Scaling-up From Leaf to Whole-plant Level for Water Use Efficiency Estimates Based on Stomatal and Mesophyll Behavior

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1 Scaling-up from leaf to whole-plant level for water use efficiency estimates based on stomatal and
2 mesophyll behavior

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8 **Abstract**

9 **Aims** Prediction of whole-plant short-term water use efficiency ($WUE_{s,p}$) is essential to indicate plant
10 performance and facilitates comparison across different temporal and spatial scales. Here, the isotope
11 model for $WUE_{s,p}$ was scaled-up from the leaf to the whole-plant level.

12 **Methods** For $WUE_{s,p}$ modelling, leaf gas exchange information, plant respiration and “unproductive”
13 water loss were taken into account. Specifically, in shaping the expression of the $WUE_{s,p}$, we emphasized
14 the role of both stomatal (g_{sw}) and mesophyll conductance (g_m).

15 **Results** The verification showed that estimates of g_{sw} from the coupled photosynthesis ($P_{n,L}$)- g_{sw} model
16 accounting for the effect of soil water stress slightly outperformed the model neglecting the soil water
17 status effect, and the established coupled $P_{n,L}$ - g_m model proved more effective in the estimation of g_m
18 than the previously proposed model. Introducing the two diffusion control functions into the whole-plant
19 model, the developed model for $WUE_{s,p}$ effectively captured its response pattern to different CO_2
20 concentration (C_a) and soil water content (SWC) conditions.

21 **Conclusions** Overall, this study confirmed that accurate estimation of $WUE_{s,p}$ requires an improved
22 predictive accuracy of g_{sw} and g_m . These results have important implications for predicting how plants

respond to climate change.

Keywords: mesophyll conductance, stomatal conductance, scaled-up, water use efficiency, whole-plant level.

1. Introduction

The ratio of carbon assimilation to water loss, water use efficiency (WUE), is essential to optimize plant water use (Medrano et al., 2015) and can be defined at different temporal and spatial scales. At the leaf level, WUE describes the leaf net photosynthetic rate ($P_{n,L}$) relative to the leaf transpiration rate (E_L). Both processes are controlled by stomatal conductance (g_{sw}). The $P_{n,L}$ is also controlled by mesophyll conductance (g_m), in addition to g_{sw} , as recent studies demonstrated that mesophyll resistance is not negligible (Flexas et al., 2006; Warren and Adams, 2006) and may be as important as stomatal conductance (Pérez-Martín et al., 2009). At the whole-plant level, although all photosynthetic and non-photosynthetic parts contribute to respiration and water loss, the canopy accounts for the largest part of carbon assimilation and transpiration water loss. Therefore, changes in g_{sw} (and or g_m) may result in a decrease or increase in whole-plant WUE, especially at smaller temporal scales.

Investigating whole-plant WUE at smaller temporal scales not only facilitates our understanding of whole-plant long-term WUE and the underlying mechanism, but also allows us to compare across different temporal and spatial scales. There have, however, been a few attempts to relate g_{sw} (and or g_m) to whole-plant WUE at smaller temporal scales, or models to predict response pattern of whole-plant short-term WUE ($WUE_{s,p}$) to environmental changes. Estimation of $WUE_{s,p}$ is frequently conducted on the assumption that leaf short-term WUE ($WUE_{s,L}$) is representative of $WUE_{s,p}$ (Hu et al., 2010). However, there may be a gap between daily integrals of leaf and whole-plant WUE, as carbon and water loss from non-photosynthetic tissue can result in a decrease in $WUE_{s,p}$ whilst not affecting WUE_{s-L} .

Therefore, it is critical to obtain adequate predictions of whole-plant WUE at smaller temporal scales.

It has been suggested that the leaf WUE model can be scaled to the whole-plant level by taking into account “unproductive” water loss and carbon use by respiration, independent of photosynthesis (Farquhar and Richards, 1984; Farquhar et al., 1989; Hubick and Farquhar, 1989). Built upon this concept, the Farquhar et al. (1989) model relates leaf gas exchange properties and carbon discrimination to whole-plant WUE, while it ignores the effect of mesophyll resistance (the inverse of g_m) on carbon discrimination (Δ). It has been pointed out that the contribution of g_m to Δ needs to be considered (Seibt et al., 2008), and consequently, g_m might have to be incorporated in the approach of Farquhar et al. (1989) to accurately predict whole-plant WUE. This hypothesis was supported by our recent findings (Zhang et al., 2019), which found that the whole-plant model emphasizing the role of g_m outperformed the Farquhar et al. (1989) model. Despite years of research, the three most widely used approaches for determining g_m , based on the high number of gas exchange properties or measurements of gas exchange combined with chlorophyll fluorescence or carbon isotope discrimination (Pons et al., 2009), use complex parameters and are associated with complicated measurements. These drawbacks limit the easy determination of g_m . In contrast, the soil water content and potential g_m (unstressed g_m , $g_{m,p}$) dependent empirical model proposed by Keenan et al. (2010), can easily be used. Unfortunately, the model is still flawed in reflecting the influence of other environmental factors and gas exchange properties on g_m . A practical and relatively simple representation of mesophyll behavior may lie at the heart of a valid and useful prediction of $WUE_{s,p}$. Furthermore, the revised whole-plant model (Zhang et al., 2019) for $WUE_{s,p}$ included the presence of g_{sw} , in addition to g_m , thereby representing the linkage between $WUE_{s,p}$ and g_{sw} . For representation of stomatal behavior, although several models have been proposed, including the simple coupled photosynthesis-stomatal conductance ($P_{n,L}$ - g_{sw}) model and its modified versions, it

remains unclear which one is the most useful approach. In general, the WUE model scaling from leaf to whole-plant level needs to be revised and improved based on well-modeled stomatal and mesophyll behavior.

In this study, we developed a model to estimate g_m based on the empirical relationship between g_m and $P_{n,L}$ (i.e., the coupled photosynthesis-mesophyll conductance model). The revised g_m model and the previously established g_{sw} model were then incorporated into the whole-plant WUE model to estimate $WUE_{s,p}$. Measurements of whole-plant net CO_2 gas exchange and transpiration were conducted concurrently, allowing us to calculate the actual $WUE_{s,p}$ and to compare the measured results with simulations obtained from the developed whole-plant WUE model. Our aim was, first, to establish a reliable model for g_m ; second, to check the applicability of the whole-plant WUE model scaled from the leaf level, based on estimations of stomatal and mesophyll behavior.

