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Resource-acquisitive species have greater plasticity in leaf functional traits than resource-conservative species in response to nitrogen addition in subtropical China

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Abstract

Evergreen broad-leaf forest is subtropical zonal vegetation in China, and their species diversity and stability are important for maintaining forest ecosystem functions. The region is generally affected by global changes such as high levels of nitrogen deposition. Therefore, it is critical to determine the adaptation strategies of subtropical dominant species under nitrogen addition. Here, we conducted two-year field experiments with nitrogen addition levels as 0 kg N ha^{-1} yr^{-1} (Control), 50 kg N ha^{-1} yr^{-1} (LN) and 100 kg N ha^{-1} yr^{-1} (HN). We investigated the effects of nitrogen addition on leaf functional traits (including nutrition, structural and physiological characteristics) of five dominant species in subtropical evergreen broad-leaf forest. We found that the effect of nitrogen addition on leaf functional traits was species-specific. The response to the HN treatment was greater in *Quercus glauca*, *Schima superba* and *Castanopsis eyrei* compared to the LN treatment, as contrary to *Rhododendron delavayi* and *Eurya muricata*. At the level of traits, the phenotypic plasticity of leaf anatomy structure was highest, and the relative effect of leaf photosynthetic property was highest under N addition. At the level of species, the ranking of the index of plasticity for the leaf functional traits of the five species under nitrogen addition was as follows: *Schima superba* > *Quercus glauca* > *Eurya muricata* > *Castanopsis eyrei* > *Rhododendron delavayi.* The PCA analysis indicated that species with high leaf plasticity adopt resource acquisitive strategies (*Schima superba* and *Quercus glauca*), whereas species with low leaf plasticity adopt resource conservative strategies (*Eurya muricata*, *Castanopsis eyrei* and *Rhododendron delavayi*). Collectively, differences in the adaptation of leaf functional traits to nitrogen addition in different dominant species may contribute to understanding and predicting uncertainties in community composition under elevated N deposition.

Keywords: N addition, leaf functional traits, dominant species, phenotypic plasticity, photosynthesis

1. Introduction

Nitrogen (N) is one of the important nutrients in the ecosystem and the main limiting factor for plant growth and photosynthesis (Elser et al., 2007; Erisman et al., 2013). However, the increase in human activities and the evolution of the natural environment have led to an increase in the input of reactive N into the global ecosystem (Galloway et al., 2008). The continuous input of N has led to an increase in the availability of N in terrestrial ecosystems, which has important implications for ecosystem function (net primary productivity, etc.) and key processes (nutrient cycling, etc.) (Liang et al., 2020; Qin et al., 2021). Previous studies have reported that subtropical regions of China are generally affected by environmental changes such as N deposition (Qin et al., 2021; Tang et al., 2021), therefore, understanding the impact of N deposition on the balance of N uptake by forest plants and the evolution of plant community composition is extremely important to maintain the health of forest ecosystems in the region (Liu et al., 2013; Lü and Tian, 2007).

Evergreen broad-leaf forest is a typical zonal forest ecosystem in the subtropical region of China (Liu et al., 2022), which has rich species resources and complex community structure and is an important plant gene pool in this region (Lin et al., 2012). It is an important source of carbon sink and a participant in the N cycle and plays an irreplaceable role in regional and global biodiversity protection and ecosystem function maintenance (Song and Da, 2016; Yao et al., 2022). The major forest plants in the community have obvious control over the structure of the community and its habitat, on the other hand, changes in the external living environment can directly impact the major forest plants to change the composition of the community (Frieswyk et al., 2007; Yang et al., 2021). However, with the rapid development of industrialization, the evergreen broad-leaf forest in the region is generally affected by N deposition (Liu et al., 2011; Tang et al., 2021). Due to the diversity of evergreen broad-leaf forest plant species, different species differ in their sensitivity to N deposition (Mao et al., 2018; Yu et al., 2020). Previous studies revealed that continuous N deposition may increase the competitive exclusion of species in evergreen broad-leaf forest, which may lead to a reduction in the importance of some species or even their disappearance and change the community structure of the ecosystem (Liu et al., 2018; Lu et al., 2010; Mo et al., 2006). In addition, this issue has received scant attention in the research literature, therefore, it is indispensable to quantify how plants in subtropical evergreen broad-leaf forest can effectively use resources to sustain growth and survival in a N-rich environment.

