Quantifying Light Response of Leaf-Scale Water-Use Efficiency and Its Interrelationships With Photosynthesis and Stomatal Conductance in C₃ and C₄ Species

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Light intensity (I) is the most dynamic and significant environmental variable affecting photosynthesis (Aᵣ), stomatal conductance (gₛ), transpiration (Tᵣ), and water-use efficiency (WUE). Currently, studies characterizing leaf-scale WUE–I responses are rare and key questions have not been answered. In particular, (1) What shape does the response function take? (2) Are there maximum intrinsic (WUEᵢ; WUEᵢ–max) and instantaneous WUE (WUEᵢ–inst; WUEᵢ–inst–max) at the corresponding saturation irradiances (Iᵢ–sat and Iᵢ–inst–sat)? This study developed WUEᵢ–I and WUEᵢ–inst–I models sharing the same non-asymptotic function with previously published Aᵣ–I and gₛ–I models. Observation-modeling intercomparison was conducted for field-grown plants of soybean (C₃) and grain amaranth (C₄) to assess the robustness of our models versus the non-rectangular hyperbola models (NH models). Both types of models can reproduce WUEᵢ–I curves well over light-limited range. However, at light-saturated range, NH models overestimated WUEᵢ–max and WUEᵢ–inst–max and cannot return Iᵢ–sat and Iᵢ–inst–sat due to its asymptotic function. Moreover, NH models cannot describe the down-regulation of WUE induced by high light, on which our models described well. The results showed that WUEᵢ and WUEᵢ–inst increased rapidly within low range of I, driven by uncoupled photosynthesis and stomatal responsiveness. Initial response rapidity of WUEᵢ was higher than WUEᵢ–inst because the greatest increase of Aᵣ and Tᵣ occurred at low gₛ. C₄ species showed higher WUEᵢ–max and WUEᵢ–inst–max than C₃ species—at similar Iᵢ–sat and Iᵢ–inst–sat. Our intercomparison highlighted larger discrepancy between WUEᵢ–I and WUEᵢ–inst–I responses in C₃ than C₄ species, quantitatively characterizing an
important advantage of C4 photosynthetic pathway—higher $A_n$ gain but lower $T_r$ cost per unit of $g_s$ change. Our models can accurately return the wealth of key quantities defining species-specific WUE–I responses—besides $A_n$–I and $g_s$–I responses. The key advantage is its robustness in characterizing these entangled responses over a wide I range from light-limited to light-inhibitory light intensities, through adopting the same analytical framework and the explicit and consistent definitions on these responses. Our models are of significance for physiologists and modelers—and also for breeders screening for genotypes concurrently achieving maximized photosynthesis and optimized WUE.

**Keywords:** irradiance, leaf gas exchange, light response curve, maximum water use efficiency, model, plant functional type (PFT), saturation light intensity, transpiration

**INTRODUCTION**

Stomata control the balance between carbon flux driven by photosynthesis and water flux dominated by transpiration, which is characterized by water-use efficiency (WUE) at various scales (Sinclair et al., 1984; Gilbert et al., 2011; Eamus et al., 2016; Medlyn et al., 2017). WUE can thus indicate the natural selection on the balance between these fluxes (Hetherington and Woodward, 2003). Characterizing the environmental impacts on WUE among plant species and/or plant function types can advance our knowledge on differential plant adaptation strategies, and improve our prediction on consequences of environmental challenges (Avola et al., 2008; Egea et al., 2011; Zhou et al., 2014, 2016; De Kauwe et al., 2015; Köhler et al., 2016; Ahrar et al., 2017). For instance, plant species with the highest WUE would show the greatest fitness in dry habitats (Dudley, 1996; Zhou et al., 2019). WUE is also an important metric in crop breeding and genotype selection, especially for irrigated crops whose water use significantly affects crop productivity and profitability (Duursma et al., 2013; Flexas et al., 2013; Bota et al., 2016; Webster et al., 2016).

WUE can be estimated using different techniques, based on observations of leaf gas exchange, stable isotope discrimination, and eddy covariance fluxes (Medlyn et al., 2017). Among these techniques, WUE is most commonly estimated by measuring leaf gas exchange, facilitated by portable photosynthesis system allowing simultaneous measurement of leaf-scale carbon and water fluxes (Medrano et al., 2015). WUE derived from leaf gas exchange measurement is usually defined as the ratio of net CO$_2$ assimilation rate ($A_n$) to stomatal conductance for water vapor ($g_s$)—intrinsic water-use efficiency (WUE$_i$; von Caemmerer and Farquhar, 1981), or the ratio of $A_n$ to transpiration rate ($T_r$)—instantaneous water-use efficiency (WUE$_{inst}$; Fischer and Turner, 1978) (see Table 1 for a summary of parameters and units). WUE$_i$ can be used to compare photosynthetic characteristics independently of evaporative demand (Linares and Camarero, 2012). WUE$_{inst}$ is a key determinant of whole-plant WUE as it summarizes plant dry mass production per unit of water loss (Sinclair et al., 1984; Duursma et al., 2013; but see Medrano et al., 2015 for constraints). WUE$_i$ and WUE$_{inst}$ have been widely used as an index of plant and vegetation performances in response to various environmental changes, such as changed water or light availabilities, vapor pressure deficit (VPD), temperature and CO$_2$ concentration (Aranda et al., 2007; Avola et al., 2008; Linares and Camarero, 2012; Duursma et al., 2013; Bota et al., 2016).

