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MICROBIAL RESPIRATION AND NITROGEN RELEASE PATTERNS OF DECOMPOSING *ACACIA MANGIUM* LEAF LITTER FROM KEMASUL FOREST RESERVE, MALAYSIA

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WAN RASIDAH, K., VAN CLEEMPUT, O. & ZAHARAH, A. R. 2001. Microbial respiration and nitrogen release patterns of decomposing *Acacia mangium* leaf litter from Kemasul Forest Reserve, Malaysia. Decomposition trials under laboratory and field conditions with *Acacia mangium* leaf litter were made over a period of 140 days and several possible factors influencing this process were examined. Resource quality of *A. mangium* litter varied significantly between plant ages (12, 18 and 24 months) at young stand. The nitrogen content in the leaf litter was lowest at the 18-month-old stand, corresponding to the lower nitrogen concentration in the green leaves. Organic carbon was quite constant while litter production was highest in the drier period. The percentage of polyphenols decreased with increasing age, hemicellulose content remained constant and cellulose increased with the age of plantation. The addition of this litter to the soil enhanced the microbial respiration, measured as the rate of CO_2 evolution under laboratory condition. The pattern of CO_2 release proceeded through two phases, an initial rapid release followed by a slow release. This microbial activity resulted in immobilisation of mineral nitrogen in the litter and mineralisation in the soil. Release of mineral N from decomposing *A. mangium* litter under field condition followed three phases, i.e. leaching, immobilisation and mineralisation. The leaching period was rather short, producing a release of soluble nitrogen.

Keywords: Forest plantation - decomposition - resource quality - CO_2 efflux - nitrogen release - mineralisation

WAN RASIDAH, K., VAN CLEEMPUT, O. & ZAHARAH, A. R. 2001. Corak respirasi mikrob dan pelepasan nitrogen bagi penguraian daun gugur *Acacia mangium* dari Hutan Simpan Kemasul, Malaysia. Kajian makmal dan lapangan terhadap penguraian daun gugur *Acacia mangium* dan faktor-faktor yang mempengaruhi proses ini dijalankan

selama 140 hari. Kualiti luruhan *A. mangium* berbeza secara signifikan mengikut umur (12, 18 dan 24 bulan) untuk dirian muda. Kandungan nitrogen dalam luruhan daun adalah paling rendah pada umur dirian 18 bulan, selari dengan kandungan nitrogen yang rendah dalam daun hijau. Kandungan karbon organik adalah stabil dan penghasilan luruhan adalah tertinggi dalam cuaca kering. Peratus kandungan polifenol berkurang bila umur pokok meningkat manakala hemiselulos adalah stabil dan selulos meningkat bila umur pokok meningkat. Penambahan luruhan ini kepada tanah meningkatkan respirasi mikrob yang diukur berasaskan kadar evolusi CO_2 dalam kajian di makmal. Kadar pembebasan CO_2 berlaku dalam dua fasa, fasa awal yang melibatkan kadar pembebasan yang tinggi dan pembebasan perlahan pada fasa akhir. Aktiviti mikrob ini menyebabkan pengambilan nitrogen daripada luruhan dan mineralisasi nitrogen dalam tanah. Pembebasan N mineral daripada penguraian luruhan *A. mangium* di lapangan berlaku dalam tiga fasa, iaitu larut lesap, imobilisasi dan mineralisasi. Tempoh masa larut lesap adalah pendek dan menghasilkan nitrogen larut.

Introduction

Acacia species at the age group of 4–11 years old accumulate 3.0–14.8 t ha^{-1} y^{-1} of litter in the tropical plantations of India, Malaysia and Congo (O'Connell & Sankaran 1997). In Malaysia, a mature *A. mangium* plantation produces about 8.8 t ha^{-1} of litter annually on an infertile Ultisol soil (Y. Adzmi, FRIM, personal communication). This is equal to the litterfall produced by a dipterocarp forest in Sarawak but is lower than the values obtained for the alluvial and limestone forests in the same area (Proctor *et al.* 1983). At the Pasoh Forest Reserve in Negri Sembilan, Lim (1978) reported litter production of 8.9 t ha^{-1} y^{-1}.