Plant functional traits are essential for exploring the relationship between plants and environment, which are key to predicting plant growth strategies, community composition and ecosystem responses to global environmental change (Li et al., 2015; Liu et al., 2020). Leaf is an organ with a high degree of plasticity during the long-term evolution of plants (Mao et al., 2018), serves as energy converter for primary producers in ecosystems (Wright et al., 2005). They are prone to perceive environmental changes and form adaptive mechanisms under different habitat stresses. Thus, it is very convincing to reflect the influence of environmental factors through changes in leaf characteristics (Sakschewski et al., 2015; Zhang et al., 2015). Leaf functional traits (LFTs) are known as the predictors of individual species performance and are commonly used to assess plant-environment interactions or to quantify specific responses of plants to ecosystem processes (Damián et al., 2018; Jones et al., 2013). Therefore, the strategies that plants adopt to cope with environmental changes may be revealed mainly from leaf functional traits (Wigley et al., 2016). Previous studies have suggested that plants respond to changes in the external environment not only in the adjustment of single or partial functional traits such as leaf morphology, photosynthesis and nutrients concentrations and ratios, but also in the integration of traits (Jones et al., 2013; Liu et al., 2020). The change in plant functional traits is generally an important strategy for plants to adapt to their environment. Leaf economic spectrum (LES) combines a range of interrelated leaf functional traits to quantitatively reflect resource use and life-history trade-offs (Valladares et al., 2000a). The leaf economic spectrum (LES) highlights trade-offs in plants between the acquisition and conservation of resources (Gorné et al., 2022; Wright et al., 2005). For instance, specific leaf area (SLA), leaf dry matter content (LDMC) and leaf nitrogen content per leaf mass (Nmass) are usually good indicators for characterizing resource use of individual species within a community (Reich et al., 2003). It has also been suggested that thicker leaves, with greater physical toughness, tend to select for lower leaf photosynthetic rates to achieve the trade-off (Gorné et al., 2022; Luo and Zhou, 2019). Therefore, the study of leaf functional traits can help to predict the dynamics of plant community composition and feedback in ecosystem functions.

The enzymes used by leaves for photosynthesis are constrained and influenced by nitrogen availability (Nakaji et al., 2001). The surplus and deficit of N influence nutrient uptake and transformation capacity of leaves, which in turn affects the productivity of the whole community (Lu et al., 2010; Nakaji et al., 2001). Exploring the responses of leaf functional traits to nitrogen addition at the species level is important to understand the evolution of plant communities (May, 1974). There are thresholds for N uptake by different plants, both redundancy and deficiency of N can alter the photosynthetic carbon assimilation capacity and affect the carbon allocation pattern in plants (Luo and Zhou, 2019; Mengesha, 2021). The response of leaf functional traits to N addition is highly dependent on the physiological characteristics of the plant species itself (Bauer et al., 2000; Elvir et al., 2006). Indeed, many forest studies have indicated that different species would respond and adjust their leaf functions in different ways to optimize the excess nitrogen input (Ye et al., 2022). However, it is unclear how the response of dominant species in evergreen broad-leaf forest to fertilizer application is accompanied by changes in functional leaf characteristics (e.g., nutritional and physiological characteristics), and effects of N addition on the local adaptation of individual tree species are often neglected, leaf anatomy and chlorophyll fluorescence are usually not considered.

Numerous studies have reported that environmental variation is the main driver of phenotypic plasticity, thereby influencing differences in plasticity between species or traits (Arnold et al., 2022; Valladares et al., 2000b). Plasticity itself involves trade-offs and differences in plasticity among species provide strong evidence for adopting different resource strategies (Weih et al., 2021). Accordingly, plasticity of different trait types respond differently to environmental changes, White (1979) argued that the physiological characteristics of leaves are the most plastic. The experimental essays have shown that deciduous tree species have high leaf plasticity and tend to adopt a rapid acquisition strategy (Zhang et al., 2020). Under changes in N availability, changes in resource use strategies of different species as indicated by changes in leaf functional traits may lead to further changes in community composition (Van Houtven et al., 2019). However, it is unclear to know how the plasticity of different leaf functional trait types will respond differently to N addition and what strategies different species will adopt in response to changes in N addition.

In the present study, an experiment on the response of N addition to dominant species was initiated in 2017 using a random block design in a secondary evergreen broad-leaf forest in subtropical China. Five dominant species (*Castanopsis eyrei*, *Quercus glauca*, *Schima superba*, *Rhododendron delavayi* and *Eurya muricata*), typical of subtropical evergreen broad-leaf forest in China, were selected to study the effects of N additions on 25 leaf functional traits including leaf morphology, nutrient, photosynthetic physiology and anatomical structure. We proposed the following three hypotheses: (1) the effects of HN treatment on the leaf functional traits of different species would greater than with LN treatment, (2) leaf functional traits with high plasticity would respond more significantly to N addition, and (3) the effect of N addition on leaf functional traits would be species-specific, and species with high plasticity of leaf functional traits would adopt acquisitive strategy. Overall, the results will contribute to understanding and forecasting the species-specific response in leaf functional traits to N deposition.

