

Height-related changes in leaf photosynthetic traits in diverse Bornean tropical rain forest trees

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Abstract Knowledge of variations in morphophysiological leaf traits with forest height is essential for quantifying carbon and water fluxes from forest ecosystems. Here, we examined changes in leaf traits with forest height in diverse tree species and their role in environmental acclimation in a tropical rain forest in Borneo that does not experience dry spells. Height-related changes in leaf physiological and morphological traits [e.g., maximum photosynthetic rate (A_{\max}), stomatal conductance (gs), dark respiration rate (R_d), carbon isotope ratio ($\delta^{13}\text{C}$), nitrogen (N) content, and leaf mass per area (LMA)] from understory to emergent trees were investigated in 104 species in 29 families. We found that many leaf area-based physiological traits (e.g., $A_{\max\text{-area}}$, R_d , gs), N, $\delta^{13}\text{C}$, and LMA increased linearly with tree height, while leaf mass-based physiological traits (e.g., $A_{\max\text{-mass}}$) only increased slightly. These patterns differed from other biomes such as temperate and tropical dry forests, where trees usually show decreased photosynthetic

capacity (e.g., $A_{\max\text{-area}}$, $A_{\max\text{-mass}}$) with height. Increases in photosynthetic capacity, LMA, and $\delta^{13}\text{C}$ are favored under bright and dry upper canopy conditions with higher photosynthetic productivity and drought tolerance, whereas lower R_d and LMA may improve shade tolerance in lower canopy trees. Rapid recovery of leaf midday water potential to theoretical gravity potential during the night supports the idea that the majority of trees do not suffer from strong drought stress. Overall, leaf area-based photosynthetic traits were associated with tree height and the degree of leaf drought stress, even in diverse tropical rain forest trees.

Keywords Carbon isotope ratio · Hydraulic limitation · Leaf water potential · LMA · Respiration · Sarawak

Introduction

Forest microenvironments, which strongly affect leaf morphophysiological traits, differ significantly from the understory to the canopy (Kumagai et al. 2001; Lloyd et al. 2010). Leaves that develop in the upper canopy layer in the forest are exposed to higher irradiances, air temperatures,

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and wind speeds, and must cope with microclimatic conditions that potentially lead to desiccation, while leaves from the lower stratum canopy experience milder conditions, such as lower irradiance, constant temperature, and higher humidity (Sanches et al. 2010). In addition, an increasing path length for water to travel to reach transpiring leaves from soil and increasing gravitational potential can increase drought stress in the upper canopy (Koch et al. 2004; Woodruff et al. 2004; Ryan et al. 2006). Leaves acclimate to this vertical environmental gradient by changing their morphological and biochemical properties. Leaf nitrogen (N) and leaf mass per area (LMA) are the primary driving factors for leaf photosynthetic acclimation and drought tolerance, respectively (Niinemets 2001, 2002). Traditionally, this acclimation was considered the sun- versus shade-leaf adaptation; leaves under canopy conditions with high light intensity show higher photosynthetic capacity and drought tolerance due to the high allocation of leaf N and larger LMA, whereas leaves under lower light conditions show reduced LMA and N with decreased dark respiration rates (R_d s) to maintain low photosynthetic light compensation points (Körner 1994; Larcher 2003).

However, previous studies on leaf photosynthesis, morphology, and biochemistry across a wide range of tree heights (which have mainly been performed in tall temperate trees) revealed that leaf photosynthetic capacity and N content usually decrease at the upper canopy, while LMA linearly increases with tree height (Niinemets 2002; Koch et al. 2004; Ryan et al. 2006; Steppe et al. 2011). This reduction in leaf photosynthesis and increase in LMA is thought to be a response to increased leaf drought stress (i.e. more negative leaf water potentials) imposed by increased hydraulic constraints, and is affected by leaf morphophysiological traits rather than changes in the light intensity with height (Koch et al. 2004; Burgess and Dawson Bruggess and Dawson 2007; Ishii et al. 2008). In addition to temperate trees, trees growing on semiarid land and in tropical dry forests, which experience severe seasonal drought and/or cold stresses, showed decreasing photosynthetic capacity with increasing tree height (Kenzo et al. 2012; Su et al. 2013). In contrast, trees in a tropical rain forest that do not experience extended dry and cold spells showed an increased capacity with height, even in emergent trees over 50 m tall (Carswell et al. 2000; Rijkers et al. 2000; Kenzo et al. 2006; Kosugi et al. 2012). Differences in height-related changes between forest types may reflect the degree of environmental stress such as drought and cold between forest types. These environmental stresses may accelerate the reduction in photosynthetic capacity due to stomatal closure and hydraulic constraints with tree height.

However, previous studies on changes in leaf gas exchange with height in tropical rain forests examined a limited number of species that did not encompass the

extremely high tropical species diversity (Peacy 1987; Barker and Booth 1996; Thomas and Bazzaz 1999; Rijkers et al. 2000; Kenzo et al. 2006; Sanches et al. 2010; Kosugi et al. 2012), excluding the Amazonian rain forest with a well-characterized dry season (Domingues et al. 2005, 2007). In addition, Thomas and Bazzaz (1999) found that canopy tree species may have higher photosynthetic capacity during the seedling and juvenile stages compared with non-canopy trees, even at the same tree height. These variations between species have not been examined in previous studies, which focused mostly on canopy tree species. Because tropical rain forests play an important role in carbon dioxide (CO_2) fixation and primary production in global ecosystems, understanding changes in leaf physiological characteristics within the forest profile can improve modeling of CO_2 exchange and primary production in forest environments (Chapin et al. 2002).

In this study, we explored the effect of tree height on leaf gas exchange properties, LMA, and N to derive a general relationship between these variables for a diversity of tree species in the tropical rain forest of Borneo, which is located in the wettest area in Southeast Asia (Malhi and Wright 2004; Kume et al. 2011; Kumagai et al. 2013). We examined specimens including more than 100 species ranging from understory trees grown under the dark forest floor to emergent or canopy trees under strong light conditions, for which we used an 85-m-high canopy crane system (Kenzo et al. 2006).

