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Effects of soil nitrogen (N) deficiency on photosynthetic N-use efficiency in N-fixing and non-N-fixing tree seedlings in subtropical China

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Soil nitrogen (N) deficiencies can affect the photosynthetic N-use efficiency (PNUE), mesophyll conductance (g_m), and leaf N allocation. However, lack of information about how these physiological characteristics in N-fixing trees could be affected by soil N deficiency and the difference between N-fixing and non-N-fixing trees. In this study, we chose seedlings of two N-fixing (*Dalbergia odorifera* and *Erythrophleum fordii*) and two non-N-fixing trees (*Castanopsis hystrix* and *Betula alnoides*) as study objects, and we conducted a pot experiment with three levels of soil N treatments (high nitrogen, set as Control; medium nitrogen, MN; and low nitrogen, LN). Our results showed that soil N deficiency significantly decreased the leaf N concentration and photosynthesis ability of the two non-N-fixing trees, but it had less influence on two N-fixing trees. The LN treatment had lower g_m in *D. odorifera* and lower leaf N allocated to Rubisco (P_R), leaf N allocated to bioenergetics (P_B), and g_m in *B. alnoides*, eventually resulting in low PNUE values. Our findings suggested that the *D. odorifera* and *E. fordii* seedlings could grow well in N-deficient soil, and adding N may increase the growth rates of *B. alnoides* and *C. hystrix* seedlings and promote the growth of artificial forests.

Nitrogen (N) is one of the most important biological elements for plants because it is a component of amino acids, proteins, genetic materials, pigments, and other key organic molecules[1–3]. A shortage of N results in a marked decrease in plant photosynthesis in many crops, and the leaf N content has a good correlation with the photosynthetic capacity[4] because up to 75% of leaf N is present in the chloroplasts, with most of it in the photosynthetic apparatus[5]. The photosynthetic N-use efficiency (PNUE, the ratio of the photosynthetic capacity to the leaf N) is frequently used as an important leaf trait for characterizing leaf photosynthetic economics, physiology and strategy[6]. Many researchers have attempted to improve our understanding of the inherent variation in PNUE under soil N deficiency[1,7,8].

Mesophyll conductance to CO_2 and N allocation in the photosynthetic apparatus of a leaf cell are important factors that explain the differences in the PNUE[9,10]. Mesophyll conductance affects the CO_2 contents of the carboxylation site, thus influencing the photosynthetic capacity and PNUE[11,12]. The N used in the photosynthetic apparatus could be divided into three parts, namely Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), bioenergetics, and light-harvesting components[13]. Rubisco is involved in carbon reduction reactions, and it is the most abundant enzyme in photosynthesis[14,15]. N is invested in bioenergetics, limiting the capacity for electron

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transport and photophosphorylation, and N is also invested in the contents of chlorophyll a/b protein complexes associated with photosystems I (PSI) and II (PSII), influencing light harvesting[13].

Furthermore, N is involved in other components of the leaf cell apart from the photosynthetic apparatus. Cell walls play an important role in the mechanical toughness of plant tissues[16] and they accumulate a significant amount of N compounds, at up to 10% of cell wall materials[17,18]. Trade-offs might occur for N allocation to cell walls versus Rubisco[16,18]. However, some researchers have suggested that these trade-offs might only be intraspecific[19] and present in species lacking leaf N[20,21]. N is also involved in carbonic anhydrases and aquaporins[22], with carbonic anhydrases accounting for 0.5–2% of the total soluble leaf protein[23]. These proteins play a role in mesophyll conductance (g_m) by changing the nature of the diffusing molecule[24] and facilitating CO_2 diffusion through membranes[25]. Cell walls could account for >50% of the total resistance and a variable proportion of CO_2 diffusion in the mesophyll, significantly affecting the variation of the g_m[26].

Soil N deficiency could affect the leaf N content, photosynthesis, PNUE, g_m, and leaf N allocation in many species. Many researchers have found that the A_{max}' (light-saturated net CO_2 assimilation rate) and N_{area} (leaf N concentration per area) were decreased in N-deficient soil[1,11,12,27]. However, the changes in the PNUEs of different species under soil N deficiency were uncertain; the PNUE values increased[1,28], decreased[27,29], or showed no marked change[7] along the N addition gradients. The g_m was also usually decreased with soil N deficiency[11,12,30]. A lower soil N content could result in smaller chloroplasts[31], leading to a decreased chloroplast surface area facing the intercellular air spaces[32] and an increased distance between the intercellular space and the catalytic site of Rubisco[12]. Adding N to the soil could improve the leaf N content in the Rubisco, bioenergetics, and light-harvesting components[7,33–35], but the changes in the proportion of N in these components were unclear[1,11].

Dalbergia odorifera, *Erythrophleum fordii*, *Betula alnoides*, and *Castanopsis hystrix* are suitable species for forestation in southern subtropical China, and they have high economic value[36–39]. *D. odorifera* and *E. fordii* are N-fixing trees and *B. alnoides* and *C. hystrix* are non-N-fixing trees. Recent studies have found that Leguminosae trees with a higher N_{area} did not have a higher A_{max}' than other non-N-fixing species[40,41]. One possible explanation was that the Leguminosae tree species might allocate less N to Rubisco and bioenergetics than nonlegumes, as shown in previous studies[40–42]. However, there is a lack of information on how the leaf N content, leaf N allocation, mesophyll conductance to CO_2 and PNUE of N-fixing trees could be affected by a low soil N content[43].

In this study, we investigated the PNUE, photosynthesis, leaf N allocation and mesophyll conductance to CO_2 in *D. odorifera*, *E. fordii*, *B. alnoides* and *C. hystrix* seedling leaves that were exposed to different soil N treatments. The objectives of our study were to 1. understand the effects of soil N deficiency on the PNUE, photosynthesis, leaf N allocation, and g_m of these trees; and 2. explore the different plant metabolism response modes between N-fixing and non-N-fixing woody species under soil N deficiency. We assumed that the photosynthetic capacity, PNUE and g_m of these trees might be reduced under a soil N deficiency, but the N-fixing trees were less affected.

Results

Effects of soil N treatments on A_{max}', N_{area}, leaf N content per mass (N_{mass}), leaf mass per area (LMA), and PNUE.

The seedling leaf N_{area} and N_{mass} values were significant higher in *D. odorifera* and *E. fordii* than they were in *C. hystrix* and *B. alnoides* under all the soil N treatments, and the PNUE was significantly lower in *D. odorifera* and *E. fordii* than it was in *C. hystrix* and *B. alnoides* (Fig. 1). The higher N_{area} and N_{mass} were direct causes of the lower PNUE in the two N-fixing tree seedlings. A significant decrease was observed in the A_{max}', N_{mass}, and PNUE in the *D. odorifera*, *C. hystrix*, and *B. alnoides* seedling leaves under the low N treatments when compared with the high N conditions, and a significant decrease was observed in the N_{area} in the *C. hystrix* and *B. alnoides* seedling leaves (Fig. 1). The A_{max}', N_{mass}, N_{area}, LMA and PNUE of *E. fordii* were less affected by the soil N deficiency (for more details, see Supplementary Table S1). The A_{max}' had a significantly positive correlation with the N_{area} in these tree seedling leaves ($P < 0.001$; Fig. 2), which showed the importance of N on photosynthesis.

Effects of soil N treatments on stomatal conductance (g_s), g_m, CO_2 concentration in substomatal cavities (C_i), CO_2 concentration at the carboxylation site (C_c), and C_i–C_c.

The g_s, g_m, C_i, and C_c in the *B. alnoides* seedling leaves were higher than they were in the other three species under any soil N treatments, except for the g_m under Control, and the C_i–C_c of *B. alnoides* seedling leaves was lower than that of the other three species, except under Control (Fig. 3). This finding may be related to the fact that *B. alnoides* is a deciduous tree. The g_m and C_c of *D. odorifera* were significantly lower under LN than Control (−55.5% and −9.7%, respectively), but the C_i–C_c was significantly higher in the LN treatment than under Control (+56.3%). No significant changes were observed in the g_s, g_m, C_i, C_c, and C_i–C_c between Control and LN for *E. fordii*. The g_s and g_m of *C. hystrix* were significantly lower under LN than Control (−24.3% and −44.4%, respectively), but the C_i and C_i–C_c were significantly higher under LN than Control (+5.6% and +14.8%, respectively). The g_m of *B. alnoides* was significantly lower under LN than Control (−38.0%), but the C_i and C_c were significantly higher under LN than Control (+14.2% and +21.7% Fig. 3). Different species have different response characteristics to the soil N conditions (More details see Supplementary Table S2).

Effects of soil N treatments on maximum carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}).