2. Material and methods

2.1. Site description

Our study area was conducted at Yangjifeng Nature Reserve with an area of approximately 10946 ha in Guixi (27°51′10″- 28°02′20″N, 117°11′30″- 117°28′40″E), Jiangxi Province, China (Figure 1). The region has a high forest cover of up to 99.7% and altitude ranges from 690 m to 785 m, it is a typical representative of subtropical evergreen broad-leaf forest in China. The site has a subtropical monsoon climate with the mean annual temperature is 14.4 °C and a natural nitrogen deposition rate of about 43 kg N ha^{-1} yr^{-1}. The mean annual precipitation and frost-free period per year are 2114 mm and 268 days, respectively. The area of natural evergreen broad-leaf forest accounts for 68% of the entire protected area. The trees, shrubs, and herb species are diverse and mainly consist of species of Fagaceae, Theaceae and Ericaceae. Evergreen broad-leaf forest average diameter at breast height (DBH) values and tree heights were 6.54 cm and 13.7 m, respectively. Our previous studies showed that the soil is predominantly typical red soils and the basic characteristics of the surface soils (0-10 cm) are as follows: soil pH: 4.33±0.07; soil total N: 4.44±0.65 g kg^{-1}; soil total P: 0.33±0.02 g kg^{-1}; soil total C: 74.56±13.20 g kg^{-1}.

2.2. Experimental design

A nitrogen addition experiment was initiated in July 2017, including three N addition treatments (in three replicates): 0 kg N ha^{-1} yr^{-1} (Control), 50 kg N ha^{-1} yr^{-1} (Low-N) and 100 kg N ha^{-1} yr^{-1} (High-N), excluding natural N deposition. The N addition gradient was based on both the present N deposition level and its further increase in the future. Totally, nine 15 × 15 m plots were established in a completely randomized design. Each plot was surrounded by at least a 10 m wide buffer strip to the next plot. For the N addition, $CO(NH_2)_2$ dissolved with water, at the beginning of every three months, and a double-nozzle sprayer was used to spray the forest floor manually and uniformly back and forth in all sample plots from July 2017, continuous N addition for 2 years. The control plots only received the same amount of water.

2.3. Plant species and sampling

Samples were collected in July 2019. Five dominant species were selected for this study in subtropical evergreen broad-leaf forest. At the study site, relative importance values (RIV) for each species within the plot were measured as the average of relative density, relative frequency and relative base area at breast height (Mao et al., 2018), RIV were 0.1468 for *C. eyrei*, 0.1218 for *S. superba*, 0.1099 for *Q. glauca*, 0.0316 for *R. delavayi* and 0.0245 for *E. muricata* and the five species are the most abundant tree and shrub species in the evergreen broad-leaf forest in the nature reserve. Three representative individuals from each species were selected in each plot, 20- 30 healthy, fully expanded and sun-exposed leaves were selected from the crown of the tree from each individual. The collected leaves were immediately placed in plastic bags and stored at 4°C, transported to the laboratory with numbers for further analysis.

2.4.Measurements of leaf morphology and chemical

After photosynthetic characterization were measured, 20 collected leaves were used for leaf morphological traits and element analysis. Leaf thickness was measured as the mean of three measurements (top, middle, and bottom), avoiding the major veins, using a vernier caliper (LT, mm). Leaf area (LA, cm^2) was measured using Epson V370 (Epson). The saturated fresh weight (Lsmass, g) was determined after submerging a leaf in deionized water for 24 h. All the samples were oven-dried at 65°C for 48 h and then weighed (Ldmass, g) to calculate leaf dry matter content (LDMC, g/g) and specific leaf area (SLA, cm^2/g). Leaf tissue density (LTD, g/cm^3) was calculated as Ldmass/ (LA × LT).

The leaf carbon and nitrogen concentration per unit mass (LCC and LNC, mg/g) were determined on the same dried leaves with the Element Analyzer (VARIO EL III Element Analyzer, Elementar). The leaf phosphorus concentration per unit mass (LPC, mg/g) was measured using the continuous flow analyzer (San++, Skalar) after H_2SO_4-$HClO_4$ (4:1, v: v) digestion.

Isotope values were reported using conventional δ-notation according to the following formula: $\delta^{13}C = [(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{standard}]/(^{13}C/^{12}C)_{standard}$ (Grams et al., 2007).

2.5.Measurements of leaf photosynthetic parameters

Gas-exchange measurements were conducted with a Li-6800 portable gas-exchange system (Li-Cor, Lincoln, USA) in July 2019. In each sample plot, three standard trees were selected for each five dominant tree species, six to eight leaves of each standard tree with the upper and middle canopy expanded, healthy and fully matured were measured from 9:00 to 11:00 a.m. on a clear and windless day. The gas exchange parameters of species in the same treatment were measured on the same day. Net photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Gs) were obtained from the five species. Photosynthetic N use efficiency (PNUE) was estimated as the ratio of Pn to leaf N concentration, and photosynthetic P use efficiency (PNUE) was estimated as the ratio of Pn to leaf P concentration, as recommended by Qin et al., 2021 (Qin et al., 2021).

