

Article

Variations in Leaf Photosynthesis and Its Limitations at Different Canopy Positions in Mature Camphor Trees

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Abstract: Urban forests play important roles in carbon sequestration and climate change mitigation. However, their adaptive mechanisms and limitations on photosynthesis throughout the canopy are poorly understood. This study takes the most widely distributed 50-year-old camphor plantations (*Cinnamomum camphora*) in Shanghai as the research objects. We investigated the variations in leaf morphology and photosynthetic physiology and biochemistry at six different canopy positions during a summer and an autumn period. We discovered that on account of leaf nitrogen loss and water deficit, light-saturated photosynthesis ($A_{\max}$) declined in upper sunlit leaves despite being exposed to high sunlight in the same fashion as stomatal and mesophyll conductance (g_{sw} , g_m), photochemical quenching coefficient and actual photochemical efficiency of PSII (Φ_{PSII} , q_P), and maximum rate of electron transport and carboxylation ($J_{\max}$, $V_{c\max}$) during the growing season. Although seasonal change had little effect on $A_{\max}$, the relative importance of limitations varied temporally. Mesophyll and biochemical limitation were the major contributors to the decline in the $A_{\max}$ in upper sunlit leaves between summer and autumn, respectively. Our study highlights the constraints of carbon fixation capacity in dense stands of mature camphor trees and offers technical support for the accurate prediction of canopy photosynthesis and the enhancement of carbon sequestration management in urban forests.

Keywords: light heterogeneity of canopy; photosynthetic traits; resource allocation; mesophyll conductance; *Cinnamomum camphora*



Academic Editors: Lazar Kesić and Janusz Zwiazek

Received: 22 February 2025

Revised: 18 March 2025

Accepted: 25 March 2025

Published: 27 March 2025

Citation: Leng, H.; Zhou, L.; Yan, W. Variations in Leaf Photosynthesis and Its Limitations at Different Canopy Positions in Mature Camphor Trees. *Forests* **2025**, *16*, 581. <https://doi.org/10.3390/f16040581>

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1. Introduction

The forest canopy, as the main place of photosynthesis, is the primary functional interface for matter and energy exchange between trees and the atmosphere [1]. In the canopy, light availability—which is affected by stand structure and foliage distribution—often differs more than 50-fold from the top of the canopy to the bottom [2]. Concomitantly, air and leaf temperatures, humidity, and vapor pressure deficits also vary dramatically along the canopy [3,4]. This environmental variability generates strong gradients in canopy leaf anatomical, morphological, and functional traits [5]. Thus, the distinct phenomenon of sun leaves and shade leaves is produced, driving leaf carbon gain dynamics in the whole tree [6]. Compared with leaves grown in shade, sun leaves have been found to have thicker palisade tissue, higher leaf mass per area (LMA) and area-based nitrogen content (N_a), as well as greater rate of photosynthesis (A_n) [7,8], yet they also suffer more sun exposure stress due to excessive light absorption [9,10].

The scaling up of photosynthesis from leaves to the canopy is often simulated and performed using the biochemical model established by Farquhar et al. [11]. The initial

optimality model (i.e., big leaf model) proposed that the photosynthetic capacity and N distribution is proportional to the light extinction coefficient in the canopy so that canopy carbon gain could be maximized [12,13]. Nevertheless, the actual N distribution in the canopy has been shown to be much less steep than the light gradient [14]. And also the photosynthetic rate usually levels off at 30%–50% of above-canopy light, implying a certain limit for high-light acclimation [15,16]. Peltoniemi et al. [17] developed a simple ‘toy’ two-leaf canopy model, which suggests that hydraulic constraints to water supply can explain shallow N gradients in the canopy. The hydraulic limitation theory states that the hydraulic conductance of the soil–leaf pathway is inversely related to the length of the water transport path. Thus, the sun leaves of the upper canopy have lower water potentials than the shaded ones of the lower canopy which may lead to lower N allocation in the upper leaves [18,19], but more N is partitioned to Calvin–Benson cycle enzymes to accelerate the photosynthetic capacity [20]. However, there is also evidence that plants can overcome this path-length limitation and can even construct superior water transport efficiency in sun branches at the top canopy as a plastic response of xylem anatomy to the light environment [21,22]. Since high photosynthetic capacity tends to be sustained by high stomatal conductance and high hydraulic conductance, the distribution optimization of hydraulic conductance and photosynthetic nitrogen within the canopy could achieve the maximization of tree primary production in the whole canopy [23]. As a matter of fact, better additional structural, chemical, and physiological constraints can be likewise used to introduce more reality into optimality models like LMA and g_{sw} [17]. Whether these factors are included depends on their roles in the regulation of photosynthesis. But to date, there is very limited evidence on physiological and biochemical factors incorporated into canopy models of photosynthesis in urban forests of subtropical region.

Under saturated light, photosynthetic rates in terrestrial C_3 plants are limited by CO_2 diffusion resistance and by CO_2 fixation velocity (i.e., biochemical limitation), which are expressed by stomatal and mesophyll conductances (g_s and g_m), and by maximum rates of Ribulose-1,5-Bisphosphate (RuBP) carboxylation and regeneration in the Calvin cycle (V_{cmax} and J_{max}), respectively [24,25]. Generally, g_s and g_m are found to be constrained by the leaf anatomical structure and biochemical factors, such as aquaporins and carbonic anhydrase [26,27], while V_{cmax} and J_{max} are mainly associated with N concentration and the amount (and activity) of the relevant enzymes [28,29]. Any decrease in CO_2 diffusion or utilization would lead to a direct reduction in the photosynthesis rate [24]. g_s , g_m , V_{cmax} , and J_{max} vary temporally and spatially under the influence of species, leaf ontogeny, and environment conditions, e.g., light, CO_2 , temperature, as well as water and nutrition supply [26,30–32]. Thus, the relative importance of these photosynthetic limitations may be highly responsive to both the intra-canopy resource gradient and seasonal variability. Several studies have reported on limitations to carbon uptake throughout the canopy. For instance, in mature *Eucalyptus tereticornis* trees, Crous et al. [33] found stronger electron transport and carboxylation limitations to photosynthesis in lower shading leaves, while Campany et al. [34] suggested that the lower A_n in shade leaves was primarily associated with reduced g_m . It was further discovered that seasonal changes impacted the relative importance of limitations across the entire canopy. According to Cano et al. [35], moderate summer drought lowered A_n in upper and lower beech leaves and in lower oak leaves by reducing the g_s and g_m . These discrepancies may be determined by the degree of variability in biochemical demand and CO_2 supply among canopy positions. The preceding studies, however, did not investigate how these inequalities throughout the canopy vary with the changing seasons, especially with regard to temperature and sunlight, and a lack of empirical data hinders us from interpreting their coupled effects on the diffusive and biochemical components.

Cinnamomum camphora is one of the most important urban tree species in the subtropical region of China and is commonly cultivated as dense pure plantations. Currently, its application frequency has been found to be as high as 80% in Shanghai [36]. However, very little is understood about the adaptive mechanisms that regulate photosynthesis in the canopy of *C. camphora* grown in the urban greenery. Here, we conducted a field study in monospecific camphor stands in a subtropical monsoon climate to assess the effects of canopy position on key leaf photosynthetic characteristics and associated functional traits at two times during the growing season. We further investigated the correlation of leaf photosynthetic capacity with resource partition and physiological and biochemical components, as well as the seasonal variability of quantitative limitations on photosynthesis along the canopy. Several questions were asked: Do photosynthetic traits increase as light increases in the canopy? Are there significant differences in photosynthetic physiology and biochemistry in the canopy between the two growing seasons? Which components significantly affect canopy photosynthesis? The present study aims to predict canopy photosynthesis more accurately using optimality-based models and guide the carbon sequestration management in urban forests.

