

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

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**Abstract** The leaf nitrogen (N) content ( $N_L$ ) and its allocation are major factors that determine the photosynthetic characteristics and photosynthetic N-use efficiency (PNUE) of tree species. In this study, we investigated the allocation of  $N_L$  to the photosynthetic apparatus ( $N_P$ ) and its effects on the photosynthetic characteristics and PNUE of three tropical tree species (*Magnolia montana* Blume, *Swietenia macrophylla* King, and *Acacia mangium* Willd), which were grown in western Java, Indonesia. *A. mangium* (the only N-fixing species among three studied species) exhibited a greater  $N_L$  and  $N_P$  than other two species, resulting in higher capacities of photosynthetic parameters such as the light-saturated photosynthetic rate ( $P_{max}$ ), maximum carboxylation rate ( $V_{Cmax}$ ), and maximum electronic transport rate ( $J_{max}$ ). However, *S. macrophylla* and *A. mangium* displayed a similar fraction of leaf N

allocated to  $N_P$  ( $N_P/N_L$ ) and showed a higher  $N_P/N_L$  ratio than *M. montana*. Accordingly, *S. macrophylla* presented a higher PNUE than *M. montana*. Although *A. mangium* exhibited a similar PNUE to *S. macrophylla*, *A. mangium* showed a higher  $N_L$  than the other two species, which resulted in a lower PNUE at a given  $N_P$ . In addition, stomatal conductance was positively correlated with PNUE, but the slopes of the correlations between species presented a negative relationship with the leaf mass per area (LMA), which caused a longer pathway to the photosynthetic apparatus. Our results suggested that the variation of PNUE between species was affected by various factors, including the fraction of  $N_L$  allocated to  $N_P$  and morphological characteristics such as LMA.

**Keywords** Gas exchange · Leaf nitrogen · Leaf nitrogen allocation · Nitrogen-fixing · Tropical species

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## Introduction

During the last several decades, the economics of nitrogen (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 ultimately determines the productivity and succession of ecosystems (Evans 1989; Poorter and Evans 1998; Hikosaka and Shigeno 2009). The importance of N originates from CO<sub>2</sub> assimilation, which is largely controlled by the supply and demand of N at the leaf or cellular level. The N efficiency, which is defined as the amount of CO<sub>2</sub> assimilated per unit of N content, is

commonly expressed as the photosynthetic N-use efficiency [PNUE ( $\mu\text{mol}(\text{CO}_2)\text{mol}^{-1}(\text{N})\text{s}^{-1}$ )]. Therefore, PNUE could be the key plant trait that determines the survival and adaptation abilities of particular species, especially under progressive N limitation associated with changing climate conditions due to increasing atmospheric  $\text{CO}_2$  concentrations (Luo et al. 2004; Finzi et al. 2006; Schlesinger 2009; Xu et al. 2012).

Within a leaf, N is allocated primarily to structural and functional N, which is further divided into photosynthetic, respiratory, and storage N (Poorter and Evans 1998; Takashima et al. 2004; Xu et al. 2012; Funk et al. 2013). Each species exhibits a different pattern of N allocation to various components, and these differences cause variation in the photosynthesis–N relationship and PNUE among species (Poorter and Evans 1998; Hikosaka and Shigeno 2009; Novriyanti et al. 2012). In addition, these variations in N allocation are determined not only by inherent species-specific characteristics but also the plant growth environment. For example, leaves growing under different light conditions, such as sun versus shade leaves, exhibit differences in N allocation to various components, resulting in different photosynthesis–N relationships (Funk et al. 2013). Furthermore, Feng and Fu (2008) reported that many species showed altered N allocation patterns when they were introduced to exotic environmental conditions compared with their native conditions. Thus, a number of recent studies have highlighted the importance of obtaining information on N allocation in various species as well as under different growth conditions to assess how the variation of N allocation affects photosynthetic characteristics and PNUE, especially regarding allocation to the photosynthetic apparatus (Poorter and Evans 1998; Takashima et al. 2004; Feng and Fu 2008; Funk 2008; Simon et al. 2010; Novriyanti et al. 2012; Funk et al. 2013).

In this study, leaf N allocation and its effects on photosynthetic characteristics and PNUE in three tropical species (*Magnolia montana* Blume, *Swietenia macrophylla* King, and *Acacia mangium* Willd) were investigated using 2-year-old seedlings grown in pots under a sun screen in western Java, Indonesia. To the best of our knowledge, this is the first study to investigate the physiological leaf traits of *M. montana*, which is an indigenous species in Malaysia and Indonesia. *S. macrophylla*, which is better known as ‘big leaf mahogany,’ has been popularly used for making furniture and musical instruments, and there have been many studies on its physiological traits (e.g., Grogan et al. 2003; Cernusak et al. 2009). However, few such studies have been conducted in Indonesia. Lastly, *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* is the only N-fixing species among the three species investigated, and it was therefore expected to exhibit a distinct mechanism and ability of N utilization compared with other two non-N-fixing species (Cleveland et al. 1999; Vitousek et al. 2013). Thus, the objectives of our study were as follows: (1) to investigate the differences in the leaf N content and its allocation within the leaf between species and (2) to assess the effect of leaf N content ( $N_L$ ) allocation to the photosynthetic apparatus on photosynthetic characteristics and N-use efficiency.

