Rapid Light-Response Curve of Chlorophyll Fluorescence in Terrestrial Plants: Relationship to CO₂ Exchange among Five Woody and Four Fern Species Adapted to Different Light and Water Regimes

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Abstract: The rapid light response of electron transport rate ($ETR_R$), obtained from chlorophyll fluorescence parameters by short illumination periods (10–30 s) at each light level, can provide a rapid and easy measurement of photosynthetic light response in plants. However, the relationship between $ETR_R$ and the steady-state light response of CO₂ exchange rate ($A_S$) of terrestrial plants has not been studied in detail. In this study, we compared the $ETR_R$ and $A_S$ for five woody and four fern species with different light and/or water adaptations. Under well-watered conditions, a constant temperature (25 °C) and with stomatal conductance ($g_s$) not being a main limiting factor for photosynthesis, $ETR_R$ and $A_S$ were closely related, even when merging data for regression analysis for a species grown under different light conditions and measured under different light intensity and air humidity. However, when Alnus formosana was treated with low soil water and air humidity, because of the decrease in $A_S$ mainly due to stomatal closure, the $ETR_R$–$A_S$ relation was not so close. In addition, at both 100 and 2000 μmol m⁻² s⁻¹ photosynthetic photon flux density (PPFD), $ETR_R$ and $A_S$ were significantly correlated within a plant group (i.e., woody plants and ferns) regardless of the broad difference in $A_S$ due to different species or environmental factors. The results indicate that the relationship between the $ETR_R$ and $A_S$ is varied by species. We concluded that 1) $ETR_R$ could reflect the variation in $A_S$ at each irradiance level within a species under well-watered conditions and 2) $ETR_R$ at 100 μmol m⁻² s⁻¹ PPFD (as the efficiency of light capture) or 2000 μmol m⁻² s⁻¹ PPFD (as a maximum photosynthetic parameter) could be used to compare the photosynthetic capacity within a plant group, such as woody plants and ferns.

Keywords: electron transport rate; fern; photosynthetic rate; rapid light curve; stomatal conductance; tree

1. Introduction

Photosynthesis is a major determinant of biomass production and terrestrial carbon budgets [1]. Sunlight is the energy source of plant photosynthesis; however, the response of photosynthesis to light intensity varies by species and environmental conditions. Plants adapted or acclimated to high light often have a high light compensation point, light saturation point, and maximal photosynthetic rate [1–3]. Light-response curves (LC) reveal the photosynthetic properties of plants. They can be used to characterize CO₂ assimilation, photochemistry, photoacclimation, photoinhibition, and photoprotective mechanisms in different light conditions. LC are widely used to describe the physiological plasticity of plants. Thus, the LC of photosynthesis is fundamental for plant ecophysiological research [1–4].

Traditionally, the LC of photosynthesis has been measured by the rate of steady-state photosynthesis under a range of relevant light intensity. Thus, the measurement is limited by the long measurement time and cumbersome leaf gas exchange techniques, especially
in the field [5]. Recently, chlorophyll fluorescence quenching analysis has been found to be a fast, simple, non-invasive, and reliable method to assess changes in photosystem II (PSII) function under different environmental and physiological conditions [6–8]. Among chlorophyll fluorescence parameters, electron transport rate (ETR), calculated from the product of PSII efficiency and absorbed light, expresses the relative rate of electron transport through PSII [9,10]. Two ways to obtain light-response data for ETR are steady-state light curve (SLC) and rapid light curve (RLC) methods.