After remaining in complete darkness overnight, we measured minimal fluorescence yield (Fo) and maximal fluorescence yield (Fm) in dark-adapted leaves. The leaves were continuously illuminated for 20 min with an actinic light to record the steady-state yield of fluorescence (Fs) and maximal light-adapted fluorescence yield (Fm'). The actinic light was turned off, and minimal fluorescence yield (Fo') in light-adapted state was determined after 5s of far-infrared illumination. The difference between the measured values of Fm and Fo is the variable fluorescence (Fv). The chlorophyll fluorescence parameters were calculated using the following formulas (Kramer et al., 2004):

$$Fv/Fm = (Fm - Fo)/Fm \tag{1}$$

$$Fv'/Fm' = (Fm' - Fo')/Fm' \tag{2}$$

$$\Phi PSII = (Fm' - Fs)/Fm' \tag{3}$$

$$NPQ = Fm/Fm' - 1 \tag{4}$$

$$qP = (Fm' - Fs)/(Fm' - Fo') \tag{5}$$

$$ETR = \Phi PSII \times PPFD \times 0.5 \times 0.85 \tag{6}$$

where Fv/Fm is maximal photochemical efficiency of photosystem II (PSII), Fv'/Fm' is excitation energy capture efficiency of PSII reaction centers, ΦPSII is actual photochemical efficiency of PSII, and qP and NPQ are photochemical and non-photochemical quenching, respectively, ETR is the electron transfer rate, where the coefficient 0.85 was the assumed average leaf absorbance and the coefficient 0.5 is the assumed proportion of absorbed photon allocated to PSII.

2.6. Measurements of leaf anatomical structure

The healthy and mature leaf sections (5mm×4mm) were collected from the middle of the leaf near the vein, and fixed at 4°C in an FAA solution (38% formaldehyde, glacial acetic acid, 70% alcohol, 5:5:90, v/v/v) for 24 h. Then, the leaf sections were dehydrated in a graded ethanol series. Sections were performed using microtome and stained for 3 min in 0.05% toluidine blue solution (Falcioni et al., 2017). Photographs were observed under a light photographic microscope (ZEISS Imager A1). Leaf up-epidermal thickness (UET), down-epidermal thickness (DET), palisade tissue thickness (PT) and spongy tissue thickness (ST) were measured using the software tools provided in Axio Vision Release 4.5 SP1 and averaged over multiple measurements. The palisade and spongy parenchyma ratio are as follows: PT/ST.

2.7. Data processing and analysis

Modeling of linear mixed effects was carried out to test the effects of N addition, species and their interaction on leaf functional traits. Fixed effects included N addition, species and their interaction, while different individuals were considered random effects. Linear mixed effects model analysis was performed in R version 4.2.2, using the lme function in lmerTest packages.

One-way analysis of variance followed by multiple least significant difference comparisons was used to compare the indicators above for different N addition treatments in the evergreen broad-leaf forest. An independent T-test was used to compare the indicators above between five dominant species. All the results reported were significant at $P < 0.05$. The data are presented as the mean ± standard error (SE). The statistical analysis was performed in SPSS 22.0 software, and graphs were created with Origin 2022 software. An index of phenotypic plasticity was calculated for each trait and species as the difference between the minimum and the maximum mean values among the N addition treatments divided by the maximum mean value (Cheplick, 1995). The relative effect (RE) of leaf functional traits on N addition were calculated as the difference between the mean value of the treatment sample and the mean value of the control sample divided by the value of the control sample (Duval et al., 2011), RE_{LN} is the relative effect under low N treatment and RE_{HN} is the relative effect under high N treatment.

Principal component analysis (PCA) using the “factoextra” package in R (version 4.2.2). Permutational multivariate analysis of variance analysis (PERMANOVA) was performed on the traits of different species using the “adonis” function of the “vegan (Oksanen et al., 2019)” package. The results are visualized through packages “ggplot2” and “ggpubr”. Structural equation modeling (SEM) was carried out to analyze the effects on the photosynthetic capacity of different species through pathways between leaf functional traits under the influence of N addition. SEM analyses were performed in R version 4.2.2 (R Development Core Team).

3. Results

3.1. Leaf morphological and nutrient traits

With the pooled data of the evergreen broad-leaf forest by N addition experiments, mixed linear model analysis showed that SLA and LTD significantly responded to the N addition treatment ($P < 0.05$), LTD and LDMC significantly varied among all study species ($P < 0.05$), LDMC was significantly affected by the interaction of N addition and species (Fig. 2). When analyzing by species, relative to CK, the SLA of *S. superba* was significantly increased under LN treatment (Fig. 2A). We found that LTD significantly decreased in *C. eyrei and S. superba* under N addition (Fig. 2B). Relative to CK, LDMC in *Q. glauca* was increased in response to HN treatment (Fig. 2C).

