plants

MDPI

Article

The Effect of Low Irradiance on Leaf Nitrogen Allocation and Mesophyll Conductance to CO_2 in Seedlings of Four Tree Species in Subtropical China

Jingchao Tang [1,2], Baodi Sun [1], Ruimei Cheng [2,3], Zuomin Shi [2,3,4,*], Da Luo [2], Shirong Liu [2] and Mauro Centritto [4]

1 School of Environmental and Municipal Engineering, Qingdao University of Technology, Qingdao 266525, China; tjc@qut.edu.cn (J.T.); sunbaodi0927@126.com (B.S.)
2 Key Laboratory of Forest Ecology and Environment of National Forestry and Grassland Administration, Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing 100091, China; chengrm@caf.ac.cn (R.C.); luoda2010@163.com (D.L.); liusr@caf.ac.cn (S.L.)
3 Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China
4 Institute for Sustainable Pant Protection, National Research Council of Italy, Strada delle Cacce 73, 10135 Torino, Italy; mauro.centritto@cnr.it
* Correspondence: shizm@caf.ac.cn; Tel.: +86-010-62888308

Citation: Tang, J.; Sun, B.; Cheng, R.; Shi, Z.; Luo, D.; Liu, S.; Centritto, M. The Effect of Low Irradiance on Leaf Nitrogen Allocation and Mesophyll Conductance to CO_2 in Seedlings of Four Tree Species in Subtropical China. *Plants* **2021**, *10*, 2213. https://doi.org/10.3390/plants10102213

Academic Editors: Byung Bae Park, Lerma S. J. Maldia and Fermin Morales

Received: 27 August 2021
Accepted: 15 October 2021
Published: 18 October 2021

Abstract: Low light intensity can lead to a decrease in photosynthetic capacity. However, could N-fixing species with higher leaf N contents mitigate the effects of low light? Here, we exposed seedlings of *Dalbergia odorifera* and *Erythrophleum fordii* (N-fixing trees), and *Castanopsis hystrix* and *Betula alnoides* (non-N-fixing trees) to three irradiance treatments (100%, 40%, and 10% sunlight) to investigate the effects of low irradiance on leaf structure, leaf N allocation strategy, and photosynthetic physiological parameters in the seedlings. Low irradiance decreased the leaf mass per unit area, leaf N content per unit area (N_{area}), maximum carboxylation rate (V_{cmax}), maximum electron transport rate (J_{max}), light compensation point, and light saturation point, and increased the N allocation proportion of light-harvesting components in all species. The studied tree seedlings changed their leaf structures, leaf N allocation strategy, and photosynthetic physiological parameters to adapt to low-light environments. N-fixing plants had a higher photosynthesis rate, N_{area}, V_{cmax}, and J_{max} than non-N-fixing species under low irradiance and had a greater advantage in maintaining their photosynthetic rate under low-radiation conditions, such as under an understory canopy, in a forest gap, or when mixed with other species.

Keywords: leaf nitrogen allocation; mesophyll conductance; photosynthetic nitrogen use efficiency; low irradiance; N-fixing tree species

1. Introduction

Radiation is a source of energy for plants. Through photosynthesis, green plants use light to synthesize carbohydrates from water and CO_2, which are necessary for maintaining growth and development. The low radiation conditions in the understory canopy of subtropical forests affect the survival and growth of forest tree seedlings [1]. Low light intensity can lead to a decrease in photosynthetic capacity, forcing plants to change their leaf photosynthesis system and structure to increase their light-harvesting ability [2–5]. Under low irradiance, plants usually adjust their leaf nitrogen (N) allocation strategies, such as increasing the fraction of leaf nitrogen (N) allocated to light-harvesting (P_L) [5–8], and some plants may also change the fraction of leaf N allocated to Rubisco (P_R) and bioenergetics (P_B) to balance the light reaction with carbon assimilation and achieve optimal photosynthetic efficiency [8,9]. However, some plants do not adjust their P_R and P_B [10], which may be because some plants store many compounds containing N, such as free amino acids [11],

inorganic N (NO_3^-, NH_4^+) [12], and some inactive Rubisco [12,13], and allocate these N sources to light-harvesting systems under low light levels.

Under low irradiance, the leaf thickness may decrease and leaf area may increase, resulting in a lower leaf mass per unit area (LMA) [1,4,14], which increases the area receiving light [8]. Low irradiance could also result in a reduction in the surface area of mesophyll cells per unit leaf area, as well as a smaller area of mesophyll cells through which CO_2 can diffuse into the chlorophyll [15,16]. These changes subsequently affect the mesophyll conductance to CO_2 (g_m) and, in turn, affect the CO_2 concentration in chloroplasts (C_c) [17,18]. Low irradiance decreased g_m [19,20] or did not significantly affect g_m in different species [21,22]. Therefore, changes in g_m in different species should be further studied.

The allocation of N in photosynthetic systems and g_m are common and important factors affecting photosynthetic N use efficiency (PNUE) [23,24], which is the ratio of the photosynthetic rate to the leaf N content [25,26] and reflects the N resources used for photosynthesis, an important leaf trait. Many authors have studied the PNUE of various plants under changing light intensities, and the changes in the PNUE of different plant species under different light intensities were inconsistent; some studies found that, under low irradiance, the PNUE increased [5,20,27], while others found that it decreased [28] or remained unchanged [7,29]. However, few studies have been conducted on whether low-irradiance treatment can affect the PNUE of N-fixing trees and the relevant internal control mechanisms of leaf N allocation and g_m. We suspect that N-fixing species with sufficient N in their leaves could increase their P_R, P_B, and P_L to increase the PNUE under low-irradiance treatment, and maintain photosynthetic capacity and growth better than non-N-fixing species under low-irradiance environments.

In this study, we exposed *Dalbergia odorifera* and *Erythrophleum fordii* (N-fixing trees), and *Castanopsis hystrix* and *Betula alnoides* (non-N-fixing trees) seedlings to three levels of irradiance (100%, 40%, and 10% sunlight irradiance) and estimated their photosynthesis, PNUE, leaf N allocation, and g_m values. These species are locally vital broad-leaved trees with high economic value, which are commonly used to change *Pinus massoniana* and *Cunninghamia lanceolata* pure forests into mixed broadleaf-conifer forests or mixed broad-leaved forests. This requires the selected species to be planted in forest gaps, mixed with other species, or directly on bare ground; therefore, their tolerance under low light conditions (e.g., in the understory canopy) will affect their survival and growth. Full light conditions of 10% and 40% are common in forest gaps as well as with mixed planting conditions, while 100% light conditions are typical for direct planting on bare land.

The aim of this study was to (1) determine the effects of low irradiance on leaf structure, leaf N allocation strategy, and photosynthetic physiological parameters (e.g., g_s, g_m, and photosynthetic rate) and (2) evaluate whether N-fixing plants are better able to maintain their photosynthetic rate under low-radiation conditions compared to non-fixing plants.

2. Results

N_{area} and N_{mass} in *D. odorifera* and *E. fordii* seedling leaves were significantly higher than those in *C. hystrix* and *B. alnoides* under each irradiance treatment (Table 1). There was a significant decrease in N_{area} and the LMA of all four species under the 10% and 40% irradiance treatments when compared with the 100% treatment (Table 1). A_{sat} of *E. fordii* under the 40% irradiance treatment was significantly higher than that under the other treatments; however, A_{sat} of *C. hystrix* under the 10% and 40% irradiance treatments, and A_{sat} of *B. alnoides* under the 10% irradiance treatment were significantly lower than that under the 100% treatment (Table 1). N_{mass} of *E. fordii C. hystrix* and *B. alnoides* was significantly higher under the 10% irradiance treatment than that under the 100% treatment (+25.6%, +33.8%, and +23.6%, respectively; Table 1). The $PNUE_{sat}$ of *D. odorifera* under the 10% irradiance treatment, and of *E. fordii* under the 10% and 40% irradiance treatments were significantly higher than that under the 100% treatment; however, the $PNUE_{sat}$ of

C. hystrix under the 10% and 40% irradiance treatments was significantly lower than that under the 100% treatment (Table 1).

Table 1. PPFD-saturated net CO_2 assimilation rate (A_{sat}); leaf mass per unit area (LMA); leaf nitrogen (N) content per unit of leaf area (N_{area}); leaf N concentration (N_{mass}) and PPFD-saturated photosynthetic N use efficiency ($PNUE_{sat}$) in *Dalbergia odorifera*, *Erythrophleum fordii*, *Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments. Data are means of seven plants per treatment ±SE. Lower case letters indicate significant difference at 0.05 levels among the irradiance treatments, whereas capital letters indicate significant difference at 0.05 levels among species under same irradiance treatment. *F*-ratios with statistically significant values denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ among irradiance treatment.