The V_{cmax} values of *E. fordii* were significantly higher than those of the other three tree species under the Control and MN treatments. The J_{max} values of *E. fordii* were higher than those of the other three tree species only under MN treatment (Fig. 4). No significant difference was observed in the V_{cmax} and J_{max} of the *D. odorifera* and *E. fordii* seedling leaves between the different N treatments. The V_{cmax} and J_{max} of *C. hystrix* in the LN treatments were 30.5 and 38.1% significantly lower than those obtained from the Control treatment, and the V_{cmax} and J_{max} of *B. alnoides* were 43.7 and 43.7% significantly lower than those obtained under the Control
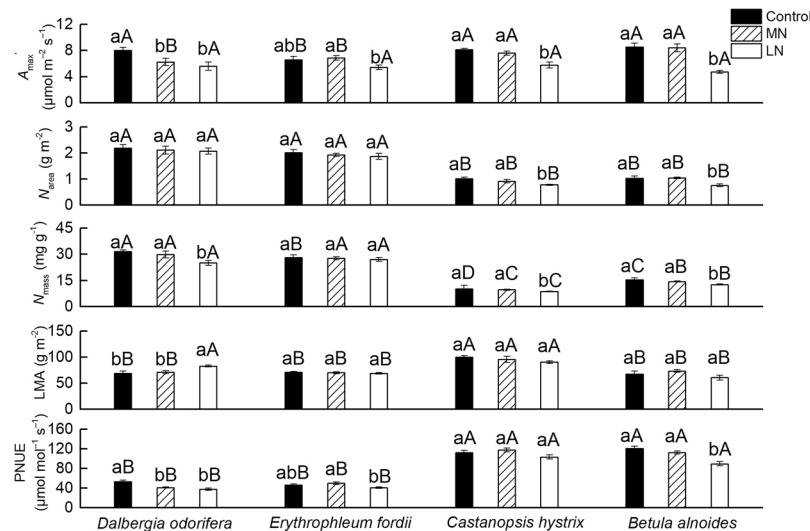
Figure 1. Light-saturated photosynthesis (A_{max}'), leaf N content per area (N_{area}), leaf N content per mass (N_{mass}), leaf mass per area (LMA), and photosynthetic-N use efficiency (PNUE) in the seedling leaves from the four studied tree species after exposure to different soil nitrogen (N) treatments. The statistical differences between each characteristic of the different species under three N treatments (mean ± SE) are the results of a one-way analysis of variance (ANOVA) (n = 7). The lowercase letters indicate significant differences at the 0.05 level between different N treatments, and the uppercase letters indicate significant differences at the 0.05 level between the species under the same N treatment. Control, high N; MN, medium N; and LN, low N.
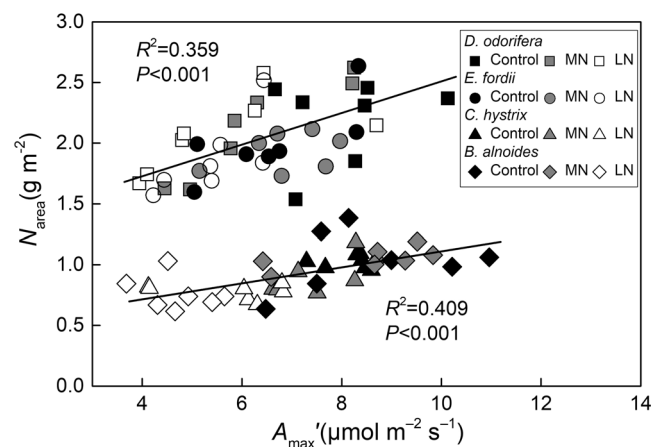

Figure 2. Regression analysis of the leaf nitrogen (N) concentration per area (N_{area}) and light-saturated photosynthesis (A_{max}') of the seedling leaves from the four studied tree species. The determination coefficients (R^2) and P-values are shown. The lines fitted for N-fixing and non-N-fixing trees are significantly different ($P < 0.05$) according to the result of a one-way analysis of covariance (ANCOVA) with A_{max}' as a dependent variable, whether it could fix N as a fixed factor, and N_{area} as a covariate

treatment (Fig. 4). The V_{cmax} and J_{max} of the two N-fixing tree seedlings were less affected by the soil N deficiency (More details see Supplementary Table S3).

Effects of soil N treatments on leaf N allocation proportion of the Rubisco (P_R), bioenergetics (P_B), light-harvesting components (P_L), photosynthetic system (P_P), cell wall (P_{CW}), and other parts (P_{Other}). The P_R, P_B, P_P, and P_{CW} values of *C. hystrix* were higher than the corresponding values obtained for the other three species under any soil N treatments (Fig. 5). No significant change was observed in the P_R, P_B, P_L, P_P, and P_{Other} values of *D. odorifera* under any N treatment; the P_{CW} of *D. odorifera* in the LN treatment was 71.4% higher than that in the Control treatment. No significant change was observed in the P_R, P_B, P_P, P_{CW}, and P_{Other} values of *E. fordii* under any N treatments, and the P_L of *E. fordii* was 33.3% higher in the LN treatment than in the Control treatment. The LN treatment significantly decreased the P_B (−28.6%) and P_{Other} (−41.2%), and it increased the P_{CW} (+66.7%) of *C. hystrix* when compared with the corresponding values obtained under the Control conditions. The LN treatment significantly decreased the P_R (−38.5%), P_B (−42.9%), P_L (−33.3%),
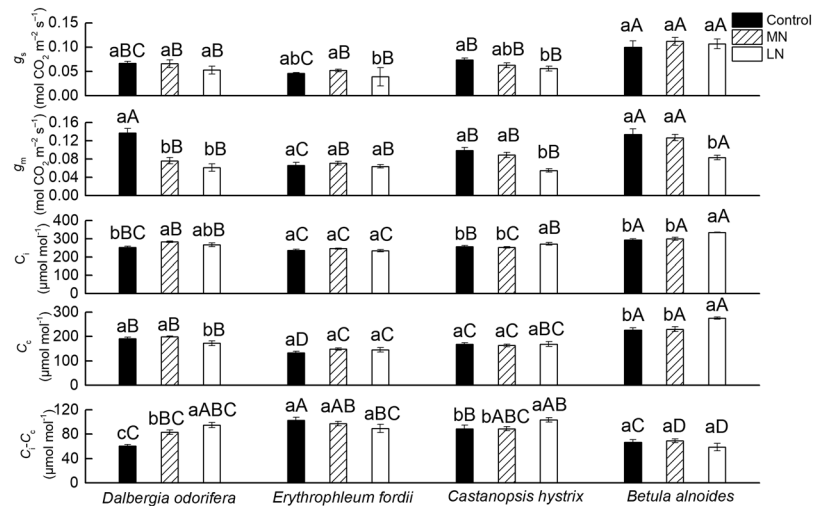
Figure 3. Stomatal conductance (g_s), mesophyll conductance (g_m), CO_2 concentration in substomatal cavities (C_i), CO_2 concentration at the carboxylation site (C_c), and C_i–C_c in the seedling leaves of the four tree species after exposure to different soil nitrogen (N) treatments. The statistical differences between each characteristic of the different species under the three N treatments (mean ± SE) are the results of a one-way analysis of variance (ANOVA) (n = 7). The CO_2 conductance data were measured under light saturated conditions, and the leaf chamber CO_2 concentration was 380 μmol mol^{-1}. The lowercase letters indicate significant differences at the 0.05 level between different N treatments, and the uppercase letters indicate significant differences at the 0.05 level between the species under the same N treatment. Control, high N; MN, medium N; and LN, low N.
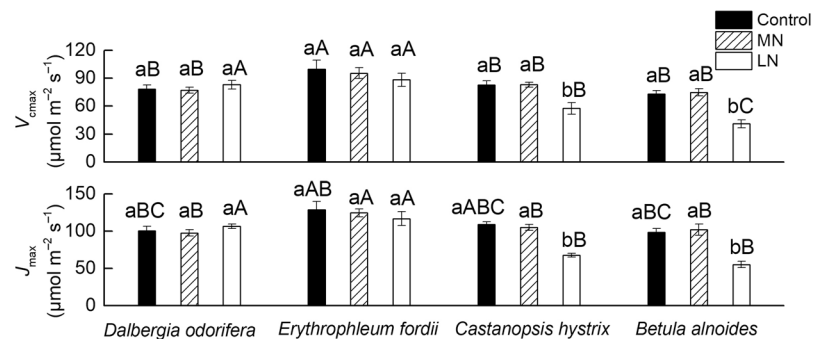

Figure 4. Maximum carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) in the seedling leaves of the four tree species after exposure to different soil nitrogen (N) treatments. The statistical differences between each characteristic of the different species under the three N treatments (mean ± SE) are the results of a one-way analysis of variance (ANOVA) (n = 7). The lowercase letters indicate significant differences at the 0.05 level between different N treatments, and the uppercase letters indicate significant differences at the 0.05 level between the species under the same N treatment. Control, high N; MN, medium N; and LN, low N.

and P_P (−34.1%), and it increased the P_{CW} (+33.3%) of *B. alnoides* (Fig. 5). Overall, the N allocation of the two N-fixing tree seedlings changed little, but there was a large change for the two non-N-fixing tree seedlings (for more details, see Supplementary Table S4).

Relationships between parameters. The P_R, P_B, and P_P values showed a significant positive correlation with the PNUE in these tree seedling leaves ($P < 0.01$; Fig. 6a,b,d). No significant correlation was observed between the P_L and PNUE in these trees (Fig. 6c). Significant positive relationships were observed between the g_m and PNUE in these tree seedling leaves ($P \leq 0.001$; Fig. 7). The changes in P_R, P_B, and g_m were important physiological factors influencing the PNUE.

