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Studies on Nitrogen in Malaysian Soils V. Mineralisation of Leaf Litter Nitrogen and Its Availability to Rubber Seedlings

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Leaf litter of Pueraria phaseoloides, Calopogonium caeruleum *and* Hevea brasiliensis *was incorporated in four different soils and incubated at optimum temperature and moisture conditions in the laboratory. While leaf litter N of* P. phaseoloides *and* C. caeruleum *was readily mineralised during incubation, immobilisation of available mineral-N was observed when leaf litter of* H. brasiliensis *was incubated in these soils. The comparatively high C/N ratio (33) and low N content (1.36%) in the leaf litter of* H. brasiliensis *were suggested to be possible causes of such observations.*

Leaf litter-N of P. phaseoloides *was mineralised faster than that of* C. caeruleum *upon incubation in spite of similar N content and other chemical composition. Results of glasshouse investigations indicated leaf litter-N of both legume species was readily available to rubber seedlings. However, the leaf litter of* P. phaseoloides *was generally found to be slightly more effective in promoting growth and nutrition (particularly N) of the young seedlings compared with that of* C. caeruleum.

Apart from the applied nitrogenous fertilisers and inherent soil N, the senescent plant tissues such as leaves, roots, *etc.* are also important sources of N in the cultivation of rubber *(Hevea brasiliensis).* In young rubber plantings, establishment of leguminous cover in between tree rows is a common agronomic practice. It has been estimated that under field conditions in Peninsular Malaysia, as much as 353 kg of N was added to 1 ha of rubber land through the deposition of litter of mixed conventional leguminous creepers comprising *Pueraria phaseoloides. Centrosema pubescens* and *Calopogonium mucunoides* over five years of the immaturity period[1]. Where a shade-tolerant legume *(C. caeruleum)* is planted together with *P. phaseoloides* their leaf litter returned a total of about 694 kg per hectare of N to the soil from the third to eighth year after planting[2]. In mature rubber fields, however, the rubber trees and the shade-tolerant undergrowth are principal sources of organic litter N. Tan[3] estimated that the leaf litter fall of a well-manured mature rubber planting could return 53 kg per hectare of organic leaf litter N annually to the soil.

In view of the importance of leaf litter N from leguminous covers during immaturity and from the annual leaf fall of mature rubber trees, it is of interest to find

COMMUNICATION 761

out how readily this organically-bound N is available to rubber upon biological mineralisation.

This paper reports some results on the mineralisation of leaf litter N of two common leguminous cover plants (*P. phaseoloides* and *C. caeruleum*) and rubber under laboratory incubation conditions. The comparative availability of leaf litter N of these two legumes to young rubber seedlings was also evaluated in glasshouse studies.

EXPERIMENTAL

Mineralisation of Leaf Litter Nitrogen in Soil (Laboratory Investigations)

Soils. Air dried and sieved samples (0–30 cm depth) of Batu Anam, Holyrood, Munchong and Serdang series soils were used. The details of sampling and some of their chemical, physical as well as mineralogical properties have already been described in an earlier paper[4].

Leaf litter and their chemical composition. Leaf litter samples of *P. phaseoloides, C. caeruleum* and *H. brasiliensis* (GT 1 clone) were used. After collection from the field, they were oven-dried (80°C) and finely ground (< 0.5 mm size). Their chemical composition is shown in *Table 1.*

Incubation procedures and determination of ammonium and nitrate in soil. Twenty-gramme samples of the four soil series were placed in 250 ml incubation bottles. Appropriate weights of finely-ground leaf litter of *P. phaseoloides, C. caeruleum* and *H. brasiliensis* containing 2 mg of N (*i.e.* 100 μg N per gramme soil) were then added and mixed with the soil. A control without any addition of leaf litter was also included. The soils and mixtures were then brought to 65% water-holding capacity (WHC) by adding appropriate amounts of distilled water. The bottle was loosely closed with a screw cap to allow free movement of air. The soils were incubated in an incubation room of constant temperature of 32°C and they were maintained at 65% WHC by periodically weighing the bottles and adding appropriate amounts of water to compensate moisture loss through evaporation. Samples were taken at two, six and ten weeks after incubation. At each sampling date, 100 ml of 2*M* KCl was added to the incubated soil and the mixture was shaken for 60 min. An aliquot of extract was taken to determine ammonium and nitrate N by the method described by Bremner[5].

