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Community Structure of Sporulating Fungi on Decaying Litters of *Shorea* spp.

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The community structure of sporulating fungi on decaying branch and leaf litters of *Shorea* spp. were studied to reveal the common saprobic fungi. The study was mainly based on morphological observation. Twenty-nine species of the sporulating fungi were found on *Shorea* spp. litters at Situ Gede and Bubulak forest area, Bogor, West Java. The fungi included seven species of Ascomycetes (*Annulohypoxylon purpureonitens*, *Diatrype chlorosarca*, *Didymosphaeria epidermidis*, *Lophiostoma* sp., *Lophodermium* sp., *Pemphidium* sp., and *Valsa* sp.) and 22 species of anamorphic taxa that consisted of 12 Coelomycetes (*Coniella musaiaensis*, *Coryneum betulinum*, *Hendersoniopsis thelebola*, *Lasiodiplodia theobromae, Lasmeniella guaranitica*, *Leptodothiorella* sp., *Massariothea themedae*, *Pestalotia guepinii*, *Pestalotiopsis* sp., *Pseudolachnea hispidula*, *Septoriella* sp., and unidentified species of Coelomycetes) and 10 Hyphomycetes (*Beltraniella portoricensis*, *Cryptophialoidea fasciculata*, *Hermatomyces spaerichus*, *Kiliophora ubiensis*, *Minimidochium setosum*, *Monodisma fragilis*, *Nodulisporium* sp., *Stilbella fimetaria*, *Virgatospora echinofibrosa*, and unidentified Hyphomycetes). The most common taxa occuring on decaying leaf litter were *B. portoricensis* and *Pemphidium* sp., while those on decaying branch material were *L. theobromae* and *C. fasciculata*. The fungal community was subtrate specific. The community on decaying branch litter was more diverse than that on leaf litter. The C/N ratio of the substrate was closely related to the structure of the community.

Key words: community structure, frequency of occurrence, fungi, *Shorea* spp.

Struktur komunitas cendawan berspora pada serasah ranting dan daun *Shorea* spp. dipelajari untuk mengetahui cendawan saprob yang umum pada serasah tersebut. Penelitian dilakukan berdasarkan pada pengamatan morfologi. Sebanyak 29 spesies cendawan berspora ditemukan pada serasah *Shorea* spp. yang dikoleksi dari areal hutan yang terletak di Situ Gede dan Bubulak, Bogor. Jawa Barat. Cendawan terdiri dari 7 spesies askomiset (*Annulohypoxylon purpureonitens*, *Diatrype chlorosarca*, *Didymosphaeria epidermidis*, *Lophiostoma* sp., *Lophodermium* sp., *Pemphidium* sp., dan *Valsa* sp.) dan 22 spesies cendawan anamorfik yang terdiri dari 12 soelomiset (*Coniella musaiaensis*, *Coryneum betulinum*, *Hendersoniopsis thelebola*, *Lasiodiplodia theobromae*, *Lasmeniella guaranitica*, *Leptodothiorella* sp., *Massariothea themedae*, *Pestalotia guepinii*, *Pestalotiopsis* sp., *Pseudolachnea hispidula, Septoriella* sp., dan soelomiset yang tidak teridentifikasi) dan 10 hifomiset (*Beltraniella portoricensis*, *Cryptophialoidea fasciculata*, *Hermatomyces spaerichus*, *Kiliophora ubiensis*, *Minimidochium setosum*, *Monodisma fragilis*, *Nodulisporium* sp., *Stilbella fimetaria*, *Virgatospora echinofibrosa*, dan hifomiset yang tidak teridentifikasi). Spesies cendawan yang umum ditemukan pada serasah daun adalah *B. portoricensis* dan *Pemphidium* sp., sedangkan pada serasah ranting adalah *L. theobromae* dan *C. fasciculata*. Komunitas cendawan bersifat spesifik substrat. Komunitas cendawan pada serasah ranting lebih beragam dibandingkan pada serasah daun. Rasio C/N pada substrat mempengaruhi struktur komunitas cendawan tersebut.

