The Light Dependence of Mesophyll Conductance and Relative Limitations on Photosynthesis in Evergreen Sclerophyllous *Rhododendron* Species

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Abstract: Mesophyll conductance \(g_m\) limits CO\(_2\) diffusion from sub-stomatal internal cavities to the sites of RuBP carboxylation. However, the response of \(g_m\) to light intensity remains controversial. Furthermore, little is known about the light response of relative mesophyll conductance limitation \(l_m\) and its effect on photosynthesis. In this study, we measured chlorophyll fluorescence and gas exchange in nine evergreen sclerophyllous *Rhododendron* species. \(g_m\) was maintained stable across light intensities from 300 to 1500 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) in all these species, indicating that \(g_m\) did not respond to the change in illumination in them. With an increase in light intensity, \(l_m\) gradually increased, making \(g_m\) the major limiting factor for area-based photosynthesis \(A_N\) under saturating light. A strong negative relationship between \(l_m\) and \(A_N\) was found at 300 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) but disappeared at 1500 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\), suggesting an important role for \(l_m\) in determining \(A_N\) at sub-saturating light. Furthermore, the light-dependent increase in \(l_m\) led to a decrease in chloroplast CO\(_2\) concentration \(C_c\), inducing the gradual increase of photorespiration. A higher \(l_m\) under saturating light made \(A_N\) more limited by RuBP carboxylation. These results indicate that the light response of \(l_m\) plays significant roles in determining \(C_c\), photorespiration, and the rate-limiting step of \(A_N\).

Keywords: CO\(_2\) diffusion; chloroplast CO\(_2\) concentration; photorespiration; photosynthesis; relative limitations; *Rhododendron*

1. Introduction

In southwest China, mountain forest ecosystems are characterized by the presence of evergreen sclerophyllous angiosperms. This vegetation type includes many species of the genus *Rhododendron* (Ericaceae) that exhibit low mesophyll conductance \(g_m\) [1]. Leaf CO\(_2\) diffusion mainly includes diffusion from air to the intercellular cavity (stomatal conductance, \(g_s\)) and diffusion from the intercellular cavity to the site of RuBP carboxylation \(g_m\). Many previous studies indicated that sclerophyllous plants have relatively lower \(g_m\) values than herbaceous plants [1–5]. Such low levels of \(g_m\) in sclerophyllous plants limit \(A_N\) to a large extent when exposed to high light. Consequently, \(g_m\) is the major limiting factor for \(A_N\) under constant high light in sclerophyllous tree species, such as Mediterranean evergreens [4,6–8], the sclerophyllous genus *Banksia* [2], *Eucalyptus camaldulensis* [9,10], and sclerophyllous *Rhododendron* species [1]. Therefore, the response of \(g_m\) to the environment plays a critical role in controlling photosynthesis in sclerophyllous angiosperms.

Under natural field conditions, leaves experience rapid changes in light intensities on timescales of seconds or minutes [11,12]. \(g_s\) usually increases with increasing light in angiosperms, which raises...
the question whether \( g_m \) changes rapidly in response to the change in light intensity. Some studies observed that \( g_m \) increased with increasing irradiance in *Eucalyptus* species [13,14], *Nicotiana tabacum* [15], *Camellia* species [16], *Triticum durum* and *Arbutus × ‘Marina’* [17], and rice (*Oryza sativa*) grown under high nitrogen concentrations [18]. Furthermore, a recent study found that with increasing irradiance, \( g_m \) gradually increased in all six studied angiosperms [19]. In contrast, other studies reported that light intensity did not affect \( g_m \) in *Triticum aestivum* [20], *N. tabacum* [21], and rice grown under low nitrogen concentrations [18]. Therefore, the response of \( g_m \) to increases in light intensity in angiosperms is species dependent and can be affected by nitrogen nutritional conditions. Previous studies mainly focused on the light response of \( g_m \) in herbaceous plants. However, the response of \( g_m \) to light intensity in sclerophyllous evergreen species is not well known.

Many previous studies have analyzed the quantitative relative stomatal, mesophyll, and biochemical limitations of \( A_N \) under saturating light conditions [4,22–25]. As we know, the value of \( A_N \) under saturating light largely determines the growth rate of plants [26–28]. However, some shade-tolerant plant species and leaves in lower parts of canopies may experience moderate light. As a result, the value of \( A_N \) at moderate light can significantly affect plant growth and crop productivity [29]. Therefore, understanding the relative limitations of \( A_N \) at moderate light may have broad applications in angiosperms and crops in particular. However, light response changes in the relative limitations of \( A_N \) are poorly understood.

In addition to RuBP carboxylation, Rubisco catalyzes RuBP oxygenation under current atmospheric environmental conditions [30–32]. The photorespiratory pathway converts phosphoglycolate (2PG) to 3-phosphoglycerate (3PGA), allowing the Calvin–Benson cycle to operate in the presence of molecular oxygen [33,34]. Under low light, \( A_N \) is mainly limited by a lack of light energy, and the resulting high chloroplast CO\(_2\) concentration (\( C_c \)) restricts the rate of RuBP oxygenation (\( V_o \)). Under high light, the increased rate of RuBP carboxylation (\( V_c \)) leads to a decrease in \( C_c \) [9,21], increasing the \( V_o/V_c \) ratio and thus enhancing photorespiration [35,36]. Therefore, photorespiration usually increases with increasing light intensity in \( C_3 \) plants [16,37–40]. Meanwhile, either an increase in \( g_s \) or \( g_m \) can partially compensate for the CO\(_2\) consumption in CO\(_2\) fixation. However, it is unclear whether the light response of photorespiration is mainly determined by \( g_s \) or \( g_m \).