Significant negative relationships were found between the P_{CW} and g_m in *D. odorifera*, *E. fordii*, and *C. hystrix* ($P < 0.001$; Fig. 8a,c,d); no significant relationships were observed in *B. alnoides* (Fig. 8b). Significant positive relationships were observed between P_{CW} and C_i–C_c in *D. odorifera* ($P = 0.002$; Fig. 9a). Significant negative relationships were noted between the P_{CW} and C_i–C_c in *E. fordii* ($P = 0.004$; Fig. 9b), and no significant relationships were observed in *C. hystrix* and *B. alnoides* (Fig. 9c,d). The improved P_{CW} in *D. odorifera* might relate to its thicker cell walls, but in *E. fordii*, it might relate to the higher cell wall density.
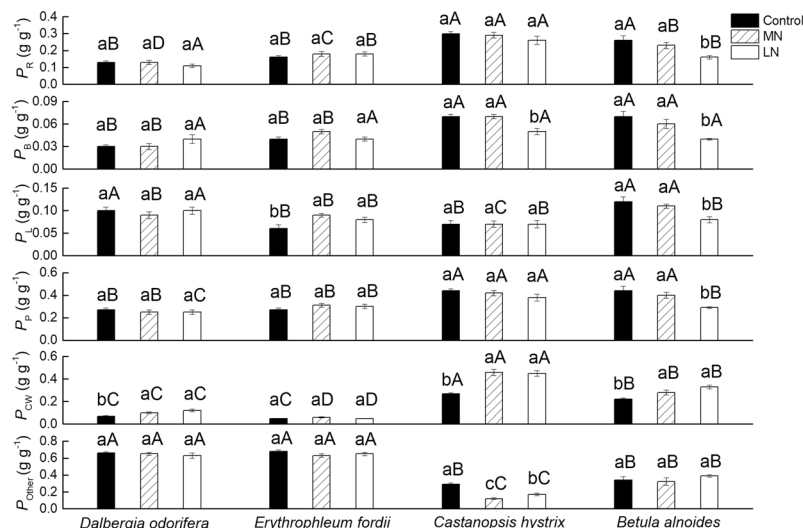
Figure 5. Nitrogen (N) allocation proportion of the Rubisco (P_R), bioenergetics (P_B), light-harvesting components (P_L), photosynthetic system (P_P), cell wall (P_{CW}), and other parts (P_{Other}) in the seedling leaves of the four tree species following exposure to different soil N treatments. The statistical differences between each characteristic of the different species under three N treatments (mean $\pm$ SE) are the results of a one-way analysis of variance (ANOVA) (n = 7). The lowercase letters indicate significant differences at the 0.05 level between different N treatments, and the uppercase letters indicate significant differences at the 0.05 level between the species under the same N treatment. Control, high N; MN, medium N; and LN, low N.
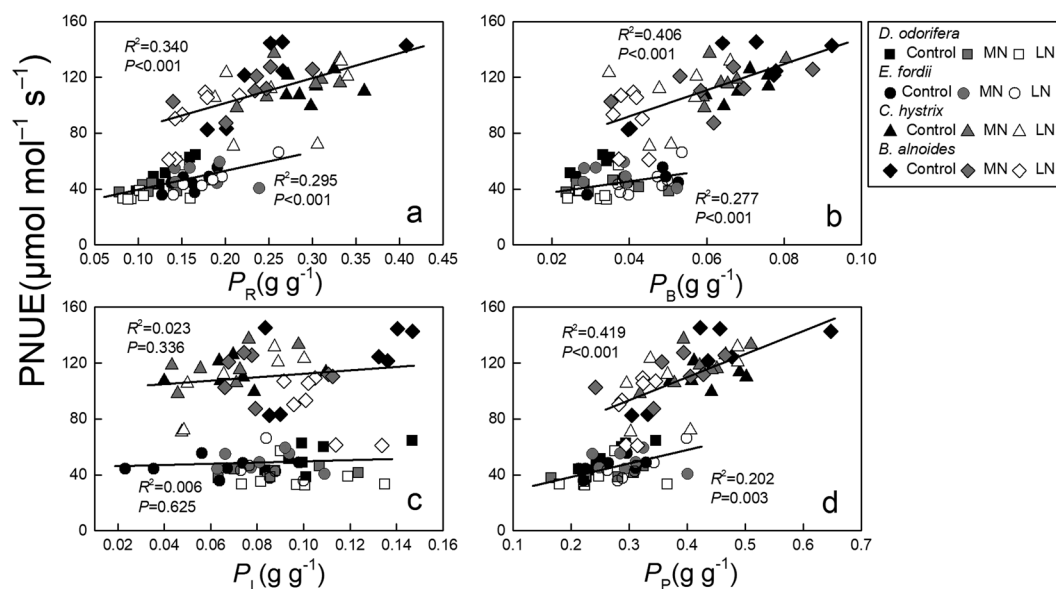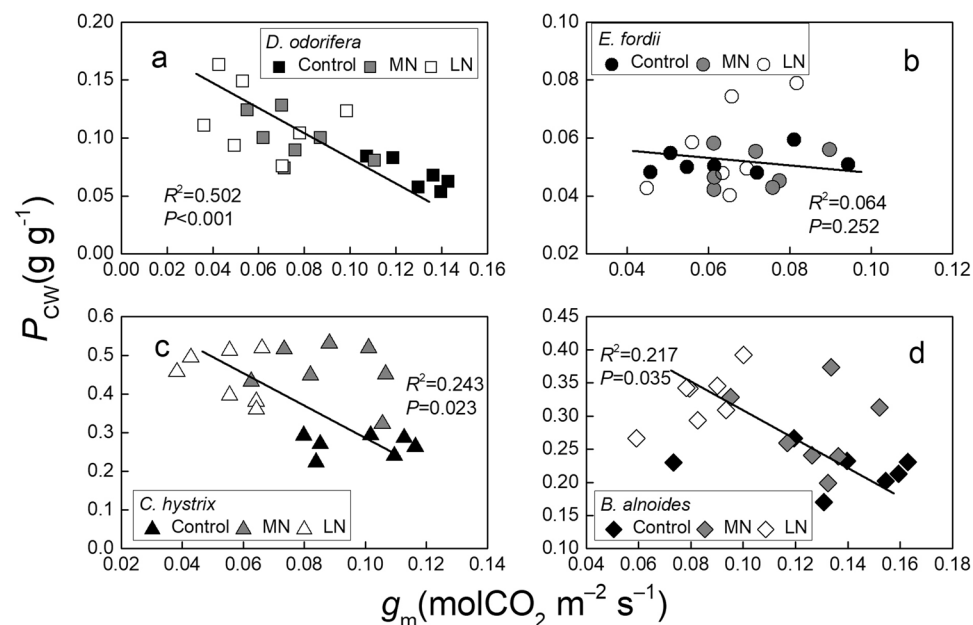

Figure 6. Regression analysis of nitrogen (N) allocation proportions in the photosynthetic system (P_P), light-harvesting components (P_L), Rubisco (P_R), and bioenergetics (P_B) with the photosynthetic N use efficiency (PNUE) in the seedling leaves of the four tree species after exposure to different soil N treatments. The determination coefficients (R^2) and P-values are shown. The lines fitted for the N-fixing and non-N-fixing trees are significantly different ($P < 0.05$) according to the results of a one-way analysis of covariance (ANCOVA) with the PNUE as a dependent variable, whether it could fix nitrogen as a fixed factor, and P_P, P_R, P_B, and P_L as covariates.

No significant relationships were observed between the P_{CW} and P_R in *D. odorifera* and *E. fordii*, but significant negative relationships were observed in *B. alnoides* and *C. hystrix* ($P \leq 0.002$). The cell wall N might influence the variation in N in the Rubisco, thus influencing the photosynthetic capacity in these two non-N-fixing tree seedlings. A regression analysis of the P_{CW} with P_R in the *B. alnoides* seedling leaves under the LN treatment was obtained within the shaded zone. Most Control and MN treatment parameters for *B. alnoides* and *C. hystrix*
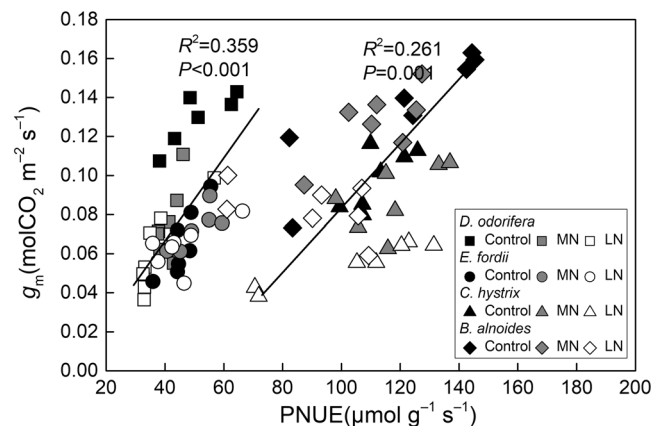
Figure 7. Regression analysis of g_m (mesophyll conductance) with the PNUE (photosynthetic nitrogen [N] use efficiency) in the seedling leaves of four tree species following exposure to different soil N treatments. The determination coefficients (R^2) and P-values are shown. The lines fitted for the N-fixing and non-N-fixing trees are significantly different ($P < 0.05$) according to the results of a one-way analysis of covariance (ANCOVA) with the PNUE as a dependent variable, whether it could fix nitrogen as a fixed factor, and g_m as a covariate.
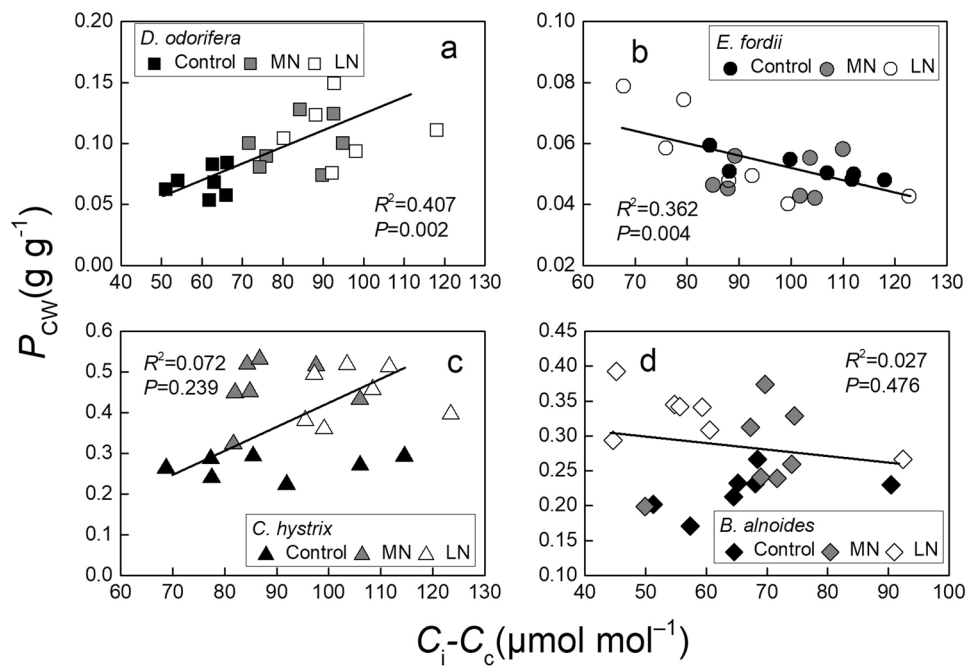

Figure 8. Regression analysis of the g_m (mesophyll conductance) with the P_{CW} (nitrogen [N] allocation proportion of cell wall) in the seedling leaves of four tree species under exposure to different soil N treatments. The determination coefficients (R^2) and P-values are shown.

were in the shaded zone, and *D. odorifera* and *E. fordii* were found under the shaded zone (Fig. 10). Low soil N increased the competition between the Rubisco and cell wall N.

