A DISSERTATION FOR THE DEGREE OF MASTER OF SCIENCE

Effects of leaf nitrogen allocation on photosynthetic nitrogen-use efficiency of seedlings of three tropical species in Indonesia

잎의 질소 분배가 세 가지 열대 수종의
광합성질소이용효율에 미치는 영향

BY

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A DISSERTATION FOR THE DEGREE OF MASTER OF SCIENCE

Effects of leaf nitrogen allocation on photosynthetic nitrogen-use efficiency of seedlings of three tropical species in Indonesia

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ABSTRACT

Leaf N content (N_L) and its allocation, which shows a wide range of variation among species, are major factors that determine the photosynthetic characteristics and photosynthetic nitrogenuse efficiency (PNUE) of tree species. In this study, Iinvestigated the allocation of N_L to a photosynthetic apparatus (N_P) and itseffectson the photosynthetic characteristics and PNUE of two-year-old pot-growing seedlings of three tropical tree species (*Magnolia montana*Blume, *Swieteniamacrophylla* King, and *Acacia mangium*Willd), which were grown at an open nursery in western Java, Indonesia. *A. mangium* (i.e. the only N-fixing species among three studied species) had greater $N_L$with higher capacities in photosynthetic characteristics, that is, light-saturated photosynthetic rate (P_{max})and dark respiration rate (R_d) than the other two species, although the N fractions allocated to N_P, namely, Rubisco (N_R) and bioenergetics (N_B), were greater in *S. macrophylla*. In all species, PNUE exhibited a positive relationship with N_P; however, the PNUE of N-fixing *A. mangium* was lower than those of non N-fixing *M. montana* and *S. macrophylla* at a given N_P. In addition, stomatal conductance was also positively correlated with PNUE, but the slopes of the correlation were steeper in *M. montana* and *S. macrophylla* than *A. mangium*. These result was explained by the differences in leaf mass per unit area between *A. mangium*and other species.My research implies that there are different strategies in N use between *A. mangium* and other species.

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i

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. Literature review

Although nitrogen (N) concentration of the foliage is only a small fraction of the total dry weight, it plays essential roles in biochemical and physiological processes. These important roles of N can be attributed to a fact that large fraction of leaf N is invested into photosynthetic apparatus such as ribulose1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Evans 1989). Thus, there are the strong relationships betweenleaf N contentand photosynthetic characteristics such as the light-saturated photosynthetic rate (P_{max}), the dark respiration rate (R_d), the maximum carboxylation rate (V_{Cmax}), and the maximum electron transport rate (J_{max})(Evans 1989, Reich et al. 1994, Poorter and Evans 1998, Wilson et al. 2000, Ripullone et al. 2003, Novriyanti et al. 2012). These correlations are also important in practice, since the use of these correlations greatly simplifies the estimation of carbon gain of leaves in field, especially considering the fact that gas exchange measurements are time-consuming and not always applicable to every leaf of the canopy(Hikosaka 2004, Wright et al. 2004, Kattge et al. 2009).

However, there are large variation of their relationships, becausethese relationships are influenced by a variety of environmental conditions and phonological characteristics of plants (Reich et al. 1994, Ellsworth et al. 2004, Hikosaka 2004, Reich et al. 2006b, Kattge et al. 2009, Reich et al. 2009, Chu et al. 2011). During the growing season, leaf photosynthesisvaries as a result of changes in both leaf ontogeny and the environment(Grassi et al. 2005). Xu and Baldocchi (2003) reported that the maximum value of V_{Cmax} was reached shortly after leaf expansion in early summer, when air temperautre was moderate and soil water availability was high. Thereafter, V_{Cmax} declied as the soil water porfile became

depleted and the trees were under high air temaperature (Xu and Baldocchi 2003).In addition, these relationships also cloud be affected by the other nutrient conditions, for example, Reich et al. (2009) reported that the limitation of phosphrous (P) in tropical forests could alter the photosynthesis-N relationship, and also claimed thatin P-limited ecosystems, the phtosynthesis–N relationship may be constrained by low P.

One of the most popular approaches to reveal the variation of photosynthesis-N relationship is by analyzing the partitioning of leaf N between various components. Within leaves, N occurs in soluble and membrane-bound proteins which are involved in carbon assimilation and light harvesting, respectively, as well as non-photosynthetic compounds such as cell wall proteins, amino acids, nucleic acids, defense compounds, and inorganic N (Funk et al. 2013). Plant species vary greatly in how N is partitioned among these pools. For example, plants may allocate 5~32% of leaf N to Rubisco and 2~30% to cell walls, with higher amount of cell wall protein in longer-lived leaves (Funk et al. 2013). Leaves with a long life span tend to have a high leaf mass per area and low photosynthetic N-use efficiency, which are positively correlated with structural toughness and biomass (Onoda et al. 2004). Thus this study suggested that there is a trade-off between N allocation to cell walls and to Rubisco in across leaves with different life spans (Onoda et al. 2004). Takashima et al. (2004) also reported that there may be a trade-off in N partitioning between components pertaining to productivity (i.e. photosynthetic protein) and those pertaining to persistence (i.e. structural proteins). They concluded that this trade-off may result in the convergence of leaf trait, where species with a longer leaf life-span have a greater leaf mass per area, lower photosynthetic capacity, and lower photosynthetic N-use efficiency regardless of

life form, phylogeny, and biome (Takashima et al. 2004). However, Harrison et al. (2009) reported that although increased allocation of N to structure was accompanied by a reduced investment in Rubisco for some species, other species showed that allocation of N to cell walls increased early in the season without any concomitant change to Rubisco and then during autumn, N released from Rubisco degradation was also not associated with any change in cell wall N, that is, there was no internal trade-off in N allocation between cell wall and Rubisco through the lifespan of these leaves.

