Article

Differentiation in Leaf Physiological Traits Related to Shade and Drought Tolerance Underlies Contrasting Adaptations of Two *Cyclobalanopsis* (Fagaceae) Species at the Seedling Stage

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Abstract: Adaptation and acclimation of tree seedlings to different combinations of light and water conditions can determine the species-specific patterns of distribution along environmental gradients and the underlying physiological mechanisms are fundamental to the understanding of such patterns. Seedlings of two *Cyclobalanopsis* species naturally occurring in southwest China, with distinct distribution and regeneration characteristics, were grown under 100%, 50% and 4% sunlight conditions and traits related to shade and drought tolerance were studied. Particularly, we investigated whether leaf hydraulics, photosynthetic traits and their functional coordination play an important role in determining seedling environmental adaptation and acclimation of the two species. Seedlings of *C. helferiana* showed characteristics adapted to high irradiance while *C. rex* had traits adapted to partially shaded environments. *Cyclobalanopsis helferiana* had significantly higher maximum net photosynthetic rate (A_{max}), light compensation point and light saturation point than *C. rex* and the contrasts were particularly large when they were grown under full sunlight. *Cyclobalanopsis helferiana* showed the highest A_{max} when grown under 100% sunlight, while *C. rex* exhibited the highest A_{max} at 50% sunlight. Similarly, under full sunlight conditions *C. helferiana* showed significantly higher leaf hydraulic conductance (K_{leaf}) than *C. rex*, i.e., 13.37 vs. 7.09 mmol m^{-2} s^{-1} MPa^{-1} ($p < 0.01$). The correlation between K_{leaf} and A_{max} followed a unified positive correlation across different light treatments of both species. Moreover, leaves of *C. helferiana* showed greater resistance to drought-induced hydraulic dysfunction and to desiccation than *C. rex*. The contrasts in functional traits between the two *Cyclobalanopsis* species are consistent with the hypothesis that there is a trade-off between shade tolerance and drought tolerance. Findings of the present study contribute to a deeper understanding of mechanisms of divergence between closely related (congeneric) species with respect to key ecophysiology associated with natural regeneration.

Keywords: drought tolerance; functional traits; leaf hydraulic conductance; photosynthesis; shade tolerance

1. Introduction

The distribution patterns of plants along environmental gradients often show significant interspecific differences, and the underlying mechanisms are important topics of plant ecophysiological investigations [1–5]. Changes in different environmental factors are often coupled in nature; for example, with the change of water conditions, the light environment of forests can change significantly. In dry forests, water deficiency is often accompanied by strong light and high temperature stresses, while in humid dense forests, trees, especially seedlings, are often subject to low light stress [6,7]. With the coupled changes of environmental factors, the environmental adaptation strategies of trees often change significantly. For example, trees in dry forests have evolved a series of adaptive characteristics in structure, physiology, phenology, etc. to resist or avoid drought, and at the same time, they have evolved characteristics to prevent physiological injury of leaves under high light [2,8–10]. Differently, trees in humid forests usually have strong shade tolerance at the seedling stage, but are sensitive to drought [11,12]. There is a synergistic or trade-off relationship between the adaptability of plants to different environmental limiting factors. The investigation into the interactive effects between different environmental limiting factors would contribute to a more comprehensive understanding of the environmental adaptability of plants [13,14].

At the seedling stage, trees are more sensitive to environmental stresses. It is of great significance to understand the adaptation and acclimation of tree seedlings to environmental factors in order to understand forest regeneration and hence to inform the protection and restoration of forest ecosystems. Light is an important limiting environmental factor for the regeneration of tree seedlings in forests by affecting their carbon economy [15–17]. Sunlight is absorbed by multiple layers of plants before it reaches the forest ground through the canopy, and the intensity of sunlight decreases exponentially. Generally, the irradiance level in the understory of dense tropical rainforests is less than 5% of full sunlight [18–20]. The structural and physiological characteristics of leaves play important roles in determining their adaptation and acclimation to low light environments. Shade tolerant plants usually have a series of characteristics favoring adaptation to low light environment, such as large specific leaf area, enhanced apparent quantum efficiency, lower light compensation point and lower respiratory rate, in order to maintain carbon balance and growth in the forest understory [14,21,22].

Drought is another important limiting factor for the survival and growth of tree seedlings. At the seedling stage, the root system is shallower and trees are thus more sensitive to drought, and the failure of water transport function caused by drought is an important reason for the death of tree seedlings [23,24]. It has been shown that leaves are the 'bottleneck' of the whole plant water transport pathway, and the resistance of leaves to water conduction accounts for about 30%–90% of the resistance of the whole plant [11,25,26]. In addition, the water transport function of leaves is generally more sensitive to drought stress than that of stems. A growing body of evidence shows that the water conduction of leaves is an important factor for plants to adapt to different environments [2,26,27]. It has been found that leaf hydraulic conductance (K_{leaf}) is closely related to the light environment of plant growth, that is, plants growing in high light conditions usually have significantly higher K_{leaf} values than those growing in low light environments [28,29]. The characteristics of leaf hydraulic conductance can be an important reference physiological characteristic to study the response of plants to the coupled changes of light and water conditions.

In this study, the seedlings of two species of *Cyclobalanopsis* (*C. helferiana* and *C. rex*) distributed in Yunnan Province of China with contrasting water and light conditions in their natural habitats were selected to carry out a comparative study on their ecophysiology related to regeneration. *Cyclobalanopsis helferiana* is widely distributed and has strong adaptability to arid environment. It is commonly found in the Savanna type of vegetation in Yuanjiang River, while *C. rex* is only distributed in the dense tropical rainforests in Yunnan Province. This study focuses on investigating the intrinsic differences between the two congeneric species of contrasting adaptations with a focus on leaf-level physiology related to shade and drought tolerance. The use of species within the same genus that are expected to have distinct adaptations to water and light conditions provide an ideal species pair for ecophysiological comparative

study [2,5,30], which would contribute to a better understanding of the underlying physiological mechanisms of species divergence in adaptation. Specifically, we tested the three following hypotheses: (1) Seedlings of *C. helferiana* have leaf structural and photosynthetic characteristics suitable for survival under high light conditions, while *C. rex* has characteristics related to stronger shade tolerance. (2) Leaves of *C. helferiana* have higher hydraulic conductance, particularly under high sunlight conditions, and exhibit stronger resistance to drought-induced hydraulic dysfunction than that of *C. rex*. (3) Photosynthetic carbon assimilation and leaf hydraulic conductance both have relatively high plasticity in response to growth light conditions and the two characteristics show a tight coordination in both species.

2. Materials and Methods

2.1. Overview of the Study Site and Species

The experiment was carried out in Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences (21°55′ N, 101°15′ E, 570 m elevation). The region is located in the north edge of Southeast Asia tropics, belonging to the north tropical monsoon climate zone, with an annual average temperature of 21.5 °C, the average temperature of the hottest month (June) of 25.5 °C, and the average temperature of the coldest month (January) of 14.8 °C. The record high and low temperatures were 40.5 °C and 2 °C, respectively. There is no frost all year round. There are distinct dry and wet seasons in the year, with an annual average rainfall of 1557 mm, including 1335 mm in the rainy season (May–October), 87% of the whole year, and 202 mm in the dry season (November–April), only 13% of the whole year (data from Ecological Station of XTBG, http://bnf.cern.ac.cn/ accessed on 22 July 2020).

