

Article

The Effect of Low Irradiance on Leaf Nitrogen Allocation and Mesophyll Conductance to CO₂ in Seedlings of Four Tree Species in Subtropical China

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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_{max}), 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_{max} , 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 CO₂, 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 *Erythrophloeum 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 CO₂ 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 \pm 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} ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	N_{area} ($\text{g}\cdot\text{m}^{-2}$)	N_{mass} ($\text{mg}\cdot\text{g}^{-1}$)	LMA ($\text{g}\cdot\text{m}^{-2}$)	PNUE _{sat} ($\mu\text{mol}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$)
<i>Dalbergia odorifera</i>	100%	8.04 \pm 0.46 aA	2.19 \pm 0.13 aA	31.7 \pm 0.76 aA	69.0 \pm 3.90 aB	52.6 \pm 3.78 bB
	40%	8.30 \pm 0.76 aA	1.62 \pm 0.04 bA	31.2 \pm 0.65 aA	51.8 \pm 0.65 bB	72.3 \pm 7.03 bB
	10%	6.88 \pm 0.30 aA	0.97 \pm 0.04 cB	33.0 \pm 1.11 aA	29.3 \pm 0.67 cC	101.0 \pm 7.12 aA
	<i>F</i>	1.967	54.700 ***	1.196	73.752 ***	15.533 ***
<i>Erythrophleum fordii</i>	100%	6.60 \pm 0.50 bB	2.01 \pm 0.12 aA	28.1 \pm 1.49 bB	71.4 \pm 0.89 aB	45.9 \pm 2.24 cB
	40%	9.34 \pm 0.49 aA	1.75 \pm 0.03 bA	33.0 \pm 0.46 bA	53.1 \pm 0.99 bB	75.0 \pm 4.56 aB
	10%	6.87 \pm 0.50 bA	1.56 \pm 0.04 bA	35.3 \pm 0.88 aA	44.3 \pm 1.47 cB	61.6 \pm 3.72 bB
	<i>F</i>	9.042 **	9.223 **	12.658 ***	145.227 ***	15.877 ***
<i>Castanopsis hystrix</i>	100%	8.16 \pm 0.18 aA	1.02 \pm 0.06 aB	10.2 \pm 1.80 bD	100.1 \pm 2.60 aA	112.0 \pm 4.62 aA
	40%	4.57 \pm 0.23 bB	0.75 \pm 0.05 bB	9.6 \pm 0.50 bC	78.8 \pm 1.11 bA	87.0 \pm 7.26 bB
	10%	4.18 \pm 0.25 bB	0.79 \pm 0.03 bC	13.7 \pm 0.49 aC	57.9 \pm 1.29 cA	74.4 \pm 4.59 bB
	<i>F</i>	95.630 ***	20.060 ***	28.220 ***	138.877 ***	12.868 ***
<i>Betula alnoides</i>	100%	8.55 \pm 0.60 aA	1.03 \pm 0.09 aB	15.4 \pm 1.04 bC	67.6 \pm 5.45 aB	120.5 \pm 5.18 aB
	40%	7.42 \pm 0.30 aA	0.75 \pm 0.04 bB	15.4 \pm 0.45 bB	49.1 \pm 3.36 bB	140.3 \pm 8.02 aA
	10%	4.26 \pm 0.52 bB	0.56 \pm 0.04 bD	19.0 \pm 0.62 aB	29.6 \pm 2.14 cC	105.3 \pm 8.33 bA
	<i>F</i>	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 CO₂ drawdown ($C_i - C_c$) in any of the four tree species studied (Table 2).