Materials and methods

Study site

Our study was performed at the Crane Plot (4 ha; 200 m \times 200 m) in a lowland mixed dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia (4°20'N, 113°50'E; 150–250 m a.s.l.). The mean height of the canopy in the stand was ~30–40 m, and some emergent trees reached 50 m in height. An 85-m-tall canopy crane with a 75-m-long rotating jib was constructed in the center of this plot to provide three-dimensional access from close to the forest floor to the canopy (Kenzo et al. 2006).

The study sites are in a humid tropical area with no distinct dry season. The annual precipitation at the study site averaged 2,600 mm from 2000 to 2009, and the average annual temperature from 2000 to 2009 was 25.8 °C (Kume et al. 2011). In the canopy and emergent layers, the daily maximum temperature and maximum photosynthetic photon flux density (PPFD; $\mu\text{mol m}^{-2} \text{s}^{-1}$) were sometimes above 35 °C and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Hiromi et al. 2012).

Plant materials and light conditions

We selected 104 species in 59 genera and 28 families for a total of 204 individuals from the understory to emergent trees (ESM Appendix). All trees were evergreens with different mature tree heights such as emergent, canopy, sub-canopy, and understory trees. The measurement points ranged from 0.5 to 53 m, and we measured a total of 220 points from 204 individuals. We also measured the relative active PPFD at different forest heights (~1–53 m) and the top of canopy crane (~75 m) using a quantum sensor with ultrasmall memory (MDS-MkV/L; Alec, Kobe, Japan). We measured integrated PPFD at each height for 10 days which included cloudy and sunny days. All data was collected at 1-min intervals. Radiance data consisted of 0.5-nm-unit data in the 350- to 1,050-nm wavelength range. Data corresponding to PAR wavelengths (400–700 nm) were extracted from the radiance data and integrated into the PAR wavelengths (Yoshimura and Yamashita 2012). By comparing light intensities at different heights with the top of canopy crane, relative integrated PPFDs were calculated. In total, 58 measurement points from 53 individuals with measured photosynthetic traits were located at the same position as the PPFD measurement. These data were used to analyze the relationship between light intensity and leaf morphophysiological traits.

Gas exchange measurements

A portable photosynthesis apparatus (LI-6400; Li-Cor, Lincoln, NE, USA) was used to measure the leaf gas exchange rate. All measurements were performed in the morning between 0800 and 1100 hours to avoid the mid-day depression in photosynthesis (Ishida et al. 1996; Kenzo et al. 2003). We used fully expanded and apparently non-senescing leaves. Gas exchange measurements were made on the third to fifth leaf from the top of the shoots (Kenzo et al. 2004). The relationship between the PPFD and carbon assimilation rate was determined for three leaves from each individual tree or measurement point. For the maximum photosynthetic rate at light saturation (A_{\max}) for all trees, conditions inside the chamber were controlled to maintain leaf temperature at 30 °C, relative humidity at ~60 %, CO_2 concentration of 360 ppm, and saturating levels of active PPFD ($800 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ for understory trees and $1,700 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ for mid- and top-canopy trees, respectively). Light intensity was controlled using an internal light-emitting diode (LED) light source (Li-640B; Li-Cor). We also measured the stomatal conductance and transpiration rate (Tr) at A_{\max} . We measured leaf R_d under full darkness at 30 °C. The measurement was performed for 119 individuals from 57 species. Most physiological measurements were conducted in September 2005, March 2006

and July 2007. Following the gas exchange measurements, leaves were collected to measure N and LMA. Stable carbon isotope composition was also measured for 153 individuals from 92 species.

Leaf N and stable carbon isotope composition determination

Leaf N and carbon content were determined using an NC analyzer (Sumigraph NC-900; Shimadzu, Kyoto, Japan) after all leaves were dried at 60 °C for 3 days. LMA was also measured from these leaf samples. The stable carbon isotope composition of the leaf dry samples was determined using an isotope ratio mass spectrometer (Delta Plus System; Thermo Quest, San Jose, CA, USA). All carbon isotope ratios were expressed in delta notation relative to the PD Belemnite standard: $\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000 (\text{‰})$, where R_{sample} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and R_{standard} is the ratio of the standard (Tanaka-Oda et al. 2010a).

Leaf water potential

The leaf water potential at predawn (Ψ_{pd}) and midday (Ψ_{mid}) was measured using a pressure chamber (model 1002; PMS Instrument, Albany, OR, USA). We selected 117 individuals from 56 species. The predawn potentials were determined at early morning before transpiration activity (e.g., 0400–0630 hours), and midday leaf water potential was measured in the afternoon on sunny days (e.g., 1200–1500 hours; Hiromi et al. 2012).

Statistical analysis

Scatterplots of leaf morphological and physiological properties with tree height and relative PPFD (RPPFD) were fitted using a regression analysis (Rijkers et al. 2000). We used an ordinary least-squares regression to investigate changes relative to height and RPPFD. Differences in the slope of linear regression between predawn and midday water potential with tree height were examined using an analysis of covariance (ANCOVA). All statistical analyses were performed with PASW statistics v.17.0 for Windows software (SPSS Japan, Tokyo, Japan).

Results

Relationship of light conditions to tree height

The RPPFD increased exponentially with tree height (Fig. 1). The PPFD varied from 0.1 to 5 % at the understory (1–5 m height) to more than 60 % at the canopy (above

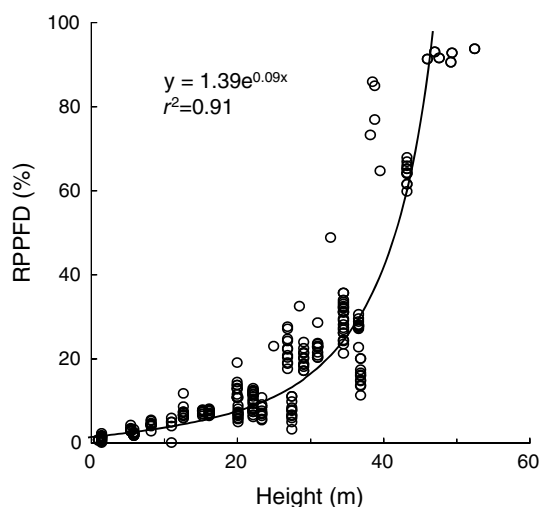


Fig. 1 Changes in the relative photosynthetic photon flux density (RPPFD) with tree height

40 m height). Based on our measurements, light intensities on sunny days ranged from 5 to 20 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ at the forest understory and were $\sim 1,800 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ at the canopy layer.