Recently, a great attention has beengiven to tropical forest.Because, it plays a role as the most important services to not only humankind, but also natural ecosystem, even though their relative amount of the coverage on the Earth' land surface is less than 10%, andthis biome contains up to 55% of global terrestrial carbon stocks and accounts for at least one-third of global terrestrial net primary production (Zhou et al. 2013). In addition, global scale models have suggested that humid tropical forest alone contributed one-third of global terrestrial net primary production, and field data also support, which would be up to one-half of the estimated terrestrial net primary production (Körner 2009). However, despite the well-recognized importance of this biome to biogeochemistry as well as biological diversity, fundamental uncertainties remain in our understanding that underlie the productivity and dynamics of these forests worldwide (Hedin et al. 2009).For example, the unawareness of why relatively more tropical species has comparativelyhigh N concentration is caused by the lack of mechanistic resolution on plant-nutrient interaction at the level of ecosystems versus at the level of physiology and ecology of individual N-fixing species (Hedin et al. 2009).

2. Introduction

During last several decades, the economics of N use in photosynthesis have become a key issue in ecophysiology because N is the most essential and limiting component of many natural ecosystems (LeBauer and Treseder 2008). Thus, the efficient use of this resource greatly affects the growth, survival, competition and distribution of plant species and eventually determines the productivity and succession of ecosystems (Evans 1989, Poorter and Evans 1998,Hikosaka and Shigeno 2009). The importance of N originates from CO_2 assimilation, which is largely controlled by the supply and demand of N at the leaf or cellular levels. The efficiency of N, which is defined as the amount of CO_2 assimilated per unit N content, is commonly expressed as the photosynthetic N-use efficiency (PNUE [μmol (CO_2) mol^{-1} (N) s^{-1}]). Consequently, PNUE could be the key plant trait that determines the survival and adaption abilities of specific species under progressive N limitation with changing climate conditions due to increasing atmospheric CO_2 concentrations (e.g. Luo et al. 2004, Finzi et al. 2006, Schlesinger 2009).

Nitrogen is allocated to various components in a leaf such as enzymes, membrane proteins and the cell wall. This allocation of N to various components is quite different among species, and these differences cause the variation in PNUE among various species (Poorter and Evans 1998, Hikosaka and Shigeno 2009,Novriyanti et al. 2012). Although the variation of PNUE is not determined by only one or two factors such as N_L, intercellular CO_2 concentration (C_i), stomatal conductance (g_s) and the allocation of N_L(Hikosaka and Shigeno 2009), a number

of recent studies have highlighted the importance of the N allocation within leaf, especially the allocation to the photosynthetic apparatus, to the variation of PNUE within and among species (Poorter and Evans 1998, Takashima et al. 2004, Feng and Fu 2008, Simon et al. 2010, Novriyanti et al. 2012, Funk et al. 2013).

As mentioned above, the leaf N content (N_L) is a key element that governs leaf physiological traits. As a result, the 'leaf economics' of physiological traits over the world are highly correlated with N_L, and they are distributed within a general spectrum (Wright et al. 2004). For example, the positive correlation between leaf photosynthetic capacities and N_L is based on the fact that approximately a half of N_L is invested into components of the photosynthetic apparatus such as ribulose1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Evans 1989). Although, it is very useful to describe the general consensus of leaf traits; however, there is significant variation within the leaf economic spectrum. For instance, the relationship between leaf photosynthetic capacities (e.g. CO_2 assimilation, carboxylation and RuBP regeneration rate) and N_L is not always conserved under different environmental conditions (Kattge et al. 2009, Reich et al. 2009, Xiang et al. 2013). Reich *et al.* (2009) reported that these relationships also significantly varied among plant functional types (e.g. tropical and temperate trees) and soil nutrient conditions such as phosphorus availability, particularly for tropical species. Furthermore, Kattage *et al.* (2009) claimed that the variation in the relationships could be attributed to the differences in the fraction of leaf N allocated to the photosynthetic apparatus. Several studies suggested that consideration of the variation in leaf economics would improve the precision of future predictions of the amount of CO_2 assimilation or plant growth by the global-scale model (Reich et al. 2006a, Thornton et al. 2007, Verheijen et al. 2013).

In this study, the leaf N allocation and its effects on photosynthetic characteristics and the N-use efficiency of three tropical species (*Magnolia montana*Blume, *Swieteniamacrophylla* King, and *Acacia mangium*Willd) were investigated using two-year-old seedlingsgrown in pots under a sun screen at an open nursery in western Java Indonesia. As far as I know, this is the first study that investigates the morphological and physiological leaf traits of *M. montana*,which is an indigenous species in Malaysia and Indonesia. *S. macrophylla*, which is better known as the 'big leaf mahogany', has been popularly used for furniture and musical instruments, and there have been many studies of its physiological traits (e.g. Grogan et al. 2003, Cernusak et al. 2009). However, few studies were conducted in Indonesia. Lately, *A. mangium* has been the most popular species used for reforestation in Indonesia because this species grows quickly and relatively well in unfavorable conditions such as during drought or in infertile soil (Broich et al. 2013, Ramdani and Hino 2013). In addition, *A. mangium* was only N-fixing species among the three species examined, so it was expected to exhibit a distinguishable mechanism and ability to utilize N compared to the other two non N-fixing species (Cleveland et al. 1999, Vitousek et al. 2013).

The objectives of my study were as follows: (1) to investigate the differences in leaf N content and its allocation within the leaf among species, and (2) to assess the effect of N_L allocation to the photosynthetic apparatus on photosynthetic characteristics and N-use efficiency.

3. Materials and methods

Site description

The experiment was conducted at Rumpin Seed Source and Nursery Center (RSSNC), which is located in Bogor, western Java Indonesia (6°26'S, 106°38'E).The altitude of the site is approximately 180 m above sea level, and the average annual rainfall and air temperature were 2,750 mm and 26.3 °C, respectively. The monthly average temperature was stable, so their variation was lower than the diurnal temperature variation. The precipitation had a marked seasonal cycle. The rainy season starts in October and is centered on January. After the rainy season, the dry season lasts from May to September. The meteorological variables were measured from May 1st to 12th, 2013. The temperature and relative humidity were measured with an HMP45C (Campbell Scientific, Utah, USA), and the vapour pressure deficit (VPD) was calculated using the temperature and relative humidity. The precipitation was measured with a rain gauge (03319-00, Cole-Parmer, Illinois, UAS). The maximum, minimum and average temperature during the period were 33.3, 22.2 and 26.6 °C, respectively, and the maximum and average VPD were 2.15 and 0.47 kPa, respectively. During the study period, precipitation occurred for every days, and the total amount of precipitation was 265 mm.

