Fungal succession and lignin decomposition on *Shorea obtusa* leaves in a tropical seasonal forest in northern Thailand

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Fungal succession and decomposition processes of *Shorea obtusa* (*Dipterocarpaceae*) leaves were studied in a tropical seasonal forest in northern Thailand. The study focused on bleached and nonbleached area of leaves, attributable to fungal colonization and the variable decomposition of lignin. A field incubation of *S. obtusa* leaf litter using a litterbag method showed that the bleached leaf area accounted for 1% of the remaining total leaf area during the first month of decomposition and increased thereafter to reach 30% at 9 months. Total hyphal length in litter increased from 830 m/g to 4050 m/g during the first 5 months and then decreased to 2500 m/g at 9 months. Eighty fungal taxa were isolated from *S. obtusa* leaves, and successional changes in fungal assemblages were observed during the decomposition. *Trichoderma asperellum* and *Aspergillus* sp. occurred most frequently throughout the decomposition process. The prevalence of *Nigrospora* sp., *Cladosporium oxysporum*, and *Talaromyces* sp. decreased, whereas an unidentified species in the *Amphisphaeriaceae* increased during decomposition. The frequency of *Nigrospora* sp. was greater in the bleached than in the nonbleached leaf area. Endophytic and epiphytic phyllosphere fungi played relatively minor roles in the development of decomposer fungal assemblages and the decomposition process. Fungal succession was related to the decomposition processes of leaves. Loss of leaf mass during the first month was attributed to the leaching and/or decomposition of polyphenols and soluble carbohydrates and to the decomposition of lignin and holocellulose. Lignin was decomposed during that period more selectively in bleached than in nonbleached areas. The decomposition from 1 to 9 months was primarily due to the decomposition of lignin and holocellulose in the bleached area. On the other hand, mass loss of lignin and holocellulose during that period was slower in the nonbleached leaf area. Pure culture decomposition tests showed that an unidentified species in the *Lachnocladiaceae*, isolated from the bleached leaf area, had the ability to cause selective loss of lignin and was responsible for the bleaching. *Amphisphaeriaceae* sp. had a cellulolytic ability, whereas the other major species caused negligible loss of litter mass in pure culture.

Key words: bleaching, decomposing ability, *Dipterocarpaceae*, fungi, lignin, nitrogen, tropical forest

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Introduction

Fungi play a central role in the decomposition of leaf litter because they are primary decomposers of lignin, which is a major structural component of litter and often limits decomposition processes (Berg and McClaugherty, 2003; Osono, 2007). Fungal decomposition of lignin often results in the production of bleached portions in leaf litter (Hintikka, 1970). Bleached leaf litter has been regularly encountered in tropical and temperate forest soils (Osono and Takeda, 2001a; Koide *et al*., 2005a,b; Osono *et al*., 2008a,b). Few studies, however, have examined the changes in bleached leaf area during decomposition in

tropical soils, and related them to fungal colonization and succession and to changes in chemical composition of the litter.

Decomposition of leaf litter in tropical forest soils is characterized by rapid disappearance of litter and accompanied release of nutrients (Swift *et al*., 1979; Swift and Anderson, 1989; Promputtha *et al*., 2002; Thongkantha *et al*., 2008). Loss of leaf litter mass on tropical soils is generally twice as fast as that in temperate forests (Takeda, 1998; Hirobe *et al*. 2004a; Kurokawa and Nakashizuka 2008). Nutrients such as nitrogen (N) and phosphorus are rapidly released from the litter, with little or no net gain in the nutrients (Swift *et al*., 1981; Anderson *et al*., 1983; Arunachalam *et al*., 1998; Torreta and Takeda, 1999; Hirobe *et al*., 2004a,b; Pandey *et al*., 2007). The rapid decomposition of leaf litter is associated with the removal of lignin (Musvoto *et al*., 2000; Hirobe *et al*., 2004b), attributable to the activity of decomposer organisms including fungi and termites (Lodge, 1997; Yamada *et al*., 2005). It is thus hypothesized that the colonization and bleaching of leaf litter by ligninolytic fungi is closely associated with the removal of lignin in tropical litter.

Hudson (1968) reviewed the ecology of saprobic fungi on leaf litter and found that species of *Nigrospora* and *Curvularia* are more commonly encountered as primary saprobes in the tropics than in temperate regions. These fungi appear on living and senescent leaves and become initial colonizers at leaf death, in advance of other saprobes that colonize following leaf fall. The patterns of fungal succession in the tropics, however, are generally similar to those in temperate regions. Since Hudson's work, several authors have investigated the succession of fungal assemblages in tropical leaves (Promputtha *et al*., 2002; Tokumasu and Aoki, 2002; Tang *et al*., 2005; Paulus *et al*., 2006; Duong *et al*., 2008; Kodsueb *et al*., 2008) and confirmed his findings.

The abilities of fungi to decompose leaf litter have been examined by pure culture tests, and temperate fungi have been grouped into ligninolytic, cellulolytic, and sugar fungi according to their abilities to cause mass loss of litter and lignin (Osono and Takeda, 2002, 2006; Osono *et al*., 2003a, 2006). In contrast, only a few studies have examined the functional biodiversity of tropical fungi in leaf litter decomposition (Pointing *et al*., 2003; Osono *et al*., 2008b), and we still know little about potential abilities of tropical fungi to decompose leaf litter. Understanding the decomposing ability of fungi is crucial to estimate causal relationships between fungal succession and decomposition of lignin in tropical leaf litter.

The purpose of the present study is to investigate the 9-month decomposition process of leaf litter of *Shorea obtusa* Wall. ex Blume in a tropical seasonal forest in northern Thailand. Litterbags were set up to examine simultaneously the succession of fungal assemblages and the changes in chemical composition. This enabled us to estimate possible relationships between fungal colonization and decomposition processes. Fungal succession and decomposition were followed in bleached and nonbleached leaf areas to examine the effect of colonization by ligninolytic and cellulolytic fungi in the overall process of decomposition. Leaf litter of *S. obtusa* was characterized by the frequent occurrence of bleached portions on the surface (Fig. 1) and was useful for the purpose of the present study. Fungi were also isolated from living leaves of *S. obtusa* to test the possibility that endophytic and epiphytic phyllosphere fungi on living leaves can persist after litterfall and take part in decomposition. Fungal species were then tested for their ability to decompose lignin and holocellulose in *S. obtusa* leaf litter under a pure culture condition to verify their contribution to decomposition in the field.

Materials and methods

Study site

Sampling was conducted at the Naresuan University Phayao Campus (14˚03'N, 100˚00'E), Phayao, in northern Thailand. The mean annual temperature is approximately 25˚C. This region experiences a dry season dominated by a typical tropical monsoon climate: a cool, dry season from November to February; a hot, dry season from March to May; and a warm, rainy season from May to October. The annual precipitation is 1155 mm of

Fig. 1. Bleached areas on a decomposing leaf of *Shorea obtusa*. Bar = 1 cm.

which more than 80% occurs during the rainy season. An experimental plot (100 × 100 m) was laid out within a dry dipterocarpus forest where *Dipterocarpus tuberculatus* Roxb., *D. turbinatus* Gaertn., *S. obtusa*, and *S. siamensis* Miq. dominated the basal area. Ten subplots (3 × 3 m) were randomly allocated in the plot and used for the litterbag experiment.