Each treatment of the above incubation experiment was carried out in triplicate.

TABLE 1. CHEMICAL COMPOSITION OF LEAF LITTER USED IN MINERALISATION STUDIES

Leaf litter	C (%)	N (%)	C/N	P (%)	K (%)	Ca (%)	Mg (%)
P. phaseoloides	36.4	2.56	14	0.11	1.09	0.98	0.37
C. caeruleum	37.4	2.36	15	0.11	0.92	1.20	0.24
H. brasiliensis	47.1	1.36	33	0.03	0.34	1.00	0.16

Availability of Leguminous Leaf Litter Nitrogen to Rubber Seedlings (Glasshouse Experiment)

Soil and leguminous leaf litter. In the glasshouse experiment, only Serdang series soil previously employed in incubation studies was used. The surface 0–30 cm depth of this particular soil was collected from the interrow area of a mature rubber field *(Field 19)* in the Rubber Research Institute of Malaysia Experiment Station at Sungai Buloh, bulked, air-dried and sieved (< 2 mm).

The same leguminous leaf litter of *P. phaseoloides* and *C. caeruleum* employed in the earlier laboratory investigations was used. Their chemical composition has already been presented in *Table 1.*

Filling of pots and planting. Large earthenware pots with an upper surface diameter of 35 cm and with their interior coated with bituminous paints were used as experimental units. To each pot, 24 kg of air-dried and sieved soil was added and it was then uniformly mixed with 56 g of CIRP (Christmas Island rock phosphate). Before planting, deionised water was added to bring the soil to field capacity.

The RRIM 600 selfed seedlings of two weeks old and of uniform height and vigour were selected from the seed bed. Each pot was planted with three of these seedlings. After one week of growth, the weakest of the three seedlings in each pot was removed.

Experimental treatments and design. The experiment was a simple randomised block design with four treatments replicated four times. The four treatments were as follows:

- Control without any N added
- *P. phaseoloides* leaf litter as N source
- *C. caeruleum* leaf litter as N source
- Ammonium sulphate as N source.

Separate samples, each containing 9 g of N, of the ground leaf litter of the two legumes were thoroughly mixed with soil immediately after addition of CIRP and prior to planting. Ammonium sulphate was applied monthly and the total amount applied in six applications was 9 g of N per pot. For all the treatments, uniform application of CIRP, muriate of potash and kieserite was carried out monthly according to the schedule given in *Table 2.*

Maintenance. Soil moisture was maintained at field capacity during the first month after planting. After each monthly fertiliser application, watering was temporarily ceased for two to three days and it was followed by daily application of 500 ml deionised water per pot (equivalent to 0.69 cm rainfall).

Prophylactic sprays of insecticides and fungicides were carried out to control pests and diseases.

Measurements and harvesting. The height of the seedlings was measured monthly from the first month after planting while stem diameter (for girth estimation) was obtained at the end of the experiment after seven months' growth.

The seedlings were harvested after seven months. Dry weights of leaves, stems and roots were recorded. During the experimental period, fallen leaves

TABLE 2. FERTILISER SCHEDULE AND TOTAL AMOUNT OF FERTILISERS APPLIED

Month after planting	Uniform application (g/pot)			N applied in fertiliser (g/pot)
	CIRP[a]	Muriate of potash	Kieserite	
At planting	56	–	–	–
1	2.8	0.84	0.56	0.59
2	5.6	1.68	1.12	1.18
3	5.6	1.68	1.12	1.18
4	8.8	2.64	1.75	1.85
5	8.8	2.64	1.75	1.85
6	11.2	3.36	2.24	2.35

[a]CIRP = Christmas Island rock phosphate with about 36% P_2O_5

were collected, dried, retained and were included in the final estimation of total dry matter yield.

Plant tissue analyses. Prior to chemical analyses, all plant materials were oven dried (80°C) and subsequently ground in a clean laboratory mill (G and N Junior) to less than 0.5 mm size.

