Nutrients obtained from leaf litter can improve the growth of dipterocarp seedlings

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Summary

• In tropical rain forests the rate of litterfall is high, and is the most important nutrient cycling pathway in these ecosystems. We tested two hypotheses using seedlings of dipterocarp species: (1) addition of leaf litter improves growth; (2) and litter addition affects both ectomycorrhizal (ECM) colonization and community structure.

• Three dipterocarp species with contrasting ecologies (*Parashorea tomentella*, *Hopea nervosa* and *Dryobalanops lanceolata*) were grown in a nursery in forest soil with or without the addition of litter.

• Litter addition improved the growth of all three species. There was no effect of litter addition on total percentage ECM colonization but ECM diversity and percentage colonization by *Cenococcum geophilum* were lower with litter addition. Foliar $\delta^{15}N$ was lower in two of the three species grown in the presence of litter, reflecting the lower $\delta^{15}N$ of the litter compared with the soil. There was a negative correlation between $\delta^{15}N$ and percentage ECM, suggesting a role for ECMs in accessing litter-derived N sources.

• This study shows that litter addition improved the growth of dipterocarp seedlings and that the ECM associations of dipterocarps facilitated access to this organic nutrient source. This has implications for the successful regeneration of seedlings in the rain forest understorey.

Key words: Borneo, $\delta^{15}N$, dipterocarps, ectomycorrhizas, fine root proliferation, leaf litter, seedling growth, nitrogen isotope discrimination, tropical rain forest.

Introduction

Plant litter can influence patterns of seedling regeneration in tropical rain forests through a number of processes affecting both the physical and chemical environment (Facelli & Pickett, 1991). At the seed germination stage, litter can intercept light, which will inhibit germination by altering the red/far-red ratio (Vázquez-Yánes *et al.*, 1990); it can act as a physical barrier to seedling emergence (Molofsky & Augspurger, 1992), especially for small-seeded species which do not have a large supply of resources (Metcalfe & Turner, 1998), and may prevent newly germinated radicles from reaching the soil. Litter can also prevent seed detection by seed predators, thereby increasing the chances of successful germination (Cintra, 1997). For plants at the seedling stage, litter can create different environmental microsites by releasing nutrients or phytotoxic compounds during its decomposition, by reducing soil erosion and evapotranspiration (but it may also intercept rainfall) and by reducing maximum soil temperatures. Litter may also act as a mechanical factor, damaging or killing seedlings as it falls to the ground (Clark & Clark, 1989; Scariot, 2000). There can also be indirect effects of leaf litter, for example, the higher humidity in the litter layer may promote growth of fungal pathogens which can then attack seedlings (García-Guzmán & Benitez-Malvido, 2003).

In tropical rain forests the rate of litterfall is high, and it is the most important nutrient cycling pathway in these ecosystems (Vitousek & Sanford, 1986; Proctor, 1987). There can be considerable spatial and temporal heterogeneity in litterfall (Burghouts *et al.*, 1994) which may be further accentuated by factors such as strong winds, forest clearance and forest fragmentation. Litter heterogeneity can also be increased by differing rates of decomposition of the leaves of different species. This heterogeneity in litter on the forest floor may create different regeneration niches (*sensu* Grubb, 1977) and hence

help contribute to the exceptionally high species diversity in tropical rain forests.

There has been considerable recent work on the mineral nutrition of dipterocarp species and on the nature of nutrient limitation in these ecosystems (Burslem *et al.*, 1995, 1996; Gunatilleke *et al.*, 1997; Bungard *et al.*, 2000, 2002; Yap *et al.*, 2000). Although phosphorus is often considered to be the major limiting nutrient (Vitousek, 1984), there is also evidence for the importance of magnesium (Burslem *et al.*, 1996; Gunatilleke *et al.*, 1997) and nitrogen, especially following simulated gap creation (Bungard *et al.*, 2000). However, one criticism which may be directed at these studies is that they have all altered the nutrient status of the growth medium by using inorganic nutrient sources which are unlikely to vary greatly in natural tropical forest ecosystems. The vegetation of tropical rain forests is much more reliant upon the decomposition of litter for nutrients as the nutrient supply from weathering of the parent material is low. Therefore it is likely that, in the forest, the main variation in nutrient status will be due to variation in the input of organic nutrients, much of which will be comprised of leaf litter.