Decomposition experiment

Decomposition processes of *S. obtusa* leaves were studied over 9 months from May 2004 to February 2005 using the litterbag method (Osono and Takeda, 2001b). Newly shed leaves without obvious fungal or faunal attack were collected from the forest floor around the study plot in February 2004. The leaves were taken to the laboratory, oven-dried to a constant mass at 40˚C, and preserved in a vinyl bag until the experiment started. Five grams of leaves was enclosed in a litterbag (18 × 24 cm) made of polyethylene with a mesh size of approximately 2 mm. Eighty litterbags were prepared; 20 of these were oven-dried again to a constant mass at 40˚C and used to determine the initial mass and chemical composition. The remaining 60 litterbags were placed on the surface of the litter layer in May 2004, six bags per subplot. Litterbags were attached to the forest floor with metal pins to prevent movement or loss and to ensure good contact between the bags and litter layer. Sampling of the bags took place three times: at 1 (June 2004), 5 (October 2004), and 9 months (February 2005) after placement. On each sampling occasion, 20 bags were retrieved from the 10 subplots. The bags were placed in paper bags and taken to the laboratory. Leaves in 10 of the 20 bags were weighed, pressed between layers of plywood and paper, and dried to a constant mass at 40˚C. The remaining mass of the leaves was recorded as a percentage of the original mass. Moisture content of the leaves was determined as the amount of water divided by the oven-dried mass of the leaves and expressed as a percentage. Mean values of remaining mass and moisture content were calculated on each sampling occasion. The leaves were then used to determine the leaf area and chemical composition. Leaves enclosed in the other 10 bags were preserved at room temperature for 3 to 6 days and used for hyphal length estimation and fungal isolation as described below.

Measurement of leaf area and leaf mass per area

The leaves for leaf area measurement were photocopied and scanned with a photo scanner (EPSON GT-8000, Seiko Epson, Tokyo, Japan). Total leaf area and proportion of bleached leaf area were measured (Koide *et al*., 2005b) by image analysis on a Macintosh computer using public domain NIH image software (written by Wayne Rasband, US NIH). The proportion of bleached area versus total leaf area was expressed as a percentage and mean values were calculated on each sampling occasion. A 6 mm diam. cork borer was used to excise leaf disks from the bleached area and surrounding nonbleached area of the same leaves, excluding the primary vein. Three to 40 disks (16 disks on average) were taken from the leaves of each litterbag. The disks were oven-dried again to a constant mass at 40°C and weighed to calculate leaf mass per area (LMA). The remaining mass of bleached and nonbleached leaf tissue was calculated as LMA on each sampling occasion with respect to the initial LMA of undecomposed leaves, expressed as a percentage.

Chemical analysis

To compare chemical composition, the disks from 10 litterbags on each sampling occasion were combined to make one sample for each bleached and nonbleached area, ground in a laboratory mill to pass through a 0.5 mm screen, and used for chemical analysis. Lignin concentration of the ground leaf samples was estimated by gravimetry according to a standardized method using hot sulphuric acid digestion (King and Heath, 1967). Samples were extracted with alcohol-benzene at room temperature (15-20°C), and the residue was treated with 72% sulphuric acid (v/v) for 2 hours at room temperature with occasional stirring. The mixture was diluted with deionized water to make a 2.5% sulphuric acid solution and autoclaved at 120°C for 60 min. After cooling, the residue was filtered and washed with water through a porous crucible (G4), dried at 105°C, and weighed as acid-insoluble residue. The filtrate (autoclaved sulphuric acid solution) was used for total carbohydrate analysis. Total carbohydrate concentration was estimated using the phenol-sulphuric acid method (Dubois *et al*., 1956). One milliliter of 5% phenol (v/v) and 5 ml of 98% sulphuric acid (v/v) were added to the filtrate. The optical density of the solution was measured with a spectrophotometer at 490 nm, using known concentrations of D-glucose as standards. Soluble carbohydrates and polyphenols were extracted with 50% methanol, and their concentrations were estimated using the phenol-sulphuric acid and the Folin-Ciocalteau methods (Waterman and Mole, 1994), respectively. The holocellulose fraction was not determined by direct analysis, but was calculated as the difference between total carbohydrates and soluble carbohydrates. Total N concentration was measured using a combustion method with an automatic gas chromatograph (NC analyser SUMIGRAPH NC-900, Sumitomo Chemical, Osaka, Japan). The mass of organic chemical components and N per leaf area was calculated for bleached and nonbleached portions as the product of LMA and their concentrations. The remaining mass of each component was calculated as the mass of the component per area on each sampling occasion with respect to the initial mass of the component per area in undecomposed leaves and expressed as a percentage.

Hyphal length estimation

Hyphal lengths of the leaves were estimated using the agar-film method of Jones and Mollison (1948) but with several modifications (Osono *et al*., 2003b). Samples (1 g fresh weight per litterbag) were homogenized in a blender at 10, 000 rev/min in 49 ml of deionized water for 3 minutes. The suspension (20 ml) was diluted with 20 ml of molten agar solution (final concentration 1.5% (w/v)) and mixed at low speed on a magnetic stirring plate. Three agar films were prepared for each suspension in a haemocytometer (0.1 mm depth), transferred to a glass slide, and dried for 24 hours. The films were stained with fluorescent brightener (FB) for 1 hour. The FB binds to chitin in fungal cell walls (West, 1988) and allows all hyaline hyphae to be seen.

The stained films were mounted between slides and coverslips with one drop of immersion oil (type DF, Cargille Laboratories, Cedar Grove, NJ, USA) and examined with an epifluorescent microscope (Nikon Microphot-SA, Nikon, Tokyo, Japan) equipped with a high-intensity mercury light source. A Nikon UV-1A filter cube was used to examine FB-stained hyphae. Darkly pigmented hyphae that were not stained with FB were observed by bright-field microscopy. Microscope fields were selected randomly and 25 fields were observed for each slide at 1000× magnification. Hyphal lengths were estimated using an eyepiece grid and the grid-intersection method (Olson, 1950). Total hyphal length was calculated as the sum of FB-stained and darkly pigmented hyphal lengths.

Fungal isolation

A surface disinfection method (Kinkel and Andrews, 1988) and a modified washing method (Harley and Waid, 1955; Tokumasu, 1996) were applied to bleached and nonbleached portions and fungi were isolated following the methods of Osono (2005). Twenty leaves were chosen from 10 litterbags, two leaves per litterbag, on each sampling occasion. A sterile 6-mm diam. cork borer was used to excise leaf disks from the bleached and

nonbleached portions of the same leaves, making a total of 80 disks (40 bleached and 40 nonbleached disks) from the 20 leaves. Twenty bleached and 20 nonbleached disks were used for the surface disinfection method and the other 20 bleached and 20 nonbleached disks for the washing method.

Living leaves of *S. obtusa* were collected and used for fungal isolation to determine phyllosphere mycobiota and their roles in fungal succession and decomposition. Sampling of living leaves took place three times, in June and October 2004 and February 2005, on the same dates as the litterbag collections. On each sampling occasion, a total of 20 living leaves were retrieved at a height of 5 m, from five mature trees. The leaves were placed in paper bags and taken to the laboratory. A sterile 6 mm diam. cork borer was used to excise 40 leaf disks from the 20 living leaves. Twenty disks were used for the surface disinfection method and the other 20 disks for the washing method.