## Materials and methods

### Site description and plant materials

The study was conducted at the Rumpin Seed Source and Nursery Center (RSSNC), which is located in Bogor, western Java, Indonesia ( $6^{\circ}26'S$ ,  $106^{\circ}38'E$ ). The altitude of the site is approximately 180 m above sea level, and the average annual rainfall and air temperature are 2750 mm and  $26.3^{\circ}\text{C}$ , respectively. The monthly average temperature is stable, and its variation is therefore lower than the diurnal temperature variation. Precipitation exhibits 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.

All of the plant materials were grown from seeds collected at the RSSNC and were germinated in September 2011. After germination, the seedlings were individually planted in 0.5 L pots, and then transplanted to bigger pots based on the size of the seedlings. At the time of the study, *M. montana* was growing in 8 L pots, and *S. macrophylla* and *A. mangium* were growing in 4 L pots. Each pot contained 80 % dark brown topsoil and 20 % air-dried rice husks by volume. The 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.

### Gas exchange measurements

Gas exchange measurements were performed for 6 days beginning on May 1, 2013, using a portable photosynthesis measurement system (LI-6400, LI-COR, USA). Light and the photosynthetic  $\text{CO}_2$  response curve were measured for each species on six mature leaves, which were randomly selected from the crowns of different individuals. All of the measurements were carried out during in the morning and completed before 14:00 h, prior to any symptoms of water stress. The light-saturated photosynthetic rate ( $P_{\text{max}}$ ) and

the dark respiration rate ( $R_D$ ) were measured at  $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$  and under a  $0 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density (PPFD) at the leaf level, respectively, under steady-state conditions. To achieve steady-state conditions, we waited at least 30 min at each light intensity. The values of intercellular  $\text{CO}_2$  concentration ( $C_i$ ) and stomatal conductance ( $g_s$ ) were derived from  $P_{\text{max}}$  measurements. During all of the measurements, the cuvette  $\text{CO}_2$  concentration, temperature, and vapor pressure deficit (VPD) were maintained at 380 ppm,  $27^\circ\text{C}$ , and 1.0 kPa, respectively. To obtain the photosynthetic  $\text{CO}_2$  response curve, we established 13 cuvette  $\text{CO}_2$  concentration values, and the cuvette conditions, such as the temperature, VPD, and PPFD at the leaf level, were maintained at levels similar to those used for performing  $P_{\text{max}}$  measurements, except for the  $\text{CO}_2$  concentration. The maximum rate of carboxylation ( $V_{\text{Cmax}}$ ) and the maximum rate of electron transport ( $J_{\text{max}}$ ) were estimated by fitting the photosynthetic  $\text{CO}_2$  response curves to a biochemical model using a Microsoft Excel worksheet (Sharkey et al. 2007; Ver. 2007.1). Among the 13 points in each photosynthetic  $\text{CO}_2$  response curve, the points below 200 ppm and above 300 ppm of  $C_i$  were assigned as Rubisco and RuBP regeneration limited, respectively, and the points that remained constant or declined with the  $\text{CO}_2$  concentration were excluded in the determination of  $J_{\text{max}}$  estimates. The  $V_{\text{Cmax}}$  and  $J_{\text{max}}$  values adjusted to  $25^\circ\text{C}$  were applied to the calculations.

#### Leaf morphology and nitrogen contents

After the gas exchange measurements, all 18 of the leaves were harvested to measure the leaf area and dry mass. Leaf areas were measured immediately after harvesting with a leaf area meter (LI-3100, LI-COR, USA), after which the leaves were dried at  $80^\circ\text{C}$  for at least 48 h in a drying oven. Leaf mass was measured after drying. The leaf dry mass per unit leaf area (LMA) was calculated by dividing the leaf dry mass by its area. Finally,  $N_L$  was measured in all of the dried leaves using an elemental analyzer (Flash EA 1112, Thermo Scientific, USA).

#### Leaf nitrogen allocation

N allocation to the photosynthetic apparatus was categorized into the following three components: (1) Rubisco ( $N_R$ ), (2) bioenergetics ( $N_B$ ), and (3) the light-harvesting complex (LHC) and photosystems (Novriyanti et al. 2012). Although the N cost allocated to the LHC and photosystems is greater than that allocated to  $N_B$  (i.e., the N cost of the former is almost twice as high as that of the latter) (Funk et al. 2013),  $N_R$  and  $N_B$  account for most of the photosynthetic capacity under light-saturated conditions

(Westbeek et al. 1999). Thus, in this study, we 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 as the sum of  $N_R$  and  $N_B$ .