Light is often viewed as the most significant environmental variable affecting photosynthesis, stomatal behavior and WUE (Knapp and Smith, 1987; Aranda et al., 2007; McAusland et al., 2016). Plants in most ecosystems experience rapid short-term variability in light resource (Smith et al., 1989), which can cause continual transition of $A_n$, $g_s$, $T_r$, WUE$_i$, and WUE$_{inst}$ throughout the growing season (Knapp and Smith, 1990; Knapp, 1993). However, studies characterizing the light response of WUE are rare (McAusland et al., 2016). It is largely unknown whether there is a maximum WUE$_i$ or WUE$_{inst}$—and the corresponding saturation irradiance—for plants under dynamic irradiance conditions, or how plant species or plant function types (PFTs) would differ in their light responses of WUE$_i$ and WUE$_{inst}$.

Characterization of the interrelationships among light responses of $A_n$, $g_s$, $T_r$, WUE$_i$ and WUE$_{inst}$—which can be simultaneously measured—will be fundamental to the scaling-up modeling of WUE–I responses at the whole-plant and ecosystem scale. The foremost step toward this direction calls for a robust model, with which (1) the WUE$_i$ and WUE$_{inst}$ responses to a gradient of irradiance intensity (I) levels (WUE–I and WUE$_{inst}$–I response curve, respectively) can be characterized, and (2) the key quantities defining the response curves—such as the initial slope of the response curve, the maximum WUE and the corresponding saturation irradiance—can be quantified. Ideally, the model can accurately represent the differential WUE$_i$ and WUE$_{inst}$ responses among plant species or PFTs, such as that reported between C3 and C4 species with contrasting light responses of photosynthesis, stomatal functioning, and WUE (Pearcy and Ehleringer, 1984; Knapp, 1993). For a given $A_n$, $g_s$ and $T_r$ are higher in C3 than C4 plants, leading to higher WUE$_i$ and WUE$_{inst}$ in C3 plants, which has higher utilization efficiency of CO$_2$ at relatively lower intercellular CO$_2$ concentration (Pearcy and Ehleringer, 1984). The objectives of this study were to develop a leaf-scale WUE–I model and assess the model performance against experimental field observations of C3 and C4 species in order to answer key questions of how best to model the light response of WUE$_i$ and WUE$_{inst}$. In particular: (1) What shape does the leaf-scale WUE–I response function
TABLE 1 | List of major model parameters defining the light response curves of photosynthesis (A), stomatal conductance (gs), intrinsic water use efficiency (WUEi), and instantaneous water use efficiency (WUEinst).