Plant litter plays a crucial role in forest ecosystems, being a substrate for the biological processes that enhance the recycling of nutrients. A limited review by Vitousek and Sanford (1986) revealed that on moderately fertile sites, the N content in litterfall is higher compared to that in litter collected from infertile soils. This is due to the inherent characteristic that trees on moderately fertile sites have a higher N concentration in the leaves. Average litterfall production is also higher on fertile sites (10.5 t ha^{-1} y^{-1}) compared to the mean production of 8.8 t ha^{-1} y^{-1} on infertile soils.

Decomposition of litter and other plant biomass is controlled by three interacting variables: the organisms (microorganisms and fauna), physical environmental factors such as soil temperature and soil moisture, and resource quality (Anderson & Ingram 1989). It is now widely accepted that during decomposition, soluble components are the first to degrade, followed by hemicellulose and cellulose, and lastly lignin and recalcitrant fractions. Many authors even suggest lignin as a rate determining factor in the litter decomposition process, which overshadows the role of N in some cases (Fogel & Cromack 1977, Gower & Son 1992). Nitrogen enhances decomposition at the beginning while at the later stage, the high lignin accumulation slows down the rate. Nevertheless, the lignin content shows a somewhat inconsistent relationship with litter

decomposition in moist tropical forests in Sarawak (Anderson *et al.* 1983). A comparative study by Palm and Sanchez (1990) showed that polyphenolic compounds influence the rate of leaf decomposition of three tropical legumes more than nitrogen and lignin. Their argument is that polyphenolics bind to the nitrogen-containing compounds in the plant material to form resistant complexes that are difficult to decompose.

Release of N from decomposing litter usually proceeds through three different phases, i.e. leaching, accumulation and final release (Berg & Staff 1981). However, it might be possible that not all of these three phases are encountered with all species. Usually those species having a high N content do not accumulate nitrogen. The aim of the present study is to determine the rate of *A. mangium* leaf litter decomposition and factors that contribute to the process such as rainfall.

Materials and methods

Site description

The area where samples were collected was under an immature *A. mangium* plantation established in December 1991 under the Compensatory Forest Plantation Programme, within the vicinity of Kemasul Forest Reserve in Pahang (28 °N, 103°E; 60 m above sea-level). The degree of site heterogeneity is very high judging from the high variation in the tree growth and the undulating landform of the area. Mean annual temperature is 26 °C with the hottest period between April and May. The rainfall distribution is seasonal with the highest amount received during the monsoon season (October to December). Total rainfall received in 1993 was 2257 mm while in 1994 it was 2211 mm. The period between January and March is usually hot with less rainfall. Details on climatic data are shown in Figure 1. The soil of the study area was developed over shales parent material and is classified as a fine, mixed, isohyperthermic, Aquoxic Tropudult, according to the Soil Taxonomy Classification.

Litter and soil collection

Litter, mainly leaves, was collected at three different stand ages (12, 18 and 24 months) of the same stand to characterise its properties. Six months before each collection, the area underneath the trees of which litter would be collected was cleared of any remaining litter. Therefore, the collected litter represented more or less litter that was produced within the six months period, i.e. July–December 1992 for the collection at the 12-month-old stand, January–June 1993 for the collection at the 18-month-old stand, and July–December 1993 for the collection at the 24-month-old stand. At the same time, green leaves from the standing trees were also sampled to quantify their total N content. These samples were taken to the laboratory, dried and ground into fine powder For the laboratory incubation

experiment, samples were collected at stand age of 18 months whereby at this time onwards litter was produced in abundance. Collection was made at random and only fresh litter was sampled. After clearing away the litter, the first 10 cm mineral soil was sampled for the laboratory incubation study. Litter samples for the decomposition study were collected at stand age of 34 months when the canopy almost closed.

