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# Leaf $\delta^{15}N$ in diverse tree species in a lowland dipterocarp forest, Lambir Hills National Park, Sarawak, Malaysia

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**Abstract** Nitrogen is one of most limiting nutrients of plants' growth. Lowland tropical rainforests in Southeast Asia are characterized by high species diversity despite limited soil nitrogen conditions. We analyzed $\delta^{15}N$ values and nitrogen content (N %) in leaves and roots of 108 woody species with different types of symbiotic microorganisms, of different life forms (emergent, canopy, subcanopy, understory, and canopy gap species), and from different families in a Bornean lowland dipterocarp forest to gain more insight into the diversity of nitrogen uptake strategies in the rhizosphere. The plant nitrogen isotope ratio ($\delta^{15}N$) changes depending on soil nitrogen source or root symbiotic fungi. Leaf $\delta^{15}N$ values in the species studied varied widely, from −7.2 to 5.0 ‰, which is comparable to the values of known Asian trees, including those from temperate, subtropical, and tropical mountain forests. Leaf $\delta^{15}N$ also varied significantly among both life forms and families, though the phylogenetically independent contrast (PIC) relationships were not statistically significant among life form, family, and symbiotic types. Some families showed specific leaf $\delta^{15}N$ values; Dipterocarpaceae, the dominant family in the canopy layer with symbiotic ectomycorrhiza in Southeast Asia, had small intraspecific variation and higher leaf $\delta^{15}N$ values (0.03 ‰) compared with species exhibiting arbuscular mycorrhiza, whereas several families such as Burseraceae, Euphorbiaceae, and Phyllanthaceae showed large interspecific variation in leaf $\delta^{15}N$ (e.g., from –7.2 to 5.0 ‰ in Euphorbiaceae). These variations suggest that tropical species may have family- or species-specific strategies, such as root symbiotic microorganisms, for nitrogen uptake under low-nutrient conditions in tropical rainforests in Southeast Asia.

**Keywords** Dipterocarpaceae, Nitrogen acquisition, Nitrogen stable-isotope, Root symbiosis, Tropical rainforest

**Introduction**

Nitrogen (N) is a key resource for plant growth, photosynthesis, and reproduction (Houlton et al. 2006). In Southeast Asia, lowland tropical rainforests have high species diversity under low soil nutrient conditions (Ashton 2014). In such tropical rainforest ecosystems, nutrient competition and/or partitioning has been presented as one of the key mechanisms for the distribution of diverse species (Palmiotto et al. 2004). In fact, the distributions of many species in Southeast Asian tropical rainforests are closely related to soil conditions (Ashton 2014), including soil N pool quantity, e.g., composition and amount of ammonium ($NH_4^+$) and nitrate ($NO_3^-$). Under N-limited conditions, plants usually develop various species-specific N-uptake strategies (Schulze et al. 1994; Kenzo et al. 2016). Competition in the rhizosphere for those different forms of N can affect the partitioning of soil N among plant species, and such competition is thus expected to influence the distribution and coexistence of plant species (Russo et al. 2013). Understanding the N-uptake strategies in diverse tropical rainforest trees may contribute to our understanding of species distribution, N cycling and proper conservation of biodiversity in tropical rainforest plant communities. However, the difficulty of studying belowground functions such as root ectomycorrhiza symbiosis limits our understanding of N-uptake strategy in tropical rainforests (Russo et al. 2013).