ETR obtained by SLC methods (ETR$_S$) is under steady-state conditions at a given strength of illumination. Because CO$_2$ fixation (As) is a major sink for electrons from PSII, when A is inhibited by environmental and/or physiological factors, leaves may downregulate their PSII efficiency, mainly by xanthophyll-dependent non-photochemical quenching to avoid damage caused by excessively absorbed energy [11–14]. Even if electrons from PSII have several energy sinks (e.g., photorespiration and the water–water cycle) [15–17], the allocation of electron flow between A and other alternative sinks remains unchanged under many conditions. Examples are C$_4$ plants (with photorespiration mostly restricted) and C$_3$ plants under conditions with approximate temperature as well as CO$_2$ and O$_2$ concentrations but varied light intensity [3,18–20]. Because both CO$_2$ fixation and photorespiration are major sinks for electrons from PSII in C$_3$ plants, the ratio of ETR to As (or PSII efficiency/photosynthetic rate per absorbed quantum) greatly increases with decreasing CO$_2$ partial pressure [21], increasing temperature [3,22], and O$_2$ partial pressure [20] because of increased photorespiration.

In contrast to ETR$_S$, ETR obtained by RLC methods (ETR$_R$) involves short illumination periods (10–30 s) at each light level, so the RLC can be measured within 1.5–2 min, but leaves do not achieve steady-state conditions during each light step [23,24]. Nevertheless, ETR$_R$ can provide reliable information about cardinal points of photosynthesis [5,25]. It can be used to investigate short-term responses to rapid changes in the light environment [4]. Aquatic photosynthetic organisms often show a parallel change in light responses of ETR$_R$ and steady-state photosynthetic rate (As); thus, ETR$_R$ is widely used to assess the photosynthetic activity and biomass productivity [26–29] and to investigate light acclimation [30–33].

For terrestrial plants, ETR$_R$ is used to study environmental acclimation [23,34–37], stress responses [35,38–41], and estimate photosynthetic efficiency [25,42]. However, in addition to irradiance, stomatal conductance ($g_s$) is another important limiting factor in the photosynthesis of terrestrial plants. To prevent water loss and facilitate CO$_2$ diffusion to mesophyll cells, guard cells may monitor the plant water status and the CO$_2$ demand from the mesophyll [1,43]. Stomatal behavior is influenced strongly by water and light conditions. In general, A and $g_s$ may decrease with decreasing light intensity [44,45], as well as soil water content [20,46] and air moisture [2,20]. In addition, the response of stomata to environmental and physiological conditions varies among species. For example, stomata of xerophytic species are more sensitive, and those of hygrophytic species are more insensitive to water deficits than are mesophytic species [47,48]. Moreover, ferns have a lower ability to respond to increases in CO$_2$ concentration and decreases to water, for lower As/$g_s$ ratio, than angiosperms [49,50]. In higher plants grown under low light and/or in dry seasons, the maximum values of As and ETR$_R$ may decrease together [35]. However, the induction of As and $g_s$ requires several minutes (e.g., [51,52]), and the time required for these inductions were varied among species with different light-adaptation capabilities [49]. However, during ETR$_R$ measurement, leaves are exposed to only 10–30 s of actinic light at each step. Thus, the effect of $g_s$ on ETR$_R$ may not be as large as on As, and the ETR$_R$–As relation may vary among species.

Studies elucidating the relation of ETR$_R$ to As or productivity of terrestrial plants are rare [35,36], as are those investigating the effect of $g_s$ on the ETR$_R$–As relation among species across a wide taxonomic range and environmental adaptation and acclimation capability. Due to the difference of light adaptation and acclimation, plants could be broadly divided into sun- and shade-tolerant plants as well as xerophytic and hygrophytic species. Plant species adapted to different light and water regimes show differential
photosynthetic characteristics. To obtain a simple, fast, non-invasive, and reliable method to assess photosynthesis under different environmental and physiological conditions [49], we compared the $\text{ETR}_R$ and $A_S$ for five woody and four fern species with different light and/or water adaptations. In this study, we examined four fern species, three broad-leaved tree species, and two broad-leaved understory shrubs with different light and/or water adaptation capabilities to investigate these aspects.