2. Theoretical background

2.1 Coupled g_{sw} - $P_{n,L}$ model

Previous studies found that leaf stomatal conductance (g_{sw} , $\text{mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) is highly correlated with photosynthesis ($P_{n,L}$, $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Based on this, a series of models on the basis of the linear relationship between g_{sw} and $P_{n,L}$ has been proposed (Ball et al., 1987; Jarvis, 1976; Leuning et al., 1990). By incorporating the effect of leaf-to-air vapor pressure deficit (D), Leuning et al. (1995) established an alternative coupled $P_{n,L}$ - g_{sw} model based on former studies:

$$g_{sw} = g_{0,sw} + g_1 P_{n,L} \frac{f(D)}{C_s - \Gamma}, \quad (1)$$

where $g_{0,sw}$ and g_1 are fitted parameters and $g_{0,sw}$ is considered to represent the residual stomatal conductance ($\text{mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); C_s is the leaf surface CO_2 concentration ($\mu\text{mol}\cdot\text{mol}^{-1}$); Γ is the CO_2 compensation point ($\mu\text{mol}\cdot\text{mol}^{-1}$); $f(D)$ is the vapor pressure deficit-dependent function. To describe the

effect of D on stomatal behavior, numerous expressions have been introduced (Ball et al., 1987; Collatz et al., 1991; Leuning et al., 1995; Lloyd, 1991; Lohammer et al., 1980; Yu et al., 2001). Lloyd (1991) and Yu et al (2001) have consistently found that the precision of estimation was highest when imposing the function $f(D) = h_s$, with h_s referring to relative humidity at leaf surface in %. Thus, we adopted the expression $f(D) = h_s$ in the Leuning et al. (1995) model.

The model introduced by Leuning et al. (1995) has been widely used to predict gas exchange properties at the leaf scale (Egea et al., 2011a; Yu et L., 2001), albeit without taking into account the response of water stress. To overcome this limitation, Egea et al. (2011b) proposed an improved model, which incorporated a soil water stress-dependent function, $f(\theta_s)$, to describe the behavior of gas exchange properties:

$$g_{sw} = g_{0,sw} + g_1 P_{n,L} \frac{f(\theta_s)f(D)}{C_s - \Gamma} \quad (2)$$

2.2 Coupled g_m - P_n model

Models which can easily represent mesophyll behavior in response to environmental drivers are still scarce. Considering restrictions of soil water stress on g_m ($\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), Keenan et al. (2010) proposed a function to predict the linkage between g_m and soil water status:

$$g_m = f(\theta_m) g_{m,p}, \quad (3)$$

where $f(\theta_m)$ is the mesophyll conductance limitation function, which depends on soil water stress; $g_{m,p}$ is the potential (unstressed) g_m . This model has been used to represent the feedback of g_m to soil water stress (Egea et al., 2010b), but does not take into account the response of mesophyll behavior to other environmental drivers, such as C_a . In fact, g_m is affected by increases or decreases in C_a , and even changes more subtly with changes in C_a than in g_{sc} ($g_{sc} = g_{sw} / 1.6$) (Flexas et al., 2007). Previous studies have observed that the $P_{n,L}$ increased linearly with g_m (Douthe et al., 2012; Misson et al., 2010; Xu and Zhou,

2011), which prompted us to establish a coupled $P_{n,L}$ - g_m function to model g_m by imposing similar limitation functions to mesophyll behavior as that to stomatal behavior. Based on the empirical relationship between $P_{n,L}$ and g_m , the proposed model is as follows:

$$g_m = g_{0,m} + g_2 P_{n,L} \frac{f(\theta_m) f(D)}{C_s - \Gamma}, \quad (4)$$

where $g_{m,0}$ and g_2 are fitted parameters and $g_{0,m}$ is considered to represent the residual mesophyll conductance ($\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

The soil water stress-dependent limitation functions ($f(\theta_i)$) were provided previously (Keenan et al., 2010; Porporato et al., 2001):

$$f(\theta_i) = \begin{cases} 1 & \theta \geq \theta_c \\ \left[\frac{\theta - \theta_w}{\theta_c - \theta_w} \right]^{q_i} & \theta_w \leq \theta \leq \theta_c, \\ 0 & \theta \leq \theta_w \end{cases} \quad (5)$$

where θ is the actual soil water content (%); θ_c and θ_w are soil water content levels at field capacity (26.20%) and permanent wilting point (4.08%), respectively; and the subscript $i = s$ and m represent stomatal and mesophyll limitations, respectively. In this study, the selected values for tunable parameters of q_s and q_m were 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50, within the previously reported range (Egea et al., 2011b; Keenan et al., 2010).

2.3 Leaf and whole-plant WUE model

The leaf instantaneous water use efficiency ($\text{WUE}_{i,L}$, $\text{mmol} \cdot \text{mol}^{-1}$) is the ratio of leaf net photosynthetic rate ($P_{n,L}$, $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) to transpiration rate (E_L , $\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) (Farquhar and Richards 1984):

$$\text{WUE}_{i,L} = \frac{P_{n,L}}{E_L} = \frac{P_{n,L}}{g_{sw} D} \quad (6)$$

Substituting the Egea et al. (2011b) model (Eq. (2)) and the Leuning et al. (1995) model (Eq. (1)) into

Eq. (6), we obtain the following formulas, respectively:

$$\text{WUE}_{i,L} = \frac{P_{n,L}}{D} \times \frac{C_s - \Gamma}{(C_s - \Gamma)g_{0,sw} + g_1 P_{n,L} f(\theta_s) f(D)} \quad (7)$$

$$\text{WUE}_{i,L} = \frac{P_{n,L}}{D} \times \frac{C_s - \Gamma}{(C_s - \Gamma)g_{0,sw} + g_1 P_{n,L} f(D)} \quad (8)$$

The $\text{WUE}_{i,L}$ inferred from Eq. (7) with well parameterized q_s ($q_s = 0.25$, see Results 3.1) is model configuration 1, and that inferred from Eq. (8) is model configuration 2.