Biomass measurement

All of the plant materials were grown from seeds collected at the RSSNC. They were germinated in September 2011. After germination, the seedlings were individually planted in 0.5 L pots, then transplanted to bigger pots based on the size of the seedlings. When the study was conducted, *M. montana* was in 8 L pots, and *S. macrophylla* and *A. mangium* were in 4 L pots. Each pot contained 80% dark brown topsoil and 20% air-dried rice husks by volume. All examined seedlings were grown under a sun screen with no side wall, which reduced the incoming photosynthetically active radiation and precipitation by approximately 20% compared with the outside ambient conditions (Fig. 1). At the end of the experiment in May 2013, when the dry season was about to start, eight seedlings for *M. montana* and ten seedlings for *S. macrophylla* and *A. mangium* were harvested to measure the biomass. The plant materials were dried at 80 °C for at least 48 hours in a drying oven. The leaf, stem and root biomass were measured separately.

Figure 1 The view of the open nursery with sun screen at the top

Gas exchange measurements

Gas exchange measurements were taken from May1[st] to 6th, 2013 with a portable photosynthesis measurement system (LI-6400, LI-COR, Nebraska, USA). I selected six mature leaves for each species. Each leaf was randomly selected within canopy of different individuals. Light and photosynthetic CO_2 response curve measurements of individual leaves were taken during all period of the day, and at least one leaf per species was measured every day. However, the measurements in the afternoon were not stable or reliable due to the afternoon showers and mid-day stomata closure due to the increased temperature and VPD. Therefore, the first measurements from each species, which were finished before 1400 h, were used for data analysis. The light-saturated photosynthetic rate (P_{max}) and the dark respiration rate (R_d) were measured at 1300 and 0 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the leaf level, respectively, under steady-state conditions. The values of C_i and stomatal conductance (g_s) were derived from P_{max} measurements. During all of the measurements, the cuvette CO_2 concentration, temperature and VPD were maintained at 380 ppm, 27 °C and 1.0 kPa, respectively. To obtain the photosynthetic CO_2 response curve, we established 13 cuvetteCO_2 concentration values, and the cuvette conditions such as temperature, VPD and PPFD on the leaf level were maintained at levels similar to those used for P_{max} measurements except for CO_2 concentration. The maximum rate of carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}) were estimated by fitting the photosynthetic CO_2 response curves to a biochemical model using a Microsoft Excel worksheet (Sharkey et al. 2007; Ver. 2007.1).

Leaf morphology

After gas exchange measurements, all 18 of the leaves were harvested to measure the leaf area and dry mass. Leaf areas were immediately measured after harvest with a leaf area meter (LI-3100, LI-COR, Nebraska, USA) and dried at 80 °C for at least 48 h in a drying oven. Leaf mass was measured after drying. Leaf dry mass per area (LMA) was calculated by dividing the leaf dry mass by its area.

Leaf N content and allocation

N_L was measured in all of the dried leaves using an elemental analyzer (Flash EA 1112, Thermo Scientific, Massachusetts, USA). The N allocation to the photosynthetic apparatus was categorized into the following three components: (1) Rubisco (N_R), (2) bioenergetics (N_B), and (3) light-harvesting complex and photosystem. N_R and N_B account for most of the photosynthetic capacity in light-saturated conditions (Westbeek et al. 1999). Thus, in this study, I considered only N_R and N_B to focus on the effect of N rather than on other limiting factors such as light and N_L allocated to the photosynthetic apparatus (N_P) was represented by the sum of N_R and N_B. N_R and N_B were calculated using V_{Cmax} and J_{max}, respectively, with other variables according to the equations and procedures of Niinemets*et al.*(1999). TheV_{Cmax} and J_{max} values adjusted to 25 ℃ were applied to the calculations.

Statistical analysis

Analysis of variance (one-way ANOVA) was performed to compare the variation of each parameter among the three species. Where significant variation was detected ($P<0.05$), pair-wise comparisons among species was performed according to Duncan's method. Linear regression analysis was performed to show the relationships between two continuous variables. Analysis of covariance (ANCOVA) was used to determine the differences in responses among species and between N-fixing and non N-fixing species with $P<0.05$. However, there was no significant difference between the two non N-fixing species, so only the comparisons between N-fixing and non N-fixing speciesare shown. Statistical analyses were performed using SAS Version 9.3 (SAS Institute, Cary, North Carolina, USA).

4. Results and discussion

The variation in the photosynthetic characteristics between and within species was attributed to inherent and climate-induced plant morphological and physiological traits (Wright et al. 2005, Hikosaka and Shigeno 2009). In this study, I investigated how N_L allocation to the photosynthetic apparatus, especially Rubisco (N_R) and the electron transport system (N_B) affected the photosynthetic characteristics and N-use efficiency of seedlings of three tropical species in Indonesia.

Leaf nitrogen content and allocation

Even though there was no difference in plant dry mass and root/shoot ratio among the three species, there were several significant differences in the morphological and physiological characteristics at the leaf level (Table 1). All area-based N content parameters (i.e. N_L, N_R and N_B) were greater in *A. mangium*, an N-fixing species, than in *M. montana* and *S. macrophylla*, which were not different from each other. Unlike the amount of N, N_R/N_L and N_R/N_L were greater in *S. macrophylla* than in *M. montana* and *A. mangium*, while there was no difference between the latter two (Fig. 1A). However, the allocation of N to N_R and N_B among the three species was not different (Fig. 1B).