2. Results

Figure 1 shows the LCs of $A_S$, $g_s$, $\text{ETR}_R$, and intercellular and atmospheric CO$_2$ concentration ($C_i/C_a$) for three tree species measured at 80% and 40% relative humidity (RH). $A_S$ and $g_s$ for four ferns and two understory shrubs, measured under well-watered conditions and 75% RH, were described previously [3]. Thus, only the LCs of $\text{ETR}_R$ and $C_i/C_a$ for $\text{Pyrrhoa lingus}$, $\text{Asplenium antiquum}$, and $\text{Diplazium donianum}$, measured at 80% and 40% RH, were selected, as shown in Figure 2a–f. To compare the $A_S$ and $g_s$, these two variables for three ferns measured at 2000 µmol m$^{-2}$ s$^{-1}$ photosynthetic photon flux density (PPFD) are also shown in Figure 2g–i. In addition, the relation between $A_S$ and $\text{ETR}_R$ for all tested species under different PPFD, RH, and soil water conditions is shown in Figure 3. Generally, $A_S$, $g_s$, and $\text{ETR}_R$ for all tested species showed a hyperbolic increase with increasing PPFD. However, these LCs varied by species and environmental conditions during cultivation and measurement. Under well-watered conditions, a pioneer tree, $\text{Alnus formosana}$, had the highest light saturation point and maximal value of photosynthesis, followed by a hemiepiphytic tree, $\text{Ficus microcarpa}$, and a hygrophytic tree, $\text{Salix warburgii}$, then by two understory shrubs, $\text{Ardisia crenata}$ and $\text{Ardisia cornudentata}$ (Figures 1–3 and [3]). In addition, for two understory shrubs, 50% sunlight-grown plants showed a higher maximal value of photosynthesis than 10% sunlight-grown plants (Figure 3e–f). Ferns adapted or acclimated to high light always had a higher light saturation point and maximal photosynthetic rate [3]. Only three trees grown under 100% sunlight and three ferns grown under 50% sunlight were measured under both high and low RH. Under well-watered conditions, the $A_S$ for $\text{A. formosana}$ was inhibited only slightly by 40% RH but not for $\text{S. warburgii}$ and $\text{F. microcarpa}$; even the $g_s$ for these two species was largely inhibited. Both $A_S$ and $g_s$ were not affected or were decreased slightly under 50% RH for three ferns. Both $A_S$ and $g_s$ were inhibited for $\text{A. formosana}$ treated with both low soil water content and air moisture. Thus, findings for $A_S$ and $g_s$ were similar (Figure 1a,d).

In contrast to $A_S$, which for most plants was saturated at 800–1200 µmol m$^{-2}$ s$^{-1}$ PPFD, $\text{ETR}_R$ for high-light- and slight-shade-adapted species did not reach saturation until 2000 µmol m$^{-2}$ s$^{-1}$ PPFD (Figures 1 and 2). Nevertheless, when merging data from the same species measured under different light and moisture conditions, the $A_S$ for three trees (high-light-adapted) and $\text{P. lingus}$, a slight-shade-adapted fern, showed a hyperbolic relation with $\text{ETR}_R$: the $A_S$–$\text{ETR}_R$ relation could be best fitted by the equation $Y = aX/(b + X)$ ($Y = A_S$, $X = \text{PPFD}$, $r^2 = 0.943–0.985$, $p < 0.001$, Figure 3a,g–i). This relation for the other medium- to heavy-shade-adapted ferns and two understory shrubs was linear ($r^2 = 0.677–0.948$, $p < 0.001$). The $A_S$ of $\text{A. formosana}$ was inhibited largely by low soil water content and low RH, but its $\text{ETR}_R$ was not as inhibited as $A_S$; thus, the $A_S$–$\text{ETR}_R$ relation was not as close as for the other tested species. At both 100 and 2000 µmol m$^{-2}$ s$^{-1}$ PPFD, the leaves with high $A_S$ always had high $\text{ETR}_R$, regardless of species or environmental factors. However, the slope of the $A_S$–$\text{ETR}_R$ regression line was higher for woody plants than ferns (Figure 4).
Figure 1. Light-response curves of $A_s$ (a–c), $g_s$ (d–f), $ETR_R$ (g–i), and $C_i/C_a$ (j–l) for three tree species measured at 25 °C and 80% (open symbols) and 40% (closed symbols) relative humidity. $A_s$ and $g_s$ indicate the net photosynthetic rate and stomatal conductance, respectively, obtained from steady-state light response; $ETR_R$ indicates electron transport rate obtained from rapid light response; $C_i$ and $C_a$ indicate intercellular and atmospheric CO$_2$ concentration, respectively, obtained from steady-state light response. Squares, diamonds, and stars $g_s$ indicate measured under well-watered conditions, mild and severe drought, respectively. Data are mean ± SE.
Figure 2. Light-response curves for \( ETR_R \) and \( C_i/C_a \) (a–f) as well as \( A_S \) and \( g_s \) measured at 2000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) photosynthetic photon flux (PPFD; g–l) for three fern species at 25 °C and 75% (open symbols, data from [3]) and 50% (closed symbols) relative humidity. \( A_S \) and \( g_s \) indicate the net photosynthetic rate and stomatal conductance, respectively, from steady-state light response; \( ETR_R \) indicates electron transport rate from rapid light response; \( C_i \) and \( C_a \) indicate intercellular and atmospheric CO\(_2\) concentration, respectively. Squares, circles, and triangles indicate cultivated under 100%, 50%, and 10% sunlight, respectively. Data are mean ± SE.