Table 2. Stomatal conductance (g_s), mesophyll conductance (g_m), intercellular CO₂ concentration (C_i), CO₂ concentration at carboxylation site (C_c) and CO₂ 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 \pm 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 ·m ⁻² ·s ⁻¹)	g_m (molCO ₂ ·m ⁻² ·s ⁻¹)	C_i (μmol·mol ⁻¹)	C_c (μmol·mol ⁻¹)	$C_i - C_c$ (μmol·mol ⁻¹)
<i>Dalbergia odorifera</i>	100%	0.067 \pm 0.004 bBC	0.137 \pm 0.010 aA	251.5 \pm 6.44 bBC	190.8 \pm 6.92 bB	60.8 \pm 2.21 aC
	40%	0.091 \pm 0.009 aA	0.107 \pm 0.005 bA	288.5 \pm 3.93 aA	210.0 \pm 8.82 bA	78.6 \pm 7.50 aAB
	10%	0.089 \pm 0.003 aA	0.100 \pm 0.007 bA	302.6 \pm 1.94 aA	231.3 \pm 6.20 aA	71.2 \pm 5.27 aB
	F	6.562 **	6.823 **	34.333 ***	7.536 **	2.698
<i>Erythrophleum fordii</i>	100%	0.046 \pm 0.002 bC	0.066 \pm 0.007 bC	235.6 \pm 6.19 bC	132.6 \pm 6.90 bD	103.0 \pm 4.83 aA
	40%	0.075 \pm 0.005 aA	0.096 \pm 0.004 aA	254.1 \pm 3.81 abB	156.8 \pm 4.09 aB	97.3 \pm 2.37 aA
	10%	0.060 \pm 0.005 abB	0.074 \pm 0.004 bB	264.4 \pm 4.18 aB	171.7 \pm 4.45 aB	92.7 \pm 1.56 aA
	F	11.744 **	9.789 **	7.982 **	13.855 ***	2.553
<i>Castanopsis hystrix</i>	100%	0.074 \pm 0.004 aB	0.099 \pm 0.006 aB	256.8 \pm 5.24 bB	168.0 \pm 6.04 bC	88.8 \pm 6.26 aB
	40%	0.039 \pm 0.004 bB	0.053 \pm 0.006 bB	280.1 \pm 3.48 aA	196.4 \pm 5.84 aA	83.7 \pm 4.14 aAB
	10%	0.036 \pm 0.001 bC	0.053 \pm 0.005 bB	268.0 \pm 5.65 abB	186.5 \pm 6.27 abB	81.5 \pm 5.03 aAB
	F	38.06 ***	22.353 ***	5.711 *	5.691 *	0.511
<i>Betula alnoides</i>	100%	0.100 \pm 0.013 aA	0.134 \pm 0.012 aA	292.9 \pm 5.94 aA	226.4 \pm 9.57 aA	66.5 \pm 4.64 aC
	40%	0.095 \pm 0.011 aA	0.104 \pm 0.009 aA	297.1 \pm 7.04 aA	222.9 \pm 12.19 aA	74.2 \pm 5.69 aB
	10%	0.063 \pm 0.005 bB	0.056 \pm 0.009 bB	312.3 \pm 4.73 aA	232.1 \pm 9.07 aA	80.3 \pm 6.14 aAB
	F	4.195 *	16.261 ***	2.93	0.198	1.568

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 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 \pm 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	V_{cmax} (μmol·m ⁻² ·s ⁻¹)	J_{max} (μmol·m ⁻² ·s ⁻¹)
<i>Dalbergia odorifera</i>	100%	78.1 \pm 4.59 bB	100.7 \pm 5.80 aBC
	40%	95.2 \pm 8.01 aB	118.5 \pm 7.39 aB
	10%	68.6 \pm 3.96 cB	79.1 \pm 2.76 bB
	F	5.405 *	12.154 ***
<i>Erythrophleum fordii</i>	100%	99.8 \pm 9.37 bA	128.8 \pm 11.20 bAB
	40%	141.4 \pm 5.24 aA	168.9 \pm 3.36 aA
	10%	80.1 \pm 4.07 cA	99.8 \pm 3.83 cA
	F	22.233 ***	23.930 ***
<i>Castanopsis hystrix</i>	100%	82.8 \pm 4.47 aB	109.3 \pm 3.40 aABC
	40%	46.5 \pm 2.51 bC	57.6 \pm 4.49 bC
	10%	47.7 \pm 2.92 bC	66.5 \pm 3.80 bC
	F	75.031 ***	49.677 ***

