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 (Narea), maximum carboxylation rate (Vcmax), maximum electron transport rate (Jmax), 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, Narea, Vcmax, and Jmax 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_P [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 | $A_{sat}$ (μmol m$^{-2}$ s$^{-1}$) | $N_{area}$ (g m$^{-2}$) | $N_{mass}$ (mg g$^{-1}$) | LMA (g m$^{-2}$) | PNUE$_{sat}$ (μmol mol$^{-1}$ s$^{-1}$) |
|----------------------|----------------------|-----------------------------------|------------------------|--------------------------|----------------|-------------------------------------|
|                      | 100%                 | 8.04 ± 0.46 $^{aA}$                | 2.19 ± 0.13 $^{aA}$    | 31.7 ± 0.76 $^{aA}$      | 69.0 ± 3.90 $^{aB}$ | 52.6 ± 3.76 $^{bB}$                |
|                      | 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 $^{bB}$    | 33.0 ± 1.11 $^{aA}$      | 29.3 ± 0.67 $^{cC}$ | 101.0 ± 7.12 $^{aA}$               |
|                      | F                    | 1.967                              |                        | 1.196                    | 73.752 $^{**}$ | 15.533 ***                         |
| *Erythrophleum fordi*| 100%                 | 6.60 ± 0.50 $^{bB}$                | 2.01 ± 0.12 $^{aA}$    | 28.1 ± 1.49 $^{bB}$      | 71.4 ± 0.89 $^{bB}$ | 45.9 ± 2.24 $^{bB}$                |
|                      | 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 $^{bB}$                |
|                      | 10%                  | 6.87 ± 0.50 $^{aA}$                | 1.56 ± 0.04 $^{bA}$    | 35.3 ± 0.88 $^{aA}$      | 44.3 ± 1.47 $^{cB}$ | 61.6 ± 3.72 $^{bB}$                |
|                      | F                    | 9.042 $^{aA}$                      |                        | 9.223 $^{aA}$            | 145.227 $^{***}$ | 15.877 ***                         |
| *Castanopsis hystrix*| 100%                 | 8.16 ± 0.18 $^{aA}$                | 1.02 ± 0.06 $^{aA}$    | 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 $^{bB}$ | 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 $^{aB}$               |
|                      | 40%                  | 7.42 ± 0.30 $^{aA}$                | 0.75 ± 0.04 $^{bB}$    | 15.4 ± 0.45 $^{aB}$      | 49.1 ± 3.36 $^{cB}$ | 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% irradiation 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% irradiation 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% irradiation treatment were higher than that under 100% irradiance, and in E. fordii, $C_i$ under 10% irradiation 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 $f_{max}$ of D. odorifera, E. fordii, and B. alnoides under the 10% irradiance treatments, and $V_{cmax}$ and $f_{max}$ of C. hystrix under the 10% and 40% irradiation treatments were lower than those under 100% irradiation (Table 3). In contrast, $V_{cmax}$ of D. odorifera and $V_{cmax}$ of E. fordii under the 40% irradiation treatment were higher than those under 100% irradiation.
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 ($C_c$−$C_c$) 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 | $g_s$ (molCO₂·m⁻²·s⁻¹) | $g_m$ (molCO₂·m⁻²·s⁻¹) | $C_i$ (µmol·mol⁻¹) | $C_c$ (µmol·mol⁻¹) | $C_c$−$C_c$ (µmol·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 bB     | 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     | 71.3 ± 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 abB    | 97.3 ± 2.37 aA           |
|                   | 10%                  | 0.060 ± 0.005 bB        | 0.074 ± 0.004 bB       | 264.4 ± 4.18 abB   | 171.7 ± 4.45 abB    | 92.7 ± 1.56 aA           |
|                   | F                    | 11.744 **               | 9.789 **               | 7.982 **           | 13.855 **           | 2.553                    |
| Castanopsis hystrix | 100%                 | 0.074 ± 0.004 bB        | 0.099 ± 0.006 bB       | 256.8 ± 5.24 bB    | 168.0 ± 6.04 bC     | 88.8 ± 6.26 bA           |
|                   | 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 aB           |
|                   | 10%                  | 0.036 ± 0.001 bB        | 0.053 ± 0.005 bB       | 268.0 ± 5.65 abB   | 186.5 ± 6.27 abB    | 81.5 ± 5.03 aB           |
|                   | F                    | 38.06 **                | 22.333 ***             | 5.711              | 6.591 *             | 0.511                    |
| Betula alnoides    | 100%                 | 0.100 ± 0.013 aA        | 0.134 ± 0.012 aA       | 292.9 ± 5.94 aA    | 226.1 ± 9.57 aB     | 66.6 ± 4.64 bC           |
|                   | 40%                  | 0.095 ± 0.011 aA        | 0.104 ± 0.009 aA       | 297.1 ± 7.04 aA    | 229.2 ± 12.19 aB    | 74.2 ± 5.69 aB           |
|                   | 10%                  | 0.063 ± 0.005 bB        | 0.056 ± 0.009 bB       | 312.3 ± 4.73 aB    | 232.1 ± 9.07 aB     | 80.3 ± 6.14 aAB          |
|                   | F                    | 4.195 *                 | 16.261 ***             | 2.93               | 0.198               | 1.568                    |