The nitrogen isotope ratio ($\delta^{15}N$) of plants is known to reflect their N source and N-uptake strategies (Houlton et al. 2006). Plants access multiple soil N sources, such as organic N and inorganic N ($NH_4^+$ and $NO_3^-$), and take up these forms mostly through root symbiotic microorganisms such as ectomycorrhizal (EcM) and arbuscular mycorrhizal (AM) fungi. Species-specific symbiotic microorganisms and preferences for inorganic nitrogen sources such as $NH_4^+$ or $NO_3^-$ are considered potentially important factors that drive leaf $\delta^{15}N$ variations (Mayor et al. 2012). For example, N fixing or N uptake through mycorrhizal fungi significantly changes leaf $\delta^{15}N$ values because $^{15}N$ is depleted when N is translocated from mycorrhizal fungi to plants (Hobbie and Högberg 2012). Generally, EcM fungi work as a typical $^{15}N$-depleter compared with AM fungi, though Mayor et al. (2015) found the opposite trend in tropical forests, where EcM trees were found to have $^{15}N$-enriched leaves. To understand plant N-uptake strategies and N cycling in forests, comparing plant $\delta^{15}N$ values among species is one of the important approaches in many ecosystems (Shulze et al. 1994; Houlton et al. 2006).

Leaf $\delta^{15}N$ values in tropical rainforests may be associated with tree life-form types, such as canopy, understory, and gap species. Trees in the upper canopy environment with high photosynthetic production may facilitate EcM fungi symbiosis, because the carbon demand of EcM fungi is assumed to be greater than that of AM fungi (Smith and Read 2008). In contrast, understory trees with small carbon production under limited light conditions may be associated with AM fungi. In gap species, high photosynthetic production under high light conditions may allow EcM symbiosis, and some species have unique ant symbiosis (ant-plant), which may use N from ant dung. In fact, in other biomes, including Amazonian seasonal dry tropical forest, plant life forms significantly affect leaf $\delta^{15}N$ values (Schulze et al. 1994). Although lowland tropical rainforest in Southeast Asia shows extremely high tree species diversity (Ashton 2014), little is known about the N sources and N-uptake strategies that are related mainly to root symbiotic microorganisms of most tree species, except for upper canopy dipterocarp species (Dipterocarpaceae) that are characterized by EcM root symbiosis (Brearley 2012). Thus we hypothesized that tree species in tropical rainforest in Southeast Asia have diverse leaf $\delta^{15}N$ values,

because high competition for the uptake of soil N among diverse tree species may promote various strategies of nitrogen acquisition from different N sources. To test this hypothesis, we determined root and leaf $\delta^{15}N$ in various tree species (108 species in 28 families) and compared $\delta^{15}N$ values of the plant species with different 1) types of symbiosis, root symbiosis and ant symbiosis, 2) life-form types, and 3) taxonomic affiliations (families) in a species-hyper-rich tropical rainforest in Borneo. We also paid particular attention to the difference in leaf $\delta^{15}N$ between EcM and AM symbiotic trees, because Mayor et al. (2015) recently found that EcM symbiotic plants had more highly $^{15}N$-enriched leaves compared with AM symbiotic plants in tropical forests. Thus, we also tested Mayor's hypothesis that nitrogen isotope discrimination by EcM symbiotic trees might not occur in tropical forests.

## Materials and methods

### *Study sites and soil sampling*

The study was conducted at the Canopy Crane Plot (4 ha, 200 × 200 m) in a lowland mixed dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia (4°20'N, 113°50'E) in 2008 and 2012. This area has a humid tropical climate, without large seasonal changes in rainfall or temperature. The mean canopy height in the stand was about 30–40 m; some emergent trees reached 50 m in height (Kenzo et al. 2015b).

Litter and bulk soil $\delta^{15}N$, nitrogen and carbon content, and the C/N ratio of the studied plot were measured. Five litter samples and three soil cores were collected from each of seven randomly selected points in the plot, and soil cores were taken from depths of 0–5, 20–25, and 80–85 cm at each point by using a soil core sampler.

