Colonization and decomposition of leaf litter by ligninolytic fungi in *Acacia mangium* plantations and adjacent secondary forests

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Abstract

Colonization of leaf litter by ligninolytic fungi and relationships between mass loss and chemical qualities of surface leaf litter were examined in *Acacia mangium* plantations and adjacent secondary forests in southern Sumatra Island, Indonesia. Leaves were collected from eight *A. mangium* plantations of different ages and three secondary forests. Partly decomposed leaves beneath the surface leaf litter were used to measure the bleached area which indicated colonization by ligninolytic fungi. Surface leaf litter was used to measure initial chemical content and subjected to the pure culture decomposition test. Bleached area was greater in secondary forests than in *A. mangium* plantations. Nitrogen content was higher in all the *A. mangium* plantations than in the secondary forests, and acid unhydrolyzable residue (AUR) content was generally higher in the *A. mangium* plantations than in the secondary forests. Bleached area of leaf litter was negatively correlated with nitrogen content of surface leaf litter at all sites, indicating an inhibition of the colonization by ligninolytic fungi of leaves with higher nitrogen content. In pure culture decomposition test inoculating a ligninolytic fungus to surface leaf litter, mass loss of leaves was negatively correlated with AUR content of surface leaf litter. Mass loss of leaves and AUR was not significantly related to nitrogen content. These results

suggested that higher nitrogen content in *A. mangium* leaf litter had a negative effect by colonization of ligninolytic fungi, but the effect of high N in *A. mangium* leaf litter on the decomposition of leaf litter and AUR remained unsolved.

Key words

Acacia mangium, bleach, decomposition, ligninolytic fungi, nitrogen

1 Introduction

2

3 *Acacia mangium* is a legume tree that is symbiotic with rhizobia and has an
4 ability to fix atmospheric nitrogen (N). Because of its rapid growth and high
5 quality timber production, *A. mangium* has commonly been planted in
6 tropical regions. *Acacia mangium* produces N-rich leaves (Tilki and Fisher
7 1998, Akinnifesi et al. 2002) leading to changes in soil N dynamics in
8 plantations. For example, Yamashita et al. (2008) reported that soil acidity
9 was higher in *A. mangium* plantations compared with grasslands, in relation
10 to the base cation loss from the soil profile associated with high leaching of
11 nitrate anions (Binkley and Giardina 1997). Arai et al. (2008) and Konda et
12 al. (2008, 2010) reported that N_2O emission from forest soil was greater in an
13 *A. mangium* plantation than in secondary, intact, or selectively cut forests.
14 Such changes in soil N status in *A. mangium* plantations can
15 influence the colonization and decomposition of leaf litter by
16 litter-decomposing fungi that play central role in decomposition process in
17 soils. Previous studies have demonstrated that excess N supply can reduce
18 the abundance and activity of fungi associated with the decomposition of
19 recalcitrant compounds such as acid unhydrolyzable residue (AUR) (Reid
20 1991, Osono 2007). Thus, it is hypothesized that the colonization and

decomposition of *A. mangium* leaf litter by ligninolytic fungi are suppressed as a result of high N content. Although the negative or positive relationship between N deposition and decomposition rate of leaf litter has been suggested in other tree species, climate zones, and laboratory experiments (Fog 1988, Berg and Matzner 1997, Knorr et al. 2005, Hobbie 2008), a few studies have examined the decomposition process of leaf litter in *A. mangium* plantations (Xiong et al. 2008, Kunhamu et al. 2009). Moreover, to our knowledge, no studies have evaluated the effects of high N status of *A. mangium* leaf litter on the fungal colonization and AUR decomposition.

The colonization of leaf litter by ligninolytic fungi results in the occurrence of bleached portions on the surface (Osono 2007). The content of recalcitrant compounds is generally lower in a bleached leaf area than in adjacent non-bleached areas of the same leaf due to the decomposition by ligninolytic fungi (Osono and Takeda 2001, Koide et al. 2003, 2005). Therefore, measurement of bleached area on the surface of *A. mangium* leaf litter will be useful for evaluating the colonization of leaf litter by ligninolytic fungi. Comparing the bleached area of leaf litter in *A. mangium* plantations with that in adjacent forests of non-N-fixing trees will provide insights into the possible effects of excess N availability on the colonization of leaf litter by ligninolytic fungi.