Discussion

The leaf N contents of two non-N-fixing tree seedlings, *B. alnoides* and *C. hystrix*, were significantly affected by the soil N content (Fig. 1, Supplementary Table S5), which was consistent with previously published studies[1,11,12,27]. However, the leaf N content of *E. fordii* was not significantly affected by the soil N content. This finding might be due to its strong N fixation capacity and its maintenance of the N content stability in leaves. Different N treatments significantly affected the N_{mass} of *D. odorifera* seedling leaves, but the N_{area} of *D. odorifera* was not affected by the soil N content (Fig. 1). Because the N_{area} was influenced by the N_{mass} and LMA, the LMA of *D. odorifera* changed with the soil N gradient (Fig. 1); the maintenance of the N_{area} at a steady state showed good leaf morphological plasticity. The low soil N content decreased the A_{max}' in *D. odorifera*, *B. alnoides*, and *C. hystrix* (Fig. 1) for different reasons. In *D. odorifera*, the low soil N content primarily decreased its C_c (Fig. 3), which is one of the important raw materials for photosynthesis[44], and the CO_2 partial pressure is important for Rubisco activity because O_2 is a competitive inhibitor of the C assimilatory reaction of Rubisco for promoting the Rubisco

Figure 9. Regression analysis of $C_i–C_c$ (the difference between the CO_2 concentration in the substomatal cavities [C_i] and carboxylation site ([C_c]) with the P_{CW} (nitrogen [N] allocation proportion of cell wall) in the seedling leaves of the four tree species under exposure to different soil N treatments. The determination coefficients (R^2) and P-values are shown.
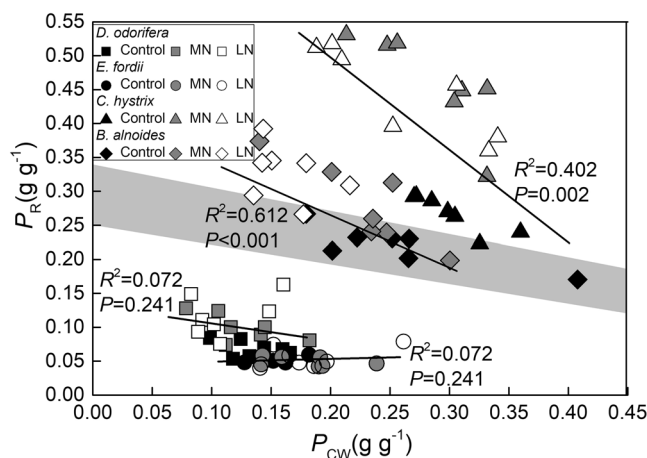
Figure 10. Regression analysis of the P_R (nitrogen [N] allocation proportion of Rubisco) and P_{CW} (N allocation proportion of cell wall) in the seedling leaves of the four tree species after exposure to different soil N treatments. The determination coefficients (R^2) and P-values are shown. The shaded zone represents the distribution area of the P_{CW} and P_R in the presence of the trade-off[20].

oxidation reaction[12]. For the two non-N-fixing tree seedlings, the low soil N content decreased their V_{cmax} and J_{max} values (Fig. 4), which are the key biochemical parameters of the photosynthetic capacity[14,45].

The fraction of the total leaf N allocated to the photosynthetic apparatus[46], especially to Rubisco and bioenergetics, could influence the variation in the PNUE[1,3,16]. The g_m could also influence the PNUE[32,47] by affecting the C_c[11,12]. In this study, the P_R and P_B showed a significant positive correlation with the PNUE ($P < 0.001$, Fig. 6a,b), and the g_m significantly affected the PNUE in the seedling leaves of the four studied tree species (Fig. 7), although the effect of the g_m on the PNUE was different among the species[48]. The LN treatment significantly decreased the g_m in *D. odorifera* and the P_R, P_B, and g_m in *B. alnoides* (Figs 1 and 5), leading to lower PNUEs in the LN treatment. It has been reported that low soil N could decrease the g_m[12,49] and N allocation[3,29]. However, Chen *et al.* (2014) found an improvement in the P_R and P_B of female *Populus cathayana* with improved soil N, but the P_R and P_B of the males decreased[1]. Warren (2004) also found that an improvement in the soil N could decrease the P_R in *Eucalyptus globulus*. Some plants might have a different strategy for adapting to the soil N[11].

The PNUEs of the two non-N-fixing tree seedlings were significantly higher than those of the two N-fixing tree seedlings under any soil treatment (Fig. 1, Supplementary Table S5), which was first attributed to their relatively low N_{area} and N_{mass} (Figs 1, 2, Table S7). The N-fixing species, which could gain N from air through legume bacteria, usually have a higher leaf N content than the non-N-fixing species[43,50]. High P_R and P_B (Fig. 4, Supplementary Table S5) were the primary biochemical factors leading to their higher PNUEs. These results were also consistent with other studies[40–42]. The leaves are the photosynthetic organs of plants, and plants have roughly two survival strategies, namely, quick investment-return and slow investment-return[51]. Two N-fixing trees might belong to the slow investment-return species and use a different strategy to use N, such as compensation for their low productivity through a long leaf lifespan[18] and storing N for other processes, such as reproduction[1]. Two N-fixing tree seedlings might grow well in N-deficient soil, and applying N could increase the growth rates of the two non-N-fixing tree seedlings and promote the growth of artificial forests. Of course, some N-fixing trees have the same N utilization and distribution strategies as non-N-fixing trees, such as *Acacia mangium*[52].

A decrease was observed in the g_m of the *D. odorifera*, *C. hystrix*, and *B. alnoides* seedlings under the LN treatment, but the reasons for this decline were different. The changes in A_{max}' or $C_i–C_c$ could influence the value of g_m. In these tree seedlings, the A_{max}' decreased under the LN treatment, but the changes in the $C_i–C_c$ were different. *D. odorifera* and *C. hystrix* showed an increased $C_i–C_c$ in the LN treatment, but *B. alnoides* showed no change in its $C_i–C_c$ value (Fig. 3). After entering through the stomata, the CO_2 diffuses through air spaces, cell walls, cytosol, and chloroplast envelopes and finally reaches the chloroplast stroma, where it is fixed by Rubisco[26,53]. Generally, cell walls account for >50% of the total cell CO_2 diffusion resistance and a variable proportion of respiration[26]. *D. odorifera*, *C. hystrix*, and *B. alnoides* showed improved P_{CW} values in the LN treatment (Fig. 5). Mu *et al.* (2016) also found an increase in the P_{CW} of maize growing under low-N stress[29]. *D. odorifera* showed no significant reduction in its N_{area} in the LN treatment, and thus there was an increase in the N contents in the cell wall (Q_{CWarea}) of *D. odorifera* (+62.4%, Supplementary Table S6). The percentage of N in the cell wall showed a slight variation in the same species[16]. An improvement in the N_{CW} of *D. odorifera* under the LN treatment indicates the high dry mass of the cell wall, resulting in improved LMA[16,54], and it might improve the thickness of the cell wall, thereby improving its $C_i–C_c$ value[16]. However, *B. alnoides* and *C. hystrix* showed a reduction in their N_{area} values in the LN treatment, leading to a smaller change in the Q_{CWarea} (+5.9% and +29.6%, respectively, Supplementary Table S6). Thus, there were no significant changes in their LMA and $C_i–C_c$ values. An improvement in the P_{CW} of *D. odorifera* therefore significantly decreased its $C_i–C_c$ and g_m, and no significant relationship was observed between the P_{CW} and $C_i–C_c$ in *B. alnoides* and *C. hystrix* (Figs 8, 9).

The P_{CW} did not influence the variation in the $C_i–C_c$, but it showed a significant negative correlation with the g_m in two non-N-fixing trees (Fig. 8). The cell wall N might influence the N variation in Rubisco, thus influencing the V_{cmax} and A_{max}' values. Onoda *et al.* (2004) and Takashima *et al.* (2004) observed a trade-off between the cell wall and Rubisco N in *Polygonum cuspidatum* and in *Quercus* species, respectively[16,18]. Zhang *et al.* (2016) also found this trade-off in *Mikania micrantha* and *Chromolaena odorata*[28]. Hikosaka and Shigeno (2009) considered this relationship unlikely to hold as a general rule; the allocation of N to the cell walls did not explain the variation in the Rubisco[19]. Harrison *et al.* (2009) and Qing *et al.* (2012) believed that this relationship might occur during N leaf deficiency[20,21]. *B. alnoides* and *C. hystrix* showed high P_R and P_{CW} values (Fig. 5), and a part of the distribution area in or on the shade zone (Fig. 10; for a further explanation of the shade zone, please see Harrison *et al.*[20]) indicates that the free amino acid, NO_3^-, and NH_4^+ in the leaves were not sufficient (appearing as low P_{Other}) to supply N to both Rubisco and the cell wall[20], which explained the existence of a trade-off between the P_R and P_{CW} (Fig. 10). It is important to note that the regression analysis of the P_{CW} with the P_R in the *B. alnoides* seedling leaves exposed to the LN treatment was found in the shaded zone; most Control and MN treatments of *B. alnoides* and *C. hystrix* were in the shaded zone (Fig. 10). Low soil N increased the competition between the Rubisco and cell wall N.