### *Plant materials*

We collected leaves from 229 individuals of 108 tree species in 28 families, including forest understory species, emergent species, and canopy gap species. We divided the studied species into 5 N-uptake categories (AM and EcM mycorrhizal, legume, ant-plant, and unknown) by their N-uptake strategies, such as root symbiotic microorganisms and ant symbiosis. Of 108 studied tree species, 22 species (Dipterocarpaceae and Fagaceae) were associated with EcM (Tanaka-Oda et al. 2016). The total number of AM species was 49; there were three legume species, four ant-plant species, and 32 species with undetermined root symbiosis (unknown). All species studied were classified into four life-form groups by mature height: forest understory (< 12.5 m), sub-canopy (12.5–27.5 m), canopy (> 27.5–42.5 m) and emergent (> 42.5 m) species. Tree species that grow mainly in canopy gaps were classified independently as "gap species" regardless of their height (Kenzo et al. 2007). To check the effect of isotope discrimination in root symbiotic fungi, we took a leaf and a fine root with its tip from each of 53 individuals of 20 species in nine families; EcM species (four species), AM species (eight species), and unknown (eight species). We analyzed the fine root and its tip (root diameter less than 2 mm) as an indicator of the $\delta^{15}N$ value of mycorrhizal fungi.

### *Supply rates and $\delta^{15}N$ of inorganic N in soil and measurement of stable N isotope*

We also determined the inorganic N supply rate and $\delta^{15}N$ value of $NH_4^+$ and $NO_3^-$ using Plant Root Simulator (PRS) probes (Western Ag Innovations Inc., Saskatoon, Canada). The probes were buried in the soil surface (2–3 cm deep) and at a 10–12 cm depth for 2 months at 10 points in the

**Fig. 1** The Plant Root Simulator (PRS) probes buried in soil for 2 months.

plot (Fig. 1). The $\delta^{15}N$ values of $NH_4^+$ and $NO_3^-$ were measured using the ammonia diffusion and denitrifier method (Koba et al. 2010) and using an isotope ratio mass spectrometer (Sercon 20-22; Sercon Ltd., Cheshire, UK). The stable N isotope composition of the soil, leaves, and fine roots was determined by using a conventional method with an elemental analyzer /isotope rate mass spectrometer (EA/IRMS, Thermo Finnigan, Delta V Advantage interfaced with FlashEA1112 HT, Thermo Fisher Scientific, Waltham, MA).

*Statistical analysis*

A one-way ANOVA with Tukey's HSD test was used to determine the relationship between leaf N characteristics and life forms, families, and types of N-uptake strategy. A one-way ANOVA of phylogenetically independent contrasts (PICs) was also performed to examine the effects of phylogenetic constraints on the relationship between leaf N characteristics and life forms, families, and N-uptake types (Inoue et al. 2015). A phylogram for the 108 taxa (species) in this study was constructed by using PHYLOMATIC (ver. 3). Branch lengths of the phylogenetic tree were assigned by using the BLADJ function of PHYLOCOM. For all statistical analyses, we used R version 3.1.2 and SPSS Ver. 11.5 (IBM, Armonk, NY, USA).

## Results

*Litter and soil $\delta^{15}N$ values*

Soil nitrogen content was extremely low even in surface soil (0.14 %). In contrast, nitrogen content in the litter layer was relatively high, varying from 1 to 1.5 %. The litter layer had a negative $\delta^{15}N$ value of about –1 ‰, while the $\delta^{15}N$ value of bulk soil was significantly higher than that of litter (Table 1). Soil $\delta^{15}N$ values increased slightly and N content decreased with soil depth. The rate of ammonium ($NH_4^+$) supply in shallow soil (2–3 cm depth) was relatively higher than that in deep soil (12–13 cm depth), though there was no statistically significant difference. The rate of nitrate ($NO_3^-$) supply in shallow soil was lower than that in deep soil (Table 2). The $\delta^{15}N$ value of $NH_4^+$ showed positive values and did not vary with soil depth, ranging from 6.7 ‰ in

**Table 1** Litter and soil $\delta^{15}N$ values, nitrogen and carbon contents, and C/N ratios at different depths from the soil surface (mean ± SE).