For surface disinfection, the leaf disks were submerged in 70% ethanol (v/v) for 30 seconds to wet the surface, and then surface-disinfected for 15 seconds in a solution of 15% hydrogen peroxide (v/v) and submerged for 30 seconds in 70% ethanol. The disks were rinsed with sterile, deionized water, transferred to sterile filter paper in 9 cm diam. Petri dishes, and dried for 24 hours to suppress vigorous bacterial growth after plating (Widden and Parkinson, 1973). The disks were placed (two per plate) on 9 cm diam. Petri dishes containing lignocellulose agar (LCA) modified by Miura and Kudo (1970). LCA contains glucose 0.1%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.02%, KCl 0.02%, $NaNO_3$ 0.2%, yeast extract 0.02%, and agar 1.3% (w/v). Note that LCA modified by Miura and Kudo (1970) does not contain lignin or other recalcitrant compounds.

For modified washing, the leaf disks were washed in a sterile test tube and agitated in a vertical shaker for 1.5 min. The disks were washed serially in five changes of 0.005% Aerosol-OT (di-2-ethylhexyl sodium sulfosuccinate) solution (w/v) and rinsed five times with sterile distilled water. The washed disks were treated in the same manner as that used in the plating-out procedure described for the surface disinfected leaves.

Plates were incubated at 20°C in darkness and observed at 3 days and at 1, 4, and 8 weeks after surface disinfection or washing (Osono and Takeda, 1999a). Any hyphae or spores on the plates were subcultured on fresh LCA plates, incubated, and identified. Pure stock cultures of isolates were maintained on slants of 1% malt-extracted agar (malt extract 1% (w/v) and agar 2% (w/v)). Identification was based on micromorphological observations, with reference to Domsch *et al*. (1980) and Ellis (1971, 1976). Two fungal morphotaxa were identified by DNA analysis by the method described by Hirose and Osono (2006). The taxa of *Lachnocladiaceae* species 5WS151 and *Amphisphaeriaceae* species 9BS21 were determined on the basis of the DNA sequences of rDNA ITS regions (ITS) amplified using PCR primers ITS5 and ITS4 (White *et al*., 1990) and of the 28S rRNA gene D1/D2 region using primers D1 (Peterson, 2000) and NL4 (O'Donnell, 1993). The DNA Data Bank of Japan (DDBJ) accession number for *Lachnocladiaceae* sp. 5WS151 is AB365532 for ITS and AB365539 for 28S, and that for *Amphisphaeriaceae* sp. 9BS21 is AB365534 for ITS.

The frequency of individual species was calculated as a percentage of incidences based on the number of leaf disks with the species on the 20 disks tested, on living leaves or bleached or nonbleached portions of decomposing leaves. A fungal taxon was arbitrarily regarded as a frequent taxon when the frequency of incidence of the taxon determined by either of the methods was more than or equal to 40% on living leaves or on the bleached or nonbleached area of decomposing leaves. Fungal taxa, other than the frequent species, were mentioned only when they were of special interest.

Pure culture decomposition test

Thirty-seven isolates of 28 species were used to assay individual decomposition rates under the pure culture decomposition test (Table 1). Thirty-four of the 37 isolates were obtained from living leaves or the bleached or nonbleached area of

Table 1. Mass loss of *Shorea obtusa* leaf litter, lignin, and total carbohydrates in the litter incubated with fungi at 20˚C for 12 weeks and lignin to carbohydrate loss ratio (L/C). Values indicate means ± standard errors (n = 4). nd not determined. *Bleaching was noticeable on leaf surfaces after the incubation.

Fungus	Code	Mass loss (% original mass)			
		Litter	Lignin	Total carbohydrates	L/C
Frequent fungi on *Shorea obtusa* leaves					
Amphisphaeriaceae sp.	9BS21	9.3±2.1	-14.0	15.5	-0.9
Pestalotiopsis sp.1	1WW131	4.1±0.7	nd	nd	nd
Colletotrichum gloeosporioides	6GS11	3.5±0.6	nd	nd	nd
Colletotrichum gloeosporioides	10GS191	2.3±0.9	nd	nd	nd
Nigrospora sp.	1WS72	2.6±1.0	nd	nd	nd
Nigrospora sp.	5WS161	1.4±1.2	nd	nd	nd
Talaromyces sp.	1WS15	1.9±0.9	nd	nd	nd
Clonostachys rosea	1WW43	1.6±0.5	nd	nd	nd
Clonostachys rosea	5BW84	0.5±0.6	nd	nd	nd
Aspergillus sp.	9WW61	0.9±2.4	nd	nd	nd
Aspergillus sp.	5WW61	0.1±2.5	nd	nd	nd
Aspergillus sp.	1WW73	-0.4±1.1	nd	nd	nd
Trichoderma asperellum	1WW101	0.7±0.9	nd	nd	nd
Trichoderma asperellum	5WS191	-1.0±0.8	nd	nd	nd
Cladosporium oxysporum	1WW21	0.2±0.7	nd	nd	nd
Infrequent fungi on *Shorea obtusa* leaves					
Lachnocladiaceae sp.*	5WS151	23.8±1.8	45.5	10.7	4.3
Eurotium sp.	5BS12	10.9±1.3	6.2	22.2	0.3
Xylaria sp.	0S112	10.3±1.6	-0.2	12.1	0.0
Xylaria sp.*	1BS142	9.3±0.6	7.4	14.5	0.5
Beltrania rhombica	9BW41	9.7±0.1	-4.6	21.4	-0.2
Geniculosporium sp.1*	10GS11	8.3±2.3	4.5	10.3	0.4
Beltraniella portoricensis	5BW105	5.3±0.2	-2.5	4.8	-0.5
Beltraniella portoricensis	1BS61	4.5±2.0	nd	nd	nd
Geniculospolium sp.2	0S53	2.8±1.8	nd	nd	nd
Geniculospolium sp.2	0S292	2.5±1.5	nd	nd	nd
Coelomycete sp.2	9BS31	2.8±0.7	nd	nd	nd
Cladosporium cladosporioides	0W222	2.8±2.1	nd	nd	nd
Trichoderma hamatum	9WW41	1.8±0.7	nd	nd	nd
Nodulisporium sp.1	0S52	1.7±1.0	nd	nd	nd
Mucor sp.	9WW103	0.9±1.0	nd	nd	nd
Eupenicillium cf. *senticosum*	5WS22	0.8±1.4	nd	nd	nd
Gliocladium virens	1WW44	0.7±1.1	nd	nd	nd
Gliocephalotrichum sp.	5WW142	-0.9±0.9	nd	nd	nd
Chaetomium globosum	5WS182	-1.7±0.7	nd	nd	nd
Fungi from culture collection					
*Trametes versicolor**	IFO30340	33.8±1.7	64.0	34.2	1.9
*Lentinula edodes**	IFO30721	19.4±3.0	18.4	21.6	0.9
Mycena polygramma	IFO33011	4.9±0.5	nd	nd	nd

decomposing leaves collected during the study period. The other three isolates *Trametes versicolor* (L.) Lloyd, *Lentinula edodes* (Berk.) Pegler (wood-decay fungi) and *Mycena polygramma* (Bull.) Gray (litter-decomposing fungi) were obtained from a culture collection (IFO/NBRC, Chiba, Japan). The three isolates were used in previous decomposition tests (Osono and Takeda, 2002, 2006; Osono *et al*., 2003a, 2006) and included in the present study for comparisons. Newly shed leaves of *S. obtusa* without obvious fungal or faunal attack were collected from the forest floor around the study plot in February 2004. The leaves were cut into strips 1 cm wide, oven-dried at 40°C to a constant mass, and preserved in a vinyl bag until the experiment started.