Two evergreen tree species in the genus of *Cyclobalanopsis* (Fagaceae) are studied in the present investigation. The distribution range, habitat type and environmental adaptations of the two species are distinctly different. *Cyclobalanopsis helferiana* (A. de Candolle) Oers is naturally distributed in India, Myanmar, Thailand, Laos and Vietnam at elevations of 900–2000 m [31]. In China, it is naturally distributed in Guangdong, Guangxi, southern Guizhou, southern and southwest of Yunnan. In Yunnan province, it is commonly found in the Savanna type vegetation in the dry and hot valley of Yuanjiang River, from where the seeds used for seedling propagation in the present study were collected. *Cyclobalanopsis rex* (henmsley) Schottky is found in northwest India, Laos, Myanmar and Vietnam. In China, it is only distributed in the dense tropical rainforests with an elevation of 1100–1800 m in the west and south of Yunnan province. It is an endangered species with poor natural reproduction ability [31]. The seeds used in this experiment were collected from the tropical rainforest in Xishuangbanna, Yunnan Province. These two tree species were chosen because they belong to the same genus but are adapted to contrasting environmental conditions (Figure 1). Seeds of the two species were collected from multiple trees tens to hundreds of meters apart from each other at the abovementioned two sites, respectively.
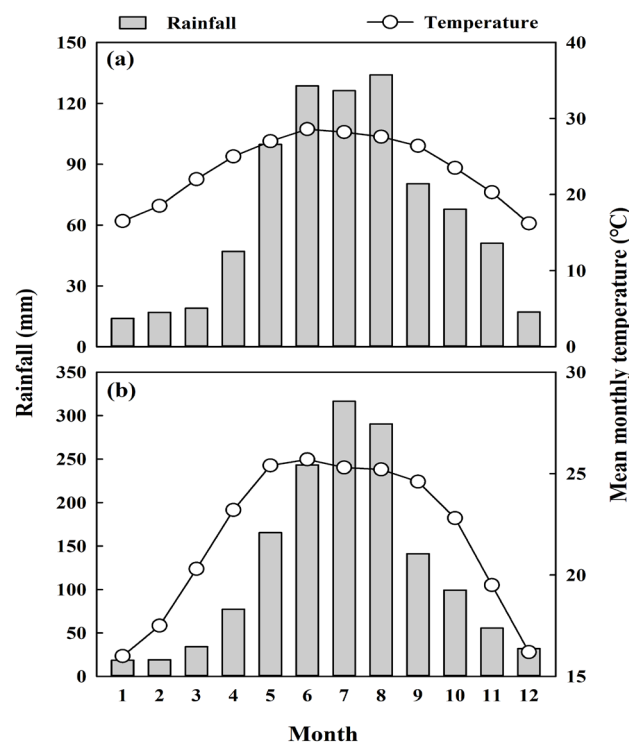
Figure 1. Monthly mean rainfall and temperature of Yuanjiang Savanna Valley (**a**) and Xishuangbanna Tropical Rainforest (**b**), Yunnan, SW China. Data from Yuanjiang Meteorological Station (396 m elevation) and Xishuangbanna Tropical Rainforest Ecosystem Station (570 m elevation), respectively.

2.2. Experimental Treatments

Seeds of the *Cyclobalanopsis* species were sown on a sand bed with about 50% sunlight intensity in July of 2005. Fine textured river sand was used and the sand had a depth of about 50 cm. The sand bed was watered daily to field capacity and weeding was done weekly. After the seedlings grew to a height of about 20 cm, they were transplanted into individual 15-L pots containing a mixture of local forest surface soil (2/3 in volume) and river sand (1/3 in volume) and were placed in a shade shed with 50% sunlight intensity in the nursery. By July of the next year, they were moved to conditions of full sunlight (100% SL), 50% sunlight (50% SL) and 4% sunlight (4% SL), respectively. The latter two sunlight levels were realized by using different shading nets for the shade houses. The daily maximum photosynthetic photon flux density (PPFD) values measured in the three nurseries were in the ranges of 1200–1300, 600–700, and 20–60 μmol m^{-2} s^{-1}, respectively. Under each light level, there were eight plants for each species and 48 individuals in total (2 species × 3 light levels × 8 replicates). Except for rainy days, plants were watered to full soil saturation once a day and compound fertilizer was applied once a month. Weeding operation and pest control were carried out in a timely manner throughout the whole experimental period. Physiological measurements were conducted during the period March–May of 2008 after about one and a half years of different light treatments. The two *Cyclobalanopsis* species, particularly *C. rex*, have relatively large seeds and thus newly germinated seedlings can obtain carbohydrates from the attached seeds for a relatively long time. This can potentially have a large effect on measurements related to shade tolerance. The one-year growing period before the start of the experiment guaranteed that the shading treatment effects were not confounded by this 'feeding' effect of seeds. When physiological measurements were carried out the plants for both species in 100% and 50% sunlight conditions were about 1 m in height and the ones grown in 4% sunlight were about 0.5 m in height.

2.3. Measurement of Leaf Photosynthetic Parameters

The light response of leaves was measured in March between 8:00 and 11:30 a.m. on clear days with a portable photosynthesis system (Li-6400, Li-COR Inc., Lincoln, NE, USA) in four seedlings under each light condition for each species. When the light intensity was weak in foggy conditions, the whole plant was placed in an artificial light source (high-pressure sodium lamps) of 1000 µmol $m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD) for 30 min for photoinduction. A heat filtering apparatus (a water reservoir) constructed using transparent plexiglass containing circulating water was placed between the plants and the light source to prevent overheating of the leaves. After the photoinduction treatment under this light source, the plant was moved away to natural sunlight conditions for photosynthetic gas exchange measurements. Mature leaves from the middle part of the terminal branches were selected for light response measurements. The PPFD of the gas exchange cuvette of the L-6400 photosynthesis system was set to change in a decreasing manner as 1700, 1500, 1000, 800, 500, 300, 200, 150, 100, 50, 20, 0 µmol $m^{-2} s^{-1}$ using the LI-6400-04A light source (Li-COR). The leaf cuvette temperature was maintained at 30 °C, the CO_2 at ambient levels, and the vapor pressure deficit ranged from 0.8 to 1.7 kPa. The data were recorded at each light level after the gas exchange parameters stabilized (about 3–5 min). The light response curves were fitted using the algorithm function $Y = Y_{max} - ae^{-bx}$ [32], where Y is net photosynthetic CO_2 assimilation rate per leaf area (A_n), x is PPFD and a×b is the initial slope of the light response curve, and Y_{max} is the maximum photosynthetic assimilation rate (A_{max}). Light compensation point (LCP) and the dark respiration rate (R_d) were obtained by fitting a linear regression to the initial part of the curve with PPFD ranged between 0 and 100 µmol $m^{-2} s^{-1}$, i.e., the LCP and R_d were calculated as the x- and y-axis intercepts, respectively. The light saturation point (LSP) was calculated as the PPFD leading to 90% of A_{max}.