Leaf gas exchange properties and tree height

Both area- and mass-based A_{max} and R_d increased significantly with tree height (Fig. 2a–d), although the regression coefficient of mass-based values were lower than area-based values. The area-based photosynthetic rate ($A_{\text{max-area}}$) of several canopy trees such as *Shorea crassa*, *Shorea beccariana*, *Shorea kunstleri* (Dipterocarpaceae), *Lithocarpus luteus* (Fagaceae), *Artocarpus obtusus* (Moraceae), and *Pentace adenophora* (Tiliaceae) reached $15 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$. In contrast, many seedlings and saplings of canopy trees and understory trees such as *Anisophyllea disticha* (Anisophylleaceae), *Gomphandra cumingiana* (Icacinaceae), and *Pavetta axillaris* (Rubiaceae) showed a low $A_{\text{max-area}}$ ($\sim 5 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$). The leaf A_{max}/R_d ratio decreased slightly with increasing tree height, although the regression line was nearly level ($A_{\text{max}}/R_d = -0.05 \text{ height} + 10.96$, $r^2 = 0.06$, $P < 0.05$, $n = 118$; data not shown). The g_s and T_r increased linearly with tree height (Fig. 2e; $r^2 = 0.52$, $P < 0.001$; Fig. 2f; $r^2 = 0.42$, $P < 0.001$), although a relatively large variation in values were observed under canopy conditions.

LMA, N contents, and leaf $\delta^{13}\text{C}$ values in relation to tree height

The LMA increased significantly with tree height (Fig. 3a; $r^2 = 0.63$, $P < 0.001$). The N content per unit area (N_{area})

increased linearly with tree height (Fig. 3b; $r^2 = 0.71$, $P < 0.001$), although the N content per unit leaf dry mass did not change with tree height (Fig. 3c; $r^2 = 0.00$, $P > 0.05$). The leaf $\delta^{13}\text{C}$ also significantly increased with tree height (Fig. 3d; $r^2 = 0.77$, $P < 0.001$).

Photosynthetic rate, LMA, and N content in relation to the light environment

The relationship between leaf area-based photosynthetic rate ($A_{\text{max-area}}$) and RPPFD is more accurately approximated using a simple allometric function of the form $y = ax^b$ than a linear regression (Fig. 4a). LMA and N_{area} also showed similar patterns with $A_{\text{max-area}}$ (Fig. 4c, d). In contrast, the leaf mass-based photosynthetic rate did not have a significant relationship with the RPPFD (Fig. 4b).

Predawn and midday leaf water potential in relation to tree height

The predawn (Ψ_{pd}) and midday (Ψ_{mid}) leaf water potential decreased linearly with tree height (Fig. 5). The decreasing rate of Ψ_{pd} was similar to the theoretical value associated with the gravitational potential ($-0.098 \text{ Mpa}/10 \text{ m}$). In contrast, midday leaf water potential (Ψ_{mid}) showed a much greater decrease with increasing height than Ψ_{pd} (ANCOVA, $P < 0.05$).

Relationship between A_{max} and leaf characteristics

A significant correlation was found between A_{max} and LMA (Fig. 6a; $r^2 = 0.59$, $P < 0.001$), between A_{max} and g_s (Fig. 6b; $r^2 = 0.66$, $P < 0.001$), and between $A_{\text{max-area}}$ and N_{area} (Fig. 6c; $r^2 = 0.67$, $P < 0.001$). In contrast, no significant relationship was detected between $A_{\text{max-area}}$ and N_{mass} ($r^2 = 0.00$, $P > 0.05$; data not shown). $A_{\text{max-mass}}$ and N_{mass} also showed positive relationships, although regression coefficients were lower than area-based coefficients (Fig. 6d; $r^2 = 0.23$, $P < 0.001$).

Discussion

Changes in photosynthetic capacity and respiration rate with tree height

A simple linear increase in area-based photosynthetic capacity ($A_{\text{max-area}}$) with height was observed in tropical rain forest trees, whereby trees maintained their physiological activity such as photosynthesis and growth through large amounts of seasonal rainfall without cold stress. In contrast, decreases and plateaus in $A_{\text{max-area}}$ with tree height or diameter were usually observed in temperate, semiarid,

