Leaf Temperature and Vapour Pressure Deficit (VPD) Driving Stomatal Conductance and Biochemical Processes of Leaf Photosynthetic Rate in a Subtropical Evergreen Coniferous Plantation

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Abstract: Photosynthesis is arguably the most important biochemical process on Earth, which is dramatically influenced by environmental conditions. How environmental factors drive stomatal conductance and biochemical processes of leaf photosynthetic rate has not been sufficiently investigated in subtropical China. In this study, we analysed the effects of stomatal and biochemical parameters on the photosynthetic rate of native Masson's pine (*Pinus massoniana* Lamb.) and exotic slash pine (*Pinus elliottii* Engelm.) in response to leaf temperature and vapour pressure deficit (VPD) in subtropical China, based on leaf gas exchange measurements in 2016. Our results showed that there was no significant difference in the light-saturated photosynthetic rate (A_{sat}) between native Masson's pine and exotic slash pine. The seasonal patterns of maximum rate of the carboxylation (V_{cmax25}) were basically consistent with seasonal patterns of A_{sat} for both species. The positive effect of leaf temperature on A_{sat} was mainly produced through its positive effect on V_{cmax25}. Leaf temperature had no significant effect on stomatal conductance. V_{cmax25} and g_s simultaneously affected A_{sat} in response to VPD. Our results highlighted the importance of biochemical processes in limiting leaf photosynthetic rate in response to environmental conditions in subtropical evergreen coniferous plantations.

Keywords: seasonal variations; photosynthetic rate; V_{cmax25}; J_{max25}; stomatal conductance; climate

1. Introduction

Forest ecosystems contain more than 85% of the total plant carbon (C) on Earth [1] and thus play a major role in the global C cycle. Subtropical forests, which are widely distributed across China's southern regions, are considered to have high C sequestration capacities [2]. Conifers were selected to establish most forest plantations in subtropical China since the 1980s due to their fast growth [3,4]. Masson's pine (*Pinus massoniana* Lamb.) (MP) is a dominant native species in subtropical China, which covers approximately area of 5.8 million ha [4]. Slash pine (*Pinus elliottii* Engelm.) (SP), native to the south-eastern United States, covers more than 1 million ha of subtropical China [4,5]. As photosynthesis is arguably the most important biochemical process on Earth and a key source of uncertainty in C cycle modelling [6,7], the direct role of environmental conditions in photosynthetic

rate changing had been fully documented [8–10]. However, how the environmental factors drive stomatal conductance and biochemical processes of leaf photosynthetic rate has not been sufficiently investigated in subtropical evergreen coniferous plantations. Studying the internal mechanisms of environmental factors affecting photosynthesis helps us better understand the physiological processes of plants under a changing climate and then provides an explanation of the physiological mechanisms for plants growth.

Temperature and humidity are the most important environmental factors in influencing leaf photosynthetic rate [11–13]. The key processes of photosynthesis include CO_2 diffusion processes (stomatal conductance (g_s), mesophyll conductance (g_m)) and biochemical processes [14–16]. The effect of g_s on photosynthesis is mainly through CO_2 diffusion from the atmosphere to the substomatal cavities [17,18]. Biochemical processes mainly include the carboxylation of ribulose-1, 5-bisphophate (RuBP) (i.e., the maximum rate of carboxylation at 25 °C, V_{cmax25}) and regeneration of RuBP (i.e., the maximum rate of electron transport at 25 °C, J_{max25}) [19–21]. Temperature influences the carboxylation rate along with stomatal conductance, and photosynthesis is recognized as a very temperature-sensitive process [13]. Leaf temperature directly determines the microenvironment in which the plants are located, and thus is also an important factor in influencing leaf photosynthetic rate. Leaf temperature is influenced by air temperature, and also controlled by leaf traits [22]. Thus, leaf temperature among different plants may be very different even under the same air temperature condition. Humidity, i.e., vapour pressure deficit (VPD), also influences the photosynthetic rate through leaf stomatal conductance. It is widely recognized that leaf stomatal conductance decreases with decreasing humidity [23]. Indeed, g_s and V_{cmax25} simultaneously regulate the photosynthetic rate in response to environmental conditions. It is necessary to evaluate the effects of leaf temperature and VPD on the stomatal and biochemical processes, and then on the leaf photosynthetic rate [15,24,25]. However, the effects of temperature and humidity on leaf photosynthetic rate through key processes have not been sufficiently investigated in subtropical China.