2.4. Measurement of Leaf Anatomical Characteristics

Mature leaves of the two *Cyclobalanopsis* species grown under three different light treatments were collected in early April, and the leaf tissues were taken from the middle part of the lamina to make freehand sections. The thickness of each layer on the cross section of the leaf lamina was measured under an optical microscope. Two leaves were randomly sampled from each plant and two sections were made for each leaf. Four plants for each species under each of the three light conditions were measured. The nail polish impression method was used to measure stomatal density and guard cell length. Because there were dense pubescences on the underside of *C. helferiana* leaves, leaves were brushed gently to remove pubescences before applying the nail polish. In addition, six leaves were sampled from each of the four plants and their areas were determined using a leaf area meter (LI-3000A, Li-COR Inc., Lincoln, NE, USA). The leaves were then oven dried at 70 °C for 48 h and the dry masses were determined using a four-digit electronic balance. Leaf mass per area (LMA) was calculated as the dry mass divided by the area.

2.5. Determination of Maximum Leaf Hydraulic Conductance (K_leaf)

For both species, two leaves were randomly collected in April from each of four plants under each light treatment, and K_{leaf} was determined by the high-pressure gas pressurization method [33]. Leaves were collected before sunrise, sealed in individual ziplock bags with wet paper towels, stored in an insulated box, and taken back to the laboratory (*ca.* 1 km from the nursery) for immediate measurement. A leaf was installed to a PMS1000 pressure chamber (PMS Instrument, Corvallis, OR, USA) to pressurize until water emerges from the xylem of the petiole [34]. The balancing pressure was maintained for about two minutes. After wiping off the water drop from the petiole, the pressure was increased rapidly by 0.3 MPa and then maintained constant. Xylem sap flowing out within 10 s was collected using a piece of pre-weighed (to the nearest 0.0001 g) absorbent paper, which was weighed again after sap collection. The leaf hydraulic conductance was calculated as follows:

$$K_{\text{leaf}} = \frac{WM/M}{\Delta t \; \Delta P \; LA} \tag{1}$$

where *WM* is the mass of collected water (g), *M* is the molar mass of water, Δt (s) is the time duration for sap collection, ΔP is the pressure difference before and after the increase in chamber pressure (MPa), and LA is the leaf area (m^2).

2.6. Drought Treatment and Construction of Leaf Hydraulic Vulnerability Curves

The leaf hydraulic vulnerability to drought was determined by measuring the declining trend of leaf hydraulic conductance in the process of a plant drought treatment for seedlings grown under the 100% and 50% sunlight treatments. The plants grown under the 4% sunlight condition were not used for leaf hydraulic vulnerability determination due to their small sizes that did not provide enough leaves for such measurements. To allow plants to reach different water potentials needed for the hydraulic vulnerability curve construction, a drought treatment was applied after all other measurements had been finished. In both the 100% and the 50% sunlight nurseries, rain shelters made of transparent plastic cloth were placed *ca.* 1 m above the plants to exclude rainfall during the process of plant dehydration in mid-April. For each species and light level combination, four plants were used for the measurement. At the beginning of the experiment, the soil surface in the pot was covered with plastic film to reduce the evaporation of water on the soil surface. The leaf water potential (Ψ_L) and K_{leaf} were measured on the 1st, 3rd, 5th, 7th and 10th days after watering was withheld. During the experiment, the leaves were randomly collected in the early morning of each day, sealed in plastic ziplock bags containing wet paper towels, and then taken back to the laboratory for determination of Ψ_L and K_{leaf}. The K_{leaf} values at different water conditions were measured using the same method as described above for measuring maximum K_{leaf}. The sigmoid equation was used to fit the relationship between K_{leaf} and Ψ_L, from which the water potential corresponding to 50% loss of leaf hydraulic conductance ($P50_{\text{leaf}}$) was obtained.

2.7. Determination of Leaf Dehydration Curves

The stomata usually close within 30 min after a leaf is excised, and a relatively low and basically constant water loss rate, i.e., cuticle transpiration rate, is maintained in the following hours [30,35]. In mid-April, terminal branches of the two *Cyclobalanopsis* species grown under 100% and 50% sunlight conditions were collected in the evening between 19:00 to 20:00. Branches were quickly inserted into water and segments about 2 cm in length were cut off from the base under water, so that the water column in the xylem conduits of the branches could be connected with the water body in the container. Four branches each from a different individual were sampled for each species and light level treatment combination. The branches were taken back to the laboratory with the cutting ends kept in water and the upper parts wrapped in black plastic bags. The branches were allowed to rehydrate for about two hours and then mature leaves were randomly selected for leaf dehydration curve construction. Briefly, after drying the leaf surface with tissue paper the saturated mass was measured with an electronic balance. The leaf was then allowed to dehydrate on the lab bench under dim light and the mass was determined periodically. Mass was determined every two minutes in the first 30 minutes and every 10 minutes later on. The duration of the dehydration process ranged between 6 and 7 h. At the end, leaves were dried using an oven at 70 °C for 48 h for dry mass determination. The relative water content (RWC) of leaves at different stages of the dehydration process can then be calculated. Four replicates were measured for each species grown under each light condition. During the experiment, the PPFD of the laboratory is about 3 $\mu mol \; m^{-2} \; s^{-1}$, and the temperature was maintained at about 20 °C.

2.8. Data Analysis

Two-way analyses of variance (two-way ANOVAs) followed by LSD post-hoc tests were used to test the differences between species and the effects of different light treatments on functional traits.

Sigmaplot 10.0 was used to analyze the correlations between functional traits. For the leaf vulnerability curves, sigmoid functions were fitted to the data.

3. Results

The results of light response measurements in seedlings of the two *Cyclobalanopsis* species grown under different light conditions showed that photosynthetic characteristics of the two species were significantly different, and the differences between them were most significant under 100% sunlight (Table 1, Figure 2). Under 100% sunlight, A_{max}, LCP and LSP of *C. helferiana* were 107%, 57% and 59% higher, respectively, than those of *C. rex*, while R_d was 49% of *C. rex*. There was no significant difference in A_{max}, LCP, LSP and R_d between the two species under 50% sunlight. Under 4% sunlight, A_{max} and LSP of *C. rex* were significantly higher than those of *C. helferiana*, which were 2.1 and 2.9 times higher, respectively, but there was no significant difference in LCP and R_d (Table 1). In general, the maximum net photosynthetic rate, light compensation point and light saturation point of *C. helferiana* were higher than those of *C. rex* with few exceptions under the deep shade treatment.

Table 1. Results of photosynthetic light response curves of *C. helferiana* and *C. rex* seedlings grown under three different light conditions. Data are means ± 1SE ($n = 4$). Different capital letters following the values indicate significant difference ($p < 0.05$) between species under the same light intensity, and different lowercase letters indicate significant difference ($p < 0.05$) between different light intensities within the species. A_{max}, maximum photosynthetic net CO_2 assimilation rate; LCP, light compensation point; LSP, light saturation point; R_d, dark respiration rate.