The two non-N-fixing tree seedling leaves showed improved P_{CW}, and the *D. odorifera* seedling leaves improved both the LMA and P_{CW} values under the LN treatment (Figs 1, 5). The LMA is the product of leaf thickness and density, and it is positively correlated with leaf toughness[55] and is a fundamental defensive trait of plants[56,57]. The cell wall also directly functions as a defense organ[58]. We observed that the N_{mass} values of these trees were affected by the soil N content (Fig. 1). Low nutrient availability limits the growth rate of seedlings and might damage the seedlings during the growing season[59]. The LN treatment might pose a threat to these seedling leaves; thus, plants need to have tougher leaves to survive[16], as shown by the relatively high P_{CW} and LMA in *D. odorifera* seedling leaves and high P_{CW} in the *B. alnoides* and *C. hystrix* seedling leaves. Givnish (2002) hypothesized that soil fertility is the primary driver of the leaf lifespan[60], and a high LMA leads to a long leaf lifespan[51]. Therefore, an improvement in the LMA might also increase the leaf lifespan of *D. odorifera* seedling leaves, ultimately maximizing the carbon assimilation per unit of nutrient over the lifespan of the leaf[61–63]. Different species have different response characteristics to the soil N conditions.

To understand the changes in the various parameters under low soil N in the four species, we drew a process diagram (Fig. 11). Generally, we found fewer parameter changes in the two N-fixing tree seedlings and more parameter changes in the two non-N-fixing tree seedlings. The physiological and ecological characteristics of these two N-fixing tree seedlings are more stable, and these two N-fixing tree seedlings could be good tree species for afforestation in N-poor areas. We also performed Between-Subjects effects tests on the tree varieties and N treatments for the variables in the four species (Supplementary Table S8). In general, varieties of the trees were more important than the N treatment interaction effect, but the N treatment interaction effect was more important in influencing the A_{max}' and g_m. More trees and more variables must be further studied.
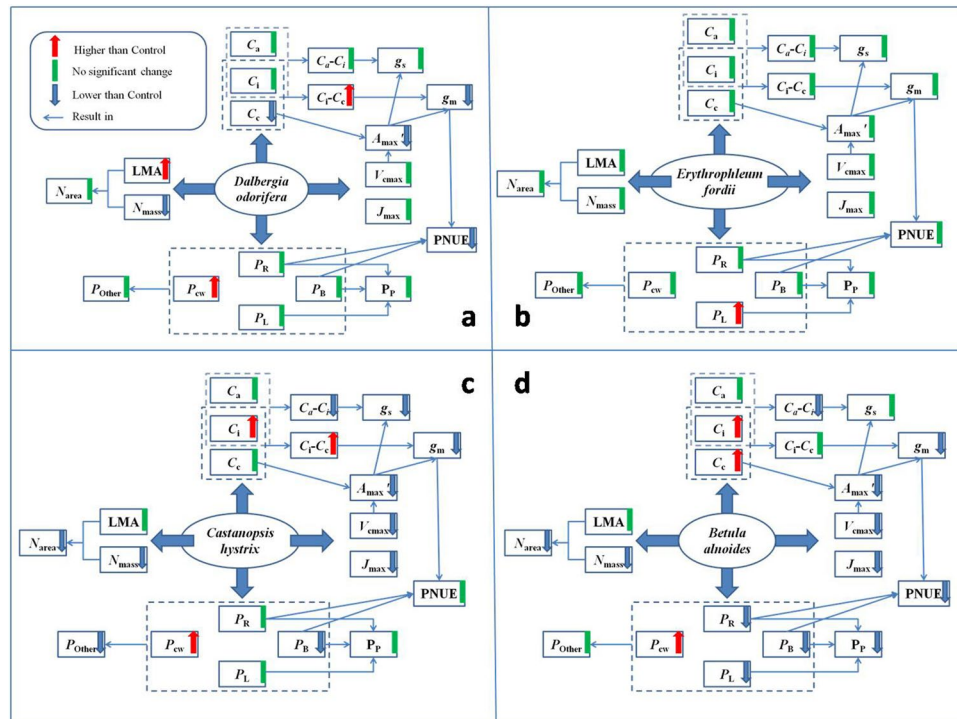
Figure 11. Changes in the variables under low soil nitrogen in four species.

Conclusions

In revisiting our questions, we concluded that (1) soil N deficiency significantly decreased the leaf N concentration and photosynthesis ability in two non-N-fixing trees, but it had less influence on these indices in the two N-fixing trees. (2) The LN treatment had a lower g_m in *D. odorifera* and had lower P_R, P_B and g_m in *B. alnoides*, eventually resulting in their low PNUE values. (3) *D. odorifera*, *B. alnoides*, and *C. hystrix* seedling leaves showed improved P_{CW} and (or) LMA to adapt to a low-N soil environment. These findings were important for understanding the ecophysiological changes in plants under low soil N conditions. Our findings suggested that the two N-fixing tree seedlings could grow well in N-deficient soil, and they could be good tree species for the afforestation of N-poor areas. Adding N may increase the growth rates for the two non-N-fixing tree seedlings and promote the growth of artificial forests. Because these species live in the same area, it is possible to mix non-N-fixing with N-fixing tree seedlings for afforestation, and mix N-fixing trees in non-N-fixing pure forest after intermediate cutting or selective cutting in non-N-fixing pure forest, which could improve soil N utilization efficiency.

Materials and Methods

Study area and plant material.   This study was performed in the Experimental Center of Tropical Forestry ($22°7'19''$–$22°7'22''$N, $106°44'40''$–$106°44'44''$E) at the Chinese Academy of Forestry located in Pingxiang, Guangxi Province, China. This location has a subtropical monsoon climate with distinct dry and wet periods, and the mean annual temperature is 21 °C. The mean monthly minimum and maximum temperatures are 12.1 and 26.3 °C, respectively. The mean annual precipitation, which takes place primarily from April to September, is 1400 mm. The active accumulated temperature above 10 °C is 6000–7600 °C. The total annual sunshine duration is 1419 hours[64,65].

The seeds of *D. odorifera*, *E. fordii*, and *C. hystrix* were collected separately from the mother trees, and the *B. alnoides* seedlings were somaclones. The *D. odorifera*, *E. fordii*, and *C. hystrix* seeds were germinated in a seedbed in February of 2014, and *B. alnoides* was budding at the same time. When the seedlings were approximately 20 cm tall, 90 similarly sized seedlings per species were transplanted to pots (5.4 L, filled with washed river sand) and established in an open site at the Experimental Center of Tropical Forestry in March, 2014.

From April to June, three levels of soil N treatments were set up (Hyponex M. Scott & Sons, Marysville, OH, USA, dissolved in the water from the aqueous solution preparation). Nitrogen fertilizer was applied ten times, once per week. A total of 0.2 (low nitrogen, LN), 0.7 (medium nitrogen, MN), and 1.5 g (set as Control) of available N were applied per pot, with each treatment including 30 seedlings per species. The forms of N that were applied in this study were mixed N (both NH_4^+ and NO_3^-), and the NH_4^+ to NO_3^- ratio was 1:1. We chose these forms because we used washed river sand as a culture substrate with a pH value of approximately 7, and only using NH_4^+ or NO_3^- might cause the soil to become more acid or alkaline, respectively, affecting the plant growth. Wu *et al.* (2012) found that the proper amounts of N applications for *D. odorifera* seedlings were 1.74–2.15 g N per pot[66]. Li *et al.* (2003) found that the appropriate N applications for *E. fordii* seedlings were approximately 1.39–1.86 g N per pot[67]. Although the purpose of this research is to understand the effects of soil N deficiency on plant metabolism, we also want to explore the plant physiological process from a comparatively

sufficient to a lack of soil N, because non-N-fixing woody species might be more sensitive to changes in the soil N gradient, and the different ecophysiological processes between a comparatively sufficient to a lack of soil N could help us to understand the effects of soil N deficiency on plant metabolism. Therefore, we set up a high N treatment as Control. The seedlings in each treatment were watered every day to keep the soil moist. Natural light (100% light in the field) was used for illumination.

Determination of gas exchange parameters. Fifteen days after the last N fertilization, on sunny days from 9:00 to 11:00 h in July and August of 2014, seven healthy and similarly sized seedlings were chosen per treatment, per species. One healthy and mature leaf per seedling that was exposed to the sun was chosen to determine the gas exchange parameters. These parameters were determined with a LiCor-6400 portable photosynthesis system (LI-COR, Lincoln Nebraska, USA), and the photosynthetic response to the photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) and C_i (μmol mol^{-1}) were determined. Under 380 μmol mol^{-1} of leaf chamber CO_2 concentration (the average air CO_2 concentration in the day time), the photosynthetic rates were measured under 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}. Under a saturated PPFD, the photosynthetic rates were detected using the same leaf-under leaf chamber CO_2 concentrations of 380, 200, 150, 100, 80, 50, 380, 600, 800, 1000, 1200, 1500, 1800 and 2000 μmol mol^{-1}[28,47]. We started at a 380 μmol mol^{-1} concentration because this is the average air CO_2 concentration during the day time that could reduce the plant activation time[28]. The relative humidity of the air in the leaf chamber was maintained at 60–70%, and the leaf temperature was set to 30 °C. The values for the following data or parameters were determined: the net photosynthetic rate (A_n, μmol m^{-2} s^{-1}), A_{max}' (μmol m^{-2} s^{-1}), g_s (mol CO_2 m^{-2} s^{-1}), and dark respiration (R_n, μmol m^{-2} s^{-1}). The light- and CO_2-saturated net CO_2 assimilation rate (A_{max}, μmol m^{-2} s^{-1}) was calculated according to Farquhar *et al.*[14]. The relative humidity of the air in the leaf chamber was maintained at 60–70%, and the leaf temperature was set to 30 °C.