Photosynthetic rates are a key source of uncertainty in modelling C cycles [6]. Many studies have demonstrated strong seasonal variations in photosynthetic parameters, and V_{cmax25} is different even for the same PFT [6,26]. However, due to a lack of field observation data, the key parameters (V_{cmax25} and J_{max25}) are still assumed to be constant over time in most models [27–29]. Exploring the seasonal variations of photosynthetic parameters over the entire growing season can provide data-based support for key model parameters for evergreen coniferous forest in subtropical China. In addition, V_{camx25} and g_s are the important parameters influencing the photosynthetic rate of plants [18–20]. Studying leaf temperature and VPD driving stomatal conductance and biochemical processes of leaf photosynthetic rate substantially improves modelling accuracy and predicting vegetation responses to environmental conditions [27,30], which is crucial for reducing the uncertainty of modelling.

V_{camx25} and g_s are key parameters characterizing the photosynthetic capacity of plants [18–20]. Here we hypothesized that leaf temperature and VPD significantly affected the stomatal and biochemical processes, and then influenced the leaf photosynthetic rate in subtropical evergreen coniferous forest. To evaluate how leaf temperature and VPD affected leaf photosynthetic physiological process in subtropical evergreen coniferous plantation, the seasonal variabilities of photosynthetic parameters were measured using a portable photosynthesis system (LI-6400; Li-Cor, Inc., Lincoln, NE, USA). We compared the effect of leaf temperature and VPD on leaf photosynthetic rate of subtropical forest through stomatal and biochemical parameters. The objective of this study is to (1) investigate seasonal variations of g_s and leaf photosynthetic capacities; and (2) evaluate how the leaf temperature and VPD drive stomatal conductance and biochemical processes of leaf photosynthetic rate in a subtropical evergreen coniferous plantation.

2. Materials and Methods

2.1. Study Area

The experimental site is located at Qianyanzhou (QYZ) Ecological Research Station (26°44′ N, 115°03′ E) in Jiangxi Province of subtropical China (Figure 1). The soils belong to a typical red soil, which developed from red sandstone, glutenite, and fluvial sediment. The study site has a typical subtropical monsoon climate. The mean annual temperature is 17.9 °C (1985–2008), with the highest daily mean temperature of 28.8 °C occurring in July and the lowest of 6.4 °C occurring in January. The average annual precipitation is 1469 mm (1985–2008), which mainly occurs from March to June. The mean air temperature and total precipitation of 2016 were 18.9 °C and 1946 mm, respectively. The major species at the QYZ site are the slash pine (*Pinus elliottii*), Masson's pine (*Pinus massoniana*) and Schima (*Schima superba*). Slash pine and Masson's pine are the pioneer species, which were mainly established in 1980s. The initial tree density of both species was about 750 stem ha^{-1} [31].
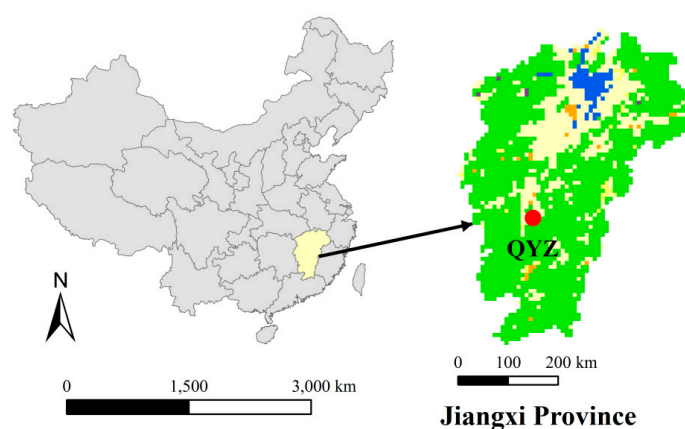

Figure 1. The location of the study site. The green areas represent forests. The red solid circle represents the Qianyanzhou (QYZ) Ecological Station.