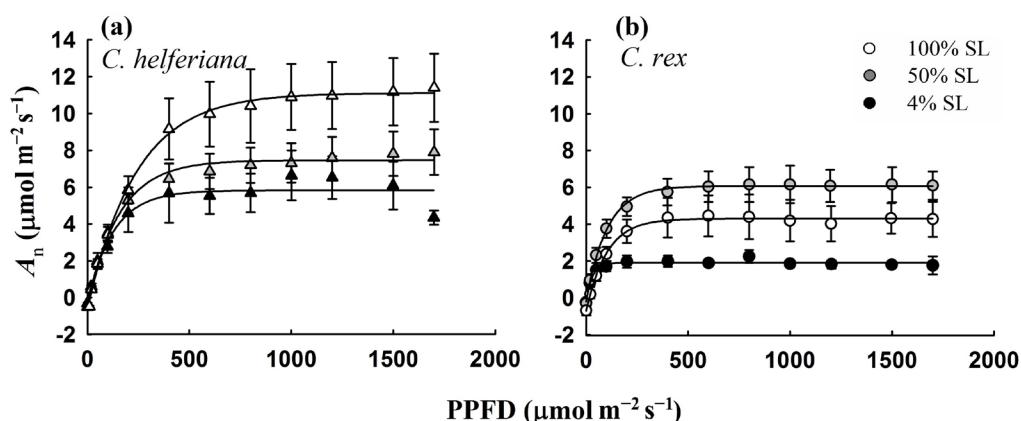| Title	Irradiance	A_{max} (µmol m^{-2} s^{-1})	LCP (µmol m^{-2} s^{-1})	LSP (µmol m^{-2} s^{-1})	R_d (µmol m^{-2} s^{-1})
	100%	9.98 ± 1.65Aa	19.4 ± 2.64Aa	517.1 ± 35.2Aa	0.47 ± 0.10Ba
C. helferiana	50%	7.52 ± 0.98Ab	7.13 ± 0.76Ab	336.7 ± 15.5Ab	0.33 ± 0.03Aa
	4%	5.52 ± 0.34Ac	7.70 ± 0.65Ab	232.4 ± 9.4Ac	0.45 ± 0.02Aa
	100%	4.82 ± 0.68Bb	12.3 ± 0.90Ba	325.4 ± 8.2Ba	0.96 ± 0.10Aa
C. rex	50%	7.05 ± 0.44Aa	6.58 ± 1.27Ab	227.6 ± 7.2Bb	0.40 ± 0.03Ab
	4%	1.78 ± 0.12Bc	5.98 ± 1.01Ab	59.7 ± 3.3Bc	0.45 ± 0.01Ab

Figure 2. Photosynthetic response curves of (**a**) *C. helferiana* and (**b**) *C. rex* seedlings grown under three different light conditions, i.e., 100%, 50% and 4% sunlight (SL). Values are means ± 1SE ($n = 4$).

There were also significant differences in leaf anatomical traits between the two species (Table 2). Under the same light intensity, the leaf thickness and LMA of *C. helferiana* were significantly larger than those of *C. rex*. The thickness of palisade tissue had no significant difference between the two species, but the thickness of sponge tissue of *C. rex* was significantly larger than that of *C. helferiana*, which meant that the former species had a significantly higher sponge-to-palisade thickness ratio than

the later species. Compared with *C. rex*, the leaf stomatal density of *C. helferiana* is significantly smaller, but the length of guard cells is significantly larger ($p < 0.01$).

Table 2. Leaf structural traits of *C. helferiana* and *C. rex* seedlings grown under three different light conditions. Data are means ± 1SE ($n = 4$). Different capital letters indicate significant difference ($p < 0.05$) between different species under the same light intensity, and different lowercase letters indicate significant difference ($p < 0.05$) between different growth light conditions within the species. SD, stomatal density; GCL, guard cell length; LMA, leaf mass per area.

Title	Irradiance	Palisade Thickness (μm)	Spongy Thickness (μm)	Palisade/ Spongy	SD (mm^{-2})	GCL (μm)	LMA (g m^{-2})
C. helferiana	100%	32.91 ± 1.43Aa	26.7 ± 2.2Aab	0.81 ± 0.03Bb	386 ± 27.1Ba	28.2 ± 0.9Ab	159 ± 8.1Aa
	50%	29.54 ± 0.62Aa	28.4 ± 2.7Aa	0.96 ± 0.18Bab	487 ± 60.1Ba	22.8 ± 0.9Aa	116 ± 6.3Ab
	4%	19.94 ± 1.27Abc	22.7 ± 8.4Abc	1.14 ± 0.17Ba	411 ± 4.6 Ba	23.6 ± 0.9Aa	62.5 ± 1.8Ac
C. rex	100%	26.64 ± 1.20Aa	15.2 ± 1.3Ba	0.57 ± 0.01Ab	955 ± 44.2Aa	13.9 ± 0.5Ba	104 ± 2.1Ba
	50%	26.29 ± 1.18Aa	15.8 ± 1.7Ba	0.60 ± 0.07Ab	743 ± 29.1Ab	13.6 ± 0.2Ba	96.9 ± 3.3Aa
	4%	18.21 ± 0.92Ab	15.8 ± 2.0Aa	0.87 ± 0.03Aa	535 ± 17.7Ac	13.9 ± 0.5Ba	82.3 ± 4.8Ab

The K_{leaf} of *C. helferiana* was significantly (89%) higher than that of *C. rex* under the full sunlight growth condition ($p < 0.05$). Under the 50% and 4% sunlight conditions, K_{leaf} of *C. helferiana* was 18% and 26% higher than that of *C. rex*, but the differences were not significant statistically (Figure 3). Both g_s and A_{max} showed strong positive correlations with K_{leaf} (Figure 4). According to the leaf hydraulic vulnerability curves, the ability of *C. helferiana* to resist drought induced decline of leaf hydraulic conductance was significantly higher than that of *C. rex* grown under the same light treatment (Figure 5). The P50$_{leaf}$ of *C. helferiana* and *C. rex* grown under 100% sunlight was −2.5 and −1.0 MPa, respectively, and it was −2.2 and −0.6 MPa under the 50% sunlight treatment, respectively, (Figure 5).
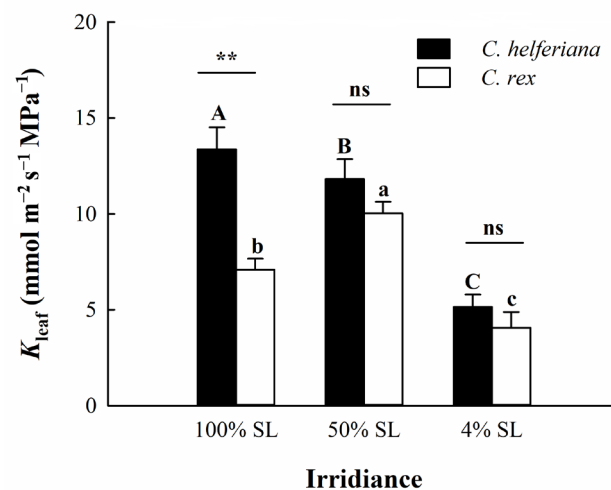