Determination of the chlorophyll fluorescence, mesophyll conductance, V_{cmax}, and J_{max}. The fluorescence yield was measured using a LiCor-6400 leaf chamber fluorometer (6400–40, LI-COR, Lincoln, Nebraska, USA) on the same leaf and with seven repetitions for each species. The chamber relative humidity and leaf temperature were controlled under the same conditions as described in the gas exchange parameters. The leaf chamber CO_2 concentration was set to 380 μmol mol^{-1}. The fluorescence yield ($\Delta F/F_m'$) was subsequently determined. The photosynthetic electron transport rate (J_f, μmol m^{-2} s^{-1}) was calculated according to the equation described by Loreto *et al.*[68] as follows:

$$J_f = PPFD \times \frac{\Delta F}{F_m'} \times Leafreflu \times PARDistPhotosys$$

(1)

where *PPFD* is the photosynthetic photon flux density; *Leafreflu* is the leaf absorptance valued between 0.82–0.85[69] (we used 0.85 in this paper); and *PARDistPhotosys* is the fraction of quanta absorbed by photosystem II (valued as 0.5)[68]. The mesophyll conductance (g_m, mol CO_2 m^{-2} s^{-1}) was calculated using three different methods to obtain a more accurate value. The variable *J* method was described by Harley *et al.*[70], and it has been commonly used in recent years[71–73]. The *A*-C_i curve fitting method was described by Ethier and Livingston[74], and Sharkey *et al.*[75] developed a software package to estimate the g_m and other parameters based on this method. The exhaustive dual optimization (EDO) method described by Gu *et al.*[76] could estimate up to eight parameters, including the g_m, and we obtained an automated analysis of *A*-C_i curves through a website (http://www.leafweb.org) by uploading our data to determine the value of the g_m. Subsequently, the g_m calculated by these three methods was used to calculate C_c (μmol mol^{-1}) as follows:

$$C_C = C_i - \frac{A_{max}'}{g_m}$$

(2)

The C_c and g_m calculated using the three methods are shown in Supplementary Table S9. The mean value of C_c was used to fit the A_n-C_c curve, followed by the calculation of V_{cmax} (μmol m^{-2} s^{-1}) according to Farquhar *et al.*[14] and the J_{max} (μmol m^{-2} s^{-1}) according to Loustau *et al.*[77]. The running fitting model used in the *in vivo* Rubisco kinetics parameters (i.e., K_o, K_c, and their activation energy) was measured according to Niinemets and Tenhunen[13].

Determination of additional leaf traits. After the gas exchange parameters and fluorescence yield were determined, the leaf samples and nearby leaves (30–50 leaves per seedling in total, the sizes of which were similar to those of the leaves used to determine the photosynthesis, healthy and mature characteristics, and sun-exposed parameters) were collected from each pot. The surface areas of 10–20 leaves were measured using a scanner (Perfection v700 Photo, Epson, Nagano-ken, Japan). The leaves were subsequently oven-dried to a constant weight at 80 °C for 48 h. The dry weight was measured using an analytic balance, and then the LMA (g m^{-2}) was calculated. The dried leaf samples were ground into dry flour. The organic carbon (C) concentration was determined by potassium dichromate-sulfuric acid oxidation method (C_{mass} mg g^{-1}, Supplementary Table S10). The N concentration was determined using a VELP automatic Kjeldahl N determination apparatus (UDK-139, Milano, Italy), and then the N_{mass} (mg g^{-1}) and N_{area} (g m^{-2}) values were calculated. Then, PNUE (μmol mol^{-1} s^{-1}) was calculated using the following formula:

$$PNUE = \frac{A_{max}'}{N_{area}} \times 14$$

(3)

where 14 is the atomic mass of nitrogen.

The remaining 20–30 leaves were frozen and kept for laboratory analysis. The frozen leaves (0.2 g, 5–10 leaves) were cut into small 5–10-mg pieces. The leaves were placed in a volumetric flask and brought to a consistenttant volume of 25 mL using 95% (v/v) alcohol. The volumetric flask was protected from light for 24 h, and then the chlorophyll contents were determined using a Shimadzu ultraviolet-visible spectrophotometer (UV 2250, Fukuoka, Japan). For the chlorophyll contents, please see Supplementary Table S10.

The remaining frozen leaves were used to determine the cell wall N content according to the method of Onoda et al.[16] as follows: 1 g of leaves was powdered in liquid N and suspended in sodium phosphate buffer (pH 7.5, 25 mL), the homogenate was centrifuged at 2500 g for 5 min, and the supernatant was discarded. The pellet was washed with 3% (w/v) SDS, amyloglucosidase (35 units ml^{-1}, Rhizopus mold, Sigma, St Louis, USA) and 0.2 M KOH and then heated and centrifuged, and the remaining pellet was washed with distilled water and ethanol and then dried in an oven (75 °C) for 2 days (for more details see Onoda et al.)[16]. The nitrogen content of the rest of the pellet (cell wall N) was determined using a VELP automatic Kjeldahl N determination apparatus. The P_{CW} represents the ratio of the cell wall N content to the total N content.

Calculation of the N allocation in the photosynthetic apparatus.

The N allocation fractions of each component in the photosynthetic apparatus were calculated according to Niinemets and Tenhunen[13], which has been widely used in recent years[1,45,78].

$$P_R = \frac{V_{cmax}}{6.25 \times V_{cr} \times LMA \times N_{mass}} \tag{4}$$

$$P_B = \frac{J_{max}}{8.06 \times J_{mc} \times LMA \times N_{mass}} \tag{5}$$

$$P_L = \frac{C_{Chl}}{C_B \times N_{mass}} \tag{6}$$

where C_{Chl} is the chlorophyll concentration (mmol g^{-1}), V_{cr} is the specific activity of Rubisco (μmol CO$_2$ g^{-1} Rubisco s^{-1}), J_{mc} is the potential rate of photosynthetic electron transport (μmol electrons μmol^{-1} Cyt f s^{-1}), and C_B is the ratio of leaf chlorophyll to leaf N during light-harvesting (mmol Chl (g N)$^{-1}$). The V_{cr}, J_{mc}, and C_B were calculated according to Niinemets and Tenhunen[13].

Statistical analysis.

The differences between the seedling leaves of the four tree species, the N-fixing and non-N-fixing tree seedlings, and the three levels of soil N were analyzed by performing a one-way analysis of variance (ANOVA), and a post-hoc test (Tukey's test) was conducted to determine if the differences were significant. The effects of the tree varieties and N treatments on the variables in the four species were analyzed by two-way ANOVA and Tukey's test. The significance of the correlation between each pair of variables was tested with a Pearson's correlation (two-tailed). All the analyses were performed using the Statistical Product and Service Solutions 17.0 program (SPSS17.0, Chicago, USA).

Data Availability

All the relevant data are in the paper and its Supporting Information files.

References

1. Chen, L. H., Dong, T. F. & Duan, B. L. Sex-specific carbon and nitrogen partitioning under N deposition in Populus cathayana. Trees 28, 793–806, https://doi.org/10.1007/s00468-014-0992-3 (2014).
2. Ji, D. H., Mao, Q. Z., Watanabe, Y., Kitao, M. & Kitaoka, S. Effect of nitrogen loading on the growth and photosynthetic responses of Japanese larch seedlings grown under different light regimes. J. Agric. Meteorol. 71, 232–238, https://doi.org/10.2480/agrmet.D-14-00027 (2015).
3. Liu, N. et al. Alterations in leaf nitrogen metabolism indicated the structural changes of subtropical forest by canopy addition of nitrogen. Ecotox environ safe 160, 134–143, https://doi.org/10.1016/j.ecoenv.2018.05.037 (2018).
4. Warren, C. R. & Adams, M. A. Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen in photosynthesis. Plant Cell Environ. 29, 192–201, https://doi.org/10.1111/j.1365-3040.2005.01412.x (2006).
5. Field, C., & Mooney, H. A. The photosynthesis nitrogen relationship in wild plants in On the Economy of Plant Form and Function (ed. Givnish, T. J.) 25–55 (Cambridge, UK: Cambridge University Press,1986).
6. Hikosaka, K. Interspecific difference in the photosynthesis–nitrogen relationship: patterns, physiological causes, and ecological importance. J. Plant Res. 117, 481–494, https://doi.org/10.1007/s10265-004-0174-2 (2004).
7. Boussadia, O. et al. Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars 'Meski' and 'Koroneiki'. Sci. Hort. 123, 336–342, https://doi.org/10.1016/j.scienta.2009.09.023 (2010).
8. Zhang, R. et al. Nitrogen deposition enhances photosynthesis in moso bamboo but increases susceptibility to other stress factors. Front. Plant Sci. 8, 1975, https://doi.org/10.3389/fpls.2017.01975 (2017).
9. Hikosaka, K. Mechanisms underlying interspecific variation in photosynthetic capacity across wild plant species. Plant Biotechnol. 27, 223–229, https://doi.org/10.5511/plantbiotechnology.27.223 (2010).
10. Wu, C. C. et al. Physiological responses of Abies faxoniana seedlings to different non-growing-season temperatures as revealed by reciprocal transplantations at two contrasting altitudes. Can. J. For. Res. 41, 599–607, https://doi.org/10.1139/X10-225 (2011).
11. Warren, C. R. The photosynthetic limitation posed by internal conductance to CO$_2$ movement is increased by nutrient supply. J. Exp. Bot. 55, 2313–2321, https://doi.org/10.1093/jxb/erh239 (2004).
12. Li, Y., Gao, Y. X., Xu, X. M., Shen, Q. R. & Guo, S. W. Light-saturated photosynthetic rate in high-nitrogen rice (Oryza sativa L.) leaves is related to chloroplastic CO$_2$ concentration. J. Exp. Bot. 60, 2351–2360, https://doi.org/10.1093/jxb/erp127 (2009).