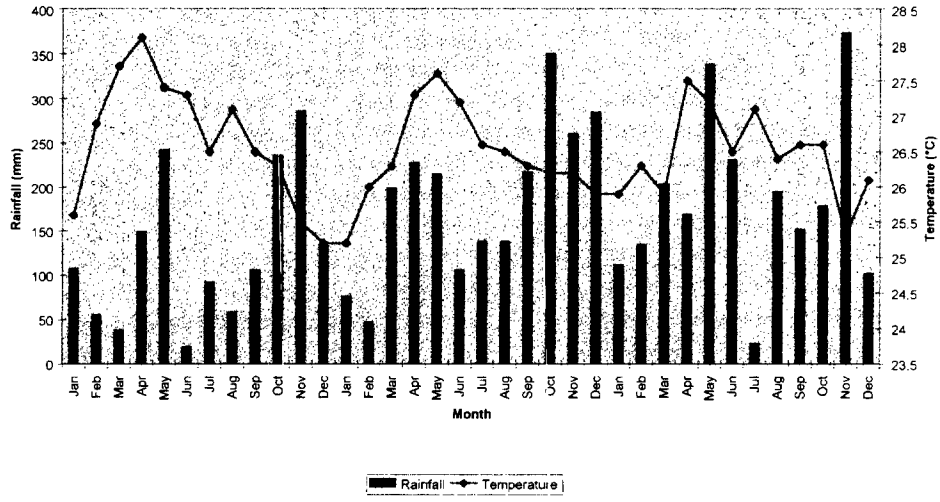

Figure 1. Temperature and rainfall distributions from 1992 to 1994 recorded at the meteorological station nearest to the study area

Characterisation of litter quality

Chemical analysis for litter samples was carried out for total N, organic C, lignin, cellulose, hemicellulose and polyphenolics. Nitrogen was analysed using the micro-Kjeldahl digestion (Bremner & Mulvaney 1982), while determination of organic C was based on the Walkley and Black method (Walkley & Black 1934). Lignin and cellulose were quantified via the acid detergent fibre preparation, while acid and neutral detergent solutions were used in the quantification of the hemicellulose content. These methods are from Van Soest (1963) and Van Soest and Wine (1967) and are described in detail by Anderson and Ingram (1989), and Allen *et al.* (1974). Acid detergent solution (AD) was prepared by dissolving 20 g cetyltrimethylammoniumbromide (CTAB) in 1000 ml 1 N H_2SO_4. The role of CTAB was to dissolve nitrogenous constituents while the acid served to hydrolysed starch. Removing cellulose with 72% H_2SO_4 solution separated lignin. The analysis of polyphenolics is based on the method described by Anderson and Ingram (1989) which was extracted from King and Heath (1967). Folin-Denis reagent was used to develop the blue colour solution to measure polyphenolics concentration.

Laboratory incubation

In the laboratory incubation experiment, the top 10-cm mineral soil layer was used. Prior to the experiment, this soil was air dried, crushed and passed through a 2-mm sieve. Two sets of treatments plus a blank (without addition of litter samples) were established: (1) the soil was preincubated at 1/2 field capacity for one week to restore the microbial activity, then the moisture was raised to 2/3 field capacity, and the soil amended with litter and incubated; (2) the soil was wetted to 2/3 field capacity, amended with litter and incubated. Litter was applied at a rate of 8.8 kg ha^{-1}. Twenty-five gram of soil was placed into a 100-ml plastic vial and mixed with litter before being incubated at 30 °C for 2, 4, 7, 14, 28, 56, 84, 112 and 140 days. This vial was put inside a 600-ml beaker together with 25 ml 0.1 N KOH solution and distilled water (to maintain soil moisture). The amount of CO_2 trapped in the KOH solution was quantified by titrating with 0.1 N HCl solution in the presence of saturated barium chloride solution. Results were expressed as mg CO_2 kg^{-1} dry sample per incubation time. The data were also calculated for the breathed C and compared statistically between the different treatments using analysis of variance and Duncan's multiple range test. Mineral-N content in the incubated soil was extracted with 1 N KCl solution and determined based on the Nessler distillation procedure.

Litter decomposition

One of the objectives of this experiment was to study the influence of rainfall on the decomposition of *A. mangium* leaf litter. For more accurate measurement, this experiment was initiated at the beginning of the monsoon season (in October 1994) and continued throughout the hot weather season that started from January onwards until May 1995. Forty-five litter bags made out of nylon net with 1-mm mesh size were used in this study. Each bag was filled with 20 g fresh litter and placed randomly in the experimental area in contact with the surface soil. The initial dry weight of litter was calculated using the moisture content of fresh litter. Litter weight loss was measured every fortnightly until the middle of February 1995 with five litter bags being withdrawn at random at each measurement. This was done by calculating the difference between the initial dry weight and the dry weight at sampling time. Before weighing, roots and unwanted materials were removed manually by hand and the remaining litter in the bag was oven dried. These samples were also analysed for their total N content. The collated data were calculated for standard error value.