Figure 3. Leaf hydraulic conductance (K_{leaf}) of *C. helferiana* and *C. rex* seedlings grown under three different light conditions. Data are means ± 1SE ($n = 4$). Different capital and lowercase letters on top of bars indicate significant differences ($p < 0.05$) between different growth light intensities within the species. "**" and "ns" indicate significant difference ($p < 0.01$) and non-significant difference, respectively, between different species under the same growth light treatment.
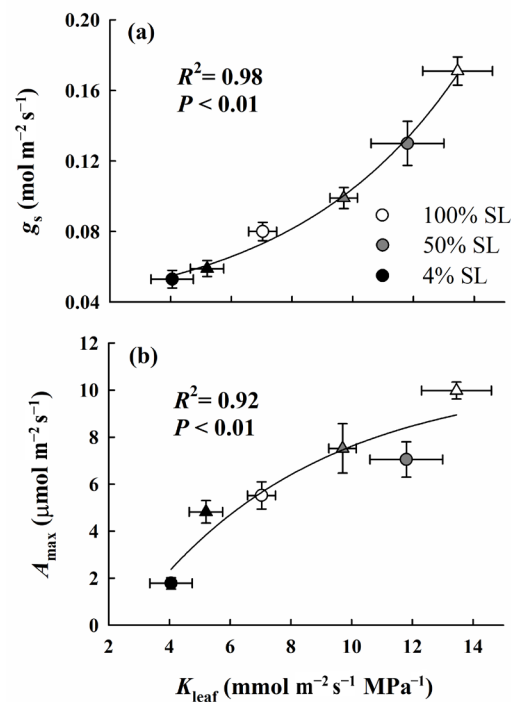
Figure 4. Correlations between leaf maximum hydraulic conductance and photosynthetic gas exchange traits, i.e., (**a**) stomatal conductance (g_s) and (**b**) maximum photosynthetic assimilation rate (A_{max}). Triangles and circles represent *C. helferiana* and *C. rex*, respectively. Values are means ± 1SE ($n = 4$). Exponential equations were used to fit the curves: $y = 0.03 + 0.01e^{0.18x}$ and $y = -5.76 + 16.37 (1 - e^{-0.17x})$.
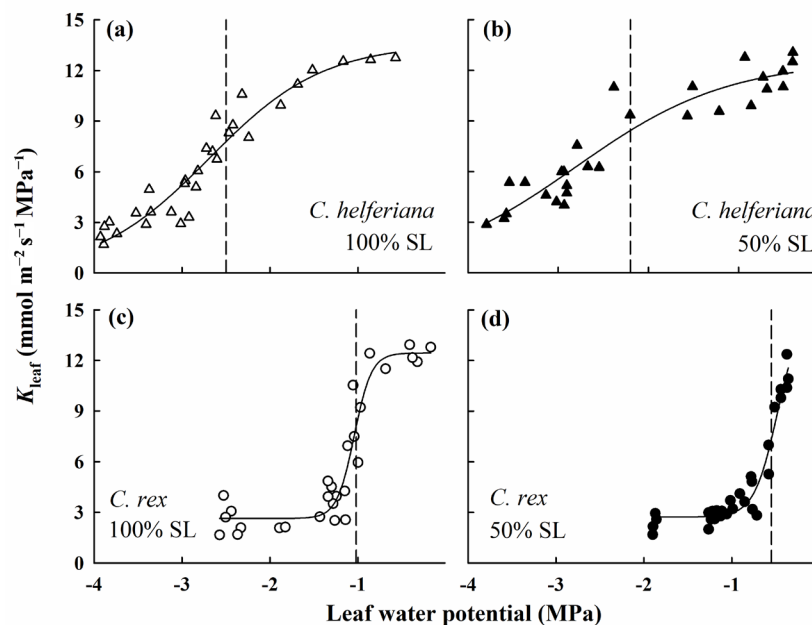
Figure 5. Changes of leaf hydraulic conductance (K_{leaf}) with the decrease in leaf water potentials (Ψ_L) during the water withholding treatment in seedlings of *C. helferiana* (**a,b**) and *C. rex* (**c,d**) grown under 100% and 50% sunlight conditions. A sigmoid function was fitted to the data in each panel ($y = a/(1 + \exp(-(x-x_0)/b))$). The vertical dashed lines mark water potentials at 50% loss of leaf hydraulic conductance ($P50_{leaf}$).

The leaf dehydration curves showed that the rate of relative water content decrease was significantly slower in *C. helferiana* than in *C. rex* for plants grown under both 100% and 50% sunlight conditions,

indicating lower cuticle conductance in the former species (Figure 6). The time needed for leaf relative water content to decrease from full saturation to 70% was 150 min and 320 min in *C. helferiana* and *C. rex* (*p* < 0.01), respectively, but there was no significant difference in dehydration rate between leaves of the same species growing under different levels of light intensity (*p* > 0.05).
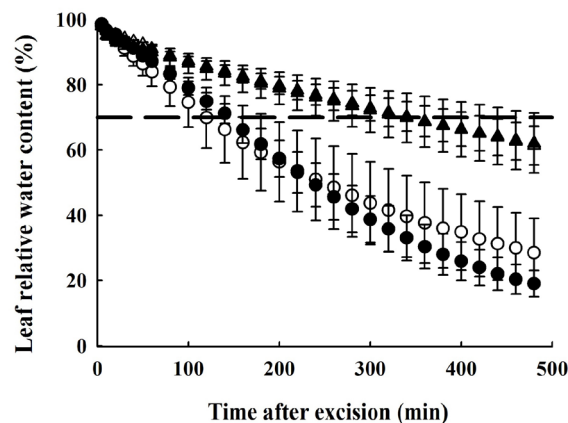

Figure 6. Leaf dehydration curves of *C. helferiana* (triangles) and *C. rex* (circles) grown under 100% (open symbols) and 50% (filled symbols) sunlight conditions, showing changes of relative water content (RWC) with time after excision. The horizontal dashed line shows RWC of 70%. Values are means ± 1SE (*n* = 4). Note, the curves of *C. helferiana* in the two light conditions overlapped.

4. Discussion

Our results showed that the two *Cyclobalanopsis* species diverged significantly in photosynthetic, leaf anatomical and hydraulic characteristics. Under the same light conditions, the maximum photosynthetic rate of *C. helferiana* was higher with the difference particularly large under high growth light treatments. *Cyclobalanopsis rex* had physiological characteristics of greater shade tolerance, but its photosynthetic carbon assimilation capacity under high light was lower. Similar to the significant difference in photosynthetic characteristics, the two species also diverged substantially in water-related functional traits. *Cyclobalanopsis helferiana* had a higher maximum leaf hydraulic conductance, stronger resistance to drought-induced hydraulic dysfunction, and greater ability to withhold leaf water under drought stress. There was a strong coordination between leaf hydraulic conductance and photosynthetic gas exchange. The differences in photosynthetic and water-related physiological functions in seedlings of the two *Cyclobalanopsis* species reflect large differences in their growth environment under natural conditions. The results of this study contribute to a deeper understanding in the physiological ecology related to natural regeneration of the two tree species.

4.1. Differences in Leaf Traits and Responses to Variation in Growth Light Conditions between the Two Species

Cyclobalanopsis helferiana has obvious xerophytic structural characteristics, such as thicker leaves, higher LMA, dense pubescence on the underside of leaves, which is consistent with plants adapted to the dry and hot habitats in their natural distributions [36]. The anatomical structure of the leaves of the two *Cyclobalanopsis* species varied significantly with the change of light environment, i.e., showing a relatively high degree of plasticity in response to growth light conditions as indicated by significant intraspecific differences across light levels for seedling growth. With the increase in light intensity, the thickness of leaf and palisade tissue increased, and the sponge-to-palisade mesophyll ratio decreased, which is conducive to the more effective use of light by plants [36]. The stomatal density of the two species under the condition of high light intensity was significantly higher than that under the condition of low light intensity, which was beneficial to the enhancement of CO_2 diffusion efficiency and the increase in mesophyll conductance [37]. Under the low light environment, the LMA of both species decreased so that the light harvesting area per unit dry mass of leaves increased [14].