Table 3. Maximum carboxylation rate ($V_{max}$) and maximum electron transport rate ($I_{max}$) 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 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ among irradiance treatment.

| Tree Species       | Irradiance Treatment | $V_{max}$ (µmol·m⁻²·s⁻¹) | $I_{max}$ (µmol·m⁻²·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 aB           | 79.1 ± 2.76 bB           |
|                   | F                    | 5.405 *                  | 12.154 **                |
| Erythrophleum fordii | 100%                 | 99.8 ± 9.37 bA           | 128.8 ± 11.2 bAB         |
|                   | 40%                  | 141.4 ± 5.24 aA          | 168.9 ± 3.36 aA          |
|                   | 10%                  | 80.1 ± 4.07 aC           | 99.8 ± 3.83 aA           |
|                   | F                    | 22.233 ***               | 23.930 ***               |
| Castanopsis hystrix | 100%                 | 82.8 ± 4.47 abB          | 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 bB           |
|                   | F                    | 75.031 ***               | 49.677 ***               |
| Betula alnoides    | 100%                 | 73.0 ± 3.51 aB           | 98.4 ± 5.37 aB           |
|                   | 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 $C$.
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Table 4. Nitrogen allocation proportion of Rubisco (P<sub>R</sub>), bioenergetics (P<sub>B</sub>), light-harvesting components (P<sub>L</sub>), photosynthetic system (P<sub>PS</sub>), cell wall (P<sub>CW</sub>) and other parts (P<sub>Other</sub>) in *Dalbergia odorifera*, *Erythrophleum hystrix*, *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<sub>R</sub> (g g<sup>−1</sup>) | P<sub>B</sub> (g g<sup>−1</sup>) | P<sub>L</sub> (g g<sup>−1</sup>) | P<sub>PS</sub> (g g<sup>−1</sup>) | P<sub>CW</sub> (g g<sup>−1</sup>) | P<sub>Other</sub> (g g<sup>−1</sup>) |
|-----------------------|----------------------|-------------------------------|--------------------------------|-------------------------------|---------------------------------|---------------------------------|----------------------------------|
| *Dalbergia odorifera* | 100%                 | 0.135 ± 0.009                 | 0.030 ± 0.002                 | 0.105 ± 0.008                 | 0.269 ± 0.016                   | 0.068 ± 0.004                   | 0.663 ± 0.015<sup>aA</sup>       |
|                       | 40%                  | 0.201 ± 0.018                 | 0.047 ± 0.003                 | 0.132 ± 0.002                 | 0.381 ± 0.021                   | 0.067 ± 0.006                   | 0.552 ± 0.017<sup>bA</sup>       |
|                       | 10%                  | 0.242 ± 0.016                 | 0.054 ± 0.003                 | 0.183 ± 0.005                 | 0.479 ± 0.021                   | 0.061 ± 0.004                   | 0.461 ± 0.023<sup>cB</sup>       |
|                       | *F*                  | 14.001 ***                   | 27.585 ***                    | 54.347 ***                    | 29.423 ***                      | 0.632 29.390 ***                |
|                       | 100%                 | 0.164 ± 0.010                 | 0.043 ± 0.003                 | 0.060 ± 0.009                 | 0.266 ± 0.018                   | 0.052 ± 0.002                   | 0.683 ± 0.019<sup>aA</sup>       |
| *Erythrophleum fordii*| 40%                  | 0.268 ± 0.011                 | 0.065 ± 0.003                 | 0.129 ± 0.004                 | 0.462 ± 0.007                   | 0.038 ± 0.001                   | 0.500 ± 0.007<sup>cA</sup>       |
|                       | 10%                  | 0.203 ± 0.011                 | 0.041 ± 0.002                 | 0.150 ± 0.008                 | 0.394 ± 0.016                   | 0.039 ± 0.002                   | 0.568 ± 0.015<sup>bA</sup>       |