Tree Species	Irradiance Treatment	A_{sat} ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	N_{area} ($g \cdot m^{-2}$)	N_{mass} ($mg \cdot g^{-1}$)	LMA ($g \cdot m^{-2}$)	$PNUE_{sat}$ ($\mu mol \cdot mol^{-1} \cdot s^{-1}$)
Dalbergia odorifera	100%	8.04 ± 0.46 ^{aA}	2.19 ± 0.13 ^{aA}	31.7 ± 0.76 ^{aA}	69.0 ± 3.90 ^{aB}	52.6 ± 3.78 ^{bB}
	40%	8.30 ± 0.76 ^{aA}	1.62 ± 0.04 ^{bA}	31.2± 0.65 ^{aA}	51.8 ± 0.65 ^{bB}	72.3 ± 7.03 ^{bB}
	10%	6.88 ± 0.30 ^{aA}	0.97 ± 0.04 ^{cB}	33.0 ± 1.11 ^{aA}	29.3 ± 0.67 ^{cC}	101.0 ± 7.12 ^{aA}
	F	1.967	54.700 ***	1.196	73.752 ***	15.533 ***
Erythrophleum fordii	100%	6.60 ± 0.50 ^{bB}	2.01 ± 0.12 ^{aA}	28.1 ± 1.49 ^{bB}	71.4 ± 0.89 ^{aB}	45.9 ± 2.24 ^{cB}
	40%	9.34 ± 0.49 ^{aA}	1.75 ± 0.03 ^{bA}	33.0 ± 0.46 ^{bA}	53.1 ± 0.99 ^{bB}	75.0 ± 4.56 ^{aB}
	10%	6.87 ± 0.50 ^{bA}	1.56 ± 0.04 ^{bA}	35.3 ± 0.88 ^{aA}	44.3 ± 1.47 ^{cB}	61.6 ± 3.72 ^{bB}
	F	9.042 **	9.223 **	12.658 ***	145.227 ***	15.877 ***
Castanopsis hystrix	100%	8.16 ± 0.18 ^{aA}	1.02 ± 0.06 ^{aB}	10.2 ± 1.80 ^{bD}	100.1 ± 2.60 ^{aA}	112.0 ± 4.62 ^{aA}
	40%	4.57 ± 0.23 ^{bB}	0.75 ± 0.05 ^{bB}	9.6 ± 0.50 ^{bC}	78.8 ± 1.11 ^{bA}	87.0± 7.26 ^{bB}
	10%	4.18 ± 0.25 ^{bB}	0.79 ± 0.03 ^{bC}	13.7 ± 0.49 ^{aC}	57.9 ± 1.29 ^{cA}	74.4 ± 4.59 ^{bB}
	F	95.630 ***	20.060 ***	28.220 ***	138.877 ***	12.868 ***
Betula alnoides	100%	8.55 ± 0.60 ^{aA}	1.03 ± 0.09 ^{aB}	15.4 ± 1.04 ^{bC}	67.6 ± 5.45 ^{aB}	120.5 ± 5.18 ^{abA}
	40%	7.42 ± 0.30 ^{aA}	0.75 ± 0.04 ^{bB}	15.4 ± 0.45 ^{bB}	49.1 ± 3.36 ^{bB}	140.3 ± 8.02 ^{aA}
	10%	4.26 ± 0.52 ^{bB}	0.56 ± 0.04 ^{bD}	19.0 ± 0.62 ^{aB}	29.6 ± 2.14 ^{cC}	105.3 ± 8.33 ^{bA}
	F	20.458 ***	13.371 ***	7.790 **	23.833 ***	3.815 *

Both g_s and g_m of *C. hystrix* under the 10% and 40% irradiance treatments, and g_s and g_m of *B. alnoides* under the 10% irradiance treatment were significantly lower than those under the 100% treatment (Table 2). In contrast, g_s of *D. odorifera* under the 10% and 40% irradiance treatments were higher than that under the 100% treatment (+32.8% and +35.8, respectively), whereas g_m of *D. odorifera* under the 10% and 40% irradiance treatments was lower than that under the 100% treatment (−27.0% and −21.9%, respectively). g_m of *E. fordii* under the 40% irradiance treatment was significantly higher than that of the other treatments (Table 2). In *D. odorifera*, C_i under the 10% and 40% irradiance treatments, and C_c under the 10% irradiance treatment were higher than that under 100% irradiance, and in *E. fordii*, C_i under 10% irradiance treatment, and C_c under 10% and 40% irradiance treatments were higher than those under 100% irradiance (Table 2). Irradiance treatments did not significantly affect the CO_2 drawdown (C_i-C_c) in any of the four tree species studied (Table 2).

V_{cmax} and J_{max} of *D. odorifera*, *E. fordii*, and *B. alnoides* under the 10% irradiance treatments, and V_{cmax} and J_{max} of *C. hystrix* under the 10% and 40% irradiance treatments were lower than those under 100% irradiance (Table 3). In contrast, V_{cmax} of *D. odorifera* and V_{cmax} and J_{max} of *E. fordii* under the 40% irradiance treatment were higher than those under 100% irradiance (Table 3).

Table 2. Stomatal conductance (g_s), mesophyll conductance (g_m), intercellular CO_2 concentration (C_i), CO_2 concentration at carboxylation site (C_c) and CO_2 drawdown from the intercellular concentration to the carboxylation site concentration (C_i-C_c) measured in PPFD-saturated conditions in *Dalbergia odorifera*, *Erythrophleum fordii, Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments. Data are means of seven plants per treatment ±SE. Lower case letters indicate significant difference at 0.05 levels among the irradiance treatments, whereas capital letters indicate significant difference at 0.05 levels among species under same irradiance treatment. *F*-ratios with statistically significant values denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ among irradiance treatment.

Tree Species	Irradiance Treatment	g_s ($molCO_2 \cdot m^{-2} \cdot s^{-1}$)	g_m ($molCO_2 \cdot m^{-2} \cdot s^{-1}$)	C_i ($\mu mol \cdot mol^{-1}$)	C_c ($\mu mol \cdot mol^{-1}$)	C_i-C_c ($\mu mol \cdot mol^{-1}$)
Dalbergia odorifera	100%	0.067 ± 0.004^{bBC}	0.137 ± 0.010^{aA}	251.5 ± 6.44^{bBC}	190.8 ± 6.92^{bB}	60.8 ± 2.21^{aC}
	40%	0.091 ± 0.009^{aA}	0.107 ± 0.005^{bA}	288.5 ± 3.93^{aA}	210.0 ± 8.82^{bA}	78.6 ± 7.50^{aAB}
	10%	0.089 ± 0.003^{aA}	0.100 ± 0.007^{bA}	302.6 ± 1.94^{aA}	231.3 ± 6.20^{aA}	71.2 ± 5.27^{aB}
	F	6.562 **	6.823 **	34.333 ***	7.536 **	2.698
Erythrophleum fordii	100%	0.046 ± 0.002^{bC}	0.066 ± 0.007^{bC}	235.6 ± 6.19^{bC}	132.6 ± 6.90^{bD}	103.0 ± 4.83^{aA}
	40%	0.075 ± 0.005^{aA}	0.096 ± 0.004^{aA}	254.1 ± 3.81^{abB}	156.8 ± 4.09^{aB}	97.3 ± 2.37^{aA}
	10%	0.060 ± 0.005^{abB}	0.074 ± 0.004^{bB}	264.4 ± 4.18^{aB}	171.7 ± 4.45^{aB}	92.7 ± 1.56^{aA}
	F	11.744 **	9.789 **	7.982 **	13.855 ***	2.553
Castanopsis hystrix	100%	0.074 ± 0.004^{aB}	0.099 ± 0.006^{aB}	256.8 ± 5.24^{bB}	168.0 ± 6.04^{bC}	88.8 ± 6.26^{aB}
	40%	0.039 ± 0.004^{bB}	0.053 ± 0.006^{bB}	280.1 ± 3.48^{aA}	196.4 ± 5.84^{aA}	83.7 ± 4.14^{aAB}
	10%	0.036 ± 0.001^{bC}	0.053 ± 0.005^{bB}	268.0 ± 5.65^{abB}	186.5 ± 6.27^{abB}	81.5 ± 5.03^{aAB}
	F	38.06 ***	22.353 ***	5.711 *	5.691 *	0.511
Betula alnoides	100%	0.100 ± 0.013^{aA}	0.134 ± 0.012^{aA}	292.9 ± 5.94^{aA}	226.4 ± 9.57^{aA}	66.5 ± 4.64^{aC}
	40%	0.095 ± 0.011^{aA}	0.104 ± 0.009^{aA}	297.1 ± 7.04^{aA}	222.9 ± 12.19^{aA}	74.2 ± 5.69^{aB}
	10%	0.063 ± 0.005^{bB}	0.056 ± 0.009^{bB}	312.3 ± 4.73^{aA}	232.1 ± 9.07^{aA}	80.3 ± 6.14^{aAB}
	F	4.195 *	16.261 ***	2.93	0.198	1.568

Table 3. Maximum carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) measured in PPFD-saturated conditions in *Dalbergia odorifera*, *Erythrophleum fordii, Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments obtained by fitting the Farquhar et al. (1980) model of leaf photosynthesis to the individual A_n-C_c response curves. Data are means of seven plants per treatment ±SE. Lower case letters indicate significant difference at 0.05 levels among the irradiance treatments, whereas capital letters indicate significant difference at 0.05 levels among species under same irradiance treatment. *F*-ratios with statistically significant values denoted by * $p < 0.05$, *** $p < 0.001$ among irradiance treatment.