Figure 3. Relationship between net photosynthetic rate from a steady-state light response (\( A_S \)) and electron transport rates from a rapid light response (\( ETR_R \)) for four ferns (a–d), two understory shrubs (e–f), and three tree (g–i) species. Squares, circles, and triangles indicate cultivated under 100%, 50%, and 10% sunlight, respectively, and measured under well-watered conditions; diamonds and stars (g) indicate cultivated under 100% sunlight and measured under mild and severe drought conditions, respectively. Open and closed symbols indicate measured under 75% and 50% relative humidity, respectively, for ferns and understory shrubs, and 80% and 40%, respectively, for trees. \( A_S \) of ferns and understory shrubs measured at 75% relative humidity were from [3]. The regression line in g was fitted for well-watered conditions only. *** is significant at \( p < 0.001 \).
Figure 4. Relationship between net photosynthetic rate from steady-state light response ($A_S$) and electron transport rates from a rapid light response ($ETR_R$) for all tested materials at 100 (a,b) and 2000 (c,d) µmol m$^{-2}$ s$^{-1}$ PPFD. □ is Alnus formosana; ∆ is Salix Warburgii; ▽ is Ficus microcarpa; ▲ is Ardisia crenata; ▼ is Ardisia cornuidentata; □ is Pyrosia lingus; ⊙ is Asplenium antiquum; • is Diplazium donianum; ● is Archangiopteris sonai. $A_S$ for ferns and understory shrubs measured at 75% relative humidity were from [3]. *** is significant at $p < 0.001$.

The $C_i/C_a$ for all measurements decreased with increasing PPFD and stabilized somewhat at about 800 µmol m$^{-2}$ s$^{-1}$ PPFD with most treatments (Figures 1j–l and 2d–f). At 2000 µmol m$^{-2}$ s$^{-1}$ PPFD, the $A_S$–$C_i/C_a$ relation could be divided into four groups: (1) four ferns, (2) two understory shrubs, (3) Ficus microcarpa and Salix Warburgii, and (4) Alnus formosana (Figure 5). The $A_S$ for ferns was decreased, and that for A. formosana was increased with increasing $C_i/C_a$. However, the $A_S$ for F. microcarpa and S. warburgii was not affected by $C_i/C_a$. As well, although the $A_S$ for two understory shrubs was inhibited by 10% sunlight during growth, their $C_i/C_a$ was not greatly affected.
Figure 5. The relationship between net photosynthetic rate ($A_s$) and the ratio of intercellular CO$_2$ concentration ($C_i$) to atmospheric CO$_2$ concentration ($C_a$) for woody plants (a), and ferns (b). All data were obtained from the steady-state light response at 2000 µmol m$^{-2}$ s$^{-1}$ PPFD. □ is Alnus formosana; △ is Salix Warburgii; ◇ is Ficus microcarpa; ▲ is Ardisia crenata; ▼ is Ardisia cornudentata; ○ is Pyrosia linguis; ⊕ is Asplenium antiquum; • is Diplazium donianum; N is Archangiopteris somai. $A_s$ for ferns and understory shrubs measured at 75% relative humidity were from [3].