| Soil depth | $\delta^{15}N$ (‰) | N (%) | C (%) | C/N |
|---|---|---|---|---|
| Litter layer | –1.37 ± 0.15 | 1.17 ± 0.06 | 46.10 ± 1.00 | 40.90 ± 2.28 |
| Soil surface (0–5cm) | 4.32 ± 0.55 | 0.14 ± 0.01 | 2.20 ± 0.24 | 15.90 ± 0.60 |
| 20–25 cm | 5.92 ± 0.29 | 0.05 ± 0.01 | 0.59 ± 0.06 | 11.86 ± 0.15 |
| 80–85 cm | 5.87 ± 0.20 | 0.03 ± 0.01 | 0.29 ± 0.07 | 9.02 ± 0.97 |

**Table 2** Ion-exchangeable ammonium ($NH_4^+$) and nitrate ($NO_3^-$) supply rates and $\delta^{15}N$ values between the shallow and deep layers of soil (mean ± SE, n = 10). Different letters denote significant differences of supply rates between soil depths (t-test, $P < 0.05$).

| Soil layer | Depth (cm) | Supply rates (μg $10cm^{-2}$ 2 $months^{-1}$) | | $\delta^{15}N$ (‰) | |
|---|---|---|---|---|---|
| | | $NH_4^+$ | $NO_3^-$ | $NH_4^+$ | $NO_3^-$ |
| Shallow | 2–3 | 182.3 ± 102.0 | 32.4 ± 12.4 [b] | 6.7 ± 0.9 | –5.6 ± 0.8 |
| Deep | 12–13 | 60.7 ± 12.4 | 70.9 ± 21.9 [a] | 7.0 ± 1.1 | –4.2 ± 0.9 |

**Table 3** Average leaf $\delta^{15}N$ values of each N-uptake strategy (mean ± SE). EcM: ectomycorrhiza; AM: arbuscular mycorrhiza; Legume: all Fabaceae trees; Ant: ant-symbiotic trees; Unknown: symbiotic mycorrhiza undetermined. The numbers of species are indicated in parentheses. Different letters denote significant differences in N-uptake strategies examined using Tukey's HSD test after ANOVA ($P < 0.05$).

| | EcM | AM | Legume | Ant-plant | Unknown |
|---|---|---|---|---|---|
| Leaf $\delta^{15}N$ (‰) | 0.29 ± 0.22 [a] (22) | –1.56 ± 0.29 [b] (47) | –0.59 ± 0.84 [ab] (3) | –3.36 ± 1.12 [b] (4) | –1.28 ± 0.48 [ab] (32) |
| Leaf N (%) | 1.54 ± 0.05 (21) | 1.60 ± 0.06 (44) | 1.59 ± 0.13 [a] (3) | 1.92 ± 0.22 (4) | 1.67 ± 0.10 (30) |

**Table 4** The $\delta^{15}N$ values, leaf nitrogen content, and number of species in each life form with N-uptake strategy (mean ± SE). Different letters denote significant differences between the life forms according to Tukey's HSD test after ANOVA ($P < 0.05$).

| | Emergent | Canopy | Sub-canopy | Understory | Gap species |
|---|---|---|---|---|---|
| $\delta^{15}N_{leaf}$ (‰) | 0.09 ± 0.27 [a] | –1.73 ± 0.43 [b] | –1.88 ± 0.46 [b] | –1.33 ± 0.60 [ab] | –0.94 ± 0.67 [ab] |
| N (%) | 1.51 ± 0.03 [b] | 1.51 ± 0.06 [b] | 1.64 ± 0.07 [ab] | 1.74 ± 0.11 [ab] | 1.83 ± 0.09 [a] |
| Number of species | 26 | 25 | 24 | 16 | 17 |
| N-uptake strategy | | | | | |
| EcM | 21 | 0 | 0 | 0 | 1 |
| AM | 1 | 20 | 13 | 7 | 6 |
| Legume | 1 | 2 | 0 | 0 | 0 |
| Ant-plant | 0 | 0 | 0 | 0 | 4 |
| Unknown | 3 | 3 | 11 | 9 | 6 |