Tree Species	Irradiance Treatment	V_{cmax} ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	J_{max} ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
Dalbergia odorifera	100%	78.1 ± 4.59^{bB}	100.7 ± 5.80^{aBC}
	40%	95.2 ± 8.01^{aB}	118.5 ± 7.39^{aB}
	10%	68.6 ± 3.96^{cB}	79.1 ± 2.76^{bB}
	F	5.405 *	12.154 ***
Erythrophleum fordii	100%	99.8 ± 9.37^{bA}	128.8 ± 11.20^{bAB}
	40%	141.4 ± 5.24^{aA}	168.9 ± 3.36^{aA}
	10%	80.1 ± 4.07^{cA}	99.8 ± 3.83^{cA}
	F	22.233 ***	23.930 ***
Castanopsis hystrix	100%	82.8 ± 4.47^{aB}	109.3 ± 3.40^{aABC}
	40%	46.5 ± 2.51^{bC}	57.6 ± 4.49^{bC}
	10%	47.7 ± 2.92^{bC}	66.5 ± 3.80^{bC}
	F	75.031 ***	49.677 ***
Betula alnoides	100%	73.0 ± 3.51^{aB}	98.4 ± 5.37^{aBC}
	40%	82.6 ± 5.46^{aB}	97.8 ± 5.39^{aB}
	10%	41.6 ± 4.80^{bC}	56.0 ± 4.59^{bC}
	F	25.05 ***	22.465 ***

In *D. odorifera*, P_R, P_B, P_L, and P_P under the 10% and 40% irradiance treatments were higher than those under 100% irradiance, but P_{Other} under the 10% and 40% irradiance treatments were lower than those under 100% irradiance (Table 4). In *E. fordii*, P_R, P_L, and P_P under 10% and 40% irradiance treatments, and P_B under 40% irradiance treatment were higher than those under 100% irradiance. However, P_{CW} and P_{Other} under the 10% and 40% irradiance treatments were lower than those under 100% irradiance (Table 4). In *C.*

hystrix, P_L under the 10% and 40% irradiance treatments, and P_{CW} under the 40% irradiance treatment were higher than those under 100% irradiance, but P_R, P_B, and P_{Other} under the 10% and 40% irradiance treatments were lower than those under 100% irradiance (Table 4). In *B. alnoides*, P_L under the 10% irradiance treatment, P_R and P_B under the 40% irradiance treatment, and P_P under the 10% and 40% irradiance treatments were higher than those under the 100% irradiance treatment, but P_{CW} under the 10% irradiance treatment was lower than that under 100% irradiance (−32.1%, Table 4).

Table 4. Nitrogen allocation proportion of Rubisco (P_R), bioenergetics (P_B), light-harvesting components (P_L), photosynthetic system (P_P), cell wall (P_{CW}) and other parts (P_{Other}) in *Dalbergia odorifera*, *Erythrophleum fordii, Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments. Data are means of seven plants per treatment ±SE. Lower case letters indicate significant difference at 0.05 levels among the irradiance treatments, whereas capital letters indicate significant difference at 0.05 levels among species under same irradiance treatment. *F*-ratios with statistically significant values denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ among irradiance treatment.

Tree Species	Irradiance Treatment	P_R (g·g^{-1})	P_B (g·g^{-1})	P_L (g·g^{-1})	P_P (g·g^{-1})	P_{CW} (g·g^{-1})	P_{Other} (g·g^{-1})
Dalbergia odorifera	100%	0.135 ± 0.009 bB	0.030 ± 0.002 bB	0.105 ± 0.008 cA	0.269 ± 0.016 cB	0.068 ± 0.004 aC	0.663 ± 0.015 aA
	40%	0.201 ± 0.018 aA	0.047 ± 0.003 aC	0.132 ± 0.002 bA	0.381 ± 0.021 bB	0.067 ± 0.006 aC	0.552 ± 0.017 bA
	10%	0.242 ± 0.016 aAB	0.054 ± 0.003 aA	0.183 ± 0.005 aA	0.479 ± 0.021 aA	0.061 ± 0.004 aC	0.461 ± 0.023 cB
	F	14.001 ***	27.585 ***	54.347 ***	29.423 ***	0.632	29.390 ***
Erythrophleum fordii	100%	0.164 ± 0.010 cB	0.043 ± 0.003 bB	0.060 ± 0.009 cB	0.266 ± 0.018 cB	0.052 ± 0.002 aC	0.683 ± 0.019 aA
	40%	0.268 ± 0.011 aB	0.065 ± 0.003 aB	0.129 ± 0.004 bA	0.462 ± 0.007 aB	0.038 ± 0.001 bC	0.500 ± 0.007 cA
	10%	0.203 ± 0.011 bB	0.041 ± 0.002 bB	0.150 ± 0.008 aB	0.394 ± 0.016 bB	0.039 ± 0.002 bC	0.568 ± 0.015 bA
	F	24.021 ***	25.215 ***	38.638 ***	47.577 ***	24.303 ***	40.909 ***
Castanopsis hystrix	100%	0.302 ± 0.012 aA	0.068 ± 0.003 aA	0.072 ± 0.008 bB	0.441 ± 0.018 aA	0.267 ± 0.010 bA	0.292 ± 0.019 aB
	40%	0.231 ± 0.018 bB	0.049 ± 0.005 bC	0.130 ± 0.014 aA	0.411 ± 0.032 aB	0.443 ± 0.022 aA	0.146 ± 0.023 cB
	10%	0.247 ± 0.010 bAB	0.054 ± 0.003 bA	0.164 ± 0.013 aAB	0.466 ± 0.015 aA	0.342 ± 0.028 bA	0.192 ± 0.031 bD
	F	7.010 **	6.229 **	15.540 ***	1.475	17.205 ***	4.023 *
Betula alnoides	100%	0.256 ± 0.028 bA	0.066 ± 0.007 bA	0.116 ± 0.011 bA	0.439 ± 0.042 cA	0.221 ± 0.011 aB	0.340 ± 0.042 aB
	40%	0.369 ± 0.026 aB	0.089 ± 0.006 aA	0.119 ± 0.005 bA	0.577 ± 0.033 aA	0.197 ± 0.011 aB	0.227 ± 0.031 aB
	10%	0.281 ± 0.017 bA	0.063 ± 0.003 bA	0.176 ± 0.005 aAB	0.521 ± 0.016 bA	0.150 ± 0.010 bB	0.329 ± 0.021 aC
	F	5.979 **	6.189 **	20.386 ***	4.641 *	10.826 **	3.774 *

The apparent quantum yield (AQY) and R_n of *D. odorifera*, *C. hystrix*, and *B. alnoides* were not significantly affected by low-irradiance treatment; however, the AQY of *E. fordii* under the 10% and 40% irradiance treatments were significantly higher than that under 100% irradiance, and R_n of *E. fordii* under the 10% and 40% irradiance treatments were significantly lower than those under 100% irradiance (Table 5). The light compensation point (LCP) of *D. odorifera* and *C. hystrix* under the 10% and 40% irradiance treatments, and *E. fordii* and *B. alnoides* under the 10% irradiance treatment were significantly lower than those under 100% irradiance (Table 5). The light saturation point (LSP) of *D. odorifera* and *E. fordii* under the 10% irradiance treatment, and *C. hystrix* and *B. alnoides* under the 10% and 40% irradiance treatments were significantly lower than those under 100% irradiance (Table 5).

Table 5. Apparent quantum yield (AQY), dark respiration (R_n), light compensation point (LCP) and light saturation point (LSP) in *Dalbergia odorifera*, *Erythrophleum fordii*, *Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments. Data are means of seven plants per treatment ±SE. Lower case letters indicate significant difference at 0.05 levels among the irradiance treatments, whereas capital letters indicate significant difference at 0.05 levels among species under same irradiance treatment. *F*-ratios with statistically significant values denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ among irradiance treatment.

Tree Species	Irradiance Treatment	AQY (mol·mol^{-1})	R_n (μmol·m^{-2}·s^{-1})	LCP (μmol·m^{-2}·s^{-1})	LSP (μmol·m^{-2}·s^{-1})
Dalbergia odorifera	100%	0.052 ± 0.004 ^{aA}	0.909 ± 0.050 ^{aBC}	22.1 ± 1.68 ^{aA}	822.9 ± 27.5 ^{aA}
	40%	0.059 ± 0.002 ^{aA}	0.845 ± 0.050 ^{aA}	14.4 ± 0.73 ^{bB}	724.3 ± 37.0 ^{aBA}
	10%	0.058 ± 0.002 ^{aA}	0.760 ± 0.038 ^{aB}	7.4 ± 0.43 ^{cB}	684.3 ± 23.1 ^{bA}
	F	2.300	2.587	46.127 ***	5.378 *
Erythrophleum fordii	100%	0.047 ± 0.003 ^{bA}	1.129 ± 0.051 ^{aA}	13.9 ± 0.81 ^{aB}	637.1 ± 29.6 ^{aB}
	40%	0.062 ± 0.002 ^{aA}	0.873 ± 0.050 ^{bA}	13.1 ± 1.10 ^{aB}	633.6 ± 17.1 ^{aA}
	10%	0.059 ± 0.001 ^{aA}	0.936 ± 0.030 ^{bAB}	7.5 ± 0.79 ^{bB}	522.1 ± 17.2 ^{bB}
	F	15.924 ***	8.811 **	14.464 ***	6.569 **
Castanopsis hystrix	100%	0.047 ± 0.003 ^{aA}	1.005 ± 0.067 ^{aAB}	14.0 ± 1.21 ^{aB}	632.9 ± 23.4 ^{aB}
	40%	0.054 ± 0.002 ^{aA}	0.988 ± 0.040 ^{aA}	7.3 ± 1.00 ^{bC}	307.1 ± 26.8 ^{bC}
	10%	0.054 ± 0.003 ^{aA}	1.048 ± 0.088 ^{aA}	7.4 ± 1.21 ^{bB}	262.1 ± 27.7 ^{bC}
	F	2.737	0.206	11.226 **	60.359 ***
Betula alnoides	100%	0.049 ± 0.001 ^{aA}	0.889 ± 0.039 ^{aBC}	20.8 ± 0.93 ^{aA}	886.4 ± 43.5 ^{aA}
	40%	0.055 ± 0.003 ^{aA}	0.844 ± 0.055 ^{aA}	18.6 ± 2.49 ^{aA}	519.3 ± 27.9 ^{bB}
	10%	0.051 ± 0.003 ^{aA}	0.834 ± 0.048 ^{aAB}	10.5 ± 0.94 ^{bA}	268.6 ± 30.5 ^{cC}
	F	1.415	0.384	10.989 **	80.343 ***

A_{100} and A_{400} in *D. odorifera* and *E. fordii* seedling leaves were significantly higher than those in *C. hystrix* and *B. alnoides* under 10% irradiance treatment (Table 6). A_{100}, $PNUE_{100}$ and $PNUE_{400}$ of *D. odorifera* and *E. fordii* under the 10% and 40% irradiance treatments were significantly higher than that under the 100% treatment (Table 6). A_{400} of *E. fordii* under the 40% irradiance treatment was significantly higher than that under the other treatments (Table 6). A_{100}, A_{400} and $PNUE_{400}$ of *C. hystrix* under the 10% and 40% irradiance treatments were significantly lower than that under the 100% treatment (Table 2). A_{400} of *B. alnoides* was significantly lower than that under the 40% and 100% treatments, but $PNUE_{100}$ of *B. alnoides* was significantly higher than that under the 100% treatment (Table 6).