Kata kunci: cendawan, frekuensi keberadaan, *Shorea* spp, struktur komunitas

Shorea spp. (Dipterocarpaceae) dominantly inhabit rainforests in Southeast Asia, especially in Malaysia and Indonesia. In Indonesia, these trees spread across the island of Sumatra, Bangka-Belitung, Kalimantan and several locations in Java island (The Ministry of Forestry 2007). In natural ecosystems, *Shorea* spp. posses an important role in maintaining the balance of the ecosystem because they produce abundant lignocellulosic rich substrates as a source of nutrients for the survival of microorganisms including saprobic fungi. The saprobic fungi are known as the major wood and leaf decomposing organisms which play an important role in the nutrient cycle of forest areas including *Shorea* spp. plantations. During the decomposition process, the saprobic fungi degrade lignocellulosic materials into more simple compounds (Osono 2007).

Hyde and Taylor (2003) noted that there are many benefits from studying the diversity of saprobic fungi. These include novel agents with important medicinal properties (Yang *et al*. 2011), and for other bioactive compounds (Strobel 2003), and discoveries of new

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taxa (genera and species). Ho *et al*. (2001) found ten new species and a new genus during their study on the saprobic fungi on submerged wood from streams. In addition, numerous new species have been discovered from palms (Goh and Hyde 1996; Yanna *et al*. 1997; 1998; Hidayat *et al*. 2006).

There are many fungal community studies on plants that have been reported worldwide. However, a study based on the fungal community structure of decaying litter of *Shorea* spp. has not widely been reported. Few studies were reported from Thailand (Osono *et al*. 2009) and India (Soni *et al*. 2011), and so far none from Indonesia. Therefore, research on the fungal community of *Shorea* spp. was undertaken in order to provide information regarding the fungal community inhabiting *Shorea* spp. litter.

MATERIALS AND METHODS

Collection of Samples. Samples of *Shorea* spp. litter were collected from *Shorea* plantation research area managed by the research and development centre for forest conservation and rehabilitation, located at the Situ Gede village and Bubulak village, Bogor, West Java. Samples were taken from 13 sampling sites which were designed plots (50 m x 50 m). The distance between each sampling site is 3 m. In total, 260 samples of *Shorea* spp. (130 branch and 130 leaf samples) were collected. The size of each branch is 10 cm lengths, regardless the diameter. Samples were placed in resealed plastic bags. After sealing, each bag was labeled as follows: name of the sample, collecting site, collector, and collection date. The environment parameters such as pH, light intensity, soil water content, water content of branch and leaf litter, the C/N ratio of branch and leaf litter were also measured.

Examination of Materials. On returning to the laboratory, the materials were immediately incubated and examined periodically over one month. The materials were examined for saprobic Ascomycetes, Coelomycetes, and Hyphomycetes. The materials were examined using an Olympus SZX7 dissecting microscope to determine the presence of the fungal fruiting structures, and an Olympus CX41 to determine microscopic structures.

For ascomycetes and coelomycetes, a sharp one sided razor blade or a pair of Inox 5 fine forceps were used to carefully remove the top of the fruiting body. The specimens were rehydrated if the contents were dry or crystalline by using distilled water or potassium hydroxide (KOH) 3% w/v before extraction. The contents were placed in a drop of distilled water on a slide and covered with an 18 x 18 mm coverslip. Hyphomycetes examination was prepared by using a pair of Inox 5 fine forceps. Water was used for all examinations, spore/conidia measurements, and most of the photographs/line drawings. Shear's solution was added to the slides for permanent fixation. The slides were heated to remove air bubbles in the Shear's solution and the edges of the coverslip sealed with two layers of clear nail varnish. The slides were labeled with the number of each specimen.

Detailed observations of morphological characters were carried out by means of an Olympus CX41 light microscope using an oil immersion lens (1000×). Single spore isolation of each new fungus encountered was as reported by Choi *et al*. (1999) with modification.