2. Materials and Methods

2.1. Study Site

This study was conducted in the adult stand of *C. camphora* located at Shanghai Botanical Garden (81.86 hm²) in the central region of Shanghai (31°08′44.40″ N, 121°26′50.12″ E). The garden is characterized by various broad-leaved evergreen and deciduous plantations, and *C. camphora* is one of the main tree species, covering an area of around 4 hm², with 774 individuals in total. The mean annual temperature is 17.8 °C, and the mean annual precipitation is 1660.8 mm. The soil is silty clay loamy sand with an average pH value of 7.7. The total organic matter, nitrogen, and phosphorous contents in the topsoil are 16.80, 2.67, and 1.05 g·kg^{−1}, respectively. Two sample camphor plots, with an area of 100 m² each and with trees spaced 2–3 m apart (dense stand), were established. Three approximately 50-year-old sample trees were selected in each plot, for a total of six study trees with a mean height of 17.9 m and a mean diameter at breast height of 40.5 cm.

2.2. Experimental Design

In accordance with canopy height, a three-storied bamboo platform with a height of about 14 m was built to provide access to the leaves of the three sample trees in each plot (Figure S1). Leaf–gas exchange measurements were performed during two measurement campaigns through the 2023 growing season, from 22 August to 7 September (summer) and from 17 October to 31 October (autumn). The same measurement protocol was followed during each campaign. Measurements were taken at three canopy layers: upper, corresponding to the outer edge of the canopy within three meters from the top; middle, corresponding to the mid-canopy (the halfway up the canopy, 11~12 m high); lower, corresponding to the lowest living branch (6~7 m high). At the same time, each layer within the canopy was divided into southern and northern faces, resulting in six distinct canopy positions, i.e., the southern and northern faces of the upper (US and UN), middle (MS and MN), and lower (LS and LN) layers. According to the developing locations of branches, the leaves located at the middle and lower layers were sampled on the secondary branches of the inner canopy, while the leaves at the upper layer were sampled on the branch apex. Fully expanded and undamaged current-year leaves were selected from the same branch for gas exchange measurements. Leaf–gas exchange and chlorophyll fluorescence were measured simultaneously on sunny days between 9:00 and 12:00 a.m. Over the two dates, we completed 60 A_n versus CO₂ concentration in the intercellular air space (C_i) response

curves (A_n/C_i curves) in total. On each date, we made four to six A_n/C_i curves at each canopy position. Following the gas exchange measurement on the first date, leaves attached to the same branch were collected, with one portion of leaves used to analyze the pigment concentration and the other to determine the carbon and nitrogen contents.

2.3. Environmental Measurements

Prior to gas exchange measurement on each date, the photosynthetic active radiation (PAR) incident on the selected leaves was measured with a handheld quantum sensor (LI-250A, LI-COR Inc., Lincoln, NE, USA) at each canopy position for the sample trees. PAR was performed on sunny days every two hours between 8:00 and 16:00. The daily PAR (PAR_{sum}) was calculated by the integral area of the diurnal light curves. Data on air temperature and precipitation were gathered by the microclimate station (DJ-6595A, DIANJIANG TECH Inc., Shanghai, China) located in the Shanghai Botanical Garden.

2.4. Gas Exchange and Chlorophyll Fluorescence Measurements

A_n/C_i curves were established from gas exchange measurements using an open gas exchange system (LI-6400XT and LI-6400-40, LI-COR Inc., Lincoln, NE, USA). The conditions inside the cuvette during the measurements were as follows: PAR of $1800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (saturating-light condition from previous trials, 90% red and 10% blue light), CO_2 concentration of $400 \mu\text{mol}\cdot\text{mol}^{-1}$, leaf temperature at prevailing levels ($33\text{--}40^\circ\text{C}$ and $26\text{--}32^\circ\text{C}$ at two measurement times, respectively), relative humidity of 40%–60%, and air flow rate of $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. A_n/C_i curve measurements were initiated when A_n and g_{sw} (stomatal conductance to H_2O) had stabilized under these conditions (usually within 30min after clamping onto the leaf). A series of adjustments were made to the ambient CO_2 concentration (C_a) inside the chamber: 400, 300, 250, 200, 150, 100, 50, 400, 600, 800, 1000, 1200, 1500, $1800 \mu\text{mol}\cdot\text{mol}^{-1}$. The parameters were recorded when a steady state was reached for both gas exchange and chlorophyll fluorescence.

At each C_a step, chlorophyll fluorescence was measured simultaneously. Steady-state fluorescence (F_s) was recorded under actinic light of $1800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from light-emitting diodes and maximal fluorescence (F_m') was recorded during a light-saturated pulse of ca. $8000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 630 nm by the multiphase flash protocol. Far-red light of $5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was applied at 740 nm without actinic light to obtain minimal fluorescence in the light-adapted leaf (F_o'). The photochemical quenching coefficient of PSII (q_p) and the actual photochemical efficiency of PSII (Φ_{PSII}) were calculated according to the gas exchange system: $q_p = (F_m' - F_s)/(F_m' - F_o')$, $\Phi_{PSII} = (F_m' - F_s)/F_m'$. Electron transport rate (ETR) was calculated as $\Phi_{PSII} \times PAR \times \alpha \times \beta$, where α is the leaf absorbance (taken as 0.85) [37] and β is the distribution of light between PSI and PSII (taken as 0.5).

Finally, light-saturated gas exchange (A_{max} , g_{sw} , and C_i) and chlorophyll fluorescence parameters (q_p , Φ_{PSII} , ETR) were determined at a C_a of $400 \mu\text{mol}\cdot\text{mol}^{-1}$ and a PAR of $1800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, which were used in the following calculations and analyses.

2.5. Estimation of Mesophyll Conductance and Biochemical Parameters

The maximum carboxylation rate ($V_{cmax}(C_i)$), potential maximum electron transport rate at saturating-light levels ($J_{max}(C_i)$), and mitochondrial respiration rate in the light (R_{day}) were estimated based on A_n/C_i curves according to Farquhar et al. [11].

Mesophyll conductance (g_m) was estimated by combining gas exchange and chlorophyll fluorescence measurements via the variable J method of Harley et al. [38], as follows:

$$g_m = \frac{A_n}{C_i - \frac{\Gamma^* \times (ETR + 8(A_n + R_{day}))}{ETR - 4(A_n + R_{day})}}$$

where g_m was judged to be reliable only when C_i ranged from 100 to 300 $\mu\text{mol}\cdot\text{mol}^{-1}$ [38]. Hence, the values of A_n , C_i , and ETR at C_a of 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ were taken from the gas exchange and chlorophyll fluorescence measurements. R_{day} was calculated and derived for the A_n/C_i curve. Γ^* represents the CO_2 compensation point in the absence of R_{day} , taken from Bernacchi et al. [39]. The CO_2 concentration at the site of carboxylation (C_c) was calculated following Fick's law, which can be described as $C_c = C_i - A_n/g_m$. Therefore, using estimates of g_m , A_n/C_i curves could be converted to A_n/C_c curves. The values of $V_{\text{cmax}}(C_c)$ and $J_{\text{max}}(C_c)$ were calculated from A_n/C_c curves and then standardized to 25 °C using the temperature-dependent equation reported by Bernacchi et al. [40]. As the effects of temperature on g_m are controversial—a positive effect was observed until 35 °C in *Nicotiana* while an obvious decline was observed from 26.9 °C to 40 °C in beech trees [30,35]— g_m estimates were not scaled with temperature. C_c -based V_{cmax} and J_{max} estimates at 25 °C were used in the subsequent analyses and comparisons. This method has stronger operability and higher reliability, which is conducive to the observation and analysis of multi-processing and multi-repetitive samples in our study.