The $PNUE_{sat}$ was significantly, linearly related to P_R, P_B, and P_P in all four tree species in all treatments ($p < 0.001$, Figure 1a,b,d). In contrast, the $PNUE_{sat}$ of all four tree species was significantly positively related to P_L only under the 10% irradiance treatment ($p < 0.001$, Figure 1c). There was no significant positive correlation between $PNUE_{sat}$ and g_m in these four species (Figure 2).

Table 6. Net CO_2 assimilation rate at PPFD of 100 umol·m^{-2}·s^{-1} (A_{100}); net CO_2 assimilation rate at PPFD of 400 umol·m^{-2}·s^{-1} (A_{400}); photosynthetic N use efficiency at PPFD of 100 umol·m^{-2}·s^{-1} ($PNUE_{100}$); photosynthetic N use efficiency at PPFD of 400 umol·m^{-2}·s^{-1} ($PNUE_{400}$) in *Dalbergia odorifera*, *Erythrophleum fordii*, *Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments. Data are means of seven plants per treatment ± SE. Lower case letters indicate significant difference at 0.05 levels among the irradiance treatments, whereas capital letters indicate significant difference at 0.05 levels among species under same irradiance treatment. F-ratios with statistically significant values denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ among irradiance treatment.

Tree Species	Irradiance Treatment	A_{100} (μmol·m^{-2}·s^{-1})	A_{400} (μmol·m^{-2}·s^{-1})	$PNUE_{100}$ (μmol·mol^{-1}·s^{-1})	$PNUE_{400}$ (μmol·mol^{-1}·s^{-1})
Dalbergia odorifera	100%	2.78 ± 0.41 ^{bA}	7.38 ± 0.45 ^{abAB}	20.7 ± 1.36 ^{cB}	48.2 ± 3.65 ^{cB}
	40%	4.06 ± 0.08 ^{aA}	8.02 ± 0.30 ^{aA}	35.2 ± 0.66 ^{bB}	69.8 ± 3.35 ^{bB}
	10%	4.03 ± 0.17 ^{aA}	6.65 ± 0.36 ^{bA}	59.2 ± 4.12 ^{aAB}	97.4 ± 7.32 ^{aA}
	F	7.114 *	6.206 *	58.81 ***	23.261 ***
Erythrophleum fordii	100%	3.42 ± 0.16 ^{bA}	6.07 ± 0.24 ^{bB}	24.2 ± 1.38 ^{bB}	42.7 ± 1.48 ^{cB}
	40%	4.04 ± 0.08 ^{aA}	8.53 ± 0.22 ^{aA}	32.3 ± 0.72 ^{aB}	68.4 ± 2.42 ^{aB}
	10%	3.98 ± 0.18 ^{aA}	6.31 ± 0.43 ^{bA}	35.8 ± 1.29 ^{aC}	56.6 ± 3.11 ^{bB}
	F	5.426 *	16.016 ***	26.501 ***	22.155 ***
Castanopsis hystrix	100%	3.57 ± 0.12 ^{aA}	7.67 ± 0.42 ^{aAB}	49.1 ± 1.83 ^{aA}	105.4 ± 6.18 ^{aA}
	40%	3.00 ± 0.15 ^{bB}	3.98 ± 0.29 ^{bB}	57.3 ± 4.57 ^{aA}	76.2 ± 8.00 ^{bB}
	10%	2.92 ± 0.19 ^{bB}	3.68 ± 0.31 ^{bB}	51.8 ± 3.22 ^{aBC}	65.3 ± 4.99 ^{bB}
	F	5.107 *	41.202 ***	1.506	10.123 **
Betula alnoides	100%	3.27 ± 0.15 ^{aA}	7.95 ± 0.52 ^{aA}	46.4 ± 4.09 ^{bA}	113.7 ± 12.66 ^{aA}
	40%	3.52 ± 0.28 ^{aAB}	7.16 ± 0.68 ^{aA}	67.3 ± 7.62 ^{abA}	135.9 ± 14.91 ^{aA}
	10%	2.92 ± 0.32 ^{aB}	3.91 ± 0.66 ^{bB}	72.9 ± 6.33 ^{aA}	95.6 ± 11.85 ^{aA}
	F	1.358	12.425 ***	5.074 *	2.852

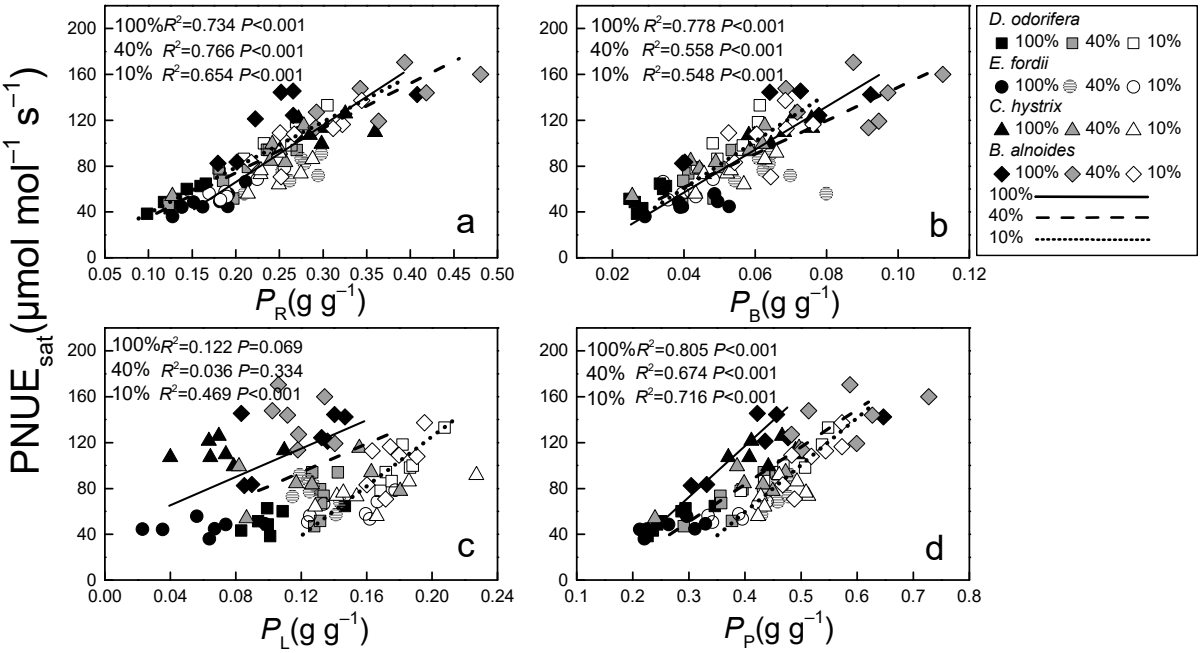

Figure 1. Relationship between PPFD-saturated photosynthetic N use efficiency ($PNUE_{sat}$) and (**a**) N allocation proportion of Rubisco (P_R), (**b**) bioenergetics (P_B), (**c**) light-harvesting components (P_L) and (**d**) photosynthetic system (P_P) in *Dalbergia odorifera*, *Erythrophleum fordii*, *Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments. The determination coefficient (R^2) and *p*-value are shown.

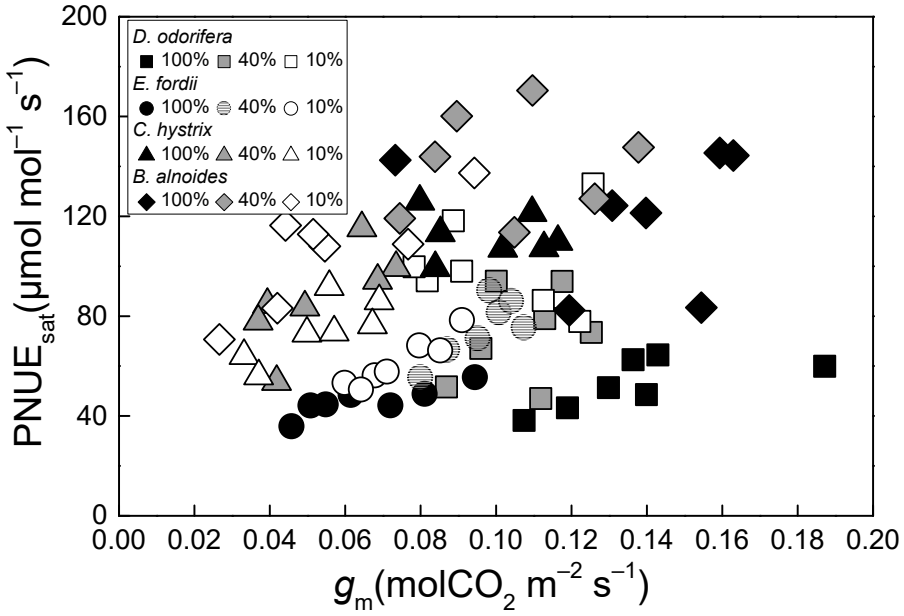

Figure 2. Relationship between PPFD-saturated photosynthetic N use efficiency ($PNUE_{sat}$) and mesophyll conductance (g_m) in *Dalbergia odorifera*, *Erythrophleum fordii*, *Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments.