| Symbol | Definition | Unit |
|--------|------------|------|
| \(A_n\) | Net photosynthetic rate | \(\mu\text{mol }\text{CO}_2 \text{m}^{-2} \text{s}^{-1}\) |
| \(A_{n,\text{max}}\) | Maximum net photosynthetic rate | \(\mu\text{mol }\text{CO}_2 \text{m}^{-2} \text{s}^{-1}\) |
| \(g_s\) | Stomatal conductance | \(\text{mol }\text{H}_2\text{O} \text{m}^{-2} \text{s}^{-1}\) |
| \(g_{s,\text{max}}\) | Maximum stomatal conductance | \(\text{mol }\text{H}_2\text{O} \text{m}^{-2} \text{s}^{-1}\) |
| \(I\) | Light intensity | \(\mu\text{mol }\text{photons} \text{m}^{-2} \text{s}^{-1}\) |
| \(I_{\text{sat}}\) | Saturation light intensity corresponding to maximum net photosynthetic rate | \(\mu\text{mol }\text{photons} \text{m}^{-2} \text{s}^{-1}\) |
| \(I_{g,\text{sat}}\) | Saturation light intensity corresponding to maximum stomatal conductance | \(\mu\text{mol }\text{photons} \text{m}^{-2} \text{s}^{-1}\) |
| \(I_{\text{inst,sat}}\) | Saturation light intensity corresponding to maximum instantaneous water-use efficiency | \(\mu\text{mol }\text{photons} \text{m}^{-2} \text{s}^{-1}\) |
| \(R_d\) | Mitochondrial \(\text{CO}_2\) release in the dark | \(\mu\text{mol }\text{CO}_2 \text{m}^{-2} \text{s}^{-1}\) |
| \(T_r\) | Transpiration rate | \(\text{mol }\text{H}_2\text{O} \text{m}^{-2} \text{s}^{-1}\) |
| \(WUE_i\) | Intrinsic water-use efficiency | \(\mu\text{mol }\text{CO}_2 \text{mol}^{-1} \text{H}_2\text{O}\) |
| \(WUE_{i,\text{max}}\) | Maximum intrinsic water-use efficiency | \(\mu\text{mol }\text{CO}_2 \text{mol}^{-1} \text{H}_2\text{O}\) |
| \(WUE_{\text{inst}}\) | Instantaneous water-use efficiency | \(\mu\text{mol }\text{CO}_2 \text{mol}^{-1} \text{H}_2\text{O}\) |
| \(WUE_{\text{inst, max}}\) | Maximum instantaneous water-use efficiency | \(\mu\text{mol }\text{CO}_2 \text{mol}^{-1} \text{H}_2\text{O}\) |
| \(\alpha\), \(\alpha_0\), \(\alpha_1\), \(\alpha_2\) | Initial slope of light response curve of \(A_n\), \(g_s\), \(WUE_i\) and \(WUE_{\text{inst}}\) | \(\text{mol }\text{CO}_2 \text{mol}^{-1} \text{H}_2\text{O}\) |
| \(\beta\), \(\beta_0\), \(\beta_1\), \(\beta_2\) | Inhibitor coefficient of light response curve of \(A_n\), \(g_s\), \(WUE_i\) and \(WUE_{\text{inst}}\) | \(\text{mmol }\text{H}_2\text{O} \text{m}^{-2} \text{s}^{-1}\) |
| \(\gamma\), \(\gamma_0\), \(\gamma_1\), \(\gamma_2\) | Saturation coefficient of light response curve of \(A_n\), \(g_s\), \(WUE_i\) and \(WUE_{\text{inst}}\) | \(\text{mmol }\text{H}_2\text{O} \text{m}^{-2} \text{s}^{-1}\) |
| \(K_i\) | Residual intrinsic water-use efficiency | \(\text{mmol }\text{H}_2\text{O} \text{mol}^{-1}\) |
| \(K_{\text{inst}}\) | Residual instantaneous water-use efficiency | \(\text{mmol }\text{H}_2\text{O} \text{mol}^{-1}\) |

Take? Is there a maximum WUEi and/or WUEinst—and the corresponding saturation irradiances for plants under dynamic irradiance conditions? (2) Can the model well represent the differential WUEi–I and/or WUEinst–I response characteristics between C3 and C4 species? By integrating the published \(A_n–I\) (Ye, 2007; Ye et al., 2013) and \(g_s–I\) (Ye and Yu, 2008) response function, we developed an explicit WUE–I modeling framework and hypothesized that the species-specific light response curves of WUEi and WUEinst can be quantitatively characterized using the same non-asymptotic function. The hypothesis was tested using an observation-modeling intercomparison on WUEi–I and WUEinst–I responses for field-grown C3 [soybean (Glycine max L.)] and C4 species [grain amaranth (Amaranthus hypochondriacus L.)] under high I condition in the growing season. Model performance against that of the non-rectangular hyperbola model was also evaluated.

MATERIALS AND METHODS

Analytical Models

A non-asymptotic model has been previously developed and tested to well characterize the light response of photosynthesis (Ye, 2007; Ye et al., 2013), with its simplified form as follows:

\[
A_n = \alpha \frac{1 - \beta I}{1 + \gamma I} I - R_d
\]  

(1)

where \(\alpha\) is the initial slope of light response curve of photosynthesis, \(I\) is the irradiance, and \(\beta\) and \(\gamma\) are the photoinhibition coefficient and saturation coefficient, respectively, and \(R_d\) is the dark respiratory rate. The key model parameters are listed in Table 1.