2.6. Quantitative Limitation to the Light-Saturated Photosynthesis (A_{max})

According to Grassi and Magnani [30], the contributions to the decreased A_{max} were partitioned into g_{sc} (stomatal conductance to CO_2), g_m , and V_{cmax} . The photosynthesis limitations were determined as follows:

$$\frac{dA_n}{A_n} = S_L + M_L + B_L = l_s \times \frac{dg_{\text{sc}}}{g_{\text{sc}}} + l_m \times \frac{dg_m}{g_m} + l_b \times \frac{dV_{\text{cmax}}}{V_{\text{cmax}}}$$

$$l_s = \frac{g_{\text{tot}}/g_{\text{sc}} \times \partial A_n / \partial C_c}{g_{\text{tot}} + \partial A_n / \partial C_c}$$

$$l_m = \frac{g_{\text{tot}}/g_m \times \partial A_n / \partial C_c}{g_{\text{tot}} + \partial A_n / \partial C_c}$$

$$l_b = \frac{g_{\text{tot}}}{g_{\text{tot}} + \partial A_n / \partial C_c}$$

where S_L , M_L , and B_L are the contributions of g_{sc} , g_m , and V_{cmax} to dA/A , respectively. l_s , l_m , and l_b are the corresponding relative limitations to photosynthesis, respectively. g_{sc} is calculated as $g_{\text{sc}} = g_{\text{sw}}/1.6$. g_{tot} is total conductance to CO_2 between the leaf surface and carboxylation site ($1/g_{\text{tot}} = 1/g_{\text{sc}} + 1/g_m$). $\partial A_n / \partial C_c$ was determined as the slope of A_n/C_c curve at $C_c = 50\text{--}120$ $\mu\text{mol}\cdot\text{mol}^{-1}$.

To investigate how the canopy position limits A_{max} between the two seasons, we compared the contributions of g_{sc} , g_m , and V_{cmax} to the reduced A_{max} (i.e., S_L , M_L , and B_L). The maximum values of A_{max} , g_{sc} , g_m , and V_{cmax} were regarded as reference values, regardless of the season in which they occurred.

2.7. Concentration of Foliar Carbon, Nitrogen, and Photosynthetic Pigments

At each canopy position of sample trees in each plot, around 20 leaves were collected, scanned to measure surface area (cm^2) using a leaf area meter (Scan Maker i800 plus, MICROTEK Inc., Shanghai, China), oven-dried at 80 °C for 48 h, and subsequently weighed to determine the leaf dry mass (g). Then, the leaf mass per area (LMA , $\text{g}\cdot\text{m}^{-2}$) was calculated. Afterward, the leaf samples were milled to assess the carbon (C_m , %) and nitrogen contents (N_m , %) using an organic element analyzer (Vario EL Cube, Elementar Inc., Hanau, Germany). Leaf nitrogen per area (N_a , $\text{g}\cdot\text{m}^{-2}$) was calculated by using the leaf LMA .

Similarly, four leaves were collected, cut into small pieces with scissors (avoiding the main vein of the leaves), and soaked in 80% acetone for 48 h until whitened. The absorbance of the extract was measured at 470, 646, and 663 nm using an ultraviolet-visible

spectrophotometer (L6S (765), INESA Inc., Shanghai, China) following Lichtenthaler and Wellburn [41]. The concentration of leaf chlorophyll a (Chl_a , $\text{mg}\cdot\text{g}^{-1}$), chlorophyll b (Chl_b , $\text{mg}\cdot\text{g}^{-1}$), and carotenoid (Car , $\text{mg}\cdot\text{g}^{-1}$) were determined.

2.8. Statistical Analysis

All analyses and graphics were performed using SPSS18.0 and Origin Pro 9.1, respectively. Before analysis, Shapiro–Wilk’s and Levene’s tests were conducted to check normality and homogeneity. The variables that did not meet the assumption were log-transformed. Repeated measures analysis of variance with Duncan’s post hoc test were performed to test the effects of canopy position and date. The relationships among photosynthetic traits were assessed using linear regressions. Curve fitting analyses were performed to assess the correlation between PAR_{sum} and leaf physiological characteristics. Effects were considered significant at 0.05. All data were represented as mean $\pm$ SE (standard error).

3. Results

3.1. Growth Environment

Precipitation and air temperature varied significantly between the summer and autumn periods. The mean daily maximum temperature was 30.57°C and 25.43°C , and the total precipitation was 98.2 mm and 1.2 mm, respectively (Figure 1a). The first campaign experienced higher temperatures but more rainfall, which suggests that camphor trees did not suffer from summer drought stress.

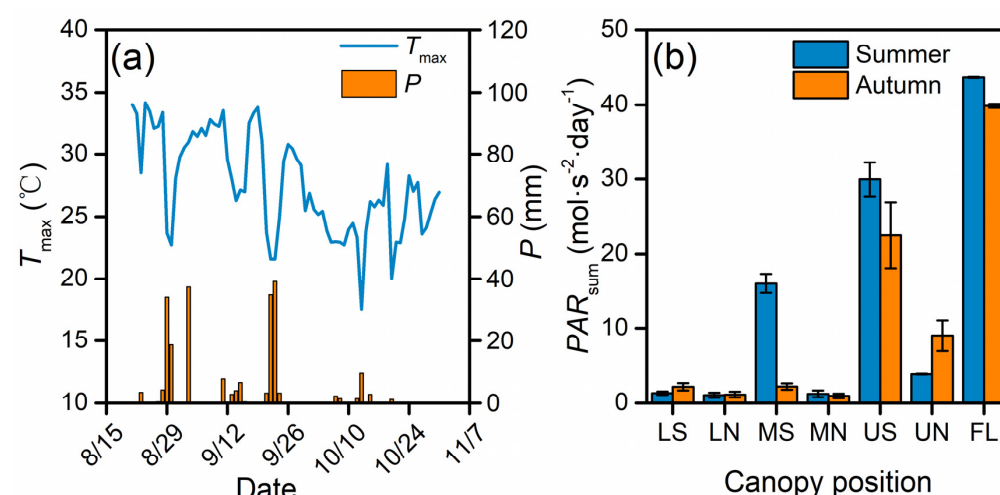


Figure 1. (a) Maximum air temperature (T_{max}) and daily precipitation (P) during measurement dates from late August to November of 2023. (b) Daily photosynthetic active radiation (PAR_{sum}) across camphor canopy positions during two campaigns. US and UN, MS and MN, LS and LN represent the southern and northern faces at the upper, middle, and lower canopy layers, respectively. FL represents full light above the canopy.

The diurnal variations in PAR in the canopy of camphor trees were measured above the canopy and at each canopy position on typical sunny days (Figure S2). The maximal daily PAR (PAR_{sum}) throughout the canopy was observed on the southern side of the upper layer (US position, $29.94\text{ mol}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ in summer and $22.49\text{ mol}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ in autumn), accounting for 68.56% and 56.41% of above-canopy light (full light), respectively (Figure 1b). A close second was the southern side of the middle layer in summer (MS position, $16.05\text{ mol}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ of PAR_{sum} ; Figure 1b). Affected by solar elevation angle and crown profile, there was an obvious effect of seasonality on PAR_{sum} incident in leaves developing at the MS positions, where light availability was 7.32-fold higher in summer than in autumn, respectively. At other canopy positions, PAR_{sum} was relatively lower

over both seasons ($<10 \text{ mol} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$; Figure 1b). Therefore, the leaves grown at the US position were considered upper sunlit leaves.

3.2. Effect of Canopy Position and Season on Leaf Photosynthetic Traits

Between the two campaigns, g_{sw} , g_{m} , C_i , and J_{max} showed significant date effects (Table 1). Mean g_{sw} and C_i were reduced by 12.32% and 7.84%, respectively, and mean g_{m} and J_{max} increased by 19.89% and 44.79%, respectively, from summer to autumn (Figures 2c–h and 3c,d). Seasonal variation was more marked in the g_{sw} of leaves grown in the MS position, in the g_{m} at the lower layer, and in the C_i and J_{max} in most positions except for US and MN positions, respectively (Figures 2c–h and 3c,d). Other variables did not vary seasonally (Table 1). Meanwhile, no date effects were detected along with the fluctuation pattern of all photosynthetic traits throughout the canopy (Table 1).