The N addition significantly affected the LNC ($P < 0.05$), all nutrient traits in this study were significantly different between species, and the interaction effect between N addition and species was significant on LCC, LNC, LPC and C/N. In terms of the responses of individual species to N addition treatments, LNC in *S. superba* and *E. muricata* were elevated in response to LN, but *R. delavayi* had significantly lower LNC in HN treatment (Fig. 3B). N addition significantly decreased LPC in *C. glauca* and *R. delavayi*, relative to CK, the LPC of *S. superba* was increased by 24.1% in LN treatment (Fig. 3C). C/N was significantly decreased in *S. superba* and *E. muricata* under LN treatment (Fig. 3D), but increased N/P and $\delta^{13}C$ in *S. superba* (Fig. 3E, F).

3.2. Leaf photosynthetic performance

The mixed linear model indicated that N addition significantly affected the Pn and PPUE, all photosynthetic characteristics significantly varied among all study species, the interaction of N addition and species affected all indicators except Tr (Table 2). Relative to the CK, Pn and Tr were significantly increased in *S. superba* and *E. muricata* under HN treatment. Gs of *C. eyrei* and *E. muricata* were increased in response to HN, in contrast, *R. delavayi* had significantly lower Gs in HN treatment. The analysis showed that PNUE of *Q. glauca* and *S. superba* were significantly increased by 27.9% and 72.8% in LN and HN treatment, respectively. PPUE of *C. eyrei* and *S. superba* was increased in HN treatment, while the PPUE of *R. delavayi* was decreased by 29.4% in LN treatment.

3.3. Leaf chlorophyll fluorescence Parameters

We found significant effects of N addition and species, and also significant interactions of them, on ΦPSII, ETR and qP (Table 3). The Fv/Fm of the five species did not show significant differences among all N addition treatment. For *C. eyrei*, the Fv'/Fm' increased slightly (11.5%) treated by LN compared with the CK treatment. ΦPSII and ETR significantly increased in *Q. glauca, S. superba* and *R. delavayi* treated by HN, while for *C. eyrei*, ETR was significantly increased by 37.2%. NPQ in *C. eyrei* was decreased by 29.0% in response to LN treatment, and qP of *Q. glauca* was increased by 16.8% in HN treatment. N addition had no effect on chlorophyll fluorescence parameters of *E. muricata*.

3.4. Leaf anatomical structure

N addition, species and their interaction had significant effects on UET and ST (*P* < 0.01), DET, UET and ST were varied by species, the interaction of N addition and species influenced all five indicators (Fig. 4, Fig S2). UET of *C. eyrei* and *Q. glauca* declined under HN treatment, but *E. muricata* is the opposite (Fig. 4A). Compared with the CK, the DET of *R. delavayi* and *E. muricata* decreased but that of *Q. glauca* increased in response HN (Fig. 4B). Compared to CK treatment, ST of *S. superba* and *R. delavayi* decreased by 37.7% and 29.5%, respectively (Fig. 4C). With increasing N addition, the values of PT in *C. eyrei* and *S. superba* were decreased (Fig. 4D). LN treatment greatly increased PT and PT/ST in *E. muricata* (Fig. 4D, E).

3.5. Phenotypic plasticity and relative effects of different species

We divided the 25 leaf functional traits into five trait categories, at the trait level, in which the leaf anatomical structure traits had the highest plasticity (0.246) and the least interspecific variation, in contrast, the leaf morphological traits had the greatest interspecific variation, lowest plasticity of leaf nutrient traits (0.128) (Fig. 5F). Species differed in their phenotypic plasticity. Overall, the average plasticity index ranking of the five species: *S. superba* > *Q. glauca* > *E. muricata* > *C. eyrei* > *R. delavayi*. In this order, the highest plasticity indicators for different species were PNUE, qP, DET, PT/ST and LTD. The highest plasticity was observed for leaf morphological traits of *S. superba* and leaf anatomical structure traits of the other four species.

Relative to RE_{LN}, RE_{HN} for leaf functional traits of *S. superba*, *Q. glauca* and *C. eyrei* was greater, whereas the opposite results were observed in *E. muricata* and *R. delavayi* (Fig. 6F). Moreover, the leaf functional traits of *R. delavayi* had negative relative effects on N addition and the consistent results with *S. superba* under LN treatment. The RE_{LN} of the leaf functional traits was the highest in *E. muricata* (7.99) and RE_{HN} of the leaf functional traits was the highest in *Q. glauca* (11.59). For all five species, the maximum values of RE_{LN} or RE_{HN} were found in leaf photosynthesis and chlorophyll fluorescence variables, indicating that leaf photosynthesis was more responsive to N addition.