The carbon content of the ground plant material was determined by Walkly and Black's rapid titration method[6].

N was analysed by the classical Kjeldahl method of digestion with subsequent colorimetric determination on the Technicon Auto-Analyser.

For the analyses of other nutrient contents, a 2 g subsample of the ground plant material was first oven dried at 110°C for 5–6 h. It was then 'dry ashed' and the product was digested with 8 ml of 20% nitric acid on a steam bath for 1 h. The filtrate was analysed for its K content on the Technicon Auto-Analyser fitted with flame photometer module. Phosphorus was determined colorimetrically by the yellow phosphovanadate complex on the Technicon Auto-Analyser. Calcium and magnesium in the diluted solution of filtrate were determined by the atomic absorption method using a Techron Atomic Absorption Spectrophotometer (Type AA-4).

Details of analytical techniques are outlined in the 'Manual of Laboratory Methods of Plant Analysis' published by the Rubber Research Institute of Malaysia[7].

All the results were expressed as percentage of dry weight.

RESULTS AND DISCUSSION

Mineralisation of Freshly Added Leaf Litter Nitrogen in Soils

The results on the mineralisation of freshly-added rubber and leguminous (*P. phaseoloides* and *C. caeruleum*) leaf litter N in Batu Anam, Holyrood, Munchong and Serdang series soils after two, six and ten weeks of incubation are given in *Table 3*.

TABLE 3. MINERALISATION OF FRESHLY-ADDED LEAF LITTER NITROGEN IN FOUR SOILS DURING INCUBATION

Soil series	Leaf litter	Mineralisation (μg N/g soil)					
		2 weeks		6 weeks		10 weeks	
		NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
Batu Anam	Control[a]	30	3	46	0	56	2
	H. brasiliensis	9	0	4	0	5	0
	P. phaseoloides	47	2	64	0	77	0
	C. caeruleum	47	2	55	0	70	0
Holyrood	Control[a]	43	0	32	21	22	38
	H. brasiliensis	5	2	0	2	0	5
	P. phaseoloides	38	2	55	16	43	54
	C. caeruleum	38	4	50	18	23	48
Munchong	Control[a]	65	6	70	17	54	46
	H. brasiliensis	40	18	35	25	6	55
	P. phaseoloides	84	20	98	34	88	63
	C. caeruleum	85	20	92	30	75	57
Serdang	Control[a]	19	4	24	7	25	10
	H. brasiliensis	7	5	7	0	0	2
	P. phaseoloides	43	21	42	17	22	60
	C. caeruleum	34	19	33	21	15	45

[a]Without litter

Incorporation of finely ground rubber leaf litter in all the four soils caused nett immobilisation of mineral-N as indicated by the lower ammonium and nitrate-N content in these soils compared with the corresponding untreated control samples *(Table 3)*. This observation was consistent throughout the ten weeks' incubation period. On the other hand, except for the slight initial immobilisation in Holyrood series soil after two weeks' incubation, soils to which leaf litter of *P. phaseoloides* or *C. caeruleum* was added generally accumulated considerably more mineral-N (ammonium plus nitrate-N) than the control samples without any leaf litter addition. Generally, higher mineral-N accumulation was also found in soils mixed with *P. phaseoloides* than those with *C. caeruleum* leaf litter. In the earlier studies[2] *P. phaseoloides* leaf litter was found to decompose faster and its N content declined more rapidly than *C. caeruleum* leaf litter under field conditions. Since both legumes had very similar N content and C/N ratio *(Table 1)*, the variation in the amounts of N mineralised may partly be attributed to the differences in the nature and composition of these two plant materials. Vallis and Jones[8] found that N mineralisation of leaf litter of two tropical legume species containing similar concentrations of N and lignin, was related to their differences in polyphenol concentration.