The leaf litter (ca. 300 mg) was sterilized by exposure to ethylene oxide gas at 60°C for 6 hours. The sterilized litter was placed on the surface of 9 cm diam. Petri dishes containing 20 mL of 2% agar. Using a sterile 6 mm diam. cork borer, inocula for each assessment were excised from the margin of a colony actively growing on previously inoculated Petri dishes containing 2% malt-extracted agar. Inocula were placed on the agar adjacent to litter strips, one plug per plate. The plates were sealed with paraffin film and incubated for 12 weeks at 20°C in darkness. After incubation, the litter strips were retrieved, oven-dried at 40°C to a constant mass, and weighed. The undecomposed initial litter was also sterilized, oven-dried at 40°C to a constant mass, and weighed to determine original mass. Four plates were prepared for each isolate, and four uninoculated plates served as a control. Occurrence of bleaching on the surface of decomposed litter was observed under a binocular microscope (40×). Loss of litter mass was determined as a percentage of the original mass, taking the mass loss of control litter into consideration. Duplicated litter strips were combined and used for chemical analyses as described above. Chemical analyses were performed for those fungi that caused more than 5% loss of litter mass.

Lignin/carbohydrate loss ratio (L/C) is a useful index of substrate-use patterns in fungal species (Osono *et al*., 2006) and was calculated according to the equation:

L/C = loss of lignin mass (percentage original lignin mass) / loss of total carbohydrate mass (percentage original total carbohydrate mass)

Data analysis

The decomposition rate of litter was calculated as Olson's *k* (Olson, 1963) according to the following equation:

$W_t = W_0 \times \exp(-kt)$

where W_t is the litter mass after a given period, W_0 is the original litter mass, t is the time in year, and *k* is the decomposition rate.

One-way analysis of variance was used to evaluate the difference in remaining mass of litter, moisture content of litter, and total hyphal length between sampling dates. The Tukey's honestly significant difference (HSD) test was used for multiple comparisons. A χ^2 test was applied to examine the difference in frequency of individual fungal taxa between bleached and nonbleached area of decomposing leaves because the data were in the form of percentages.

Results

Overall patterns of decomposition, bleached area, and hyphal length

In the field incubation of leaf litter, approximately 57% of the original mass of the whole litter remained at the end of the 9-month study (Table 2). The decomposition rate (Olson's *k*) over the period was 0.738 (r^2 = 0.876, n = 4). The moisture content of litter was significantly greater at 1 month in the rainy season than at 5 and 9 months in the dry season (Table 2). Bleached leaf area accounted for 1% of total leaf area during the first month and then increased thereafter to 30% at the end of 9 months (Table 2). Total hyphal length increased fivefold, from 826 m/g to 4045 m/g, during the first 5 months and then decreased significantly to 2498 m/g at the end of the period (Table 2).

Fungal succession

Eighty fungal taxa were isolated from living and decomposing leaves of *S. obtusa* (Table 4). The number of fungal taxa was generally lower on living leaves than on decomposing leaves (Fig. 2). In general, the number of fungal taxa recorded from 20 leaf disks with the washing method was greater than that with the surface disinfection method (Fig. 2). The number of fungal taxa on living leaves isolated with the washing method remained relatively constant on three sampling dates, whereas, with the surface disinfection method, there were fewer fungal taxa in June than in October and February. The number of fungal taxa decreased during decomposition of litter after the washing method, whereas it increased during decomposition after the surface disinfection method (Fig. 2). The number of fungal taxa was similar on the bleached and nonbleached area of decomposing leaves (Fig. 2).

Table 2. Changes in remaining mass, moisture content, bleached area, and total hyphal length in decomposing leaves of *Shorea obtusa*. Values indicate means ± standard errors (n = 10). The same letters are not significantly different at 5% level by Turkey's HSD test. nd not determined.

	Date and month of decomposition			
	May 04	**Jun 04**	**Oct 04**	**Feb 05**
	0	**1**	**5**	**9**
Remaining mass (% original mass)	100	83±3 a	77±5 a	57±7 b
Moisture content (%)	nd	146±8 a	31±6 b	35±3 b
Bleached area (% remaining area)	0	1±0 c	15±2 b	30±3 a
Total hyphae length (m/g dry litter)	826±86 c	3391±273 a	4045±236 a	2498±164 b

Cluster analysis revealed a primary difference in fungal assemblages between living (Group I) and decomposing leaves (Groups II and III) (Fig. 3). The fungal assemblages on decomposing leaves were further divided into those examined with the washing method (Group II) and those with the surface disinfection method (Group III). Each of Groups II and III was further divided into fungal assemblage after 1 month of decomposition and those at 5 and 9 months. Fungal assemblages were relatively similar on the bleached and nonbleached area of decomposing leaves.

Nine frequent species were recorded on the living and decomposing leaves of *S. obtusa* (Fig. 4). Five of these were common on living leaves (Fig. 4). *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. was frequently isolated from leaves treated with the surface disinfection and washing methods, *Nigrospora* sp. was isolated more frequently from leaves treated with the surface disinfection method, and *Pestalotiopsis* sp.1, *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams, and *Cladosporium oxysporum* Berk. & M.A. Curtis were isolated more frequently from the washing method treatment (Fig. 4).

Six species were common on decomposing leaves (Fig. 4). *Trichoderma asperellum* Samuels, Lieckfeldt & Nirenberg and *Aspergillus* sp. were occurred most commonly with the washing method treatment on the bleached and nonbleached area. The frequencies of *Nigrospora* sp., *Cladosporium oxysporum*, and *Talaromyces* sp. decreased, whereas that of an unidentified species of the *Amphisphaeriaceae*, designated as *Amphisphaeriaceae* sp., increased during decomposition. The frequency of *Nigrospora* sp. on decomposing leaves was significantly greater (χ^2 test, $P < 0.05$) on bleached area than on nonbleached area, whereas no significant ($P > 0.05$) differences were found for the other five major species on decomposing leaves between the bleached and nonbleached area. An unidentified species of the *Lachnocladiaceae*, designated as *Lachnocladiaceae* sp., was isolated from bleached area after 5 months but with a mean frequency of 2% (Appendix).

Decomposition and chemical changes in bleached and nonbleached portions

In the field incubation of leaf litter, loss of LMA and loss of organic chemical components and N mass were generally faster in the bleached than in the nonbleached leaf area (Fig. 5). Relatively rapid loss of LMA was observed in both the bleached and the nonbleached area during the first month compared with the rest of the period. This rapid loss was attributable to the leaching and/or decomposition of polyphenols and soluble carbohydrates and the decomposition of lignin and holocellulose. During the first month, lignin was decomposed more and holocellulose was decomposed less in the bleached than in the nonbleached area, and loss of N mass was similar for the bleached and nonbleached area. During 1 to 9 months, both lignin and holocellulose were decomposed in the bleached area, whereas net increase of lignin mass and negligible loss of holocellulose mass occurred in the nonbleached area. During that period, there was a net loss of N mass in the bleached area, whereas a net increase (1-5 months) and net decrease (5-9 months) of N mass occurred in the nonbleached area.