At saturating light, \( A_N \) can be limited by RuBP carboxylation and/or RuBP regeneration [41–43]. Once the operating \( C_c \) is lower than the chloroplast CO\(_2\) concentration (\( C_{\text{trans}} \)) at which the transition from RuBP carboxylation limitation to RuBP regeneration limitation occurs, \( A_N \) is limited by RuBP carboxylation. When \( C_c \) is higher than \( C_{\text{trans}} \), then \( A_N \) tends to be limited by RuBP regeneration. The major rate-limiting step of \( A_N \) is species dependent and can be affected by leaf nitrogen content and measurement temperature [21,41,43]. However, the effect of \( g_m \) on the rate-limiting step of \( A_N \) is poorly understood. The leaf \( g_m \) is positively correlated to leaf nitrogen content [18,38,43]. Furthermore, \( A_N \) tends to be limited by RuBP regeneration in plants grown under high nitrogen concentrations but is limited by RuBP carboxylation in plants grown under nitrogen-deficient concentrations [43]. Therefore, we hypothesize that the rate-limiting step of \( A_N \) under saturating light is largely determined by \( g_m \).

In this study, we measured light responses of gas exchange and chlorophyll fluorescence in nine evergreen sclerophyllous *Rhododendron* species. The aims of this study were (1) to investigate the light response changes in \( g_m \) and the relative limitations of \( A_N \); (2) to assess whether the increase in \( V_o/V_c \) ratio under high light is mainly determined by \( g_s \) or \( g_m \); and (3) to examine the effects of \( g_m \) and \( l_m \) on the rate-limiting step of \( A_N \).

2. Results

2.1. Light Intensity Dependence of Photosynthesis and Mesophyll Conductance

The \( A_{800}/A_{1500} \) ratios ranged from 0.86 to 1.02, suggesting that \( A_N \) were saturated or almost saturated at 800 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\). Under a high light of 1500 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\), \( A_N \) were saturated in all *Rhododendron* species. The light-saturated \( A_N \) ranged from 10.3 (R. *cilicalyx*) to
17.8 μmol CO₂ m⁻² s⁻¹ (R. glanduliferum), a total variation of 60% between species (Figure 1). Under such saturating light, the PSII electron transport rate (J_PSII) ranged from 151.9 (R. decorum subsp. diaprepes) to 205.2 μmol electrons m⁻² s⁻¹ (R. delavayi), leading to a variation of 35% in J_PSII (Figure 1).

![Figure 1. Responses of the leaf CO₂ assimilation rate (A_N) and electron transport rate (J_PSII) to incident photosynthetic photon flux density (PPFD) in nine Rhododendron species. Symbols represent means ± SE (n = 4–5).](image)

We calculated g_m under different light intensities according to the method of [44]. The value of g_m at 300 μmol photons m⁻² s⁻¹ varied between species, ranging from 0.040 (R. ciliicalyx) to 0.20 mol m⁻² s⁻¹ (R. decorum subsp. diaprepes) (Figure 2). At 1500 μmol photons m⁻² s⁻¹, g_m ranged from 0.049 (R. ciliicalyx) to 0.16 mol m⁻² s⁻¹ (R. decorum subsp. diaprepes) (Figure 2). All species showed no significant change in g_m between 300 and 1500 μmol photons m⁻² s⁻¹ and g_m was maintained stable over the light intensity change (Figure 2). The average light response values of g_m ranged from 0.048 (R. ciliicalyx) to 0.18 mol m⁻² s⁻¹ (R. decorum subsp. diaprepes) (Figure 2).

### 2.2. Light Intensity Dependence of Relative Limitations of Photosynthesis

The relative limitations of photosynthesis were significantly affected by the light intensity. Under low light, the rate of photosynthesis was largely limited by biochemical capacity (l_b) (Figure 3), owing to the lack of ATP and NADPH. With an increase in light intensity, l_b gradually decreased (Figure 3). Meanwhile, mesophyll conductance limitation (l_m) gradually increased and stomatal conductance limitation (l_s) changed slightly (Figure 3). At a moderate light intensity of 500 μmol photons m⁻² s⁻¹, the major limiting factor for photosynthesis shifted from l_b to l_m, except for the species with the highest g_m: R. decorum subsp. diaprepes (Figure 3). Under the saturating light of 1500 μmol photons m⁻² s⁻¹, l_m ranged from 0.37 to 0.71, l_b from 0.15 to 0.37, and l_s from 0.12 to 0.26 (Figure 3). Therefore, g_m is the major limiting factor for CO₂ assimilation at saturating light in Rhododendron species, followed by biochemical capacity and stomatal conductance.
The relationships between $g_m$, $l_m$, and $A_N$ at sub-saturating and saturating light intensities were also analyzed in these studied species (Figure 4). As expected, the values of $g_m$ were significantly positively correlated to $A_N$. Interestingly, a closer relationship between $g_m$ and $A_N$ was found at 300 µmol photons m$^{-2}$ s$^{-1}$ than that at 1500 µmol photons m$^{-2}$ s$^{-1}$ (Figure 4A). Furthermore, a close negative correlation was found between $l_m$ and $A_N$ at 300 µmol photons m$^{-2}$ s$^{-1}$ (Figure 4B). However, this significant relationship disappeared at 1500 µmol photons m$^{-2}$ s$^{-1}$ (Figure 4B). These results
indicate that $g_m$ and $l_m$ play more important roles in determining $A_N$ at sub-saturating light than at saturating light.