Table 1 Morphological and physiological characteristics of each species. N_L – leaf nitrogen content, N_R – leaf nitrogen allocated to Rubisco; N_B – leaf nitrogen allocated to bioenergetics; P_{max} – light-saturated photosynthetic rate; R_d – dark respiration rate; C_i – intercellular CO_2 concentration. Means ± SD ($n \geq 6$). Different letters within a row indicate a significant difference with $P<0.05$

	M. montana	*S. macrophylla*	*A. mangium*
Plant dry mass [g]	161 ± 28[a]	131 ± 37[a]	142 ± 32[a]
Root/shoot ratio [g g^{-1}]	0.26 ± 0.05[a]	0.24 ± 0.06[a]	0.22 ± 0.05[a]
Leaf mass per area [g m^{-2}]	67.4 ± 8.8[b]	54.5 ± 5.4[c]	79.9 ± 4.3[a]
N_L [mmol m^{-2}]	60 ± 13[b]	60 ± 8[b]	166 ± 19[a]
N_R [mmol m^{-2}]	13 ± 4[b]	19 ± 6[b]	31 ± 9[a]
N_B [mmol m^{-2}]	2.0 ± 0.5[b]	2.6 ± 0.5[b]	4.5 ± 0.8[a]
N_R/N_L [%]	21.9 ± 6.0[b]	31.4 ± 8.1[a]	19.4 ± 7.6[b]
N_B/N_L [%]	3.4 ± 0.9[ab]	4.5 ± 0.7[a]	2.7 ± 0.8[b]
C_i [ppm]	314±8.8[a]	275±20.8[b]	292± 18.1[ab]
P_{max} [µmol m^{-2} s^{-1}]	4.1 ± 1.8[b]	6.1 ± 1.6[b]	14.9 ± 4.6[a]
R_d [µmol m^{-2} s^{-1}]	0.24 ± 0.11[b]	0.32 ± 0.18[b]	1.05 ± 0.18[a]
R_d/P_{max} [%]	6.20 ± 2.31[a]	5.53 ± 2.90[a]	7.47 ± 2.30[a]
g_s [mmol m^{-2} s^{-1}]	102 ± 55[b]	107 ± 49[b]	304 ± 146[a]
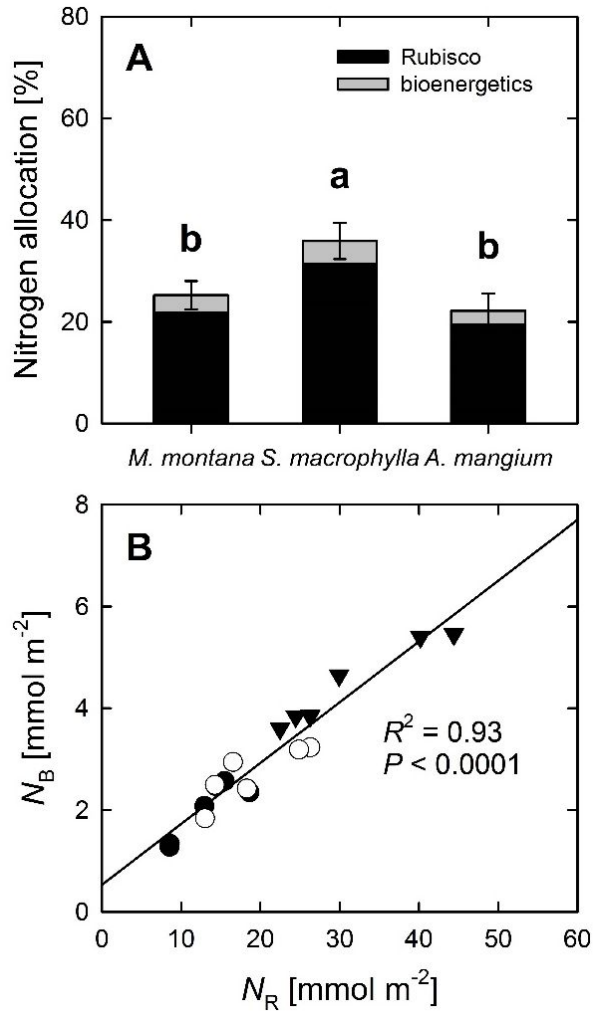
Figure 2 (A) The fraction of nitrogen content allocated to Rubisco (N_R, black bars), bioenergetics (N_B, gray bars) and (B) the relationship between N_R and N_B in *M. montana* (●), *S. macrophylla* (○), *A. mangium* (▼). Different letters in panel A represent significant differences in the sum of the fraction of N_R and N_B among species with $P<0.05$ and error bars represent standard errors in those of them. Regression line: (B) $y = 0.12x + 0.53$

CO_2 assimilation and respiration rate and their relationship

P_{max} and R_d showed positive correlations with N_L, N_R and N_B (Fig. 2).In terms of all N content, *A. mangium* exhibited greater P_{max} and R_d than *M. montana* and *S. macrophylla* (Table 1). However, the variations in P_{max} were better explained by N_R and N_B than by N_L, while the variation of R_d was better explained by N_L than N_R or N_B. These results are in agreement with the well-known fact that there is segmentation between photosynthetic and respiration components within leaf, although N_R and N_B could also explain the variation in R_d relatively well (Fig. 2). In particular, P_{max} of *A. mangium* had a negative relationship with N_L, which is an uncommon relationship between A_{max} and N_L, but it showed a strong positive relationship with N_R and N_B (Fig.2A).

Figure 3 shows the ratio of R_d to P_{max} as a function of N_L, N_P and N_P/N_L. There was a significant negative correlation between the ratio and N_P/N_L (Fig. 3C), whereas there was no significant correlation with N_L and N_P (Fig. 3A and B). Recently, there have been many studies about the relationship between P_{max} and R_d because of their importance as indicators of the leaf carbon balance (Chu et al. 2011, Smith and Dukes 2013). Chu *et al.*(2011) emphasized the incorporation of variable R_d/P_{max} in global-scale vegetation growth models would improve the prediction of rate of photosynthesis compared to models using a constant R_d/P_{max} (e.g. Cox 2001, Thornton et al. 2009, Friend 2010). These results showing a systematic change in R_d/P_{max} with N_P/N_L imply that the differences in N_L allocation are one factor that contributes to the variation of R_d/P_{max} among biomes and plant functional groups.