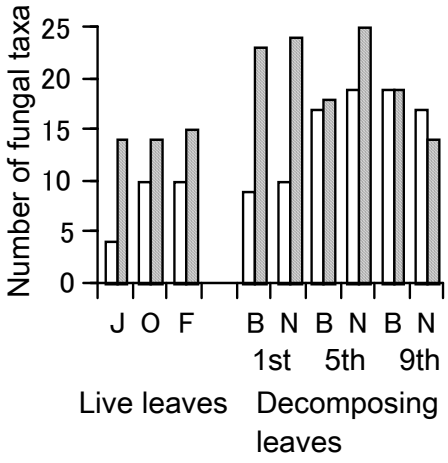

Fig. 2. Changes in taxa number on living and decomposing leaves of *Shorea obtusa*. *Open bars* surface disinfection method, *shaded bars* washing method. J June, O October, F February. B bleached portions, N nonbleached portions. The 1st, 5th, and 9th indicate the months of decomposition on the forest floor.

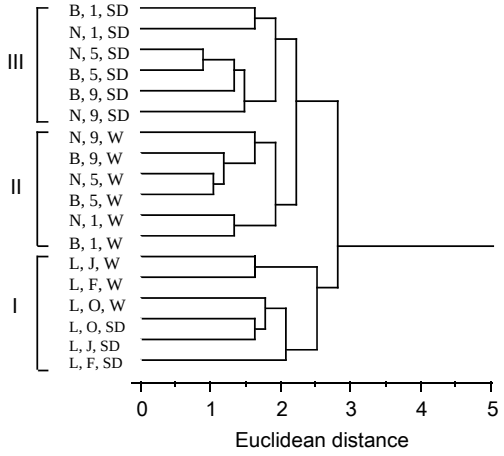

Fig. 3. Dendrogram of fungal assemblages on *Shorea obtusa* leaves by the average method using the pair-group method using arithmetic average with Euclidean distance. Groups identified are depicted by brackets. L living leaves, B bleached area of decomposing leaves, N nonbleached area of decomposing leaves. J June, O October, F February. The numbers 1, 5, and 9 indicate the months of decomposition on the forest floor. SD surface disinfection method, W washing method. 'L, F, SD', for example, indicates the fungal assemblage on living leaves examined in February with the surface disinfection method; 'B, 1, W' indicates the fungal assemblage on bleached area of decomposing leaves collected at 1-month decomposition and examined with the washing method.

Initial concentrations of lignin, holocellulose, polyphenols, soluble carbohydrates, and N were 359, 338, 102, 77, and 6 mg/g, respectively (Table 3). Lignin concentration decreased in the bleached area during decomposition, whereas it increased in the nonbleached area. Holocellulose concentration decreased in the nonbleached area during decomposition, whereas it increased during the first month in the bleached area and then decreased to reach a similar level to the nonbleached area at 9 months. Generally, concentrations of polyphenols and soluble carbohydrates were lower in the bleached than in the nonbleached area and decreased rapidly during the first month and decreased slowly thereafter. Nitrogen concentration showed an increasing trend during decomposition and was greater in the bleached than in the nonbleached area.

Decomposing ability of individual fungal species

The loss of leaf litter mass caused by 37 isolates ranged from -1.7% to 33.8% (Table 1). Of 15 isolates of the nine frequent species on *S. obtusa* leaves, *Amphisphaeriaceae* sp. 9BS21 showed 9.3% loss of litter mass, but the other 14 isolates of the frequent species showed less than 5% loss of litter mass. Seven isolates of six infrequent species, i.e. *Lachnocladiaceae* sp. 5WS151, *Eurotium* sp. 5BS12, *Xylaria* sp. 0S112 and 1BS142, *Beltrania rhombica* Penz. 9BW41, *Geniculosporium* sp. 1 10GS11, and *Beltraniella portoricensis* (F. Stevens) Piroz. & S.D. Patil 5BW105, caused a loss of litter mass between 5% and 26%. Bleaching of the litter was noticeable on leaves inoculated with *Lachnocladiaceae* sp. 5WS151, *Xylaria* sp. 1BS142, and *Geniculosporium* sp.1 10GS11,

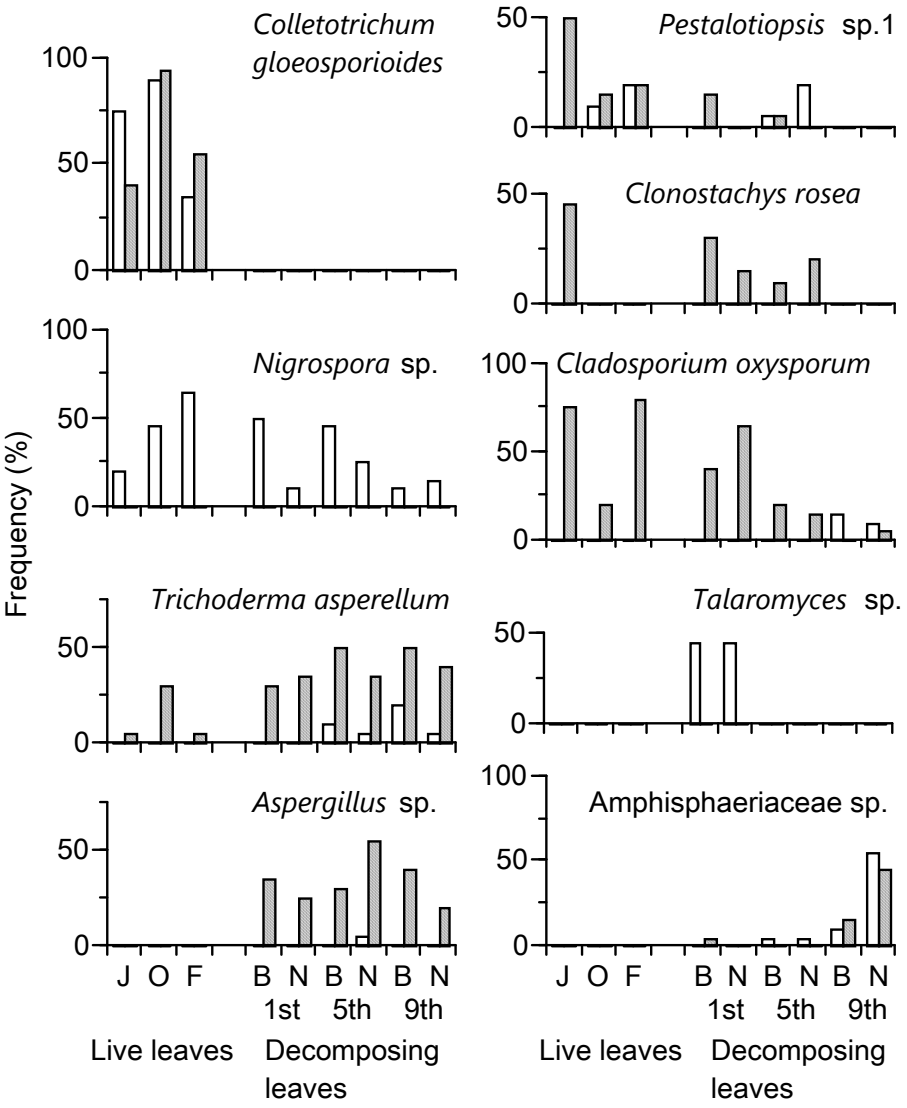

Fig. 4. Changes in frequency of major fungal species on living and decomposing leaves of *Shorea obtusa*. Symbols are as in **Fig. 2.**