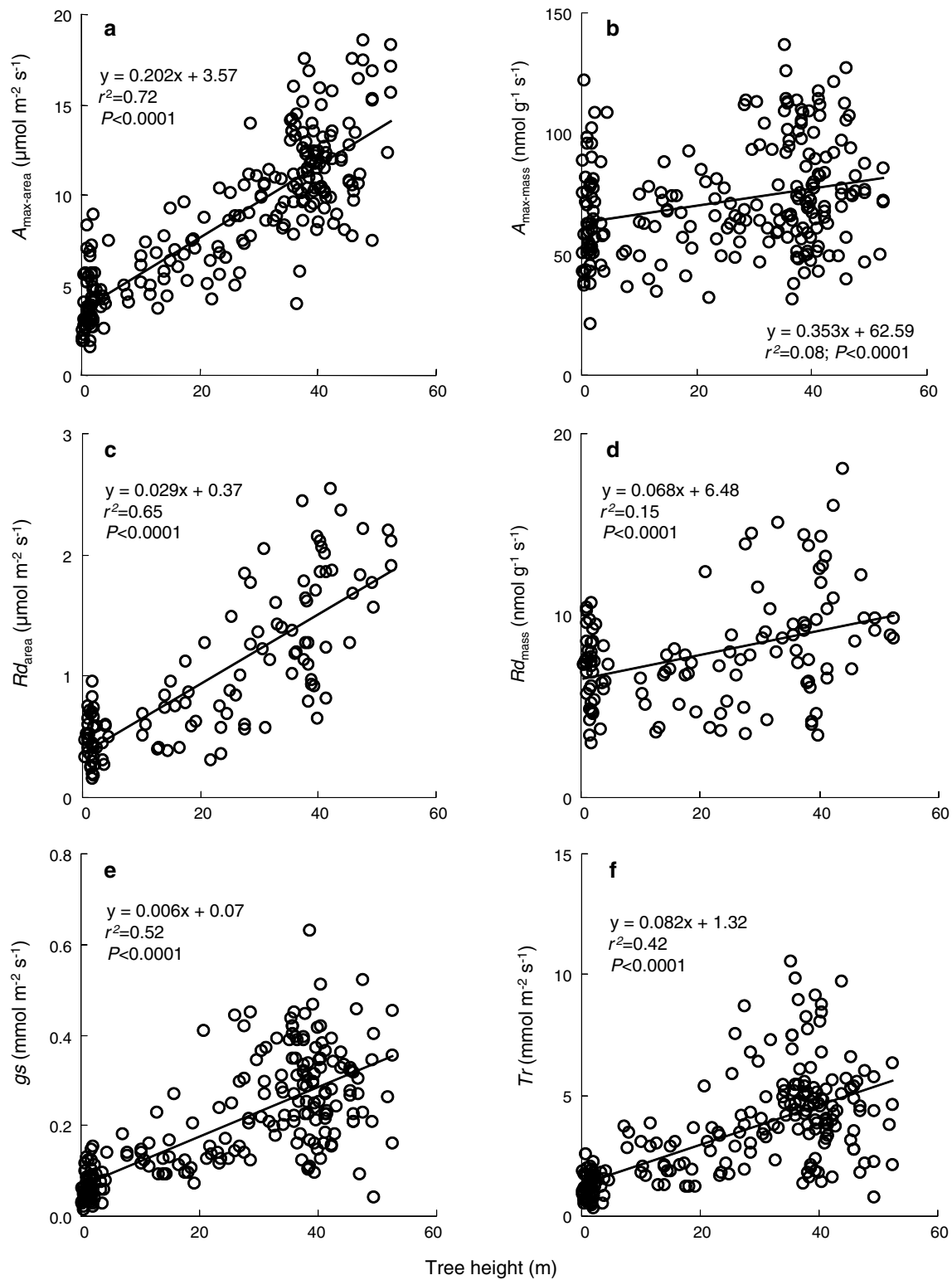


Fig. 2 Relationship between tree height and light-saturated area- and mass-based photosynthetic rates, $A_{\text{max-area}}$ (a) and $A_{\text{max-mass}}$ (b), area- and mass-based dark respiration rates, $R_{\text{d-area}}$ (c) and $R_{\text{d-mass}}$ (d), maxi-

um stomatal conductance, g_s (e), and maximum transpiration rate, T_r (f). Values represent the means for each measurement points across all replicate leaves