The purposes of the present study were to investigate the colonization and decomposition of *A. mangium* leaf litter by ligninolytic fungi and to examine whether higher N content in *A. mangium* leaf litter that was compared to leaf litter of non-N fixing tree would have inhibitory effect onto the appearance of bleached portion on the leaf litter and would also have inhibitory effect onto the decomposition of the leaf litter by pure culture decomposition test. Samples were collected from eight *A. mangium* plantations of different ages (0 to 17 years old) located in southern Sumatra Island, Indonesia. Colonization by ligninolytic fungi was evaluated as the area of bleached portions on the surface of leaf litter collected from the forest floor. Chemical properties of surface leaf litter were analyzed, and effects of the properties on fungal decomposition were evaluated with a bioassay under pure culture conditions, using a ligninolytic fungus *Trametes versicolor* as an agent of decomposition. The data from *A. mangium* plantations were compared with those from three adjacent forests of non-N-fixing trees (one regenerating coppice of *Schima wallichii* and two mature secondary forests) to examine the possible effect of high N content in *A. mangium* leaf litter on the colonization and decomposition by ligninolytic fungi.

Materials and Methods

Study site

Study sites were located in the southern part of Sumatra Island, Indonesia (3º 52´ S and 103º 58´ E). The mean annual temperature and annual precipitation of the area in 2004 were 29°C and 2520 mm (Yamashita et al. 2008). A total of 11 forest stands were chosen from three regions that were Sodong, Gemawang, and Merbau, for the present study. The distance between Sodong and Gemawang was 33 km, Sodong and Merbau was 6 km and Gemawang and Merbau was 28 km, respectively. The stands included eight *A. mangium* plantations of different ages (0-, 3- and 5-year-old stands in the Sodong region and in the Gemawang region, and 8- and 17-year-old stand in the Sodong region), and three secondary forests (a secondary regenerating stand of *S. wallichii* in the Sodong region, and two mature secondary forests in Sodong and Merbau regions) (Table 1). The original vegetation in 0-year-old *A. mangium* plantations was 7-year-old *A. mangium* plantations which were cut one to six months prior to leaf litter collection. The planting interval of *A. mangium* in 3 and 5-years-old plantations was 3 m by 3 m (1089 individuals/ha), and was 2 m by 4 m (1250 individuals/ha) in

8-years-old plantation. The mean DBH in 3-years-old *A. mangium* plantations was 12.1 - 13.5 cm, that in 5-years-old plantations was 13.7 - 16.0 cm, and that in secondary forests was 17.3 - 19.0 cm. No DBH data were available in 8 and 17-years-old *A. mangium* plantations and *S. wallichii* regeneration. The mean canopy height in 3-years-old plantations was 13.3 - 17.0 m, that in 5-years-old plantations was 18.8 - 20.1 m, that in 8-years-old plantation was 24 - 25m, and that in secondary forests was 17.5 - 18.9 m. No height data were available in 17-years-old plantation and *S. wallichii* regeneration. *A. mangium* is fast growing pioneer species, and the canopy was already closed even in 3-years-old plantation. Tree families in the secondary forests included Moraceae, Fabaceae, Myrtaceae, Proteaceae, Dipterocarpaceae, and Lecythidaceae. Fabaceae, the only family that has N fixing ability, accounted 7.8% of the total number of stems in the secondary forests. Thus we regarded the leaf litter in the secondary forests as being consisted of those derived from non-N fixing trees for the sake of simplicity.

Samples were collected in September 2007. Ten quadrats (20 cm × 20 cm) were set on the forest floor along a 9-m line transect at 1-m intervals inside of each site, which was about 1 to 1.5 ha in size. Surface leaf litter accumulated on the surface of the forest floor was collected within the quadrats and used for chemical analysis and pure culture decomposition

tests. Partly decomposed leaves that decomposition had been undergone but more than half of the original leaf area had been remained were collected beneath surface leaf litter and used for leaf area measurement. Numbers of partly decomposed leaves that we collected ranged 20 to 97 pieces in each quadrat and were summed up to 389 to 548 pieces in each site. The collected leaves only included those of the respective species in the *A. mangium* plantations and the *S. wallichii* regeneration because they were pure stands, but we did not distinguish individual tree species in the mature secondary forests. Partly decomposed leaves were not collected at two 0-year-old *A. mangium* plantations because no bleached leaves were seen there.