Figure 4. The relationship between $g_m$ and $A_N$ (A), $l_m$ and $A_N$ (B) at 300 and 1500 µmol photons m$^{-2}$ s$^{-1}$ in nine Rhododendron species. Symbols represent means ± SE ($n = 4$–5).

2.3. Light Intensity Dependence of Chloroplast CO$_2$ Concentration and Photorespiration

With an increase in light intensity, intercellular CO$_2$ concentration ($C_i$) and chloroplast CO$_2$ concentration ($C_c$) gradually decreased in all Rhododendron species (Figure 5A,B). At 1500 µmol photons m$^{-2}$ s$^{-1}$, $C_i$ ranged from 253 (R. decorum subsp. diaprepes) to 317 µmol mol$^{-1}$ (R. glanduliferum) (Figure 5A), and $C_c$ ranged from 76 (R. hancockii) to 144 µmol mol$^{-1}$ (R. decorum subsp. diaprepes) (Figure 5B). Further analysis found that the drop in $C_c$ was tightly correlated with an increase in $l_m$ (Figure 5C). Therefore, the light dependence change in $C_c$ was mainly caused by an increase in $l_m$. $C_c$ is known to be a key factor affecting the affinity of Rubisco to CO$_2$ and O$_2$, and thus determines the value of $V_o/V_c$. With an increase in illumination, the $V_o/V_c$ ratio gradually increased (Figure 6A). Furthermore, the light dependence of $V_o/V_c$ was positively correlated to $l_m$ (Figure 6B). These results indicate that the increased $V_o/V_c$ with increasing irradiance was mainly caused by the enhanced $l_m$. 
Figure 5. (A,B) Responses of intercellular and chloroplast CO$_2$ concentrations ($C_i$ and $C_c$, respectively) to incident light intensity in nine *Rhododendron* species. (C) Relationship between $I_m$ and $C_c$ across light response curves in these nine *Rhododendron* species. Symbols represent means ± SE ($n=4$–5). Species abbreviations: DEC, *R. decorum*; GLA, *R. glanduliferum*; HAN, *R. hancockii*; DED, *R. decorum* subsp. *disprepes*; CIL, *R. ciliicalyx*; DEL, *R. delavayi*; DAV, *R. davidii*; FOR, *R. fortunei*; PAC, *R. pachypodum*.

At 1500 µmol photons m$^{-2}$ s$^{-1}$, $C_{\text{trans}}$ was calculated to analyze the rate-limiting step of $A_N$. The operating $C_c$ values are significantly lower than the $C_{\text{trans}}$ values in these studied species, except for *R. decorum* subsp. *disprepes* (Figure 7). Therefore, the saturating $A_N$ at current atmospheric CO$_2$ concentration was mainly limited by RuBP carboxylation in these *Rhododendron* species. In *R. decorum* subsp. *disprepes*, $A_N$ was limited by RuBP carboxylation and regeneration.
Figure 6. (A) Light intensity dependence of the $V_o/V_c$ ratio in nine Rhododendron species. (B) Relationship between $l_m$ and $V_o/V_c$ across light response curves in these nine Rhododendron species. Symbols represent means ± SE ($n = 4–5$). Species abbreviations: DEC, R. decorum; GLA, R. glanduliferum; HAN, R. hancockii; DED, R. decorum subsp. disprepes; CIL, R. ciliicalyx; DEL, R. delavayi; DAV, R. davidii; FOR, R. fortunei; PAC, R. pachypodum.

Figure 7. Comparison of $C_c$ and $C_{trans}$ at 1500 μmol photons m$^{-2}$ s$^{-1}$ in nine Rhododendron species. Symbols represent means ± SE ($n = 4–5$). Significant differences at a 5% confidence level are indicated with an asterisk. Species abbreviations: DEC, R. decorum; GLA, R. glanduliferum; HAN, R. hancockii; DED, R. decorum subsp. disprepes; CIL, R. ciliicalyx; DEL, R. delavayi; DAV, R. davidii; FOR, R. fortunei; PAC, R. pachypodum.
3. Discussion

3.1. Rapid Response of $g_m$ to Changes in Light Intensity is Consistent among Rhododendron Species

With an increase in light intensity, stomatal conductance gradually increased to enhance the CO$_2$ diffusion from air to sub-stomatal internal cavities and thus to favor photosynthetic CO$_2$ assimilation [45–47]. Subsequently, mesophyll conductance limits the diffusion of CO$_2$ from intercellular cavities to the sites of carboxylation, and may respond rapidly to changes in light intensity. Some previous studies observed that $g_m$ rapidly increased with increasing irradiance in *N. tabacum* [15], *Triticum durum*, and *Arbutus × ‘Marina’* [17], and three *Eucalyptus* species *E. globules*, *E. saligna*, and *E. sieberi* [13,14]. A recent study reported that $g_m$ rapidly responded to changes in light intensity in six studied angiosperms [19]. By contrast, some authors found that $g_m$ was not responsive to irradiance in *Triticum aestivum* [20] and *N. tabacum* [21]. Therefore, the response of $g_m$ to light intensity highly differs between species, presenting an important photosynthetic response to environmental change. However, the light response of $g_m$ in evergreen sclerophyllous angiosperms is poorly understood.