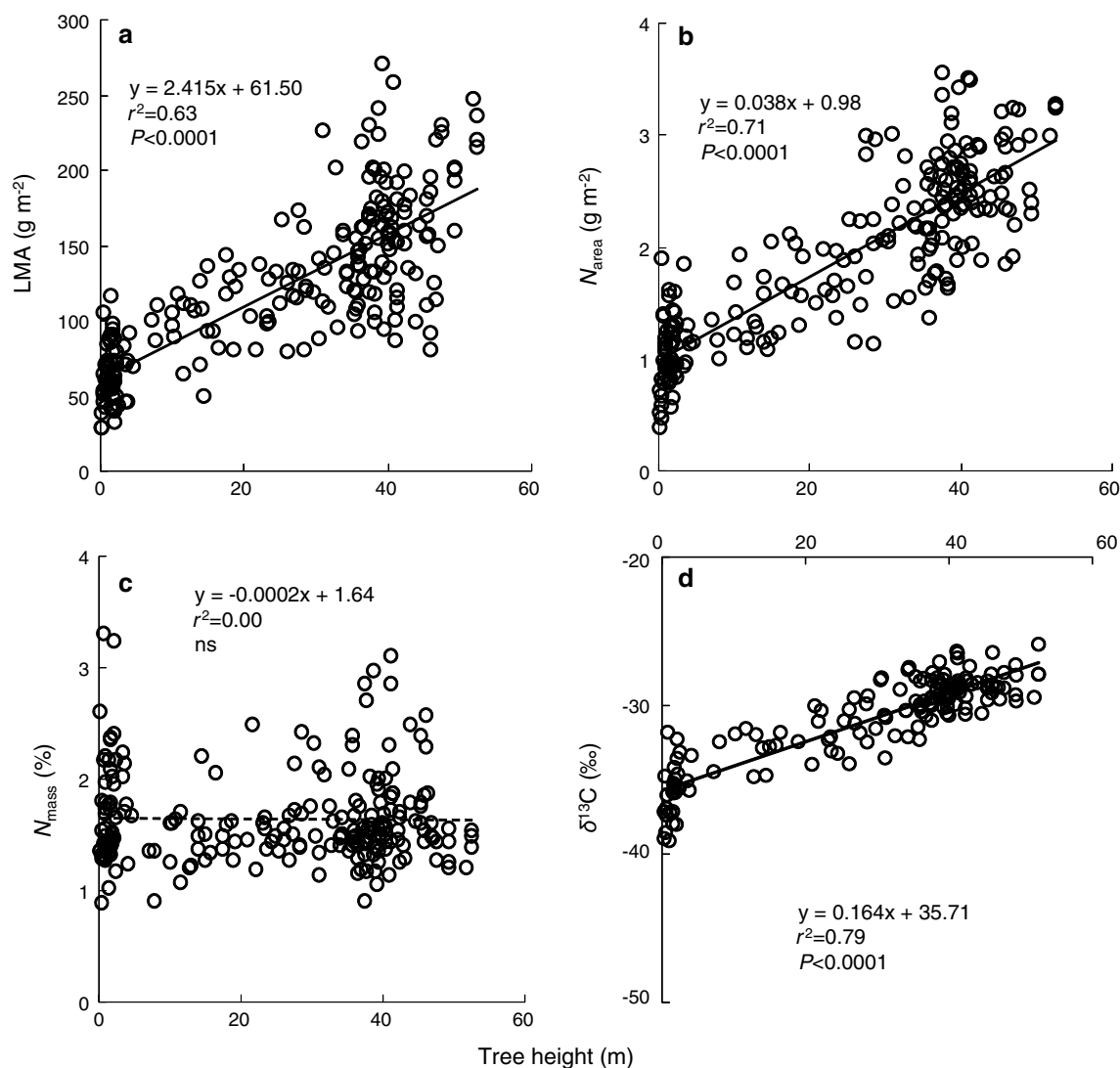


Fig. 3 Changes in leaf mass per area, LMA (**a**), leaf area-based nitrogen, N_{area} (**b**) and mass-based nitrogen, N_{mass} , content (**c**), and carbon isotope ratio $\delta^{13}\text{C}$ (**d**) with tree height. Values represent the means of each measurement point across all replicate leaves

and dry tropical forests (Fredericksen et al. 1996; Ishii et al. 2008; Nabeshima and Hiura 2008; Thomas 2010; Kenzo et al. 2012; Sendall and Reich 2013; Su et al. 2013). The majority of studies on tropical rain forests showed a significant linear increase in $A_{\text{max-area}}$ with tree height, including upper canopy trees over 40 m tall (Rijkers et al. 2000; Kenzo et al. 2006). Even the $A_{\text{max-area}}$ of saplings of canopy trees grown under gap conditions with direct sunlight (canopy openness of 18.2 ± 1.0 %) were ~ 50 % lower than canopy and emergent trees. In addition, the mass-based photosynthetic rate ($A_{\text{max-mass}}$) also increased weakly with tree height. This increase was not consistent with previous studies on tall canopy trees in other biomes, which showed a significant reduction with increasing tree height (Koch et al. 2004; Ishii et al. 2008; Steppe et al. 2011; Kenzo et al. 2012). The cause of these results remains unclear,

but relatively low soil moisture deficits due to year-round rainfall in these forests (rainfall exceeded 100 mm/month) compared with temperate and tropical dry forests (Kume et al. 2011) may increase photosynthetic capacity in the upper canopy. In fact, nearly zero predawn water potential in understory trees implies that substantial soil water exists in this forest. Modeling approaches also support the idea that trees maintaining high photosynthesis with less stomatal closure (anisohydric plants) are more successful under moist conditions in the Bornean tropical rain forest (where little risk of hydraulic failure exists) than trees that regulate transpiration through sensitive stomatal closure to maintain a constant midday leaf water potential (isohydric plants; Kumagai and Porporato 2012). On the other hand, relatively large variation of leaf midday water potential among canopy trees (tree height >27.5 m; Kenzo et al. 2007)