The use of nutrients from decomposing litter may be facilitated by the ectomycorrhizal (ECM) associations of dipterocarp seedlings. Many ECM fungi can utilize organic nitrogen and phosphorus sources (Abuzinadah & Read, 1986; Hilger & Krause, 1989; Finlay *et al.*, 1992; Turnbull *et al.*, 1995; Chalot & Brun, 1998; Sangtiean & Schmidt, 2002) and subsequently transfer these nutrients to their host plant (Finlay *et al.*, 1992; Turnbull *et al.*, 1995; Perez-Moreno & Read, 2000, 2001; Tibbett & Sanders, 2002). There is also clear evidence of the use of nitrogen and phosphorus obtained directly from litter patches added to soil in experimental settings using birch (*Betula pendula* Betulaceae) seedlings (Perez-Moreno & Read, 2000). In this experiment, and the experiment of Bending and Read (1995), there was a spectacular proliferation of fungal hyphae in the litter patches indicating the importance of the ECM association in accessing the nutrients within them. However, there are reports of extracts from leaf litter causing an inhibition of ECM growth *in vitro* (Rose *et al.*, 1983; Baar *et al.*, 1994; Koide *et al.*, 1998) and litter addition also led to a reduction in ECM formation on Douglas fir (*Pseudotsuga menziesii* Pinaceae) (Rose *et al.*, 1983) and red pine (*Pinus resinosa* Pinaceae) seedlings (Koide *et al.*, 1998).

Most litter studies in tropical rain forest regions have considered the effects of litter on seed germination and early establishment; studies examining the effects on established seedlings are fewer. In this paper we examine the potential role of nutrients contained within litter on the growth of dipterocarp seedlings. Specifically, we test two hypotheses: does the addition of leaf litter to the growth medium improve the growth of dipterocarp seedlings; and does litter addition affect the ectomycorrhizal colonization or community structure of dipterocarp seedlings? In addition, we report foliar $\delta^{15}N$ values as a potential measure of litter nitrogen usage.

Materials and Methods

Study site

The study was carried out in the nursery of the Sabah Forestry Department's Forest Research Centre. The Forest Research Centre is situated adjacent to the 4294 ha Kabili-Sepilok Forest Reserve in eastern Sabah (a state of Malaysia on the island of Borneo) (5°52′N, 117°56′E). The area experiences a wet tropical climate and receives *c.* 3000 mm of rainfall per annum. Most months receive > 100 mm of rain, but in some years there is a pronounced dry spell around April and the climate could be considered weakly seasonal. The mean daily temperature range at Sandakan Airport (*c.* 11 km to the east) is from 31.3°C to 23.8°C and there is greater diurnal than annual variation.

Study species

Three species, with contrasting ecologies, were chosen for the study. *Parashorea tomentella* (Symington) Meijer (Urat mata beludu) is a relatively light-demanding species, which is common in the lowland forests (< 200 m) of north-east Borneo. It is a very large, light hardwood tree and its timber is used extensively. *Hopea nervosa* King (Selangan jangkang) is a shade-tolerant, medium hardwood species. It is common in northern Borneo, but is rarely used for timber because of its comparatively small stature. *Dryobalanops lanceolata* Burck (Kapur paji) is a shade-tolerant species, although it can survive under high light conditions due to effective dissipation of excess light energy (Scholes *et al.*, 1997). It can grow to a very large size where it is found on relatively more fertile soils in northern Borneo. It is a medium hardwood, which is also commonly used for timber (see Meijer & Wood 1964 and Newman *et al.* 1996, 1998 for further details on the study species).

Growth conditions

Two-year-old seedlings of *H. nervosa* and *D. lanceolata* and one-year-old seedlings of *P. tomentella* (grown from seed obtained from Danum Valley Conservation Area or Kabili-Sepilok Forest Reserve, Sabah) were planted into 1.2-l plastic pots containing alluvial soil from Kabili-Sepilok Forest Reserve (sieved to *c.* 1 cm). All seedlings were ECM at the time of planting. They were placed on four replicate shade-tables in the nursery of the Forest Research Centre under neutral density shade cloth allowing transmission of 20% of full sunlight (up to 8.1 mol m^{-2} day^{-1}). There were three replicates of each species/treatment combination per table, giving a total of 12 replicates. The seedlings were grown for 10 months and watered by natural rainfall. On days when there was no rainfall, the seedlings were watered until the soil in the pots was saturated.

Table 1 Nutrient concentrations of the litter added and of the leaves of three dipterocarp species following growth for 10 months in soil with or without litter addition (all values are mean ± SE)

		Parashorea tomentella		*Hopea nervosa*		*Dryobalanops lanceolata*	
	Litter	– Litter	+ Litter	– Litter	+ Litter	– Litter	+ Litter
Nitrogen (%)	0.91 ± 0.05	1.34 ± 0.09	1.35 ± 0.07	1.07 ± 0.03	1.09 ± 0.04	0.99 ± 0.08	0.99 ± 0.06
Phosphorus (mg g^{-1})	0.20 ± 0.04	0.79 ± 0.03	0.78 ± 0.04	0.81 ± 0.03	0.77 ± 0.03	0.85 ± 0.07	0.80 ± 0.01
Potassium (mg g^{-1})	6.55 ± 0.71	5.72 ± 0.43	4.95 ± 0.24	9.08 ± 0.50	8.77 ± 0.38	10.53 ± 0.39	11.02 ± 0.54
Calcium (mg g^{-1})	6.32 ± 0.61	14.93 ± 0.61	14.35 ± 0.83	7.74 ± 0.37	7.12 ± 0.36	10.06 ± 1.19	9.00 ± 1.30
Magnesium (mg g^{-1})	2.93 ± 0.32	0.84 ± 0.09	0.65 ± 0.08	0.80 ± 0.12	0.89 ± 0.13	2.11 ± 0.11	2.06 ± 0.16

Litter collection, addition and analysis

Litter was collected from the alluvial forest of Kabili-Sepilok Forest Reserve in August 2001, immediately before the start of the experiment. Freshly fallen leaves of a mixed, and random, species selection were collected directly from the ground, air-dried and chopped into pieces of *c.* 3 cm^2. Ten g of the litter was added to half the seedlings of each species; it was added to the top half of each pot in four bands across the pot. Litter was buried within the soil to prevent desiccation and encourage its microbial decomposition.