Identification Procedures. The following texts were consulted for basic identification such as Hyde *et al*. (2000) and Hyde and Taylor (2003) for Ascomycetes; Nag Raj (1993) and Sutton (1980) for Coelomycetes; and Ellis (1971, 1976), Carmichael *et al.* (1980) and Seifert *et al*. (2011) for Hyphomycetes. Further identification of fungal specimens was by reference to the recent publications in various journals of mycology. The following fungal database websites were also used such as Index Fungorum (), Mycobank (http://www. mycobank.org) and USDA fungus-host database (http://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm.

Data Analysis. Frequency of occurence (FO) of a taxon was calculated according to the following formula:

$$\text{Frequency of occurrence of taxon A} = \frac{\text{Occurrence of taxon A}}{\text{Total number of samples examined}} \times 100\%$$

The total number of species and the number of fungi per sample were recorded and calculated. Based on the percentage occurrence of different species, grouping was done as follows: very frequent (>10%), frequent (>5%-10%), less frequent >1%<5%), and rare (<1%).

Shannon-Wiener diversity (H') and evenness indices (E) were calculated for each sampling point along with Margalef's species richness. Calculations were carried out according to Magurran (1988). The Margallef index on diversity (D_{Mg}) was calculated as follows:

$$\text{Margallef index } D_{Mg} = (S-1)/\ln N;$$

D_{mg} = Margalef Index

S = the number of species
N = the total number of fungal occurrences

Shannon index (H') = $-\sum_{i=1}^{s} pi \cdot \ln pi$

pi = the proportional abundance of the i^{th} species = (ni/N)

$$\text{Evenness (E)} = \frac{H}{\ln S}$$

H' = Shannon index,
S = total species number

The relationship between assemblage of the fungal community and different organ type of *Shorea* spp. was also analysed using a simple correspondence analysis (CA). The analysis were performed using Minitab 15 software.

RESULTS

Community Structure of Sporulating Fungi on Decaying Litters of *Shorea* spp. Examination of decaying branch and leaf litter of *Shorea* spp., indicated that only 50% of the litter containing fungi. In those litter, 29 of the sporulating fungal taxa, comprising seven species of Ascomycetes (representing 24.1% of all taxa), and 22 species of anamorphic fungi (representing 75.9% of all taxa) were found. The anamorphic fungi were composed of 12 Coelomycetes (41.4%) and 10 Hyphomycetes (34.5%) (Fig 1).

The number of fungal taxa found on decaying branch litter was higher than those on leaf litter (Table 1). Twenty-two species of fungi were recorded for the branch litter, and seven species were found in the leaf litter. *Beltraniella portoricensis* and *Pemphidium* sp. appeared to be the most common taxa inhabiting leaf litter with FO of 16.9 and 11.5% respectively (Table 1). During the process of decaying branch litter, *Lasiodiplodia theobromae* and *Cryptophialoidea fasciculata* were determined to be the most frequent fungi found during this study. The remaining fungi were found either less frequently or were rare based on their FO on branch or leaf litter (Table 1).

Based on fungal groups, Coelomycetes appeared as the most dominant group found on decaying branch litter with 11 species (total abundance 36), followed by Hyphomycetes with 6 species (total abundance 28), and Ascomycetes with 5 species (13 total abundance) (Fig 2, Fig 3). In the case of leaf litter, Hyphomycetes were found to be the highest fungal group in species richness and abundance, with 4 species and score of 55, respectively. Ascomycetes appeared as the second highest in species richness with 2 taxa recorded and score of 32, followed by Coelomycetes taxa with 1 species and score of 1 (Fig 2, Fig 3).

The Shannon-Wiener index was measured on thirteen sites in the sampling location (Fig 4). The diversity of sporulating fungi on branch litter was higher than those on leaf litter. This data was also supported by the Margalef index calculation (Table 1). Evenness was higher on the branch litter than on the leaf litter (Table 1). This means that every fungal species in branch litter has a higher frequency of occurrence than for leaf litter.