|                       | *F*                  | 24.021 ***                   | 25.215 ***                    | 38.638 ***                    | 47.577 ***                      | 24.303 ***                     |
|                       | 100%                 | 0.302 ± 0.012                 | 0.068 ± 0.003                 | 0.072 ± 0.008                 | 0.441 ± 0.018                   | 0.267 ± 0.010                   | 0.292 ± 0.019<sup>aB</sup>       |
| *Castanopsis hystrix* | 40%                  | 0.231 ± 0.018                 | 0.049 ± 0.005                 | 0.130 ± 0.014                 | 0.411 ± 0.032                   | 0.443 ± 0.022                   | 0.146 ± 0.023<sup>cB</sup>       |
|                       | 10%                  | 0.247 ± 0.010                 | 0.054 ± 0.003                 | 0.164 ± 0.013                 | 0.466 ± 0.015                   | 0.342 ± 0.028                   | 0.192 ± 0.031<sup>bD</sup>       |
|                       | *F*                  | 7.010 **                     | 6.229 **                      | 15.540 ***                    | 1.475                            | 17.205 ***                     |
|                       | 100%                 | 0.256 ± 0.028                 | 0.066 ± 0.007                 | 0.116 ± 0.011                 | 0.439 ± 0.042                   | 0.221 ± 0.011                   | 0.340 ± 0.042<sup>bB</sup>       |
|                       | 40%                  | 0.369 ± 0.026                 | 0.089 ± 0.006                 | 0.119 ± 0.005                 | 0.577 ± 0.033                   | 0.197 ± 0.011                   | 0.227 ± 0.031<sup>cB</sup>       |
|                       | 10%                  | 0.281 ± 0.017                 | 0.063 ± 0.003                 | 0.176 ± 0.005                 | 0.521 ± 0.016                   | 0.150 ± 0.010                   | 0.329 ± 0.021<sup>cB</sup>       |
|                       | *F*                  | 5.979 **                     | 6.189 **                      | 20.386 ***                    | 4.641 *                         | 10.826 **                      |

The apparent quantum yield (AQY) and R<sub>n</sub> 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<sub>n</sub> 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 ($R_o$), 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$^{-1}$) | $R_o$ (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 bA      | 0.845 ± 0.050 aA             | 14.4 ± 0.73 bB               | 724.3 ± 37.0 aB              |
|                       | 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 ***                   |                               |
| *Erythrophleum fordii*| 100%                 | 0.047 ± 0.003 bA      | 1.129 ± 0.051 aA             | 13.9 ± 0.81 aB               | 637.1 ± 29.6 bB              |
|                       | 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 ± 0.002 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 ab             | 20.8 ± 0.93 aA               | 886.4 ± 43.5 ab              |
|                       | 40%                  | 0.055 ± 0.003 aA      | 0.844 ± 0.055 aA             | 18.6 ± 2.49 aA               | 519.3 ± 27.9 bc              |
|                       | 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 6). $A_{400}$ 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 $P_R$, $P_S$, 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 CO₂ assimilation rate at PPFD of 100 umol·m⁻²·s⁻¹ ($A_{100}$); net CO₂ 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 (PNUE₁₀₀); photosynthetic N use efficiency at PPFD of 400 umol·m⁻²·s⁻¹ (PNUE₄₀₀) 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.