Proctor (1984) and Bagchi (2002) present data for 20 south-east Asian lowland rain forest sites, which have a mean value of 9.4 t ha^{-1} $year^{-1}$ for total litterfall. Ten t ha^{-1} $year^{-1}$ is equivalent to 1 kg m^{-2}. As the surface area of the pots used in the experiment was 50 cm^2, we would expect this area to receive 5 g of litter per annum in nature. Therefore, the addition of 10 g is approximately double that which would be seen in nature but, given the highly variable nature of litterfall, is certainly not unrealistic.

Twenty random samples of litter were analysed for P, K, Ca and Mg concentrations following digestion in a salicylic/sulphuric acid mix (33 g l^{-1}) with a lithium sulphate/copper sulphate (10 : 1 ratio) catalyst (Table 1). Phosphorus was analysed on an auto-analyser (Tecator 5042 Detector and 5012 Analyser, Foss UK Ltd, Didcot, UK) using the ammonium molybdate-stannous chloride method (Tecator Ltd, 1983). Potassium, Ca and Mg were analysed by atomic absorption spectrophotometry (Perkin-Elmer 2100 Atomic Absorption Spectrophotometer, Beaconsfield, UK).

Ten samples of *c.* 1 mg of mixed litter were analysed for percentage nitrogen and $\delta^{15}N$ after being ground in liquid nitrogen (PDZ Europa ANCA-GSL preparation module connected to a 20-20 isotope ratio mass spectrometer, Northwich, UK). Eight composite soil samples from Kabili-Sepilok Forest Reserve were also analysed for $\delta^{15}N$. Isotope ratios were calculated as:

$$\delta^{15}N\ (‰) = (R_{sample}/R_{standard}) \times 1000$$

where R is the isotope ratio of $^{15}N/^{14}N$ of either the sample or the standard (atmospheric nitrogen).

Seedling measurements

At the end of the experiment, after 10 months, the seedlings were harvested, divided into leaf, main stem and branches, tap root and fine root fractions, dried at 80°C for 48 h and each fraction was weighed. Leaf area was calculated by measuring the length and width of every leaf in mm and using regression equations to determine the leaf area in cm^2: *P. tomentella* leaf area = 0.762 + (0.00670 × length × width) (r^2 = 98%); *H. nervosa* leaf area = 0.00751 × length × width (r^2 = 97%; Leakey, 2002); *D. lanceolata* leaf area = −0.28 + (0.00700 × length × width) (r^2 = 99%; Bungard *et al.*, 2002).

The three youngest fully expanded leaves were removed from each seedling and N, P, K, Ca and Mg were measured on a bulked sample of a small section from each leaf as described above. The leaves were also analysed for $\delta^{15}N$ as above. Approximately one third of the leaf samples were analysed in duplicate for $\delta^{15}N$ and the mean relative standard deviation was 0.22‰. Specific leaf area of the same three leaves was calculated by dividing their areas by their individual weights.

Ectomycorrhizas

Percentage ectomycorrhizal colonization (% ECM) of the fine roots was calculated as a percentage of the number of ECM root tips out of a total of *c.* 150–200 root tips for every seedling. The ECM community was examined on eight seedlings per species/treatment combination. Morphotypes were recognized from gross morphological features (branching patterns, colour, mantle texture, presence of emanating hyphae, etc.), the mantle and hyphal characteristics were examined microscopically using squashing (Ingleby *et al.*, 1990) and scraping (Agerer, 1991) techniques.

The Shannon–Wiener diversity index (H') was calculated for the ECM community on each seedling using the equation:

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

where s = number of morphotypes and p_i = abundance of the i^{th} morphotype expressed as a proportion of the total colonised root tips.

The Berger–Parker evenness index (d) was calculated for the ECM community on each seedling using the equation:

$$d = 1 - \left(\frac{N_{max}}{N} \right)$$

where N_{max} is the percentage of the root tips with the most abundant morphotype and N is the total percentage of colonized root tips.