Correspondence Analysis (CA) of the fungal community on branch and leaf litter showed that taxa such as *Annulohypoxylon purpureonitens*, *Diatrype chlorosarca*, *Didymosphaeria epidermidis*, *Lophiostoma* sp., *Valsa* sp., *Coniella musaiaensis*, *Coryneum betulinum*, *Hendersoniopsis thelebola*, *Lasiodiplodia theobromae*, *Lasmeniella guaranitica*, *Leptodothiorella* sp., *Massariothea themedae*, *Pestalotia guepinii*, *Pseudolachnea hispidula*, *Septoriella* sp., *Cryptophialoidea fasciculata*, *Hermatomyces spaerichus*, *Minimidochium setosum*, *Monodisma fragilis*, *Nodulisporium* sp., *Virgatospora echinofibrosa*, and *Coelomycetes* sp.1 were common on branch litter. However, on leaf litter, taxa such as *Lophodermium* sp., *Pemphidium* sp., *Pestalotiopsis* sp., *Beltraniella portoricensis*., *Kiliophora ubiensis*, *Stilbella fimetaria*, and *Hyphomycetes* sp. 1 were more common (Fig 5).

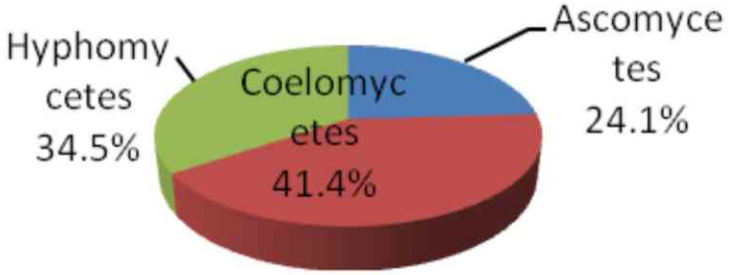

Fig 1 Comparison of fungal community on *Shorea* spp. based on number of species.

Table 1 Frequency of occurence (FO) of sporulating fungi on branch and leaf litter of *Shorea* spp.

Taxa	Branch	Leaf	Total	FO (%)
Ascomycetes				
Annulohypoxylon purpureonitens (Aap)	2	0	2	0.7
Diatrype chlorosarca (Adc)	5	0	5	1.9
Didymosphaeria epidermidis (Ade)	3	0	3	1.1
Lophiostoma sp. (Als)	2	0	2	0.7
Lophodermium sp. (Ald)	0	2	2	0.7
Pemphidium sp.(Apd)	0	30	30	11.5
Valsa sp.(Avs)	1	0	1	0. 3
Anamorphic fungi				
Coelomycetes				
Coniella musaiaensis (Ccm)	3	0	3	1.1
Coryneum betulinum (Ccb)	1	0	1	0.3
Hendersoniopsis thelebola (Cht)	1	0	1	0.3
Lasiodiplodia theobromae (Clt)	17	0	17	6.5
Lasmeniella guaranitica (Clg)	1	0	1	0.3
Leptodothiorella sp. (Cld)	1	0	1	0.3
Massariothea themedae (Cmt)	2	0	2	0.7
Pestalotia guepinii (Cpg)	2	0	2	0.7
Pestalotiopsis sp. (Cps)	0	1	1	0.3
Pseudolachnea hispidula (Cph)	1	0	1	0.3
Septoriella sp.(Cst)	6	0	6	2.3
Coelomycetes sp. 1 (Csp)	1	0	1	0.3
Hyphomycetes				
Beltraniella portoricensis (Hbp)	0	44	44	16.9

*FO = Frequency of occurence

DISCUSSION

The present study is one amongst a few fungal community studies on branch and leaf litter of *Shorea* spp., and it is the first study in Indonesia. Osono *et al*. (2009) and Soni *et al*. (2011) studied the fungal community on leaf litter only. Several studies of fungal community on other host plants, such as palmae, bamboo, mangrove, etc. have been carried out in several areas of the tropics (Huhndorf and Lodge 1997; Hyde and Alias 2000; Hyde and Sarma 2001; Maria and Sridhar 2003; Hyde and Taylor 2003; Karamachand *et al*. 2009).