isolated from *S. obtusa* leaf litter, and *Trametes versicolor* and *Lentinula edodes* from a culture collection. In contrast, no isolates of the nine frequent species from fresh *S. obtusa* leaves caused bleaching on leaf litter. *Lachnocladiaceae* sp. 5WS151 caused the greatest loss of lignin mass of fungi from *S. obtusa* leaves, followed by *Trametes versicolor* (Table 1). *Eurotium* sp. 5BS12, *Xylaria* sp. 1BS142, and *Geniculosporium* sp.1 10GS11 also caused loss of lignin mass. *Trametes versicolor* caused the greatest loss of total carbohydrate mass, followed by *Lentinula edodes*, *Eurotium* sp. 5BS12, *Beltrania rhombica* 9BW41, and *Amphisphaeriaceae* sp. 9BS21 (Table 1). *Lachnocladiaceae* sp. 5WS151 showed the greatest amount of L/C (4.3), which was greater than that for *Trametes versicolor* (L/C of 1.9) (Table 1). The other isolates that caused more than 5% loss of litter mass showed L/C values between -0.9 and 0.9 (Table 1).

Discussion

Fungi responsible for the bleaching

In the pure culture test, *Lachnocladiaceae* sp. 5WS151 caused selective loss of lignin from *S. obtusa* leaves (Table 1). Its L/C value of 4.3 is similar to that of some basidiomycetes and ascomycetes that cause selective decomposition of lignin over holocellulose and significant loss of litter mass in other litter types (Lindeberg, 1946; Osono and Takeda, 2002, 2006; Osono *et al*., 2003a,

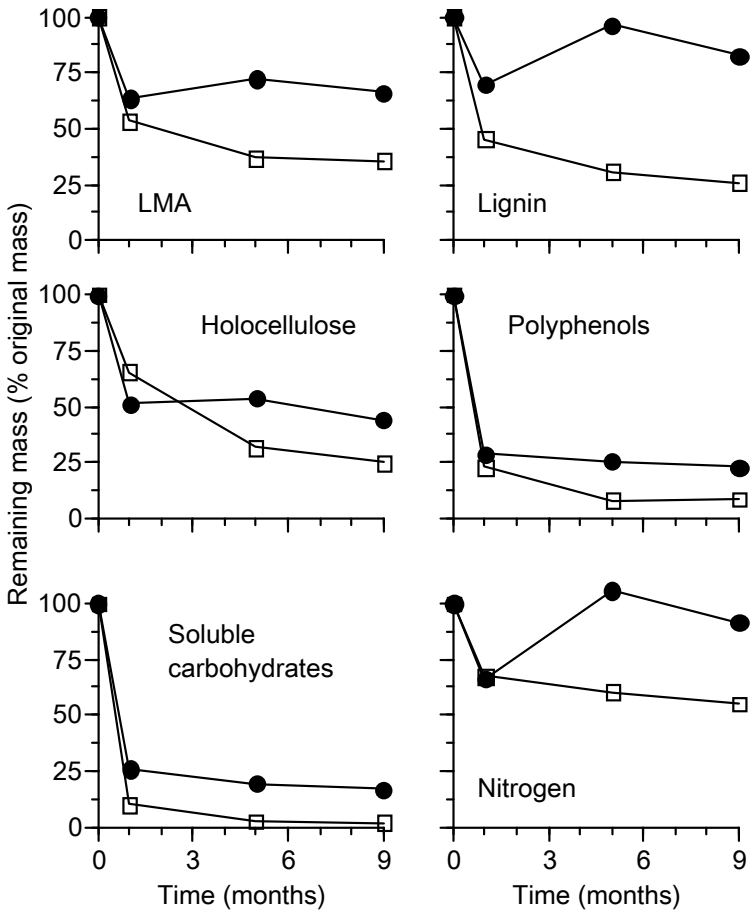

Fig. 5. Changes in leaf mass per area (LMA) and mass per area of lignin, holocellulose, polyphenols, soluble carbohydrates, and nitrogen in bleached (open box) and nonbleached portions (filled circle) of decomposing leaves of *Shorea obtusa*.

2006, 2008a,b; Koide *et al*., 2005b). The ability of *Lachnocladiaceae* sp. 5WS151 to cause selective loss of lignin and the occurrence of this taxon on bleached leaf area (Appendix) suggest that this fungus was responsible for the bleaching of *S. obtusa* leaves. The increase of bleached leaf area during decomposition may also confirm that lignin decomposition was associated with colonization by basidiomycetes in the later stages of decomposition (Hudson, 1968). The low frequency of occurrence of this fungus is possibly due to the method of fungal isolation which favors the fast-growing anamorphic and ascomycetous species. The same *Lachnocladiaceae* sp. was also frequently isolated from bleached portions of *Ptercarpus macrocarpum* Kurz. leaves in a dry diptero-carpus forest in north-eastern Thailand, approximately 500 km from the study site (T. Osono, unpublished data). Osono *et al*. (2008b) reported that another unidentified species of *Lachnocladiaceae* was also responsible for the bleaching of *Castanopsis sieboldii* (Makino) Hatusima leaf litter in a subtropical forest in southern Japan. This subtropical species belongs to the same family as the Thai species, but is probably a different species, because the homology of the DNA sequences of the 28S rRNA gene D1/D2 region between them was 95%.

Xylaria sp. 1BS142, *Geniculosporium* sp. 1 10GS11, and *Eurotium* sp. 5BS12 also caused loss of lignin mass under the pure culture (Table 1). Species in *Xylaria* and *Geniculosporium* have the potential ability to bleach litter but generally decompose holocellulose in preference to lignin (Osono and Takeda, 2002, 2006; Osono *et al*., 2003a,

Table 3. Concentrations of lignin, holocellulose, polyphenols, soluble carbohydrates, and nitrogen in the bleached and nonbleached portions of decomposing leaves of *Shorea obtusa*. Values are in mg/g dry litter. na not applicable.

	Months of decomposition			
Component	**0**	**1**	**5**	**9**
Lignin (mg/g)				
Bleached portion	na	305	300	261
Nonbleached portion	359	393	477	450
Holocellulose (mg/g)				
Bleached portion	na	414	291	237
Nonbleached portion	338	272	250	226
Polyphenols (mg/g)				
Bleached portion	na	44	23	25
Nonbleached portion	102	46	36	35
Soluble carbohydrates (mg/g)				
Bleached portion	na	14	5	4
Nonbleached portion	77	31	20	20
Nitrogen (mg/g)				
Bleached portion	na	7.6	9.8	9.3
Nonbleached portion	6.0	6.2	8.8	8.3

2006, 2008a,b; Koide *et al*., 2005b).and Takeda, 2002, 2006; Osono *et al*., 2003a, 2006, 2008b; Koide *et al*., 2005b). As these three fungal taxa occurred on the bleached leaf area (Appendix), they may take part in the lignin decomposition and the bleaching of *S. obtusa* leaves, but their roles in lignin decomposition may be minor compared with that of *Lachnocladiaceae* species.

Amphisphaeriaceae sp. 9BS21, *Beltrania rhombica* 9BW41, and *Beltraniella portoricensis* 5BW105 cause selective loss of holocellulose and are regarded as cellulolytic (Table 1). The other eight frequent fungi on *S. obtusa* leaves, except *Amphisphaeriaceae* sp., caused negligible loss of litter mass (Table 1), indicating that these fungi may play a minor role in loss of litter mass during decomposition, despite their high frequency of occurrence. Functionally, these fungi may be regarded as 'sugar fungi' and their growth may depend on readily available energy sources such as soluble carbohydrates (Hudson, 1968).