Figure 3 The relationship between the light-saturated photosynthetic rate (P_{max}) and (A) leaf nitrogen content (N_L), (B) leaf nitrogen allocated to Rubisco (N_R), and (C) leaf nitrogen allocated to bioenergetics (N_B). The relationship between the dark respiration rates (R_d) and (D) leaf nitrogen content (N_L), (E) leaf nitrogen allocated to Rubisco (N_R), and (F) leaf nitrogen allocated to bioenergetics (N_B). Symbols are the same as in Fig. 1. Regression lines: (A) $y = 0.083x + 0.48$; (B) $y = 0.52x - 2.6$; (C) $y = 4.3x - 4.8$; (D) $y = 0.0068x - 0.12$; (E) $y = 0.031x - 0.12$; (F) $y = 0.28x - 0.32$
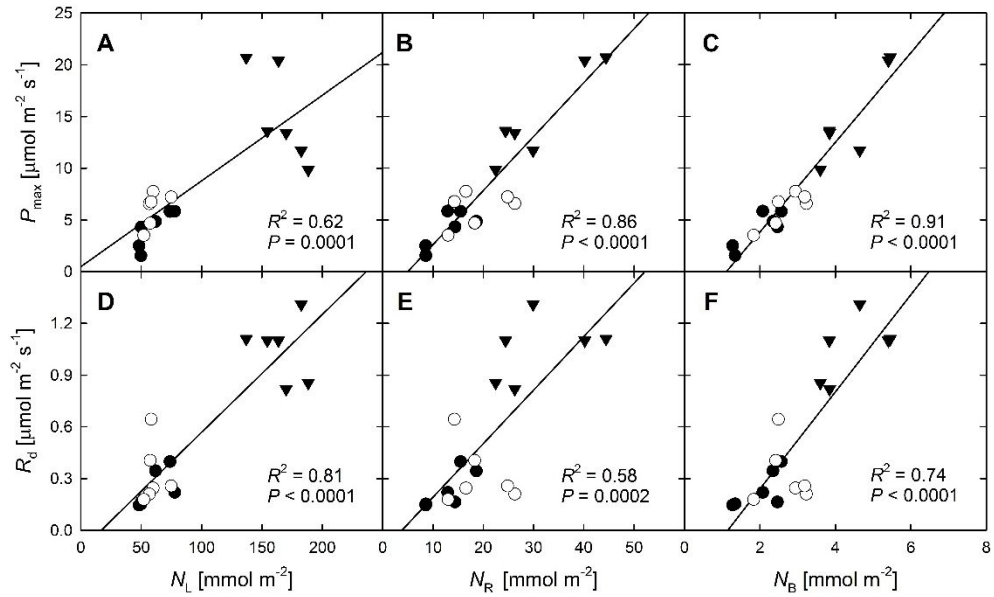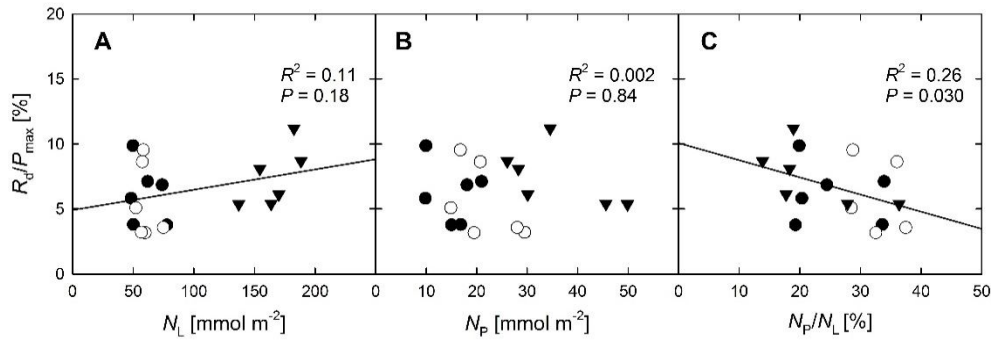
Figure 4 The relationship between the ratio of the dark respiration to the light-saturated photosynthetic rate (R_d/P_{max}) and (A) leaf nitrogen content (N_L), (B) leaf nitrogen allocated to the photosynthetic apparatus (N_P), and (C) the fraction of leaf nitrogen allocated to the photosynthetic apparatus (N_P/N_L). Symbols are the same as in Fig. 1. Regression line: C) $y = -0.13x + 10$

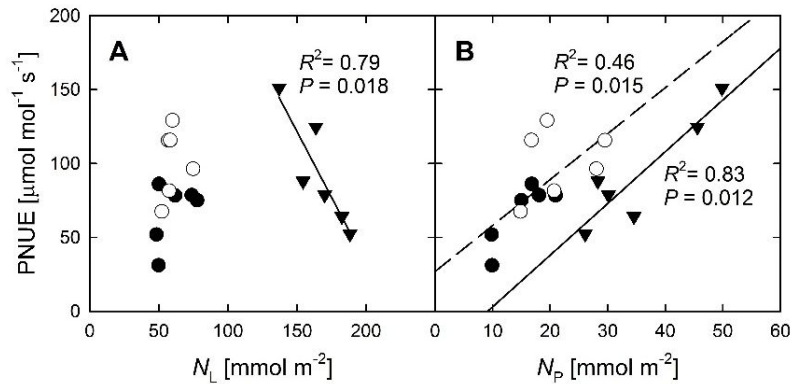**Figure 5** The relationship between photosynthetic nitrogen-use efficiency (PNUE) and (A) leaf nitrogen content (N_L) and (B) leaf nitrogen allocated to the photosynthetic apparatus (N_P). Symbols are the same as in Fig. 1. The dashed lines refer to *M. montana* and *S. macrophylla* (i.e. non N-fixing), and the solid lines refer to *A. mangium* (i.e. N-fixing). The parameters of the regression lines are provided in Table 2

19

Variation in photosynthetic nitrogen-use efficiency

Generally, variations in PNUE are attributed to the variation of N_L and its allocation to different functional and structural components, such as C_i and g_s(Poorter and Evans 1998, Hikosaka and Shigeno 2009). In this study, PNUE varied from 31.1 to 151.1 μmol mol^{-1} s^{-1} for all species. They showed significant positive correlations with N_P and a negative correlation with N_L in *A. mangium*(Fig. 4). N_P was the major independent variable in PNUE, which explained 46 and 83% of the variation of PNUE in N-fixing and non N-fixing species, respectively, while the regression lines were significantly different in N-fixing and non N-fixing species. ANCOVA analysis showed that the intercepts of regression lines for N-fixing species (i.e.*A. mangium*) were significantly lower than those of non N-fixing species (i.e.*M. montana* and *S. macrophylla*) ($P<0.001$). These results imply that *A. mangium* exhibited lower PUNE than *M. montana* and *S. macrophylla* at a given N_P. Table 2 shows the linear regression parameters for these relationships.