Results and discussion

Resource quality parameters

Results of the chemical analysis of litter, sampled at various plantation ages, are shown in Table 1. It appears that N concentration was lowest between the stand

ages of 13 and 18 months corresponding to the first six months of the year. This corresponds also to a period of lower N mineralisation rate in the soil of the area (Wan Rasidah *et al.* 1999). The organic carbon contents were significantly higher during the period of 7–12 months but were rather constant throughout the second year of growth. Thus, C/N ratio was influenced mainly by the percentage of N. Overall, the N content was rather high and the relation C/N ratio quite low. Litterfall production also had a similar trend but in opposite direction to the N content. High leaf-fall was observed at 18 months old, which covers the litter produced between January to June 1993. This could be the effect of higher temperature and less rainfall during this period. According to Staff and Berg (1981), evergreen plants in tropical rain forests also show a seasonal variation in leaf litter production with the highest input rate occurring during the dry period. Changes in litter N concentration follow the fluctuation in the N concentration in green leaves.

Table 1. The characteristics of *Acacia mangium* litter at different stand ages

Parameter	Stand age (months)		
	7–12	13–18	19–24
Nitrogen (%)	1.42 ± 0.10 (3.01)	1.00 ± 0.20 (2.44)	1.28 ± 0.04 (3.04)
Carbon (%)	32.47 ± 0.98	28.88 ± 0.65	29.75 ± 0.87
C/N ratio	22.87	28.88	23.24
Dry matter yield (g tree^{-1} 6 months^{-1})	174.4 ± 59.2	704.3 ± 153.4	499.2 ± 61.0
Polyphenols (%)	2.97 ± 0.42	2.39 ± 0.76	1.74 ± 0.07
Cellulose (%)	n.d.	4.03 ± 2.37	8.35 ± 0.80
Hemicellulose (%)	n.d.	16.07 ± 1.34	16.29 ± 0.80
Lignin (%)	n.d.	58.5 ± 5.3	59.2 ± 7.1

Results are means of five samples ± standard error. Figures in parentheses are % N in the corresponding green leaves; n.d. - not determined (due to insufficient samples).

The polyphenols data (Table 1), which were based on the tannic acid equivalents, revealed another interesting finding. Litter from *A. mangium* had the highest polyphenols concentration during the initial growth but it decreased with increasing age. In contrast, the polyphenols content measured in *Khaya ivorensis* litter increased with increasing age (0.71% at stand age of 7–12 months, 1.07% at stand age of 13–18 months and 1.36% at stand age of 19–24 months) (Wan Rasidah 1995). Rasadah *et al.* (1988) discovered a significant correlation between the total phenolic content in ten mature *Shorea* species estimated by the Folin-Denis and Vanillin methods and their resistance to termite attack. The percentage of phenolic compounds was also highest in the species known to have a moderate to very durable timber. Perhaps analysing the total phenolic content in the *A. mangium* stem and bark might help in explaining the susceptibility of *A. mangium* to heartrot

disease. A study by Zakaria *et al.* (1994) on the different possible factors related to heartrot incidence in *A. mangium* showed that a positive correlation between this disease and the age of trees. The incidence level increased from 49% at the age of three years to 98% when the tree was eight years old. The polyphenols value in *A. mangium* litter was low as compared to that in leguminous leaves (Palm & Sanchez 1990) but was comparable with that in litter from matured lowland forests in Sarawak, Malaysia (Anderson *et al.* 1983).