Chemical analyses

Surface leaf litter from 10 quadrats was combined to make one sample for each forest stand and oven-dried at 40°C for 1 week. Surface leaf litter were then ground in a laboratory mill to pass through a 0.5-mm screen and used for chemical analysis. The AUR content in the samples was estimated by gravimetry, according to a standardized method using hot sulfuric 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 % (v/v) sulfuric acid for 2 h at room temperature with occasional stirring. The mixture was diluted with distilled water to furnish a 2.5 % sulfuric 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. Total N content was measured by automatic gas chromatography (Sumigraph NC-900 NC analyzer; Sumitomo Chemical, Osaka, Japan). The methods are described in detail elsewhere (Osono and Takeda 2005). Throughout this paper we use AUR to refer to the final residual fraction remaining after proximate analysis. AUR fraction contains a mixture of organic compounds in various proportions, including condensed tannins, phenolic compounds, carboxylic compounds, alkyl compounds such as cutins, and true lignin (Preston et al. 1997).

Leaf area measurement

Partly decomposed leaves were pressed between broad papers and oven-dried at 40°C for 1 week, then photocopied and scanned with a scanner (EPSON GT-8000). The area of bleached portions, where the color of leaf litter was defined clearly paler than the surrounding leaf area by the

software, and total leaf area were measured by image analysis performed on a Windows computer using Scion image software (version 4.0.3, windows version of NIH image, Scion Corporation). For each sample that was collected from a quadrat, the bleached area was defined as a proportion of total leaf area and expressed as a percentage to the total leaf area. The mean value of bleached area was calculated for each forest stand.

Pure culture decomposition test

Surface leaf litter were cut into pieces 1 cm in width and preserved in a PVC bag until the experiment started. The leaves (300 mg) were sterilized by exposure to ethylene oxide gas at 60°C for 6 h. The sterilized litter was placed on the surface of petri dishes (9 cm in diameter) containing 20 ml of 2 % agar. *Trametes versicolor* is a white rot fungus of wood possessing an ability to decompose recalcitrant compounds such as AUR. The strain IFO30340 used in the present study was the one that was registered in Institute of Fermentation, Osaka (IFO), Osaka, Japan and had been used as a standard one for the decomposition test of timber under Japanese Industrial Standards (JIS). Moreover, the strain had been repeatedly used in bioassays of leaf litter decomposition (Osono et al. 2003, Osono and Takeda.

2006, Osono 2010). Thus, we used this strain in the pure culture decomposition test in the present study. The inoculum was cut from the margin of the previously inoculated Petri dishes on 2 % malt-extracted agar (malt extract 2 % and agar 2 % (w/v)) with a sterile cork borer (6 mm in diameter) and placed on the agar adjacent to the litter, one plug per plate. The plates were incubated for 12 weeks at 25 °C in darkness. The plates were sealed firmly with laboratory film during incubation so that moisture did not limit decomposition on the agar. After incubation the leaves were retrieved, oven-dried at 40°C for 1 week, and weighed. The undecomposed initial litter was also sterilized, oven-dried at 40°C for 1 week, and weighed to determine the original mass. Four plates were prepared for each forest stand, and four uninoculated plates served as a control. Mass loss of leaves was determined as a percentage of the original mass, taking the mass loss of control litter into consideration. Duplicated samples of surface leaf litter after the incubation were combined to make one sample per forest stand and were also analyzed for AUR content with the method as above. Mass loss of AUR was determined as a percentage of the original AUR amount, taking the mass loss of AUR of control litter into consideration.