This article investigated the rapid response of $g_m$ to light intensity in nine sclerophyllous *Rhododendron* species. We found that the values of $g_m$ did not change between 300 and 1500 µmol photons m$^{-2}$ s$^{-1}$ in either of these species (Figure 2). Therefore, these nine *Rhododendron* species showed the same response model of $g_m$ to changes in light intensity. Consistently, the three *Eucalyptus* species *E. globules*, *E. saligna*, and *E. sieberi* also showed the same trend in the light response of $g_m$ [13,14]. Therefore, we propose that the rapid response of $g_m$ to the change in light intensity may be a conservative photosynthetic trait in a given genus. This conclusion is applicable only under normal growth conditions because water and/or nutrition stresses can alter the rapid response of $g_m$ to irradiance.

3.2. Light Response Changes in Relative Limitations of Photosynthesis

Many studies have documented that plant growth and crop productivity are largely linked to $A_N$ under high light [26–28]. However, global rice productivity in agricultural fields was not determined by the maximum $A_N$ under saturating light but was linked to the $A_N$ under low light [29]. Therefore, in order to optimize the cultivation strategy, we should take into consideration the relative limitations of photosynthesis under different light intensities. While the relative limitations of $A_N$ under high light are widely studied, information on the light response of relative limitations is very limited. In this article, we analyzed the relative limitations of $A_N$ under different light intensities in nine *Rhododendron* species. The stomatal conductance limitation was the most insignificant factor for $A_N$ in these species, irrespective of light intensity (Figure 3). Under low light, the light-dependent production of ATP and NADPH was restricted by limiting light energy, and $A_N$ was mainly limited by biochemical factors (Figure 3). With an increase in light intensity, ETRII rapidly increased (Figure 1), leading to the increased production rates of ATP and NADPH. In contrast, the value of $C_c$ gradually decreased (Figure 5B). Therefore, the change in $C_c$ was out-of-step with the change in energy supply, generating an imbalance between CO$_2$ supply and production of ATP and NADPH. When exposed to a moderate light of 300 µmol photons m$^{-2}$ s$^{-1}$, the most limiting factor for $A_N$ shifted from biochemical capacity to $g_m$ (Figure 3). Therefore, $g_m$ was the major limiting factor for $A_N$ in these species when exposed to high light. Furthermore, the values of $A_N$ at 300 µmol photons m$^{-2}$ s$^{-1}$ were largely correlated to the values of $g_m$ and $l_m$ (Figure 4), indicating that increasing $g_m$ has a significant potential to increase $A_N$ under sub-saturating light. Taking into consideration that different plants have different light saturating points, measuring the light dependence changes in relative limitations of $A_N$ may have broad applications in tree breeding and crop improvement.

With an increase in light intensity, electron transport for photorespiration gradually increased [16,37–39]. However, it is unclear whether the light response of photorespiration was caused by the change of $l_m$ or $l_s$. In this study, we found that $l_s$ showed only small responses to changes
in light intensity (Figure 3). By comparison, \( I_m \) gradually increased with light intensity (Figure 3). Meanwhile, \( C_c \) gradually decreased (Figure 5B) and the ratio of \( V_o/V_c \) gradually increased (Figure 6A). Furthermore, with an increase in light intensity, the decrease in \( C_c \) was tightly correlated to an increased \( I_m \) (Figure 5C). Thus, the stable \( g_m \) was insufficient to compensate for CO\(_2\) consumption by \( A_N \) under high light, increasing the affinity of Rubisco to O\(_2\), and thus enhancing photorespiration. Therefore, the light response of photosynthesis is more determined by \( I_m \) rather than \( I_s \) (Figure 6B).

### 3.3. CO\(_2\) Assimilation under High Light Tends to be Limited by RuBP Carboxylation in Rhododendron Species

In C\(_3\) plants, \( A_N \) under saturating light can be limited by RuBP carboxylation and/or RuBP regeneration. Once the value of \( C_c \) is lower than the value of \( C_{trans} \), \( A_N \) tends to be limited by RuBP carboxylation. When \( C_c \) is higher than \( C_{trans} \), \( A_N \) is then limited by RuBP regeneration. However, the rate-limiting step of \( A_N \) in sclerophyllous species is poorly understood. In this study, we found that the values of operating \( C_c \) under saturating light were significantly lower than the values of \( C_{trans} \) in these species, apart from in \( R. decorum \) subsp. disprepes (Figure 7). Therefore, \( A_N \) under saturating light in these Rhododendron species was mainly limited by RuBP carboxylation.

In general, the rate-limiting step of \( A_N \) can be influenced by temperature and leaf nitrogen content. For example, at normal growth temperatures, \( A_N \) is limited by RuBP carboxylation under nitrogen deficiency but tends to be limited by RuBP regeneration at high nitrogen conditions in C\(_3\) crop species. In this article, we found that the rate-limiting step of \( A_N \) by RuBP carboxylation in Rhododendron species was mainly caused by a relatively lower \( g_m \) (Figures 2 and 7). In rice (\( Oryza sativa \)), wheat (\( Triticum aestivum \)), spinach (\( Spinacia oleracea \)), and tobacco (\( Nicotiana tabacum \)), values for \( g_m \) at saturating light were higher than 0.2 mol m\(^{-2}\) s\(^{-1}\) \([43,48]\). By comparison, the studied Rhododendron species displayed \( g_m \) values ranging from 0.048 to 0.18 mol m\(^{-2}\) s\(^{-1}\) (Figure 2). Such low \( g_m \) in these Rhododendron species limited the diffusion of CO\(_2\) from intercellular cavities to chloroplasts, resulting in \( C_c \) being lower than \( C_{trans} \). Therefore, \( g_m \) has the potential to alter the rate-limiting step of \( A_N \) under high light.