The saturation irradiance \(I_{\text{sat}}\) corresponding to the light-saturated photosynthetic rate \(A_{n,\text{max}}\) can be calculated as follows:

\[
I_{\text{sat}} = \frac{\sqrt{\beta + \gamma}}{\gamma} - 1
\]  

(2)

\[
A_{n,\text{max}} = \alpha \left( \frac{\sqrt{\beta + \gamma} - \sqrt{\gamma}}{\gamma} \right)^2 - R_d
\]  

(3)

Eq. 1 has been widely used to characterize photosynthetic light response curves of various plant species under different environmental conditions, highlighting its better performance than that of rectangular (Baly, 1935) and non-rectangular hyperbolic models (Thorley, 1976; Wargent et al., 2011; Xu et al., 2012a,b; Song et al., 2015; Chen et al., 2016). The rectangular and the non-rectangular hyperbolic models have been reported to overestimate \(A_{n,\text{max}}\) (dos Santos et al., 2013), and cannot quantify \(I_{\text{sat}}\) (Gomes et al., 2006; dos Santos et al., 2013; Song et al., 2015; Chen et al., 2016).

Meanwhile, a model of the same non-asymptotic form as Eq. 1 has been developed and tested to well characterize the light response of stomatal conductance (Ye and Yu, 2008), as follows:

\[
g_s = \alpha_0 \frac{1 - \beta_0 I}{1 + \gamma_0 I} I + g_{s0}
\]  

(4)

where \(\alpha_0\) is the initial slope of light response curve of stomatal conductance, \(g_{s0}\) is the residual stomatal conductance, and \(\beta_0\) and \(\gamma_0\) are two coefficients that are independent of \(I\) (Ye and Yu, 2008). Most existing stomatal conductance models cannot quantify the \(g_{s,\text{max}}\) or the corresponding \(I_{g,s,\text{sat}}\) under changing irradiance conditions (Dewar, 2002; Buckley et al., 2003; Buckley and Mott, 2013; Flexas et al., 2013).
The $g_s$–$I$ model developed by Ye and Yu (2008) can well characterize the $g_s$–$I$ response, from which key parameters defining the $g_s$–$I$ response—such as $g_{s\text{-max}}$ and $I_{g\text{-sat}}$—can be easily obtained.

The saturation irradiance ($I_{g\text{-sat}}$) corresponding to the light-saturated stomatal conductance ($g_{s\text{-max}}$) can be calculated as follows:

$$I_{g\text{-sat}} = \frac{\sqrt{\beta_0 + \gamma_0}}{\gamma_0} - 1 \tag{5}$$

$$g_{s\text{max}} = a_0 \left(\frac{\sqrt{\beta_0 + \gamma_0} - \sqrt{\beta_0}}{\gamma_0}\right)^2 + g_0 \tag{6}$$

Here, we hypothesize that the light response of WUE$_i$ can be characterized using the same non-asymptotic form as that of the $A_n$–$I$ (Eq. 1) and $g_s$–$I$ (Eq. 4) response functions, as follows:

$$WUE_i = \alpha_1 \frac{1 - \frac{\beta_1}{1 + \gamma_1} I - K_i}{I} \tag{7}$$

where $\alpha_1$ represents the initial slope of light response curve of WUE$_i$, $\beta_1$, and $\gamma_1$ are coefficients that are independent of $I$, and $K_i$ is the residual intrinsic water-use efficiency. The saturation irradiance ($I_{i\text{-sat}}$) corresponding to the maximum WUE$_i$ ($WUE_{i\text{-max}}$) can be calculated as follows:

$$I_{i\text{-sat}} = \frac{\sqrt{\beta_1 + \gamma_1}}{\gamma_1} - 1 \tag{8}$$

$$WUE_{i\text{-max}} = \alpha_1 \left(\frac{\sqrt{\beta_1 + \gamma_1}}{\gamma_1} - \sqrt{\beta_1}\right)^2 - K_i \tag{9}$$

**FIGURE 1** | Irradiance ($I$) responses of net photosynthetic rate ($A_n$) (A,B), stomatal conductance ($g_s$) (C,D) and transpiration rate ($T_r$) (E,F) for $C_3$ [soybean (*Glycine max*)] and $C_4$ species [grain amaranth (*Amaranthus hypochondriacus*)], respectively. In plots (A) and (B), solid lines were fitted using Eq. 1 and dashed lines were fitted using the non-rectangular hyperbola model (Eq. S1). In plots (C) and (D), solid lines were fitted using Eq. 4. Data are the mean ± SE (n = 4).
TABLE 2 | Fitted (Eq. 1) and measured (Obs.) values of parameters defining the light response curve of photosynthesis for C\textsubscript{3} species (grain amaranth).

| Species           | \( \alpha \) | \( \beta \) | \( \gamma \) | \( \alpha_{\text{max}} \) | \( \beta_{\text{max}} \) |
|-------------------|-------------|-------------|-------------|----------------|----------------|
| Soybean           | -21.25      | 4.36        | 1.20        | 21.25 ± 0.05   | 1.20 ± 0.10   |
| Grain amaranth    | -21.86      | 4.90        | 0.81        | 21.56 ± 0.10   | 0.81 ± 0.15   |

The parameters are initial slope of the A\textsubscript{I} curve (\( \alpha_{\text{max}} \)), the maximum net photosynthetic rate (\( \beta_{\text{max}} \)), and the corresponding saturation irradiance (\( \gamma \)), respectively. Different letters denote statistically significant differences (P < 0.05) between soybean and grain amaranth within each column of fitted (Eq. 1) or measured (Obs.) values. For details of abbreviations, see Table 1.