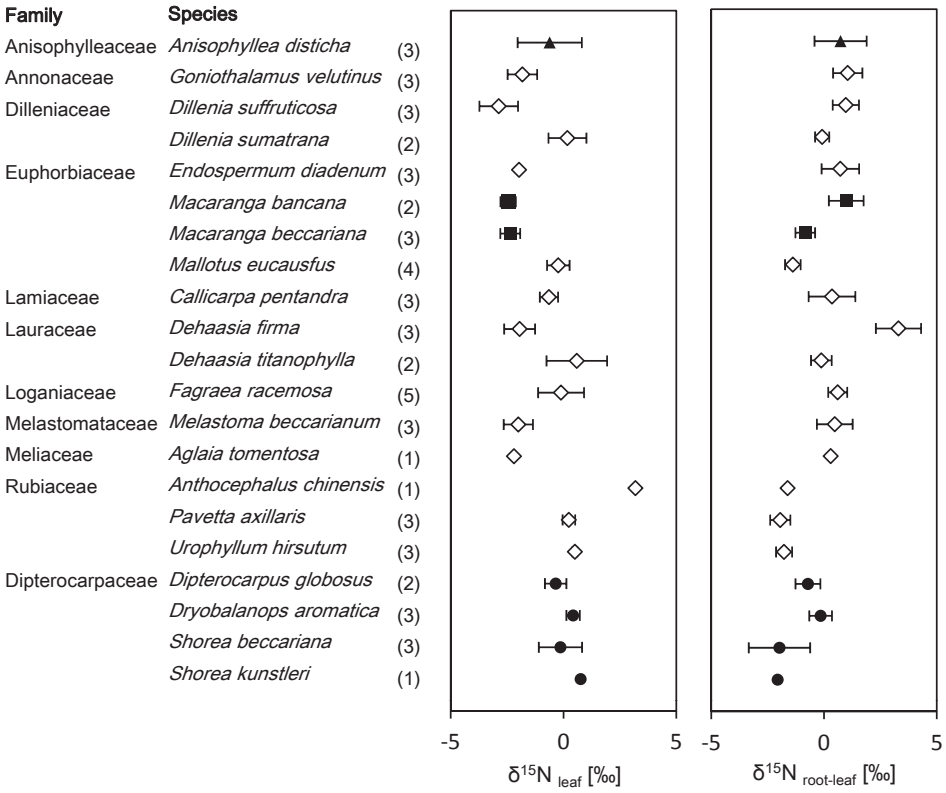


**Fig. 2** Average leaf and root-leaf $\delta^{15}N$ values of each species (Mean ± SE). The numbers of individuals are indicated in the parentheses. Triangles, rhombuses, squares and circles are Unknown, AM, Ant-plant and EcM species, respectively.

shallow soil to 7.0 ‰ in deep soil. In contrast, the $\delta^{15}N$ of $NO_3^-$ showed negative values ranging from –5.6 ‰ in shallow soil to –4.2 ‰ in deep soil (Table 2).

*Leaf $\delta^{15}N$ values according to different N-uptake strategies and life forms*

Among the different N-uptake strategies, EcM species had the highest average leaf $\delta^{15}N$ values and the smallest variation (Table 3). The leaf $\delta^{15}N$ of most AM species had significantly negative values (Table 3). The leaf $\delta^{15}N$ of legume species showed the second highest values, ranging from 0 to –2 ‰. All ant-plant species had negative leaf $\delta^{15}N$ values (–7.2 ‰). In the Unknown category, leaf $\delta^{15}N$ values varied widely. The difference in $\delta^{15}N$ between leaves and roots ($\delta^{15}N_{root\text{-}leaf}$) varied among species and root mycorrhizal types (Fig. 2). The lowest $\delta^{15}N_{root\text{-}leaf}$ values among them were found in EcM species (Fig. 2), and the $\delta^{15}N$ in their leaves was higher compared with that within their roots. There was a positive correlation between leaf N content and leaf $\delta^{15}N$ (data not shown).