Statistics

Two-way ANOVAs, generated by general linear modelling, were carried out using species and treatment as the main factors. Data were transformed using the results from a Box-Cox analysis where necessary. The species by treatment interaction was initially included in all the models; when it did not explain a significant proportion of the variation it was removed and is therefore not reported. Due to heterogeneous variances among species, foliar nutrient concentrations and $\delta^{15}N$ were analysed using t-tests within in each species (a one-tailed t-test was used for $\delta^{15}N$ as we hypothesized a decrease in $\delta^{15}N$ with litter addition; this was because litter $\delta^{15}N$ was significantly more negative than soil $\delta^{15}N$). Correlation between $\delta^{15}N$ and percentage ECM was carried out using Pearson's product moment correlation coefficient. All statistical analyses were performed using Minitab 12.2 (Minitab Inc., State College, Pennsylvania, USA).

Results

Litter addition increased the biomass of *H. nervosa* by 60%, *P. tomentella* by 20% and *D. lanceolata* by 10% ($F_{1,59} = 9.74$, $P = 0.003$; Fig. 1a). Litter addition also increased the leaf area of all three species by 55%, 40% and 25% for *P. tomentella*, *H. nervosa*, and *D. lanceolata*, respectively ($F_{1,59} = 16.79$, $P < 0.001$; Fig. 1b). These increases in growth were not accompanied by any changes in specific leaf area (Table 2).

The addition of litter had a small impact on the allocation of biomass among leaf, stem and root tissue (Table 2), although the direction of response varied among species, and no overall impact on root : shoot ratio was observed (Table 2). Of most interest here was the increase in the proportion of biomass allocated to fine roots, which although a small fraction of total biomass, showed a significant increase in response to litter addition by 35%, 25% and 15% for *H. nervosa*, *D. lanceolata* and *P. tomentella*, respectively ($F_{1,59} = 7.71$, $P = 0.007$; Table 2).

The addition of litter had no significant impact on the concentrations of N, P, K, Mg or Ca in the leaves of the seedlings (Table 1). Species differed in foliar nutrient concentrations, sometimes by more than two-fold (Table 1).

$\delta^{15}N$ measurements revealed a significant difference in the isotope signature of the soil and the litter used in the experiment, with values of 8.54‰ and 4.48‰ respectively

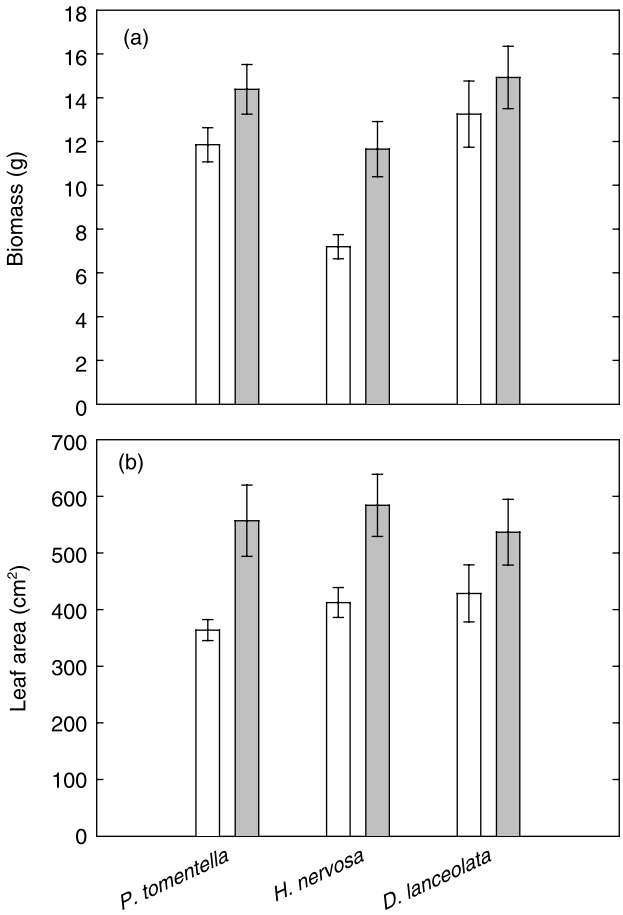

Fig. 1 (a) Biomass, and (b) leaf area of three dipterocarp species following growth for 10 months in soil with (shaded bars) or without (unshaded bars) litter addition (all bars are mean ± SE).

($t_{16} = 3.35$, $P = 0.040$; Fig. 2). Analysis of leaf material showed that for both *D. lanceolata* and *H. nervosa*, the addition of litter resulted in significantly lower $\delta^{15}N$ values, by 1.49‰ and 0.73‰, respectively (*D. lanceolata*: $t_{17} = 2.47$, $P = 0.012$; *H. nervosa*: $t_{14} = 1.69$, $P = 0.057$; Fig. 2), suggestive of a significant acquisition of nitrogen from the added litter. By contrast, no such difference was observed in the leaves of *P. tomentella* ($t_{21} = 0.05$, $P = 0.48$; Fig. 2).