The fractions of  $N_L$  in  $N_R$  and  $N_B$  ( $N_R/N_L$  and  $N_B/N_L$ , respectively) were calculated using  $V_{\text{Cmax}}$  and  $J_{\text{max}}$ , respectively, together with other variables according to the equations and procedures of Novriyanti et al. (2012) as follows:

$$N_R/N_L = V_{\text{Cmax}} / (6.25 V_{\text{cr}} \text{LMA} N_m) \times 100, \quad (1)$$

where  $V_{\text{cr}}$  is the specific activity of Rubisco, which is equal to  $20.5 \mu\text{mol CO}_2 (\text{g Rubisco})^{-1} \text{s}^{-1}$  at  $25^\circ\text{C}$  for purified Rubisco enzyme from *Spinacia oleracea* (Jordan and Ogren 1984); LMA is the leaf dry mass per unit leaf area ( $\text{g m}^{-2}$ );  $N_m$  is the N content per unit leaf mass ( $\text{mg g}^{-1}$ ); and the value of 6.25 converts N contents into protein contents [ $\text{g Rubisco} (\text{g N in Rubisco})^{-1}$ ].

$$N_B/N_L = J_{\text{max}} / (156 \times 9.53 \text{LMA} N_m) \times 100, \quad (2)$$

where 156 is the ratio of  $J_{\text{max}}$  to the cytochrome *f* content [ $\text{mmol electrons} (\text{mol cyt } f)^{-1} \text{s}^{-1}$ ], and 9.53 is N in bioenergetics per unit cytochrome *f*.

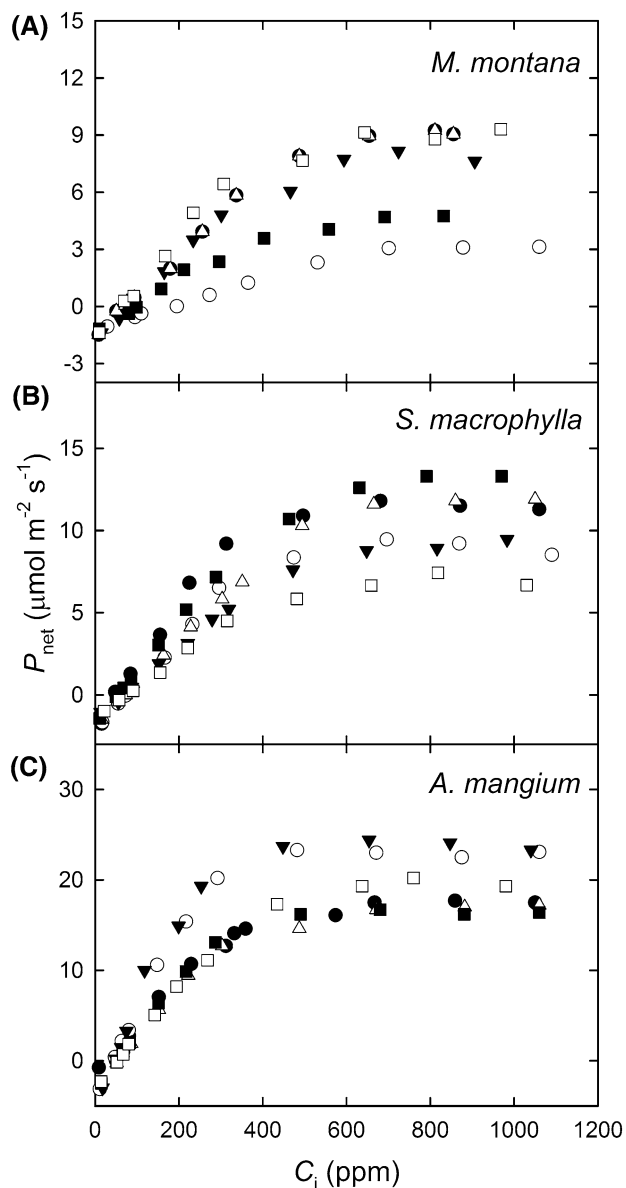
#### Statistical analysis

Analysis of variance (ANOVA) was performed to compare the variation of each parameter among the three species. When significant variation was detected ( $p < 0.05$ ), pair-wise comparisons between species were performed according to Duncan's method. The non-parametric Mann–Whitney test was conducted for comparisons of  $N_P$  fractions (Ruxton 2006). Linear regression analysis was performed to show the relationships between two continuous variables. Analysis of covariance (ANCOVA) was applied to determine the differences in responses between species and between N-fixing and non-N-fixing species (Feng 2008). However, there was no significant difference between the two non-N-fixing species; thus, only the comparisons between N-fixing and non-N-fixing species are shown. Statistical analyses were performed using SAS Version 9.3 (SAS Institute, USA).