2.2. CO$_2$ Response Curve

Leaf gas exchanges of both species were measured on the top of a bamboo tower. The height and size of the bamboo tower are approximately 14 m and 5 m × 10 m, respectively. An open gas-exchange system (Li-6400; Li-Cor, Inc., Lincoln, NE, USA) with a red-blue light source (6400-02B) was used to measure the CO$_2$ responses of the leaf photosynthesis of two species (SP and MP) over the growing season. The seasonal variability of photosynthetic parameters was measured over four periods in the 2016 growing season (i.e., 31 March–4 April (Apr), 4–9 June (Jun), 2–7 September (Sep), and 10–15 November (Nov)). The observations of each tree species were repeated for 3 sunny days in each period (excepting the observations for SP in April, for which the available observation days included only one day). The photosynthetic parameters of each day were measured from 8:00 to 16:00, with a measuring interval of 80 min. Twelve needles of each SP and MP sample were put into a 2 × 3 cm^2 leaf cuvette, and the cuvette was plugged with plasticine to prevent leakage [15]. Seven leaf samples (12 needles in each leaf sample) of each species were measured each day. Each CO$_2$ response curve lasted for approximately 40 min. We tagged the sampled branches to repeat measurements of the same leaf position over the growing season. CO$_2$ response curves were measured under saturating light conditions, at photosynthetic photon flux density (PPFD) level of 1500 μmol m^{-2} s^{-1}. The CO$_2$ concentrations (C$_a$) were initially set to range from 400 μmol CO$_2$ mol^{-1} air to 50 μmol CO$_2$ mol^{-1} air and then to range from 400 μmol CO$_2$ mol^{-1} air to 1400 μmol CO$_2$ mol^{-1} air with a total of 10 points (400, 300, 200, 100, 50, 400, 600, 900, 1000, 1400 μmol CO$_2$ mol^{-1} air). Prior to measuring CO$_2$ response curves, twelve leaves were acclimated in 2 × 3 cm^2 leaf cuvette at saturated light, ambient temperature and a CO$_2$ concentration of 400 μmol CO$_2$ mol^{-1} air for 20 min. All measurements were carried out

under ambient temperature. The relative humidity was set to be within 60–80%, and the flow rate through the chamber was set at 500 mmol s^{-1} for photosynthesis. Leaf temperature was measured by the energy balance method, and logged based on the leaf gas-exchange system (Li-Cor 6400).

The A–Ci curves were fitted using a tool developed by Kevin Tu (www.landflux.org/Tools.php). The photosynthesis parameters, including V_{cmax} and J_{max}, were estimated by plotting the intercellular CO_2 concentration with the net photosynthetic rate. V_{cmax} and J_{max} were normalized at a standard temperature of 25 °C by using the Arrhenius equation [20,32]. The A_{sat} value used in this study was the net photosynthetic rate with a CO_2 concentration of 400 µmol mol^{-1} under saturated light intensity. The corresponding $g_{s,w}$ (stomatal conductance to water, mol H_2O m^{-2} s^{-1}) was logged. $g_{s,w}$ was 1.6 times higher than the $g_{s,c}$ (stomatal conductance to CO_2, mol CO_2 m^{-2} s^{-1}) and we converted the $g_{s,w}$ to $g_{s,c}$ in this study. Vapour pressure deficit (VPD) was also logged based on the gas-exchange system. The specific leaf area (SLA) was calculated using the leaf area and leaf dry weights. As suggested by Gower et al. [33], the needle was approximated as a cylinder, and leaf area was calculated on the basis of half the total surface area [34].

For MP (two-needle leaves), the leaf area enclosed in the cuvette was calculated as Li et al. [35]:

$$\text{leaf area} = [(2h + d)\pi/4 + d] \times 1 \times n \tag{1}$$

For SP (three-needle leaves), the leaf area enclosed in the cuvette was calculated as Li et al. [35]:

$$\text{leaf area} = \left[\left(\sqrt{3}d/6 + h/2\right)\pi + \sqrt{3}d\right] \times 1 \times n \tag{2}$$

where d (cm) and h (cm) were the width and thickness of leaves, respectively, l was the length of leaf samples enclosed in leaf cuvette (i.e., 3 cm), and n was the number of leaf samples enclosed in the leaf cuvette.

2.3. Statistical Analysis

The photosynthesis parameters were calculated by fitting the A–C_i curve using a tool developed by Kevin Tu. Independent sample *t*-tests with a 95% confidence level were used to evaluate the differences among the variables. Prior to analysis, we used Levene's test ($p < 0.05$) to assess the homogeneity of variables. The performances of relationships between variables were evaluated by the coefficient of determination (R^2). Structural equation modelling (SEM) was applied to analyse the effect of leaf temperature and VPD on the stomatal conductance and biochemical processes of leaf photosynthetic rate. We used AMOS 17.0 statistical analysis software to establish our model and used the root mean square error of approximation (RMSEA) to examine the performance of our model. All the statistical figures were plotted in Origin 8.5.1.