The current study shows that species diversity (H') and species richness (D_{Mg}) of the fungal community on the branch litter was higher than for leaf litter (Table 1). The higher the C/N ratio and density of wood, rather than that of leaves best supported the growth of fungi on the wood (Kodsueb *et al*. 2008). On palm, Pinnoi *et al*. (2006) suspected that thicker cell walls may yield more nutrients, in particular cellulose and starch. These nutrients could sustain the growth most of the fungi. They found that the palmicolous fungi were more prevalent on palm petioles (53%) than on rachides

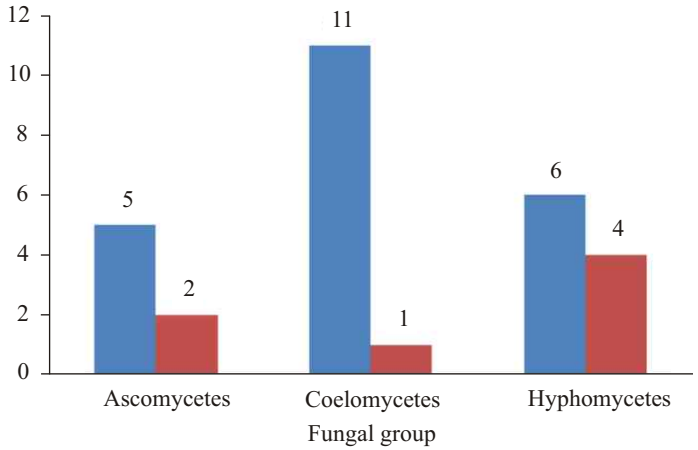

Fig 2 Distribution of all taxa recorded from of *Shorea* spp. The graphics are presented based on qualitative data of species richness. : branch litter; :leaf litter.

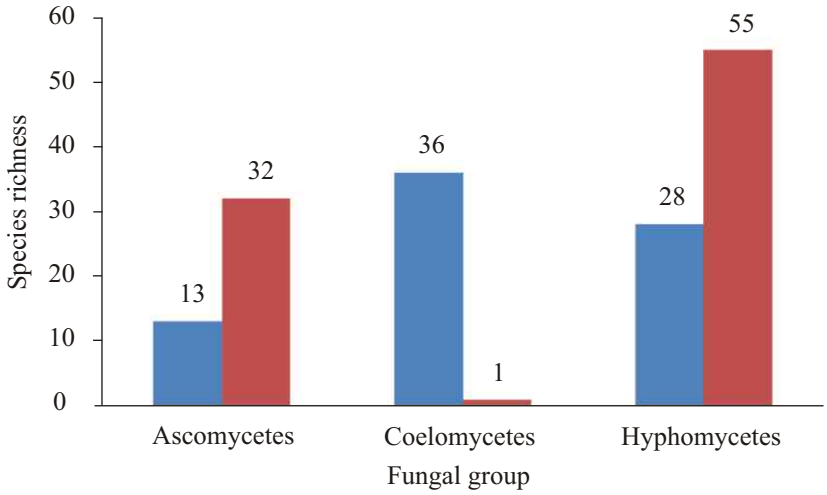

Fig 3 Distribution of all taxa recorded from of *Shorea* spp. The graphics are presented based on quantitative data (total abundance). : branch litter; : leaf litter.