3. Discussion

Under the 10% and 40% irradiance treatments, plants consistently reduced their LMAs (Table 1), that is, they reduced their leaf thickness to improve the transmittance of light and increase the leaf area to increase the area receiving light [1,4,8,14]. Leaves may change their arrangements of mesophyll cells and chloroplasts to increase their light capture efficiency, which allows their light-harvesting capacity to be increased and sustain photosynthesis [3,4]. The N_{mass} of all four tree species included in this study was higher under the 10% irradiance treatment. Although the increase in N_{mass} in *D. odorifera* was not significant, it was significant in the other three species (Table 1). N is an important component of chlorophyll, and plants increase the concentration of N to increase chlorophyll synthesis under low irradiance [7,8,20]. As N_{area} = LMA × N_{mass}, the significant decrease in LMA led to a decrease in N_{area} under the 10% and 40% irradiance treatments, indicating that thinner leaves had a lower concentration of N per unit area [1,10,30]. We hypothesized that N-fixing trees could fix nitrogen from the air; therefore, the reduction in N_{area} under low light may be smaller than that of non-N-fixing tree species. However, our results indicate that the decrease in the proportion of N_{area} was not lower in N-fixing trees than that in non-N-fixing trees (*D. odorifera:* −55.70%, *E. fordii:* −22.39%, *C. hystrix:* −22.54%, and *B. alnoides:* −45.63%). The N fixation capacities of *D. odorifera* and *E. fordii* did not limit the reduction in N_{area} under low light treatment.

In this study, A_{sat} significantly reduced in two non-N-fixing tree species, *C. hystrix* under the 10% and 40% irradiance treatments, and *B. alnoides* under the 10% irradiance treatment (Table 1). Reduced A_{sat} under low light treatment has been observed in many other studies [20,31], and many researchers have reported that C_c, V_{cmax}, and J_{max} are important factors affecting A_{sat}. CO_2 is a key material for photosynthesis [32], and V_{cmax} and J_{max} are key biochemical parameters of the photosynthetic capacity [33]. In this study, the C_c of *C. hystrix* and *B. alnoides* did not change significantly from the 10% to 100% irradiance treatments (Table 2), but the V_{cmax} and J_{max} of *C. hystrix* under the 10% and 40% irradiance treatments, and V_{cmax} and J_{max} of *B. alnoides* under the 10% irradiance treatment were lower than those under 100% irradiance (Table 3), which were the main reasons for the reduction in A_{sat} in these two species (Table 1). Although V_{cmax} and J_{max} of two N-fixing tree species, *D. odorifera* and *E. fordii*, were reduced under 10% irradiance (Table 3), the C_c of these species was significantly increased under 10% irradiance (Table 2),

which resulted in the absence of significant changes in A_{sat} (Table 1). The A_{sat}, V_{cmax}, and J_{max} of *E. fordii* were highest under 40% irradiance, suggesting that moderate shading may be more beneficial to its growth (Tables 1 and 3).

g_m in *D. odorifera*, *C. hystrix*, and *B. alnoides* seedlings decreased under 10% irradiance, which was consistent with previous studies [19,20] (Table 2). g_m could be affected by leaf anatomical differences, such as cell wall thickness, surface area of mesophyll cells, number of mesophyll layers, and leaf stomata density [17,18]. Variations in LMA could be driven by several anatomical traits, such as the cell wall thickness and number of mesophyll layers [34], and changes in LMA always influence g_m [35]. If a lower LMA is the result of mesophyll cell wall thinning, it will increase g_m [36,37]; if it is the result of a lower number of mesophyll layers, it will decrease g_m [38]. In this study, the LMA of *D. odorifera*, *C. hystrix*, and *B. alnoides* decreased under the 10% irradiance treatment, indicating that low light may decrease the number of mesophyll layers in these tree seedlings.

There was no significant change or increase in A_{sat}, but N_{area} was significantly reduced in the two N-fixing tree species under 10% and 40% irradiance treatments, which caused an increase in the $PNUE_{sat}$ in these trees. The $PNUE_{sat}$ in *C. hystrix* decreased under 10% and 40% irradiance treatments (Table 1). The $PNUE_{sat}$ of different tree species can respond differently to low light treatment, and may increase [5,20,27], decrease [28], or show no marked change [7,29]. This is related to the functional characteristics of different tree species. Many scholars have suggested that P_R and P_B are the main factors affecting $PNUE_{sat}$ [39,40]. In this study, P_R and P_B were the main factors affecting the variation in the $PNUE_{sat}$ under the 100% and 40% irradiance treatments; however, under the 10% irradiance treatment, the effects of P_L on $PNUE_{sat}$ became significant, and the effects of P_R and P_B on $PNUE_{sat}$ decreased (Figure 1). The ability to harvest light under low light treatment is a key factor limiting photosynthesis, and the importance of carboxylation and electron transport capacity decreases under such treatment, but persists [8,31]. We speculated that changes in g_m may affect $PNUE_{sat}$, based on the role of N in mesophyll conductance [41,42]. However, our results indicated that the effect of g_m on $PNUE_{sat}$ was not significant under varying light treatments in all tree species (Figure 2).

As these species are commonly used to plant in gaps or mixed with other species, their tolerance to low light conditions will affect the growth effect after planting. All four species decreased the LMA to increase the area receiving light (Table 1) [1,4,8,14], increased P_L to increase their light-harvesting capacity and sustain photosynthesis (Table 4) [3,4], and decreased the LCP and LSP to increase the ability to use low light (Table 5) under low light conditions. Meanwhile, N-fixing plants exhibited some other adaptations to low light conditions, such as increased A_{100}, $PNUE_{100}$ and $PNUE_{400}$ under the 10% and 40% irradiance treatments (Table 6). N-fixing plants also had higher A_{100}, A_{400}, N_{area}, V_{cmax} and J_{max} than non-N-fixing species under the 10% irradiance treatment (Tables 1, 3 and 6). Overall, these two N-fixing plant seedlings had higher photosynthetic rates, photosynthetic ability and higher adjustment ability of photosynthetic N use under low light conditions. AQY refers to the ability to use low light [43]. *E. Fordii* exhibited improved AQY under the 10% and 40% irradiance treatments, and also reduced R_n under the 10% and 40% irradiance treatments to reduce respiratory expenditure (Table 5). In conclusion, these results suggest that the adaptability of these two N-fixing species to low light environments is better than that of non-N-fixing species.

We previously studied the interspecific differences between *D. odorifera* and *E. fordii* (N-fixing trees), and *C. hystrix* and *B. alnoides* (non-N-fixing trees) [44], and how they are affected by soil N deficiencies [45]. The data obtained under high light intensity in this manuscript are the same as those used by Tang et al. [44] and the high nitrogen condition reported by Tang et al. [45], which were used as the "Control group." In [44], N-fixing trees had higher N_{area} and N_{mass}, but lower P_R, P_B, and PNUE than non-N-fixing trees. In [45], soil N deficiency had less influence on the leaf N concentration and photosynthetic ability in the two N-fixing trees. Combined with the results of this study, we consider that

nitrogen-fixing plants are suitable species for afforestation, and could be independently planted in poor soil, mixed with non-N-fixing species, or planted in gaps.

The P_L of all four species increased to improve their light-trapping ability under low irradiance treatments (Table 4), which was consistent with previous studies [31,46]. However, different tree species employ different strategies to increase their P_L: *D. odorifera* seedling leaves decreased P_{Other} to increase P_R, P_B, and P_L; *E. fordii* seedling leaves decreased P_{Other} and P_{CW} to increase P_L and P_R; *C. hystrix* seedling leaves decreased P_R, P_B, and P_{Other} to increase P_L; and *B. alnoides* seedling leaves decreased P_{CW} to increase P_L under 10% irradiance (Table 4). Many studies have also observed changes in N allocation under low light treatment [4,5,8,9,47]. These different strategies are related to the ecological characteristics of each tree species, but the goal is the same (reducing some other N components and increase light-harvesting N components under low light treatment). However, why these tree species reduce the corresponding N components requires further study.

4. Materials and Methods

4.1. Study Area and Plant Material

This study was conducted at the Experimental Center of Tropical Forestry, Chinese Academy of Forestry (22°719″–22°722″ N, 106°4440″–106°4444″ E), located in Guangxi Pingxiang, China. This area experiences a subtropical monsoon climate, with long summers and abundant rainfall. The average annual temperature of Pingxiang is 19.5–21.41 °C. Rainfall mainly occurs from April to September, and the annual precipitation is approximately 1400 mm [48,49].