The whole-plant instantaneous water use efficiency ($\text{WUE}_{i,p}$, $\text{mmol} \cdot \text{mol}^{-1}$) is the ratio of whole-plant net photosynthetic rate ($P_{n,p}$, $\mu\text{mol} \cdot \text{h}^{-1}$) to transpiration rate (E_p , $\text{mmol} \cdot \text{h}^{-1}$) (Farquhar et al., 1989). Considering respiration and water loss from non-photosynthetic organs, the ratio of instantaneous net photosynthesis to transpiration can be scaled from the leaf to the whole-plant level:

$$\text{WUE}_{i,p} = \frac{P_{n,p}}{E_p} = \frac{P_{n,L}}{E_L} \times \frac{(1 - \phi_{c,i})}{(1 + \phi_{w,i})} = \frac{P_{n,L}}{g_{sw} D} \times \frac{(1 - \phi_{c,i})}{(1 + \phi_{w,i})}, \quad (9)$$

where $\phi_{c,i} = (3.6P_{n,L} \times LA - P_{n,p}) / (3.6P_{n,L} \times LA)$, with LA referring to plant total leaf area in m^2 is the proportion of respiration from non-photosynthetic parts (twigs and stem) during the daytime, and $\phi_{w,i} = (E_p - 3.6E_L \times LA) / (3.6E_L \times LA)$ is the proportion of water loss from non-photosynthetic parts during the daytime. Similarly, we substituted the simulated g_{sw} , calculated via the Egea et al. (2011b) model (Eq. 2) and the Leuning et al. (1995) model (Eq. 1) into Eq. (9), obtaining the following formulas, respectively:

$$\text{WUE}_{i,p} = \frac{P_{n,L}}{D} \times \frac{C_s - \Gamma}{(C_s - \Gamma)g_{0,sw} + g_1 P_{n,L} f(\theta_s) f(D)} \times \frac{(1 - \phi_{c,i})}{(1 + \phi_{w,i})} \quad (10)$$

$$\text{WUE}_{i,p} = \frac{P_{n,L}}{D} \times \frac{C_s - \Gamma}{(C_s - \Gamma)g_{0,sw} + g_1 P_{n,L} f(D)} \times \frac{(1 - \phi_{c,i})}{(1 + \phi_{w,i})} \quad (11)$$

The $\text{WUE}_{i,p}$ inferred from Eq. (10) with the tunable parameter $q_s = 0.25$ is model configuration 1, while that inferred from Eq. (11) is model configuration 2.

The whole-plant short-term water use efficiency ($\text{WUE}_{s,p}$) is the ratio of whole-plant cumulative

CO₂ assimilation to water loss. At the diel time scale, not only the role of respiration and water loss from non-photosynthetic parts (twigs and stem) during the daytime need to be included, but also respiration and water loss from whole parts (leaf, twigs and stem) during the nighttime contribute substantially to WUE_{s,p}. When all these processes are taken into account, the time-integrated WUE_{s,p} is as follows:

$$WUE_{s,p} = \frac{\int P_{n,p}}{\int E_p} = \frac{\int P_{n,L}}{\int E_L} \times \frac{(1 - \phi_{c,s})}{(1 + \phi_{w,s})} = \frac{\int P_{n,L}}{g_{sw} D} \times \frac{(1 - \phi_{c,s})}{(1 + \phi_{w,s})}, \quad (12)$$

where $\phi_{c,s} = (3.6P_{n,L} \times LA - P_{n,p} + R_p) / (3.6P_{n,L} \times LA)$, with R_p referring to nighttime respiration in mmol·h⁻¹ is the proportion of respiration from non-photosynthetic parts (twigs and stem) during the whole time and from leaves during the nighttime; $\phi_{w,s} = (E_p - 3.6E_L \times LA + E_d) / (3.6E_L \times LA)$, with E_d referring to nighttime transpiration in mol·h⁻¹ is the proportion of water loss from non-photosynthetic parts (twigs and stem) during the whole time and from leaves during the nighttime. The above time integral is denoted as $\int$. According to Fick's law:

$$\frac{P_{n,L}}{g_{sw}} = \frac{C_a}{1.6} \times \left(1 - \frac{C_i}{C_a}\right), \quad (13)$$

where C_i is the leaf intercellular CO₂ concentration (μmol·mol⁻¹). The photosynthetic ¹³C discrimination (Δ, ‰) reflects the physiological properties over short time scales (Gessler et al., 2004; Hu et al., 2009; Kodama et al. 2008). From the variant of the Farquhar et al. (1982) classical model, including the effect of g_m on Δ, the short-term C_i / C_a ratio can be written as follows:

$$\frac{C_i}{C_a} = \frac{\Delta - a + (b - a_m) \frac{g_{sw}}{1.6g_m}}{b - a + (b - a_m) \frac{g_{sw}}{1.6g_m}}, \quad (14)$$

where a is the fractionation associated with the atmospheric CO₂ diffusion at the boundary layer (2.9‰); a_m is the fractionation of CO₂ diffusion and dissolution in the liquid phase (1.8‰); b is the fractionation during carboxylation (29‰); $\Delta = (\delta^{13}C_a - \delta^{13}C_i) / (1 + \delta^{13}C_i)$, with $\delta^{13}C_a$ and $\delta^{13}C_i$ referring to $\delta^{13}C$ of

atmospheric CO₂ and water-soluble organic materials (WSOM, fast-turn-over carbohydrates) in leaves, respectively.