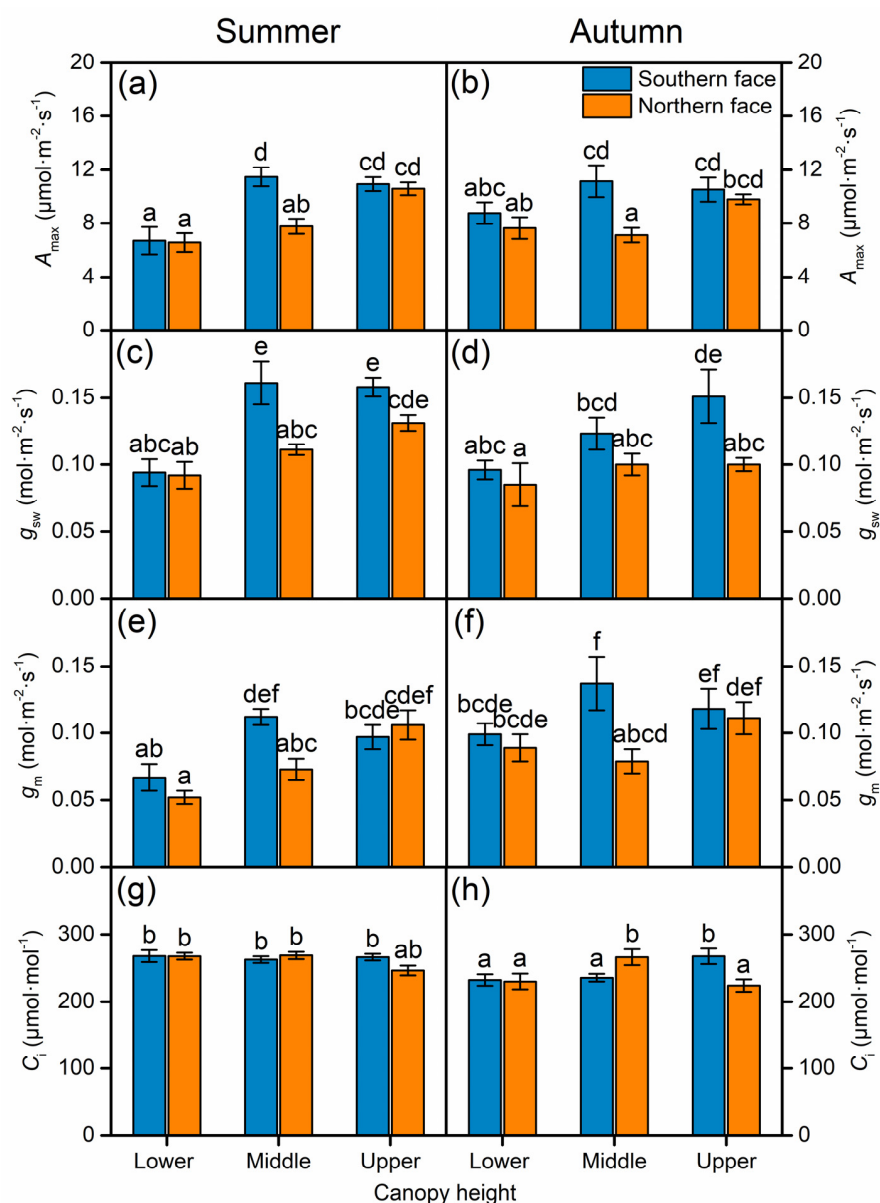


Figure 2. Effects of canopy position and season on gas exchange parameters for mature *C. camphora* trees. (a,b) Light-saturated photosynthesis (A_{max}), (c,d) stomatal conductance (g_{sw}), (e,f) mesophyll conductance (g_{m}), and (g,h) CO_2 concentration in the intercellular air spaces (C_i) are given as mean $\pm$ SE ($n = 4-6$). Letters represent a significant difference across canopy positions and seasons according to Duncan's test ($\alpha = 0.05$).

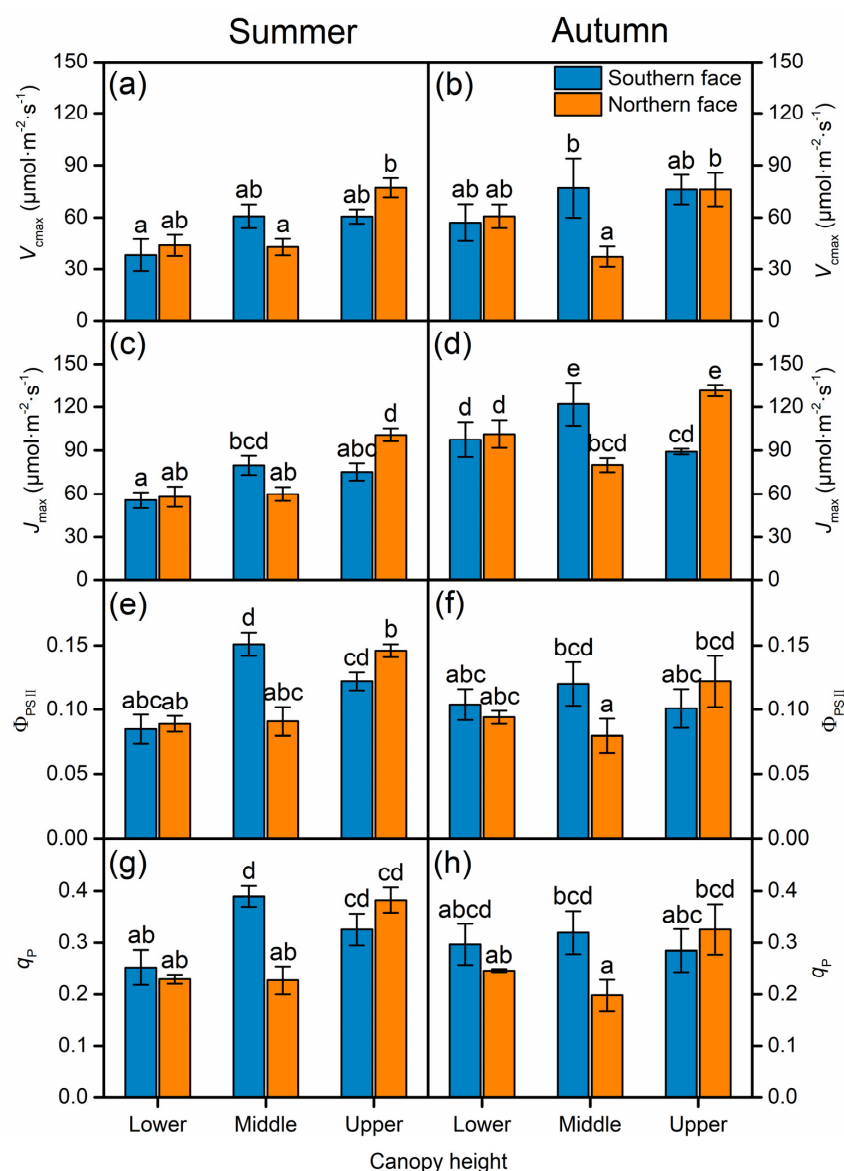


Figure 3. Effects of canopy position and season on biochemical traits for mature *C. camphora* trees. (a,b) Maximum carboxylation rate at 25 °C (V_{cmax}), (c,d) potential maximum electron transport rate at 25 °C (J_{max}), (e,f) actual photochemical efficiency of PSII (Φ_{PSII}), and (g,h) photochemical quenching coefficient of PSII (q_P) are given as mean $\pm$ SE ($n = 4-6$). Letters represent a significant difference across canopy positions and seasons according to Duncan's test ($\alpha = 0.05$).

Significant effects of both canopy height and orientation on the A_{max} , g_{sw} , g_m , and q_P were observed across canopy positions, while the V_{cmax} , J_{max} , and Φ_{PSII} only exhibited prominent differences across canopy heights (Table 1). Accordingly, most leaf photosynthetic traits (except for g_{sw} and C_c) showed significant interactive effects (Table 1). Contrasting two canopy orientations over two seasons, the A_{max} , g_m , V_{cmax} , J_{max} , Φ_{PSII} , and q_P all increased pronouncedly as height increased but only for the north-facing canopy. The south-facing leaves instead showed a tendency to decline at the upper layer relative to the middle layer (Figures 2a,b,e,f and 3a–h). Nevertheless, this pattern only occurred for the g_{sw} in summer, while the value increased significantly with height for both-side leaves in the autumn (Figure 2c,d). In summary, the highest photosynthetic traits were shown in leaves grown in the MS or UN position rather than the US position, which implies that the scaling of photosynthetic traits with the PAR_{sum} was not linear. In terms of the C_i , conspicuous increases of 16.03% and 15.44% from summer to autumn were shown in the

US and MN positions, respectively, whereas no disparities existed among canopy positions at the first sampling (Figure 2g,h). In addition, no change was detected in the C_c across heights or orientations over the two campaigns (Table 1).