Figure 5 shows the correlations between PNUE and C_i and g_s. Although several studies reported a positive correlation between PNUE and C_i(e.g. Hikosaka et al. 1998, Warren and Adams 2004), I did not find a correlation between PNUE and C_i, which was also reported by Pons et al. (2004). Instead, C_i was explained well by the combination of $g_s$and N_P. The multiple regression of C_i and these variables showed that C_i increased with g_s, but it decreased with N_P (maximum $P<0.01$, data not shown). The differing behaviour of C_i with g_s and N_P makes C_i a controversial variable in the explanation of PNUE.
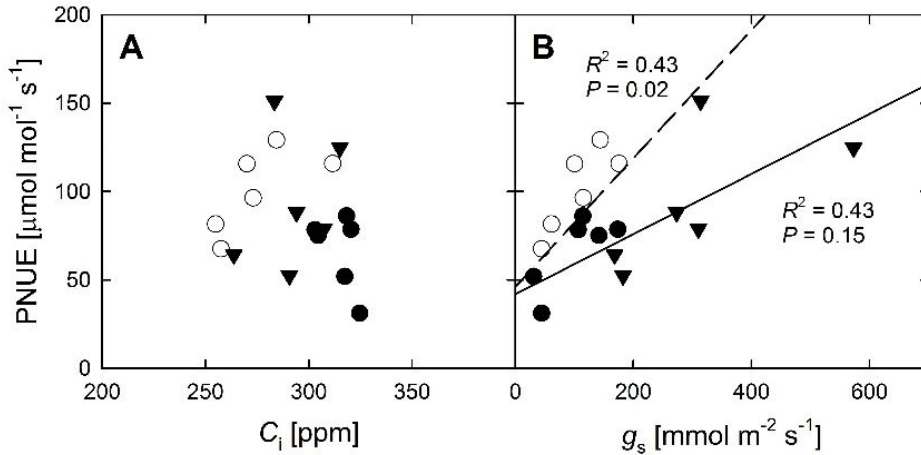
Figure 6 The relationship between photosynthetic nitrogen-use efficiency (PNUE) and (A) intercellular CO_2 concentration (C_i) and (B) stomatal conductance (g_s). Symbols are the same as in Fig. 1. The dashed line refers to *M. montana* and *S. macrophylla* (i.e. non N-fixing), and the solid line refers to *A. mangium*(i.e. N-fixing). The parameters of the regression lines are provided in Table 2

The slope of the relationship between PNUE non N-fixing species was higher than in N-fixing species (Table 2 and Fig.5B). The differences in the slopes between N-fixing and non N-fixing species could be attributed to their morphological differences (Fig. 6). The higher LMA of N-fixing species could have significantly increased the distance of the pathway from intercellular space to CO_2 assimilation sites compared to non N-fixing species (Table 1). This increased distance could decrease mesophyll conductance (e.g. Terashima et al. 2006, Hikosaka and Shigeno 2009) and eventually cause the reduced slope of the PNUE and g_s relationship.
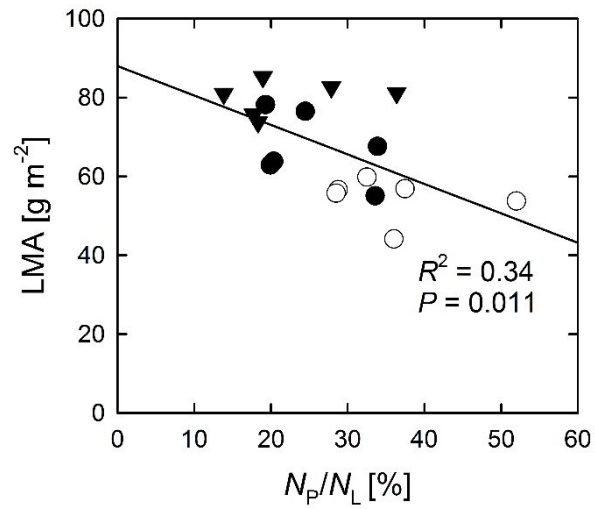
21

Figure 7 The relationship between the leaf dry mass per area (LMA) and the fraction of leaf nitrogen allocated to photosynthetic apparatus (N_P). Symbols are the same as in Fig. 1. Regression line: $y = -0.75x + 88$

Table 2 Details of linear regression results for the relationships between photosynthetic nitrogen-use efficiency (PNUE) and other variables. N_L – leaf nitrogen content, N_P – leaf nitrogen allocated to photosynthetic apparatus; C_i – intercellular CO_2 concentration; g_s – stomatal conductance. ANCOVA was conducted only for N_P vs. PNUE because the other relationships with PNUE were not significant for at least one of the two groups. Different letters indicate a difference at the level of $P<0.01$

Relationship (x- vs. y-axis)	Group	Intercept	Slope	R^2	P
N_L[mmol m^{-2}] vs. PNUE[µmol mol^{-2} s^{-1}]	nonN-fixing	41.7	0.70	0.07	0.42
	N-fixing	388	-1.8	0.79	**0.018**
N_P[mmol m^{-2}] vs. PNUE [µmol mol^{-2} s^{-1}]	nonN-fixing	**26.9[a]**	3.11 a	0.46	**0.015**
	N-fixing	**-32.0[b]**	3.50 a	0.83	**0.012**
C_i[ppm]vs. PNUE [µmol mol^{-2} s^{-1}]	nonN-fixing	198	-0.388	0.12	0.253
	N-fixing	-62.1	0.531	0.065	0.624
g_s[mmol m^{-2} s^{-1}] vs. PNUE [µmol mol^{-2} s^{-1}]	nonN-fixing	46.1	0.363	0.43	**0.021**
	N-fixing	41.9	0.170	0.43	0.158

5. Conclusion

I examined the effect of leaf nitrogen allocation on photosynthetic nitrogen-use efficiency (PNUE) of seedlings of three tropical species. In conclusion, *A. mangium*, which had a greater leaf nitrogen (N_L) than *S. macrophylla* and *M. montana* and the high leaf mass per unit area (LMA) species, showed a lower PNUE at given leaf nitrogen in photosynthetic apparatus (N_P), while exhibited a greater capacities in photosynthetic characteristics such as light-saturated photosynthetic rate (P_{max}) and dark respiration rate (R_d).