Substituting Eq. (13) and Eq. (14) into Eq. (12), we obtain the following equation:

$$WUE_{s,P} = \frac{C_a}{1.6D} \times \frac{b - \Delta}{b - a + (b - a_m) \frac{g_{sw}}{g_m}} \times \frac{(1 - \phi_{c,s})}{(1 + \phi_{w,s})} \quad (15)$$

Similar to the simulation of WUE_{i,L}, two model configurations were applied in Eq. (15), and we obtained the following equations:

$$WUE_{s,P} = \frac{C_a}{1.6D} \times \frac{(1 - \phi_{c,s})}{(1 + \phi_{w,s})} \times \frac{b - \Delta}{b - a + (b - a_m) \times \frac{(C_s - \Gamma)g_{0,sw} + g_1 P_{n,L} f(\theta_s) f(D)}{(C_s - \Gamma)g_{0,m} + g_2 P_{n,L} f(\theta_m) f(D)}} \quad (16)$$

$$WUE_{s,P} = \frac{C_a}{1.6D} \times \frac{(1 - \phi_{c,s})}{(1 + \phi_{w,s})} \times \frac{b - \Delta}{b - a + (b - a_m) \times \frac{(C_s - \Gamma)g_{0,sw} + g_1 P_{n,L} f(\theta_s) f(D)}{(C_s - \Gamma) f(\theta_m) g_{m,p}}} \quad (17)$$

Obviously, in model configuration 1 (Eq. (16)), g_m was calculated by Eq. (4) with well parameterized q_m ($q_m = 0.25$, see Results 3.2), and g_{sw} was calculated by Eq. (2) with well parameterized q_s ($q_s = 0.25$, see Results 3.1). In model configuration 2 (Eq. (17)), g_m was calculated by Eq. (3) with well parameterized q_m ($q_m = 0.50$, see Results 3.2), and g_{sw} was calculated by Eq. (1).

3. Material and Methods

3.1 Experimental design and management

The experiment was carried out in April 2018 at the Chinese Forest Ecosystems Research Network (116°05'E, 40°03'N), situated at Western Hill, Beijing, North China. In the experiment, 7-year-old *Platycladus orientalis* saplings with the same genotype of a temperate origin were transplanted into 15.51-L pots (one sampling per pot) containing soil collected from a local *Platycladus orientalis* stand. These samplings, with similar growth status and canopy structure (approximately 1.4 m high), were

grown in a greenhouse. After acclimation in the greenhouse for 2 months, saplings were moved to growth chambers (FH-230, Taiwan Hipoint Corporation, Kaohsiung City, Taiwan) and subjected to a nested design with three C_a levels and five SWC regimes.

The controlled environment (light, air temperature, and relative humidity) in the growth chambers was set to simulate natural growth conditions. From 07:00 to 19:00 (simulating daytime), all lights were turned on, with 60% relative humidity and 25°C. From 19:00 to 07:00 (simulating nighttime), all lights were turned off, with 80% relative humidity and 18°C. To realize orthogonal treatments, two growth chambers were used: one with ambient C_a (400 $\mu\text{mol}\cdot\text{mol}^{-1}$) and another one with elevated C_a (600 and 800 $\mu\text{mol}\cdot\text{mol}^{-1}$) levels. Each C_a treatment was subjected to five SWC regimes: 1) 35%-45% of field capacity, FC, (simulating severe drought), 2) 50%-60% of FC (moderate drought), 3) 60%-70% of FC (mild drought), 4) 70%-80% of FC (well-watered), and 5) 95%-100% of FC (excessively watered). The FC of the potting soil was 26.20%. For the sake of calculative simplicity, we assumed that the SWC gradient was: 1) 10.48%, 2) 14.41%, 3) 17.03%, 4) 19.65%, and 5) 26.20%, respectively. To reduce soil evaporation, the surface of the potting soil was covered with an approximately 2-cm layer of perlite. The desired SWC regimes were achieved following the method from Zhang et al. (2019). Each treatment ($C_a \times \text{SWC}$) lasted for 30 days and had three pot-grown saplings as replicates. One growth chamber was able to hold five pots, and the experiment was performed progressively from June to November 2018. The pots were rearranged frequently to exclude position effects.

3.2 Measurements

3.2.1 Whole-plant carbon balance and measurement

After the saplings had been subjected to the 30-day $C_a \times \text{SWC}$ treatment, whole-plant carbon balance was measured inside the growth chambers using the static chamber as designed by Jasoni et al.

(2005). The static chamber measured $50 \times 50 \times 150$ cm, and in its interior, a pocket weather meter was incorporated (Kestrel 5500, Nielsen-Kellerman, Boothwyn, PA, USA) to monitor air temperature and pressure. To avoid soil respiration, the substrate surface was tightly sealed with airtight plastic film as described by Escalona et al. (2013). Prior to each measurement, the sapling was enclosed in the static chamber, and the fan on the top of the static chamber was turned on for 30 seconds to ensure that the flux was mixed well. The C_a in the static chamber was measured by an infrared gas analyzer (Li-8100, Li-Cor, Lincoln, NE, USA), starting after the flux was well mixed (initial C_a , i.e., C_0) and finishing after the measurement had lasted for 3 minutes (final C_a , i.e., C_1). Measurements for each sapling were repeated three times and conducted at 9:00, 13:00, and 17:00 during daytime and at 22:00, 2:00, and 6:00 during nighttime. The whole-plant daytime net photosynthetic rate ($P_{n,p}$) and the nighttime respiratory rate (R_p) were calculated as follows:

$$P_{n,p} = \frac{V}{\Delta t} \times \frac{273.15}{T_a} \times \frac{P}{101325} \times \frac{1}{22.41} \times (C_0 - C_1) \times \frac{60}{1000}, \quad (18)$$

$$R_{n,p} = \frac{V}{\Delta t} \times \frac{273.15}{T_a} \times \frac{P}{101325} \times \frac{1}{22.41} \times (C_1 - C_0) \times \frac{60}{1000}, \quad (19)$$

where V is the chamber volume (L) and $\Delta t = 3$ min is the time duration. The $P_{n,p}$ and R_p were calculated from values measured during daytime and nighttime, respectively.

3.2.2 Whole-plant transpiration measurements

The whole-plant daytime transpiration rate (E_p) was measured from the beginning until the end of the experiment by a Flow 32-1K system (Dynamax, Houston, TX, USA). The Flow 32-1K system includes gauges installed at approximately 25 cm above the stem base and a CR1000 logger (Campbell Scientific, Logan, UT, USA), which continuously collected E_p data every 15 min.

The whole-plant nighttime transpiration rate (E_d) was measured by mass loss during the night. Total plant nighttime transpiration was obtained from the difference in pots weight at the onset (19:00) and

end of night (7:00). During plant nighttime transpiration measurements, the substrate surface was tightly sealed with airtight plastic film as described by Escalona et al. (2013) to avoid soil evaporation. Measurements were made every 3 days.

The measured $WUE_{i,p}$ was the ratio between $P_{n,p}$ to E_p ($P_{n,p} / E_p$), and the measured $WUE_{s,p}$ was the ratio between accumulative carbon gain and cumulative water loss, that is, $WUE_{s,p} = (P_{n,p} - R_p) / (E_p + E_d)$.

