is an evergreen tree and has essential oils in the leaves, which are used for flavoring sweets and confectionery (Jantan et al. 1995). It has been reported that leaf extracts exhibit some biological and pharmaceutical activity (Jantan et al. 1992, Zaridah et al. 2006). In Thailand, there have been some studies concerning endophytic fungi from *Cinnamomum* species (Worapong et al. 2001, 2003, Lumyong et al. 2002, Suwannarach et al. 2010). Worapong et al. (2003) and Suwannarach et al. (2010) discovered new fungal species and strains that can produce volatile compounds. However, there has been no study on the fungal diversity on decaying *C. iners* leaves. It is therefore interesting to study these fungi to understand the relationship between fungi and host.

Most previous studies on saprobic fungi are based on the wet season when the number of fungi on the forest floor is abundant (Hyde et al. 2001, Parungao et al. 2002, Paulus et al. 2006). In this study we focused on saprobic fungi on *Magnolia liliifera* and *Cinnamomum iners* in the dry season at Doi Suthep-Pui, Chiang Mai, Thailand. The purposes of the present study were to examine fungal communities and compare fungal species between two hosts and to establish whether fungal saprobes are host-specific or host-recurrent (Zhou & Hyde 2001). In addition, the saprobic fungal communities on each host were evaluated for the effect of different stages of decomposition.

Methods

Study site and sample collection

The study site was located in an evergreen forest in Doi Suthep-Pui, Chiang Mai, northern Thailand (N 18º 48' 18.73", E 98 º 54' 47.28", elev., 107 m) and samples were collected in the dry season between November 2009 and April 2010, when there was low humidity; the forest floor was damp but not wet. Ten decaying leaves were randomly collected from each individual tree of *Magnolia liliifera* and *Cinnamomum iners* in 100 m^2 plots and returned to the laboratory.

Fungal examination and isolation

Leaves were divided into two stages of decomposition (five leaves for each stage); stage I were recently green or yellow fallen leaves and stage II were mostly decaying brown leaves. Samples were incubated in 15 cm diameter sterile Petri dishes with a tissue paper moistened with sterile distilled water at room temperature (Manoch 2004). Leaves were examined for fungi over six weeks of incubation. Fungi were identified based on morphological characters using relevant references (e.g. Ellis 1971, 1976, Carmichael et al. 1980, Sutton 1980, Sivanesan 1984, Hanlin 1990, 1998a, 1998b, Nag Raj 1993, Seifert et al. 2011). Single spore isolation method was used in fungal isolation (Choi et al. 1999;

Chomnunti et al. 2011). All cultures were grown on potato dextrose agar (PDA) and malt extract agar (MEA) and deposited in MFLU Culture Collection (MFLUCC) and BIOTEC Culture Collection (BCC).

Data analysis

The percentage occurrence of fungi was calculated and fungal taxa with a percentage occurrence higher than 10 are regarded as 'common species' in this study. The formula of fungal percentage occurrence was measured by using the following formula:

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

Species diversity were compared at each stage of decomposition (stage I and II) using diversity indices to demonstrate the result of species diversity of a community (Shannon & Weaver 1949). Species richness means the number of fungal species in a community and species evenness means the contribution (relative equability) of individuals (McCune & Grace 2002). The Shannon index (H') refers to the abundance of species diversity of a community and the Shannon evenness (E') refers to the equability of species diversity, which ranges from 0 to 1. If the Shannon evenness is equal to 1, then every species in the community has the same frequency of occurrence. The Shannon index is calculated according to the equation:

$$H' = -\sum_{i=1}^{n} P_i \log_e P_i \quad \text{, and} \quad P_i = \frac{N_i}{N}$$

Where, N_i is individual number of *i* species, N is individual number of all species, P_i is the proportion of *i* species, n is the number of species.

The Shannon evenness is calculated according to the equation:

E' = H/lnS (S: total species number).

Sørensen's index of similarity was used to compare the similarity of the species on different hosts (Magurran 1988). Sørensen's similarity index = 2c/a + b, where a = the number of species in host A, b = the number of species in host B, c = the number of species in common in both hosts. Similarity is expressed with values between 0 (no similarity) and 1 (absolute similarity).