The cellulose content was quite low, only 4% at stand age of 18 months but increased to 8.4% at stand age of 24 months. The value obtained for mature *Dipterocarpus baudii* leaf litter was 24.5% (Yamashita & Takeda 1988). The percentage of hemicellulose, however, was higher. The last parameter, lignin, was higher compared to other species. The lignin content in *D. baudii* leaf litter was 42.2% (Yamashita & Takeda 1988), 27–40% in mixed litter collected from Gunung Mulu Forests in Sarawak (Anderson *et al.* 1983), 20–27% in 5–9-y-old *A. mangium* bark and 22–46% in mature *Rhizophora mucronata* bark (Chew *et al.* 1992). The content varies greatly between localities and ages. The lignin content in *A. mangium* litter measured in our study was between 58 and 59%.

Carbon and nitrogen mineralisation potentials

Mean CO_2 evolutions from the soil and the soil amended with litter are presented in Figure 2. This CO_2 was solely produced through microbial respiration as the study was conducted under controlled conditions without any influence from living roots. The amount of CO_2 produced was rather high compared with the values obtained by Kachaka (1993) on residues of several fast-growing nitrogen fixing trees. The patterns of CO_2 release followed those reported by Jedidi *et al.* (1993) on the mineralisation of soils amended with organic material where the curves showed two important stages of C mineralisation. Stage one is a rapid release of CO_2, which is due to the intense microbial activity following rewetting of the soil. Stage two corresponds to the reduced microbial activity caused by the reduced supply of the easily biodegradable organic matter and hence the lower rate of CO_2 released.

Carbon dioxide evolution was slightly low for the preincubated soil and this corroborated with the postulation stated by Kachaka (1993) that preincubation of the soil results in the decomposition of part of the labile soil organic matter. Thus, this soil has less soluble carbon supply for microbial activity. Nevertheless, both incubated and preincubated soils reached stage two almost at the same time which was four weeks after litter amendment. Incorporation of litter led to a higher CO_2 release but the difference was not significant within both the preincubated and the rewetted soils. The calculated data for breathed C showed quite a consistent trend up to 28 days of incubation (Table 2). Higher rate was always obtained for rewetted soil with litter and rewetting of the soil (either with or without litter) resulted in higher microbial activity.

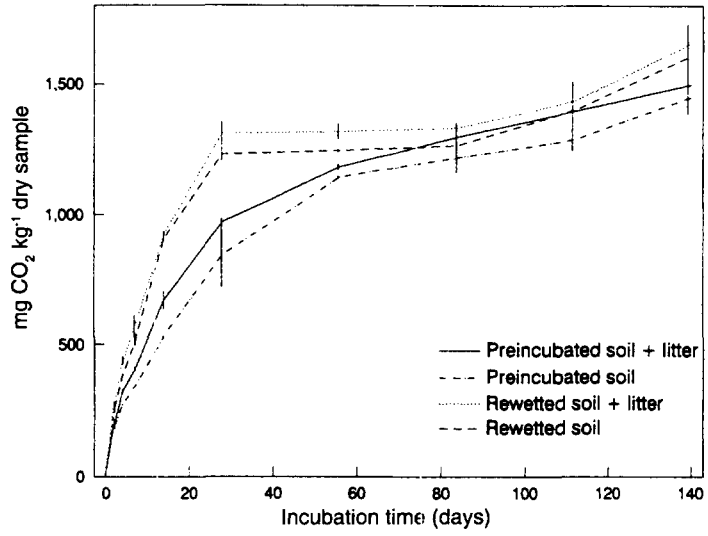

Figure 2. Cumulative CO_2 production (mean value ± S.E.) from soil and soil amended with litter

Table 2. The total value of breathed C (mg $C.CO_2$ kg^{-1} soil day^{-1}) from the laboratory incubation experiment

Measurement period (days)	Preincubated soil		Rewetted soil	
	With litter	Without litter	With litter	Without litter
0–2	26.6 b	25.8 b	37.7 a	34.8 a
3–4	17.1 b	11.1 c	22.4 a	17.1 b
5–7	8.0 b	6.6 b	11.7 a	11.8 a
8–14	10.3 b	7.4 b	13.9 a	15.7 a
15–28	5.9 b	6.1 b	7.5 a	6.2 b
29–56	1.8 b	3.3 a	0.0 c	0.0 c
57–84	0.6 ab	1.1 a	0.6 ab	0.3 b
85–112	0.2 b	0.5 ab	0.7 ab	1.4 a
113–140	2.5 a	1.2 a	2.1 a	2.0 a

Means with the same letter across the rows are not significantly different at 5% probability level.