## Results

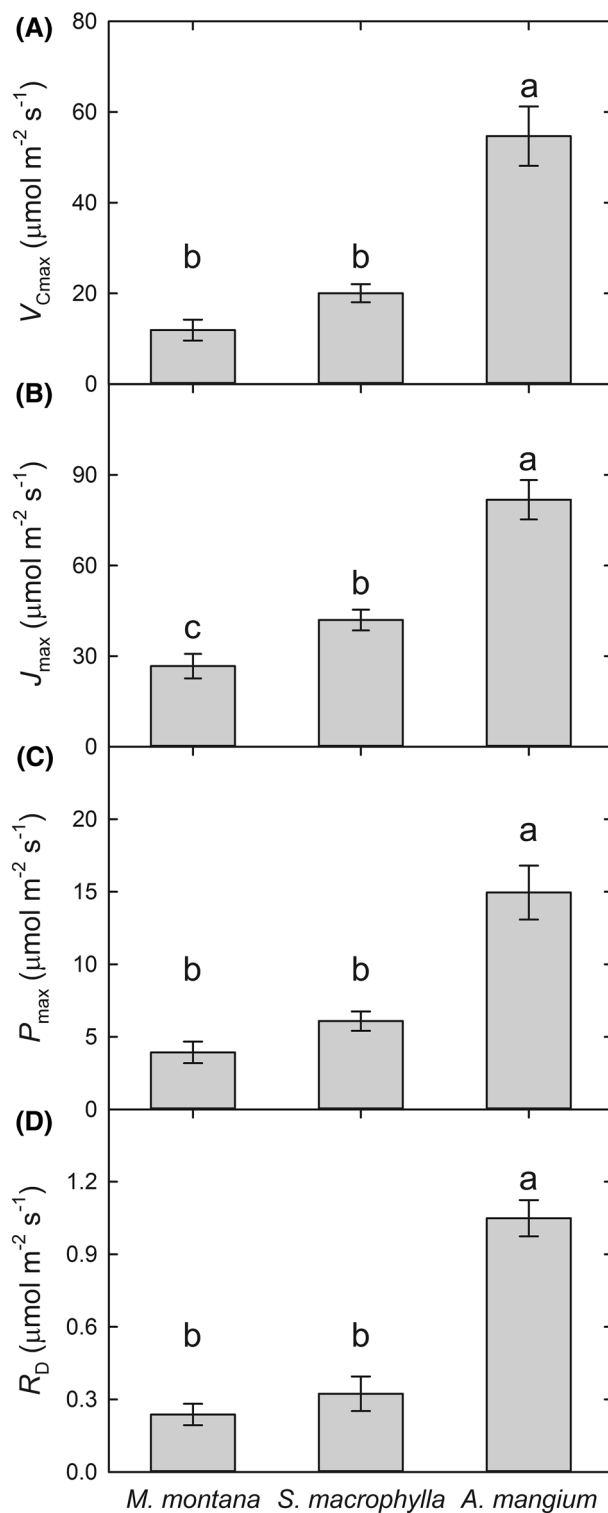
#### Photosynthetic characteristics

To deduce  $V_{\text{Cmax}}$  and  $J_{\text{max}}$ , six photosynthetic  $\text{CO}_2$  response curves were fitted for each species from six leaves (Fig. 1). For *M. montana* and *S. macrophylla*, the net photosynthetic rates reached approximately 9 and  $14 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively, where  $C_i$  was above

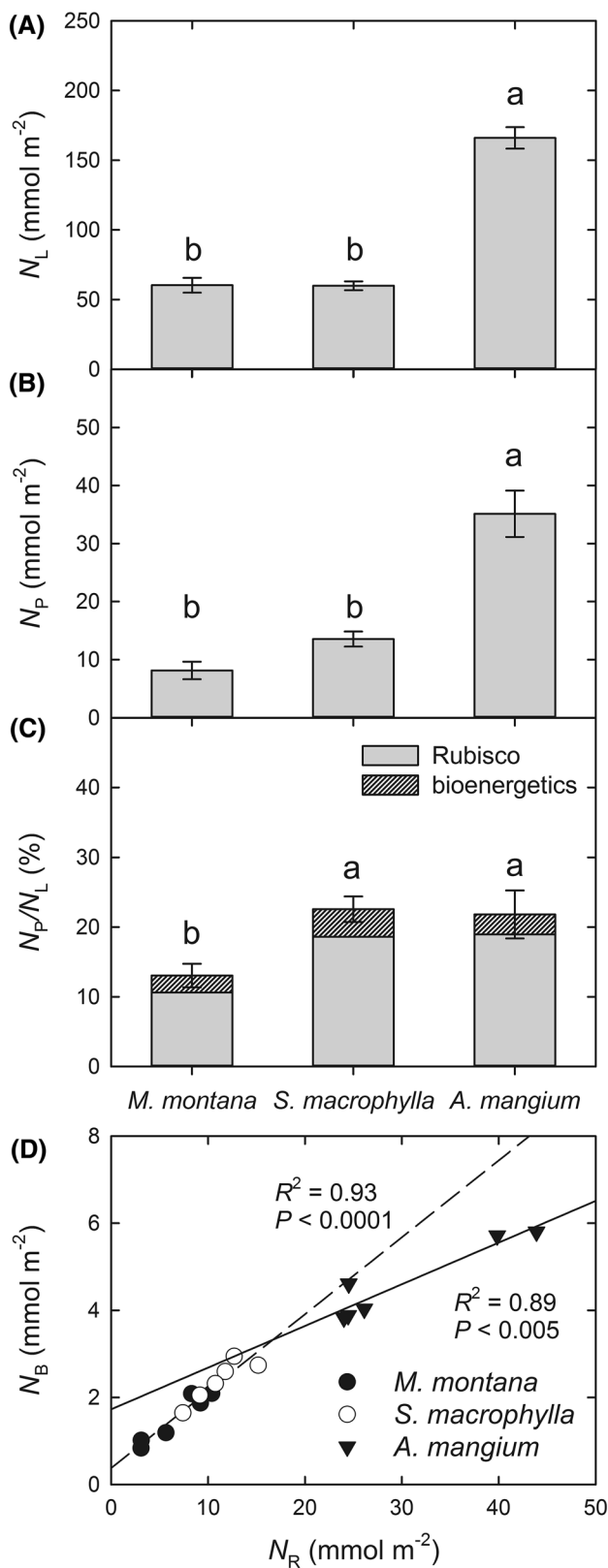


**Fig. 1** Photosynthetic  $\text{CO}_2$  response curves for each species. **A** *Magnolia montana* Blume, **B** *Swietenia macrophylla* King, **C** *Acacia mangium* Willd. Each symbol represents each sample