Fungal succession

Fungal assemblages on living leaves (Group I) were characterized by the frequent occurrence of *Colletotrichum gloeosporioides*, *Pestalotiopsis* sp. 1, *Nigrospora* sp., *Clonostachys rosea*, and *Cladosporium oxysporum* (Fig. 4). Species in these five genera are common components of tree phyllosphere mycobiota in temperate (Osono, 2002; Osono *et al*., 2004; Koide *et al*., 2005a) and tropical forests (Cannon and Simmons, 2002; Suryanarayanan *et al*. 2002; Murali *et al*., 2007; Osono *et al*., 2008b). Promputtha *et al*. (2005) also reported the occurrence of *Glomerella* (the teleomorph of *Colletotrichum*) on living leaves of *Magnolia liliifera* in Chiang Mai, Thailand, approximately 70 km from the study site. *Nigrospora* sp. and *Cladosporium oxysporum* are 'primary saprobes' of tropical tree leaves (Hudson, 1968; Osono, 2006), as they occurred frequently on recently fallen leaf litter but disappeared as decomposition progressed (Fig. 4) and had negligible ability to cause loss of litter mass (Table 1). The frequency of *Nigrospora* sp. was greater in the bleached than in the nonbleached leaf areas, suggesting that this fungus was an associate species relying for survival on carbohydrates delignified by *Lachnocladiaceae* sp. and other ligninolytic fungi in decomposing leaves. However, cluster analysis showed a primary difference in fungal assemblages between living and decomposing leaves (Fig. 3), suggesting relatively minor roles for endophytic and epiphytic phyllosphere fungi in the development of decomposer fungal assemblages and the decomposition processes of *S. obtusa* leaf litter. Using molecular phylogenetic analyses, in contrast, Promputtha *et al*. (2007) showed the incidence of the species in *Colletotrichum*, *Fusarium*,

Phomopsis, and *Phyllosticta* as both endophytes and saprobes. More studies are necessary regarding the importance of phyllosphere fungi on living leaves in the decomposition of leaf litter in tropical forests.

The cluster analysis showed a secondary difference in fungal assemblages on decomposing leaves between the washing and surface disinfection methods (Fig. 3). These methods are considered suitable for isolating fungi colonizing the surface and interior of substrata, respectively (Osono and Takeda, 1999b), suggesting that the overall patterns of succession were different between the surface and the interior of *S. obtusa* leaves (Fig. 3). This is consistent with fungal successions studied with these isolation methods (Tokumasu, 1998a,b; Osono and Takeda, 2001b; Osono, 2005). The frequent occurrence of species in such genera as *Trichoderma* and *Aspergillus* are repeatedly isolated in the study of fungal succession of decomposing leaves (Hudson, 1968). This may be due to the isolation method used in this study as these fungi are not common when direct studies of leaves are followed (Promputtha *et al*., 2002, 2004; Hyde and Soytong, 2007; Duong *et al*., 2008; Kodsueb *et al*., 2008; Thongkantha *et al*., 2008). The succession of the major species on the bleached and nonbleached leaf area of *S. obtusa* possibly plays a minor role in decomposition as they had the ability to cause a negligible loss of litter mass (discussed above). The exception is *Amphisphaeriaceae* sp., which had the potential to contribute significantly to the loss of holocellulose, on the bleached and nonbleached leaf area, at the 9 month of decomposition.

Decomposition processes

A decomposition rate of 0.738 year^{-1} is within the range of litter decomposition rates previously reported from tropical forests (Takeda *et al*., 1984). Decomposition is often inhibited during dry seasons compared with rainy seasons in tropical seasonal forests (Swift and Anderson, 1989), but such a retarding effect of seasonal drought was not obvious in the present study. The 9 months of decomposition of *S. obtusa* leaves was divided into two stages characterized by different, dominant organic chemical constituents. The relatively rapid loss of soluble carbohydrates and polyphenols during the first months of decomposition was consistent with previous studies (Hirobe *et al*., 2004b; Osono and Takeda, 2005). The loss of lignin and holocellulose during the first few months of decomposition contrasts to a general model of litter decomposition in temperate forests where holocellulose was decomposed more rapidly than lignin in the early stages of decomposition (Berg, 1986; McTiernan *et al*., 2003; Osono and Takeda, 2005) but was consistent with the results of decomposition studies carried out in tropical forests (Musvoto *et al*., 2000; Hirobe *et al*., 2004b).

The decomposition of *S. obtusa* leaf litter from 1 to 9 months was due to the expansion of the bleached portions and the decomposition of lignin and holocellulose in the bleached area, whereas loss of leaf tissue mass was slower in the nonbleached leaf area during that period. To our knowledge, the study of Koide *et al*. (2005b) is the only one that examined the changes in bleached leaf area during decomposition of *Camellia japonica* L. leaves in a temperate forest. Contrary to *S. obtusa* leaves, the bleached area in *C. japonica* leaves increased rapidly to reach 17% during the first 2 months and decreased gradually thereafter to 2% after 18 months (Koide *et al*., 2005b). Endophytic ascomycetes capable of colonizing freshly fallen leaves as prior colonizers and removing lignin selectively were responsible for the bleaching of *C. japonica* leaves (Koide *et al*., 2005a). The difference in the patterns of occurrence of bleached portions between *S. obtusa* and *C. japonica* can be attributed to the taxonomic group and life cycle strategy of ligninolytic fungi. In *S. obtusa* leaves, *Lachnocladiaceae* sp. colonizing litter after litterfall was probably responsible for the bleaching (discussed above). Termites also take part in the disappearance and loss of leaf mass in tropical seasonal forests (Yamada *et al*., 2005). The contribution of termites to the decomposition of *S. obtusa* leaves is not evaluated in the present study, but Torreta and Takeda (1999) estimated that termites accounted for 10-15% of leaf area loss in a tropical hill evergreen forest in northern Thailand.

Table 4. Mean frequency (%) of fungal taxa isolated from livingand decomposing leaves of *Shorea obtusa* with surface disinfection (SD) and washing methods (W).