Seedlings of *D. odorifera*, *E. fordii*, *C. hystrix*, and *B. alnoides* were selected from nurseries in March 2014, with 90 seedlings per species. The seedlings were healthy and similar in size (approximately 20-cm tall), and were transplanted into pots filled with 5.4 L of washed river sand outdoors. From April to June 2014, three levels of irradiance, that is, 100%, 40%, and 10% of sunlight irradiance, were applied using neutral black polypropylene frames with a covering film of black polyolefin resin fine mesh. The irradiation treatment lasted for three months. Illumination was measured using an MT-4617LED-C monochromator spectroradiometer (Pro's Kit Ltd., Shanghai, China); the average sunny midday illumination in the 100%, 40%, and 10% irradiance treatments were 78,000, 31,000, and 7800 lux, respectively.

There were three different randomized blocks per irradiance treatment, with each block consisting of 10 seedlings per species (30 seedlings per species per irradiance treatment), which were frequently moved within each block in order to avoid their position affecting the results. Each seedling was watered every day to pot water capacity and supplied with Hyponex's nutrient solution (0.125 g N and 0.11 g P) once a week at free-access rate.

4.2. Determination of Gas Exchange and Fluorescence Parameters

The experiment was conducted between 09:00 a.m. and 11:00 a.m. on sunny days, on newly fully expanded leaves of seven seedlings per treatment from July to August 2014, lasting for two months. An LI-6400-40 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) was used to determine the photosynthetic light and CO_2 response curves. The photosynthetic response to the photosynthetic photon flux density (PPFD, $\mu mol \cdot m^{-2} \cdot s^{-1}$) was determined under a leaf chamber CO_2 concentration of 380 $\mu mol\ mol^{-1}$, and the net photosynthetic rate (A_n, $\mu mol \cdot m^{-2} \cdot s^{-1}$), CO_2 concentration at sub-stomatal cavities (C_i, $\mu mol\ mol^{-1}$), and stomatal conductance (g_s, mol $CO_2 \cdot m^{-2} \cdot s^{-1}$) were measured at photon flux densities of 1500, 1200, 1000, 800, 600, 400, 200, 150, 100, 80, 50, 30, 20, 10, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (see Figure S1). The PPFD-saturated net CO_2 assimilation rate (A_{sat}, $\mu mol \cdot m^{-2} \cdot s^{-1}$), net CO_2 assimilation rate at PPFD of 100 $umol \cdot m^{-2} \cdot s^{-1}$ (A_{100}), net CO_2 assimilation rate at PPFD of 400 $umol \cdot m^{-2} \cdot s^{-1}$ (A_{400}), dark respiration (R_n, $\mu mol \cdot m^{-2} \cdot s^{-1}$), LSP ($\mu mol \cdot m^{-2} \cdot s^{-1}$), and LCP ($\mu mol \cdot m^{-2} \cdot s^{-1}$) were then measured from the light response curves. (100 and 400 $umol \cdot m^{-2} \cdot s^{-1}$ were in the range of the growth irradiance in the 10% and 40% light conditions, respectively). The AQY ($mol \cdot mol^{-1}$) was measured as the initial slope of the light response curves (PPFD $\leq$ 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

The CO_2 response curve was determined under saturated PPFD, and C_i and g_s were measured under leaf chamber CO_2 concentrations of 380, 200, 150, 100, 80, 50, 380, 600, 800, 1000, 1200, 1500, 1800, and 2000 μmol·mol^{-1} (see Figure S2). The light- and CO_2-saturated net CO_2 assimilation rate (A_{max}, μmol·m^{-2}·s^{-1}) was then measured from the CO_2 response curve. The fluorescence yield ($\Delta F/F_m$) was measured under leaf chamber CO_2 concentrations of 380 μmol·mol^{-1} and saturated PPFD. Meanwhile, the relative humidity of the leaf chamber was maintained at 50 ± 5%, and the leaf temperature was maintained at 25 ± 2 °C.

4.3. Determination of Mesophyll Conductance, V_{cmax}, and J_{max}

To better measure the mesophyll conductance to CO_2 (g_m, molCO_2 m^{-2}·s^{-1}), three methods were used: the variable *J* [50], exhaustive dual optimization [51], and A_n–C_i curve fitting methods [52,53]. The CO_2 concentration in chloroplasts (C_c, μmol·mol^{-1}) was then calculated as:

$$C_C = C_i - \frac{A_{sat}}{g_m} \quad (1)$$

The C_c and g_m values are listed in Table S1. The mean value of C_c calculated by the three methods was used to obtain the A_n–C_c curves, which were then used to calculate the maximum carboxylation rate (V_{cmax}, μmol·m^{-2}·s^{-1}) and electron transport rates (J_{max}, μmol·m^{-2}·s^{-1}) [54].

4.4. Determination of Additional Leaf Traits

After the determination of the gas exchange and fluorescence parameters, 20–30 leaves from each seedling used for gas exchange measurements were selected, which were healthy and similar in size. Ten to 15 of these leaves were selected, and the area of each leaf was measured using a scanner. Each leaf was then oven-dried at 80 °C for 48 h until the weight became constant, and the dry weight of each leaf was recorded. The LMA (g·m^{-2}) was calculated as the ratio of the dry weight to leaf area.

Subsequently, dries leaves were ground into powder and the leaf N per unit mass (N_{mass}, mg·g^{-1}) was determined following the micro-Kjeldahl method (UDK-139, Milano, Italy). The leaf N per unit area (N_{area} g·m^{-2}) values were then determined as N_{mass} × LMA/1000, while the PNUE (μmol·mol^{-1}·s^{-1}) was calculated as:

$$PNUE = \frac{A_n}{N_{area}} \times 14 \quad (2)$$

where $PNUE_{sat}$ was calculated by A_{sat} and N_{area}, $PNUE_{100}$ was calculated by A_{100} and N_{area} and $PNUE_{400}$ was calculated by A_{400} and N_{area}, respectively.

The remaining 10–15 leaves from each seedling were frozen with liquid nitrogen; 0.2-g of the leaves were weighed and cut into small pieces, and then added to a volumetric flask along with 95% (*v*/*v*) alcohol to a volume of 25 mL. The flasks were then stored under darkness for 24 h. The chlorophyll content (C_{Chl}, mmol·g^{-1}) was then determined by spectrophotometry. The cell wall N concentrations (Q_{CWmass} mg·g^{-1}) were measured following the method proposed by Onoda et al. [55], and the fraction of leaf N allocated to cell walls (P_{CW} g·g^{-1}) was determined as Q_{CWmass}/N_{mass}.

4.5. Calculation of N Allocation in the Photosynthetic Apparatus

The N allocation proportions in Rubisco (P_R, g·g^{-1}), bioenergetics (P_B, g·g^{-1}), and light-harvesting components (P_L, g·g^{-1}) were calculated according to Niinemets and Tenhunen [56]:

$$P_R = \frac{V_{cmax}}{6.25 \times V_{cr} \times LMA \times N_{mass}} \quad (3)$$

$$P_B = \frac{J_{max}}{8.06 \times J_{mc} \times LMA \times N_{mass}} \quad (4)$$

$$P_L = \frac{C_{Chl}}{C_B \times N_{mass}} \quad (5)$$

where C_{Chl} is the chlorophyll concentration (mmol·g^{-1}), V_{cr} is the specific activity of Rubisco (μmol CO_2 g^{-1} Rubisco s^{-1}), J_{mc} is the potential rate of photosynthetic electron transport (μmol electrons μmol^{-1} Cyt f s^{-1}), and C_B is the ratio of leaf chlorophyll to leaf nitrogen during light-harvesting (mmol Chl (g·N)$^{-1}$). V_{cr}, J_{mc}, and C_B were calculated according to Niinemets and Tenhunen [56]:

$$V_{cr}(J_{mc}) = \frac{e^{\left(c - \frac{\Delta H_a}{R \times T_k}\right)}}{1 + e^{\frac{\Delta S \times T_k - \Delta H_d}{R \times T_k}}} \quad (6)$$

$$[C_B] = 1.94 + \frac{12.6}{[LMA]} \quad (7)$$

where R is the gas constant (8.314 J·K^{-1}·mol^{-1}), T_k is the leaf temperature (K), ΔH_a is the activation energy, ΔH_d is the deactivation energy, ΔS is the entropy term, and c is the scaling constant. [LMA] and [C_B] are the values of LMA and C_B, respectively. The values of ΔH_a, ΔH_d, ΔS, and c were 74,000 J·mol^{-1}, 203,000 J·mol^{-1}, 645 J·K^{-1}·mol^{-1}, and 32.9 when calculating V_{cr}, and 24,100 J·mol^{-1}, 564,150 J·mol^{-1}, 1810 J·K^{-1}·mol^{-1}, and14.77 when calculating J_{mc} [56].

The leaf N allocated to the photosynthetic apparatus (P_P, g·g^{-1}) was calculated as P_R + P_B + P_L while the leaf N allocated to the other parts (P_{Other}, g·g^{-1}) was calculated as 1–P_P–P_{CW}. We also calculated the quantities of leaf N per unit area and the mass of N in the Rubisco, bioenergetics, light-harvesting components, photosynthetic apparatus, cell wall, and other parts (Tables S2 and S3).

4.6. Statistical Analysis

The differences between the four seedling species and different irradiance treatments were analyzed using one-way analysis of variance (ANOVA), and a post-hoc test (Tukey's test) was conducted to determine if the differences were significant. The *F*-ratio in the tables is the ratio of the mean squares between groups and within groups, and p is the confidence interval of F. The significance of the linear relationships between each pair of variables was tested by Pearson's correlation (two-tailed). All analyses were conducted using Statistical Product and Service Solutions 17.0 (version 17.0; SPSS, Chicago, IL, USA).