3. Results

3.1. Seasonal Variations in Environmental Condition, Leaf Traits, and Leaf Photosynthetic Rates

The mean and highest leaf temperatures for MP during the experimental period were 28.2 °C and 33.2 °C, respectively (Table 1). For SP, the mean and highest leaf temperatures were 30.5 °C and 35.3 °C, respectively. The highest leaf temperatures of both species occurred in June. The average leaf temperature of SP was significantly higher than that of MP ($p = 0.02$) during the entire experimental period. However, there was no significant difference in the average VPD values of MP and SP ($p > 0.05$). In general, high leaf temperatures were accompanied by high VPD values at the QYZ site. The maxima of VPD appeared in June for both species (Table 1). A significantly lower SLA was observed for SP ($p < 0.001$) during the measurement period (Table 1).

Table 1. The differences in the seasonal variations of T_{leaf}, VPD and leaf trait of MP and SP.

	Month	T_{leaf} (°C)	VPD (kPa)	SLA (m² kg⁻¹)
MP	Apr	28.68 ± 3.01	1.27 ± 0.10	5.66 ± 0.90
	Jun	33.19 ± 4.49	2.63 ± 0.79	5.67 ± 0.32
	Sep	31.64 ± 1.15	1.54 ± 0.20	5.83 ± 0.53
	Nov	19.15 ± 3.35	0.62 ± 0.16	5.43 ± 0.32
SP	Apr	29.45	2.21	4.00
	Jun	35.33 ± 0.93	2.75 ± 0.25	4.02 ± 0.22
	Sep	32.50 ± 0.43	1.83 ± 0.15	3.71 ± 0.17
	Nov	24.69 ± 1.57	0.88 ± 0.09	3.34 ± 0.05
p value		**0.020**	0.052	**0.000**

Note: The available observation days for SP in April included only one day and thus there was no standard deviation. The differences among the variables were evaluated using the independent sample *t*-tests. The significant values ($p < 0.05$) between the two species during the entire measurement period are shown in bold (Mean $\pm$ SD).

There exist significant differences in the A_{sat} values of MP and SP ($p < 0.001$) in September and November ($p < 0.05$) (Figure 2). However, there was no significant difference in the average A_{sat} values between MP (9.54 µmol m⁻² s⁻¹) and SP (9.28 µmol m⁻² s⁻¹) ($p > 0.05$) during the experimental period. The A_{sat} values were higher in MP than in SP in April and September. The maxima of A_{sat} occurred in September for both species, with the value of 12.73 µmol m⁻² s⁻¹ and 10.34 µmol m⁻² s⁻¹ (Figure 2).
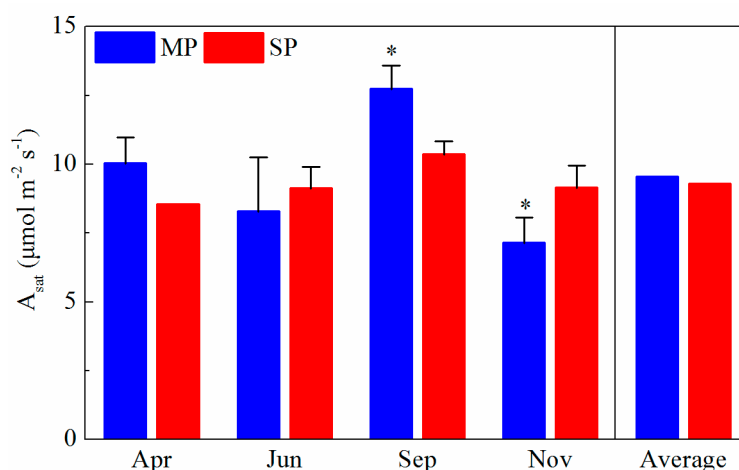

Figure 2. The seasonal variations of leaf photosynthetic rates (A_{sat}) of MP (*Pinus massoniana* Lamb.) and SP (*Pinus elliottii* Engelm.) during the experimental period. ANOVA: * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$.

3.2. Seasonal Variations in Gas Exchange Parameters

The average g_s values of MP and SP during the experimental period were 0.08 mol m⁻² s⁻¹ and 0.07 mol m⁻² s⁻¹, respectively. The seasonal patterns of g_s differed from those of leaf photosynthetic rate for both species. The maximum of g_s occurred in April for MP, with the value of 0.11 mol m⁻² s⁻¹. For SP, the maximum of g_s occurred in June, with the value of 0.08 mol m⁻² s⁻¹ (Figure 3a).