### 4. Materials and Methods

#### 4.1. Plant Materials and Growth Conditions

Nine evergreen sclerophyllous Rhododendron species from China were studied: \( R. decorum \), \( R. glandulosum \), \( R. hancockii \), \( R. decorum \) subsp. disprepes, \( R. ciliicalyx \), \( R. delavayi \), \( R. davidii \), \( R. fortunei \), and \( R. pachypodium \). All plants of these species are cultivated in a common garden at the Kunming Botanical Garden, Yunnan, China (102° 44′ 31″ E, 25° 08′ 24″ N, 1950 m of elevation). We chose fully expanded but not senescent sun leaves for photosynthetic measurements. For each species, at least four leaves from different individual plants were measured.

#### 4.2. Gas Exchange and Chlorophyll Fluorescence Measurements

Gas exchange and chlorophyll fluorescence were recorded using the 2-cm\(^2\) measuring head of LI-6400XT (Li-Cor Biosciences, Lincoln, NE, USA). All measurements were conducted at approximately 25 °C and relative air humidity near 60%. After photosynthetic induction at 1500 µmol photons m\(^{-2}\) s\(^{-1}\) for 20 min, light response curves were recorded at a 400 µmol mol\(^{-1}\) CO\(_2\) concentration, and photosynthetic parameters were monitored after exposure to each light intensity for 2 min. After light adaptation at 1500 µmol photons m\(^{-2}\) s\(^{-1}\) and 400 µmol mol\(^{-1}\) CO\(_2\) concentration for 20 min, \( A/C_i \) measurements were made at 50, 100, 150, 200, 300, 400, 600, 800, 1000, and 1200 µmol mol\(^{-1}\) CO\(_2\) concentrations. For each CO\(_2\) concentration, photosynthetic measurement was completed in 2 to 3 min. Using the \( A/C_i \) curves, the maximum rates of RuBP regeneration \( (J_{max}) \) and carboxylation \( (V_{cmax}) \) were calculated \([49]\).
The quantum yield of photosystem II (PSII) photochemistry was calculated as 
$$\Phi_{\text{PSII}} = \frac{(F_{m'} - F_s)}{F_{m'}}$$ [50],
where $F_{m'}$ and $F_s$ represent the maximum and steady-state fluorescence after light adaption, respectively [51]. The total electron transport rate through PSII ($J_{\text{PSII}}$) was calculated as follows [52]:
$$J_{\text{PSII}} = \Phi_{\text{PSII}} \times \text{PPFD} \times L_{\text{abs}} \times 0.5,$$
where PPFD is the photosynthetic photon flux density and leaf absorbance ($L_{\text{abs}}$) is assumed to be 0.84. We applied the constant of 0.5 based on the assumption that photons were equally distributed between PSI and PSII.

4.3. Estimation of Mesophyll Conductance and Chloroplast CO$_2$ Concentration

We calculated mesophyll conductance according to the following equation [44]:
$$g_m = \frac{A_N}{C_i - \Gamma^*(J_{\text{PSII}} + 8(A_N + R_d)) / (J_{\text{PSII}} - 4(A_N + R_d))},$$
where $A_N$ represents the net rate of CO$_2$ assimilation; $C_i$ is the intercellular CO$_2$ concentration; and $\Gamma^*$ is the CO$_2$ compensation point in the absence of daytime respiration [41,48], and we used a typical value of 40 umol mol$^{-1}$ in our current study [19]. Respiration rate in the dark ($R_d$) was considered to be the half of the dark-adapted mitochondrial respiration rate as measured after 10 min of dark adaptation [23].

Based on the estimated $g_m$, we then calculated the chloroplast CO$_2$ concentration ($C_c$) according to the following equation [49,53]:
$$C_c = C_i - \frac{A_N}{g_m}.$$

To identify the rate-limiting step of CO$_2$ assimilation, we subsequently estimated $C_{\text{trans}}$ (the chloroplast CO$_2$ concentration at which the transition from RuBP carboxylation to RuBP regeneration occurred) [21,43]:
$$C_{\text{trans}} = \frac{K_c(1 + O/K_o)/\text{max}}{1 - \text{Jmax}/4V_{\text{cmax}}} - 2\Gamma^*$$
where $K_c$ (µmol mol$^{-1}$) and $K_o$ (mmol mol$^{-1}$) are assumed to be 407 µmol mol$^{-1}$ and 277 mmol mol$^{-1}$ at 25 °C, respectively (Long and Bernacchi 2003); $O$ was assumed to be 210 mmol mol$^{-1}$ (Farquhar et al., 1980). The rate-limiting step for CO$_2$ assimilation was analyzed by comparing the values of $C_c$ and $C_{\text{trans}}$. $A_N$ tends to be limited by RuBP carboxylation when $C_c$ is lower than $C_{\text{trans}}$ and tends to be limited by RuBP regeneration when $C_c$ is higher than $C_{\text{trans}}$.