The patterns for nitrogen mineralisation in the soil after addition of litter were rather inconsistent, probably due to the heterogeneity of the soil (Figure 3). Within the first week of incubation, there were sharp increases in the mineral-N concentration. After this period, the concentration still increased, but at a slower rate. Addition of litter in both incubated and preincubated soils resulted in lower N mineralisation. In fact, there was an immobilisation of nitrogen from the soil. Carbon used by microbes from plant residues and mineral N from the soil for the synthesis of microbial biomass results in immobilisation of soil N. A slightly higher mineral-N concentration observed during the first four days in the

incubated soil with litter could be due to the release of leachable N from the litter after the dried soil was rewetted. Similar observation has also been reported by Kachaka (1993). In the preincubated soil, addition of litter resulted in the withdrawal of soil N even after five months of incubation.

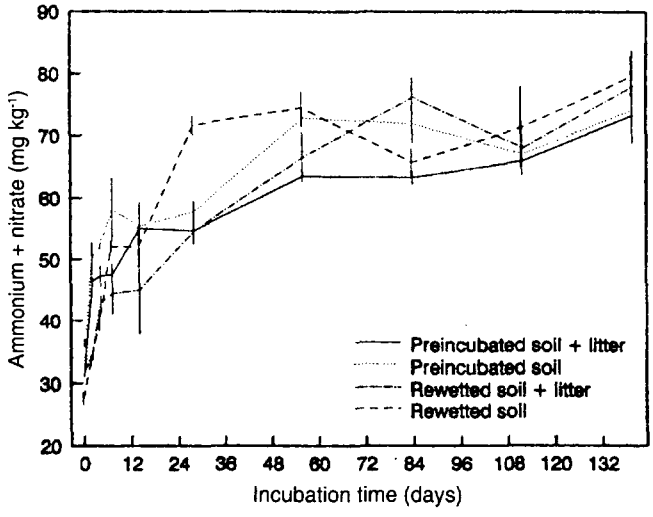

Figure 3. Changes in mineral N concentration (mean value ± S.E.) in the soil following litter amendment

Litter decomposition

Figure 4 represents changes in litter decomposition rate and nitrogen release by the decomposing *A. mangium* leaf litter. Decomposition is described by the loss in dry matter weight against time. As shown by the standard error values, the variability of biomass loss within the site became higher at later sampling periods and this corresponded mainly to the heterogeneity of the site. The decomposition rate constant was calculated based on Olson's single exponential model decay equation (Olson 1963),

$$Biomass_t \times 100 \,/\, Biomass_0\ [\%] = 100 \times \exp(-kt)$$

where $Biomass_t$ is the dry matter weight at time t (in years), $Biomass_0$ is the initial dry matter weight, k is the decay constant. The decay constant for *A. mangium* litter at stand age of about three years was 3.05 y^{-1}. The decay constant reported for the litter from lowland rain forests of Malaysia was in the range of 1.4–5.1 y^{-1} (O'Connell & Sankaran 1997).

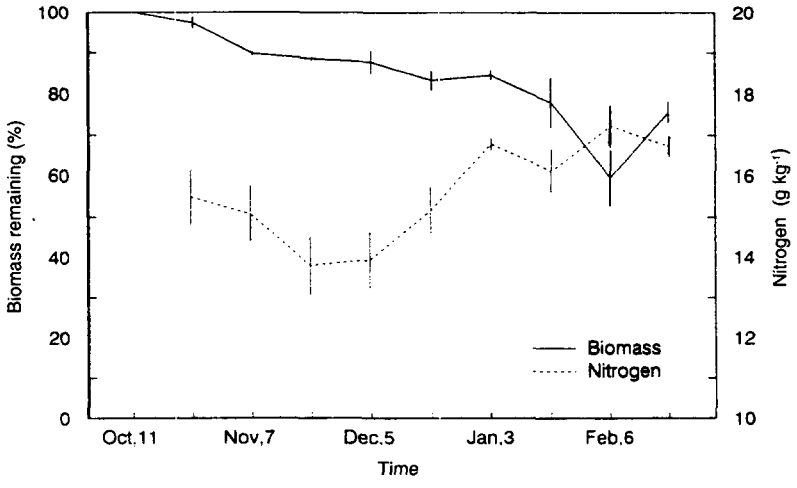

Figure 4. Weight loss and changes in the mineral-N content of *A. mangium* litter following decomposition. Bar values represent standard error.