The seasonal patterns of V_{cmax25} were consistent with the seasonal patterns of leaf photosynthetic rate for both species (Figures 2 and 3b). The average V_{cmax25} values of MP and SP were 67.10 and 69.64 µmol m⁻² s⁻¹, respectively. V_{cmax25} reached maxima in September for both species, with the values of 95.73 and 85.05 µmol m⁻² s⁻¹. For MP, V_{cmax25} was higher in April, with the value of 63.16 µmol m⁻² s⁻¹. V_{cmax25} decreased by 4.5% in June compared to that in April, and then recovered in September. V_{cmax25} declined markedly in November. For SP, V_{cmax25} was 56.89 µmol m⁻² s⁻¹ in April and V_{cmax25} was 22.3% higher in June than that in April. V_{cmax25} reached its maximum in September, and then declined slightly in November. V_{cmax25} values were higher in MP than that in SP in April and September, which were consistent with the patterns of leaf photosynthetic rates (Figures 2

and 3b). The seasonal patterns of J_{max25} were basically consistent with those of V_{cmax25} for both species (Figure 3c). J/V ratios of MP and SP reached maxima of 2.11 and 2.17 in April and minima of 1.71 and 1.80 in November and September, respectively (Figure 3d).
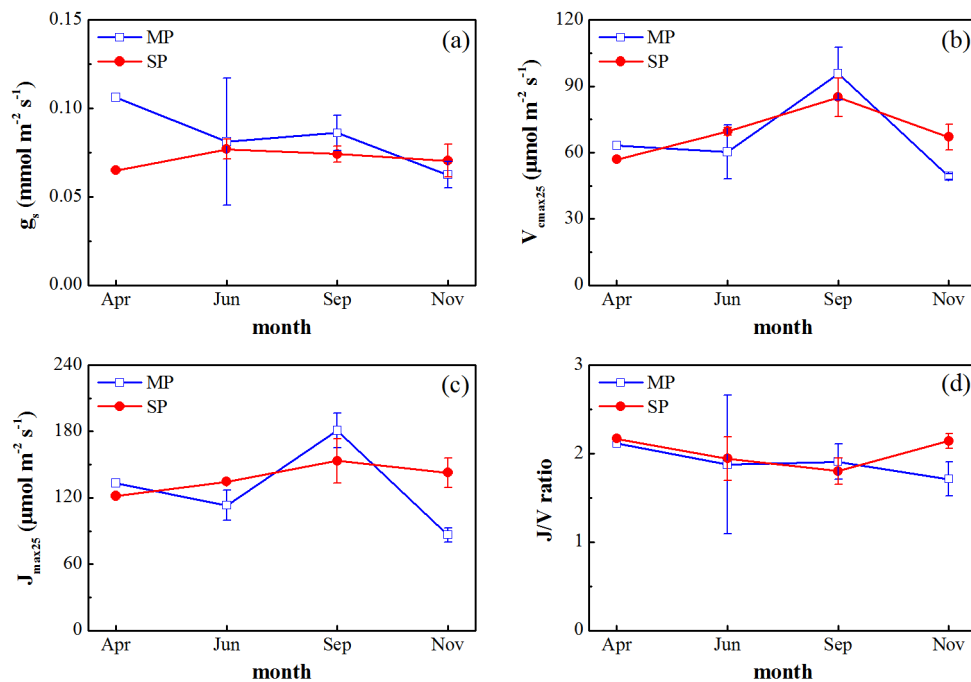

Figure 3. Seasonal patterns in (**a**) g_s, (**b**) V_{cmax25}, (**c**) J_{max25}, and (**d**) J/V ratio, ±SD, for MP (blue line) and SP (red line) during the experimental period (April, June, September, and November). Each value represents the average of the total measured data for three days or for one day (only SP in April).