4.4. Quantitative Limitation Analysis of $A_N$

Relative photosynthetic limitations were assessed as follows [22]:
$$l_s = \frac{g_{\text{tot}} / g_s \times \partial A_N / \partial C_c}{g_{\text{tot}} + \partial A_N / \partial C_c}$$
$$l_m = \frac{g_{\text{tot}} / g_m \times \partial A_N / \partial C_c}{g_{\text{tot}} + \partial A_N / \partial C_c}$$
$$l_b = \frac{g_{\text{tot}}}{g_{\text{tot}} + \partial A_N / \partial C_c}$$
where $l_s$, $l_m$, and $l_b$ represent the relative limitations of stomatal conductance, mesophyll conductance, and biochemical capacity, respectively, in setting the observed value of $A_N$. $g_{\text{tot}}$ is the total conductance of CO$_2$ between the leaf surface and sites of RuBP carboxylation (calculated as $1/g_{\text{tot}} = 1/g_s + 1/g_m$).
4.5. Modeling of $V_c$ and $V_o$

The rates of RuBP carboxylation ($V_c$) and oxygenation ($V_o$) were calculated as follows [36]:

$$V_c = \frac{A_n + R_d}{1 - (\Gamma^*/C_c)}$$

and:

$$V_o = \frac{A_n + R_d}{(C_c/2\Gamma^*) - 0.5}$$

4.6. Statistical Analysis

Data were displayed as means ± SE ($n = 4–5$). After testing for normality and homogeneity of variances, one-Way ANOVA tests were used at $\alpha = 0.05$ significance level to determine whether significant differences existed between different averages.

5. Conclusions

In this study, we examined the light response of $g_m$ and its effect on photosynthesis in nine evergreen sclerophyllous *Rhododendron* species. The results indicated that all species showed no significant response of $g_m$ to variations in illumination. Therefore, the response of $g_m$ to rapid changes in irradiance may be a conservative photosynthetic trait in a given genus, at least in the genus *Rhododendron*. Furthermore, we found that the light response of photorespiration was mainly determined by $g_m$ limitation rather than $g_s$ limitation. At saturating light, $g_m$ limitation significantly affected the differentials between $C_{\text{trans}}$ and $C_c$, thus altering the rate-limiting step of $A_N$. We propose that examining the light dependence changes in relative limitations of $A_N$ can provide some valuable means for tree breeding and crop improvement.