3. Discussion

The $ETR_R$–$A_s$ relation of terrestrial plants has not been studied in detail, especially among species across a wide taxonomic range and environmental adaptation capability. In this study, we compared the $A_s$ and $ETR_R$ for five woody and four fern species with different light and/or water adaptations. The obtained data showed a broad range of $A_s$ because of the specific differences of species and the environmental conditions under which materials were cultivated and measured. Under the steady-state, plants adapted or acclimated to high light always had high values of both light saturation point and maximal photosynthetic rate (Figures 1 and 2 and [3]), which agreed with previous results (e.g., [1,2]).

$ETR$ is calculated as the product of PSII efficiency and absorbed light. Many studies used the empirical mean of $\alpha$ (0.84) to calculate $ETR$ and compare differences in $ETR$ among species [5] and under different growth irradiances [35]. However, the $\alpha$ value may vary by leaf pigment content and anatomical structures. Previously, we examined leaves with a broad range of chlorophyll content (0.18–0.55 g m$^{-2}$) and found a similar association of $A_s$ and $ETR$ regardless of the use of $\alpha = 0.84$ or 0.80–0.89 (from an empirical regression equation between $\alpha$ and chlorophyll content) to calculate $ETR$ (Weng et al. unpublished data). In addition, our plants featured no specific anatomical structures. So we chose the empirical mean $\alpha$ of 0.84 [5].

Measurement of SLC requires light steps long enough to allow for stabilization of the photosynthetic processes under each irradiance level. RLC only requires 10 to 30 s at each light level; nevertheless, the difference in $A_s$ between high- and low-light-grown materials can be defined by $ETR_R$ [24,31,35]. However, in addition to a long-term photoacclimation status, $ETR_R$ also depends on the short-term (min) light history of photosynthetic organisms immediately before measurement as well as illumination time for each light level during measurement. Maximum $ETR_R$ value is very low after long-term dark adaptation, but when organisms are exposed to light immediately before RLC measurement, maximum $ETR_R$ increases with increasing illumination time and to a stable level within 8 to 15 min of illumination [23]. In addition, maximum $ETR_R$ increases with increasing light intensity immediately before measurement [30] but may decrease under high light (2000 µmol m$^{-2}$ s$^{-1}$ PPFD) pre-irradiance [23]. During measurement, maximum $ETR_R$ increases with increasing illumination time at each light level [24,53].
Our results indicate that the $ETR_R - A_S$ relationship varies by species. The increase in LC in the light-limiting region, as well as light-saturation and maximum photosynthetic variables, have been used for research into plant ecophysiology [24,30,31,35]. Because the $ETR_R$ for some species did not reach saturation until 2000 μmol m$^{-2}$ s$^{-1}$ PPFD, we could not compare the light-saturation variable among species. Here, we used only data obtained at 100 μmol m$^{-2}$ s$^{-1}$ PPFD for the efficiency of light capture and data obtained at 2000 μmol m$^{-2}$ s$^{-1}$ PPFD as a maximum photosynthetic variable to elucidate the interspecific relationship between $ETR_R$ and $A_S$. A significant $ETR_R - A_S$ relation could be found within a plant group (i.e., woody plants and ferns) (Figure 4). Therefore, the $ETR_R$ value obtained
at both 100 and 2000 μmol m\(^{-2}\) s\(^{-1}\) PPFD could be used to compare the photosynthetic capacity within the same plant group, regardless of a broad difference in \(A_S\) due to different species or environmental factors, with the empirical mean of \(\alpha\) (0.84) used to calculate ETR for all tested materials. Moreover, slopes were higher for woody plants than ferns for both \(AS - ETRg\) (Figure 4) and \(AS - ETR_S\) [3] on regression analysis, which might somewhat be caused by the difference of light absorptivity of leaf among species. However, even with \(\alpha\) value changed from 0.80 to 0.89 (chlorophyll content from 0.18 to 0.55 g m\(^{-2}\)), there was only a 1.1-fold difference between ETR with \(\alpha = 0.81\) and 0.89 used for calculation. However, the difference in slopes for the \(AS - ETRg\) regression between woody plants and fern was much higher than 1.1-fold (1.6- and 2.3-fold at 100 and 2000 μmol m\(^{-2}\) s\(^{-1}\) PPFD, respectively, Figure 4). Thus, we prefer to explain that tested woody species could share more electrons for CO\(_2\) fixation at a given ETR level than ferns. This finding might be due to differences in allocation portion between CO\(_2\) fixation and alternative electronic pathways [19,22], such as photorespiration [15], water–water cycle [16], and cyclic electron flow within PSI [17] as well as nitrogen [56] and sulfur [57] assimilation.