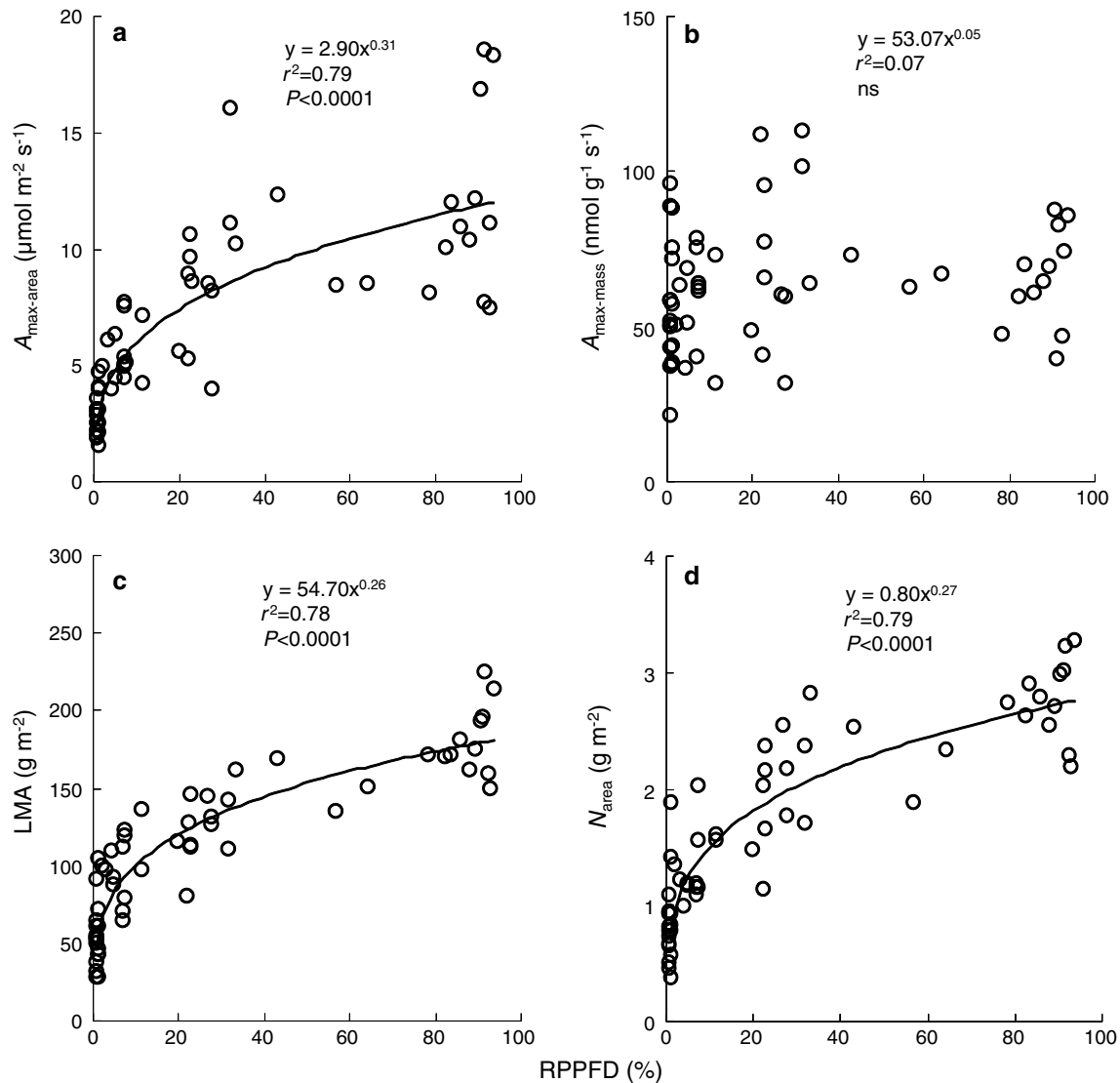


Fig. 4 Changes in leaf area- and mass-based photosynthetic rates, $A_{\text{max-area}}$ (a) and $A_{\text{max-mass}}$ (b), LMA (c), and leaf area-based nitrogen content (d) with the relative photosynthetic photon flux density

(RPPFD). Values represent the means for each measurement points across all replicate leaves

compared with subcanopy and understory trees may suggest that there is different leaf water use by different stomatal regulations such as isohydric and anisohydric behavior within the canopy species (Fisher et al. 2006; Kumagai and Porporato 2012). In fact, Hiromi et al. (2012) and Inoue et al. (2014) reported that there is a large difference on leaf midday water potential caused by different stomatal regulations between canopy trees in the same forest stand. They also suggested that leaf and xylem structure might relate to different leaf water uses. We also found that canopy trees with high LMA showed significantly lower midday water potential than those with low LMA species, though correlation coefficient was low ($P < 0.05$, $r^2 = 0.06$, $n = 81$). The higher stomatal conductance and T_r of taller trees

compared with shorter trees indicated that the water supply from deeper root systems of large trees may be adequate for a high photosynthetic capacity in the forest (Sendall and Reich 2013). Rapid recovery of leaf midday water potential to theoretical gravity potential at night in the majority of canopy trees supports the notion that large canopy trees did not suffer strong drought stress in this region, although they consume a large amount of water through their high transpiration activity (Fig. 5; Hiromi et al. 2012). However, midday photosynthetic depression occurs in canopy trees on sunny days (Ishida et al. 1996; Kenzo et al. 2003; Kitahashi et al. 2008; Kosugi et al. 2009). Although there are no data on occurrence of midday photosynthetic depression with tree height gradient, larger depression in canopy trees

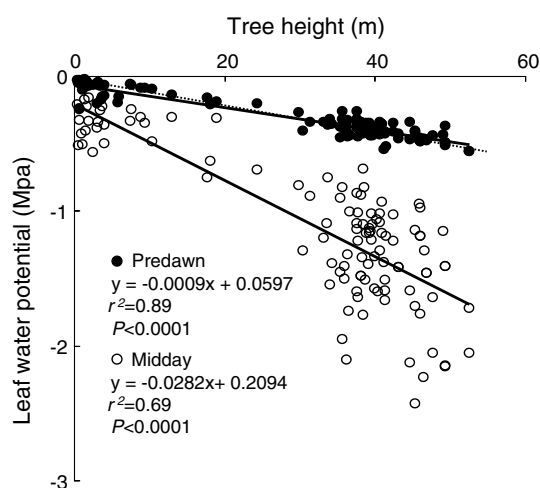


Fig. 5 Changes in leaf predawn and midday water potential with tree height. The *dotted line* represents the theoretical hydrostatic gradient of the leaf predawn potential ($-0.0098 \text{ MPa m}^{-1}$; Scholander et al. 1965). *Values* represent the means for each measurement point across all replicate leaves

may have weakened the linear relationship between tree $A_{\text{max-area}}$ and height, when we conduct the gas exchange measurement in the afternoon.

The leaf respiration rate is more strongly associated with tree height when expressed on a leaf area basis than a leaf mass basis, likely as a result of strong linear increases in LMA with tree height (Cavaleri et al. 2008). Higher leaf R_d s in the upper canopy may be the result of increased leaf physiological activities such as photosynthesis, carbohydrate export, and photoprotective mechanisms with high light intensity (Amthor 1989). In general, the photosynthetic rate is positively associated with the leaf respiration rate in plant species including tropical trees, which is consistent with the present study (Amthor 1989; Kosugi et al. 2012; van de Weg et al. 2012; Slot et al. 2013). Many tropical forest trees also showed similar increases in the respiration rate with tree height (Domingues et al. 2007; Cavaleri et al. 2008; Kosugi et al. 2012), although the rate decreased with tree size in several temperate trees (Sendall and Reich 2013). The high leaf respiration rate under canopy conditions also contributes to the high gross photosynthetic production (GPP) in tropical rain forests because total leaf respiration accounts for almost half of the total aboveground respiration in tropical rain forests of Malaysia (Yoda 1983). Our observation of simple linear increases in leaf respiration and photosynthetic capacity could be used to estimate community GPP in tropical rain forests consisting of diverse tree species (Cavaleri et al. 2008).

In recent years, there has been a debate over which leaf traits provide an informative insight into leaf functioning with emphasis on the usefulness of area- versus

mass-based expressions of leaf photosynthetic capacity (Lloyd et al. 2013; Osnas et al. 2013; Niinemets et al. 2014). In this study, area-based photosynthetic capacity showed a stronger relationship with tree height than mass-based capacity. This weak relationship of leaf mass-based photosynthesis may have been caused by much compressed leaf traits within the forest profile compared with worldwide variations in leaf traits (Niinemets et al. 2014). On the other hand, several authors suggested that appropriateness and utility of mass-based expressions when considering within-canopy light-acclimation and carbon economy in individual leaves and whole plants (Westoby et al. 2013; Niinemets et al. 2014; Poorter et al. 2014). Niinemets et al. (2014) also argued that studies attempting to resolve the control on small-scale variations, such as within-canopy variations in area-based photosynthetic capacity, should analyze variations in structural, chemical and allocational traits collectively. Further studies of these comprehensive leaf traits with tree height will provide a better understanding of the photosynthetic response of tropical rainforest trees to height gradients.