Leaf $\delta^{15}N$ values and leaf N content varied significantly among life forms (Table 4), though neither value was significantly correlated with life forms in PIC calculations. Emergent species had the highest leaf $\delta^{15}N$ values, whereas canopy and sub-canopy species had the lowest values. Sub-canopy and understory species had similar leaf nitrogen content and displayed large interspecific differences in leaf $\delta^{15}N$. Large interspecific variation in leaf $\delta^{15}N$ was also observed in gap species, with significantly higher leaf N content than in the other life forms.

**Table 5** Average, minimum and maximum leaf $\delta^{15}N$ values and leaf N % of the families (mean ± SE).

| | $\delta^{15}N_{leaf}$ (‰) | $\delta^{15}N_{leaf\text{-}min}$ (‰) | $\delta^{15}N_{leaf\text{-}max}$ (‰) | Leaf N (%) |
|---|---|---|---|---|
| Achariaceae | –0.03 | –0.03 | –0.03 | 1.28 |
| Anacardiaceae | –3.27 ± 0.25 | –4.71 | –2.27 | 1.59 ± 0.09 |
| Anisophylleaceae | –0.38 ± 0.87 | –2.04 | 2.23 | 1.24 ± 0.05 |
| Annonaceae | –2.45 ± 0.36 | –3.33 | –1.16 | 1.43 ± 0.14 |
| Burseraceae | –3.59 ± 0.74 | –7.08 | –1.16 | 1.65 ± 0.13 |
| Cannabaceae | 2.28 | 2.28 | 2.28 | 2.09 |
| Clusiaceae | –1.08 ± 0.67 | –2.95 | 0.77 | 1.15 ± 0.10 |
| Dilleniaceae | –1.45 ± 0.75 | –4.55 | 0.40 | 1.37 ± 0.05 |
| Dipterocarpaceae | 0.03 ± 0.12 | –1.77 | 3.32 | 1.49 ± 0.03 |
| Euphorbiaceae | –1.59 ± 0.46 | –7.24 | 5.04 | 2.01 ± 0.10 |
| Fabaceae | –1.60 ± 0.67 | –3.18 | 0.75 | 1.73 ± 0.16 |
| Fagaceae | 1.33 ± 1.42 | –0.09 | 2.74 | 1.34 |
| Gentianaceae | 0.24 ± 0.69 | –2.87 | 3.30 | 1.35 ± 0.20 |
| Ixonanthaceae | 0.67 ± 0.03 | 0.64 | 0.70 | 1.26 ± 0.01 |
| Lamiaceae | 0.13 ± 0.64 | –2.09 | 3.62 | 1.61 ± 0.16 |
| Lauraceae | –1.40 ± 0.63 | –3.31 | 1.66 | 1.84 ± 0.14 |
| Magnoliaceae | 1.47 | 1.47 | 1.47 | No data |
| Malvaceae | –1.68 ± 1.33 | –3.94 | 1.50 | 1.74 ± 0.03 |
| Melastomataceae | –3.43 ± 0.62 | –6.02 | –1.04 | 1.39 ± 0.09 |
| Meliaceae | –1.81 ± 0.20 | –2.20 | –1.55 | 2.19 ± 0.10 |
| Moraceae | –0.43 ± 1.11 | –3.27 | 2.09 | 1.54 ± 0.20 |
| Myristicaceae | –2.01 ± 0.50 | –3.97 | 0.33 | 1.68 ± 0.05 |
| Myrtaceae | –3.54± 0.64 | –5.33 | –1.60 | 1.25 ± 0.07 |
| Phyllanthaceae | –2.96 ± 1.48 | –8.45 | 0.37 | 1.86 ± 0.28 |
| Rubiaceae | 1.07 ± 0.31 | –0.03 | 3.98 | 2.34 ± 0.21 |
| Sapindaceae | –0.03 | –0.03 | –0.03 | No data |
| Sapotaceae | –1.60 ± 1.13 | –4.25 | 2.10 | 1.34 ± 0.15 |
| Stemonuraceae | –0.43 | –0.43 | –0.43 | 2.44 |