3.2.3 Leaf gas exchange and stable isotope analysis

On the day of whole-plant carbon balance measurements, leaf gas change properties ($P_{n,L}$, E_L , g_s , and C_i), leaf temperature (T_L), and leaf surface relative humidity (RH) were measured inside the growth chambers on fully expanded leaves, using an open gas exchange system (Li-6400, Li-Cor, Lincoln, NE, USA). The measurements were conducted at different positions (upper, middle, and lower crown) and made on at least three different leaves in each canopy layer at 9:00, 13:00, and 17:00. No significant differences ($p > 0.05$) in these measurements among different canopy layers were observed. Almost all leaves were exposed to similar light intensities, and thus, the effect of internal leaves was not considered. In this study, we assumed that a period of 30 days was long enough for saplings to be subjected to the treatments, according to our pilot experiment as described by Zhang et al. (2019). Measured leaf instantaneous water use efficiency ($WUE_{i,L}$) was calculated as the ratio between $P_{n,L}$ and E_L ($P_{n,L} / E_L$).

The leaves used for gas exchange measurements were detached, immediately wrapped in tinfoil, and preserved in liquid nitrogen. Leaf water-soluble organic matter (WSOM) was extracted using the same method as described by Zhang et al. (2019). The obtained WSOM was dried and then combusted in an elemental analyzer (Flash EA 1112, Thermo Finnigan, Germany) coupled to a continuous-flow stable isotope ratio mass spectrometer (DELTAplusXP, Thermo Finnigan, Germany). The $\delta^{13}C$ of leaf

WSOM ($\delta^{13}\text{C}_i$) was analyzed using the stable isotope ratio mass spectrometer with a precision of $\pm 0.1\%$.

In addition, at the end of each treatment, atmosphere samples from the growth chamber were also collected (at least three replicates), and the $\delta^{13}\text{C}$ of the atmosphere ($\delta^{13}\text{C}_a$) was analyzed by the stable isotope ratio mass spectrometer. Measured g_m was obtained by carbon isotope discrimination combined with gas exchange measurements as described by Zhang et al. (2019).

3.2.4 Whole-plant total leaf area measurement

At the end of the experiment, saplings were harvested and separated into different parts. Leave subsample fresh weight (FW_{sub}) and leaf area (LA_{sub}) were immediately determined, and these leaves were subsequently dried at 80°C for 48 h in an oven to obtain their dry weight (DW_{sub}). The dry weights of the remaining harvested leaves (DW_{rest}) were also determined. The whole-plant total leaf area (LA) was calculated as follows:

$$LA = R_D \times DW = (LA_{\text{sub}} / DW_{\text{sub}}) \times (DW_{\text{sub}} + DW_{\text{rest}} + DW_{\text{iso}}). \quad (20)$$

In this equation, R_D is leaf area per dry weight ($\text{m}^2 \cdot \text{g}^{-1}$), DW is whole-plant total dry weight (g), and DW_{iso} ($= FW_{\text{iso}} \times DW_{\text{sub}} / FW_{\text{sub}}$, with FW_{iso} referring to fresh weight of leaves used for isotope analysis in g) is dry weight of leaves used for isotope analysis (g).

3.3 Data analysis

All statistical analyses were conducted using SPSS 19.0. The influences of C_a and SWC on mean variables of g_{sw} , g_m , and WUE (including $\text{WUE}_{i,L}$, $\text{WUE}_{i,P}$, and $\text{WUE}_{s,P}$) were determined by two-way analysis of variance (ANOVA). Results were considered statistically significant at $p < 0.05$. Deviations of the modeled g_{sw} , g_m , and WUE from their measurements were absolute differences between the modeled and measured values. Relationships between the measured and modeled values in g_{sw} , g_m , and WUE were assessed using general linear regression analysis.

4. Results

4.1 Measured and modeled responses of g_{sw} to SWC and C_a

Changes in SWC and C_a led to significant effects on g_{sw} ($p < 0.05$), with a maximum of $0.0963 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at $C_{400} \times 19.65\%$ of SWC and a minimum of $0.0155 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Fig. 1). Irrespective of C_a , g_{sw} increased sharply as water stress was alleviated, and this effect was less evident when SWC exceeded 17.03% and even decreased when g_{sw} peaked at 19.65% of SWC. In all cases, g_{sw} increased with elevated C_a (Fig. 1).

Fig. 1 Response of measured leaf stomatal conductance (g_{sw}) to three CO_2 concentrations (C_a) $\times$ five soil water contents (SWC) for *Platycladus orientalis* saplings. Data represent mean values $\pm$ SD.

In the absence of additional parameterization associated with soil water stress, the g_{sw} simulated by the coupled $P_{n,L}$ - g_{sw} model (Eq. 1) decreased in response to elevated C_a , but increased as the soil water status improved and reached maximum values at 19.65% of SWC, with a slight decrease thereafter (Fig. 2). In contrast, when the effect of soil water stress was incorporated in the coupled $P_{n,L}$ - g_{sw} model (Eq. 2), the simulated g_{sw} generally increased as SWC increased, regardless of the value imposed by q_s . More specifically, at any given C_a , the amplification in simulated g_s by SWC was much greater as the q_s

decreased with SWC below 17.03%, while the opposite trend was observed when SWC exceeded 19.65%. When the SWC improved from 17.03 to 19.65%, the reduction of q_s led to a higher amplification in simulated g_{sw} at ambient C_a (C_{400}) (Fig. 2a), while the amplitude was similar by imposing different q_s levels at elevated C_a (C_{600} and C_{800}) (Figs. 2b and 2c).

Fig. 2 Leaf stomatal conductance (g_{sw}) estimated by different models (Eqs. (1) and (2)) of *Platycladus orientalis* saplings under five soil water contents (SWC) $\times$ three CO_2 concentrations (C_a) conditions. Data represent mean values $\pm$ SD.