Data analysis

Welch's *t*-test was used to test differences in the chemical properties of surface leaf litter between *A. mangium* plantations and secondary forest. One-way analysis of variation (ANOVA) was used to test differences in the bleached area and mass loss of leaf litter among forest stands. Tukey's honestly significant difference (HSD) test was used for multiple comparisons of means. These analyses were performed with SPSS for windows software Version 10.0.5 (SPSS, Chicago, IL, USA). Pearson's correlation coefficients were calculated for linear relationships among forest age, chemical contents of surface leaf litter, bleached leaf area, mass loss and AUR loss of surface leaf litter caused by *T. versicolor* for *A. mangium* plantations and for all forest stands.

Results

Chemical compositions

Initial AUR contents were significantly (*t*-test $P<0.05$) greater in surface leaf litter from the *A. mangium* plantations (420.5 to 519.7 mg g^{-1}) than in the secondary forests (407.0 to 460.7 mg g^{-1}) (Table 1). Initial N contents were

1 significantly (*t*-test, $P<0.01$) greater in surface leaf litter from the *A.*
2 *mangium* plantations (18.9 to 32.7 mg g^{-1}) than in the secondary forests (13.1
3 to 16.3 mg g^{-1}) (Table 1). The age of *A. mangium* plantations was not
4 significantly ($P>0.05$ n=8 r=0.255) correlated with the initial AUR or N
5 contents of newly shed leaves.
6
7 Bleached area
8
9 Mean values of the bleached area of partly decomposed leaves ranged from
10 4.7±1.4 to 10.9±1.2 % in six *A. mangium* plantations and from 10.7±0.8 to
11 27.1±2.8 % in three secondary forests (Table 1). There was no significant
12 difference in bleached area between *A. mangium* plantations and secondary
13 forests (*t*-test $P>0.05$). The bleached area of partly decomposed leaves from
14 the secondary forest in the Merbau region was significantly (ANOVA $P<0.05$)
15 greater than those in the other stands, and that in the secondary
16 regeneration stand of *S. wallichii* was significantly (ANOVA $P<0.05$) greater
17 than that in the 8-years-old *A. mangium* plantation in the Sodong region
18 (Table 1). The bleached area of partly decomposed leaves was not
19 significantly ($P>0.05$) correlated with contents of initial AUR for all nine
20 stands (r=−0.404) or for six *A. mangium* plantations (r=−0.277). The

bleached area of partly decomposed leaves was significantly (P<0.05) and negatively correlated with N content for all nine stands (r=−0.814) (Fig. 1a), whereas the correlation was not significant (P>0.05) when examined for six *A. mangium* plantations (r=−0.059). Age of the *A. mangium* plantation was not significantly (n=6, r=0.366, P>0.05) correlated with the bleached area of partly decomposed leaves.

Pure culture decomposition test

The mean values of mass loss of leaves caused by *Trametes versicolor* ranged from 16.6±0.2 to 22.4±1.4 % for eight *A. mangium* plantations and from 6.7±1.1 to 25.8±0.8 % for three secondary forests (Table 1). There was no significant difference (t-test P>0.05) of mass loss between *A. mangium* plantations and secondary forests. The lowest values of mass loss of leaves were recorded for the secondary forest in the Merbau region and for the 0-year-old *A. mangium* plantation in the Gemawang region, whereas the highest values were recorded for the secondary regeneration of *S. wallichii* and the secondary forest in Sodong region (Table 1). Mass loss of leaves was not significantly (P>0.05) correlated with initial AUR content for all 11 stands (r=−0.369), but was significantly (P<0.01) and negatively correlated

with initial AUR content when the data of the secondary forest in the Merbau region was excluded from the analysis (r=−0.845 n=10) (Fig. 1b). Mass loss of leaves was marginally significantly (P=0.055) and negatively correlated with initial AUR content when examined for the eight *A. mangium* plantations (r=−0.696). Mass loss of leaves was not significantly (P>0.05) correlated with N content for all 11 stands (r=0.157) or for the eight *A. mangium* stands (r=−0.114).