There was no effect of litter addition on total percentage ECM colonization ($F_{1,59} = 0.98$, $P = 0.33$; Table 3) although differences were observed among the three study species: *H. nervosa* showed a greater ECM colonization (*c.* 80%) than *P. tomentella* (*c.* 70%), which both showed a significantly greater colonization than *D. lanceolata* (*c.* 55%) ($F_{2,59} = 23.02$, $P = 0.001$; Table 3). Combining species and treatments resulted in a significant negative correlation between foliar $\delta^{15}N$ and percentage ECM colonization ($r = -0.467$, $P = 0.001$; Fig. 3), suggesting a role for ECMs in enhancing acquisition of litter-derived nitrogen.

A total of 11 morphotypes (and one fungal endophyte) were identified on the roots of the dipterocarp seedlings

Table 2 Biomass allocation patterns and specific leaf area of three dipterocarp species following growth for 10 months in soil with or without litter addition (all values are mean ± SE)

	Parashorea tomentella		*Hopea nervosa*		*Dryobalanops lanceolata*	
	– Litter	+ Litter	– Litter	+ Litter	– Litter	+ Litter
Leaf mass (%)	19 ± 1.4	23 ± 1.5	31 ± 1.2	27 ± 1.2	21 ± 1.3	25 ± 1.1
Stem mass (%)	38 ± 1.6	32 ± 0.9	33 ± 1.6	32 ± 1.5	46 ± 1.8	44 ± 1.3
Root mass (%)	43 ± 2.0	45 ± 1.7	36 ± 2.3	41 ± 1.4	34 ± 1.6	32 ± 1.7
Fine root mass (%)	15 ± 1.4	17 ± 1.2	12 ± 1.1	16 ± 0.9	9 ± 0.9	12 ± 2.4
Root:shoot ratio	0.76 ± 0.06	0.85 ± 0.06	0.57 ± 0.06	0.69 ± 0.04	0.51 ± 0.04	0.47 ± 0.04
Specific leaf area (g m^{-2})	65.4 ± 3.2	68.3 ± 2.6	59.4 ± 2.5	60.6 ± 2.6	70.0 ± 3.3	72.3 ± 4.4

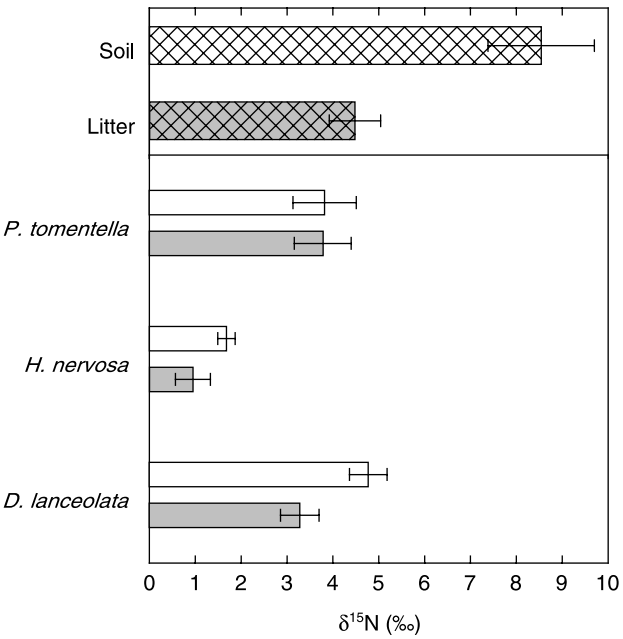

Fig. 2 $\delta^{15}N$ of soil and litter used in the experiment together with foliar values of three dipterocarp species following growth for 10 months with (shaded bars) or without (unshaded bars) litter addition (all bars are mean ± SE).

(Table 3). There were notable effects of litter addition on the ECM community structure. ECM diversity and evenness were both lower when litter was added (Diversity: $F_{1,44} = 5.21$, $P = 0.027$; Evenness: $F_{1,44} = 5.95$, $P = 0.019$; Table 3). Impacts of litter on community structure appeared to be driven mainly by changes in the proportion of root tips colonized by the second commonest species, *Cenococcum geophilum* Fr. (Elaphomycetaceae). *C. geophilum* showed a significant reduction in colonization with the addition of litter ($F_{1,44} = 21.70$, $P = 0.001$; Table 3), this reduction was over two-fold in *P. tomentella*, around two-fold in *H. nervosa* and eight-fold in *D. lanceolata*. The commonest morphotype, *Inocybe* spp. (Cortinariaceae), showed no response to litter addition ($F_{1,44} = 0.15$, $P = 0.702$; Table 3).

Discussion

How does litter addition affect seedling growth and performance?