Since \( g_p \) controls leaf \( T_e \) at a given VPD (Duursma et al., 2013), we hypothesize that the light response of WUE\textsubscript{inst} can also be characterized using the same non-asymptotic function as that of WUE\textsubscript{i}–I response function (Eq. 7), as follows:

\[
\text{WUE}_{\text{inst}} = \alpha_2 \frac{1 - \beta_2 I}{1 + \gamma_2 I} - K_{\text{inst}}
\]

where \( \alpha_2 \) represents the initial slope of light response curve of WUE\textsubscript{inst}, \( \beta_2 \) and \( \gamma_2 \) are coefficients that are independent of \( I \), and \( K_{\text{inst}} \) is the residual instantaneous water-use efficiency. The saturation irradiance (\( I_{\text{sat}} \)) corresponding to the maximum WUE\textsubscript{inst} (WUE\textsubscript{inst–max}) can be calculated as follows:

\[
I_{\text{sat}} = \frac{\sqrt{\beta_2 + \gamma_2} - 1}{\gamma_2}
\]

In this study, we tested if Eqs. 7 and 10 can well characterize the species-specific WUE–I response characteristics against model-oriented field observations and the simulations using the non-rectangular hyperbola model—in terms of the initial slope of light response curve of WUE (\( \alpha_1 \) and \( \alpha_2 \), respectively), the maximum WUE\textsubscript{inst} (WUE\textsubscript{i} and WUE\textsubscript{inst–max}, respectively), and the saturation irradiance (\( I_{\text{sat}} \) and \( I_{\text{inst–sat}} \), respectively).

**Study Site and Plant Material**

The field observations on one C\textsubscript{3} species—soybean (Glycine max L.) and one C\textsubscript{4} species—grain amaranth (A. hypochondriacus L.) were conducted at the Yucheng Comprehensive Experiment Station of the Chinese Academy of Sciences, located at the irrigation district of the Yellow River Basin in the North China Plain. This region is dominated by the warm-temperate semi-humid monsoon climate and is suitable for planting soybean and grain amaranth with high yields. This region has ample energy resource, and the light intensity in the growing season usually reaches ~2000 \( \mu \text{mol m}^{-2} \text{ s}^{-1} \) in sunny days. Soybean and grain amaranth were planted in field on May 3rd and June 15th, 2012, respectively. All plants were kept under moist condition throughout the experiment.

**Light Response Curve Measurement**

The leaf gas exchange measurements were conducted after 45 days of growth in field—June 16th for soybean and July 29th for grain amaranth. Fully expanded sun-exposed leaves of four plants for each species were measured using a portable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, NE, United States). Before each measurement, the leaf was acclimated in the chamber to achieve stable gas exchange, with reference CO\textsubscript{2} concentration maintained at 380 \( \mu \text{mol} \) CO\textsubscript{2} mol\textsuperscript{-1}, irradiance intensity maintained at 2000 \( \mu \text{mol} \) photon m\textsuperscript{-2} s\textsuperscript{-1}, and leaf temperature maintained at 35°C. After the leaf acclimated to the cuvette environment, the photosynthetic light response curve measurements were conducted with a descending gradient of irradiance intensity...
levels, as follows: 2000, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 150, 100, 80, 50, and 0 μmol m$^{-2}$ s$^{-1}$. At each irradiance level, leaf gas exchange was monitored to ensure reaching steady-state plateau before data-logging. VPD was kept stable during measurements (Supplementary Figure S1). The $A_n$–$I$, $g_s$–$I$, WUE$_i$–$I$, and WUE$_{inst}$–$I$ response curves were fitted by Eqs. 1, 4, 7, and 10, respectively. $I_{sat}$, $I_{g-sat}$, $I_{g-sat}$, and $I_{inst-sat}$ values were calculated following Eqs. 2, 5, 8, and 11, respectively. $A_{nmax}$, $g_{s-max}$, WUE$_i$–$max$, and WUE$_{inst}$–$max$ values were calculated following Eqs. 3, 6, 9, and 12, respectively.