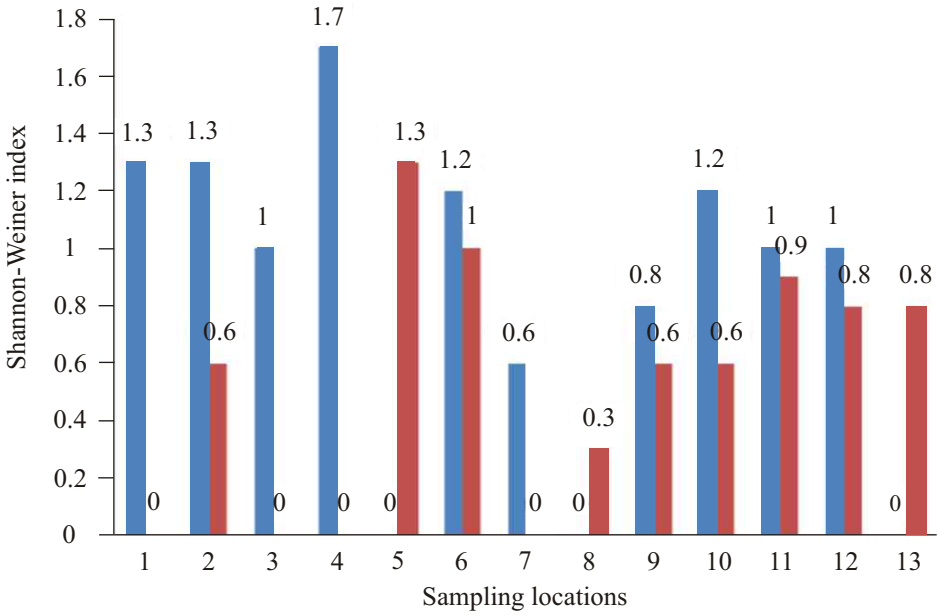

Fig 4 Histogram of Shannon-Wiener diversity index (H') showing the diversity of the fungal community on thirteen sites in the sampling location. : branch litter; : leaf litter.

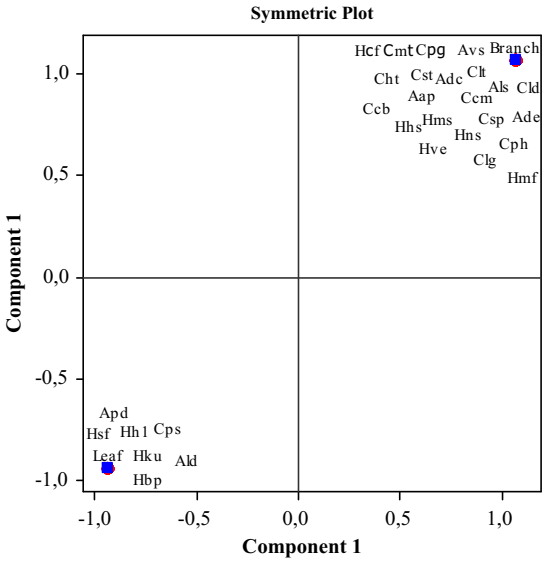

Fig 5 Correspondence analysis of fungal community on branch and leaf litter. Taxa are shown by their acronym.

(30%) and leaves (17%). Hyde and Sarma (2006) noted that branch thickness may also offer a better substrate for fungal colonization than that of leaves which were thinner. Leaves contain mainly parenchymatous cells which are thin-walled, with chloroplasts and rich in starch, while rachides and petioles have more sclerenchyma associated with their vascular bundles.

Our analyses of fungal community structure, based on the artificial taxonomical groups, showed that anamorphic fungi were more dominant than teleomorphic fungi, and this result is in agreement with previous studies carried out by Osono *et al*. (2009) and Soni *et al*. (2011). Osono *et al*. (2009) noted that hyphomycetes fungi were commonly found at the early stage of fungal community succession. However, the reasons for the higher dominance of anamorphic fungi over Ascomycetes (teleomorphic fungi) are unknown.