The values of mass loss of AUR caused by *T. versicolor* followed a similar trend to those of leaves, ranging from 12.4 to 20.8 % for eight *A. mangium* plantations and from 11.7 to 26.9 % for three secondary forests (Table 1). Mass loss of AUR was not significantly different (t-test P>0.05) between *A. mangium* plantations and secondary forests. The lowest values of mass loss of AUR were recorded for the secondary forest in the Merbau region and for 8-year-old *A. mangium* plantation, whereas the highest values were from the secondary regeneration of *S. wallichii* and the secondary forest in Sodong (Table 1). Mass loss of AUR was significantly (P<0.05) correlated with initial content of AUR of Surface leaf litter (r=0.68). Mass loss of AUR of surface leaf litter was not significantly (P>0.05) correlated with N content for neither all 11 stands (r=0.213) nor eight *A. mangium* plantations (r=0.024).

1 Discussion

2

3 The initial AUR and N contents of surface leaf litter of *A. mangium* in the
4 present study (Table 1) were similar to those reported for legume trees (e.g.
5 Ngoran et al. 2006, Siddique et al. 2008) and were relatively high when
6 compared to those of non-legume tree species in other tropical forests (e.g.
7 Aerts 1997, Kurokawa and Nakashizuka 2008).

8 The bleached area of partly decomposed leaves in *A. mangium*
9 plantations (Table 1) was generally lower than those in other tropical forests,
10 whereas those in the three secondary forests were at similar levels to those
11 in other tropical forests (7.9-29.7%, Osono 2006). The smaller area of
12 bleached area in *A. mangium* plantations and the negative relationship
13 between the bleached area and N content (Fig. 1a) suggested that high N
14 content could inhibit the colonization by ligninolytic fungi. Previous studies
15 showed that N could cause a biochemical suppression of AUR-degrading
16 enzymes of fungi (Keyser et al. 1978, Fenn et al. 1981). This might have
17 reduced competitiveness relative to other fungi and hence mycelial growth in
18 *A. mangium* leaves. Similarly, Osono et al. (2002) reported that
19 avian-derived N suppressed the colonization of ligninolytic fungi in
20 coniferous litter in a temperate forest. Thus, the present study successfully

demonstrated the inhibitory effect of high N content of *A. mangium* surface leaf litter on the appearance of bleached area that represent colonization of ligninolytic fungi. The bleached are of *A. mangium* leaf litter was not significantly correlated with the stand age between 3 and 17 years where the canopies were closed, consistent with the results of Osono et al. (2008) reporting that the bleached area on salal leaf litter was not significantly different among forest stand of different ages (50 to 324 years) after the canopy closure.

In contrast to the colonization of *A. mangium* leaves in the field, which was limited by N, the decomposition of the leaves by a ligninolytic fungus (*T. versicolor*) was limited by initial AUR content (Fig. 1b). Recalcitrant compounds in tree leaves categorized as AUR, such as lignin, have often been shown to limit the rate of decomposition in forest soils (e.g., Mellilo and Aber 1982, Osono and Takeda 2005). The factors causing the low rate of mass loss of leaves from the secondary forest in the Merbau region remained unclear, but might have been related to inhibitory compounds in tree leaves included in the leaf mixture. The lack of a significant relationship between the mass loss of leaves and AUR and N content suggested that the growth and AUR decomposition by *T. versicolor* was not suppressed by N. It was unclear from the results of the present study whether N-rich leaves of *A.*

mangium had inhibitory effects on the decomposition by other ligninolytic fungi, or whether fungi taking part in the decomposition of *A. mangium* leaves in the field could be adapted to N-rich conditions of the leaves.

In conclusion the present study demonstrated that the colonization of *A. mangium* leaf litter by ligninolytic fungi was suppressed due to high N content, but the effect of high N in *A. mangium* leaf litter on the decomposition of leaf litter and AUR remained unsolved. Further studies will be necessary regarding the ligninolytic fungi associated with the bleaching of *A. mangium* leaves and the effect of N-rich conditions on the decomposition of the leaves by these fungi.

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Table

Table 1. Stand age (in years), initial chemical content in surface leaf litter (mg g^{-1}), bleached area of partly decomposed leaves on the forest floor (% total leaf area), mass and AUR loss (% original mass) of surface leaf litter and AUR caused in the pure culture by *Trametes versicolor*. Values indicate means ± standard errors (n=10 for bleach area, n=4 for mass loss). Values that do not share a common letter are significantly different at the 5% level by Tukey's HSD test. nd not determined.