Table 1. *F* statistics from full-factorial mixed effect ANOVA for a main effect and interactions of canopy position and season on photosynthetic physiology and biochemistry. Variables analyzed were: light-saturated photosynthesis ($A_{\max}$), stomatal conductance (g_{sw}), mesophyll conductance (g_m), CO_2 concentration in the intercellular air spaces (C_i), CO_2 concentration at the site of carboxylation (C_c), maximum carboxylation rate at 25 °C (V_{cmax}), potential maximum electron transport rate at 25 °C ($J_{\max}$), actual photochemical efficiency of PSII (Φ_{PSII}), and photochemical quenching coefficient of PSII (q_P).

	Date	Canopy Height	Canopy Orientation	H × O	D × H × O
$A_{\max}$	0.07	11.73 ***	13.53 **	6.06 **	0.32
g_{sw}	6.41 *	10.34 **	12.94 **	1.74	1.98
g_m	13.23 **	5.78 *	8.29 **	3.91 *	1.13
C_i	10.55 **	2.75	0.27	14.60 ***	1.22
C_c	0.50	0.65	0.49	3.25	0.83
$C_i - C_c$	15.08 **	0.28	0.01	0.04	1.63
V_{cmax}	0.60	4.81 *	0.54	11.39 ***	0.14
$J_{\max}$	45.81 ***	9.19 **	0.06	25.65 ***	0.49
Φ_{PSII}	4.11	4.93 *	2.37	10.15 **	1.79
q_P	2.95	5.23 *	4.48 *	8.03 **	1.62

Significance is noted as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ in the table. H, O, and D represent canopy height, canopy orientation, and measuring date, respectively.

Figure 4 illustrates the PAR_{sum} responses of gas exchange and chlorophyll fluorescence parameters at the first measuring with higher light intensity. A quadratic polynomial was applied and well-fitted the $A_{\max}$, g_{sw} , g_m , Φ_{PSII} , and q_P vs. PAR_{sum} correlation ($R^2 = 0.573$, $p = 0.022$; $R^2 = 0.725$, $p = 0.003$; $R^2 = 0.556$, $p = 0.026$; $R^2 = 0.486$, $p = 0.049$; $R^2 = 0.516$, $p = 0.038$; Figure 4a–c), but it was not able to fit the relationships of V_{cmax} and $J_{\max}$ with PAR_{sum} ($p > 0.05$; Figure 4d). Still, these photosynthetic traits all followed similar trends, that is, an initial increase within a certain range of PAR_{sum} and a decline after reaching the apex.

Figure 5 and Table S1 illustrate comparisons among canopy positions in other leaf functional traits. The LMA was positively related to the PAR_{sum} ($R^2 = 0.589$, $p = 0.018$) and increased with height; hence, upper-canopy leaves showed a higher LMA than mid and lower leaves (Figure 5a). With regard to leaf stoichiometry, there were significant effects of height and height × orientation interaction on N content (including N_m and N_a ; Figure 5c,e). The N_m and N_a both varied along the canopy in the same fashion as the gas exchange and chlorophyll fluorescence (i.e., there was a decline in the US position of the canopy), but only did the N_m show a significant quadratic correlation with the PAR_{sum} ($R^2 = 0.831$, $p < 0.001$; Figure 5c). In contrast to the N_m , the C_m followed a shallower gradient throughout the canopy (only 0.91% of dispersion coefficient), though significant effects of canopy height and orientation were observed (Table S1). As a result, a significant quadratic correlation was also found between the ratio of C_m to N_m ($C:N$) and the PAR_{sum} (Figure 5f). As for pigment concentrations, chlorophyll (Chl_{a+b}) decreased markedly as canopy height increased, and they were lower in leaves growing on the southern face than on the northern face, similar to the variation in Car (Figure 5b,d). Finally, the PAR_{sum} had a significantly negative power relationships with Chl_{a+b} and Car ($R^2 = 0.648$, $p = 0.017$; $R^2 = 0.662$, $p = 0.001$). The gradual drop in the chlorophyll to carotenoid ratio ($Chl_{a+b}:Car$) was observed as canopy height increased on account of more dramatic changes in Chl_{a+b} , though there was no response to the PAR_{sum} (Table S1).

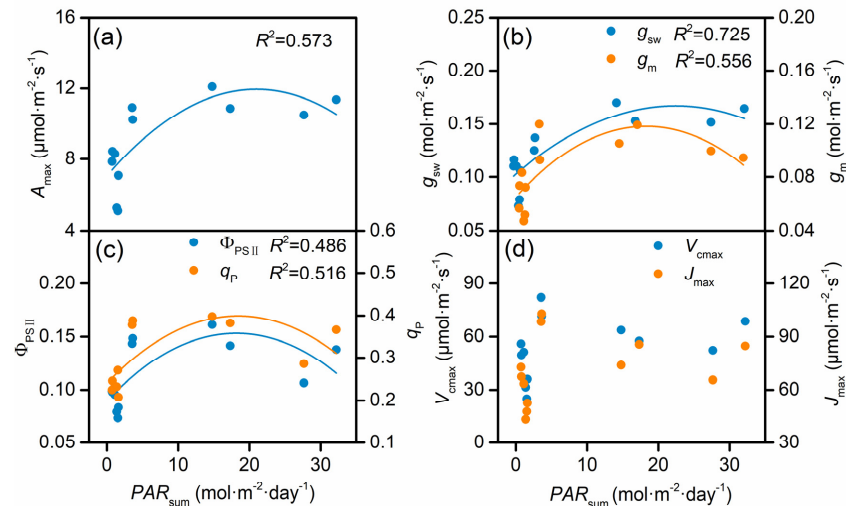


Figure 4. Correlations of daily photosynthetic active radiation (PAR_{sum}) with (a) light-saturated photosynthesis (A_{max}), (b) stomatal conductance (g_{sw}), (b) mesophyll conductance (g_m), (c) actual photochemical efficiency of PSII (Φ_{PSII}), (c) photochemical quenching coefficient of PSII (q_p) throughout the camphor tree canopy, (d) maximum carboxylation rate at 25 °C (V_{cmax}), and (d) potential maximum electron transport rate at 25 °C (J_{max}). Regression lines are only shown for significant relationships ($p < 0.05$). Blue circles indicate A_{max} , g_{sw} , Φ_{PSII} , and V_{cmax} of canopy leaves, and orange circles indicate g_m , q_p , and J_{max} .