3.6. Effect of N addition on the resource trade-off strategy of different species

Based on the autocorrelation among the photosynthetic physiological indicators, PCA analysis was first performed to screen out the indicators with significant contributions as Pn and ΦPSII (Fig S1). The results of PCA of leaf functional traits (Fig. 7) reveal the resource trade-off strategies of the different species. PC1 explained 21.4% of the variation in leaf functional traits, and showed strong positive loadings on LNC, LPC, Pn and SLA, indicating the resource acquisition strategy. By contrast, while traits representing the resource conservation strategy of LTD, LDMC and C/N showed strong negative loadings. Considering species, *S. superba* and *Q. glauca* were occupy the acquisitive side, while *E. muricata*, *C. eyrei* and *R. delavayi* distributed on the conservative side. PERMANOVA analysis showed a non-significant difference between N addition treatment (P = 0.458, Fig. 7A), whereas there was a significant difference among different species (P = 0.001, Fig. 7B).

The results of structural equation modeling revealed that N addition affected the Pn of five species through different pathways (Fig. 8). N addition could indirectly decline the Pn by lowering the allocation of foliar N/P in *C. eyrei* (Fig. 8A). For *Q. glauca*, N addition had an indirect positive effect on the Pn its negative effect on the LPC (Fig. 8B). The N addition could either directly reduce Pn or indirectly increase it by increasing $\delta^{13}C$ and PPUE in *S. superba* (Fig. 8C). N addition indirectly increased Gs and promoted Pn in *E. muricata* (Fig. 8E).

4. Discussion

4.1. Effect of N addition on leaf functional traits

4.1.1 Effect of N addition on leaf structural and nutrient traits

N effectiveness is a critical environmental factor limiting plant photosynthesis, growth and nutrient cycling (Tang et al., 2021). Thus, the potential role of key leaf functional traits is expected to change along the N availability gradients (Bauer et al., 2000; Liu et al., 2018). A set of easily measured leaf traits have been identified as important factors in response to changes in external resources (Reich et al., 2003), our results showed that leaf morphology not only varies among individual species but also react sensitively to variations in N addition (Fig. 2). N addition increased SLA but decreased LTD of *S. superba*, the increase in SLA indicates that accumulating equal amounts of dry matter can lead to a larger leaf area to capture more light energy and expands the light transmission in the leaf mesophyll tissue (Flores et al., 2014), fact that have also been confirmed in our studies of photosynthesis. Adjustment of leaf anatomical structure is a physiological adaptation in response to external environmental changes (Falcioni et al., 2017; Sun et al., 2022). The mesophyll is the main site for photosynthesis (Crous et al., 2021). The present study showed that the UET, DET and ST of *Q. glauca* were significantly increased under HN treatment (Fig. 4), indicating that the epidermal layer of *Q. glauca* was thicker under the condition of N addition and could resist the stimulation of external disturbing factors such as strong light. On the other hand, the thickening of leaf ST may reflect the increase of internal porosity of the leaf and promote the exchange of gas inside the leaf, which was also confirmed from the increase in Ci of *Q. glauca* (Table S1). Meanwhile, we found that N addition significantly increased PT and PT/ST of *E. muricata*, but significantly decreased UET and DET. This may be explained by *E. muricata* using sufficient N to extend the thickness of the chloroplasts and increasing the chlorophyll content, thus improving the light energy utilization and enhancing the Pn (Table 2). These results are consistent with those of previous studies on woody plants (He et al., 2018; Zhu et al., 2020).

In terrestrial ecosystems, the concentration of foliar elements (C, N, P) and their ratios are habitually linked to the balance between plant metabolism and nutrient supply