3.3. Correlation between Gas Exchange Parameters and Leaf Temperature and VPD

There were significant positive correlations between photosynthetic rate and both stomatal conductance and biochemical parameter (V_{cmax25}) ($p < 0.001$) (Figure 4a,b) for both species. The correlation between biochemical parameter and photosynthetic rate ($R^2 = 0.41$) was larger than the correlation between stomatal conductance and photosynthetic rate ($R^2 = 0.30$) (Figure 4a,b). The effect of leaf temperature on V_{cmax25} was significant ($R^2 = 0.35$, $p < 0.001$), but there was no significant correlation between leaf temperature and g_s ($p > 0.05$) (Figure 4c,d). VPD had significant effects on both biochemical parameter and stomatal conductance ($R^2 = 0.31$, $p < 0.001$ and $R^2 = 0.15$, $p = 0.009$) (Figure 4e,f).
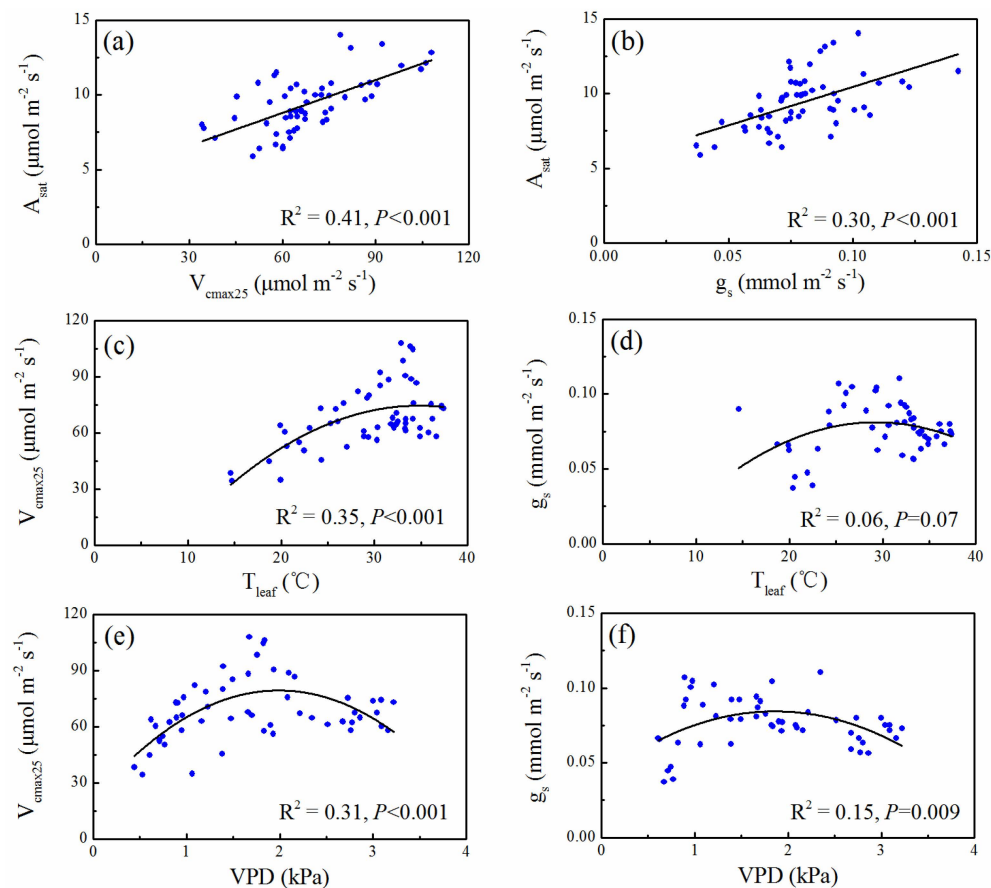
Figure 4. Relationships between leaf photosynthetic rate and photosynthetic parameters with leaf temperature and VPD during the experimental period in subtropical evergreen coniferous plantation.

In our study, J/V ratio was not constant at seasonal scale (Figure 3d), which was significantly correlated with leaf temperature (Figure 5). The J/V ratio increased with leaf temperature, and then decreased as the leaf temperature continued to rise (Figure 5). J/V ratio reached its maximum at the leaf temperature of 27.27 °C during the experimental period. The highest point of the nonlinear fitting curve was 2.14 (Figure 5).
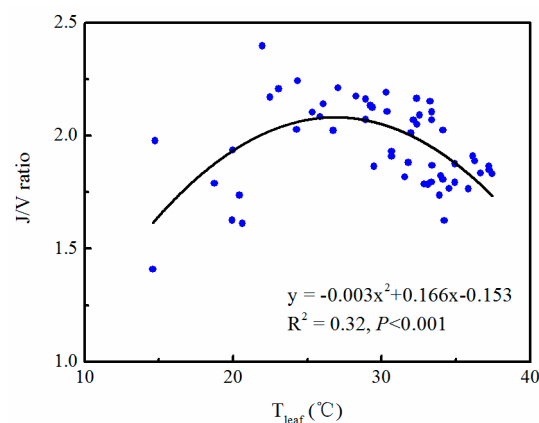

Figure 5. Relationship between J/V ratio and leaf temperature during the experimental period.