600 ppm (Fig. 1A, B). For *A. mangium*, which is the only N-fixing species among the three species, the net photosynthetic rate was a higher than for the other two species, reaching approximately  $24 \mu\text{mol m}^{-2} \text{s}^{-1}$ , where  $C_i$  was above 400 ppm (Fig. 1C). Similarly, the values of the other photosynthetic characteristics (i.e.,  $V_{\text{Cmax}}$ ,  $J_{\text{max}}$ ,  $P_{\text{max}}$ , and  $R_{\text{D}}$ ) of *A. mangium* were greater than those of *M. montana* and *S. macrophylla*, and these differences were significant (minimum  $p < 0.001$ , Fig. 2). The  $V_{\text{Cmax}}$  values of *M. montana*, *S. macrophylla*, and *A. mangium* were  $11.9 \pm$



**Fig. 2** **A** Maximum carboxylation rate ( $V_{\text{Cmax}}$ ), **B** maximum electron transport rate ( $J_{\text{max}}$ ), **C** light-saturated photosynthetic rate ( $P_{\text{max}}$ ), and **D** dark respiration rate ( $R_{\text{D}}$ ) for each species. Different letters in each panel indicate differences with  $p < 0.05$  by Duncan's tests. Error bars represent the standard error for six leaves



◀**Fig. 3** **A** Leaf nitrogen content ( $N_L$ ), **B** leaf nitrogen allocated to the photosynthetic apparatus ( $N_P$ ), **C** ratio of leaf nitrogen allocated to the photosynthetic apparatus to the leaf nitrogen content ( $N_P/N_L$ ), and **D** relationship between leaf nitrogen in bioenergetics ( $N_B$ ) and Rubisco ( $N_R$ ) for each species. Different letters in panels **A**, **B**, and **C** indicate differences with  $p < 0.05$  by Duncan's tests. Error bars represent the standard error for six leaves. In panel **D**, the dashed line refers to *M. montana* (filled circle) and *S. macrophylla* (circle):  $y = 0.18x + 0.38$ , and the solid line refers to *A. mangium* (inverted triangle):  $y = 0.10x + 1.73$

(Fig. 2B). Their  $P_{max}$  values were  $3.9 \pm 1.8$ ,  $6.1 \pm 1.6$ , and  $14.9 \pm 4.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (Fig. 2C). Their  $R_D$  values were  $0.24 \pm 0.11$ ,  $0.32 \pm 0.17$ , and  $1.05 \pm 0.18 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (Fig. 2D).

### Leaf nitrogen content and allocation

Regarding the leaf N content,  $N_L$ , the patterns among species were similar to those of the photosynthetic characteristics; that is, the N-fixing species *A. mangium* showed an approximately three times greater  $N_L$  than the non-N-fixing species *S. macrophylla* and *M. montana* ( $166.0 \pm 18.8$ ,  $60.3 \pm 13.0$ , and  $59.9 \pm 7.8 \text{ mmol m}^{-2}$ , respectively,  $p < 0.001$ , Fig. 3A). The amount of N allocated to the photosynthetic apparatus, including Rubisco ( $N_R$ ) and the electron transport system (bioenergetics,  $N_B$ ), was also a greater in *A. mangium* than in *S. macrophylla* and *M. montana* ( $35.1 \pm 0.7$ ,  $13.5 \pm 0.4$ , and  $8.1 \pm 0.8 \text{ mmol m}^{-2}$ , respectively,  $p < 0.001$ , Fig. 3B). However, the fraction of  $N_L$  to  $N_P$  ( $N_P/N_L$ ) was not significantly different between *A. mangium* and *S. macrophylla* ( $21.8 \pm 8.4$  and  $22.7 \pm 4.5 \%$ , respectively,  $p = 0.85$ , Fig. 3C), and these fractions were more than 50 % greater than that recorded in *M. montana* ( $13.1 \pm 4.1 \%$ ,  $p < 0.01$ ). In addition, there were differences in the partitioning of  $N_P$  into  $N_B$  and  $N_R$  among species. Figure 3D shows that there were two strong positive relationships between  $N_B$  and  $N_R$ , and the slope of the regression line for *A. mangium* was significantly lower than those for *S. macrophylla* and *M. montana* ( $p < 0.01$ ). These differences imply that *A. mangium* partitioned more  $N_P$  into  $N_R$  than  $N_B$  compared with *S. macrophylla* and *M. montana*.