The effect of leaf litter addition on seedling growth and performance has been studied in neotropical seedlings in genera which possess arbuscular mycorrhizas (AM); tropical ECM genera have received much less attention in this regard. Our results are consistent with the other studies of AM species which have found the effects of litter to be species specific (Guzmán-Grajales & Walker, 1991; Molofsky & Augspurger, 1992; Benitez-Malvido & Kossmann-Ferraz, 1999; Ganade & Brown, 2002). All of these studies showed an improvement in growth with the addition of litter to some of their study species, whereas other species did not show as great a response. It also appears that successional status has a bearing on the response of the seedlings to litter addition, with late successional species generally showing a more positive response. This is likely to be because late successional species generally have larger seeds and hence greater reserves to emerge from deep litter layers. The only litter addition study using a dipterocarp species is that of Suhardi *et al.* (1992) who found that addition of litter of the grass, alang-alang (*Imperata cylindrica* Poaceae), reduced the percentage ECM (especially under higher irradiances) and overall growth of *Shorea bracteolata*, but this may have been due to the allelopathic nature of *I. cylindrica* (Brook, 1989; Suhardi, 2000).

Our results can be compared with other inorganic nutrient addition experiments with dipterocarp species. A number of studies have shown an increase in the biomass of *Dryobalanops* species by at least 30% with additions of N, P and K (Sundralingham, 1983; Yap & Moura-Costa, 1996; Yap *et al.*, 2000; F. Q. Brearley, unpublished data) to > 200% (Nussbaum *et al.*, 1995) on degraded soils. Bungard *et al.* (2002) did not see a growth response when N, P and K were added to *D. lanceolata* in the forest understorey, but there was a change in the photosynthetic physiology, with an increased rate of photosynthetic induction. In the studies of Yap & Moura-Costa (1996), Yap *et al.* (2000) and Bungard *et al.* (2002), it appeared that nitrogen was the primary limiting

Table 3 Percentage colonization of three dipterocarp species by 11 ectomycorrhizal morphotypes (and one fungal endophyte) following growth for 10 months in soil with or without litter addition (all values are mean ± SE)

	Parashorea tomentella		*Hopea nervosa*		*Dryobalanops lanceolata*	
	– Litter	+ Litter	– Litter	+ Litter	– Litter	+ Litter
Mycorrhizal	68.4 ± 3.4	69.2 ± 4.7	80.5 ± 3.8	80.7 ± 2.6	49.8 ± 4.0	60.1 ± 4.3
Nonmycorrhizal	31.6 ± 3.4	30.8 ± 4.7	19.5 ± 3.8	19.3 ± 2.6	50.2 ± 4.0	39.9 ± 4.3
Morphotypes per seedling	2.6 ± 0.2	2.1 ± 0.2	2.9 ± 0.2	2.5 ± 0.2	1.9 ± 0.2	2.3 ± 0.2
Shannon–Wiener index	0.70 ± 0.06	0.56 ± 0.04	0.76 ± 0.07	0.57 ± 0.10	0.58 ± 0.07	0.50 ± 0.08
Berger–Parker index	0.29 ± 0.06	0.20 ± 0.04	0.34 ± 0.06	0.20 ± 0.06	0.25 ± 0.06	0.14 ± 0.06
Inocybe spp.*	27.8 ± 8.8	34.5 ± 11.4	51.8 ± 7.9	53.2 ± 10.7	40.3 ± 5.5	40.5 ± 8.8
Cenococcum geophilum Fr.	31.2 ± 6.6	12.5 ± 2.4	17.7 ± 3.0	8.9 ± 6.3	13.1 ± 3.8	1.5 ± 0.6
Riessiella sp.	–	–	6.1 ± 4.3	13.9 ± 7.7	0.7 ± 0.7	0.2 ± 0.2
Boletales sp.	10.9 ± 6.1	–	0.3 ± 0.3	2.0 ± 2.0	–	7.9 ± 7.9
Basidiomycete sp. 1	0.1 ± 0.1	12.4 ± 8.2	–	–	–	1.6 ± 1.6
Thelephorales sp. 1	–	–	3.1 ± 3.1	–	–	5.3 ± 5.2
Thelephorales sp. 2	–	–	1.1 ± 1.1	2.5 ± 1.8	–	3.0 ± 3.0
Basidiomycete sp. 2	–	5.2 ± 5.2	–	–	–	–
cf. Russulaceae sp.	0.5 ± 0.5	–	–	–	–	–
Basidiomycete sp. 3	0.4 ± 0.4	–	–	–	–	–
cf. T20 (Lee *et al*., 1997)	–	0.1 ± 0.1	–	–	–	–
Endophyte sp.	–	1.1 ± 1.1	0.3 ± 0.3	1.2 ± 0.7	1.4 ± 1.3	1.6 ± 1.2

*There may have been up to three *Inocybe* species but, due to difficulties in making positive identifications, these were combined to form one morphotype grouping.