13. Niinemets, Ü. & Tenhunen, J. D. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant Cell Environ.* **20**, 845–866, https://doi.org/10.1046/j.1365-3040.1997.d01-133.x (1997).

14. Farquhar, G. D., von Caemmerer, S. & Berry, J. A. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149**, 78–90, https://doi.org/10.1007/BF00386231 (1980).

15. Tazoe, Y., Noguchi, K. O. & Terashima, I. Effects of growth light and nitrogen nutrition on the organization of the photosynthetic apparatus in leaves of a C_4 plant, *Amaranthus cruentus*. *Plant Cell Environ.* **29**, 691–700, https://doi.org/10.1111/j.1365-3040.2005.01453.x (2006).

16. Onoda, Y., Hikosaka, K. & Hirose, T. Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-use efficiency. *Funct. Ecol.* **18**, 419–425, https://doi.org/10.1111/j.0269-8463.2004.00847.x (2004).

17. Reiter, W. D. The molecular analysis of cell wall components. *Trends Plant Sci.* **3**, 27–32, https://doi.org/10.1016/S1360-1385(97)01169-2 (1998).

18. Takashima, T., Hikosaka, K. & Hirose, T. Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. *Plant Cell Environ.* **27**, 1047–1054, https://doi.org/10.1111/j.1365-3040.2004.01209.x (2004).

19. Hikosaka, K. & Shigeno, A. The role of Rubisco and cell walls in the interspecific variation in photosynthetic capacity. *Oecologia* **160**, 443–451, https://doi.org/10.1007/s00442-009-1315-z (2009).

20. Harrison, M. T., Edwards, E. J., Farquhar, G. D., Nicotra, A. B. & Evans, J. R. Nitrogen in cell walls of sclerophyllous leaves accounts for little of the variation in photosynthetic N-use efficiency. *Plant Cell Environ.* **32**, 259–270, https://doi.org/10.1111/j.1365-3040.2008.01918.x (2009).

21. Qing, H., Cai, Y., Xiao, Y., Yao, Y. H. & An, S. Q. Leaf nitrogen partition between photosynthesis and structural defense in invasive and native tall form *Spartina alterniflora* populations: effects of nitrogen treatments. *Biol. Invasions* **14**, 2039–2048, https://doi.org/10.1007/s10530-012-0210-4 (2012).

22. Buckley, T. N. & Warren, C. R. The role of mesophyll conductance in the economics of nitrogen and water use in photosynthesis. *Photosynth. Res.* **119**, 77–88, https://doi.org/10.1007/s11120-013-9825-2 (2014).

23. Momayyezi, M. & Guy, R. D. Substantial role for carbonic anhydrase in latitudinal variation in mesophyll conductance of *Populus trichocarpa* Torr. & Gray. *Plant Cell Environ.* **40**, 138–149, https://doi.org/10.1111/pce.12851 (2017).

24. Flexas, J. *et al.* Mesophyll diffusion conductance to CO_2: an unappreciated central player in photosynthesis. *Plant Sci.* **193–194**, 70–84, https://doi.org/10.1016/j.plantsci.2012.05.009 (2012).

25. Nakhoul, N. L., Davis, B. A., Romero, M. F. & Boron, W. F. Effect of expressing the water channel aquaporin-1 on the CO_2 permeability of *Xenopus* oocytes. *Am. J. Physiol.* **274**, C543–C548 (1998).

26. Evans, J. R., Kaldenhoff, R., Genty, B. & Terashima, I. Resistances along the CO_2 diffusion pathway inside leaves. *J. Exp. Bot.* **60**, 2235–2248, https://doi.org/10.1093/jxb/erp117 (2009).

27. Cao, B., Dang, Q. L. & Zhang, S. R. Relationship between photosynthesis and leaf nitrogen concentration in ambient and elevated CO_2 in white birch seedlings. *Tree Physiol.* **27**, 891–899, https://doi.org/10.1093/treephys/27.6.891 (2007).

28. Zhang, L., Chen, X. & Wen, D. Interactive effects of rising CO_2 and elevated nitrogen and phosphorus on N allocation in invasive weeds *Mikania micrantha* and *Chromolaena odorata*. *Biol. Invasions* **18**, 1391–1407, https://doi.org/10.1007/s10530-016-1089-2 (2016).

29. Mu, X. H., Chen, Q. W., Chen, F. J., Yuan, L. X. & Mi, G. H. Within-leaf nitrogen allocation in adaptation to low nitrogen supply in maize during grain-filling stage. *Front. Plant Sci.* **7**, 699, https://doi.org/10.3389/fpls.2016.00699 (2016).

30. Von, Caemmerer, S. & Evans, J. R. Determination of the average partial pressure of CO_2 in chloroplasts from leaves of several C_3 plants. *Aust. J. Plant Physiol.* **18**, 287–305, https://doi.org/10.1071/PP9910287 (1991).

31. Muller, O., Oguchi, R., Hirose, T., Werger, M. J. A. & Hikosaka, K. The leaf anatomy of a broad–leaved evergreen allows an increase in leaf nitrogen content in winter. *Physiol. Plant.* **136**, 299–309, https://doi.org/10.1111/j.1399-3054.2009.01224.x (2009).

32. Li, Y. *et al.* Does chloroplast size influence photosynthetic nitrogen use efficiency? *PLoS One* **8**, e62036, https://doi.org/10.1371/journal.pone.0062036 (2013).

33. Warren, C. R., Dreyer, E. & Adams, M. A. Photosynthesis-Rubisco relationships in foliage of *Pinus sylvestris* in response to nitrogen supply and the proposed role of Rubisco and amino acids as nitrogen stores. *Trees* **17**, 359–366, https://doi.org/10.1007/s00468-003-0246-2 (2003).

34. Ibrahim, M. H., Jaafar, H. Z., Rahmat, A. & Rahman, Z. A. The relationship between phenolics and flavonoids production with total non structural carbohydrate and photosynthetic rate in *Labisia pumila* Benth. under high CO_2 and nitrogen fertilization. *Molecules* **16**, 162–174, https://doi.org/10.3390/molecules16010162 (2010).

35. Akita, R., Kamiyama, C. & Hikosaka, K. *Polygonum sachalinense* alters the balance between capacities of regeneration and carboxylation of ribulose-1, 5-bisphosphate in response to growth CO_2 increment but not the N allocation within the photosynthetic apparatus. *Physiol. Plant.* **146**, 404–412, https://doi.org/10.1111/j.1399-3054.2012.01631.x (2012).

36. Luo, W. Y., Luo, P. & Liu, Y. J. Choice and development of the fine and valuable hardwood tree species in tropical and south subtropical regions of China. *Chin. J. Trop. Agric.* **30**, 15–21 (2010).

37. Pang, Z. H. The study progress of *Betula alnoides* in China. *Chin. J. Guangxi Acad. Sci.* **27**, 243–250, https://doi.org/10.13657/j.cnki.gxkxyxb.2011.03.002 (2011).

38. Yang, B. G., Liu, S. L., Hao, J., Pang, S. J. & Zhang, P. Research advances on the rare tree of *Erythrophleum fordii*. *Guangxi. For. Sci.* **46**, 165–170, https://doi.org/10.19692/j.cnki.gfs.2017.02.009 (2017).

39. You, Y. *et al.* Positive interactions between *Pinus massoniana* and *Castanopsis hystrix* species in the uneven-aged mixed plantations can produce more ecosystem carbon in subtropical China. *For. Ecol. Manag.* **410**, 193–200, https://doi.org/10.1016/j.foreco.2017.08.025 (2017).

40. Novriyanti, E. *et al.* Photosynthetic nitrogen and water use efficiency of acacia and eucalypt seedlings as afforestation species. *Photosynthetica* **50**, 273–281, https://doi.org/10.1007/s11099-012-0033-7 (2012).

41. Zhu, J. T., Li, X. Y., Zhang, X. M., Yu, Q. & Lin, L. S. Leaf nitrogen allocation and partitioning in three groundwater-dependent herbaceous species in a hyper-arid desert region of north-western China. *Aus. J. Bot.* **60**, 61–67, https://doi.org/10.1071/BT11181 (2012).

42. Tang, J. C. *et al.* Fagaceae tree species allocate higher fraction of nitrogen to photosynthetic apparatus than Leguminosae in Jianfengling tropical montane rain forest, china. *PLoS One* **13**, e0192040, https://doi.org/10.1371/journal.pone.0192040 (2018).

43. Chen, L. Y., Zhao, J., Zhang, R. Y., Wang, S. M. & Zhao, G. Effects of nitrogen and phosphorus fertilization on legumes in *Potentilla fruticosa* shrub in alpine meadow. *Ecol. Sci.* **29**, 512–517, https://doi.org/10.3969/j.issn.1008-8873.2010.06.003 (2010).

44. Niinemets, Ü., Flexas, J. & Peñuelas, J. Evergreens favored by higher responsiveness to increased CO_2. *Trends Ecol. Evol.* **26**, 136–142, https://doi.org/10.1016/j.tree.2010.12.012 (2010).

45. Bahar, N. H. *et al.* Leaf-level photosynthetic capacity in lowland Amazonian and high-elevation Andean tropical moist forests of Peru. *New Phytol.* **214**, 1–17, https://doi.org/10.1111/nph.14079 (2016).