### Variation in the photosynthetic nitrogen-use efficiency

To explain photosynthetic abilities in association with the leaf N content and its allocation among species, the PNUE values of individual leaves and species were investigated in relation to the leaf N content and its allocation to the photosynthetic apparatus. PNUE showed wide variation, ranging from 31.1 to 151.1  $\mu\text{mol mol}^{-1} \text{s}^{-1}$ , and the

5.6,  $20.4 \pm 4.9$ , and  $54.7 \pm 16.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (Fig. 2A). Their  $J_{max}$  values were  $26.7 \pm 10.0$ ,  $41.9 \pm 8.4$ , and  $81.8 \pm 16.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively

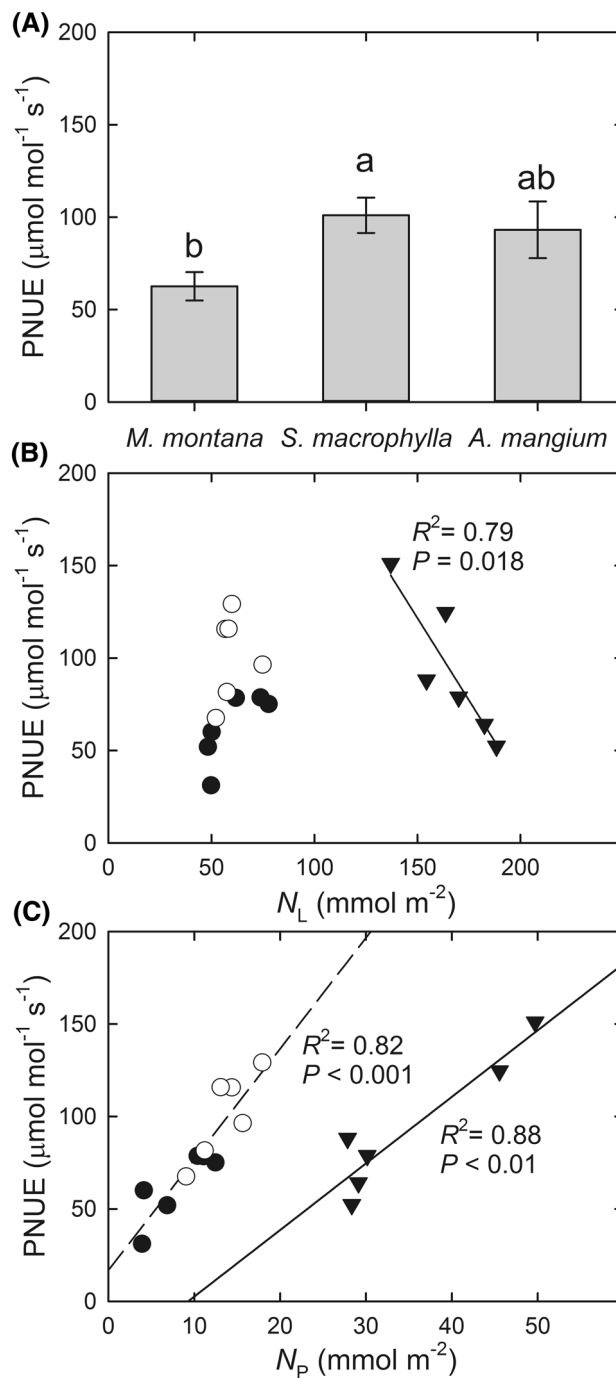
average value for *S. macrophylla* was higher than that for *M. montana* ( $p = 0.011$ ), while the PNUE of *A. mangium* was between those of the two other species ( $101.0 \pm 23.5$ ,  $62.6 \pm 18.8$ , and  $93.2 \pm 37.7 \mu\text{mol mol}^{-1} \text{s}^{-1}$ , respectively, Fig. 4A, B). With the exception of *A. mangium*, which presented a negative relationship between PNUE and  $N_L$ , there was no general relationship between PNUE and  $N_L$  observed among individual leaves and species (Fig. 4B). In contrast to  $N_L$ ,  $N_P$  was the major variable determining the variation of PNUE, explaining 88 and 82 % of the variation of PNUE for *A. mangium* (i.e., N-fixing species) and the other two species (i.e., non-N-fixing species), respectively (Fig. 4C). The slope and intercept of the regression line for *A. mangium* were significantly lower than those for *S. macrophylla* and *M. montana* ( $p = 0.049$  and  $p < 0.001$ , respectively). These results imply that *A. mangium* exhibited a lower PUNE than *S. macrophylla* and *M. montana* at a given  $N_P$ .

In addition to  $N_P$ , stomatal conductance ( $g_s$ ) also substantially explained the variation of PNUE (Fig. 5A). The slope for *S. macrophylla* was highest, followed by *M. montana* and *A. mangium*, and each line accounted for at least 60 % of the variation in PNUE within species. To describe the relationship between PNUE and  $g_s$ , the key morphological characteristic LMA was examined (Fig. 5B). LMA showed an reverse relationship with the slope between PNUE and  $g_s$ ; that is, the LMA of *A. mangium* was the highest, followed by *M. montana* and *S. macrophylla*, and the differences between species were significant ( $79.9 \pm 3.9$ ,  $67.4 \pm 8.0$ , and  $54.5 \pm 5.0$ , respectively,  $p < 0.001$ ).