The regression analysis between measured and modeled g_{sw} is presented in Figure 3. When g_{sw} was calculated from Eq. (2), the modeled values were significantly related with measurements ($p < 0.01$), and the correlation coefficient R^2 decreased from 0.88 to 0.68 as q_s increased from 0.25 to 1.50 (Fig. 3). The g_{sw} calculated from Eq. (1) was also significantly correlated with measurements ($p < 0.01$), with R^2 being equal to 0.87. However, estimates of g_{sw} from Eq. (2), using $q_s = 0.25$, showed slightly better agreements (higher R^2 and slope closer to 1) with measurements than estimates from Eq. (1), and the former caused less deviation ($0.0084 \pm 0.0053 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) from the measured results than the latter ($0.0086 \pm 0.0062 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) (Fig. 3), indicating that Eq. (2) using $q_s = 0.25$ for g_{sw} outperformed Eq. (1).

Fig. 3 Correlation analysis between measured and modeled leaf stomatal conductance (g_{sw}) values of different models (Eqs. (1) and (2)).

4.2 Measured and modeled responses of g_m to SWC and C_a

The g_m was significantly influenced by SWC and C_a treatments ($p < 0.05$), with a pattern similar to g_{sw} (Figs. 1 and 4). The g_m ranged between 0.0131 and 0.0571 $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, which was significantly lower than g_{sw} ($p < 0.05$). In all cases, elevation of C_a decreased g_m . The g_m increased rapidly with SWC; however, the amplification was substantially less evident as SWC exceeded 17.03% and even decreased as SWC increased from 19.65% to 25.55% (Fig. 4).

Fig. 4 Response of measured leaf mesophyll conductance (g_m) to three CO_2 concentrations (C_a) $\times$ five soil water contents (SWC) for *Platycladus orientalis* saplings. Data represent mean values $\pm$ SD.

The simulated g_m , calculated by the SWC- and $g_{m,0}$ -dependent function (Eq. (3)) and the coupled $P_{n,L}$ - g_m model (Eq. (4)), is presented in Fig. 5. Regardless of the model used, the simulated g_m decreased in response to an increased C_a (Fig. 5). Applying Eq. (3), the simulated g_m approximately increased linearly with increasing SWC levels and tended to be higher with decreasing levels of the imposed q_m , except at excessive-watered conditions (25.55% of SWC). In contrast, the simulated g_m calculated by Eq. (4), using various q_m values, produced a more complicated tendency to SWC. At any given C_a , the amplification was more obvious due to the rapid reduction in q_m at SWC levels below 17.03%, while the opposite pattern was observed when SWC exceeded 19.65%. When the SWC increased from 17.03 to 19.65%, the reduction in q_m induced a decrease in the amplification of the simulated g_m at ambient C_a (Fig. 5b), while the amplification was similar for different q_s levels at elevated C_a (C_{600} and C_{800}) (Figs. 5d and 5f).

Fig. 5 Leaf mesophyll conductance (g_m) estimated by different models (Eqs. (3) and (4)) under five soil water contents (SWC) × three CO_2 concentrations (C_a). Data represent mean values $\pm$ SD.

The relationships between measured and modeled g_m , calculated by Eqs. (3) and (4) using various q_m values, are shown in Figs. 6a and 6b. Both model approaches produced significant relationships between simulated and measured results ($p < 0.05$). Using Eq. (3), the highest R^2 between measured and

modeled g_m was observed when the estimation was calculated from $q_m = 0.50$ (Fig. 6a). In contrast, when g_m was calculated from Eq. (4), the R^2 between measured and modeled results decreased as q_m increased (Fig. 6b). Setting the same q_m value, Eq. (4) led to the higher R^2 (0.44 ~ 0.79) between estimated and measured results than that the Eq. (3) (0.34 ~ 0.52), and the former caused less deviation ($0.0055 \pm 0.0038 \sim 0.0097 \pm 0.0046$) from measurements than the latter ($0.0090 \pm 0.0058 \sim 0.0159 \pm 0.0078$) (Figs. 6a and 6b).

Fig. 6 Correlation analysis between measured and modeled leaf mesophyll conductance (g_m) using different models (Eqs. (3) and (4)).

4.3 Measured and modeled instantaneous WUE at leaf and whole-plant level

At the leaf level, elevated C_a significantly enhanced the measured $WUE_{i,L}$ ($p < 0.05$). Variations in SWC also significantly influenced the measured $WUE_{i,L}$ ($p < 0.05$), which increased as the severe drought was alleviated (SWC increased from 10.48% to 14.41%), followed by a decline with increasing SWC levels and by constant values when the SWC was above 19.65% (Fig. 7a). In both model configurations, the response pattern of simulated $WUE_{i,L}$ to $SWC \times C_a$ was similar to that of measured values, except

that the simulated $WUE_{i,L}$ increased as the SWC improved from 14.41 to 17.03% at C_{400} and C_{600} , departing from the observed decreasing trend (Fig. 7a).

At the whole-plant level, a significant effect of C_a and SWC on the measured instantaneous WUE ($WUE_{i,P}$) was observed ($p < 0.05$). In general, the $WUE_{i,P}$ was higher at elevated C_a levels (Fig. 7b). When the SWC increased from 10.48% to 14.41%, the percentage increase in the measured $WUE_{i,P}$ was more pronounced at C_{800} than at C_{400} and C_{600} . In response to further increases in SWC, the measured $WUE_{i,P}$ generally decreased sharply, but this impact was less evident when the soil water status was more than 19.65% of SWC. In both model configurations, the measured and simulated $WUE_{i,P}$ values were similar in their response patterns to $SWC \times C_a$, except when the SWC increased from 14.41% to 17.03% at C_{400} and C_{600} (Fig. 7b).

Fig. 7 Measured and simulated leaf ($WUE_{i,L}$; a) and whole-plant level instantaneous water use efficiency ($WUE_{i,P}$; b) in different model configurations of *Platycladus orientalis* saplings under five soil water contents (SWC) $\times$ three CO_2 concentrations (C_a) conditions. Data represent mean values $\pm$ SD.

At the leaf and the whole-plant level, the measured and modeled instantaneous WUE values were

significantly correlated in both model configurations ($p < 0.01$); however, the R^2 values in model configuration 1 (C1) were slightly higher than those in model configuration 2 (C2) (Fig. 8). Estimates of $WUE_{i,L}$ ($WUE_{i,P}$) in C1 caused a deviation of 3.12 ± 2.44 (2.59 ± 1.86) $\text{mmol} \cdot \text{mol}^{-1}$ from the measured results, which was slightly less than that caused in C2 with a deviation of 3.14 ± 2.52 (2.62 ± 1.90) $\text{mmol} \cdot \text{mol}^{-1}$ (Fig. 8). This indicates that the C1 for $WUE_{i,L}$ and $WUE_{i,P}$ behaved slightly better than the C2.