The overlap of fungi from different hosts were calculated using the Sørensen quotient:

$$\text{Overlap}(\%) = \frac{\text{number of taxa shared between host A and host B}}{\text{total number of taxa observed in host A and host B}} \times 100$$

Results and Discussion

Diversity of saprobic fungi on Magnolia liliifera and Cinnamomum iners

In this study, fungal diversity on decaying leaves of *M. liliifera* and *C. iners* in the dry season in northern Thailand was investigated. A total of 139 fungal collections were made and 35 taxa were identified from decaying leaves of *M. liliifera*. This comprised 8 ascomycetes (representing 23% of all taxa) and 27 anamorphic fungi (77%) including 11 coelomycetes (31%) and 16 hyphomycetes (46%). The most abundant species were *Sporidesmium* sp. (80%), *Colletotrichum fructicola* (70%), *Stachybotrys parvispora* (70%), *Dicyma pulvinata* (60%), *Lasiosphaeria*-like sp. 1 (60%) and *Volutella consors* (60%).

In decaying leaves of *C. iners*, 58 fungal collections were made and 17 taxa were identified. This comprised two ascomycetes (representing 11% of all taxa) and 15 anamorphic fungi (89%) including 6 coelomycetes (33%) and 9 hyphomycetes (56%). The most abundant species were anamorph of *Eutypa* sp. 2 (60%), *Pleurophragmium* sp. (60%), *Acremonium* sp. (40%), *Colletotrichum* sp. (40%) and Hyphomycetes sp. 2 (40%). Percentage occurrence of fungi occurring on *M. liliifera* and *C. iners* are shown in Table 1. Diversity indices of fungi on decaying leaves of *M. liliifera* and *C. iners* are shown in Table 2.

Fungal diversity of *M. liliifera* in the dry season can be compared with previous studies in the wet season (Promputtha et al. 2002, 2004). Twenty-two taxa of fungi were recorded in a succession study was from 110 leaf samples (Promputtha et al. 2002). Thirty-seven taxa were recorded on naturally

occurring decaying leaves from 90 leaf samples (Promputtha et al. 2004). In the present study, 35 fungal taxa were identified from 10 leaf samples. This sample number was shown to be a threshold for studying fungal diversity in *M. liliifera* (Promputtha et al. pers. obs.). It seems that leaves of *M. liliifera* in the dry season support more fungal taxa than in the wet season. Similar results were reported by Kodsueb et al. (2008) and Seephueak et al. (2010, 2011) who found that samples collected in the dry season had greater species richness and higher Shannon diversity index than samples collected in the wet season. There were no seasonal effects of fungal communities on palms in Hong Kong (Yanna et al. 2001) or on *Pandanus penetrans* in Thailand (Thongkantha et al. 2008). The reason is still unclear. Pinnoi et al. (2006) showed that the spore germination and reproduction of fungi required quite high humidity. Rayner & Todd (1979) reported that the wettest period had an unsuitable ratio between moisture content and aeration of wood with relative high moisture and low aeration. Fungal composition and dominant species reported by Promputtha et al. (2002, 2004) were different from this study. Only a few overlapping genera occurred in both studies (*Colletotrichum*, *Phaeosphaeria*, *Phomopsis*, *Stachybotrys* and *Volutella*); *S. parvispora* is the only overlapping species between the two studies. Nevertheless, the given name of taxa may be different following sexual-asexual states which were present on samples. *Clonostachys rosea* was found in this study, whereas *Bionectria ochroleuca*, which is linked to sexual states of this fungus (Schroers 2001), was reported by Promputtha et al. (2002, 2004). However, differences in the number of samples and the years of collecting, which varied in temperature, humidity and rainfall make comparisons difficult.

Only 17 fungal taxa were identified from *C. iners* leaves and most were anamorphic fungi. Lumyong et al. (2000) isolated endophytic fungi from *C. iners* collected from Doi Suthep-Pui National Park, Thailand. The genera of endophytes (Lumyong et al. 2002) were not the same as in our study except for *Colletotrichum* sp. Fungal diversity on *M. liliifera* leaves provides greater species richness and Shannon diversity indices than fungi on *C. iners* leaves (see Table 2). A reason for this may be that leaves of *M. liliifera* are larger than those of *C. iners*. Larger leaves are likely to provide higher species diversity than smaller leaves (Wong & Hyde 2001). This is consistent with previous studies on hosts such as banana, palm and *M. liliifera*, which supported higher diversity and more diverse taxa (Photita et al. 2001, 2003, Yanna et al. 2001, Promputtha et al. 2002, 2004). Chemical composition of leaves may affect fungal numbers. Leaves of *C. iners* contain essential oils such as eugenol and cinnamaldehyde as major bioactive compounds (Jantan et al. 1992). Reports have shown antimicrobial activities of essential oils from *Cinnamomum* species (Jantan et al. 1992, Ranasinghe et al. 2002, Mustaffa et al. 2011). Antifungal tests demonstrated that cinnamaldehyde and eugenol had strong inhibitory effect against wood decay fungi (Wang et al. 2005, Cheng et al. 2006), while Yen & Chang (2008) concluded that the synergy of cinnamaldehyde with eugenol could alter cell wall structure of fungi, reduce cell wall synthesis, and the addition of radical scavenging. Such factors may lead to a reduced number of fungi on *C. iners* leaves.