The present observations of nett immobilisation as a result of incorporating rubber leaf litter in soil are in accord with the earlier results of Guha and Watson[9] who attributed their findings to the high C/N ratio (about 28) in senescent rubber leaves. The C/N ratio of rubber leaf litter used in the the current investigations was about 33 which is considerably higher than the critical value of 20 above which nett N immobilisation would normally take place when it is added to the soil[10]. Besides C/N ratio, Tritini and Arnold[11] reported that incorporation of only those plant residues with a minimum of 1.66% to 1.89% N led to greater amounts of mineral N accumulation than that mineralised from native soil N. The rubber leaf litter used in present studies contains 1.36% N which is lower than the minimum value cited above.

Availability of Leguminous Leaf Litter Nitrogen to Rubber Seedlings

Growth and dry matter production. The effect of two types of leguminous leaf litter and ammonium sulphate as N sources on the height of rubber seedlings during the seven months' growth period is illustrated in *Figure 1*. Throughout the experimental period, the tallest plants consistently recorded were those supplied with ammonium sulphate as the source of N. The shortest plants were in control treatment without any N addition. Of the two types of leguminous leaf litter, *P. phaseoloides* leaf litter N appeared to produce taller plants than *C. caeruleum* leaf litter N.

While the application of either ammonium sulphate or leguminous leaf litter as N source significantly increased the girthing of seedlings compared with control, the biggest girth was recorded with ammonium sulphate-treated seedlings. Girth differences between seedlings supplied with *P. phaseoloides* or *C. caeruleum* leaf litter as the N source were not statistically significant.

Results on girth of seedlings at the seventh month of growth are presented in *Table 4*.

Results on the dry weights of leaves, petioles, stems and roots after seven months of growth are given in *Table 5*.

TABLE 4. EFFECT OF LEGUMINOUS LEAF LITTER AND AMMONIUM SULPHATE AS NITROGEN SOURCES ON GIRTH MEASUREMENT OF RUBBER SEEDLINGS

Nitrogen source	Mean girth/seedling (cm)
Control (without N)	3.14
Ammonium sulphate	5.10
P. phaseoloides leaf litter	4.86
C. caeruleum leaf litter	4.28
S.E. (±)	0.199
L.S.D. ($P < 0.05$)	0.587