## Discussion

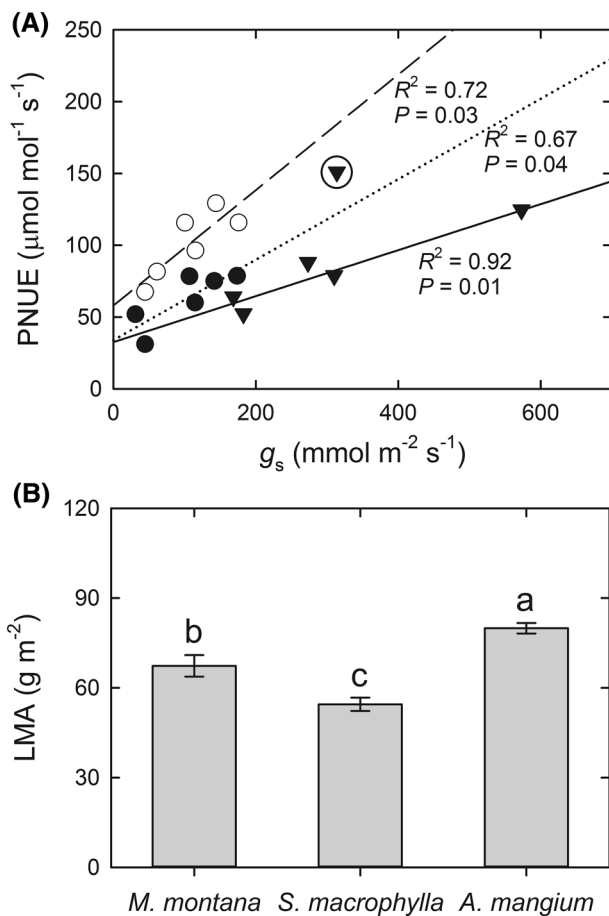
### Photosynthetic capacities and leaf N content

The greater capacities observed for all of the photosynthetic characteristics (i.e.,  $V_{C_{\max}}$ ,  $J_{\max}$ ,  $P_{\max}$ , and  $R_D$ ) of *A. mangium* were apparently due to the significantly higher  $N_L$  and  $N_P$  in this species compared with *S. macrophylla* and *M. montana* (Figs. 1, 2, 3). It is well known that there is a strong positive relationship between the photosynthesis rate and  $N_L$  (Poorter and Evans 1998; Wright et al. 2004); however, this relationship differs considerably among plant functional types and plant growth environments (Leishman et al. 2007; Kattge et al. 2009; Reich et al. 2009; Xiang et al. 2013). For example, Ripullone et al. (2003) reported that broad-leaved species (*Populus × euroamericana*) show a threefold steeper slope of the  $P_{\max}$ – $N$  relationship than conifer species (*Pseudotsuga menziesii*). Novriyanti et al. (2012) observed a higher  $N_L$  in N-fixing species (*A. mangium* and *A. auriculiformis*) than in four non-N-fixing



**Fig. 4** A Photosynthetic nitrogen-use efficiency (PNUE) for each species and the relationships between B PNUE and the leaf nitrogen content ( $N_L$ ) and C PNUE and leaf nitrogen allocated to the photosynthetic apparatus ( $N_P$ ). Different letters in panel A indicate differences with  $p < 0.05$  by Duncan's tests. Error bars represent the standard error for six leaves. In panels B and C, the symbols are the same as in Fig. 3; the dashed line refers to *M. montana* and *S. macrophylla*:  $y = 6.0x + 17$ , and the solid line refers to *A. mangium*:  $y = 3.6x - 33$

eucalyptus species, although the photosynthetic capacities (i.e.,  $P_{\text{net}}$ ,  $V_{C_{\max}}$ , and  $J_{\max}$ ) of the acacia species were lower than those of the eucalyptus species. In addition, Reich



**Fig. 5** **A** Relationship between the photosynthetic nitrogen-use efficiency (PNUE) and stomatal conductance ( $g_s$ ) and **B** the leaf dry mass per unit leaf area (LMA) for each species. In *panel A*, the symbols are the same as in Fig. 3. The *long-dashed line* refers to *S. macrophylla*:  $y = 0.40x + 58$ ; the *dotted-line* refers to *M. montana*:  $y = 0.28x + 34$ ; and the *solid line* refers to *A. mangium*, except for the *circled point*:  $y = 0.16x + 33$ . In *panel B*, different letters in each panel indicate differences with  $p < 0.05$  by Duncan's tests. Error bars represent the standard error for six leaves

et al. (2009) described the variation of the  $P_{\text{max}}-N$  relationship, showing that the slope of the  $P_{\text{max}}-N$  relationship was greater in the colder arctic and temperate biomes than in warmer tropical and subtropical biomes. They also found that the  $P_{\text{max}}-N$  relationship was affected by other nutrient gradients, such as leaf  $P$ . In the present study, we showed that N-fixing *A. mangium* displays a higher photosynthetic capacity and  $N_L$  than the two other, non-N-fixing species (Figs. 1, 2, 3).