Decomposition of this litter did not show any clear relationship with rainfall but a rather high variability was observed after fourteen weeks (January 3). As shown in Figure 1, high rainfall was experienced in November and the amount dropped tremendously in December. The period between mid-December and mid-January was a bit dry with intermittent rain and dry between January and February 1995 (personal observation). Litter weight loss was rather rapid at the beginning, with 10% of the original weight disappearing within four weeks. After this period, there was a reduction in the decomposition rate and in eighteen weeks, 70% of the original mass still remained. Yamashita and Takeda (1998), in their study on the decomposition of *Dipterocarpus baudii* litter in Malaysia, reported loss of 38% of initial weight within 6 months in Kepong and 27% within 5 months in Pasoh Forest Reserve when 0.5-mm mesh bag was used. Canopy closure (which started at stand age of 30 months) may be one of the factors leading to the above ambiguous relationship. Throughout the year after canopy closure, the area underneath was always moist. High intensity of closure and high volume of litter on the soil surface slowed down the loss of moisture from the soil.

Apart from environmental factors, resource quality has also been recognised as an important element that controls the rate of litter decomposition and N mineralisation. The activities of soil microorganisms determine the rate of organic material decomposition and respiration is the key pathway of carbon loss from organic materials. The pattern for litter decay rate observed in our study was similar to that obtained for the leaves of *Inga edulis*, *Cajanus cajan* and *Erythrina* sp. (Palm & Sanchez 1990), with a high decomposition rate during the first month and a lower rate for the subsequent period. They, however, discovered that polyphenolics influence the rate of decomposition more than the percentage of nitrogen or lignin.

Nitrogen release from leaf litter during decomposition followed the pattern described by Berg and Staff (1981). There was an initial release of N (leaching phase) followed by immobilisation and finally mineralisation after fourteen weeks. The initial release occurred for about six weeks, which is usually related to the release of leachable N. More than 2 g of N for each kg of dry weight was lost during this period. This phase was not observed with forest litter (Anderson *et al.* 1983), *Leucaena* leaves (Van Der Meersch 1992) and the three species studied by Palm and Sanchez (1990). The accumulation phase started right after leaching and continued for about ten weeks. The N concentration increased from 1.4 to 1.7%. This is a common phenomenon for litter with low N concentration. This increase could probably be the result of complex formation of mineralised N with lignin or the effect of uptake of N from the soil by microbial activity (immobilisation).

The third phase, which was the final release of N, has been reported in other decomposition experiments (Palm & Sanchez 1990, Van Der Meersch 1992). The rate of release was slower compared to the first phase and this was due to mineralisation. In the present study, the release might have started after the nitrogen level had reached 1.7%, which could be considered as the critical nitrogen level for this litter. However, long-term measurements are needed to study the trend of this release. Anderson *et al.* (1983) discovered that in over ten months of litter decomposition in moist tropical forests, nitrogen was conserved in litter regardless of the dry weight loss.

Conclusion

Litter quality and quantity showed some significant differences between the different ages particularly in the biomass and nitrogen and polyphenols contents. The nitrogen content was lowest during the drier period when litter was produced in abundance. The polyphenols content decreased with increasing age. Litter decomposition, which was based on the biomass weight loss, was comparatively rapid in the first month but proceeded at lower rates during the subsequent period. It is not clear whether the decomposition process was influenced by the rainfall. The release of mineral N from decomposing litter followed three phases, i.e. leaching, immobilisation and mineralisation. Laboratory incubation study showed that application of this litter to the soil resulted in immobilisation of nitrogen. Under field condition, leaf senescence is continuous thus leaching of soluble N is also continuous. The leached N might be taken up by plant roots or might be immobilised by microbial biomass. Thus, to determine the rate of nitrogen contribution from litter in an *A. mangium* plantation, one has to consider the interaction between all the possible factors including biological nitrogen fixation.

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