Leaf $\delta^{15}N$ values varied significantly also among families (Table 5), while they did not with regard to PIC (data not shown). Several families, including Dipterocarpaceae, Lamiaceae, and Rubiaceae, showed positive leaf $\delta^{15}N$ values, though more than half of the studied families, including Anacardiaceae and Burseraceae, showed negative values. Although the variation in leaf $\delta^{15}N$ among several families (e.g., Dipterocarpaceae) was small, internal variation within families was high in several families. For example, Euphorbiaceae had large interspecies variation, ranging from –7.2 to +5.0 ‰. Phyllanthaceae (–8.4 to +0.4 ‰), Sapotaceae (–4.3 to +2.10 ‰) and Malvaceae (–3.9 to +1.5 ‰) also displayed large interspecies variation (Table 5). Ant-plant species displayed a wide range of values, even in the same *Macaranga* genus. *Macaranga* species with ant symbiosis showed significantly negative leaf $\delta^{15}N$ values (–3.4 ‰), whereas non-symbiotic *Macaranga gigantea* had a positive value (+5.0 ‰).

**Discussion**

*Soil N traits and plant use*

The presence of significantly lower soil nitrogen content (Table 1) in the studied forest, comparable to that under sandy infertile soil conditions in Borneo (Kenzo et al. 2015a), suggests that the nitrogen for trees is mostly supplied from the litter layer. The $\delta^{15}N$ values were significantly higher in soil (6 ‰) than in litter (–1 ‰). Such a difference in $\delta^{15}N$ value between the soil and litter layers has also been reported for other forest biomes. Our findings are consistent with the global pattern reported so far (Amundson et al. 2003). Litter-derived nitrogen is hypothesized to immediately be taken up by trees, and litter decomposition is thus probably a major nitrogen source in forests. The fact that most biomass of fine roots in dipterocarp forests is distributed over the surface soil layer and/or litter layer supports this assumption (Kenzo et al. 2015a).

There was large variation in leaf $\delta^{15}N$ in the studied forest (–7.2 to 5.0 ‰), and this variation was comparable to that of all Asian tree species, which includes diverse trees of various forest types (–7.1 to +2.7 ‰, Fang et al. 2013). This large variation in leaf $\delta^{15}N$ may indicate that tropical rainforest trees have various N sources, because they have various species-specific strategies of N acquisition, such as EcM and AM mycorrhizal symbiosis and ant symbiosis, though species with high leaf-N values tend to have enriched $\delta^{15}N$ leaves.

*Effect of N isotope discrimination by root mycorrhizal fungi*

Although EcM fungi generally function as an $^{15}N$-depleter, and thus the leaves of plants that associate with EcM fungi display lower $\delta^{15}N$ values than those of plants associating with AM fungi in boreal and temperate forests (Mayor et al. 2015), tropical rainforest trees with EcM association (e.g., Dipterocarpaceae and Fagaceae) showed relatively higher leaf $\delta^{15}N$ values than trees associated with AM species. The precise mechanisms underlying these high leaf $\delta^{15}N$ values in EcM trees in the tropical forest are unknown (Mayor et al. 2015). Fine-root $\delta^{15}N$ values of EcM species (–1.2 ‰) were significantly lower than those of deep soil (+5.9 ‰) and leaves (+0.2 ‰) in this study (Fig. 2). These relatively low fine-root $\delta^{15}N$ values were also opposite to the trend found in EcM species in other forest biomes (Mayor et al. 2012) and in AM species in this study. Recently, Mayor et al. (2015) suggested that nitrogen isotope discrimination in the EcM association *per se* might not occur in tropical rainforests, including those in Africa and South America. Our results also support their suggestion.