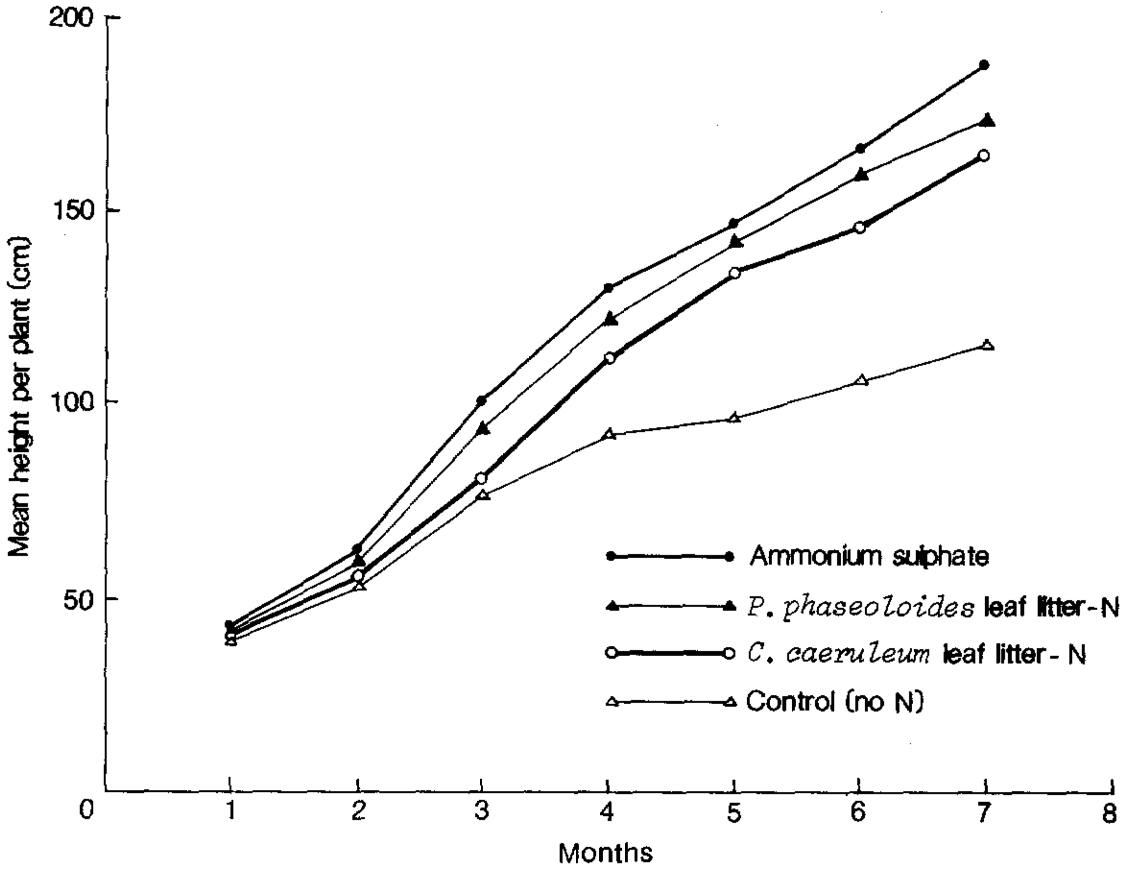

Figure 1. Effects of leguminous leaf litter-N and ammonium sulphate on height of rubber seedlings.

TABLE 5. EFFECT OF LEGUMINOUS LEAF LITTER AND AMMONIUM SULPHATE AS NITROGEN SOURCE ON DRY MATTER YIELD OF RUBBER SEEDLINGS

Nitrogen source	Dry matter yield (g/pot)			
	Leaves + petioles	Stems	Roots	Total
Control (without N)	13.3	36.5	40.5	90.3
Ammonium sulphate	87.0	138.8	69.5	295.3
P. phaseoloides leaf litter	63.0	138.5	79.3	280.8
C. caeruleum leaf litter	51.8	87.8	56.0	201.0
S.E. (±)	1.66	5.74	4.29	8.4
L.S.D. ($P < 0.05$)	5.3	18.4	13.7	26.9

Total dry matter yield of seedlings supplied with either leguminous leaf litter or ammonium sulphate as the N source was two to three times higher than that of seedlings which did not receive N. Although seedlings supplied with ammonium sulphate consistently produced the highest total dry matter the total dry weights were not significantly higher than those supplied with *P. phaseoloides* leaf litter as the N source. On the other hand, *P. phaseoloides* leaf litter-treated seedlings yielded significantly higher dry matter than the corresponding *C. caeruleum*-treated seedlings. In the control, the dry weights of stems and roots were similar while the stems contributed the largest proportion of total dry weight in other treatments. Except where ammonium sulphate was the source of N, the weight of roots was higher than the combined weight of leaves and petioles.

Uptake of Nutrients

The total uptake of major nutrients (N, P, K, Mg and Ca) by rubber seedlings as influenced by the different N sources are given in *Table 6*.

The results indicate that the amounts of all major nutrients in seedlings were the lowest where N was not applied. While seedlings supplied with ammonium sulphate contained significantly large amounts of N, higher uptake of K, Mg and Ca was found in seedlings supplied with *P. phaseoloides* leaf litter as the N source. It was also observed that seedlings supplied with *C. caeruleum* as the N source took up considerably less nutrients than seedlings which obtained their N from the mineralisation of *P. phaseoloides* leaf litter. Although higher dry matter production of seedlings fed with *P. phaseoloides* leaf litter may partly attribute to these observations, it would be of interest to do further investigations in view of the rather similar chemical composition of the two leguminous leaf litter *(Table 1)*.

However, the high N content in seedlings fed with ammonium sulphate is expected because of its immediate availability. In contrast, the lower N accumulation in seedlings supplied with leguminous leaf litter can be largely attributed to the relatively slow availability of the organic N source which is governed by the biological mineralisation process.