3.4. The Effect of Stomatal and Biochemical Parameters on A_{sat} in Response to Leaf Temperature and VPD

SEM analysis showed that V_{cmax25} played an important role in limiting leaf photosynthetic rate in response to leaf temperature and VPD. The positive effect of leaf temperature on A_{sat} was mainly

produced through its positive effect on V_{cmax25} (the path coefficient was 0.5). Leaf temperature had no significant effect on stomatal conductance ($p > 0.05$) (Figure 6a). The path coefficients between V_{cmax25} and g_s with A_{sat} were 0.57 and 0.55, respectively (Figure 6a). The effect of VPD on A_{sat} was mainly derived via its positive effect on V_{cmax25} and negative effect on g_s (the path coefficients were 0.25 and -0.20, respectively) (Figure 6b). The path coefficients between V_{cmax25} and g_s with A_{sat} were 0.56 and 0.51, respectively. The effect of biochemical parameter on A_{sat} is greater than the effect of stomatal conductance on A_{sat} (Figure 6b).
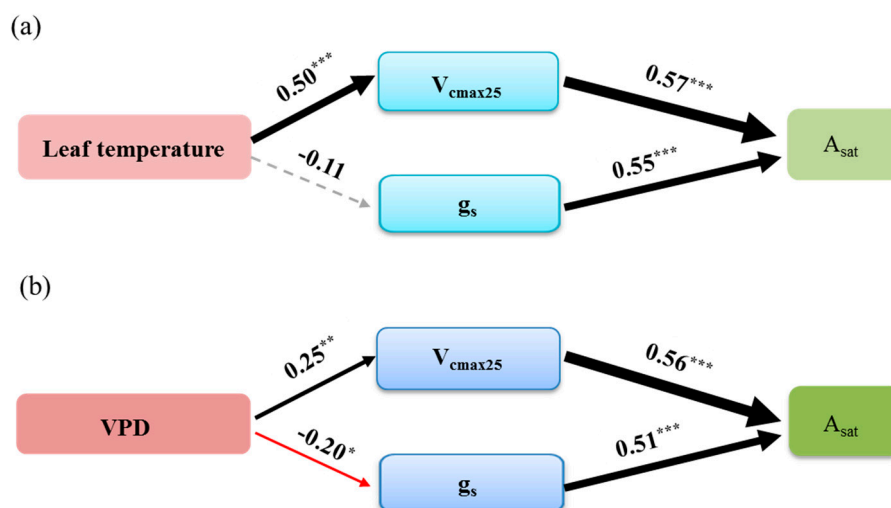

Figure 6. Structural equation models of the effects of stomatal and biochemical parameters on A_{sat} in response to (**a**) leaf temperature and (**b**) VPD in subtropical evergreen coniferous plantation. The bold numbers indicate the standard path coefficients. The grey dashed arrows indicate nonsignificant pathways, and the red and black arrows represent significant negative and positive pathways, respectively. The arrow width represents the strength of the relationship. * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$. (**a**) RMSEA = 0.00, $\chi^2 = 0.61$, $p = 0.44$, (**b**) RMSEA = 0.00, $\chi^2 = 0.39$, $p = 0.53$.

4. Discussion

Environmental conditions play an important role in stomatal conductance and biochemical processes and thus regulate leaf photosynthetic rate in forests. Our results highlighted the importance of V_{cmax25} in limiting leaf photosynthetic rate in response to leaf temperature and VPD (Figure 6), which confirmed our hypothesis. The positive effects of leaf temperature on photosynthetic rate were mainly due to its positive effect on the biochemical process (Figure 6). We attribute this phenomenon to the fact that V_{cmax25} is closely related to temperature [13,36]. Temperature directly influences the reactions catalyzed by Rubisco and the electron transport chain along with stomatal conductance, thus photosynthesis is recognized as a very temperature-sensitive process [13]. Compared with the stomatal conductance, temperature directly impacts Rubisco activity [13,37]. Elevated temperatures can enhance V_{cmax25} before reaching an optimum temperature. However, an elevated temperature will lower V_{cmax25} when the temperature exceeds its optimum value [36]. For terrestrial ecosystems, plants have adapted to the current regional climate, the highest monthly temperature are generally close to the optimal value for maximal rates of photosynthesis [38]. Thus, V_{cmax25} generally showed a positive response to increased temperature because temperatures are frequently lower than optimal (Figures 4 and 6a). Some studies showed that stomatal conductance increased in response to increasing temperature [39,40]. The possible underlying mechanism is that high temperature can decrease water viscosity and increase plant membrane permeability and thus increase water supply to guard cells [39], which will result in higher stomatal conductance. Such behavior of stomatal conductance in response to high temperature can benefit plants by cooling leaves in hot environments [41,42]. However, the increasing temperature can drive the increase of VPD, which will lead to the decrease of stomatal

conductance [23,43]. Leaf stomatal conductance could increase as VPD increases when VPD is low but decrease with increasing VPD when it is high [23,44]. Therefore, changes in stomata are the result of multiple environmental factors [45]. In our study, the effect of VPD on A_{sat} was partly through its negative effect on g_s (Figure 6b). In addition, the effect of VPD on stomatal conductance also influences carboxylation rate because vegetation absorbs CO_2 from the atmosphere through stomata [11,46]. Thus, V_{cmax25} and g_s simultaneously affect the photosynthetic rates in response to VPD (Figure 6).