Low LMA allows plants to obtain larger leaf area with equal amount of dry matter, and thus contributes to the increase in photosynthetic carbon assimilation rate per unit leaf dry mass, which is beneficial to light interception and carbon economy under low light conditions.

The comparison of the light response curves of the two species shows that their optimal growth light conditions for seedlings are substantially different. The A_{max} of *C. helferiana* under full sunlight was higher than that under the two lower light treatments, while the A_{max} of *C. rex* was the largest under 50% shading (Figure 2). Under the same light intensity, the light compensation point and light saturation point of *C. helferiana* were both higher than those of *C. rex*, which indicated that the former species has higher light demand and higher photosynthetic capacity. On the other hand, these differences in photosynthetic traits also reflect that *C. rex* has a higher degree of shade tolerance but is not adapted to high sunlight levels at the seedling stage. Under 50% and 4% sunlight treatments, there was no significant difference in dark respiration rate between the two species, but under full sunlight, dark respiration rate of *C. rex* was significantly higher than that of *C. helferiana*. Compared with 50% light treatment, *C. rex* showed lower A_{max} and higher dark respiration rate under 100% light treatment, suggesting lower efficiencies of photosynthetic carbon assimilation under high light likely due to a certain degree of photoinhibition. The intolerance to high light of *C. rex* seedlings was further confirmed by the observations that leaves of *C. rex* under 100% light treatment turned yellow and partially died, while the plants under 50% shade grew vigorously.

4.2. Difference in Leaf Hydraulic Conductance between the Two Species

Consistent with their contrasting patterns in responding to growing light conditions with respect to photosynthetic characteristics, responses of water-related leaf physiological characteristics to growth light intensity also differed significantly between the two species. For *C. helferiana*, the highest value of K_{leaf} was in full light treatment, slightly lower in 50% light treatment, and the lowest in 4% light treatment, while the highest K_{leaf} was in 50% light treatment for *C. rex*. This shows that the optimal light conditions for the growth of seedlings of the two *Cyclobalanopsis* species differ, i.e., seedlings of *C. helferiana* are adapted to the high light conditions, while seedlings of *C. rex* are adapted to partially shaded environments. This is consistent with the light conditions of the two species for regeneration under natural conditions. The seedlings of *C. helferiana* are often exposed to high light in the Savanna-like vegetation in its natural habitats, while the seedlings of *C. rex* usually grow in the dark understory of dense tropical rainforests. Such divergences in leaf hydraulic conductance between the two congeneric species reflect the results of their long-term adaptation to contrasting light environments [28]. Vascular system with higher K_{leaf} has higher construction cost, and leaves with higher K_{leaf} are usually developed only when plants have higher photosynthetic rate and carbon gain [38]. Leaves of *C. helferiana* seedlings can make better use of high light and achieve the highest photosynthetic carbon yield under full sunlight, which require high K_{leaf} for efficient water supply under high light conditions. However, the photosynthetic systems of *C. rex* seedlings are likely greatly inhibited by full sunlight that resulted in lower photosynthetic efficiency than individuals growing under 50% sunlight treatment, so that the corresponding maximum K_{leaf} value was also found under the 50% light treatment.

4.3. Coordination between Leaf Hydraulic Conductance and Photosynthetic Gas Exchange

The significant positive correlations between K_{leaf} and gas exchange parameters (g_s and A_{max}) are consistent with the significant functional coordination found among leaf photosynthesis, stomatal conductance and leaf hydraulics across other species [3,30,33,39,40]. Some studies have shown that sun species have higher A_{max} and g_s than shade species, but the high photosynthetic rate of sun species is at the cost of high transpiration rate, which requires a more efficient vascular system to support their higher water transport needs [41,42]. It seems that photosynthesis is strongly coordinated with hydraulic conductance at the leaf level. Generally, the structural characteristics of xylem directly affect the water supply potential of leaves, while the change of hydraulic conductance affects the water potential and

stomatal conductance, and then affects the intercellular CO_2 concentration, which ultimately determine the photosynthetic carbon assimilation and plant growth potential [41–43].

4.4. Difference in Drought Resistance and Water-Holding Capacity between the Two Species

The significant difference between the vulnerability curves of the two species indicates that they have different levels of hydraulic safety against drought stress. In this study, both in full sunlight and the 50% sunlight treatments, *C. helferiana* had lower $P50_{leaf}$, indicating that it has relatively strong drought resistance [36,44]. In different interspecific comparative studies, hydraulic conductance and xylem cavitation resistance are often negatively correlated [45–47], that is, there is a trade-off between hydraulic efficiency and safety. Plants distributed in better water conditions usually have high xylem water transport efficiency but lower resistance to cavitation; on the contrary, plants distributed in drought-stressed conditions usually have lower hydraulic conductance but stronger resistance to cavitation. However, in this study, *C. helferiana* distributed in arid environment has high leaf water conductivity and greater hydraulic safety, which is consistent with the result that there is no trade-off relationship between K_{leaf} and $P50_{leaf}$ in some plant groups [48]. The lack of such a trade-off here can be related to the fact that plants at the seedling stage were used in the present study. The ecophysiological characteristics of plants in the seedling stage are not only affected by water, but also closely related to the light conditions of the environment. The seedlings of *C. rex* generally grow under the humid tropical rainforests, where they have low transpirational demand and usually high environmental water availability. The construction of leaves with low water transport efficiency and resistance to embolism can reduce carbon cost and help plants maintain carbon balance in shaded environments [14]. However, xylem with high hydraulic conductance is likely to be developed to support higher photosynthetic rate in the adult stage that would contribute to enhanced competition in the highly competitive environment of the tropical rainforest canopy.

Compared with *C. rex*, *C. helferiana* has higher leaf water-holding ability, which is a typical adaptation to more arid environments. The ability of water retention under drought stress is affected by characteristics of leaf epidermis, such as cuticle thickness, waxy layer thickness and other surface characteristics. There is a well-developed cuticle on the upper epidermis of *C. helferiana* leaves, and a layer of dense pubescence on the lower side of the leaves. These structural features favor leaf water retention after stomata closure under drought-stressed conditions. Therefore, the rate of water loss of its leaves after stomata closure is slow, which is of great significance for delaying the adverse effects of severe drought [36]. For example, it can delay catastrophic hydraulic failure due to xylem embolism under drought stresses, so it is considered as an adaptive strategy of plants in drought prone habitats. The ecophysiological differences observed here between the two species of *Cyclobalanopsis* support the hypothesis that there is a trade-off between drought resistance and shade tolerance [11,13,14].