					Chemical content of surface leaf litter				Pure culture decomposition test		
Forest type		Region	Site abbr.	Stand age	AUR	Nitrogen	Bleached area		Mass loss		AUR loss
Plantations	*Acacia mangium*	Gemawang	G0	0	519.7	27.9	nd		16.2±1.2	d	13.7
		Sodong	S0	0	420.5	32.7	nd		21.3±0.8	abcd	18.5
		Gemawang	G3	3	458.8	18.9	7.6±1.2	bc	22.4±1.4	abc	19.6
		Sodong	S3	3	490.8	19.9	10.9±1.2	bc	20.2±1.6	abcd	17.5
		Gemawang	G5	5	497.5	20.1	7.8±0.8	bc	21.0±0.8	abcd	20.8
		Sodong	S5	5	517.2	20.5	8.2±0.9	bc	19.8±0.8	bcd	16.1
		Sodong	S8	8	515.6	20.1	4.7±1.4	c	16.6±0.2	cd	12.4
		Sodong	S17	17	489.7	21.4	7.4±1.0	bc	19.0±1.4	bcd	12.6
Secondary forests	*Schima wallichii* regeneration	Sodong	Sw	nd	407.0	16.3	13.0±1.6	b	25.8±0.8	a	26.9
	Mature forest	Sodong	FS	nd	431.7	15.0	10.7±0.8	bc	22.5±2.0	ab	25.0
		Merbau	FM	nd	460.7	13.1	27.1±2.8	a	6.7±1.1	e	11.7

Figure legend

Figure 1 Bleached area of partly decomposed leaves on the forest floor as a function of N content (a), and mass loss of surface leaf litter caused in the pure culture by *Trametes versicolor* as a function of initial AUR content (b) of surface leaf litter. Site abbreviations are listed in Table 1.

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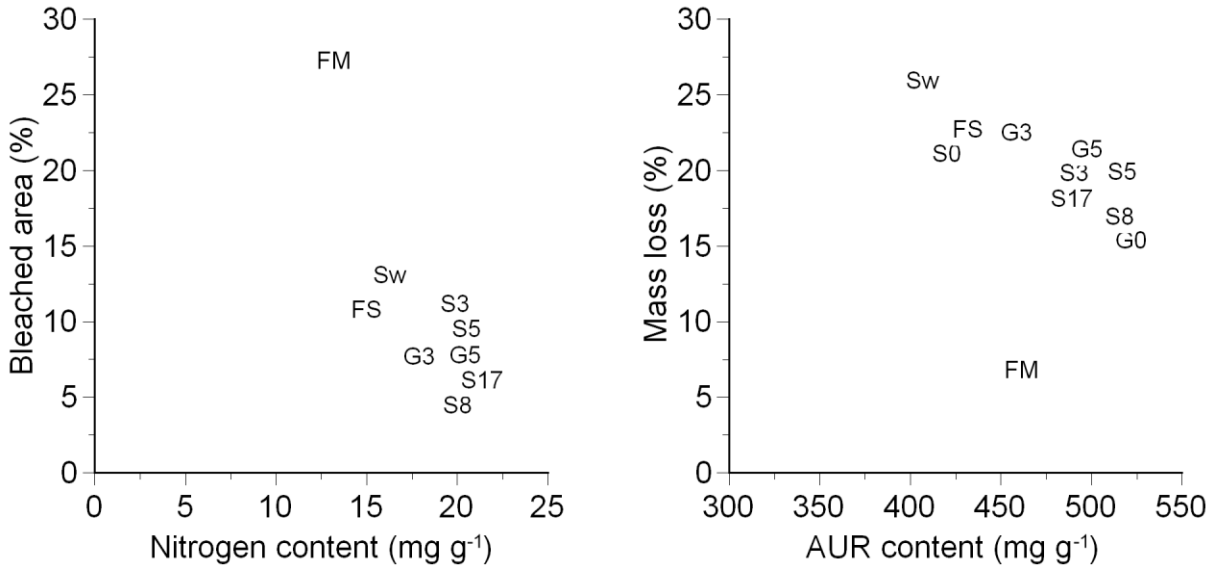

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