In the present study, we found that 1) \(ETRg\) could reflect the variation in \(A_S\) at each irradiance level within a species under well-watered conditions and 2) \(ETRg\) obtained at 100 μmol m\(^{-2}\) s\(^{-1}\) PPFD (as the efficiency of light capture) or 2000 μmol m\(^{-2}\) s\(^{-1}\) PPFD (as a maximum photosynthetic parameter) could be used to compare the photosynthetic capacity within a plant group, such as woody plants and ferns. Because \(ETRg\) can be measured within 1.5–2 min, it might be a useful tool for ecophysiological research. However, we investigated only five woody plants and four fern species. The number of species may not be enough to argue the taxonomic distinctions, and more comparisons might be needed. In addition, photorespiration is another major sink for electrons from PSII in C\(_4\) plants. The \(A_S / ETRg\) ratio may vary on changing the CO\(_2\) and O\(_2\) concentration as well as temperature because the allocation of electrons between CO\(_2\) fixation and photorespiration may vary.

4. Materials and Methods

4.1. Plant Materials

We examined 4 fern species with different light adaptation (ranked from high to low light adaptation: \(Pyrolisia lingus\) (Thunb.) Farw., \(Asplenium antiquum\) Makino, \(Diplazium donianum\) (Mett.) Tard. -Blot., and \(Archangiopteris somai\) Hayata), 3 broad-leaved tree species with different water adaptation (\(Alnus formosana\) (Burkill) Makino, a pioneer tree; \(Salix warburgii\) O. Seem., a hygrophyte, and \(Ficus microcarpa\) L., a hemiepiphyte) and 2 broad-leaved understory shrubs (\(Ardisia crenata\) Sims. and \(Ardisia cornudentata\) Mez.) in this study [48,49,58]. In addition, \(A. formosana\) and \(S. warburgii\) are usually distributed near the rivers or in gullies; \(P. lingus\) and \(F. microcarpa\) can survive in the dry environment; whereas \(D. donianum\), \(Arc. Somai\), and \(S. warburgii\) are sensitive to drought [3]. Four ferns (adult plants, about 30 cm tall), 2 understory shrubs (adult plants, about 60 cm tall), and \(A. formosana\) (1- to 2-year-old seeding, about 30–50 cm tall) were the same as we used previously, and collected from central Taiwan [3]. The other 2 trees were only used in the present study and were propagated from cutttings (about 30–50 cm tall). All plants were collected in March and then transplanted to pots (16-cm diameter, 12-cm depth, 1 plant per pot for the five woody species and \(As. antiquum\), and 1 rhizome with 3–4 leaves per pot for the other 3 ferns) filled with organic soil and maintained outdoors in the nursery of the Endemic Species Research Institute, Chichi Township, Nantou County, Taiwan (23°49\(^\prime\) N, 120°48\(^\prime\) E, 250 m a.s.l.). Materials were regularly watered and fertilized (half-strength Hoagland’s nutrient solution per month) and received up to 3 levels of light intensity (i.e., 100%, 50%, and 10% (beneath shade cloth)), according to the light condition of their habitat, i.e., 3 trees received 100% sunlight; 2 slight- to medium-shade ferns, \(P. lingus\) and \(As. antiquum\), received 100%, 50%, and 10% sunlight; 1 medium-to-heavy shade fern, \(D. donianum\), and 2 understory shrubs received 10% and 50% sunlight; and 1 heavy-shade fern, \(Arc. somai\) received 10% sunlight. The average elevation and temperature were about...
250 m and 20 °C. The average annual rainfall and air humidity were about 2200 mm and 80%. During the growth period of the materials (March–November), the average hourly values of daily maximum photosynthetic photon flux density (PPFD) ranged from 1296–1456 µmol m⁻² s⁻¹ (Mar.–Aug.) and 1150–770 µmol m⁻² s⁻¹ (Sept.–Nov.) (data from the Endemic Species Research Institute). Only A. formosana was treated with mild and severe drought immediately before photosynthetic measurement by withholding water, until AS values were reduced to about 70% and 30%, respectively, of the maximum (AS under well-watered conditions: 100%) [54].