5. Conclusions

Ecophysiological differences in phylogenetically closely related plant species make them occupy different niches in the same habitat or contribute to their different distributions, which is an important basis for species coexistence and diversity formation. When comparing the environments for seedling growth between the hot and dry valley savanna forests and the humid tropical rainforests, drought stress in combination with high sunlight are in clear contrast with a combination of low light stress and relatively sufficient water. Low light intensity in the understory of humid rainforests may cause carbon imbalance, while excessive light and drought in the savanna habitats can result in photoinhibition and hydraulic failure. Trees adapted to savanna and rainforest understory environments at the seedling stage have adopted a series of strategies to cope with drought and low light stresses, respectively. Along the precipitation gradient from savanna to rainforest there is likely a shift in plants from competing for water to competing for light and from drought tolerance to shade tolerance. However, due to some physiological constrains plants have to trade-off between different functions. Our physiological comparative study between the two *Cyclobalanopsis* species involving photosynthetic

and hydraulic measurements provides an important mechanistic explanation for the long-hypothesized functional trade-off between shade tolerance and drought tolerance [11,13,14].

Seedlings of the two congeneric tree species adapted to contrasting environments diverged substantially in characteristics related to shade and drought tolerance. Compared with that of *C. rex*, seedlings of *C. helferiana* are more adapted to high light conditions, which is of great significance for success in hot and dry habitat with sparse vegetation. Meanwhile, the higher hydraulic safety of *C. helferiana* is also an important physiological basis for adaptation to its drought prone natural habitats. Contrastingly, *C. rex* has characteristics of greater shade tolerance with too high light intensity showing a harmful effect on its seedlings. The poor drought resistance and intolerance to high light intensity of *C. rex* seedlings indicates that the regeneration of this endangered species relies heavily on the humid environment of the tropical rainforests as well as a well-preserved forest canopy for providing suitable partially shaded light environments.

References

1. Cavender, B.J.; Ackerly, D.D.; Baum, D.A.; Bazzaz, F.A. Phylogenetic overdispersion in *Floridian oak* communities. *Am. Nat.* **2004**, *163*, 823–843. [CrossRef] [PubMed]

2. Hao, G.-Y.; Hoffmann, W.A.; Scholz, F.G.; Bucci, S.J.; Meinzer, F.C.; Franco, A.C.; Meinzer, F.C.; Franco, A.C.; Cao, K.-F.; Goldstein, G. Stem and leaf hydraulics of congeneric tree species from adjacent tropical savanna and forest ecosystems. *Oecologia* **2008**, *155*, 405–415. [CrossRef] [PubMed]

3. Hao, G.-Y.; Goldstein, G.; Sack, L.; Holbrook, N.M.; Liu, Z.H.; Wang, A.-Y.; Harrison, R.D.; Su, Z.H.; Cao, K.-F. Ecology of hemiepiphytism in fig species is based on evolutionary correlation of hydraulics and carbon economy. *Ecology* **2011**, *92*, 2117–2130. [CrossRef] [PubMed]

4. Hao, G.-Y.; Lucero, M.E.; Sanderson, S.C.; Zacharias, E.H.; Holbrook, N.M. Polyploidy enhances the occupation of heterogeneous environments through hydraulic related trade-offs in *Atriplex canescens* (Chenopodiaceae). *New Phytol.* **2013**, *197*, 970–978. [CrossRef]

5. Zhang, W.-W.; Song, J.; Wang, M.; Liu, Y.Y.; Li, N.; Zhang, Y.J.; Holbrook, N.M.; Hao, G.-Y. Divergences in hydraulic architecture form an important basis for niche differentiation between diploid and polyploid *Betula* species in NE China. *Tree Physiol.* **2017**, *37*, 604–616. [CrossRef]

6. Holmgren, M. Combined effects of shade and drought on tulip poplar seedlings: Trade-off in tolerance or facilitation? *Oikos* **2000**, *90*, 67–78. [CrossRef]

7. Poorter, L.; Kitajima, K. Carbohydrate storage and light requirements of tropical moist and dry forest tree species. *Ecology* **2007**, *88*, 1000–1011. [CrossRef]

8. Müller, P.; Li, X.P.; Niyogi, K.K. Non-photochemical quenching: A response to excess light energy. *Plant Physiol.* **2001**, *125*, 1558–1566. [CrossRef]

9. Wang, A.-Y.; Jiang, Y.-J.; Hao, G.-Y.; Cao, K.-F. The effect of seasonal drought to plant hydraulics and photosynthesis of three dominant evergreen tree species in seasonal tropical rainforest of Xishuangbanna limestone area. *Acta Botanica Yunnanica.* **2008**, *30*, 325–332, (In Chinese with English Abstract).

10. Zhang, J.L.; Hao, G.-Y.; Cao, K.-F. Phenology of woody species in Yunnan dry-hot valley in Yunnan province. *J. Wuhan Bot. Res.* **2009**, *27*, 76–82, (In Chinese with English Abstract).

11. Sack, L.; Cowan, P.D.; Holbrook, N.M. The 'hydrology' of leaves: Co-ordination of structure and function in temperate woody species. *Plant Cell Environ.* **2003**, *26*, 1343–1356. [CrossRef]

12. Myers, J.A.; Kitajima, K. Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *J. Ecol.* **2007**, *95*, 383–395. [CrossRef]

13. Smith, T.; Huston, M. A theory of the spatial and temporal dynamics of plant communities. *Vegetatio* **1989**, *83*, 49–69. [CrossRef]

14. Valladares, F.; Niinemets, Ü. Shade tolerance, a key plant feature of complex nature and consequences. *Annu. Rev. Ecol. Evol. Syst.* **2008**, *39*, 237–257. [CrossRef]

15. Denslow, J.S.; Hartshorn, G.S. Treefall gap environments and forest dynamic process. In *LaSelva: Ecology and Natural History of a Neotropical Rain Forest*; McDade, L.A., Bawa, K., Hespenheide, H., Hartshorn, G.S., Eds.; University of Chicago Press: Chicago, IL, USA, 1994; pp. 120–127. [CrossRef]

16. Fetcher, N.; Oberbauer, S.F.; Chazdon, R.L. Physiological ecology of plants at La Selva. In *La Selva: Ecology and Natural History of a Neotropical Forest*; McDale, L., Bawa, K.S., Hespenheide, H., Hartson, G., Eds.; University of Chicago Press: Chicago, IL, USA, 1994; pp. 128–141. [CrossRef]

17. Chazdon, R.L. Sunflecks in the forest understory. *Adv. Ecol. Res.* **1988**, *18*, 1–63. [CrossRef]

18. Chazdon, R.L.; Pearcy, R.; Lee, D.; Fetcher, D. Photosynthetic responses of tropical plants to contrasting light environments. In *Tropical Forest Plant Ecophysiology*; Mulkey, S.S., Chazdon, R.L., Smith, A.P., Eds.; Springer: Boston, MA, USA, 1996; pp. 5–55. [CrossRef]

19. Clark, D.B.; Clark, D.A.; Rich, P.M.; Weiss, S.; Oberbauer, S.F. Landscape-scale evaluation of understory light and canopy structures: Methods and application in a neotropical lowland rain forest. *Can. J. For. Res.* **1996**, *26*, 747–757. [CrossRef]

20. Lambers, H.; Chapin, F.S.; Pons, T.L. *Physiological Plant Ecology*, 4th ed.; Springer: New York, NY, USA, 2008. [CrossRef]

21. Veneklaas, E.J.; Poorter, L. Growth and carbon partitioning of tropical tree seedlings in contrasting light environments. In *Inherent Variation in Plant Growth: Physiological Mechanisms and Ecological Consequences*; Lambers, H., Poorter, H., Van Vuuren, M.M.I., Eds.; Backhuys Publsihers: Leiden, The Netherlands, 1998; pp. 337–362.