	100%	73.0 ± 3.51 ^{aB}	98.4 ± 5.37 ^{aBC}
<i>Betula alnoides</i>	40%	82.6 ± 5.46 ^{aB}	97.8 ± 5.39 ^{aB}
	10%	41.6 ± 4.80 ^{bC}	56.0 ± 4.59 ^{bC}
	<i>F</i>	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})
<i>Dalbergia odorifera</i>	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}
	<i>F</i>	14.001 ^{***}	27.585 ^{***}	54.347 ^{***}	29.423 ^{***}	0.632	29.390 ^{***}
<i>Erythrophleum fordii</i>	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}
	<i>F</i>	24.021 ^{***}	25.215 ^{***}	38.638 ^{***}	47.577 ^{***}	24.303 ^{***}	40.909 ^{***}
<i>Castanopsis hystrix</i>	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}
	<i>F</i>	7.010 ^{**}	6.229 ^{**}	15.540 ^{***}	1.475	17.205 ^{***}	4.023 [*]
<i>Betula alnoides</i>	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}
	<i>F</i>	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 \pm 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 ($\mu\text{mol}\cdot\text{mol}^{-1}$)	R_n ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	LCP ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	LSP ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
<i>Dalbergia odorifera</i>	100%	0.052 \pm 0.004 aA	0.909 \pm 0.050 aBC	22.1 \pm 1.68 aA	822.9 \pm 27.5 aA
	40%	0.059 \pm 0.002 aA	0.845 \pm 0.050 aA	14.4 \pm 0.73 bB	724.3 \pm 37.0 aBA
	10%	0.058 \pm 0.002 aA	0.760 \pm 0.038 aB	7.4 \pm 0.43 cB	684.3 \pm 23.1 bA
	F	2.300	2.587	46.127 ***	5.378*
<i>Erythrophleum fordii</i>	100%	0.047 \pm 0.003 bA	1.129 \pm 0.051 aA	13.9 \pm 0.81 aB	637.1 \pm 29.6 aB
	40%	0.062 \pm 0.002 aA	0.873 \pm 0.050 bA	13.1 \pm 1.10 aB	633.6 \pm 17.1 aA
	10%	0.059 \pm 0.001 aA	0.936 \pm 0.030 bAB	7.5 \pm 0.79 bB	522.1 \pm 17.2 bB
	F	15.924 ***	8.811 **	14.464 ***	6.569**
<i>Castanopsis hystrix</i>	100%	0.047 \pm 0.003 aA	1.005 \pm 0.067 aAB	14.0 \pm 1.21 aB	632.9 \pm 23.4 aB
	40%	0.054 \pm 0.002 aA	0.988 \pm 0.040 aA	7.3 \pm 1.00 bC	307.1 \pm 26.8 bC
	10%	0.054 \pm 0.003 aA	1.048 \pm 0.088 aA	7.4 \pm 1.21 bB	262.1 \pm 27.7 bC
	F	2.737	0.206	11.226 **	60.359 ***
<i>Betula alnoides</i>	100%	0.049 \pm 0.001 aA	0.889 \pm 0.039 aBC	20.8 \pm 0.93 aA	886.4 \pm 43.5 aA
	40%	0.055 \pm 0.003 aA	0.844 \pm 0.055 aA	18.6 \pm 2.49 aA	519.3 \pm 27.9 bB
	10%	0.051 \pm 0.003 aA	0.834 \pm 0.048 aAB	10.5 \pm 0.94 bA	268.6 \pm 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} , PNUE₁₀₀ and PNUE₄₀₀ 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 PNUE₄₀₀ 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 PNUE₁₀₀ of *B. alnoides* was significantly higher than that under the 100% treatment (Table 6).

Table 6. Net CO₂ assimilation rate at PPFD of 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (A_{100}); net CO₂ assimilation rate at PPFD of 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (A_{400}); photosynthetic N use efficiency at PPFD of 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (PNUE₁₀₀); photosynthetic N use efficiency at PPFD of 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (PNUE₄₀₀) in *Dalbergia odorifera*, *Erythrophleum fordii*, *Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments. Data are means of seven plants per treatment \pm 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} ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	A_{400} ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	PNUE ₁₀₀ ($\mu\text{mol}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$)	PNUE ₄₀₀ ($\mu\text{mol}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$)
<i>Dalbergia odorifera</i>	100%	2.78 \pm 0.41 bA	7.38 \pm 0.45 abAB	20.7 \pm 1.36 cB	48.2 \pm 3.65 cB
	40%	4.06 \pm 0.08 aA	8.02 \pm 0.30 aA	35.2 \pm 0.66 bB	69.8 \pm 3.35 bB
	10%	4.03 \pm 0.17 aA	6.65 \pm 0.36 bA	59.2 \pm 4.12 aAB	97.4 \pm 7.32 aA
	F	7.114 *	6.206 *	58.81 ***	23.261 ***
	100%	3.42 \pm 0.16 bA	6.07 \pm 0.24 bB	24.2 \pm 1.38 bB	42.7 \pm 1.48 cB

<i>Erythrophleum fordii</i>	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 ***
	100%	3.57 ± 0.12 aA	7.67 ± 0.42 aAB	49.1 ± 1.83 aA	105.4 ± 6.18 aA
<i>Castanopsis hystrix</i>	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 **
	100%	3.27 ± 0.15 aA	7.95 ± 0.52 aA	46.4 ± 4.09 bA	113.7 ± 12.66 aA
<i>Betula alnoides</i>	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

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).