Leaf photosynthetic capacity in relation to LMA and N content

LMA is a key leaf morphological variable since variations in the area-based photosynthetic rate ($A_{\text{max-area}}$) and N content could be traced back to variations in LMA with tree height (Rijkers et al. 2000). $A_{\text{max-area}}$ is known to be strongly affected by various leaf characteristics, such as LMA (Niinemets 2002), leaf mesophyll structure [surface area of mesophyll cells per unit leaf area (A^{mes}/A_a); Nobel 1975; Kenzo et al. 2004], leaf vein structure (Sack and Holbrook 2006; Brodribb et al. 2007), and N content (Niinemets 2002; Niinemets et al. 2014). Differences in $A_{\text{max-area}}$ with height in this study are positively correlated with the LMA and area-based N content (Fig. 6). In general, height-dependent decreases in both mass- and area-based leaf N content reduce the photosynthetic capacity in temperate and tropical dry forest trees, even when the LMA is increased under tall canopy conditions (Niinemets 2002; Kenzo et al. 2012). However, our results clearly demonstrate that mass-based N content is stable with tree height and results in a high leaf area-based N content by increasing the LMA with tree height. Although mass-based N content was stable with tree height, photosynthetic N use efficiency (PNUE) increased slightly with tree height (data not shown; $\text{PNUE} = 0.02 \times H \text{ (m)} + 3.96$, $r^2 = 0.08$, $P < 0.001$). This increase may be the result of high allocation of leaf N to photosynthetic enzymes, such as Rubisco, rather than light capture components such as chlorophyll–protein complexes under canopy conditions (Hikosaka and Terashima 1995; Koike et al. 2001). In fact,

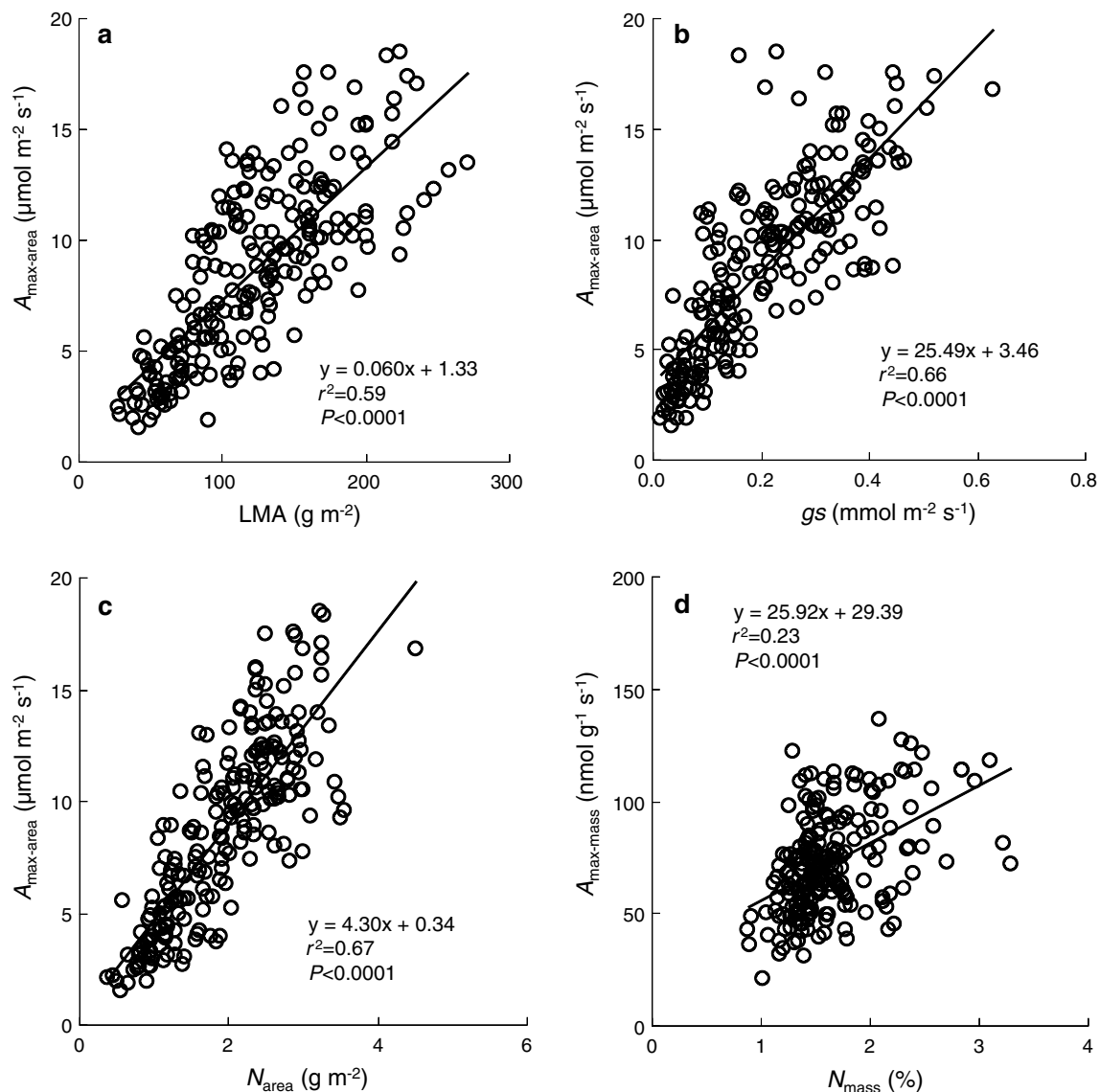


Fig. 6 Leaf area- and mass-based photosynthetic rate relationship with **a**, **b** LMA, **c** stomatal conductance, and **d** nitrogen contents. Values represent the means for each measurement point across all replicate leaves

the chlorophyll/N ratio decreased with tree height in several canopy species in the study site (Kenzo et al. 2006). A larger LMA usually facilitates the construction of leaf photosynthetic apparatus such as a thicker mesophyll structure with developed layers of palisade parenchyma and increased surface area of mesophyll cells per unit leaf area (Terashima et al. 2001; Kenzo et al. 2006). The developed mesophyll structure facilitates CO_2 diffusion in the leaves and may enhance the photosynthetic capacity in canopy leaves with larger LMA (Terashima et al. 2001; Kenzo et al. 2004). In fact, leaves in canopy trees showed highly developed mesophyll structures with high LMA and high photosynthetic capacity in Bornean tropical rain forests (Kenzo et al. 2006).