Fig. 8 Correlation analysis between measured and modeled results of leaf instantaneous water use efficiency ($WUE_{i,L}$) estimated by different model configurations (a), as well as between measured and modeled results of whole-plant instantaneous water use efficiency ($WUE_{i,P}$) estimated by different model configurations (b).

4.4 Comparison of measured and modeled $WUE_{s,P}$ values

The measured and simulated $WUE_{s,P}$ values are shown in Figure 9. At severe drought (10.48% of SWC), the measured $WUE_{s,P}$ peaked at C_{600} and was lowest at C_{800} , while the simulated $WUE_{s,P}$, in both model configurations, reached its maximum at C_{600} and its minimum at C_{400} (Fig. 9). At an improved soil water status (SWC at 14.41% ~ 25.55%), the measured and simulated $WUE_{s,P}$ values significantly increased due to elevated C_a levels ($p < 0.01$). The measured $WUE_{s,P}$ was also significantly influenced

by SWC, generally responding in a similar manner as the measured $WUE_{i,p}$ in response to SWC. When the saplings were subjected to an SWC of 14.41% ~ 25.55% of SWC, in C1, the response pattern of simulated $WUE_{s,p}$ to SWC was consistent with that of the measured values. In contrast, in C2, the simulated $WUE_{s,p}$ increased as the soil water status increased from 19.65 to 26.20% at any given C_a , deviating from the response pattern of the measured values (Fig. 9).

C_{400} —●— Measured values —●— Model Configuration 1 —●— Model Configuration 2
 C_{600} —▲— Measured values —▲— Model Configuration 1 —▲— Model Configuration 2
 C_{800} —■— Measured values —■— Model Configuration 1 —■— Model Configuration 2

Fig. 9 Measured and modeled whole-plant short-term water use efficiency ($WUE_{s,p}$) values of *Platycladus orientalis* saplings under five soil water contents (SWC) × three CO_2 concentrations (C_a) conditions. Data represent mean values $\pm$ SD.

In both model configurations, the modeled and measured WUE values were significantly correlated ($p < 0.01$); however, the R^2 values in C1 were higher than those in model C2 (Fig. 10). Estimates of $WUE_{s,p}$ in C1 led to a deviation of $2.77 \pm 2.23 \text{ mmol} \cdot \text{mol}^{-1}$ from the measured values in comparison with that caused by C2, with a deviation of $2.91 \pm 2.95 \text{ mmol} \cdot \text{mol}^{-1}$ (Fig. 10). Therefore, the observed $WUE_{s,p}$ could be better represented by C1 than by C2.

● Model Configuration 1 ● Model Configuration 2

Fig. 10 Correlation analysis between measured and modeled whole-plant short-term water use efficiency (WUE_{s,p}) values.

Discussion

Model performance for estimating g_{sw} and g_m

Soil water stress exclusion in the coupled $P_{n,L}$ - g_{sw} model (Eq. (1)) for response patterns of g_{sw} performed reasonably well under non-limiting soil water conditions (Fig. 3), which is in agreement with previous studies conducted in almond trees (Egea et al., 2011a) and in maize and soybean plants (Yu et al., 2001). In response to contrasting soil water treatments, the combination of a soil moisture-dependent function with the coupled $P_{n,L}$ - g_{sw} model (Eq (2)), if the model was well parameterized (using $q_s = 0.25$), was slightly more capable to represent the observed pattern of g_{sw} than Eq. (1) (Fig. 3). These results align with previous study highlighting the importance of the inclusion of the water stress function in the coupled $P_{n,L}$ - g_{sw} model (Sala and Tenhunen, 1996). However, the addition of the soil water stress-dependent function to the coupled $P_{n,L}$ - g_{sw} model contributed little to the improvement of model performance for g_{sw} . This can be ascribed to the fact that the measured $P_{n,L}$ incorporated the effect of the

soil water status, resulting in the estimation of g_{sw} from the coupled A_n - g_{sw} model account for the effect of soil water.

The Keenan et al. (2010) model (Eq. (3)) for g_m was insufficient to take into account the impact of C_a and was therefore less suitable to simulate g_m (Fig. 6). In contrast, the predictive accuracy improved considerably when estimating g_m using the coupled $P_{n,L}$ - g_m model (Eq. (4)) (Fig. 6). Therefore, the proposed coupled $P_{n,L}$ - g_m model is valid and promising for simulating g_m , despite its phenomenological nature and its dependence upon physiological hypotheses. Furthermore, imposing $q_m = 0.25$ in Eq. (4) provided the best fit with the measured values (Fig. 6), indicating that the limitation strength of g_m was similar to that of g_{sw} . This result conflicts with the general findings that stomatal behavior imposed a higher limitation to photosynthesis than mesophyll behavior (Egea et al., 2011a; Galmes et al., 2007; Grassi and Magnani, 2005); however, this phenomenon may not always occur. Pérez-Martín et al. (2009) observed minor difference between stomatal and mesophyll limitations and reported that stiffer and more sclerophyllous leaves would provide greater mesophyll resistance during CO_2 diffusion. The response pattern of g_m to C_a and SWC resembled that for g_{sw} (Figs. 1 and 4), leading to a tight correlation of g_m with g_{sw} ($P < 0.01$, $R^2 = 0.83$); this has also been observed for Douglas fir (Bögelein et al., 2012; Ethier et al., 2006). The ratio g_m/g_{sw} tends to be higher in drought than in irrigated conditions (Pérez-Martín et al., 2009), which was also the case in this study. This demonstrates that the previously proposed hypothesis that g_{sw} is proportional to g_m with almost a constant ratio (Lauteri et al., 1997; Ohsumi et al., 2007) is not always valid.