| Tree Species          | Irradiance Treatment | $A_{100}$ (μmol m⁻² s⁻¹) | $A_{400}$ (μmol m⁻² s⁻¹) | PNUE₁₀₀ (μmol mol⁻¹ s⁻¹) | PNUE₄₀₀ (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 AB          | 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 bB          | 68.4 ± 2.42 aB          |
|                       | 10%                  | 3.98 ± 0.18 aA           | 6.31 ± 0.43 aA           | 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 AB          | 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 BB                | 5.074*                  | 2.852                   |

Figure 1. Relationship between PPFD-saturated photosynthetic N use efficiency (PNUE₄₀₀) and (a) N allocation proportion of Rubisco ($P_R$), (b) bioenergetics ($P_L$), (c) light-harvesting components ($P_L$), and (d) photosynthetic system ($P_F$) 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.
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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 *Dalbergia 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 $c_{max}$ and $V_{cmax}$ were significantly reduced in two non-N-fixing tree species, *Erythrophleum fordii* and *Betula alnoides*, and *Castanopsis hystrix* grown under three different irradiance treatments.

![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.](image)

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

$g_{\text{m}}$ in $D. \text{odorifera}$, $C. \text{hystrix}$, and $B. \text{alnoides}$ seedlings decreased under 10% irradiance, which was consistent with previous studies [19,20] (Table 2). $g_{\text{m}}$ 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 $g_{\text{m}}$ [35]. If a lower LMA is the result of mesophyll cell wall thinning, it will increase $g_{\text{m}}$ [36,37]; if it is the result of a lower number of mesophyll layers, it will decrease $g_{\text{m}}$ [38]. In this study, the LMA of $D. \text{odorifera}$, $C. \text{hystrix}$, and $B. \text{alnoides}$ 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 $A_{\text{sat}}$, but $N_{\text{area}}$ was significantly reduced in the two N-fixing tree species under 10% and 40% irradiance treatments, which caused an increase in the PNUE$_{\text{sat}}$ in these trees. The PNUE$_{\text{sat}}$ in $C. \text{hystrix}$ decreased under 10% and 40% irradiance treatments (Table 1). The PNUE$_{\text{sat}}$ 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 $P_{\text{R}}$ and $P_{\text{B}}$ are the main factors affecting PNUE$_{\text{sat}}$ [39,40]. In this study, $P_{\text{R}}$ and $P_{\text{B}}$ were the main factors affecting the variation in the PNUE$_{\text{sat}}$ under the 100% and 40% irradiance treatments; however, under the 10% irradiance treatment, the effects of $P_{\text{R}}$ on PNUE$_{\text{sat}}$ became significant, and the effects of $P_{\text{R}}$ and $P_{\text{B}}$ on PNUE$_{\text{sat}}$ 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 $g_{\text{m}}$ may affect PNUE$_{\text{sat}}$, based on the role of N in mesophyll conductance [41,42]. However, our results indicated that the effect of $g_{\text{m}}$ on PNUE$_{\text{sat}}$ 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 $P_{\text{L}}$ 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 $A_{100}$, $A_{400}$, $N_{\text{area}}$, $V_{\text{cmax}}$ and $J_{\text{max}}$ 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]. $E. \text{fordii}$ exhibited improved AQY under the 10% and 40% irradiance treatments, and also reduced $R_{\text{n}}$ 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 $D. \text{odorifera}$ and $E. \text{fordii}$ (N-fixing trees), and $C. \text{hystrix}$ and $B. \text{alnoides}$ (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 $N_{\text{area}}$ and $N_{\text{mass}}$, but lower $P_{\text{R}}$, $P_{\text{B}}$, 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 area was 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, μmol·m$^{-2}$·s$^{-1}$) was determined under a leaf chamber CO$_2$ concentration of 380 μmol·mol$^{-1}$, and the net photosynthetic rate ($A_{n}$, μmol·m$^{-2}$·s$^{-1}$), CO$_2$ concentration at sub-stomatal cavities ($C_{i}$, μmol·mol$^{-1}$), and stomatal conductance ($g_{s}$, mol CO$_2$·m$^{-2}$·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 μmol·m$^{-2}$·s$^{-1}$ (see Figure S1). The PPFD-saturated net CO$_2$ assimilation rate ($A_{Sat}$, μmol·m$^{-2}$·s$^{-1}$), net CO$_2$ assimilation rate at PPFD of 100 μmol·m$^{-2}$·s$^{-1}$ ($A_{100}$), net CO$_2$ assimilation rate at PPFD of 400 μmol·m$^{-2}$·s$^{-1}$ ($A_{400}$), dark respiration ($R_{n}$, μmol·m$^{-2}$·s$^{-1}$), LSP (μmol·m$^{-2}$·s$^{-1}$), and LCP (μmol·m$^{-2}$·s$^{-1}$) were then measured from the light response curves. (100 and 400 μmol·m$^{-2}$·s$^{-1}$ were in the range of the growth irradiance in the 10% and 40% light conditions, respectively). The AQY (mol·mol$^{-1}$) was measured as the initial slope of the light response curves (PPFD ≤ 30 μmol·m$^{-2}$·s$^{-1}$).
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The CO₂ response curve was determined under saturated PPFD, and Cᵢ and gₛ were measured under leaf chamber CO₂ 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 CO₂-saturated net CO₂ assimilation rate (Aₘₐₓ, µmol·m⁻²·s⁻¹) was then measured from the CO₂ response curve. The fluorescence yield (ΔF/Fₘ) was measured under leaf chamber CO₂ 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, Vₑₘₐₓ, and Jₘₐₓ