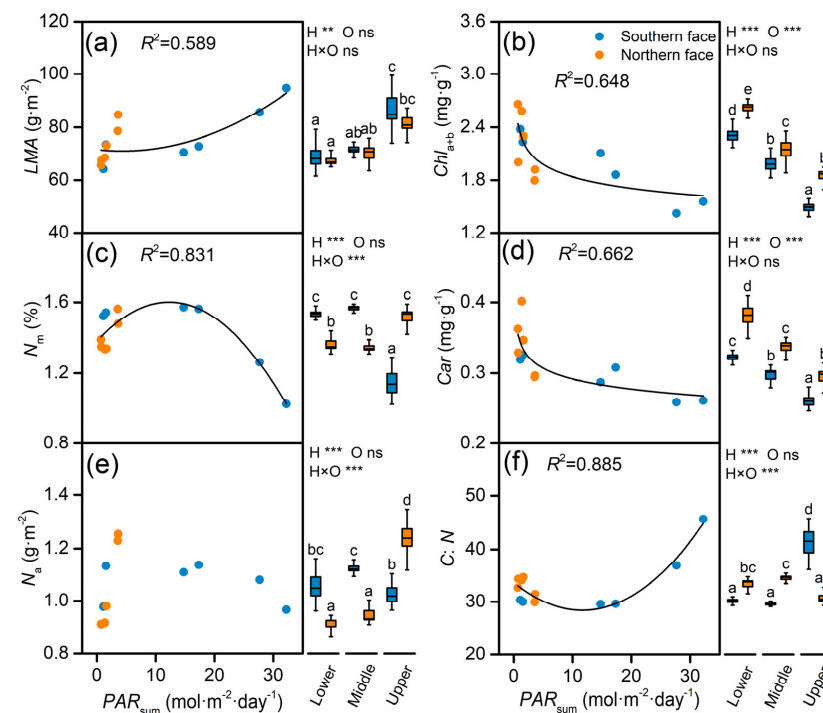


Figure 5. Correlations of daily photosynthetic active radiation (PAR_{sum}) with (a) leaf dry mass per area (LMA), (b) chlorophyll concentration (Chl_{a+b}), (c,e) mass- (N_m) and area-based (N_a) nitrogen content, (d) carotenoid concentration (Car), and (f) ratio of C_m to N_m ($C:N$) throughout the camphor tree canopy. Blue and orange circles represent the southern and northern faces of the canopy, respectively. Regression lines are only shown for significant relationships ($p < 0.05$). The boxplots of the leaf traits are included on the right side of the scatter subplots, and both share the same Y-axis. Lowercase letters represent a significant difference across canopy heights and orientations according to Duncan's test ($\alpha = 0.05$). Capital letters represent canopy height (H), orientation (O), and their interaction ($H \times O$), whose significance is noted as ns $p > 0.05$, ** $p < 0.01$, and *** $p < 0.001$. All values are mean $\pm$ SE ($n = 6$).

3.3. Correlations Among $A_{\max}$ and Other Photosynthetic Traits

Across canopy positions and sampling times, the $A_{\max}$ correlated significantly with the g_{sw} , g_m , V_{cmax} , J_{max} , Φ_{PSII} , and q_P ($R^2 = 0.664$, $p < 0.001$; $R^2 = 0.634$, $p < 0.001$; $R^2 = 0.630$, $p < 0.001$; $R^2 = 0.330$, $p = 0.001$; $R^2 = 0.755$, $p < 0.001$; $R^2 = 0.636$, $p < 0.001$; Figure 6a). Meanwhile, the g_m related negatively to the C_i - C_c drawdown and Chl_{a+b} ($R^2 = 0.278$, $p = 0.008$; $R^2 = 0.519$, $p = 0.008$; Figure S3a), and there was also a strong positive relationship between the V_{cmax} and the J_{max} ($R^2 = 0.650$, $p < 0.001$; Figure S3b). Additionally, the N_a was positively associated with the g_m , J_{max} , Φ_{PSII} , and q_P ($R^2 = 0.369$, $p = 0.036$; $R^2 = 0.364$, $p = 0.038$; $R^2 = 0.346$, $p = 0.044$; $R^2 = 0.545$, $p = 0.006$; Figure 6b,c) but had no relationships with the V_{cmax} ($p > 0.05$; Figure 6c).

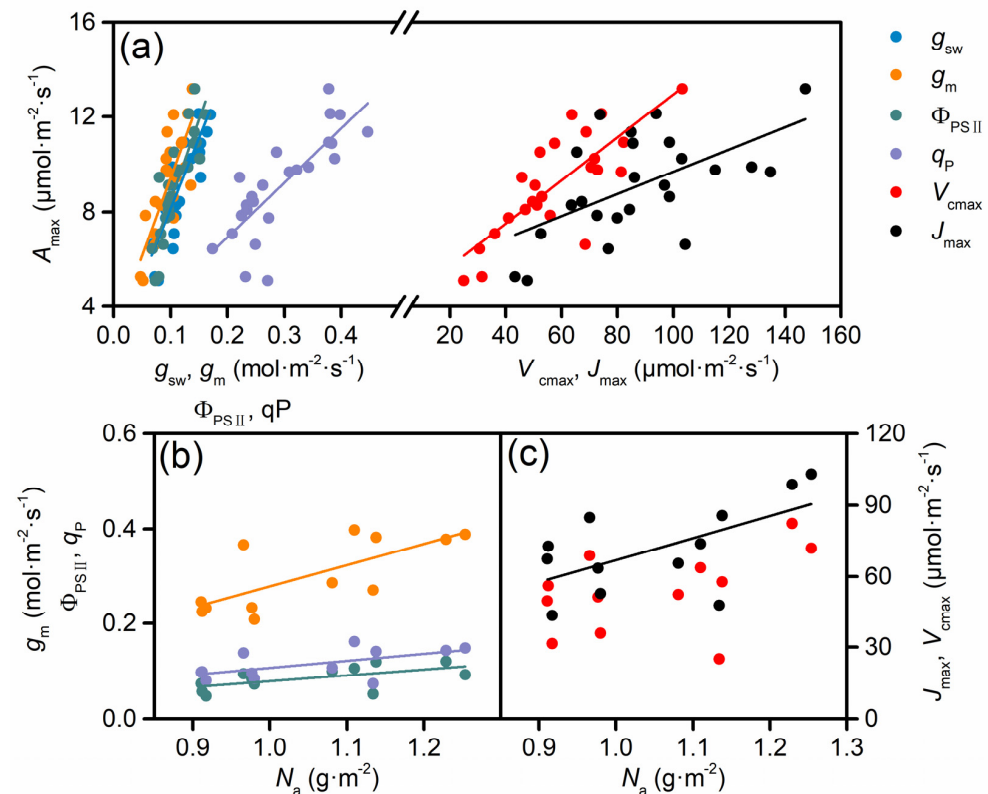


Figure 6. Linear regression relationships (a) between light-saturated photosynthesis ($A_{\max}$) and stomatal conductance (g_{sw}), mesophyll conductance (g_m), actual photochemical efficiency of PSII (Φ_{PSII}) and photochemical quenching coefficient of PSII (q_P), maximum carboxylation rate at 25 °C (V_{cmax}), potential maximum electron transport rate at 25 °C (J_{max}). (b,c) Between nitrogen content on an area basis (N_a) and Φ_{PSII} , q_P , g_m , V_{cmax} , and J_{max} across canopy positions during the summer and autumn seasons (or only during summer period). Regression lines are only shown for significant relationships ($p < 0.05$).

3.4. Quantitative Limitation to Photosynthesis Throughout the Canopy

Figure 7 presents the contribution of stomatal (S_L), mesophyll (M_L), and biochemical (B_L) limitations to relative changes in the $A_{\max}$ throughout the canopy from August to October. MS position leaves reached the maximum $A_{\max}$ with the lowest total limitation, and upper leaves were close behind, limited to a certain extent by the photosynthetic process. The weaker light decreased photosynthesis to a greater extent. In summer, g_m was the main contributor to photosynthetic limitations throughout the canopy, including for US position leaves (Figure 7a). But during the autumn period, the decline in the $A_{\max}$ was primarily triggered by stomatal and biochemical limitations due to the up-regulation in g_m .

B_L explained the major relative changes in the A_{max} of upper sunlit leaves in particular (US position; Figure 7b).