Different model configurations for estimating $WUE_{s,p}$

In the proposed short-term WUE model (Eq. (15)), scaling up from leaf to whole-plant level, there are diffusive limitation parameters. The coupled $P_{n,L}$ - g_{sw} model, incorporating the water stress-dependent

function with well parameterized q_s (Eq. (2)), slightly more accurately captured the observed g_{sw} pattern than the model excluding the soil water stress effect (Eq. (1)) (Fig. 3), and the proposed coupled $P_{n,L}$ - g_m model with well parameterized q_m (Eq. (4)) effectively improved the predictive accuracy of g_m compared to the previously introduced $g_{m,p}$ - and SWC-dependent model (Eq. (3)) (Fig. 6). Therefore, the C1 inferred from Eqs. (2) and (4) could more accurately represent the observed $WUE_{s,p}$ than the C2 inferred from Eqs. (1) and (3) (Fig. 10). This leads us to infer that the model scaling-up from leaf to whole-plant level, based on more accurate predictions of stomatal and mesophyll behavior, could be used to estimate $WUE_{s,p}$ with a high level of precision.

The developed model for estimating $WUE_{s,p}$ also contains photosynthetic parameters by introducing the coupled $P_{n,L}$ - g_{sw} and $P_{n,L}$ - g_m models. Rather than estimating $P_{n,L}$ via the photosynthesis model (Jones 1992; Yu et al., 2001), neither model configuration 1 nor model configuration 2 for $WUE_{s,p}$ simulate $P_{n,L}$ from environmental and physiological factors. The estimates of $WUE_{s,p}$ were calculated from measured $P_{n,L}$ values in order to exclude the situation that errors in the representation of g_{sw} and g_m might be compensated or overwhelmed by errors in simulated $P_{n,L}$. In such a situation, we can identify the influence of precision of stomatal and mesophyll modelling on the credibility and accuracy of the developed $WUE_{s,p}$ model. For $WUE_{i,L}$ and $WUE_{i,p}$ modelling, the C1 inferred from the more accurate g_{sw} model incorporating a soil water stress-dependent function (Eq. (2)) slightly outperformed the C2 (Figs. 8a and 8b). However, the R^2 between measured and modeled values of $WUE_{s,p}$ were lower than those of $WUE_{i,L}$ and $WUE_{i,p}$ (Figs. 8 and 10), most likely because involvement of more parameters in the isotope-inferred $WUE_{s,p}$ model could introduce more uncertainties and errors. For example, complications arising from post-photosynthetic carbon isotope fractionations are not considered, as the process is still difficult to assess and largely unknown (Tcherkez et al., 2005; Wingate et al., 2007).

Furthermore, the effects of photorespiration and mitochondrial respiration on photosynthetic ^{13}C discrimination are still subject of debate (Douthe et al., 2012) and were thus ignored.

Uncertainties of $\text{WUE}_{\text{s,p}}$ introduced by g_{sw} and g_{m}

To further determine the uncertainties of $\text{WUE}_{\text{s,p}}$ associated with simulations of stomatal and mesophyll behavior, uncertainty analysis was conducted. Using the most effective approach to reproduce g_{sw} (Eq. (2), with tunable parameter $q_{\text{s}} = 0.25$) and g_{m} (Eq. (4), with tunable parameter $q_{\text{m}} = 0.25$), the average (s.d.) uncertainties in g_{sw} and g_{m} were 17.10% (14.14%) and 15.39% (10.98%), respectively. The $\text{WUE}_{\text{s,p}}$ estimated from C1 caused average uncertainties of 24.09% (21.61%). The relative small discrepancies between mean value and standard deviation in uncertainties of g_{sw} , g_{m} , and $\text{WUE}_{\text{s,p}}$ indicate that these estimation methods were not stable, although model performance was improved. In addition, the $\text{WUE}_{\text{s,p}}$ was more sensitive to g_{sw} than to g_{m} . That is, 10% error in g_{sw} introduced 6.17% error in $\text{WUE}_{\text{s,p}}$, while 10% error in g_{m} introduced less error of 4.48% in $\text{WUE}_{\text{s,p}}$. Although the stomatal and mesophyll limitations were similar to those of the photosynthetic process in this study, the leaf transpiration is exclusively controlled by g_{sw} when the v is almost constant (Seibt et al., 2008; Zhao et al., 2017; Zhang et al., 2019), which could result in the g_{sw} being more influential for $\text{WUE}_{\text{s,p}}$ than for g_{m} .

Overall, the explored model for $\text{WUE}_{\text{s,p}}$, scaling from leaf to whole-plant level, is based on well-characterized coupled $P_{\text{n,L}}-g_{\text{sw}}$ (Eq. (2)) and $P_{\text{n,L}}-g_{\text{m}}$ models (Eq. (4)). However, we recognize that using only data of pot-grown *Platycladus orientalis* saplings acclimated in growth chambers, with relatively similar canopy components (canopy structure, light interception), is not convincing enough for a general verification of the developed modeling approach. It is therefore important to collect data of more plant species to further examine the model. In addition, the $\text{WUE}_{\text{s,p}}$ model should still be explored and

improved, especially for field-grown plants with a complex canopy structure.

Conclusions

In this study, the performances of coupled $P_{n,L}$ - g_{sw} and $P_{n,L}$ - g_m models were evaluated using leaf gas exchange measurements. We found the coupled $P_{n,L}$ - g_{sw} model incorporating the water stress-dependent function with well parameterized q_s (Eq. (2)) agreed slightly better with the measured g_{sw} values than the model excluding the soil water stress effect (Eq. (1)), and the established coupled $P_{n,L}$ - g_m model with well parameterized q_m (Eq. (4)) allowed for a more reliable estimation of g_m than the previously introduced $g_{m,p}$ - and SWC-dependent model (Eq. (3)). Based on the well characterized models describing stomatal and mesophyll behavior, an isotopic model, scaling from leaf to whole-plant level for estimating $WUE_{s,p}$ (Eq. (16)), was then established and validated using a combination of datasets of the leaf and the whole-plant level. The measured $WUE_{s,p}$ was sensitive to environmental conditions, and the developed model for $WUE_{s,p}$ proved effective at capturing response patterns to C_a and SWC. Therefore, introducing the model performing well for g_{sw} and g_m into the Farquhar et al. (1989) model was applicable and represents a promising approach for describing whole-plant WUE at smaller temporal scales.

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Author Contributions Statement

Yonge Zhang designed and performed the experiment. Yonge Zhang analyzed the data and wrote the manuscript. Guodong Jia and Lihua Chen contributed significantly to data analysis, manuscript preparation and practice of experiment. Xinxiao Yu revised the paper and finished the submission.

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