	Living leaves		**Decomposing leaves, bleached leaf area**		**Decomposing leaves, nonbleached leaf area**	
Fungus	**SD**	**W**	**SD**	**W**	**SD**	**W**
Colletotrichum gloeosporioides	67	63	0	0	0	0
Ascomycete sp.1	23	12	0	0	0	0
Ascomycete sp.2	17	13	0	0	0	0
Phomopsis sp.	10	10	0	0	0	0
Ascomycete sp.3	8	0	0	0	0	0
Geniculosporium sp.2	3	0	0	0	0	0
Chloridium lignicola	2	0	0	0	0	0
Geniculosporium sp.3	2	0	0	0	0	0
Phyllosticta sp.	2	0	0	0	0	0
Cladosporium cladosporioides	2	7	0	0	0	0
Aphanocladium sp.?	0	7	0	0	0	0
Coniothyrium sp.	0	7	0	0	0	0
Lechanicillium psalliotae	0	3	0	0	0	0
Cladosporium sp.	0	2	0	0	0	0
Nigrospora sp.	43	0	35	0	17	0
Geniculosporium sp.1	15	2	0	0	3	0
Nodulisporium sp.	15	2	12	0	13	0
Acremonium spp.	0	2	5	0	2	0
Eupenicillium cf. *senticosum*	0	2	7	0	17	0
Pestalotiopsis sp.1	10	28	2	7	7	0
Cladosporium oxysporum	0	58	5	20	3	28
Trichoderma asperellulm	0	13	10	43	3	37
Fusarium sp.2	0	2	10	7	3	5
Beltrania rhombica	0	3	5	3	2	15
Penicillium citrinum	0	2	0	0	2	2
Fusarium sp.1	0	2	0	5	2	12
Verticillium spp.	0	7	0	7	2	15
Penicillium citreonigrum	0	17	0	7	0	12
Clonostachys rosea	0	15	0	13	0	12
Penicillium corylophilum	0	8	0	2	0	13
Phoma sp.	0	3	0	0	0	2
Coelomycete sp.1	0	0	17	0	0	0
Talaromyces sp.	0	0	15	0	15	0
Coelomycete sp.2	0	0	10	0	5	0
Eurotium spp.	0	0	7	0	7	0
Penicillium sp.2	0	0	3	0	0	0
Lachnocladiaceae sp.	0	0	2	0	0	0
Nigrospora sphaerica	0	0	2	0	0	0
Phialophora sp.	0	0	2	0	0	0
Subulispora sp.	0	0	2	0	0	0
Chaetomium sp.1	0	0	2	0	2	0
Chaetomium sp.2	0	0	2	0	2	0
Xylaria sp.	0	0	0	0	5	0
Arthrinium phaeospermum	0	0	0	0	2	0
Arthroconidial sp.	0	0	0	0	2	0
Scolecobasidium humicola	0	0	0	0	2	0
Chaetomium globosum	0	0	18	0	5	2
Trichoderma longibrachiatum	0	0	10	18	3	18
Amphisphaeriaceae sp.	0	0	5	7	20	15
Trichoderma hamatum	0	0	7	20	0	20
Clonostachys sp.1	0	0	2	8	3	3
Pestalotiopsis sp.2	0	0	2	0	3	3
Penicillium miczynskii	0	0	2	0	2	2

Table 4 (continued). Mean frequency (%) of fungal taxa isolated from livingand decomposing leaves of *Shorea obtusa* with surface disinfection (SD) and washing methods (W).

	Living leaves		Decomposing leaves, bleached leaf area		Decomposing leaves, nonbleached leaf area	
Fungus	**SD**	**W**	**SD**	**W**	**SD**	**W**
Mucor spp.	0	0	2	15	0	8
Gliocephalotrichum sp.	0	0	2	12	0	3
Gliocladium virens	0	0	2	7	0	7
Beltraniella portoricensis	0	0	0	3	10	10
Aspergillus sp.	0	0	0	35	2	33
Scolecobasidium sp.	0	0	0	0	2	2
Trichoderma sp.4	0	0	0	7	0	0
Gonytrichum macrocladum	0	0	0	5	0	0
Paecilomyces variotii	0	0	0	5	0	0
Clonostachys sp.3	0	0	0	3	0	0
Absidia cylindrospora	0	0	0	2	0	0
Clonostachys sp.2	0	0	0	2	0	0
Curvularia senegalensis	0	0	0	2	0	0
Gliomastix felina	0	0	0	2	0	0
Penicillium janthinellum	0	0	0	2	0	0
Stachybotrys sp.	0	0	0	2	0	0
Trichoderma sp.5	0	0	0	2	0	0
Myrothecium verrucaria	0	0	0	10	0	7
Penicillium simplicissimum	0	0	0	7	0	3
Penicillium montanense	0	0	0	3	0	2
Penicillium sp.1	0	0	0	3	0	3
Umbelopsis isabellina	0	0	0	3	0	2
Arthrobotrys oligospora	0	0	0	2	0	2
Penicillium cf. *adametzioides*	0	0	0	2	0	2
Geniculosporium sp.4	0	0	0	0	0	2
Mucoraceous sp.	0	0	0	0	0	3
Pseudobotrytis sp.	0	0	0	0	0	2

Decomposition in bleached and nonbleached leaves

The faster decomposition of leaf tissue in bleached leaf area compared with nonbleached area is consistent with the results of *C. japonica* leaves (Koide *et al*., 2005b). Lignin protects holocellulose from the action of cellulases so that delignification enhances the hydrolysis of carbohydrates (Cooke and Whipps, 1993). Thus, the delignification of *S. obtusa* leaves can enhance the availability of holocellulose to decomposers, accounting for the further loss of both lignin and holocellulose in bleached leaf area during decomposition (Osono and Hirose 2009). The decrease in N concentration and net loss of N in the bleached leaf area support the results of previous work suggesting that N dynamics are closely associated to lignin decomposition (Berg and McClaugherty, 1989; Osono and Takeda, 2004). In contrast to the bleached leaf area, the mass of lignin increased in relation to the net immobilization of N in nonbleached leaf area, leading to negligible loss of holocellulose and whole leaf tissue (Fig. 2). This pattern is consistent with a general model of litter decomposition in temperate forests (Berg, 1986; Osono and Takeda, 2005). The comparison of decomposition between bleached and non-bleached leaf area thus showed that the removal of lignin in the bleached area was a dominant process promoting the 9-month decomposition of *S. obtusa* leaves in the study site.

The examination of decomposition on the bleached portions of *S. obtusa* leaves and the fungi responsible for the bleaching provides useful insights into the biological aspect of decomposition in this tropical forest. In *S. obtusa* leaf litter, the effective colonization of the leaves by ligninolytic fungi can promote decomposition. It appears that the colonization

of leaves by ligninolytic fungi evaluated as the bleached leaf area was generally more effective in tropical than in temperate forests (Koide *et al*., 2005a; Osono *et al*., 2008a,b). This is consistent with previous findings that decomposition was faster and lignin was decomposed more actively in tropical forests than in temperate forests (Takeda, 1998; Hirobe *et al*., 2004a,b). However, data are still lacking on the occurrence of bleached leaf area and more studies are needed regarding the ecology and functional roles of ligninolytic fungi in leaf litter decomposition in tropical forests.

Recent studies have applied molecular techniques to evaluate fungal diversity on decomposing leaves. Duong *et al*. (2006) used Denaturing Gradient Gel Electrophoresis (DGGE) analysis to assess fungal diversity on leaves of *Magnolia liliifera* and found that this approach identified fungal taxa that were not recovered from traditional cultural and morphological studies but did not detect taxa that were predominantly isolated using traditional methods. Seena *et al*. (2008) extracted DNA from stream-exposed leaves to amplified rDNA sequences and construct clone libraries and found that traditional studies can underestimate fungal diversity on leaves. Therefore, these molecular methods appear to be useful to detect unculturable fungi. Demonstrating the presence of a fungal species, whether by molecular or traditional techniques, however, does not necessarily indicate its contribution to decomposition processes. Alternatively, Luis *et al*. (2005) and Blackwood *et al*. (2007) have developed molecular methods to study the diversity in litter and soils of fungal genes encoding laccases that catalyze the oxidation of aromatic substrates and are responsible for lignin decomposition. Application of these molecular methods to, for example, bleached portion of leaf litter, coupled with the verification of ligninolytic potentialities of major fungal taxa in the laboratory experiments, will be promising in the evaluation of biodiversity and functioning of fungal assemblages on leaf litter.

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