Leaf hydrostatic conditions and environmental adaptation with height gradient

The large transpiration demand associated with high photosynthetic rates due to high stomatal conductance in canopy leaves decreases the leaf water potential at mid-day, and induces drought stress (low water potential) in tall trees at midday. More than a twofold increase in the T_r was observed in canopy leaves compared with understory trees (Fig. 2), indicative of a large water transport demand from soil to leaf based on the large difference in water potential along with tree height. The theoretical hydrostatic gradient of the leaf predawn potential ($-0.0098 \text{ MPa m}^{-1}$; Scholander et al. 1965) confirmed that strong drought stress was

not usual in studied tropical rain forest trees, even under upper canopy conditions. However, the midday leaf water potential in all trees decreased significantly; e.g., canopy trees (height >40 m) varied from -1.0 to -2.0 MPa (Fig. 5). Moreover, the degree of the reduction was steeper in tall upper canopy trees with high T_r s compared with lower understory trees, which indicated that canopy trees and trees grown in other tropical and temperate regions may suffer drought stress at midday; e.g., the variation in midday potential was in the range of previous studies with similar tree heights (-1.0 to -2.5 MPa; Myers et al. 1987; Zotz and Winter 1994; Koch et al. 2004; Woodruff et al. 2004; Burgess and Dawson 2007).

The high LMA under canopy conditions may allow a decreased midday leaf water potential with tree height because the large LMA may increase tolerance to collapse caused by a low water potential (Niinemets 2002; Thomas and Winner 2002; Koch et al. 2004; Woodruff et al. 2004). Wind exposure and physical abrasion likely increase with tree height, favoring more robust leaves with a higher LMA in the upper canopy (Sendall and Reich 2013). In contrast, the saturated relationship between LMA and relative PPFD in our study revealed that light intensity did not linearly affect LMA, $A_{\max\text{-area}}$, or N_{area} . Cavaleri et al. (2010) also reported that the tree height gradient has a stronger effect on changes in LMA than light intensity in various tree species in an Amazonian tropical rain forest. The tallest conifer tree species also showed a linear increase in LMA with tree height, and a nonlinear relationship between LMA and light intensity was observed (Woodruff et al. 2004; Ishii et al. 2008). These observations indicate that leaf drought and physical stress with tree height are more important for leaf morphological and physiological functions through changes in LMA than light intensity under tropical rain forest conditions (Steppe et al. 2011). In contrast to upper canopy conditions, lower LMA under lower canopy conditions contributes to high shade tolerance through reduction in the leaf respiration rate (Kosugi et al. 2012; Slot et al. 2013). In addition, Osada et al. (2001) found that reduced LMA with lower tree height also related to longer leaf longevity and contributed to a higher shade tolerance by reducing the annual cost of replacing senescent leaves in a Malaysian tropical rainforest.

A linear increase in $\delta^{13}\text{C}$ with tree height is advantageous to height-induced drought stress tolerance because the increase usually reflects long-term leaf photosynthetic water use efficiency (WUE) improvement with tree height (Goldstein et al. 1996; Hanba et al. 1997; Domingues et al. 2005). The combination of high light intensity and large photosynthetic rate with tree height results in a low leaf internal CO_2 concentration (C_i) and higher $\delta^{13}\text{C}$ and WUE (Hanba et al. 1997). However, the index of $\delta^{13}\text{C}$ for long-term WUE may include errors due to variations in the leaf internal CO_2 concentration (C_i) from different light

conditions (Medina and Minchin 1980; Ehleringer et al. 1986; Kumagai et al. 2001). Variations in leaf $\delta^{13}\text{C}$ (-38 to -25 ‰) in this forest's trees showed similar values with many tropical rain forest tree species (Medina and Minchin 1980; Goldstein et al. 1996; Domingues et al. 2005; Holttum and Winter 2005), although these values were significantly lower than arid and semiarid tree species, even in canopy leaves (Ehleringer and Cooper 1988; Klein et al. 2005; Tanaka-Oda et al. 2010a, b). The relatively low leaf $\delta^{13}\text{C}$ in this study forest may indicate that stomatal limitation or sensitivity to drought stress (i.e. low leaf water potential) is weaker than in arid land trees.

Conclusions

Our results suggest that the photosynthetic capacity ($A_{\max\text{-area}}$) is not limited by tree height in a tropical rain forest with diverse tree species. We also observed a simple and significant linear relationship with tree height of both leaf photosynthetic characteristics (e.g., A_{\max} , R_d , g_s) and leaf morphological and biochemical traits (e.g., LMA, N_{area}), which in turn affects the photosynthetic capacity. In contrast to the linear changes observed in relation to tree height, leaf morphological and physiological traits associated with photosynthesis did not correspond to the exponential increase in light intensity with increasing tree height. This suggests that leaf traits are more strongly associated with the tree height gradient (which reflects a linear reduction in the leaf water potential) than light conditions in tropical rain forest trees. In contrast, simple height-related changes on diverse tree species from the forest understory to canopy may allow accurate estimation of CO_2 flux and primary production in species-rich tropical rain forests. Our study also suggests that tropical rain forest trees adapt to increased drought stress and light intensity from understory to canopy by changing the morphological and functional properties of leaves, especially the LMA and leaf N content.

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