Data Analysis

All statistical tests were performed using the statistical package SPSS 18.5 statistical software (SPSS, Chicago, IL, United States). The analysis of variance (ANOVA) was used to assess species effects. Paired-sample $t$ tests were conducted to test whether there were significant differences between fitted and measured values of quantitative traits ($\alpha$, $A_{nmax}$, $I_{sat}$, $g_{s-max}$, $I_{g-sat}$, $g_1$, WUE$_i$–$max$, $I_{inst-sat}$, $g_2$, WUE$_{inst}$–$max$, $I_{inst-sat}$, etc.). Goodness of fit of the mathematical model to experimental observations was assessed using the coefficient of determination ($r^2 = 1 – \frac{SSE}{SST}$, where $SST$ is the total sum of squares and $SSE$ is the error sum of squares).

**RESULTS**

Light Response Curves of $A_n$, $g_s$, and $T_r$

The increase of $I$ led to a rapid initial increase of $A_n$ (Figures 1A,B), $g_s$ (Figures 1C,D), and $T_r$ (Figures 1E,F) for both C$_3$ and C$_4$ species. However, the initial increase rate of $A_n$ was 100-fold higher than that of $g_s$ for both species (Tables 2 and 3). The high coefficient of determination ($r^2$) values indicated that the species-specific $A_n$–$I$ response curves fitted by Eq. 1—and the $g_s$–$I$ response curves fitted by Eq. 4—were highly representative of the observations for both species (Figure 1).

Soybean exhibited a single-peaked pattern for both $A_n$–$I$ and $g_s$–$I$ responses, characterized by the increase of $A_n$ and $g_s$ with the increasing $I$ until reaching the $A_{nmax}$ and $g_{s-max}$ at the corresponding $I_{sat}$ and $I_{g-sat}$, respectively (Figures 1A,C and Supplementary Table S1). Compared with Eq. 1, the non-rectangular hyperbola model (Supplementary Eq. S1) showed similarly high $r^2$ value in simulating $A_n$–$I$ response curves but significantly overestimated the $A_{nmax}$ (Figures 1A,B and Supplementary Table S1). Paired-sample $t$ tests showed there were no significant differences between the fitted values and the measured values of $A_{nmax}$, $I_{sat}$, $g_{s-max}$, and $I_{g-sat}$ for soybean (Tables 2 and 3).

Grain amaranth kept increasing its $A_n$ and $g_s$ within the range of irradiance intensity applied during measurements (0–2000 μmol photon m$^{-2}$ s$^{-1}$), without showing an observational $A_{nmax}$, $I_{sat}$, $g_{s-max}$, or $I_{g-sat}$ (Figures 1B,D and Tables 2 and 3). Grain amaranth showed relatively higher (not significant) initial increase rate of $g_s$, characterized by an initial slope of the light response curve of $g_s$ ($a_0$) (Figure 1 and Tables 2 and 3).
Light Response Curves of $WUE_i$ and $WUE_{inst}$

Within the low range of irradiance intensity, $WUE_i$ and $WUE_{inst}$ of both species increased almost linearly with the increasing $I$. Both soybean and grain amaranth exhibited a single-peaked $WUE_{i}-I$ and $WUE_{inst}-I$ response pattern, respectively. In particular, both species showed an increase of $WUE_i$ and $WUE_{inst}$ with the increasing $I$ until reaching the species-specific $WUE_{i,max}$ and $WUE_{inst,max}$ at the corresponding species-specific saturation irradiance levels ($I_{i,sat}$ and $I_{inst,sat}$, respectively) (Figure 2 and Tables 4 and 5). However, soybean showed significantly lower observed and fitted $WUE_{i,max}$ and $WUE_{inst,max}$ ($P \leq 0.05$) than grain amaranth (Figure 2 and Tables 4 and 5). The two species showed no significant difference in $I_{i,sat}$, $I_{inst,sat}$ or the initial increase rate of $WUE_i$ or $WUE_{inst}$—characterized by a maximal slope of the light response curves ($a_1$ and $a_2$, respectively) (Figure 2 and Tables 4 and 5).

The high $r^2$ values indicated that $WUE_{i}-I$ response curves fitted by Eq. 7—and the $WUE_{inst}-I$ response curves fitted by Eq. 10—were highly representative of the observations of both species (Figure 2 and Tables 4 and 5). There were no significant differences between fitted and observed values in $WUE_{i,max}$, $WUE_{inst,max}$, $I_{i,sat}$, $I_{inst,sat}$, $K_{i}$, or $K_{inst}$ (Tables 4 and 5). Compared with Eqs. 7 and 10, the non-rectangular hyperbola model (Supplementary Eqs. S2 and S3, respectively) showed similarly high $r^2$ values but significantly overestimated $WUE_{i,max}$ and $WUE_{inst,max}$ for the two species (Supplementary Tables S2 and S3).