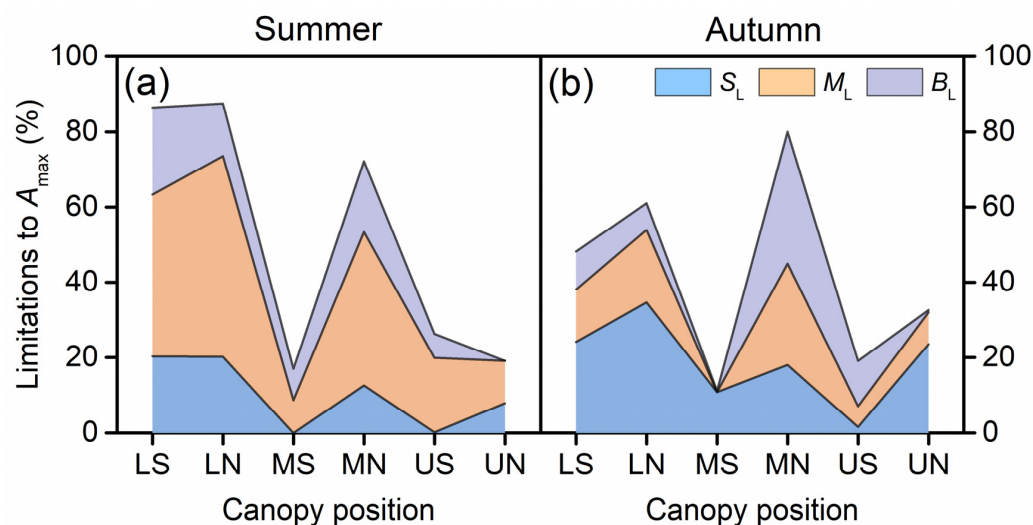


Figure 7. Quantitative limitation to light-saturated photosynthesis (A_{max}) among canopy positions during summer (a) and autumn (b). US and UN, MS and MN, LS and LN represent the southern and northern faces at the upper, middle, and lower canopy layers, respectively. S_L , M_L , and B_L represent the contribution of stomatal, mesophyll, and biochemical limitations, respectively.

4. Discussion

4.1. Foliar Physiological and Biochemical Traits Regulated by Light Availability

Our research investigated changes in leaf functional traits, such as the LMA , C and N contents, and pigment concentrations along the canopy of mature *C. camphora*. We found that the LMA was closely related to the amount of incident light, increasing significantly as canopy height increased, in accordance with previous research [35]. Conversely, the N_m was notably constrained in the sunlit leaves of the upper canopy and showed a substantial decline. While some studies suggested that the N_m remained constant regardless of spatial irradiance heterogeneity within the canopy [42], the leaves with higher LMA —as characterized by thicker, denser structures with more palisade layers—tended to ‘dilute’ the N_m . This reveals a greater investment in leaf structure (such as cell wall development) rather than in photosynthesis (such as prevention of mesophyll damage from intense light and enhancing leaf resilience), as supported by our observation of a higher $C:N$ ratio in corresponding canopy position leaves [43,44]. In contrast, the N_a did not respond to light, primarily due to a counteracted effect of variations in the LMA and N_m . It was contrary to the views of Kenichi [8] and Jin et al. [45], who found that leaf LMA increased and N_m remained unchanged with the canopy height in natural forests, leading to the positive correlation between light and N_a . This discrepancy may be attributed to species-specific differences in canopy structure and nitrogen distribution acclimating to their respective environments. We also observed an apparent intra-canopy light gradient to which pigments were responding. In line with previous studies on forest-grown trees, irradiance had significantly negative relationships with Chl_{a+b} and Car [46], thus indicating that shaded leaves require more light capture to improve photosynthetic efficiency [47,48]. In the upper-canopy leaves that were exposed to high light, more Car was invested than Chl_{a+b} (resulting in lower $Chl_{a+b}:Car$) so as to dissipate excess light energy and to protect the photosynthetic machinery [49,50].

Photosynthetic physiological and biochemical characteristics strongly varied with the light gradient, which are predetermined to scale up from the bottom to the top of

canopy [51,52]. Contrary to our expectation, however, we observed that the $A_{\max}$ peaked in the south-facing leaves of the mid canopy, and it failed to acclimate to high light as full as the LMA in the upper sunlit leaves. Other photosynthetic parameters, such as the g_{sw} , g_m , Φ_{PSII} , q_P , V_{cmax} , and J_{max} , simultaneously exhibited similar patterns to the $A_{\max}$, declining in leaves at the corresponding position, and had a positive linear correlation with the $A_{\max}$ along the light gradient. Most parameters showed quadratic correlations with light, whereas the V_{cmax} and J_{max} did not follow this pattern, mainly because the maximum values in leaves occurred under lower light conditions to avoid photoinhibition [53]. The decrease in the g_{sw} and g_m reflects the limitation of CO_2 diffusion resistance on the photosynthetic rate in leaves. Since the photosynthetic biochemistry acts as the core driver of photosynthesis, the reduced energy conversion and transport efficiency (i.e., Φ_{PSII} , q_P , and J_{max}) and limited RuBisCO (RuBP carboxylase) amount and activity (i.e., V_{cmax}) would also weaken photosynthetic efficiency. The upper sunlit canopy, as the ‘engine’ of light capture, determines the level of the whole canopy productivity. Any slight decline in photosynthetic capacity can be gradually amplified through resource allocation imbalance and hormone signal disorder, ultimately manifested as decreased biomass accumulation and abnormal growth of the plant [5].

4.2. Strategies of Canopy Resource Allocation to Photosynthetic Traits

The photosynthetic progress needs to be supported by sufficient nutrients, and leaf nitrogen is assumed to be crucial for the regulation of synthesis and the activity of photosynthetic organs [54,55]. The amount of RuBisCO that predominantly determines the V_{cmax} accounts for a large fraction of the total N in leaves, while N is allocated to electron transport system including cytochrome *f* and coupling factor [56,57]. The V_{cmax} and J_{max} are closely coordinated to ensure that the energy supply from light harvesting and electron transport meets the carboxylation demand [29]. Our data suggests that both the V_{cmax} and J_{max} were constrained by N loss in the sunlit leaves of the upper canopy due to their simultaneous decreases, which resulted in reduced energy demand for the Calvin cycle, and paralleled descents in the Φ_{PSII} and q_P as a crucial protective strategy for the photosystem reaction center. Since the J_{max} decreased more significantly than the V_{cmax} did, the lowered J_{max} to V_{cmax} ratio displayed the imbalance between light-driven electron transport and RuBisCO carboxylation capacity, and contributed to avoiding photoinhibition under high light conditions [58]. However, we found a positive relationship of N_a with the J_{max} , Φ_{PSII} , and q_P , but not with the V_{cmax} . This may be due to intra-canopy gradients in other environmental conditions (not light availability) that also influence the V_{cmax} [4,59]. Furthermore, total N in leaves is also used for non-photosynthetic opponents (e.g., cell walls), which further complicates the relationship between N concentration and the V_{cmax} [60]. Additionally, total N likewise impacts the g_m through the changes in anatomical structure and relative gene expression that regulate transmembrane transport [61–63], which concurs with our finding of a strong coupling between the N_a and g_m .

According to the hydraulic limitation hypothesis, as trees grow in stature, leaf water stress increases due to gravity and path-length resistance. Upper-canopy leaves are most suitable for absorbing sunlight, but they are also more prone to suffering from sun exposure, temperature, and wind stress especially in summer, exhibiting the greatest demand for water [42]. In our study, the upper sunlit leaves in camphor trees had a lower $A_{\max}$ and g_{sw} compared with southern leaves of mid canopy, indicating that they are probably subject to a water deficit (i.e., the reduced leaf water potential) driven by both a high transpiration rate and insufficient water availability [17,27], as the g_{sw} is regulated by hydraulic conductance [64]. Eventually, leaf growth and photosynthesis are restricted, even if the soil humidity is adequate [65]. The overgrowth in camphor tree height in overly dense

stands (up to nearly 20 m high) intensifies the burden of hydraulic transport to upper leaves and accelerate water transpiration through stomata. In light of the trade-off between xylem hydraulic efficiency and safety, the sun leaves in the upper canopy could close stomata and mitigate the water loss to prevent cavitation or embolism of the branch xylem, albeit at the expense of reduced photosynthesis [65–67]. In conclusion, the concomitant decrease of the g_{sw} and N_m in top canopy leaves reveals the optimal co-allocation of leaf nitrogen and hydraulic conductance in plant canopies, as the waste of excess nitrogen investment under the condition of hydraulic supply shortage can be avoided [17].