*Leaf $\delta^{15}N$ differences between life-form types and taxonomic groups (families)*

Significant variations in leaf $\delta^{15}N$ among life forms may reflect phylogenetic bias in the species composition of each life form, because the analysis of PICs clearly showed that there were no differences among life forms considering phylogenetic distance (PICs). Emergent species accounted for 50–60 % of the total basal area in the forest (Ashton 2014) and had the highest leaf $\delta^{15}N$ values among the life forms. The high leaf $\delta^{15}N$ values in emergent species may be a result of a bias for symbiotic EcM fungi in dipterocarp species, which showed $^{15}N$-enriched leaves (Tanaka-Oda et al. 2016). Tropical rainforest trees mainly display AM symbiosis, though species of Dipterocarpaceae typically show EcM symbiosis in Southeast Asia (Allen 1991; Ashton 2014). In other tropical, temperate, and boreal forests, dominant upper canopy species, such as Fagaceae and Pinaceae, usually associate with EcM fungi. This similarity between biomes may indicate that EcM fungi confer advantages for nutrient competition in nutrient-deficient soils (Allen 1991).

Ectomycorrhizal fungi often possess enzymes that degrade complex organic compounds, and long hyphae help to exploit nutrients (Allen 1991). The upper canopy environment with high photosynthetic productivity may allow symbiosis with EcM fungi, which may have a higher carbohydrate demand than AM fungi (Smith and Read 2008). In fact, dominant dipterocarp trees in the emergent layer showed higher photosynthetic ability under upper canopy conditions than most non-dipterocarp trees or lower trees when they were in the emergent layer (Kenzo et al. 2015b; Inoue et al. 2015). Canopy, sub-canopy, and understory species showed significantly negative leaf $\delta^{15}N$ values, with large variation. These trees mainly exhibit AM symbiosis, and none of them showed clear EcM symbiosis. Thus, most trees with AM symbiosis among those life forms may preferentially take up $NO_3^-$ rather than $NH_4^+$, because $\delta^{15}N$ of $NO_3^-$ (approx. –5 ‰) showed significantly more negative values than $NH_4^+$ (approx. +7 ‰) in the soil. In contrast, gap species showed the highest leaf N content and had large leaf $\delta^{15}N$ variation. High leaf N content contributes to high photosynthetic ability and high growth rates under the gap conditions (Kenzo et al. 2015b). Various N-uptake strategies, including ant symbiosis and EcM and AM symbioses, in the gap species may be related to the high variability of leaf $\delta^{15}N$ from different N-sources.

Diverse N-uptake strategies such as EcM and AM symbioses and ant symbiosis may cause large variation in leaf $\delta^{15}N$ values among families. Several families showed small internal variation in leaf $\delta^{15}N$ values, suggesting that their N-uptake strategy may be similar within a family. For example, Fabaceae, whose species are presumed to be nitrogen fixing, showed higher leaf $\delta^{15}N$ (near 0 ‰ of leaf $\delta^{15}N$) than AM species, except for *Koompassia malaccensis*, which had similar leaf $\delta^{15}N$ values to those of most AM species. All species in the Dipterocarpaceae with EcM association had small variation in leaf $\delta^{15}N$. In contrast, the leaf $\delta^{15}N$ of Euphorbiaceae species varied from –7.2 to +5.0 ‰; this variation was greater than the total variation in Asian tree species growing in diverse forest types (–7.1 to + 2.7 ‰, Fang et al. 2013). This large variation may reflect the varied ecological characteristics of tree species in the family. For example, the Euphorbiaceae includes tree species with various life forms, including canopy, sub-canopy, understory, and gap species. Furthermore, several species have ant symbiosis, which may supply nitrogen from symbiotic ant dung. In fact, leaf $\delta^{15}N$ values of ant-symbiotic species (–3.4 ‰) significantly differed from those of non-symbiotic species (5.0 ‰) even in the same genus (*Macaranga*).

We conclude that high leaf $\delta^{15}N$ variation suggests diverse N-uptake strategies among species and/or life forms in the tropical rainforest. Further studies investigating the varied and detailed mechanisms involved in N isotope discrimination and N sources across mycorrhiza types and/or species will provide a clearer understanding of N competition and the partitioning of N resources in trees in diverse tropical rainforests.

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