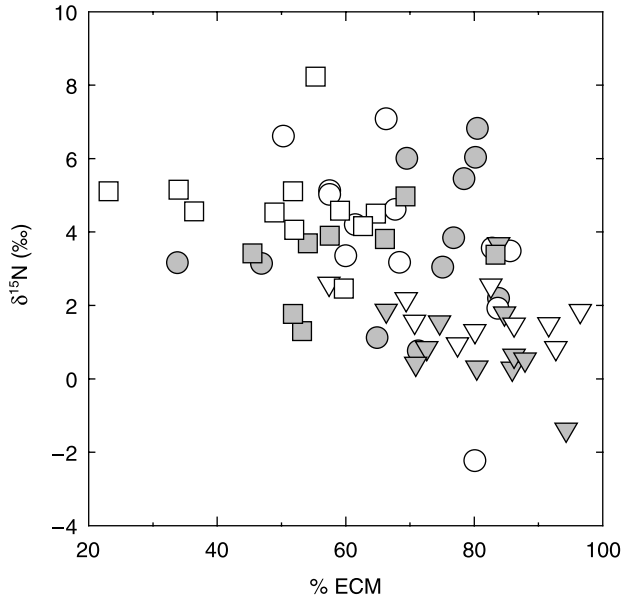

Fig. 3 Negative correlation between percentage ECM and foliar $\delta^{15}N$ for three dipterocarp species (*Parashorea tomentella*, circles; *Hopea nervosa*, triangles; *Dryobalanops lanceolata*, squares) following growth for 10 months with (shaded symbols) or without (unshaded symbols) litter addition.

nutrient to the growth of *D. lanceolata*. In our study we found no differences in foliar nutrient concentrations between the treatments, therefore we did not gain any insight into possible limiting nutrients. *Dryobalanops* species are certainly able to respond to the addition of nutrients by a large increase in growth. Possibly the lower levels of ECM infection in *D. lanceolata* do not allow effective utilization of organic matter.

The increase in allocation to fine root biomass of all three species with the addition of litter is interesting as roots often proliferate in patches enriched with organic nutrients (St. John *et al*., 1983b; Blair & Perfecto, 2001). This contrasts with the response of roots when nutrients are added in solution, which will presumably diffuse throughout the growth medium and raise the soil fertility more uniformly. Burslem *et al*. (1996) found reductions in the lateral root ratio (ratio of fine root to tap root biomass) for two dipterocarp species when nutrients were added in this fashion.

How does litter addition affect ectomycorrhizal colonization and community structure?

It was somewhat unexpected that we did not find any change in percentage ECM in the litter addition treatment as many studies have shown an increasing mycorrhizal colonization and an association of mycorrhizal hyphae with litter patches, e.g. Rose & Paranka (1987) found AM colonization to be higher in the litter and humus layer of a tropical forest in Brazil and St. John *et al*. (1983a) and Hodge *et al*. (2001) also found that AM hyphae were associated with patches of organic matter in pot experiments. Similar findings have been reported by Read (1991) where ECM hyphae associated with localized patches of organic matter but this did not happen when inorganic mineral salts were added. In the experiments of Perez-Moreno & Read (2000, 2001) there was a slight reduction in percentage ECM in the litter or pollen addition

treatments but a very clear association of hyphae with the patches of organic material. Perhaps if we had measured extramatrical hyphal length in the litter substrate, a greater amount of hyphae, being the main site of nutrient absorption, would have been found in the litter addition treatments.

However, we did find that litter addition had a clear effect on the ECM community structure, much of which was driven by changes in the abundance of *C. geophilum* which was less common in soils with litter addition. Malajczuk & Hingston (1981) and Reddell & Malajczuk (1984) also found that *C. geophilum* on the roots of *Eucalyptus marginata* (Myrtaceae) was found mainly in the mineral soil rather than in the litter layer, but this contrasts with Fransson *et al.* (2000) and Jonsson *et al.* (2000) who found *C. geophilum* to be more common in the litter layer. Very little work has been done on the ecology of various ECM fungi and it is not clear why these apparently contradictory results were obtained. *C. geophilum* is able to utilise complex organic nitrogen sources *in vitro* (Abuzinadah & Read, 1986; Lilleskov *et al.*, 2002b) but it is affected by various phenolics and volatiles which can reduce its growth rate and respiration (Pellisier, 1993; Boufalis & Pellissier, 1994; Koide *et al.*, 1998). Leaf litter is likely to retain moisture and therefore a third possibility is that *C. geophilum* associates with mineral soils as they are more likely to dry out, especially in the nursery where there may be a slightly hotter and drier environment than in the forest. Worley & Hacskaylo (1959) and Piggott (1982) have both shown that *C. geophilum* can withstand desiccation well and it may be that this species gains a competitive advantage in drier soils. It appears that there is a delicate balance between the nutrient content, phenolic content, and moisture retaining capabilities of leaf litter affecting the abundance of different ECM fungi.

A change in plant community structure and a reduction in diversity has been seen for other 'fertilization' studies where the species which are most responsive to nutrient addition out-compete and dominate the less responsive species (e.g. Huenneke *et al.*, 1990; Press *et al.*, 1998). The response of the below-ground ECM community to increased nutrients is less clear, as some studies have found a decrease in ECM diversity with increasing nitrogen deposition (Taylor *et al.*, 2000; Lilleskov *et al.*, 2002a), whereas others have found smaller changes in response to nitrogen fertilization (Kårén & Nylund, 1997; Jonsson *et al.*, 2000). Taylor *et al.* (2000) found a decrease in the abundance of protein using fungi with increasing soil inorganic nitrogen. Unfortunately, the protein using abilities of the fungi identified in our study have not been examined so we cannot predict possible changes with increasing soil organic nitrogen.