Several fungal species commonly found on decayed litter of *Shorea* spp. by Osono *et al*. (2009) and Soni *et al*. (2011), such as *Beltraniella, Pestalotiopsis*, and *Lophodermium* were also found in the current study. Shirouzu *et al*. (2009) noted that *Beltraniella* species most commonly occured on newly fallen leaves and their FO usually decreased with decay. The distributions of the fungal community on branch and leaf litter were different (Fig 5). It is apparent that several taxa only occured either on branch or leaf littter. Distinct fungal community composition occurs on different organs of *Shorea* spp. is indicated by the fact that fungi on *Shorea* litter are substrate specific. There is limited information regarding the physical structure of each organ of *Shorea* spp., therefore, it is difficult to determine the factors affecting the distinct community of each organ type.

On the contrary, the FO of fungi in leaf litter was higher than that of branch litter. This probably relates to water content of substrates. The water content of leaf litter of *Shorea* spp. was higher than that of branch litter (Table 2). Water has been recognized as an important factor in fungal growth, in particular during the germination process and the dispersal of fungal propagules. The water content is directly related to a high relative humidity required for spore germination, growth, and reproduction of fungi (Yoder and Wood 1973). Fungi are well-recognized as organisms that utilized sugar due to sugar occupying a central position in fungal metabolism. Hyde and Sarma (2001) noted that other parameters to be considered as the factors affecting the frequency of occurrence of the fungal community on different habitats included pH, incubation time, ecological niches, availability of the substrate, quality of the substrate (old or young samples/ soft or hard tissue), nutrient availability, DOD, COD, and BOD, seasonality and succession, temperature (tropical or subtropical), and host specificity/ main host samples.

Different fungal communities occured on branch and leaf litters of *Shorea* spp. indicating that substrates might play an important role in determining species composition by selectively stimulating or inhibiting the growth of specific fungi. Different parts of the *Shorea* spp. (i.e. leaf and branch) were found to support different fungi, pointing to the existence of a distinct ecological niche. This indicated that some fungi may preferentially develop on certain tissue types, as previously reported by Hyde and Alias (2000).

Table 2 Environmental parameters in samples location

Parameters	Value
pH of soil	6
Light intensity	120
Water content of soil	38.34%
Water content of	
branch litter	3.39%
leaf litter	3.98%
C/N	
branch litter	67.12
leaf litter	55.97

In relation to the fungal community on decayed plant substratum, the nature of the host substratum (chemical), minor components of the substratum, lignin/cellulose ratio, and nitrogen/carbohydrate ratio probably play important roles as selective factors determining which group of fungi colonize the substrate at different stages of decomposition. Host plants may contain low to high amounts of compounds that are toxic or inhibit the growth of fungi e.g. phenols, while wood density of a substratum may also influence the ability of fungi to colonize (Pinruan *et al*. 2007). Based on phytochemical studies of *Shorea* spp., the main secondary metabolites of this plant genus is a class of phenolic compounds, such as oligostilbenoid, flavonoids, phenyl propanoid, and phenolic acid derivates. Rohaiza *et al*. (2011) have been isolated four oligostilbenes of (-)-e-viniferin, (-)-ampelopsin E, (-)-hopeaphenol and shoreaphenol from the acetone extract of *S. hopeifolia.* Oligostilbene compounds exhibit a variety of significant bioactivities, including anti-bacterial (Nitta *et al*. 2002) and anti-fungal (Kusuma and Tachibana 2007). Stilbene derivates are known to be abundantly distributed in the plants belonging to the Dipterocarpaceae, Vitaceae, Leguminosae, and Cyperaceae (Ohguchi *et al*. 2003). Therefore, the smaller number of sporulating fungi in this study, as compared to studies on another hosts such as bamboo (Cai *et al*. 2006), palm (Pinruan *et al*. 2007), mangrove plants (Maria and Sridhar 2003), were probably due to the phenolic compound contents in the *Shorea* plants which inhibited fungal growth and thus protected plant tissues (including leaf and branch material) from the fungal colonization.

In conclusion, there were different fungal community structures which occupied decaying branch and leaf litters of *Shorea* spp. However, the factors that influenced the distinct fungal communities structures on branch and leaf litters were difficult to determine. Therefore, future studies should take into consideration all factors effecting the frequency of occurence of fungi in *Shorea* spp., in particular from the hosts point of view.

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