Discussion

Our $WUE_i-I$ and $WUE_{inst}-I$ models represented cultivarspecific response curves over a wide range of light intensities extremely well ($r^2 \geq 0.996$), including the decline of $WUE_i$ and $WUE_{inst}$ beyond the saturation irradiances which the NH models cannot represent due to the asymptotic function. Our models can also return values for $WUE_{i,max}$, $WUE_{inst,max}$, $I_{i,sat}$, and $I_{inst,sat}$, which were in very close agreement with the measured values. The NH models cannot characterize the decline in $WUE_i$ and $WUE_{inst}$ induced by high light, leading to overestimations of $WUE_{i,max}$ and $WUE_{inst,max}$ (Supplementary Tables S2 and S3).

Interrelationships of Light Responses of Photosynthesis, Stomatal Conductance, and Water-Use Efficiency

$WUE_i$ and $WUE_{inst}$ increased rapidly within low range of $I$, mainly driven by the uncoupled rapidity of photosynthetic and stomatal responses (Figures 1, 2 and Tables 4 and 5; Knapp and Smith, 1990; McAusland et al., 2016). In this study, both $C_3$ and
C₄ species showed 100-fold higher initial increase rate of Aₙ (α) than that of gₛ (α₀) (Tables 2 and 3). The rapid initial increase of WUEᵢ and WUEᵢⁿ—I—that characterized by α₁ and α₂, respectively—occurred at low gₛ (and at low I), when small increase in gₛ exerted the greatest impacts on Aₙ and Tₑ (Hetherington and Woodward, 2003). The occurrence of the greatest Aₙ and Tₑ increase at low gₛ also determined that α₁ would be much higher than α₂ for a given species (Figure 2 and Tables 4 and 5).

With the increasing I (from 0 to ~800 µmol m⁻² s⁻¹), faster photosynthesis response than stomatal response led to the decline of intercellular CO₂ concentration (Cᵢ) (Supplementary Figure S1; McAusland et al., 2016), causing further opening of stomatal pores (Mott, 1988) which allowed for diffusion of ambient CO₂ into the leaf (Hetherington and Woodward, 2003). Further increase of gₛ—beyond the low gₛ range—led to minimal increase of Aₙ and Tₑ (Hetherington and Woodward, 2003), such that WUEᵢ and WUEᵢⁿ—I flattened quickly after reaching the WUEᵢ maxx and WUEᵢⁿ—I maxx (Figure 2). Further increase of I beyond I₁sat and Iᵢⁿ—I sat led to a decrease in WUEᵢ and WUEᵢⁿ—I. To reach Aᵢ maxx, both soybean and grain amaranth would have to show a decrease of WUEᵢ (or WUEᵢⁿ—I) from WUEᵢ maxx (or WUEᵢⁿ—I maxx) (Figures 1, 2).

**Differential Light Responses of Water-Use Efficiency Between C₃ and C₄ Species**

The observation-modeling intercomparison in this study highlighted the differential single-peaked WUEᵢ—I and WUEᵢⁿ—I responses—besides differential Aₙ—I and gₛ—I responses—between C₃ and C₄ species (Figure 2 and Tables 4 and 5). C₄ species (grain amaranth) showed higher WUEᵢ and WUEᵢⁿ—I than C₃ species (soybean), suggesting its better leaf-scale optimization of carbon uptake versus water loss than C₃ species (Figures 1, 2 and Tables 2, 4, and 5). This may be due to higher photosynthetic capacity and rapidity of stomatal response (α₀) in C₄ species under changing irradiance conditions (Figure 1 and Tables 2 and 3), which facilitate relatively closer coupling between Aₙ and gₛ in C₄ species than C₃ species (McAusland et al., 2016).

Moreover, this study identifies greater interspecific difference in WUEᵢⁿ—I than that in WUEᵢ—at high I range when WUEᵢ and WUEᵢⁿ—I flatten (Figure 2 and Tables 4 and 5). C₃ species (soybean) showed larger discrepancy between its WUEᵢ—I and WUEᵢⁿ—I responses than that of C₄ species (grain amaranth). This may be due to differential water use strategies between C₃ and C₄ species—C₄ species holds smaller Tₑ change per unit of gₛ change in relative to C₃ species (Knapp, 1993). These results quantitatively demonstrate that the differential WUEᵢ—I responses between C₃ and C₄ species would not necessarily mirror their differential WUEᵢⁿ—I responses (Figure 2).