To better measure the mesophyll conductance to CO₂ (gₘ, µmolCO₂ m⁻²·s⁻¹), three methods were used: the variable J [50], exhaustive dual optimization [51], and Aᵦ₋Cᵢ curve fitting methods [52,53]. The CO₂ concentration in chloroplasts (Cₖ, µmol·mol⁻¹) was then calculated as:

\[ Cₖ = Cᵢ - \frac{Aᵦ}{gₘ} \]   \hspace{1cm} (1)

The Cₑ and gₘ values are listed in Table S1. The mean value of Cₑ calculated by the three methods was used to obtain the Aᵦ, Cₑ curves, which were then used to calculate the maximum carboxylation rate (Vₑₘₐₓ, µmol·m⁻²·s⁻¹) and electron transport rates (Jₘₐₓ, 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 (Nₘₐₓ, mg·g⁻¹) was determined following the micro-Kjeldahl method (UDK-139, Milano, Italy). The leaf N per unit area (Nₐₐₑ𝑎, g·m⁻²) values were then determined as Nₘₐₓ × LMA/1000, while the PNUE (µmol·mol⁻¹·s⁻¹) was calculated as:

\[ \text{PNUE} = \frac{Aᵦ}{Nₐₐₑ𝑎} \times 14 \]   \hspace{1cm} (2)

where PNUEᵦ was calculated by Aᵦ and Nₐₐₑ𝑎, PNUE₁₀₀ was calculated by A₁₀₀ and Nₐₐₑ𝑎, and PNUE₄₀₀ was calculated by A₄₀₀ and Nₐₐₑ𝑎, 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 (Cₜₐₚ₁, mmol·g⁻¹) was then determined by spectrophotometry. The cell wall N concentrations (Qₜₐₚ₁, mg·g⁻¹) were measured following the method proposed by Onoda et al. [55], and the fraction of leaf N allocated to cell walls (Pₜₐₚ₁, g·g⁻¹) was determined as Qₜₐₚ₁/Nₘₐₓ.