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References

1. Huang, W.; Yang, Y.-J.; Wang, J.-H.; Hu, H. Photorespiration is the major alternative electron sink under high light in alpine evergreen sclerophyllous *Rhododendron* species. *Plant Sci.* 2019, 110275. [CrossRef] [PubMed]
2. Hassiotou, F.; Ludwig, M.; Renton, M.; Veneklaas, E.J.; Evans, J.R. Influence of leaf dry mass per area, CO2, and irradiance on mesophyll conductance in sclerophylls. *J. Exp. Bot.* 2009, 60, 2303–2314. [CrossRef] [PubMed]
3. Niinemets, Ü.; Díaz-Espejo, A.; Flexas, J.; Galmés, J.; Warren, C.R. Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. *J. Exp. Bot.* 2009, 60, 2249–2270. [CrossRef] [PubMed]
4. Peguero-Pina, J.J.; Sísó, S.; Flexas, J.; Galmés, J.; García-Nogales, A.; Niinemets, Ü.; Sancho-Knapik, D.; Saz, M.A.; Gil-Pelegrín, E. Cell-level anatomical characteristics explain high mesophyll conductance and photosynthetic capacity in sclerophyllous Mediterranean oaks. *New Phytol.* 2017, 214, 585–596. [CrossRef] [PubMed]
5. Flexas, J.; Díaz-Espejo, A.; Gago, J.; Gallé, A.; Galmés, J.; Gulías, J.; Medrano, H. Photosynthetic limitations in Mediterranean plants: A review. *Environ. Exp. Bot.* 2014, 103, 12–23. [CrossRef]
6. Niinemets, Ü.; Keenan, T. Photosynthetic responses to stress in Mediterranean evergreens: Mechanisms and models. *Environ. Exp. Bot.* 2014, 103, 24–41. [CrossRef]
7. Peguero-Pina, J.J.; Sancho-Knapik, D.; Morales, F.; Flexas, J.; Gil-Pelegrin, E. Differential photosynthetic performance and photoprotection mechanisms of three Mediterranean evergreen oaks under severe drought stress. *Funct. Plant Biol.* 2009, 36, 453–462. [CrossRef]
8. Peguero-Pina, J.J.; Sisó, S.; Fernández-Marín, B.; Flexas, J.; Galmés, J.; García-Plazaola, J.I.; Niinemets, Ü.; Sancho-Knapik, D.; Gil-Pelegrin, E. Leaf functional plasticity decreases the water consumption without further consequences for carbon uptake in Quercus cocifera L. under Mediterranean conditions. *Tree Physiol.* 2016, 36, 356–367. [CrossRef]
9. Huang, W.; Tong, Y.-G.; Yu, G.-Y.; Yang, W.-X. The sclerophyllous Eucalyptus camaldulensis and herbaceous Nicotiana tabacum have different mechanisms to maintain high rates of photosynthesis. *Front. Plant Sci.* 2016, 7, 1769. [CrossRef]
10. Yang, Y.-J.; Tong, Y.-G.; Yu, G.-Y.; Zhang, S.-B.; Huang, W. Photosynthetic characteristics explain the high growth rate for Eucalyptus camaldulensis: Implications for breeding strategy. *Ind. Crops Prod.* 2018, 124, 186–191. [CrossRef]
11. Yamori, W. Photosynthetic response to fluctuating environments and photoprotective strategies under abiotic stress. *J. Plant Res.* 2016, 129, 379–395. [CrossRef] [PubMed]
12. Valladares, F.; Allen, M.T.; Peary, R.W. Photosynthetic responses to dynamic light under field conditions in six tropical rainforest shrubs occurring along a light gradient. *Oecologia* 1997, 111, 505–514. [CrossRef]
13. Drouth, C.; Dreyer, E.; Epron, D.; Warren, C.R. Mesophyll conductance to CO2, assessed from online TDL-AS records of 13CO2 discrimination, displays small but significant short-term responses to CO2 and irradiance in Eucalyptus seedlings. *J. Exp. Bot.* 2011, 62, 5335–5346. [CrossRef] [PubMed]
14. Drouth, C.; Dreyer, E.; Brendel, O.; Warren, C.R. Is mesophyll conductance to CO2 in leaves of three Eucalyptus species sensitive to short-term changes of irradiance under ambient as well as low O2? *Funct. Plant Biol.* 2012, 39, 435–448. [CrossRef] [PubMed]
15. Flexas, J.; Ribas-Carbo, M.; Diaz-Espejo, A.; Galmés, J.; Medrano, H. Mesophyll conductance to CO2: Current knowledge and future prospects. *Plant Cell Environ.* 2008, 31, 602–621. [CrossRef]
16. Cai, Y.-F.; Yang, Q.-Y.; Li, S.-F.; Wang, J.-H.; Huang, W. The water-water cycle is a major electron sink in Camellia species when CO2 assimilation is restricted. *J. Photochem. Photobiol. B Biol.* 2017, 168, 59–66. [CrossRef]
17. Théroux-Rancourt, G.; Gilbert, M.E. The light response of mesophyll conductance is controlled by structure across leaf profiles. *Plant. Cell Environ.* 2017, 40, 726–740. [CrossRef]
18. Xiong, D.; Liu, X.; Liu, L.; Drouth, C.; Li, Y.; Peng, S.; Huang, J. Rapid responses of mesophyll conductance to changes of CO2 concentration, temperature and irradiance are affected by N supplements in rice. *Plant Cell Environ.* 2015, 38, 2541–2550. [CrossRef]
19. Xiong, D.; Drouth, C.; Flexas, J. Differential coordination of stomatal conductance, mesophyll conductance, and leaf hydraulic conductance in response to changing light across species. *Plant. Cell Environ.* 2018, 41, 436–450. [CrossRef]
20. Tazoe, Y.; Von Caemmerer, S.; Badger, M.R.; Evans, J.R. Light and CO2 do not affect the mesophyll conductance to CO2 diffusion in wheat leaves. *J. Exp. Bot.* 2009, 60, 2291–2301. [CrossRef]
21. Yamori, W.; Evans, J.R.; Von Caemmerer, S. Effects of growth and measurement light intensities on temperature dependence of CO2 assimilation rate in tobacco leaves. *Plant Cell Environ.* 2010, 33, 332–343. [CrossRef]
22. Grassi, G.; Magnani, F. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant Cell Environ.* 2005, 28, 834–849. [CrossRef]
23. Carriqui, M.; Cabrera, H.M.; Conesa, M.; Coopman, R.E.; Drouth, C.; Gago, J.; Gallé, A.; Galmés, J.; Ribas-Carbo, M.; Tomás, M.; et al. Diffusional limitations explain the lower photosynthetic capacity of ferns as compared with angiosperms in a common garden study. *Plant Cell Environ.* 2015, 38, 448–460. [CrossRef] [PubMed]
24. Tosens, T.; Nishida, K.; Gago, J.; Coopman, R.E.; Cabrera, H.M.; Carriqui, M.; Lannisto, L.; Morales, L.; Nadal, M.; Rojas, R.; et al. The photosynthetic capacity in 35 ferns and fern allies: Mesophyll CO2 diffusion as a key trait. *New Phytol.* 2016, 209, 1576–1590. [CrossRef] [PubMed]
25. Yang, Z.-H.; Huang, W.; Yang, Q.-Y.; Chang, W.; Zhang, S.-B. Anatomical and diffusional determinants inside leaves explain the difference in photosynthetic capacity between Cypripedium and Paphiopedilum, Orchidaceae. *Photosynth. Res.* 2018, 136, 315–328. [CrossRef] [PubMed]
26. Yamori, W.; Kondo, E.; Sugiruda, D.; Terashima, I.; Suzuki, Y.; Makino, A. Enhanced leaf photosynthesis as a target to increase grain yield: Insights from transgenic rice lines with variable Rieske FeS protein content in the cytochrome b6/f complex. *Plant Cell Environ.* 2016, 39, 80–87. [CrossRef]

27. Yamori, W.; Sakata, N.; Suzuki, Y.; Shikanai, T.; Makino, A. Cyclic electron flow around photosystem I via chloroplast NAD(P)H dehydrogenase (NDH) complex performs a significant physiological role during photosynthesis and plant growth at low physiological temperature. *Plant J.* 2011, 68, 966–976. [CrossRef]

28. Yamori, W.; Makino, A.; Shikanai, T. A physiological role of electron transport around photosystem I in sustaining photosynthesis under fluctuating light in rice. *Sci. Rep.* 2016, 6, 20147. [CrossRef]

29. Qu, M.; Zheng, G.; Hamdani, S.; Essemime, J.; Song, Q.; Wang, H.; Chu, C.; Sirault, X.; Zhu, X.-G. Leaf photosynthetic parameters related to biomass accumulation in a global rice diversity survey. *Plant Physiol.* 2017, 175, 248–258. [CrossRef]

30. Timm, S.; Florian, A.; Fernie, A.R.; Bauwe, H. The regulatory interplay between photorespiration and photosynthesis. *J. Exp. Bot.* 2016, 67, 2923–2929. [CrossRef]