TABLE 6. EFFECT OF LEGUMINOUS LEAF LITTER AND AMMONIUM SULPHATE AS SOURCES OF NITROGEN ON THE UPTAKE OF NUTRIENTS BY RUBBER SEEDLINGS

Nitrogen source	Total nutrient uptake (mg/pot)				
	N	P	K	Mg	Ca
Control	885	140	1 652	205	537
Ammonium sulphate	6 282	479	3 005	543	1 327
P. phaseoloides leaf litter	3 126	450	3 932	622	1 936
C. caeruleum leaf litter	2 257	352	3 065	395	1 468
S.E. (±)	93.42	13.92	73.23	20.71	36.78
L.S.D. ($P < 0.05$)	298.8	44.5	243.3	66.2	117.7

Between the two legumes, leaf litter N of *P. phaseoloides* appears to be more readily available than those of *C. caeruleum* as reflected by the significantly higher N uptake by seedlings supplied with leaf litter of *P. phaseoloides (Table 6)*. This observation is not surprising because it has been reported in another study[2] that leaf litter of *P. phaseoloides* decomposed faster in the field and mineralised relatively large amounts of N during incubation compared with *C. caeruleum* leaf litter *(Table 3)*.

In the control treatment without any applied N source, the rubber seedlings had to depend mainly on the mineralisation of native soil organic N for their requirement. Results in *Table 6* indicate these seedlings were able to accumulate about 885 mg of N per pot after seven months of growth on Serdang series soil. Disregarding the amount of N present in the two-week-old seedlings and available N in soil at planting and also assuming negligible N losses from the pot as well as immediate utilisation of all mineralised N, this amount of total N uptake represents mineralisation of about 6% of the total organic N from the 24 kg of soil during the experimental period. Comparatively poorer growth and nutrient uptake in the control seedlings than those where N was supplied indicate the inability of this particular soil to mineralise sufficient inherent soil organic N to sustain healthy normal growth. Better performance could possibly be obtained through either application of ammonium sulphate or provision of leguminous leaf litter as N sources. Leguminous leaf litter of both *P. phaseoloides* and *C. caeruleum*, although slower in providing N than ammonium sulphate, has proved to be an invaluable N source for rubber.

In field experiments, the beneficial effects of leguminous cover on the growth and productivity of rubber were largely due to its direct result of addition of considerable amount of leaf litter N to the soil[1,12] as well as its indirect influence on soil physical properties[13] and rooting[1]. Present results showing growth improvement of rubber seedlings raised on soil containing ground leguminous leaf litter were likely due to the main and direct effects of its N availability because the experimental period of seven months was relatively short for the added leguminous leaf litter to have any significant influence on soil physical properties.

CONCLUSION

Laboratory studies showed that the leaf litter of the two common leguminous cover plants, *P. phaseoloides* and *C. caeruleum*, readily mineralised its organically-bound N upon incubation in soil. The leaf litter of *P. phaseoloides* appeared to be able to release relatively more mineral-N compared with that of *C. caeruleum*. On the other hand, incubation of rubber leaf litter resulted in immobilisation of available mineral N in the soil, probably due to its higher C/N ratio and lower N content than the two legumes. These findings suggest that fallen rubber leaf litter-N is not only temporarily unavailable but also likely to reduce the availability of soil mineral-N during the biological decomposition of leaf litter. It would be of interest, therefore, to know how soon it would become available to the trees by perhaps following the transformation processes of rubber leaf litter tagged with N^{15} in soil and monitoring its uptake by the trees in the field.

Results of glassware experiments further confirm field observations on the beneficial effects of leguminous cover on the growth and N nutrition of rubber trees, particularly during their immaturity period. The availability of N in the leaf litter of *P. phaseoloides* and *C. caeruleum* upon mineralisation was well reflected in the better growth and nutrition of rubber seedlings whose N source was the leaf litter of these two legumes compared with seedlings in the control treatment without any external source of N. Between the two legumes, leaf litter of *P. phaseoloides* promoted slightly better growth and nutrition (especially N) of rubber seedlings than the leaf litter of *C. caeruleum*. The higher N uptake by seedlings supplied with leaf litter of *P. phaseoloides* could probably be attributed to faster mineralisation of its N in comparison with leaf litter N of *C. caeruleum* which was shown earlier in the incubation studies. Although less efficient than ammonium sulphate, leaf litter of both legumes was shown to be a valuable complementary N source in the cultivation of rubber.

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