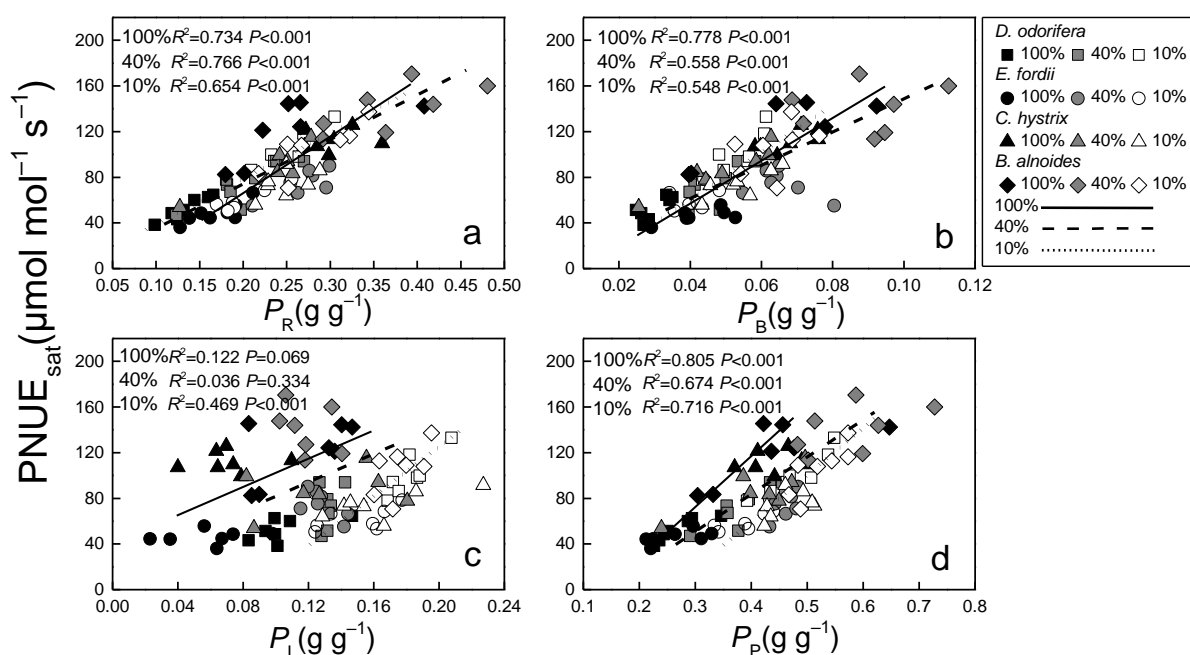


Figure 1. Relationship between PPFD-saturated photosynthetic N use efficiency (PNUE_{sat}) and (a) N allocation proportion of Rubisco (P_R), (b) bioenergetics (P_B), (c) light-harvesting components (P_L) and (d) photosynthetic system (P_P) in *Dalbergia odorifera*, *Erythrophleum fordii*, *Betula alnoides*,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

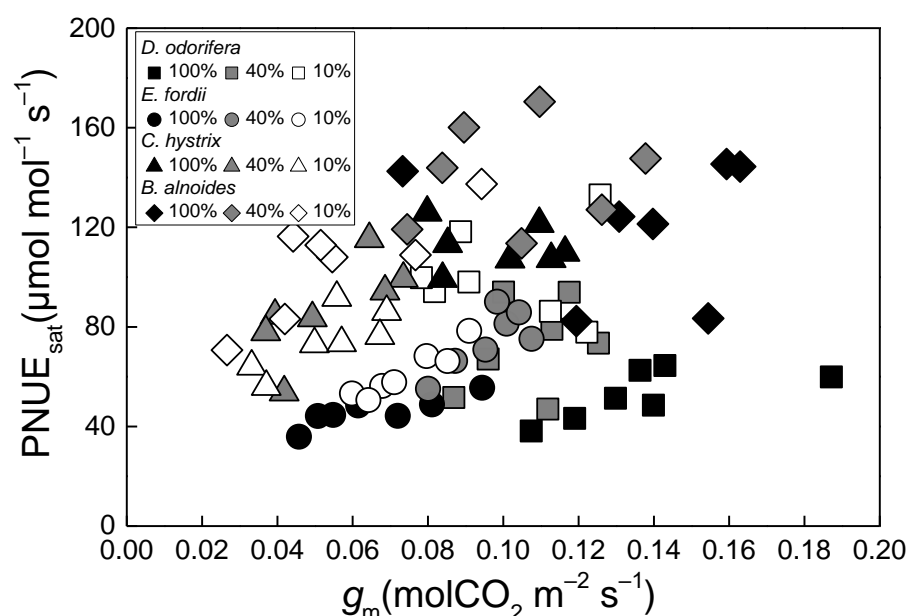


Figure 2. Relationship between PPFD-saturated photosynthetic N use efficiency (PNUE_{sat}) and mesophyll conductance (g_m) in *Dalbergia odorifera*, *Erythrophleum fordii*, *Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments.