46. Feng, Y. L., Wang, J. F. & Sang, W. G. Biomass allocation, morphology and photosynthesis of invasive and noninvasive exotic species grown at four irradiance levels. *Acta Oecol.* **31**, 40–47, https://doi.org/10.1016/j.actao.2006.03.009 (2007).

47. Broeckx, L. S., Fichot, R., Verlinden, M. S. & Ceulemans, R. Seasonal variations in photosynthesis, intrinsic water-use efficiency and stable isotope composition of poplar leaves in a short-rotation plantation. *Tree Physiol.* **34**, 701–715, https://doi.org/10.1093/treephys/tpu057 (2014).

48. Nha, B., Hayes, L., Scafaro, A. P., Atkin, O. K. & Evans, J. R. Mesophyll conductance does not contribute to greater photosynthetic rate per unit nitrogen in temperate compared with tropical evergreen wet-forest tree leaves. *New Phytol.* **218**, 1–13, https://doi.org/10.1111/nph.15031 (2018).

49. Xiong, D. *et al.* Rapid responses of mesophyll conductance to changes of CO_2 concentration, temperature and irradiance are affected by nitrogen supplements in rice. *Plant Cell Environ.* **38**, 2541–2550, https://doi.org/10.1111/pce.12558 (2015).

50. Harris, W., Baker, M. J, & Williams, W. M. Population dynamics and competition in *White Clover*, (ed. M. Baker and W. Williams) 205–297 (Wallingford, UK: CAB International,1987).

51. Wright, I. J. *et al.* The leaf economics spectrum worldwide. *Nature* **428**, 821–827, https://doi.org/10.1038/nature02403 (2004).

52. Moon, M., Kang, K. S., Park, I. K., Kim, T. & Kim, T. S. Effects of leaf nitrogen allocation on the photosynthetic nitrogen-use efficiency of seedlings of three tropical species in Indonesia. *J. Korean Phys. Soc.* **58**, 511–519, https://doi.org/10.1007/s13765-015-0074-2 (2015).

53. Tholen, D. & Zhu, X. G. The mechanistic basis of internal conductance: a theoretical analysis of mesophyll cell photosynthesis and CO_2 diffusion. *Plant Physiol.* **156**, 90–105, https://doi.org/10.1104/pp.111.172346 (2011).

54. Niinemets, Ü., Díazespejo, A., Flexas, J., Galmés, J. & Warren, C. R. Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. *J. Exp. Bot.* **60**, 2249–2270, https://doi.org/10.1093/jxb/erp036/ (2009).

55. Wright, I. J. & Cannon, K. Relationships between leaf lifespan and structural defenses in a low-nutrient, sclerophyll flora. *Funct. Ecol.* **15**, 351–359, https://doi.org/10.1046/j.1365-2435.2001.00522.x (2001).

56. Coley, P. D. Herbivory and defensive characteristics of tree species in a low land tropical forest. *Ecol. Monogr.* **53**, 209–233, https://doi.org/10.2307/1942495 (1983).

57. Ridenour, W. M., Vivanco, J. M., Feng, Y. L., Horiuchi, J. & Callaway, R. M. No evidence for trade-offs: *Centaurea* plants from America are better competitors and defenders. *Ecol. Monogr.* **78**, 369–386, https://doi.org/10.1890/06-1926.1 (2008).

58. Showalter, A. M. Structure and function of plant cell wall proteins. *Plant Cell* **5**, 9–23, https://doi.org/10.2307/3869424 (1993).

59. Maruta, E. Growth and survival of current-year seedlings of *Polygonum cuspidatum* at the upper distribution limit on Mt Fuji. *Oecologia* **60**, 316–320, https://doi.org/10.1007/BF00376845 (1983).

60. Givnish, T. J. Adaptive significance of evergreen vs. deciduous leaves: solving the triple paradox. *Silva Fenn.* **36**, 703–743, https://doi.org/10.14214/sf.535 (2002).

61. Sage, R. G. & Pearcy, R. W. The nitrogen use efficiency of C_3 and C_4 plants. II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiol.* **84**, 959–963, https://doi.org/10.1104/pp.85.2.355 (1987).

62. Funk, J. L., Jones, C. G. & Lerdau, M. T. Leaf- and shoot-level plasticity in response to different nutrient and water availabilities. *Tree Physiol.* **27**, 1731–1739, https://doi.org/10.1093/treephys/27.12.1731 (2007).

63. van Ommen Kloeke, A. E. E., Douma, J. C., Ordoñez, J. C., Reich, P. B. & van Bodegom, P. M. Global quantification of contrasting leaf life span strategies for deciduous and evergreen species in response to environmental conditions. *Global Ecol. Biogeogr.* **21**, 224–235, https://doi.org/10.1111/j.1466-8238.2011.00667.x (2012).

64. Wang, W. X., Shi, Z. M., Luo, D., Liu, S. R. & Lu, L. H. Characteristics of soil microbial biomass and community composition in three types of plantations in southern subtropical area of China. *Chin. J. Appl. Ecol.* **24**, 1784–1792, https://doi.org/10.13287/j.1001-9332.2013.0411 (2013).

65. Tang, J. C., Shi, Z. M., Luo, D. & Liu, S. R. Photosynthetic nitrogen-use efficiency of *Manglietia glauca* seedling leaves under different shading levels. *Acta Ecol. Sin.* **37**, 7493–7502, https://doi.org/10.5846/stxb201609111838 (2017).

66. Wu, G. X., Wang, L. H., Liang, H. P., Li, Y. F. & Hao, J. Fertilizer treatments for growth and physiology of *Dalbergia odorifera* seedlings. *J. Zhejiang A F Univ.* **29**, 296–300, 2095-0756(2012)02-0296-05 (2012).

67. Li, G. X., He, C. Y., Li, Y. F., Feng, P. & Song, Q. D. Cultivation techniques of *Erythrophleum fordii*. *Guangdong For. Sci. Tech.* **19**, 51–52 (2003).

68. Loreto, F., Di Marco, G., Tricoli, D. & Sharkey, T. D. Measurements of mesophyll conductance, photosynthetic electron transport and alternative electron sinks of field grown wheat leaves. *Photosynth. Res.* **41**, 397–403, https://doi.org/10.1007/BF02183042 (1994).

69. Loreto, F., Tsonev, T. & Centritto, M. The impact of blue light on leaf mesophyll conductance. *J. Exp. Bot.* **112**, 1–8, https://doi.org/10.1093/jxb/erp112 (2009).

70. Harley, P. C., Loreto, F., Di Marco, G. & Sharkey, T. D. Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. *Plant Physiol.* **98**, 1429–1436 (1992).

71. Momayyezi, M. & Guy, R. D. Blue light differentially represses mesophyll conductance in high vs low latitude genotypes of *Populus trichocarpa* Torr. & Gray. *J. Plant. Physiol.* **213**, 122–128, https://doi.org/10.1016/j.jplph.2017.03.006 (2017).

72. Pegueropina, J. J. *et al.* Cell-level anatomical characteristics explain high mesophyll conductance and photosynthetic capacity in sclerophyllous Mediterranean oaks. *New Phytol.* **214**, 585–596, https://doi.org/10.1111/nph.14406 (2017).

73. Wang, X., Du, T., Huang, J., Peng, S. & Xiong, D. Leaf hydraulic vulnerability triggers the decline in stomatal and mesophyll conductance during drought in rice (*Oryza sativa*). *J. Exp. Bot.* **69**, 4033–4045, https://doi.org/10.1093/jxb/ery188 (2018).

74. Ethier, G. J. & Livingston, N. J. On the need to incorporate sensitivity to CO_2 transfer conductance into the farquhar-von caemmerer-berry leaf photosynthesis model. *Plant Cell Environ.* **27**, 137–153, https://doi.org/10.1111/j.1365-3040.2004.01140.x (2004).

75. Sharkey, T. D., Bernacchi, C. J., Farquhar, G. D. & Singsaas, E. L. Fitting photosynthetic carbon dioxide response curves for C_3 leaves. *Plant Cell Environ.* **30**, 1035–1040, https://doi.org/10.1111/j.1365-3040.2007.01710.x (2007).

76. Gu, L., Pallardy, S. G., Tu, K., Law, B. E. & Wullschleger, S. D. Reliable estimation of biochemical parameters from C_3 leaf photosynthesis-intercellular carbon dioxide response curves. *Plant Cell Environ.* **33**, 1852–1874, https://doi.org/10.1111/j.1365-3040.2010.02192.x (2010).

77. Loustau, D., Brahim, M. B., Gaudillère, J. P. & Dreyer, E. Photosynthetic responses to phosphorus nutrition in two-year-old maritime pine seedlings. *Tree Physiol.* **19**, 707–715, https://doi.org/10.1093/treephys/19.11.707 (1999).

78. Yao, H. S. *et al.* Diaheliotropic leaf movement enhances leaf photosynthetic capacity and photosynthetic use efficiency of light and photosynthetic nitrogen via optimizing nitrogen partitioning among photosynthetic components in cotton (*Gossypium hirsutum* L.). *Plant Biol.* **20**, 213–222, https://doi.org/10.1111/plb.12678 (2018).

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Author Contributions

This study was performed as a collaboration between all the authors. J.T. contributed to the planning of the experiment, field experiment and laboratory work, data analysis, interpretation of results, and manuscript preparation; B.S. contributed to the planning of the experiment, interpretation of results, and manuscript preparation; R.C. contributed to the data analysis and the interpretation of results; Z.S. contributed to the planning of the experiment, interpretation of results and manuscript preparation; D.L. contributed to the field experiment and laboratory work; S.L. contributed to the interpretation of results; and M.C. contributed to the critical literature review and manuscript preparation. All the authors have reviewed the manuscript.

Additional Information

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