A. mangium, which is the N-fixing species, had a greater amount of N_L, N_R and N_B than *S. macrophylla* and *M. montana*, which are non N-fixing species. As results, *A. mangium* exhibited a greater capacities in P_{max} and R_d. In contrast. *S. macrophylla*, which obtained only one third of N_L compared to *A. mangium*, had a greater portion of N_P than other two species. In the case of *M. montana*, which had a similar N_L with *S. macrophylla*, showed a slightly lower capacities in P_{max} and R_d than other species. For PNUE, *A. mangium* showed a lower PNUE than *S. macrophylla* and *M. montana* at a given N_P. In addition, the slope of the relationship between PNUE and stomatal conductance (g_s) in *A. mangium* was lower than in other species. The differences in the slopes among species could be attributed to their morphological differences, that is, the higher LMA of *A. mangium* species than other species. For *A. mangium*, the higher LMA, which was the output of high investment for the structural components of leaves such as cell wall and membrane, increased the distance of the pathway from intercellular space to CO_2 assimilation sites compared to *S. macrophylla* and *M. montana*. Thus the higher LMA of *A. mangium* accounted for the inefficient use of N in terms of photosynthetic abilities.

REFERENCES

Broich M, Hansen M, Potapov P, Wimberly M (2013) Patterns of tree-cover loss along the Indonesia–Malaysia border on Borneo. Int J Remote Sens 34:5748-5760.

Cernusak LA, Winter K, Turner BL (2009) Physiological and isotopic (δ^{13}C and δ^{18}O) responses of three tropical tree species to water and nutrient availability. Plant Cell Environ 32:1441-1455.

Chu ZY, Lu YJ, Chang J, Wang M, Jiang H, He JS, Peng CH, Ge Y (2011) Leaf respiration/photosynthesis relationship and variation: an investigation of 39 woody and herbaceous species in east subtropical China. Trees-Struct Funct 25:301-310.

Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedin LO, Perakis SS, Latty EF, Von Fischer JC, Elseroad A, Wasson MF (1999) Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. Glob Biogeochem Cycle 13:623-645.

Cox PM (2001) Description of the "TRIFFID" dynamic global vegetation model. Hadley Centre technical note. Met Office, Haedley Centre, Met Office, Bracknell, Berkshire.

Ellsworth DS, Reich PB, Naumburg ES, Koch GW, Kubiske ME, Smith SD (2004) Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO2 across four free-air CO2 enrichment experiments in forest, grassland and desert 10:2121-2138.

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78:9-19.

Feng YL, Fu GL (2008) Nitrogen allocation, partitioning and use efficiency in three invasive plant species in comparison with their native congeners. Biol Invasions 10:891-902.

Finzi AC, Moore DJ, DeLucia EH, Lichter J, Hofmockel KS, Jackson RB, Kim HS, Matamala R, McCarthy HR, Oren R, Pippen JS, Schlesinger WH (2006) Progressive nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperate forest. Ecology 87:15-25.

Friend AD (2010) Terrestrial plant production and climate change. J Exp Bot 61:1293-1309.

Funk JL, Glenwinkel LA, Sack L (2013) Differential Allocation to Photosynthetic and Non-Photosynthetic Nitrogen Fractions among Native and Invasive Species. PloS One 8:e64502.

Grassi G, Vicinelli E, Ponti F, Cantoni L, Magnani F (2005) Seasonal and interannual variability of photosynthetic capacity in relation to leaf nitrogen in a deciduous forest plantation in northern Italy. Tree physiology 25:349-60.

Grogan J, Ashton MS, Galvao J (2003) Big-leaf mahogany (*Swietenia macrophylla*) seedling survival and growth across a topographic gradient in southeast Para, Brazil. For Ecol Manage 186:311-326.

Harrison MT, Edwards EJ, Farquhar GD, Nicotra AB, Evans JR (2009) Nitrogen in cell walls of sclerophyllous leaves accounts for little of the variation in photosynthetic nitrogen-use efficiency 32:259-270.

Hedin LO, Brookshire EJ, Menge DN, Barron AR (2009) The nitrogen paradox in tropical forest ecosystems 40:613-635.

Hikosaka K (2004) Interspecific difference in the photosynthesis-nitrogen relationship: patterns, physiological causes, and ecological importance. J Plant Res 117:481-94.

Hikosaka K, Hanba YT, Hirose T, Terashima I (1998) Photosynthetic nitrogen-use efficiency in leaves of woody and herbaceous species. Funct Ecol 12:896-905.

Hikosaka K, Shigeno A (2009) The role of Rubisco and cell walls in the interspecific variation in photosynthetic capacity. Oecologia 160:443-451.

Körner C (2009) Responses of humid tropical trees to rising CO2 40:61-79.

Kattge J, Knorr W, Raddatz T, Wirth C (2009) Quantifying photosynthetic capacity and its relationship to leaf nitrogen content for global-scale terrestrial biosphere models. Glob Change Biol 15:976-991.

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371-379.

Luo Y, Su B, Currie WS, Dukes JS, Finzi AC, Hartwig U, Hungate B, McMurtrie RE, Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience 54:731-739.

Niinemets U, Tenhunen JD, Canta NR, Chaves MM, Faria T, Pereira JS, Reynolds JF (1999) Interactive effects of nitrogen and phosphorus on the acclimation potential of foliage photosynthetic properties of cork oak, *Quercus suber*, to elevated atmospheric CO_2 concentrations. Glob Change Biol 5:455-470.

Novriyanti E, Watanabe M, Makoto K, Takeda T, Hashidoko Y, Koike T (2012) Photosynthetic nitrogen- and water-use efficiency of acacia and eucalypt seedlings as afforestation species. Photosynthetica 50:273-281.

Onoda Y, Hikosaka K, Hirose T (2004) Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-use efficiency 18:419-425.

Poorter H, Evans JR (1998) Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. Oecologia 116:26-37.

Ramdani F, Hino M (2013) Land use changes and GHG Emissions from tropical forest conversion by oil palm plantations in Riau province, Indonesia. PloS One 8:e70323.

Reich P, Walters M, Ellsworth D, Uhl C (1994) Photosynthesis-nitrogen relations in Amazonian tree species 97:62-72.

Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JM, Naeem S, Trost J (2006a) Nitrogen limitation constrains sustainability of ecosystem response to CO_2. Nature 440:922-925.