What are the implications of differences in the $\delta^{15}N$ values?

The nitrogen isotope composition of a plant can be influenced firstly by the isotope ratio of the nitrogen source and secondly by various physiological mechanisms during nitrogen uptake, assimilation and recycling within the plant. Further isotope discrimination as nitrogen moves from the fungus to the plant during mycorrhizal mediated uptake, can also cause the isotope ratio to deviate from the source (Evans, 2001).

Our experiment shows two important results with regard to nitrogen isotope fractionation. Firstly, two of our three study species showed a lower $\delta^{15}N$ when supplied with an organic nitrogen source (leaf litter). Secondly, seedlings with a greater degree of ECM colonization showed a more negative $\delta^{15}N$.

When nitrogen demand exceeds nitrogen supply and is therefore limiting to growth, it has been shown that the $\delta^{15}N$ of the plant is a good approximation of the $\delta^{15}N$ of the source (Högberg *et al.*, 1999; Evans, 2001). This is because under nitrogen limitation, a plant should take up all the available nitrogen, leaving little possibility for physiological fractionation. In our experiment, the soil $\delta^{15}N$ was *c.* 4‰ more negative than the litter $\delta^{15}N$, therefore we expected the seedlings grown with the addition of litter to have a more negative $\delta^{15}N$ value. The $\delta^{15}N$ of *H. nervosa* was 0.73‰ more negative when grown with the addition of litter, and the $\delta^{15}N$ of *D. lanceolata* was 1.49‰ more negative. Assuming isotope fractionation patterns to be the same in the two treatments, we can calculate that *c.* 18% of the foliar nitrogen of *H. nervosa* was obtained from the added litter, with *D. lanceolata* obtaining *c.* 37% of its nitrogen from the litter. Why only a negligible proportion of nitrogen appeared to be obtained from the litter in *P. tomentella*, probably the most nitrogen demanding species, is unclear. This value of 18–37% compares with the values of 8.5% obtained by Preston & Mead (1994) studying *Pinus contorta* (Pinaceae) and 16–21% obtained by Setälä *et al.* (1996) studying *Populus trichocarpa* (Salicaceae) although it is considerably higher than the 2% obtained by Zeller *et al.* (2000) who examined adult beech (*Fagus sylvatica* Fagaceae) trees. Presumably, adult trees have a lower demand for nitrogen than seedlings or, alternatively, the pot conditions under which the seedlings were grown led to more rapid nitrogen mineralization rates (Zeller *et al.*, 2000). That nitrogen was not supplied to excess in our experiment can be confirmed, firstly by the lack of a difference in foliar nitrogen between the two treatments, and secondly that the concentrations in our study were markedly lower than those found in wildings growing in Danum Valley Conservation Area forest in Sabah (Bungard *et al.*, 2002).

A number of studies have correlated foliar $\delta^{15}N$ with mycorrhizal status and, by inference, access to litter-derived nutrients. Michelson *et al.* (1996) suggested that the use of organic nitrogen by ECM and ericoid fungi could account for leaves depleted in ^{15}N when compared with non- and arbuscular mycorrhizal species. Our experimental results are consistent with this suggestion, as the lower foliar $\delta^{15}N$ values in the litter addition treatment, for two of the three species,

strongly suggest that the nitrogen taken up originated from this organic source. Under conditions of stronger nitrogen limitation, some species may become more reliant upon mycorrhizas. Therefore, with a greater percentage ECM, seedlings may become more depleted in ^{15}N as a greater amount of ^{15}N is sequestered in fungal tissue and isotopically lighter nitrogen is transferred to the plant (Hobbie *et al.*, 2000).

What are the community implications of this study?

Germination and establishment of seeds and seedlings are two factors in plant community organization that are particularly sensitive to the presence of litter (Facelli & Pickett, 1991). Our results, together with results from other studies, show that leaf litter variability can create a variety of regeneration niches with some species more able to utilize the extra resources provided. Furthermore, the effect of litter on seedling emergence can cause reversal in species rankings in the success of emergence (Molofsky & Augspurger, 1992). This means that if litterfall rates were increased consistently across a forest (perhaps due to fertilization, e.g. Mirmanto *et al.*, 1999) then there would be greater changes in the species composition than if all species were affected in a similar fashion. Litter may also affect the structure of plant communities through an indirect competitive fashion whereby one strongly competitive species, which is negatively affected by litter, may be prevented from out-competing other species when litter is present (Facelli, 1994). In the forest there is likely to be some interaction with light levels as this is the main limiting resource for seedlings. Gap creation and pulses of litter production are likely to be correlated to a certain degree as a tree fall is likely to carry down with it a number of leaves and other organic material, and also kill other smaller seedlings beneath it. Therefore, these changes in community structure may only be realized during the gap regeneration phase.

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