(Mao et al., 2018; Ye et al., 2022). In this study, we found empirical evidence of interspecific differences in leaf nutrients changes among five different species as a response to N addition. Tessier and Raynal (2003) reported that N addition could gradually change the elements associated with the limitation of plant growth and development. Under N addition, LNC and LPC were significantly higher in *S. superba*, but the effect on *R. delavayi* was the opposite (Figure 3B, C). The possible reason for this difference in response was that the effects of N addition on N and P uptake might vary depending on specific species (Liu et al., 2020; Ye et al., 2022). Also, ratios of C:N represent the ability of plants in assimilating C when simultaneously absorbing N, and were considered to be effective to reflect the growth status of plants (Vrede et al., 2004). We found that lower C:N ratios of *S. superba* and *E. muricata* when given the unchanged LCC and significant increase of LNC in response to N addition, however, the LNC and C: N of *R. delavayi* had the opposite variation from the above. Part of this result could be attributed to the significant effect of N addition on LNC, it also suggested that assimilated C allocation did not change, and N uptake and C assimilation in the above three plants under N addition might be decoupled (Vrede et al., 2004; Ye et al., 2022). According to the Growth Rate Hypothesis (GRH), the plant growth rate was significantly and negatively correlated with C:N (Elser et al., 2007), consistent with these earlier studies, it showed that the N addition is beneficial for *S. superba* and *E. muricata* to improve the N utilization and move towards the trend of favorable growth. N:P ratio in leaves is widely recognized as a reliable indicator to characterize nutrient limitation (Elser et al., 2007; Liu et al., 2020). Previous studies suggested that N:P ratios greater than 16 and P concentrations lower than 1.0 mg/g reflected P limitation, which was consistent with our study (17.67 ± 0.39). This is consistent with the majority of studies concluded that P rather than N is the main factor limiting plant growth in subtropical China (Vrede et al., 2004). Besides, our results showed that N addition significantly increased the N:P of *Q. glauca* and *S. superba*, however, the LNP of *S. superba* increased while the LNP of *Q. glauca* decreased under N addition, suggesting that different plants have different levels of investment on P uptake and also distinct mechanisms of P uptake, allocation and utilization under N addition (Tessier and Raynal, 2003). N addition increased the P utilization efficiency of *Q. glauca*. $\delta^{13}C$ has been used for intrinsic water use efficiency and to determine the long-term physiological regulation of plants in response to environmental changes (Liang et al., 2020). With reference to past studies, we tried to relate $\delta^{13}C$ with instantaneous water efficiency (WUEi) (Zhu et al., 2019) and the long-term water efficiency (long-WUE) calculating by $\delta^{13}C$ (Liang et al., 2020). Nonetheless, our experiment showed that N addition did not significantly change the $\delta^{13}C$, WUEi and long-WUE values of different species, only the $\delta^{13}C$ of the *S. superba* increased under HN treatment (Fig. 3F). Thus, it is surmisable that the experimental area has a relatively sufficient water supply. indicating a minor if any impact of N addition on plant carbon-water relations. Increasing N effectiveness did not cause plants to compete for water resources, but instead to compete for other resources (e.g., light resources), which agree with N addition experiment conducted on dominant woody species in an evergreen broad-leaf forest (Liu et al., 2020).

4.1.2 Effect of N addition on leaf photosynthesis traits

The N in leaves is mainly distributed in the photosynthetic protein complex and chloroplast, which can affect the intensity of photosynthesis and related parameters (Kong et al., 2016; Nakaji et al., 2001). As expected, the effect of N addition on plant photosynthesis varied according to the intrinsic characteristics of the species. Pn is an important indicator of photosynthetic productivity and strength has been reported in many previous studies (Mao et al., 2018; Zhang et al., 2015). Our results showed that N addition, species and their interaction had significant effects on Pn, which suggested that the mechanism of the effect of N addition on photosynthesis can be more clearly characterized by the high or low Pn. The variation of Gs determined the rate at which O_2, CO_2 and water vapor moved in and out of plant cells (Mengesha, 2021), a meta-analysis showed that N addition might affect leaf photosynthesis by affecting leaf stomatal conductance (Liang et al., 2020). In this experiment, HN treatment significantly increased Pn, Tr and Gs in *E. muricata*, but Pn and Tr of *S. superba* were significantly higher at HN treatment, and Gs of *S. superba* did not change (Table 2). The former is consistent with the results of many studies that have observed how N addition may have promoted stomatal opening, and accelerated transport of photosynthetic CO_2, thus exhibiting high stomatal conductance and leading to an increase in Pn (Sun et al., 2018). The latter result has not previously been described, which may be because the increase in Pn due to an increase in available N in the leaves as a result of N addition. Such as the HN treated trees had used the surplus N to synthesize more Rubisco, and thus chloroplast activity was elevated. It has been demonstrated that stomatal factors dominate when stomata open to elevate photosynthesis and transpiration (Mengesha, 2021). Non-stomatal factors were thought to act in conjunction with mesophyll conductance and leaf biochemistry, which respond to photosynthesis from the perspective of biochemical processes (Qin et al., 2021; Ye et al., 2022). We found *E. muricata* was mainly regulated by stomatal factors, on the contrary, the variation in *S. superba* was more consistent with the influence of non-stomatal factors under N addition. The partitioning of N in Rubisco and cell wall affected PNUE, N allocation to cell membrane proteins, water-soluble proteins and free amino acid content could also be factors that affected the high PNUE (Qin et al., 2021). Many studies have confirmed that plant leaves with higher PNUE and PPUE tend to be more tolerant to changes in the external environment (Byeon et al., 2021; Novriyanti et al., 2012), which enables the leaves to make full use of their internal N and P throughout the growth period, providing power for photosynthesis (Liang et al., 2020). It is generally accepted that increased N availability in plants under N addition resulted in a slight increase in leaf N and P concentration of species, thereby increasing the PNUE and PPUE of the leaves (Liu et al., 2018; Mao et al., 2018). Consistent with this view, our study revealed N addition significantly increased the PNUE and PPUE of *S. superba*, *Q. glauca* and *C. eyrei*, respectively. We hypothesize that the maximum Pn of *S. superba* at N addition is, at least in part, consequence of the increase of PNUE and PPUE. Further certified that N addition increased nutrients availability and had better uptake of N for some tree species leaves. This outcome is contrary to that of Chen et al. (2015) who found forests in Dinghushan, a subtropical area in southern China, where plant photosynthesis has been demonstrated to respond moderately even negatively to N addition.