The J/V ratio reflects resource allocation between enzymatic (i.e., Rubisco) and light-harvesting (i.e., electron transport) capabilities [47–49]. J/V ratio was assumed to be constant in many process-based models. Medlyn et al. [12] reported a J/V ratio of 1.67 across a large range of crops and tree species. However, many studies have indicated the J/V ratio varies with the season [50–52]. Our results showed that J/V ratio was significantly correlated with leaf temperature in subtropical evergreen coniferous forests. With the increase of leaf temperature, the J/V ratio increased first and then decreased (Figure 5). The seasonal variation in the J/V ratio is due to the difference in the partitioning of leaf nitrogen between different fractional pools [53]. This partitioning involves the distribution of leaf nitrogen between the Rubisco and light-harvesting fractions [54–56]. At low temperatures, More N is allocated to the light-harvesting complex to absorb more light energy for photosynthesis. At high temperatures, previous studies have suggested that more N should be invested in the RuBP carboxylase process to optimize the photosynthesis efficiency [54–57]. Thus, the response of J/V ratio to leaf temperature is parabolic, with a peak at an optimum temperature in subtropical evergreen plantation (Figure 5).

Previous studies have focused on the growth rates of exotic and native plant species [58–61]. However, these studies often show inconsistent results due to differences of regions and environments. For example, previous studies showed that the growth rates of exotic species were superior to those of native species [58,59] and have also shown that the native species have higher growth rates [62,63]. In our study, there was no significant difference in A_{sat} of MP and SP ($p < 0.05$) during the experimental period (Figure 2). SP, as an exotic species, may have showed a faster growth rate than the native species at the beginning of planting. However, there was no significant difference in growth rate between the two species after planting for 20 years [62,63]. Exotic species (SP) can be used as pioneer trees for vegetation restoration in subtropical China. But, there is no obvious growth advantage of exotic species in the late stage of planting compared with native species. This finding can provide an effective approach on how to select exotic or native species for planting in subtropical forest, which may give better suggestions for forest management.

Our study captured the seasonal variations of photosynthetic parameters based on field gas-exchange measurement and thus explored the effect of leaf temperature and VPD on stomatal conductance and biochemical processes of leaf photosynthesis. Studying the environmental factors influencing physiological process of photosynthesis allows us to better understand the mechanism of CO_2 uptake and emission by plants under a changing climate. Furthermore, the knowledge about plants' responses under changing environmental conditions can provide some suggestions for effectively mitigating greenhouse gas emissions and environmental sustainability.

In the future, the cycle of field observation of CO_2 response curves can be set for one month. This will reduce uncertainties in measurement data and enhance the reliability of result. In addition, the difficulty in accurately evaluating V_{cmax25} has made photosynthetic rate a key source of uncertainty in C cycle modelling. Recent studies have utilized leaf chlorophyll content, instead of leaf total nitrogen content, as a proxy for V_{cmax25} in croplands and deciduous forests [6,26]. However, there is limited information in the literature on the relationship between V_{cmax25} and leaf chlorophyll content in subtropical evergreen forests. We can evaluate the relationship between V_{cmax25} and leaf chlorophyll content and explore the role of leaf chlorophyll content in determining leaf photosynthetic capacity in subtropical evergreen coniferous forests in the future.

5. Conclusions

In conclusion, this study evaluated the effects of stomatal and biochemical parameters on photosynthetic rate in response to leaf temperature and VPD based on leaf gas exchange measurements in subtropical evergreen plantation. Our results showed that there was no significant difference in the light-saturated photosynthetic rate (A_{sat}) between slash pine and Masson's pine during the experimental period. The seasonal patterns of g_s differed from those of leaf photosynthetic rate for both species. However, the seasonal patterns of V_{cmax25} were basically consistent with those of A_{sat}. The positive effect of leaf temperature on A_{sat} was mainly produced through its positive effect on V_{cmax25} based on SEM. Leaf temperature had no significant effect on stomatal conductance. However, V_{cmax25} and g_s simultaneously affected A_{sat} in response to VPD. Our results highlighted the importance of V_{cmax25} in limiting leaf photosynthetic rate in response to leaf temperature and VPD in subtropical evergreen coniferous plantations.

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