5. Conclusions

In our study, we concluded that: (1) low irradiance decreased the LMA, N_{area}, V_{cmax}, J_{max}, LCP, and LSP, increased the P_L in all species; increased A_{100}, $PNUE_{100}$ and $PNUE_{400}$ in N-fixing trees and decreased A_{sat} and g_s in non-N-fixing trees. These tree seedlings changed their leaf structure, leaf N allocation strategy, and photosynthetic physiological parameters to adapt to low light environments. (2) N-fixing plants had higher A_{100}, A_{400}, N_{area}, V_{cmax} and J_{max} than non-N-fixing species under low-irradiance treatment, and were more advantageous than non-N-fixing plants in maintaining the photosynthetic rate under low-radiation conditions.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants10102213/s1, Figure S1: A_n-PPFD curves in *Dalbergia odorifera*, *Erythrophleum fordii*, *Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments. Figure S2: A_n–C_i curves in *Dalbergia odorifera*, *Erythrophleum fordii*, *Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments. Table S1: Mesophyll conductance (g_m), and CO_2 concentration at carboxylation site (C_c) calculated by three methods in four species seedling leaves under different irradiance treatments. Table S2: Quantity of leaf N per area allocated to Rubisco (Q_{Rarea}), bioenergetics (Q_{Barea}), light-harvesting components (Q_{Larea}), photosynthetic apparatus

(Q_{Parea}), cell wall (Q_{CWarea}), and other parts ($Q_{Other\text{-}area}$) in four species seedling leaves under different irradiance treatments. Table S3: Quantity of leaf N per mass allocated to Rubisco (Q_{Rmass}), bioenergetics (Q_{Bmass}), light-harvesting components (Q_{Lmass}), photosynthetic apparatus (Q_{Pmass}), cell wall (Q_{CWmass}), and other parts ($Q_{Other\text{-}mass}$) in four species seedling leaves under different irradiance treatments.

Author Contributions: Conceptualization, Z.S. and J.T.; methodology, J.T.; software, B.S.; validation, J.T., R.C. and Z.S.; formal analysis, J.T.; investigation, J.T. and D.L.; resources, R.C. and Z.S.; data curation, J.T. and B.S.; writing—original draft preparation, J.T.; writing—review and editing, J.T., Z.S. and M.C.; visualization, J.T.; supervision, S.L.; project administration, S.L.; funding acquisition, J.T. and Z.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The National Key Research and Development Program, grant number 2016YFC0502104-02, the Natural Science Foundation of Shandong Province, grant number ZR2020QC027, and the Provincial key R&D Program of Shandong, grant number 2019GGX101013.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available within this article and Supplementary Figures S1 and S2, Tables S1–S3.

Acknowledgments: The authors thank the Experimental Center of Tropical Forestry at the Chinese Academy of Forestry for providing the experimental apparatus and assisting with the measurements. We would like to thank Editage. Available online: https://www.editage.cn/ (accessed on 18 October 2021) for English language editing.

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

References

1. Tang, X.; Liu, G.; Jiang, J.; Lei, C.; Zhang, Y.; Wang, L.; Liu, X. Effects of growth irradiance on photosynthesis and photorespiration of Phoebe bournei leaves. *Funct. Plant Biol.* **2020**, *47*, 1053. [CrossRef]
2. Warren, C.R.; Adams, M. Distribution of N, Rubisco and photosynthesis in Pinus pinaster and acclimation to light. *Plant Cell Environ.* **2001**, *24*, 597–609. [CrossRef]
3. Coble, A.P.; Cavaleri, M.A. Vertical leaf mass per area gradient of mature sugar maple reflects both height-driven increases in vascular tissue and light-driven increases in palisade layer thickness. *Tree Physiol.* **2017**, *37*, 1337–1351. [CrossRef]
4. Coble, A.P.; Fogel, M.L.; Parker, G.G. Canopy gradients in leaf functional traits for species that differ in growth strategies and shade tolerance. *Tree Physiol.* **2017**, *37*, 1415–1425. [CrossRef]
5. Bachofen, C.; D'Odorico, P.; Buchmann, N. Light and VPD gradients drive foliar nitrogen partitioning and photosynthesis in the canopy of European beech and silver fir. *Oecologia* **2020**, *192*, 323–339. [CrossRef]
6. Evans, J.R.; Poorter, H. Photosynthetic acclimation of plants to growth irradiance: The relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell Environ.* **2001**, *24*, 755–767. [CrossRef]
7. Trouwborst, G.; Hogewoning, S.; Harbinson, J.; Van Ieperen, W. Photosynthetic acclimation in relation to nitrogen allocation in cucumber leaves in response to changes in irradiance. *Physiol. Plant.* **2011**, *142*, 157–169. [CrossRef] [PubMed]
8. Chen, J.-W.; Kuang, S.-B.; Long, G.-Q.; Meng, Z.-G.; Li, L.-G.; Chen, Z.-J.; Zhang, G.-H.; Yang, S.-C. Steady-state and dynamic photosynthetic performance and nitrogen partitioning in the shade-demanding plant Panax notoginseng under different levels of growth irradiance. *Acta Physiol. Plant.* **2014**, *36*, 2409–2420. [CrossRef]
9. Osada, N.; Onoda, Y.; Hikosaka, K. Effects of atmospheric CO_2 concentration, irradiance, and soil nitrogen availability on leaf photosynthetic traits of Polygonum sachalinense around natural CO_2 springs in northern Japan. *Oecologia* **2010**, *164*, 41–52. [CrossRef] [PubMed]
10. Delagrange, S. Light- and seasonal-induced plasticity in leaf morphology, N partitioning and photosynthetic capacity of two temperate deciduous species. *Environ. Exp. Bot.* **2011**, *70*, 1–10. [CrossRef]
11. Ruan, J.; Haerdter, R.; Gerendás, J. Impact of nitrogen supply on carbon/nitrogen allocation: A case study on amino acids and catechins in green tea [*Camellia sinensis* (L.) O. Kuntze] plants. *Plant Biol.* **2010**, *12*, 724–734. [CrossRef] [PubMed]
12. Funk, J.L.; Glenwinkel, L.A.; Sack, L. Differential Allocation to Photosynthetic and Non-Photosynthetic Nitrogen Fractions among Native and Invasive Species. *PLoS ONE* **2013**, *8*, e64502. [CrossRef] [PubMed]
13. Stitt, M.; Schulze, D. Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular ecophysiology. *Plant Cell Environ.* **1994**, *17*, 465–487. [CrossRef]
14. Annighöfer, P.; Petritan, A.M.; Petritan, I.C.; Ammer, C. Disentangling juvenile growth strategies of three shade-tolerant temperate forest tree species responding to a light gradient. *For. Ecol. Manag.* **2017**, *391*, 115–126. [CrossRef]