22. Walters, M.B.; Reich, P.B. Low-light carbon balance and shade tolerance in the seedlings of woody plants: Do winter deciduous and broad-leaved evergreen species differ? *New Phytol.* **1999**, *143*, 143–154. [CrossRef]

23. Urli, M.; Porte, A.J.; Cochard, H.; Guengant, Y.; Burlett, R.; Delzon, S. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. *Tree Physiol.* **2013**, *33*, 672–683. [CrossRef]

24. Lucani, C.J.; Brodribb, T.J.; Jordan, G.J.; Mitchell, P.J. Juvenile and adult leaves of heteroblastic *Eucalyptus globulus* vary in xylem vulnerability. *Trees-Struct. Funct.* **2019**, *33*, 1167–1178. [CrossRef]

25. Nardini, A.; Salleo, S. Limitation of stomatal conductance by hydraulic traits: Sensing or preventing xylem cavitation? *Trees-Struct. Funct.* **2000**, *15*, 14–24. [CrossRef]

26. Sack, L.; Tyree, M.T. Leaf hydraulics and its implications in plant structure and function. In *Vascular Transport in Plants*; Holbrook, N.M., Zweiniecki, M.A., Eds.; Elsevier/Academic Press: Oxford, UK, 2005; pp. 93–114. [CrossRef]

27. Zhu, S.-D.; Liu, H.; Xu, Q.-Y.; Cao, K.-F.; Ye, Q. Are leaves more vulnerable to cavitation than branches? *Funct. Ecol.* **2016**, *30*, 1740–1744. [CrossRef]

28. Sack, L.; Tyree, M.T.; Holbrook, N.M. Leaf hydraulic architecture correlates with regeneration irradiance in tropical rainforest trees. *New Phytol.* **2005**, *167*, 403–413. [CrossRef] [PubMed]

29. Sack, L.; Frole, K. Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. *Ecology* **2006**, *87*, 483–491. [CrossRef] [PubMed]

30. Hao, G.-Y.; Sack, L.; Wang, A.-Y.; Cao, K.-F.; Goldstein, G. Differentiation of leaf water flux and drought tolerance traits in hemiepiphytic and non-hemiepiphytic *Ficus* tree species. *Funct. Ecol.* **2010**, *24*, 731–740. [CrossRef]

31. Huang, C.C.; Zhang, Y.T.; Bartholomew, B. *Flora of China*; Science Press: Beijing, China; Missouri Botanical Garden Press: St. Louis, MO, USA, 1999; Volume 4.

32. Bassman, J.H.; Zwier, J.C. Gas exchange characteristics of *Populus trichocarpa*, *Populus deltoides* and *Populus trichocarpa* × *P. deltoides* clones. *Tree Physiol.* **1991**, *8*, 145–159. [CrossRef]

33. Franks, P.J. Higher rates of leaf gas exchange are associated with higher leaf hydrodynamic pressure gradients. *Plant Cell Environ.* **2006**, *29*, 584–592. [CrossRef]

34. Scholander, P.F.; Hammel, H.T.; Brandstreet, E.D. Sap pressure in vascular plants. *Science* **1965**, *148*, 339–346. [CrossRef]

35. Muchow, R.C.; Sinclair, T.R. Epidermal conductance, stomatal density and stomatal size among genotypes of *Sorghum bicolor* (L.) Moench. *Plant Cell Environ.* **1989**, *12*, 425–431. [CrossRef]

36. Zhang, J.L. Phenology, Leaf Structure and Function, and Seasonal Variation in Photosynthesis of Woody Plants in a Dry-hot Valley of Yuanjiang, Southwestern China. Ph.D. Thesis, Chinese Academy of Sciences, Beijing, China, 2007. (In Chinese with English Abstract).

37. Pathare, V.S.; Koteyeva, N.; Cousins, A.B. Increased adaxial stomatal density is associated with greater mesophyll surface area exposed to intercellular air spaces and mesophyll conductance in diverse C-4 grasses. *New Phytol.* **2020**, *225*, 169–182. [CrossRef]

38. McCulloh, K.A.; Sperry, J.S.; Adler, F.R. Water transport in plants obeys Murray's law. *Nature* **2003**, *421*, 939–942. [CrossRef]

39. Song, J.; Yang, D.; Niu, C.-Y.; Zhang, W.-W.; Wang, M.; Hao, G.-Y. Correlation between leaf size and hydraulic architecture in five compound-leaved tree species of a temperate forest in NE China. *For. Ecol. Manag.* **2018**, *418*, 63–72. [CrossRef]

40. Yang, D.; Zhang, Y.-J.; Song, J.; Niu, C.-Y.; Hao, G.-Y. Compound leaves are associated with high hydraulic conductance and photosynthetic capacity: Evidence from trees in Northeast China. *Tree Physiol.* **2019**, *39*, 729–739. [CrossRef] [PubMed]

41. Meinzer, F.C. Co-ordination of vapour and liquid phase water transport properties in plants. *Plant Cell Environ.* **2002**, *25*, 265–274. [CrossRef] [PubMed]

42. Santiago, L.S.; Goldstein, G.; Meinzer, F.C.; Fisher, J.B.; Machado, K.; Woodruff, D.; Jones, T. Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* **2004**, *140*, 543–550. [CrossRef]

43. Hao, G.-Y.; Wang, A.-Y.; Liu, Z.-H.; Franco, A.C.; Goldstein, G.; Cao, K.-F. Differentiation in light energy dissipation between hemiepiphytic and non-hemiepiphytic *Ficus* species with contrasting xylem hydraulic conductivity. *Tree Physiol.* **2011**, *31*, 626–636. [CrossRef]

44. Zhu, J.-J.; Zhang, J.-L.; Liu, H.-C.; Cao, K.-F. Photosynthesis, non-photochemical pathways and activities of antioxidant enzymes in a resilient evergreen oak under different climatic conditions from a valley-savanna in Southwest China. *Physiol. Plantarum.* **2009**, *135*, 62–72. [CrossRef]

45. Martínez-Vilalta, J.; Prat, E.; Oliveras, I.; Piñol, J. Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. *Oecologia* **2002**, *133*, 19–29. [CrossRef]

46. Sperry, J.S.; Meinzer, F.C.; McCulloh, K.A. Safety and efficiency conflicts in hydraulic architecture: Scaling from tissues to trees. *Plant Cell Environ.* **2008**, *31*, 632–645. [CrossRef]

47. Manzoni, S.; Vico, G.; Katul, G.; Palmroth, S.; Jackson, R.B.; Porporato, A. Hydraulic limits on maximum plant transpiration and the emergence of the safety-efficiency trade-off. *New Phytol.* **2013**, *198*, 169–178. [CrossRef]

48. Chen, J.-W.; Zhang, Q.; Li, X.-S.; Cao, K.-F. Independence of stem and leaf hydraulic traits in six *Euphorbiaceae* tree species with contrasting leaf phenology. *Planta* **2009**, *230*, 459–468. [CrossRef]