31. Peterhansel, C.; Maurino, V.G. Photorespiration Redesigned. *Plant Physiol.* 2011, 155, 49–55. [CrossRef] [PubMed]

32. Bauwe, H.; Hagemann, M.; Kern, R.; Timm, S. Photorespiration has a dual origin and manifold links to central metabolism. *Curr. Opin. Plant Biol.* 2012, 15, 269–275. [CrossRef]

33. Timm, S.; Florian, A.; Arrivault, S.; Stitt, M.; Fernie, A.R.; Bauwe, H. Glycine decarboxylase controls photosynthesis and plant growth. *FEBS Lett.* 2012, 586, 3692–3697. [CrossRef] [PubMed]

34. Timm, S.; Wittmiß, M.; Gamlien, S.; Wirtz, M.; Hell, R.; Fernie, A.R.; Bauwe, H. Mitochondrial dihydrolipoyl dehydrogenase activity shapes photosynthesis and photorespiration of Arabidopsis thaliana. *Plant Cell* 2015, 27, 1968–1984. [CrossRef] [PubMed]

35. Walker, B.J.; Strand, D.D.; Kramer, D.M.; Cousins, A.B. The response of cyclic electron flow around photosystem I to changes in photorespiration and nitrate assimilation. *Plant Physiol.* 2014, 165, 453–462. [CrossRef]

36. Walker, B.J.; VanLoocke, A.; Bernacchi, C.J.; Ort, D.R. The costs of photorespiration to food production now and in the future. *Annu. Rev. Plant Biol.* 2016, 67, 107–129. [CrossRef]

37. Zhang, W.; Huang, W.; Yang, Q.Y.; Zhang, S.B.; Hu, H. Effect of growth temperature on the electron flow for photorespiration in leaves of tobacco grown in the field. *Physiol. Plant.* 2013, 149, 141–150. [CrossRef]

38. Huang, W.; Yang, Y.J.; Hu, H.; Zhang, S.B. Response of the water-water cycle to the change in photorespiration in tobacco. *J. Photochem. Photobiol. B Biol.* 2016, 157, 97–104. [CrossRef]

39. Valentini, R.; Epron, D.; Angelis, P.D.E.; Matteucci, G.; Dreyer, E. In situ estimation of net CO2 assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Q. cerris* L.) leaves: Diurnal cycles under different levels of water supply. *Plant Cell Environ.* 1995, 18, 631–640. [CrossRef]

40. Vines, H.M.; Armitage, A.M.; Chen, S.-S.; Tu, Z.-P.; Black, C.C. A Transient Burst of CO2 from Geranium Leaves during Illumination at Various Light Intensities as a Measure of Photorespiration. *Plant Physiol.* 1982, 70, 629–631. [CrossRef]

41. Yamori, W.; Noguchi, K.; Hikosaka, K.; Terashima, I. Phenotypic plasticity in photosynthetic temperature acclimation among crop species with different cold tolerances. *Plant Physiol.* 2010, 152, 388–399. [CrossRef] [PubMed]

42. Farquhar, G.D.; von Caemmerer, S.; Berry, J.A. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. *Planta* 1980, 149, 78–90. [CrossRef] [PubMed]

43. Yamori, W.; Nagai, T.; Makino, A. The rate-limiting step for CO2 assimilation at different temperatures is influenced by the leaf nitrogen content in several C3 crop species. *Plant Cell Environ.* 2011, 34, 764–777. [CrossRef] [PubMed]

44. Harley, P.C.; Loreto, F.; Di Marco, G.; Sharkey, T.D. Theoretical considerations when estimating the mesophyll conductance to CO2 flux by analysis of the response of photosynthesis to CO2. *Plant Physiol.* 1992, 98, 1429–1436. [CrossRef]

45. De Souza, A.P.; Wang, Y.; Orr, D.J.; Carmo-Silva, E.; Long, S.P. Photosynthesis across African cassava germplasm is limited by Rubisco and mesophyll conductance at steady state, but by stomatal conductance in fluctuating light. *New Phytol.* 2020, 225, 2498–2512. [CrossRef]
46. Yamori, W.; Kusumi, K.; Iba, K.; Terashima, I. Increased stomatal conductance induces rapid changes to photosynthetic rate in response to naturally fluctuating light conditions in rice. *Plant. Cell Environ.* **2020**, *43*, 1230–1240. [CrossRef]

47. Huang, W.; Hu, H.; Zhang, S.-B. Photorespiration plays an important role in the regulation of photosynthetic electron flow under fluctuating light in tobacco plants grown under full sunlight. *Front. Plant Sci.* **2015**, *6*, 621. [CrossRef]

48. von Caemmerer, S.; Evans, J.R. Temperature responses of mesophyll conductance differ greatly between species. *Plant Cell Environ.* **2015**, *38*, 629–637. [CrossRef]

49. Long, S.P.; Bernacchi, C.J. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J. Exp. Bot.* **2003**, *54*, 2393–2401. [CrossRef]

50. Genty, B.; Briantais, J.-M.; Baker, N.R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta Gen. Subj.* **1989**, *990*, 87–92. [CrossRef]

51. Baker, N.R. Applications of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities. *J. Exp. Bot.* **2004**, *55*, 1607–1621. [CrossRef] [PubMed]

52. Krall, J.P.; Edwards, G.E. Relationship between photosystem II activity and CO2 fixation in leaves. *Physiol. Plant.* **1992**, *86*, 180–187. [CrossRef]

53. Warren, C.R.; Dreyer, E. Temperature response of photosynthesis and internal conductance to CO2: Results from two independent approaches. *J. Exp. Bot.* **2006**, *57*, 3057–3067. [CrossRef] [PubMed]

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