Reich PB, Hungate BA, Luo Y (2006b) Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide:611-636.

Reich PB, Oleksyn J, Wright IJ (2009) Leaf phosphorus influences the photosynthesis-nitrogen relation: a cross-biome analysis of 314 species. Oecologia 160:207-212.

Ripullone F, Grassi G, Lauteri M, Borghetti M (2003) Photosynthesis–nitrogen relationships: interpretation of different patterns between Pseudotsuga menziesii and Populus× euroamericana in a mini-stand experiment 23:137-144.

Schlesinger WH (2009) On the fate of anthropogenic nitrogen. Proc Natl Acad Sci U S A 106:203-208.

Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL (2007) Fitting photosynthetic carbon dioxide response curves for C_3 leaves. Plant Cell Environ 30:1035-1040.

Simon J, Gleadow RM, Woodrow IE (2010) Allocation of nitrogen to chemical defence and plant functional traits is constrained by soil N. Tree Physiol 30:1111-1117.

Smith NG, Dukes JS (2013) Plant respiration and photosynthesis in global-scale models: incorporating acclimation to temperature and CO_2. Glob Change Biol 19:45-63.

Takashima T, Hikosaka K, Hirose T (2004) Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. Plant Cell Environ 27:1047-1054.

Terashima I, Hanba YT, Tazoe Y, Vyas P, Yano S (2006) Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion. J Exp Bot 57:343-354.

Thornton PE, Doney SC, Lindsay K, Moore JK, Mahowald N, Randerson JT, Fung I, Lamarque JF, Feddema JJ, Lee YH (2009) Carbon-nitrogen interactions regulate climate-carbon cycle feedbacks: results from an atmosphere-ocean general circulation model. Biogeosciences 6:2099-2120.

Thornton PE, Lamarque JF, Rosenbloom NA, Mahowald NM (2007) Influence of carbon-nitrogen cycle coupling on land model response to CO_2 fertilization and climate variability. Glob Biogeochem Cycle 21:GB4018.

Verheijen L, Brovkin V, Aerts R, Bönisch G, Cornelissen J, Kattge J, Reich P, Wright I, van Bodegom P (2013) Impacts of trait variation through

observed trait–climate relationships on performance of an Earth system model: a conceptual analysis. Biogeosciences 10:5497-5515.

Vitousek PM, Menge DN, Reed SC, Cleveland CC (2013) Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Philos Trans R Soc B-Biol Sci 368:20130119.

Warren CR, Adams MA (2004) What determines rates of photosynthesis per unit nitrogen in *Eucalyptus* seedlings? Funct Plant Biol 31:1169-1178.

Westbeek MHM, Pons TL, Cambridge ML, Atkin OK (1999) Analysis of differences in photosynthetic nitrogen use efficiency of alpine and lowland *Poa* species. Oecologia 120:19-26.

Wilson KB, Baldocchi DD, Hanson PJ (2000) Spatial and seasonal variability of photosynthetic parameters and their relationship to leaf nitrogen in a deciduous forest. Tree Physiol 20:565-578.

Wright IJ, Reich PB, Cornelissen JH, Falster DS, Garnier E, Hikosaka K, Lamont BB, Lee W, Oleksyn J, Osada N, Poorter H, Villar R, Warton DI, Westoby M (2005) Assessing the generality of global leaf trait relationships. New Phytol 166:485-496.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JH, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas ML, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. Nature 428:821-827.

Xiang S, Reich PB, Sun SC, Atkin OK (2013) Contrasting leaf trait scaling relationships in tropical and temperate wet forest species. Funct Ecol 27:522-534.

Xu L, Baldocchi DD (2003) Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (Quercus douglasii) under prolonged summer drought and high temperature 23:865-877.

Zhou X, Fu Y, Zhou L, Li B, Luo Y (2013) An imperative need for global change research in tropical forests 33:903-912.

ABSTRACT IN KOREAN

잎의 질소분배가 세 가지 열대 수종의
광합성질소이용효율에 미치는 영향

질소의 효율적인 이용은 식물의 생장과 생존에 필수적이다. 특히 식물 잎의 질소 함량(N_L)은 식물 잎의 광합성 능력과 강한 양의 상관관계를 가지는데, 이는 식물 잎의 질소 중 거의 절반 이상이 루비스코, 엽록소 등과 같은 광합성기구에 분배되기 때문이다. 하지만 잎 내부의 질소 분배 특성은 수종 및 생육 환경에 따라 변이를 보이며, 이러한 변이는 식물의 광합성질소이용효율(Photosynthetic nitrogen−use efficiency, PNUE)에 영향을 미친다. 따라서 본 연구는 잎의 질소분배가 잎의 광합성질소이용효율에 미치는 영향에 대해 세 가지 열대 수종(*Magnolia montana*, *Swieteniamacrophylla*, *Acacia mangium*)을 대상으로 실험 하였다.

유일한 질소고정식물인 *A. mangium*이 N_L, 루비스코에 분배되는 질소(N_R), 전자전달계에 분배되는 질소(N_B)모두에서 가장 많은 양의 질소를 함유하고 있었고,이에 따라 최대광합성률(P_{max})과호흡률(R_d)모두다른 두 수종에 비해 높게 나타났다.반면에*S. macrophylla*는 *A.mangium*과비교하여 세 배 정도 적은 양의 N_L을 함유하고 있었지만,N_P의 비율은 다른 두 수종과 비교하여 높게 나타났다.PNUE는 *A. mangium*이 다른 두 수종과

32

비교하여 단위 N_P에서 더 낮은 효율을 보였고,기공전도도(stomatal conductance, g_s)또한 세 수종 모두에서 광합성질소이용효율과 양의 상관관계를 보였지만, *A. mangium*이 다른 두 수종과 비교하여 더 낮은 기울기를 보였다.이러한 차이는 질소고정 수종인 *A. mangium*이 비질소고정수종인 *M.montana, S.macrophylla*와 비교하여더 높은 단위 엽면적 당 잎의 무게(leaf mass per unit area, LMA) 가지는 데 대한 결과로써,*A. mangium*이 다른 두 수종과 비교하여 세포벽 등 구조적인 부분에 더 많은 질소를 분배한 결과로 생각된다.

주요어: 광합성, 인도네시아, 잎의 질소함량, 질소 분배

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