These results support previous studies reporting that water conservation—in terms of high WUE—is an important consequence of the C₄ photosynthetic pathway (besides high carbon gain rate) at different scales including single leaf, whole plant, and even whole communities (Ludlow and Wilson, 1972),
Model Significance

By providing (1) analytical models characterizing the single-peaked light responses of WUE and WUE\textsubscript{inst} and (2) key quantitative traits defining WUE\textsubscript{sat}–I and WUE\textsubscript{inst}–I response differences between C\textsubscript{3} and C\textsubscript{4} species, this study provides a practical and robust modeling approach—in a form potentially applicable to WUE–I models at whole-plant and/or ecosystem scale. In particular, the key quantitative traits—the initial increase rates of WUE\textsubscript{i} (\(\alpha\)) and WUE\textsubscript{inst} (\(\alpha_2\)) besides that of \(\alpha_0\) and \(g_s\) (\(\alpha_0\)), the maximum WUE\textsubscript{i} (WUE\textsubscript{max}) and WUE\textsubscript{inst} (WUE\textsubscript{inst–max}) besides that of \(\alpha_n\) (\(A_{n\text{max}}\)) and \(g_s\) (\(g_s\text{–max}\)), and the corresponding saturation irradiances—will directly help physiologists and modelers investigate the interrelationships among photosynthesis, stomatal behavior, and WUE under changing irradiance conditions.

Meanwhile, the above quantitative traits allow for easier and more extensive evaluation of light-intensity consequences on carbon and water relations among different species and/or PFTs. Such quantitative information, gathered on a wider range of species and/or PFTs, could allow (1) a deeper understanding of interspecific variation in light response strategies (Knapp, 1993; Hetherington and Woodward, 2003; McAusland et al., 2016), (2) a realistic representation of adaptive WUE–I response differences among PFTs into ecosystem modeling.

The explicit models developed in this study can be viewed as an initial step toward filling the gap between investigating the trends of interspecific variation in short-term leaf-scale WUE–I responses and translating the variation into improved process representation in models of plant and ecosystem scales. The findings in this study remain to be validated (1) with species of different growth form and PFT membership (e.g., slower-growing woody species), which could hold different light response strategies (Knapp and Smith, 1989), (2) with daily and seasonal integrals and/or whole-plant estimates of WUE that sometimes could show a low correlation with short-term leaf-scale WUE observations (Medrano et al., 2015), and (3) when leaf gas exchange is subjected to compound effects of other climatic conditions in current and future climate change scenarios.

CONCLUSION

The newly developed models (Eqs. 7 and 10, respectively) allow robust reproduction of the differential single-peaked WUE\textsubscript{sat–I} and WUE\textsubscript{inst–I} trends between C\textsubscript{3} and C\textsubscript{4} species and easy parameterization of key traits defining the trends (\(\alpha_1, I_{\text{sat}}, K_i\) and WUE\textsubscript{sat–max}, \(\alpha_2, I_{\text{inst–sat}}, K_{\text{inst}}, \) and WUE\textsubscript{inst–max}). The models can be employed for fast and accurate assessment of plant WUE\textsubscript{i} and WUE\textsubscript{inst} responses—besides that of photosynthetic and stomatal responses using a consistent modeling framework—across all light-limited, light-saturated, and photoinhibitory light intensities. These findings are useful (1) for breeders screening for ideal genotypes target with maximized photosynthesis capacity and optimized WUE, (2) for plant physiologists quantifying

| Species               | WUE\textsubscript{max} (\mu mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1}) | \(\alpha_2\) (\mu mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1}) | \(\alpha_0\) (\mu mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1}) | \(K_{\text{inst}}\) (\mu mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1}) | \(K_i\) (\mu mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1}) | \(I_{\text{sat}}\) (\mu mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1}) | \(I_{\text{inst–sat}}\) (\mu mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1}) |
|----------------------|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Soybean              | 0.035 ± 0.009 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 2.47 ± 0.10 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 6.99 ± 0.50 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 1.80 ± 0.19 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 2.83 ± 0.19 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 17.8 ± 1.00 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 244 ± 10.03 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} |
| Grain amaranth       | 0.037 ± 0.009 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 2.47 ± 0.10 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 6.99 ± 0.50 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 1.80 ± 0.19 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 2.83 ± 0.19 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 17.8 ± 1.00 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} | 244 ± 10.03 \(\mu\) mol mmol\textsuperscript{-1} m\textsuperscript{-2} s\textsuperscript{-1} |
intra- and/or inter-specific variation in leaf-scale WUE–I responses, and (3) for modelers working on better representation of the coupling between carbon and water processes under dynamic irradiance conditions.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

Z-PY and S-XZ drafted the work. All authors contributed substantially to the completion of this work and critically revised the work. Z-PY, H-JK, and Y-GL secured the funding.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00374/full/supplementary-material
Linares, J. C., and Camarero, J. J. (2012). From pattern to process: linking intrinsic water-use efficiency to drought-induced forest decline. *Glob. Change Biol.* 18, 1000–1015. doi: 10.1111/j.1365-2486.2011.02566.x

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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