4.5. Calculation of N Allocation in the Photosynthetic Apparatus

The N allocation proportions in Rubisco (Pₐᵦ, g·g⁻¹), bioenergetics (Pₐᵦ, g·g⁻¹), and light-harvesting components (Pₐᵦ, g·g⁻¹) were calculated according to Niinemets and Tenhunen [56]:

\[ Pᵦ = \frac{Vₑₘₐₓ}{6.25 \times Vₑₚ \times LMA \times Nₘₐₓ} \]   \hspace{1cm} (3)

\[ Pᵦ = \frac{Jₘₐₓ}{8.06 \times Jₚ \times LMA \times Nₘₐₓ} \]   \hspace{1cm} (4)
\[ P_L = \frac{C_{\text{Chl}}}{C_B \times N_{\text{mass}}} \]  

where \( C_{\text{Chl}} \) is the chlorophyll concentration (mmol·g\(^{-1}\)), \( V_{\text{cr}} \) is the specific activity of Rubisco (\( \mu \text{mol CO}_2 \cdot \text{g}^{-1} \cdot \text{Rubisco} \cdot \text{s}^{-1} \)), \( J_{\text{mc}} \) is the potential rate of photosynthetic electron transport (\( \mu \text{mol electrons} \cdot \mu \text{mol}^{-1} \cdot \text{Cyt f} \cdot \text{s}^{-1} \)), and \( C_B \) is the ratio of leaf chlorophyll to leaf nitrogen during light-harvesting (mmol Chl (g·N\(^{-1}\))). \( V_{\text{cr}} \), \( J_{\text{mc}} \), and \( C_B \) were calculated according to Niinemets and Tenhunen [56]:

\[ V_{\text{cr}}(J_{\text{mc}}) = \frac{e^{(c-\frac{\Delta H_d}{T_k}-\frac{\Delta S}{R}+\Delta H_a)}}{1+e^{(c-\frac{\Delta H_d}{T_k}-\frac{\Delta S}{R}+\Delta H_a)}} \]  

\[ [C_B] = 1.94 + \frac{12.6}{[\text{LMA}]} \]  

where \( R \) is the gas constant (8.314 J·K\(^{-1}\)·mol\(^{-1}\)), \( T_k \) is the leaf temperature (K), \( \Delta H_d \) is the activation energy, \( \Delta H_a \) is the deactivation energy, \( \Delta S \) is the entropy term, and \( c \) is the scaling constant. \([\text{LMA}]\) and \([C_B]\) are the values of LMA and \( C_B \), respectively. The values of \( \Delta H_d, \Delta H_a, \Delta S, \) and \( c \) were 74,000 J·mol\(^{-1}\), 203,000 J·mol\(^{-1}\), 645 J·K\(^{-1}\)·mol\(^{-1}\), and 32.9 when calculating \( \text{V}_{\text{cr}} \), and 24,100 J·mol\(^{-1}\), 564,150 J·mol\(^{-1}\), 1810 J·K\(^{-1}\)·mol\(^{-1}\), and 14.77 when calculating \( J_{\text{mc}} \) [56].

The leaf N allocated to the photosynthetic apparatus (\( P_P, \mu \text{g} \cdot \text{g}^{-1} \)) was calculated as \( P_R + P_B + P_L \) while the leaf N allocated to the other parts (\( P_{\text{Other}}, \mu \text{g} \cdot \text{g}^{-1} \)) was calculated as \( 1-P_P-P_{\text{CW}} \). 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, \( N_{\text{area}}, V_{\text{cmax}}, J_{\text{max}}, \) LCP, and LSP, increased the \( P_L \) in all species; increased \( A_{100, \text{PNUE}_{100}} \) and \( \text{PNUE}_{400} \) in N-fixing trees and decreased \( A_{\text{sat}} \) 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 \( A_{100, \text{PNUE}_{400}}, N_{\text{area}}, V_{\text{cmax}} \) and \( J_{\text{max}} \) 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/plants1002213/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 (\( g_{\text{ms}} \)), 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 (\( Q_{R\text{area}} \)), bioenergetics (\( Q_{B\text{area}} \)), light-harvesting components (\( Q_{L\text{area}} \)), photosynthetic apparatus
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