4.2. Measurements

Measurements were carried out from September to November in a laboratory at the Endemic Species Research Institute. At nightfall of 1 day before the measurement, potted materials were dark-adapted overnight (room temperature about 25 °C). On the next day, fully expanded younger leaves were selected for measurements. First, the measurement of ETRR was at dawn at room temperature and involved the software of the PAM-2000 fluorometer (Walz, Effeltrich, Germany). Nine steps of active light from about 60–2300 µmol m⁻² s⁻¹ PPFD were applied at each irradiation step for 10 s [23,34,35]. The actual (F) and maximal (Fm') levels of fluorescence were measured at the end of each irradiance level. The F was determined under each PPFD level, and the Fm' was determined by applying a 0.8-s pulse of saturating flashes of approximately 6000 µmol quanta m⁻² s⁻¹. Actual PSII efficiency (ΨPSII) was calculated as (Fm' − F)/Fm', and ETRR was calculated as ΨPSII × PPFD × 0.5 × α [8]. We used the mean value of leaf absorption (α) of 0.84 for green leaves [59] (see Discussion section). ETRR at 200, 400, 800, 1200, and 2000 µmol m⁻² s⁻¹ PPFD was calculated from linear interpolation between the 2 nearest values. After the measurement of ETRR, the measured leaves were kept in the dark until the measurement of the steady-state light response of CO₂ exchange. From 09:30 h to 15:00 h, photosynthesis and stomatal conductance were measured by using a portable, open-flow gas exchange system (LI-6400, LI-COR, Lincoln, NE, USA), and an integrated fluorescence LI-6400-40 chamber head stepwise from low to high levels of PPFD (i.e., 0, 100, 200, 400, 800, 1200, and 2000 µmol m⁻² s⁻¹). The values of AS (net photosynthetic rate), gs, and intercellular CO₂ concentration/ambient CO₂ concentration (Ci/Ca) were recorded when the gas exchange was stable (about 4 min in the dark and 10–20 min under each level of illumination). Throughout the measurements, leaf temperature and CO₂ concentration were kept at 25 °C and 350–400 µmol mol⁻¹ (no control), respectively, for all materials. Relative humidity (RH) in the chamber was taken at 75% and 50% (air entering chamber controlled by passing temperature-controlled water) for ferns and understory shrubs, and 80% and 40% for trees.

4.3. Statistical Analysis

Four to 6 fully expanded younger leaves from 4 plants of each species grown in each light condition were measured. Each leaf was taken as 1 replicate for statistical analyses. The results are expressed as the mean ± standard error (SE). The light-response curve of photosynthetic rate was fitted by sigmoidal or hyperbolic equations. Data were analyzed by linear or curve–linear regression. All statistical analyses involved the use of Sigma Plot v10.0.

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