4.3. Effect of Season on Canopy Photosynthesis and Its Limitations

The seasonal effects on leaf photosynthetic traits can be highlighted through a comparison of the summer and autumn periods, which could be characterized as a modest growing season and a near-dormant season, respectively. The notable disparities were observed only in the g_{sw} , g_m , C_i , and J_{max} , whereas the A_{max} remained unchanged. It was primarily due to no change in the CO_2 supply in the carboxylation site (i.e., C_c) and the RuBisCO carboxylation rate that Calvin cycle was limited (i.e., V_{cmax}) in spite of the significant increase of the g_m and J_{max} . Many studies have documented a marked decrease in photosynthetic traits in woody species throughout the whole growing season due to leaf ontogeny and the severity of summer drought [35,53,68]. Our study, nonetheless, only focused on the current-year mature leaves of camphor trees, a broad-leaved evergreen species, which did not suffer from leaf senescence prior to defoliation nor from obvious summer drought from August to October. We therefore speculate that the seasonal fluctuations in the g_{sw} , g_m , C_i , and J_{max} may be ascribed to the different rates (i.e., time scale) at which these photosynthetic traits acclimated to changes in the temperature and PAR , consistent with the findings of Yu et al. [60]. Actually, distinguishing between the contributions of leaf ontogenetic development and of climatic impacts at the seasonal scale remains a challenge because of their mutual dependence [69].

In our study, the g_{sw} declined in camphor tree leaves as the air cooled, and this has also been reported by some researchers who have proposed that stomata close at lower temperatures to prevent water loss and energy consumption [70]. Because mid-canopy leaves are better protected from temperature and wind extremes and can transport water more effectively, some researchers have suggested that their increased g_{sw} outperforms upper sunlit leaves, particularly when exposed to sunflecks [34,71]. This finding is corroborated by our results, where this phenomenon occurred during the summer period when apparent sunflecks persisted 2–4 h from mid-morning to noon each day (Figure S2). As the sunflecks diminished in autumn, due to the decreasing solar zenith angle, a corresponding stronger stomatal closure was observed. Our observations display the adaptability and sensitivity of stomata in camphor leaves to high-light conditions across a certain seasonal temperature range.

The g_m is influenced by leaf anatomical traits (e.g., cell wall thickness and chloroplast surface area exposed to intercellular air space, S_c) and biochemical mechanisms (e.g., the quantity and activity of aquaporins, AQP, and carbonic anhydrase, CA) [26]. AQP, located in the chloroplast envelope and plasma membrane, facilitate CO_2 diffusion into the stroma, whereas CA regulates pH value inside the chloroplast by interconversion between CO_2 and HCO_3^- to enhance CO_2 diffusion to the carboxylation site [72,73]. Previous studies suggest that the LMA increases gradually as season progresses, and a higher LMA could lead to an increased cell wall thickness and a reduced g_m [74]. Instead, our study revealed the marked enhancement in g_m from summer to autumn, indicating that membrane permeabilities and cytosol probably dominate the variation in g_m [75,76]. Bernacchi [39] suggested that the g_m gradually declined at temperatures over 35 °C in *Nicotiana*, whereas other researchers

found the reduced g_m from around 27–29 °C to 40 °C in tree species [77–79]. Although our measurements were conducted at a mean maximum air temperature of 30.38 °C in summer, many sampling leaves were subjected to leaf temperatures exceeding 35 °C inside the cuvette, which may generate the deactivation of AQPs and CA proteins and finally lead to the significant reduction in g_m of canopy leaves. The lower leaves with thicker chloroplasts were more prone to the decline in CA activity [80], consistent with our finding of the negative link between g_m and Chl_{a+b} (Figure S3a).

In terms of photosynthetic biochemical capacity, we found that only the J_{max} was significantly suppressed along the camphor canopy profile in summer, whereas the V_{cmax} did not vary seasonally. It indicates that the electron transport chain is more sensitive to high temperatures, thereby causing ATP shortage and RuBP regeneration limitation. In other words, the J_{max} is more susceptible to environmental changes than the V_{cmax} , as was also supported by Kumarathunge [81]. The value of C_i is determined by an equilibrium between the CO_2 supply from the atmosphere and the CO_2 demand in the chloroplast. Its dependence on the simultaneous changes in stomatal opening (e.g., g_{sw}) and photosynthetic capacity (e.g., J_{max}) thereby accounts for the differences observed between the two seasons.

Based on the observations above, a quantitative limitation analysis was conducted to assess the effects of canopy position and seasonal climate on stomatal (S_L), mesophyll (M_L), and biochemical limitations (B_L) to the A_{max} in camphor trees. It was found that the limitation to photosynthesis decreased with within-canopy light intensity. However, the relative contributions were distinct between summer vs. autumn periods, though small differences were observed in the A_{max} . During the summer, characterized by higher temperatures, the g_m was the main contributor to the reduction in the A_{max} of canopy leaves, whereas S_L and B_L became predominant when the weather grew cooler in autumn because of the rising g_m . With regard to the decline in A_{max} of upper sunlit leaves, M_L and B_L could explain the main cause in summer and autumn, respectively. Bachofen et al. [4] reported stomatal limitation of photosynthesis in top European beech canopy leaves during spring, while Cano et al. [35] identified both the g_s and g_m as the key limitations during summer. Instead, Crous et al. [33] did not detect canopy differences in the g_m and g_s of mature eucalyptus leaves. It is possible that the differences in photosynthetic limitations along the canopy can be attributed to species-specific physiological responses to climate.

5. Conclusions

Here, we showed the significant effect that canopy position and season have on most of the leaf photosynthetic properties during the summer and autumn seasons in dense stands of mature camphor trees (*Cinnamomum camphora*). Different from the variations in the LMA and C content, the key gas exchange and chlorophyll fluorescence parameters increased along the canopy merely within a certain range of irradiance. In spite of being exposed to high light, the upper sunlit leaves exhibited a limited photosynthetic process (i.e., lower A_{max} , g_{sw} , g_m , q_P , Φ_{PSII} , J_{max} and V_{cmax}), which was generated by nitrogen loss and water deficit. On the other hand, despite the significant differences in the g_{sw} , C_i , g_m , and J_{max} between two seasons, the A_{max} did not respond to seasonality due to there being no variation in CO_2 supply in the carboxylation site (C_c) and RuBisCO carboxylation rate that limits the Calvin cycle (V_{cmax}). From the perspective of quantitative limitation analysis, our findings highlight that mesophyll and biochemical limitation contribute the most to the A_{max} decrease in summer and autumn, respectively. It is recommended to conduct the moderate regulation and management of stand density for mature forests. These findings are vital for the accurate prediction of carbon sink capacity of urban vegetation in the region.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f16040581/s1>, Figure S1: Bamboo experimental platform established in Shanghai Botanical Garden; Figure S2: Diurnal variation in photosynthetic active radiation (PAR) across canopy positions between summer and autumn. US and UN, MS and MN, LS and LN represent the southern and northern faces at the upper, middle, and lower canopy layers, respectively. FL represents full light above the canopy; Figure S3: Linear regression relationships (a) between mesophyll conductance (g_m) and the CO_2 concentration difference from stomatal cavity to carboxylation site ($C_i - C_c$), chlorophyll content (Chl_{a+b}), and (b) between maximum carboxylation rate of RuBisCO at 25 °C (V_{cmax}) and potential maximum electron transport rate at 25 °C (J_{max}) across canopy positions during summer and autumn seasons (or only during summer period); Table S1: Leaf morphology, stoichiometry and pigment across different canopy positions for mature *C. camphor* trees.

Author Contributions: Conceptualization, L.Z. and H.L.; methodology, L.Z. and H.L.; formal analysis, H.L.; data curation, H.L.; writing—original draft preparation, H.L.; writing—review and editing, H.L., L.Z. and W.Y.; visualization, H.L. and W.Y.; supervision, W.Y.; project administration, W.Y.; funding acquisition, W.Y. All authors have read and agreed to the published version of the manuscript.

Funding: The study has been funded by the Natural Science Foundation of Shanghai (22ZR1461000) and the National Natural Science Foundation of China (32471683, 31600352).

Data Availability Statement: The data that underlie this study are available upon request.

Acknowledgments: We thank Zhang Chao for helpful suggestions on the manuscript. Special thanks to Guo Jiankang for providing meteorological data. Finally, we thank Jiang Kai for English editing.

Conflicts of Interest: The authors declare no conflicts of interest.

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