Host specificity

Overlapping genera on both hosts were anamorph of *Eutypa*, *Colletotrichum*, *Lasiodiplodia* and *Ophioceras*. Only one species, *Lasiodiplodia theobromae*, overlapped on both hosts. Sørensen's similarity index, overlap (%) of fungi from the two hosts are shown in Table 3. Few taxa overlapped between the two hosts, and this resulted in a low similarity index suggesting that saprobic fungi may be host-specific. Fungal composition between hosts in different families has been shown to be varied (Wang & Hyde 2001) and *M. liliifera* and *C. iners* are in different families. Saprobic fungi were less specific at host species level (Wang et al. 2008). Other factors such as season, location and number of samples might also affect the host specific of saprobic fungi. Further studies in wet season with other hosts and/or other locations should be done to determine host-specificity of saprobic fungi.

Effect of decomposing stages on fungal communities

The percentage occurrence of fungi on *M. liliifera* and *C. iners* at each stage of decomposition are given in Table 1. On *M. liliifera* dominant species at stage I of decomposition were *Dicyma pulvinata* (100%), *Sporidesmium* sp. (100%) and *Volutella consors* (100%). The most abundant species at stage II of decomposition were anamorph of *Eutypa* sp. 1 (60%), *Colletotrichum fructicola* (60%), *Sporidesmium* (60%), *Ophioceras* cf. *leptosporum* (60%) and *Stachybotrys parvispora* (60%). For *C. iners*, the most abundant species at stage I of decomposition were *Acremonium* sp. (60%), *Pleurophragmium* sp. (60%), anamorph of *Eutypa* sp. 2 (40%), Coelomycete sp. 5 (40%), *Colletotri-chum* sp. (40%), Hyphomycete sp. 2 (40%), *Lasiodiplodia theobromae* (40%) and *Pyricu-laria costina* (40%). The most abundant species in stage II of decomposition were anamorph of *Eutypa* sp. 2 (80%), *Pleurophragmium* sp. (60%), *Colletotrichum* sp. (40%), *Graphium* sp. (40%) and Hyphomycete sp. 2 (40%).

Species richness, species evenness, Shannon indices (H') and Shannon evenness (E') at each stage of decomposition of fungi on *M. liliifera* and *C. iners* were calculated (Table 2). On *M. liliifera*, the number of species at stage I of decomposition was higher than that at stage II of decomposition, but stage I of decomposition has a lower diversity of fungi than stage II of decomposition. On *C. iners*, leaves in stage I of decomposition supported a greater diversity of fungi than leaves in stage II and the number of species in stage I was higher than the number of species in stage II of decomposition.

The replacement of fungal species sequentially throughout the decomposing process relies on the capability of decomposers to utilize organic matter and nutrients, which are particular to each substrate or host (Frankland 1992, Tang et al. 2005). In the early stage, decomposer fungi might switch their roles from endophytes or pathogens (Lodge & Cantrell 1995, Duong et al. 2008). The evidence that endophytic fungi change to be saprobes has been reported in many previous studies (Osono et al. 2004, 2009, Koide et al. 2005, Tang et al. 2005, Promputtha et al. 2010, Purahong & Hyde 2011). Promputtha et al. (2010) also showed that endophytes can produce various degrading enzymes in succession process, which is an important activity for their adaptation to a saprobic lifestyle. The primary enzymes degrade small soluble carbon-based molecules, such as hemicelluloses and the most complex cellulose and lignin are then degraded at the late stage of decomposition (McClaugherty & Berg 1987, Promputtha et al. 2010). In addition, soilborne and airborne fungi can colonize fallen leaves (Duong et al. 2008) so that leaves which have been on the forest floor for a long time might have higher fungal diversity than leaves which have recently fallen. In this study, decaying leaves of *M. liliifera* and *C. iners* at stage I of decomposition supported a greater fungal diversity than in stage II of decomposition suggesting that endophytic fungi might play an important role in the decay of dead leaves and these fungi are different at different stages of decay. Shanthi & Vitthal (2010) studied leaf litter fungi of *Pavetta indica* on freshly fallen senescent leaves and on leaves already undergoing active decomposition and found more taxa on the former than on the latter. Seephueak et al. (2010, 2011) studied fungi at different stages of decaying leaf and branch litter of the rubber tree. They showed that the number of taxa on middle stage decaying branches was higher than new and old decaying fallen branches.