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 $N_{\text{area}} = \text{LMA} \times N_{\text{mass}}$, 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 mid-day 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 am and 11:00 am 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 CO₂ response curves. The photosynthetic response to the photosynthetic photon flux density (PPFD, $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was determined under a leaf chamber CO₂ concentration of 380 $\mu\text{mol}\cdot\text{mol}^{-1}$, and the net photosynthetic rate (A_n , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), CO₂ concentration at sub-stomatal cavities (C_i , $\mu\text{mol}\cdot\text{mol}^{-1}$), and stomatal conductance (g_s , $\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) were measured at photon flux densities of 1500, 1200, 1000, 800, 600, 400, 200, 150, 100, 80, 50, 30, 20, 10, and 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (see Figure S1). The PPFD-saturated net CO₂ assimilation rate (A_{sat} , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), net CO₂ assimilation rate at PPFD of 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (A_{100}), net CO₂ assimilation rate at PPFD of 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (A_{400}), dark respiration (R_n , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), LSP ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$),

and LCP ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) were then measured from the light response curves. (100 and 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were in the range of the growth irradiance in the 10% and 40% light conditions, respectively). The AQY ($\text{mol}\cdot\text{mol}^{-1}$) was measured as the initial slope of the light response curves ($\text{PPFD} \leq 30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

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 $\mu\text{mol}\cdot\text{mol}^{-1}$ (see Figure S2). The light- and CO_2 -saturated net CO_2 assimilation rate (A_{max} , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) 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 $\mu\text{mol}\cdot\text{mol}^{-1}$ and saturated PPFD. Meanwhile, the relative humidity of the leaf chamber was maintained at $50 \pm 5\%$, and the leaf temperature was maintained at $25 \pm 2^\circ\text{C}$.

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

To better measure the mesophyll conductance to CO_2 (g_m , $\text{mol}\text{CO}_2 \text{ m}^{-2}\cdot\text{s}^{-1}$), 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 , $\mu\text{mol}\cdot\text{mol}^{-1}$) was then calculated as:

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

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} , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and electron transport rates (J_{max} , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) [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 ($\text{g}\cdot\text{m}^{-2}$) was calculated as the ratio of the dry weight to leaf area.

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

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

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} , $\text{mmol}\cdot\text{g}^{-1}$) was then determined by spectrophotometry. The cell wall N concentrations (Q_{CWmass} , $\text{mg}\cdot\text{g}^{-1}$) were measured following the method proposed by Onoda et al. [55], and the fraction of leaf N allocated to cell walls (P_{CW} , $\text{g}\cdot\text{g}^{-1}$) was determined as $Q_{\text{CWmass}}/N_{\text{mass}}$.

4.5. Calculation of N Allocation in the Photosynthetic Apparatus

The N allocation proportions in Rubisco (P_R , $\text{g}\cdot\text{g}^{-1}$), bioenergetics (P_B , $\text{g}\cdot\text{g}^{-1}$), and light-harvesting components (P_L , $\text{g}\cdot\text{g}^{-1}$) 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 ($\text{mmol} \cdot \text{g}^{-1}$), V_{cr} is the specific activity of Rubisco ($\mu\text{mol CO}_2 \text{ g}^{-1} \text{ Rubisco s}^{-1}$), J_{mc} is the potential rate of photosynthetic electron transport ($\mu\text{mol electrons } \mu\text{mol}^{-1} \text{ Cyt f s}^{-1}$), and C_B is the ratio of leaf chlorophyll to leaf nitrogen during light-harvesting ($\text{mmol Chl (g N)}^{-1}$). 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 ($8.314 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$), 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 $74,000 \text{ J} \cdot \text{mol}^{-1}$, $203,000 \text{ J} \cdot \text{mol}^{-1}$, $645 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$, and 32.9 when calculating V_{cr} , and $24,100 \text{ J} \cdot \text{mol}^{-1}$, $564,150 \text{ J} \cdot \text{mol}^{-1}$, $1810 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$, and 14.77 when calculating J_{mc} [56].

The leaf N allocated to the photosynthetic apparatus (P_P , $\text{g} \cdot \text{g}^{-1}$) was calculated as $P_R + P_B + P_L$ while the leaf N allocated to the other parts (P_{Other} , $\text{g} \cdot \text{g}^{-1}$) 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 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 ($Q_{Other-area}$) 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 ($Q_{Other-mass}$) in four species seedling leaves under different irradiance treatments.

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