15. Oguchi, R.; Hikosaka, K.; Hirose, T. Leaf anatomy as a constraint for photosynthetic acclimation: Differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. *Plant Cell Environ.* **2005**, *28*, 916–927. [CrossRef]
16. Terashima, I.; Hanba, Y.T.; Tazoe, Y.; Vyas, P.; Yano, S. Irradiance and phenotype: Comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion. *J. Exp. Bot.* **2005**, *57*, 343–354. [CrossRef]
17. Evans, J.R.; Kaldenhoff, R.; Genty, B.; Terashima, I. Resistances along the CO_2 diffusion pathway inside leaves. *J. Exp. Bot.* **2009**, *60*, 2235–2248. [CrossRef]
18. Niinemets, Ü.; Díaz-Espejo, A.; Flexas, J.; Galmés, J.; Warren, C.R. Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. *J. Exp. Bot.* **2009**, *60*, 2249–2270. [CrossRef]
19. Fini, A.; Loreto, F.; Tattini, M.; Giordano, C.; Ferrini, F.; Brunetti, C.; Centritto, M. Mesophyll conductance plays a central role in leaf functioning of Oleaceae species exposed to contrasting sunlight irradiance. *Physiol. Plant.* **2016**, *157*, 54–68. [CrossRef]
20. Gu, J.; Zhou, Z.; Li, Z.; Chen, Y.; Wang, Z.; Zhang, H. Rice (*Oryza sativa* L.) with reduced chlorophyll content exhibit higher photosynthetic rate and efficiency, improved canopy light distribution, and greater yields than normally pigmented plants. *Field Crop. Res.* **2017**, *200*, 58–70. [CrossRef]
21. Rocha, J.S.; Calzavara, A.K.; Bianchini, E.; Pimenta, J.A.; Stolf-Moreira, R.; Oliveira, H.C. Nitrogen supplementation improves the high-light acclimation of Guazuma ulmifolia Lam. seedlings. *Trees* **2018**, *33*, 421–431. [CrossRef]
22. Yang, Y.-J.; Hu, H.; Huang, W. The Light Dependence of Mesophyll Conductance and Relative Limitations on Photosynthesis in Evergreen Sclerophyllous *Rhododendron* Species. *Plants* **2020**, *9*, 1536. [CrossRef]
23. Hikosaka, K. Mechanisms underlying interspecific variation in photosynthetic capacity across wild plant species. *Plant Biotechnol.* **2010**, *27*, 223–229. [CrossRef]
24. Hou, W.; Tränkner, M.; Lu, J.; Yan, J.; Huang, S.; Ren, T.; Cong, R.; Li, X. Interactive effects of nitrogen and potassium on photosynthesis and photosynthetic nitrogen allocation of rice leaves. *BMC Plant Biol.* **2019**, *19*, 302. [CrossRef] [PubMed]
25. Wright, I.J.; Reich, P.B.; Westoby, M.; Ackerly, D.D.; Baruch, Z.; Bongers, F.; Cavender-Bares, J.; Chapin, T.; Cornelissen, J.H.C.; Diemer, M.; et al. The worldwide leaf economics spectrum. *Nature* **2004**, *428*, 821–827. [CrossRef]
26. Yin, L.; Xu, H.; Dong, S.; Chu, J.; Dai, X.; He, M. Optimised nitrogen allocation favours improvement in canopy photosynthetic nitrogen-use efficiency: Evidence from late-sown winter wheat. *Environ. Exp. Bot.* **2019**, *159*, 75–86. [CrossRef]
27. Wang, S.; Cai, Y.F.; Li, Z.L.; Li, S.F. Effect of different light intensities on photosynthetic characteristics of rhododendron 'Furnivall's Daughter'. *Acta Bot. Boreali-Occident. Sin.* **2012**, *32*, 2095–2101. [CrossRef]
28. Zhang, Y.-J.; Feng, Y.-L. The relationships between photosynthetic capacity and lamina mass per unit area, nitrogen content and partitioning in seedlings of two ficus species grown under different irradiance. *J. Plant Physiol. Mol. Boil.* **2004**, *30*, 269–276.
29. Wyka, T.P.; Oleksyn, J.; Żytkowiak, R.; Karolewski, P.; Jagodzinski, A.; Reich, P. Responses of leaf structure and photosynthetic properties to intra-canopy light gradients: A common garden test with four broadleaf deciduous angiosperm and seven evergreen conifer tree species. *Oecologia* **2012**, *170*, 11–24. [CrossRef]
30. Catoni, R.; Gratani, L.; Sartori, F.; Varone, L.; Granata, M.U. Carbon gain optimization in five broadleaf deciduous trees in response to light variation within the crown: Correlations among morphological, anatomical and physiological leaf traits. *Acta Bot. Croat.* **2015**, *74*, 71–94. [CrossRef]
31. Townsend, A.J.; Retkute, R.; Chinnathambi, K.; Randall, J.W.P.; Foulkes, J.; Carmo-Silva, E.; Murchie, E.H. Suboptimal Acclimation of Photosynthesis to Light in Wheat Canopies. *Plant Physiol.* **2018**, *176*, 1233–1246. [CrossRef] [PubMed]
32. Niinemets, Ü.; Flexas, J.; Penuelas, J. Evergreens favored by higher responsiveness to increased CO_2. *Trends Ecol. Evol.* **2011**, *26*, 136–142. [CrossRef] [PubMed]
33. Bahar, N.H.A.; Ishida, F.Y.; Weerasinghe, L.K.; Guerrieri, R.; O'Sullivan, O.S.; Bloomfield, K.J.; Asner, G.P.; Martin, R.E.; Lloyd, J.; Malhi, Y.; et al. Leaf-level photosynthetic capacity in lowland Amazonian and high-elevation Andean tropical moist forests of Peru. *New Phytol.* **2017**, *214*, 1002–1018. [CrossRef]
34. Peguero-Pina, J.J.; Sisó, S.; Flexas, J.; Galmés, J.; Niinemets, Ü.; Sancho-Knapik, D.; Gil-Pelegrín, E. Coordinated modifications in mesophyll conductance, photosynthetic potentials and leaf nitrogen contribute to explain the large variation in foliage net assimilation rates across Quercus ilex provenances. *Tree Physiol.* **2017**, *37*, 1084–1094. [CrossRef] [PubMed]
35. Cousins, A.B.; Mullendore, D.L.; Sonawane, B.V. Recent developments in mesophyll conductance in C3, C4, and crassulacean acid metabolism plants. *Plant J.* **2020**, *101*, 816–830. [CrossRef] [PubMed]
36. Warren, C.R. Stand aside stomata, another actor deserves centre stage: The forgotten role of the internal conductance to CO_2 transfer. *J. Exp. Bot.* **2007**, *59*, 1475–1487. [CrossRef]
37. Scafaro, A.P.; VON Caemmerer, S.; Evans, J.R.; Atwell, B.J. Temperature response of mesophyll conductance in cultivated and wild Oryza species with contrasting mesophyll cell wall thickness. *Plant Cell Environ.* **2011**, *34*, 1999–2008. [CrossRef]
38. Niinemets, U.; Cescatti, A.; Rodeghiero, M.; Tosens, T. Leaf internal diffusion conductance limits photosynthesis more strongly in older leaves of Mediterranean evergreen broad-leaved species. *Plant Cell Environ.* **2005**, *28*, 1552–1566. [CrossRef]
39. Qing, H.; Cai, Y.; Xiao, Y.; Yao, Y.; An, S. Leaf nitrogen partition between photosynthesis and structural defense in invasive and native tall form Spartina alterniflora populations: Effects of nitrogen treatments. *Biol. Invasions* **2012**, *14*, 2039–2048. [CrossRef]
40. Chen, L.; Dong, T.; Duan, B. Sex-specific carbon and nitrogen partitioning under N deposition in Populus cathayana. *Trees* **2014**, *28*, 793–806. [CrossRef]

41. Flexas, J.; Barbour, M.M.; Brendel, O.; Cabrera, H.M.; Carriquí, M.; Diaz-Espejo, A.; Douthe, C.; Dreyer, E.; Ferrio, J.P.; Gago, J.; et al. Mesophyll diffusion conductance to CO_2: An unappreciated central player in photosynthesis. *Plant Sci.* **2012**, *193–194*, 70–84. [CrossRef]
42. Buckley, T.N.; Warren, C.R. The role of mesophyll conductance in the economics of nitrogen and water use in photosynthesis. *Photosynth. Res.* **2014**, *119*, 77–88. [CrossRef]
43. Olsson, T.; Leverenz, J.W. Non-uniform stomatal closure and the apparent convexity of the photosynthetic photon flux density response curve. *Plant Cell Environ.* **1994**, *17*, 701–710. [CrossRef]
44. Tang, J.; Sun, B.; Cheng, R.; Shi, Z.; Luo, D.; Liu, S.; Centritto, M. Seedling leaves allocate lower fractions of nitrogen to photosynthetic apparatus in nitrogen fixing trees than in non-nitrogen fixing trees in subtropical China. *PLoS ONE* **2019**, *14*, e0208971. [CrossRef] [PubMed]
45. Tang, J.; Sun, B.; Cheng, R.; Shi, Z.; Luo, D.; Liu, S.; Centritto, M. Effects of soil nitrogen (N) deficiency on photosynthetic N-use efficiency in N-fixing and non-N-fixing tree seedlings in subtropical China. *Sci. Rep.* **2019**, *9*, 4604. [CrossRef] [PubMed]
46. Fotis, A.T.; Curtis, P.S. Effects of structural complexity on within-canopy light environments and leaf traits in a northern mixed deciduous forest. *Tree Physiol.* **2017**, *37*, 1426–1435. [CrossRef] [PubMed]
47. Hikosaka, K.; Hanba, Y.T.; Hirose, T.; Terashima, I. Photosynthetic nitrogen-use efficiency in leaves of woody and herbaceous species. *Funct. Ecol.* **1998**, *12*, 896–905. [CrossRef]
48. Wang, W.-X.; Shi, Z.-M.; Luo, D.; Liu, S.-R.; Lu, L.-H. Characteristics of soil microbial biomass and community composition in three types of plantations in southern subtropical area of China. *J. Appl. Ecol.* **2013**, *24*, 1784–1792.
49. Tang, J.C.; Shi, Z.M.; Luo, D.; Liu, S.R. Photosynthetic nitrogen-use efficiency of Manglietia glauca seedling leaves under different shading levels. *Acta Ecol. Sin.* **2017**, *37*, 7493–7502. [CrossRef]
50. Harley, P.C.; Loreto, F.; Di Marco, G.; Sharkey, T. Theoretical Considerations when Estimating the Mesophyll Conductance to CO_2 Flux by Analysis of the Response of Photosynthesis to CO_2. *Plant Physiol.* **1992**, *98*, 1429–1436. [CrossRef]
51. Gu, L.; Pallardy, S.G.; Tu, K.; Law, B.E.; Wullschleger, S.D. Reliable estimation of biochemical parameters from C3 leaf photosynthesis-intercellular carbon dioxide response curves. *Plant Cell Environ.* **2010**, *33*, 1852–1874. [CrossRef] [PubMed]
52. Ethier, G.J.; Livingston, N.J. On the need to incorporate sensitivity to CO_2 transfer conductance into the Farquhar-von Caemmerer-Berry leaf photosynthesis model. *Plant Cell Environ.* **2004**, *27*, 137–153. [CrossRef]
53. Sharkey, T.D.; Bernacchi, C.J.; Farquhar, G.D.; Singsaas, E.L. Fitting photosynthetic carbon dioxide response curves for C3leaves. *Plant Cell Environ.* **2007**, *30*, 1035–1040. [CrossRef] [PubMed]
54. Farquhar, G.D.; Von Caemmerer, S.; Berry, J.A. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. *Planta* **1980**, *149*, 78–90. [CrossRef]
55. Onoda, Y.; Hikosaka, K.; Hirose, T. Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-use efficiency. *Funct. Ecol.* **2004**, *18*, 419–425. [CrossRef]
56. Niinemets, U.; Tenhunen, J.D. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species Acer saccharum. *Plant Cell Environ.* **1997**, *20*, 845–866. [CrossRef]