The results presented here show that the fungi on the two plants are hyperdiverse and that there is very little overlap between the fungi occurring on the two host species. The data provides evidence to suggest that the number of fungal species worldwide (Hawksworth 1991, 2001) are at least at great as estimated (Hawksworth & Rossman 1997, Hyde et al. 2007).

Table 1 Percentage occurrence of fungi occurring on *Magnolia liliifera* and *Cinnamomum iners* at two stages of decomposition.

Fungal species	*Magnolia liliifera*			*Cinnamomum iners*		
	Stage I*	Stage II*	Overall	Stage I*	Stage II*	Overall
Acremonium sp.				60	20	40
Anamorph of *Eutypa* sp. 1		60	30			
Anamorph of *Eutypa* sp. 2				40	80	60
Ascomycete sp. 1		20	10			
Ascomycete sp. 2		20	10			
Beltrania rhombica	40		20			
Botryosphaeria sp.	20	20	20			
Canalisporium caribense		20	10			
Chaetomium sp.				20		10
Cladosporium sp. 1	20		10			
Cladosporium sp. 2		20	10			
Clonostachys compactiuscula	20		10			
Clonostachys rogersoniana	20		10			
Clonostachys rosea	20		10			
Coelomycete sp. 1	20		10			
Coelomycete sp. 2		20	10			
Coelomycete sp. 3		20	10			
Coelomycete sp. 4				20		10
Coelomycete sp. 5				40		20
Colletotrichum fructicola	80	60	70			
Colletotrichum sp.				40	40	40
Dicyma pulvinata	100	20	60			
Ellisiopsis occulta				20		10
Fusicoccum aesculi	40	20	30			
Gliocladium sp.				20		10
Graphium penicillioides				20		10
Graphium sp.					40	20
Hyphomycete sp. 1	20		10			
Hyphomycete sp. 2				40	40	40
Hyphomycete sp. 3					20	10
Lasiodiplodia theobromae	60		30	40	20	30
Lasiosphaeria-like sp.	80	40	60			
Montagnula sp.		40	20			
Nodulisporium sp.		20	10			
Ophioceras cf. *commune*					20	10
Ophioceras cf. *leptosporum*	20	60	40			
Pestalotiopsis sp.	20		10			
Phaeosphaeria sp.	40		20			
Phialophora sp.		20	10			
Phoma sp. 1	60	40	50			
Phoma sp. 2	20		10			
Phomatospora sp.	20		10			
Phomopsis sp.	20		10			
Pleurophragmium sp.				60	60	60
Pyrenochaeta sp.				20		10
Pyricularia costina				40		20
Sporidesmium sp.	100	60	80			
Stachybotrys parvispora	80	60	70			
Stachylidium bicolor		20	10			
Volutella consors	100	20	60			
Zygosporium sp.		20	10			

*Stage I = Stage I of decomposition,
–Stage II = Stage II of decomposition

Table 2 Diversity indices of fungi from *Magnolia liliifera* and *Cinnamomum iners* at two stages of decomposition.

	Magnolia liliifera		*Cinnamomum iners*	
	Stage I*	**Stage II***	**Stage I***	**Stage II***
Species richness	23	21	14	9
Species evenness	88	44	24	17
Shannon indices	2.84	2.9	2.56	2.1
Shannon evenness	0.74	0.84	0.92	0.9

*Stage I = Stage I of decomposition, Stage II of decomposition

Table 3. Sørensen's similarity index and, overlap (%) of fungi from *Magnolia liliifera* and *Cinnamomum iners.*

	Overlapping genera	**Overlapping species**
Sørensen's similarity index	0.17	0.04
Overlap (%)	8.51	1.92

Acknowledgements

This work was supported by The Thailand Research Fund BRG5280002. The authors thank Scientific and Technological Instruments Center, Mae Fah Luang University for providing facilities. Appreciation is extended to Prof. D.J. Bhat for helping with identification of fungi.

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