#### Leaf N allocation among species

Although there are several reasons for the variation in photosynthetic characteristics, the photosynthesis–N relationship, and PNUE observed among different species and environments, the allocation pattern of  $N_L$ , especially to

$N_P$ , is the most important factor explaining the variation of these physiological traits (Poorter and Evans 1998; Westbeek et al. 1999; Ripullone et al. 2003; Hikosaka and Shigeno 2009; Novriyanti et al. 2012). Eucalyptus species, which are commonly used for pulp production and reforestation due to their rapid growth ability, allocate more than 60 % of  $N_L$  to  $N_P$  (Novriyanti et al. 2012). These species exhibit a higher  $P_{\text{max}}$  than acacia species, which allocate only approximately 18 % of  $N_L$  to  $N_P$ , even though the  $N_L$  of acacia species is higher than that of eucalyptus. Similarly, Feng and Fu (2008) reported that invasive species, which showed higher allocation of  $N_L$  to  $N_P$ , presented a higher  $P_{\text{max}}$  than native species. In accord with other studies, *A. mangium*, which is a species characterized by high  $N_L$  and  $N_P$ , exhibited a higher  $P_{\text{max}}$  than the other two species. In addition, *S. macrophylla*, which showed a higher allocation of  $N_L$  to  $N_P$  than *M. montana*, presented  $J_{\text{max}}$  values that were approximately 60 % higher than those of *M. montana*, even though these species exhibited approximately the same  $N_L$  (Fig. 2B). Furthermore, the  $V_{\text{Cmax}}$  and  $P_{\text{max}}$  values of *S. macrophylla* were more than 50 % higher than those of *M. montana*, even though they were not significantly different (Fig. 2A, C).

The partitioning of  $N_P$  to  $N_R$  and  $N_B$  also differs between species. Feng and Fu (2008) reported that invasive species partitioned a higher fraction of  $N_P$  to  $N_R$ , while their native congeners partitioned a higher fraction of  $N_P$  to light-harvesting components. These authors suggested that the higher partitioning of  $N_P$  to  $N_R$  in the invasive species was associated with their greater specific leaf area (i.e., lower LMA). However, in the present study, *A. mangium* partitioned more  $N_P$  to  $N_R$  than the other two species (Fig. 3D) and displayed a higher LMA (Fig. 5B). These differences could be attributed to the distinct N acquisition ability of *A. mangium* in comparison with the other species. Because *A. mangium* obtains N from the environment through symbiosis with N-fixing microbes, it can utilize relatively greater amounts of N compared with non-N-fixing species. Consequently, *A. mangium* can partition more  $N_P$  to  $N_R$  while retaining its advantage of physical strength with a higher LMA.

#### Effect of leaf N allocation on PNUE

Figure 4C shows the relationship between PNUE and  $N_P$  for each species. For *S. macrophylla* and *M. montana*, the slope and intercept of the regression line were significantly higher than those for *A. mangium*. These results imply that *A. mangium* showed a lower PNUE than other two species at a given  $N_P$ . Thus, *A. mangium* was not able to use  $N_P$  for photosynthesis as efficiently as the other species. However, by increasing its total  $N_P$  compared with the other species (Fig. 4B), *A. mangium* was able to compensate PNUE to a similar or greater extent compared with the other species

(Fig. 4A). The low efficiency of  $N_p$  in *A. mangium* was partially due to its leaf morphological characteristics, such as LMA. The higher LMA of *A. mangium* could have significantly increased the distance of the pathway from the intercellular space to  $CO_2$  assimilation sites compared with *S. macrophylla* and *M. montana*, and this increased distance could decrease mesophyll conductance (Terashima et al. 2006; Hikosaka and Shigeno 2009). Low mesophyll conductance might result in inefficiency of photosynthesis because low mesophyll conductance means a low delivery rate of substances required for photosynthesis (Niinemets et al. 2002; Hikosaka 2004; Onoda et al. 2004; Flexas et al. 2008; Funk et al. 2013). The relationships between PNUE and  $g_s$  obtained for each species also support our hypothesis that a higher LMA, which implies greater investment of resources in the leaf structure, including N, causes inefficient use of N in terms of photosynthesis. The slope of the relationship between PNUE and  $g_s$  was highest for *S. macrophylla*, followed by *M. montana* and *A. mangium* (Fig. 5A), which was the reverse order of that found for LMA (Fig. 5B). The lower slope observed for *A. mangium* indicates that this species exhibits more obstacles between the ambient air and the specific site where carboxylation occurs within the leaf compared with other two species at a given  $g_s$ . Consequently, *A. mangium* presents higher internal resistance due to thicker and longer structures, such as the cell wall, and these factors lead to less efficient use of  $N_p$  in photosynthesis in this species. Although *A. mangium* presented a lower PNUE at a given  $N_p$ , this disadvantage could be compensated by ecologically advantageous characteristics. In general, species characterized by a high LMA show high resistance not only to drought but also to insect damage, as well as a longer leaf lifespan (Reich et al. 1998; Wright et al. 2004; Hikosaka and Shigeno 2009). Many studies have demonstrated that acacia species show such characteristics under field or plantation growth conditions (Yamashita et al. 2008; Leroy et al. 2009; Forrester et al. 2010; Voigtlaender et al. 2012).

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