Chlorophyll fluorescence during photosynthesis can be considered as an excellent and effective parameter for characterizing plant adaptations (Melo et al., 2017; Stirbet et al., 2018). However, its response to external environmental changes depends on the genotype of the plant itself (Larcher, 1995; Lovelock et al., 1994). ΦPSII characterizes the actual photochemical quantum yield, higher ΦPSII indicates the higher ability of leaves to convert photon energy into chemical energy (Larcher, 1995; Porcar-Castell et al., 2008). ETR characterizes the fast and slow electron transport rate in leaves, increase in ETR means more fast electron transport and greater proportion of captured light energy is transferred to photosynthesis (Melo et al., 2017). Our study suggested that

ΦPSII and ETR of *Q. glauca*, *S. superba* and *R. delavayi* were significantly increased under HN treatment, indicating that HN treatment promotes the light energy conversion efficiency of the above three dominant plants. Through accelerating electron capture and delivery capability to further stimulate the potential active center of PSII thus to enhanced photochemical efficiency of the reaction center, which is consistent with Qin et al. (2021).

Our results partly supported hypothesis (1) that the effects of HN treatment on the leaf functional traits of different species would be greater than with LN treatment (Fig. 6F). The positive relative effect of HN treatment on leaf functional traits was greater in *Q. glauca*, *S. superba* and *C. eyrei*, indicating a higher threshold of N availability for these species. It can rapidly convert excess N into energy substances that promote growth and development when the availability of N increases (Elvir et al., 2006; Liu et al., 2020). Moreover, the functional traits of some species (e.g., *R. delavayi*) may be saturated at the background of N addition, therefore the response to N addition was not significantly increased or even negative. This difference is not only a result of the rate of uptake, but also depends on the difference in the ability of the plant species to absorb N during growth (Chen et al., 2015; Weih et al., 2021) reported that the enhanced photosynthetic capacity of leaves is usually associated with high N availability, explaining the relative effect of leaf photosynthetic traits were greater than other leaf functional traits among the five species in this study (Fig. 6), and indicating that changes in photosynthesis may be the main response of plants to N addition.

4.2. Effect of N addition on plasticity and trade-off strategy of different plants

Leaf functional traits elucidate the mechanisms that drive the responses of plants to resource addition, and plants with various would develop different coordination strategies in response to environmental changes (Valladares et al., 2000b; Zhu et al., 2020). However, even if the same plants are in different habitats, their structure and characteristics will also show differences, reflecting the adaptation of plants to specific habitats (Mao et al., 2018). The flexible mechanism by which plants adapt to variable resource environments is phenotypic plasticity, which is the expression of different phenotypes of individual genotypes in response to environmental changes (Valladares et al., 2000b). In our study, since we found that the average plasticity of leaf anatomical structures was greatest rather than the leaf photosynthetic traits that responded strongly to N addition, we have to reject our second hypothesis. Further, it indicates that leaf anatomical structures were crucial in the adaptation of different species to N addition, but leaf photosynthetic traits are more sensitive to the response of N addition.

The interconnections and trade-offs between leaf functional traits provide the maximum information about resource-use strategies in plants (Fortunel et al., 2012). We found relationships among leaf functional traits under N addition in the sampled species that support the existence of two opposing strategies (conservative versus acquisitive) in the LES (Gorné et al., 2022; White, 1979). As expected, we found that the expression of leaf functional traits in *S. superba* and *Q. glauca* was at one extreme of that LES equivalent to species adopting an acquisition strategy, both leaves with larger, thinner (higher SLA), with higher LNC and LPC, and with stronger photosynthetic capacity (higher Pn and ΦPSII). In contrast, *E. muricata*, *C. eyrei* and *R. delavayi* exhibited the other extreme of leaf functional traits with high C/N, high LDMC and high defense investment, which has been demonstrated for species adopting a conservative strategy in interspecific comparisons. In addition to the influence of their own genotypes, leaf functional traits are plastic and thus contribute to the adaptation of plants to their environment (Cheplick, 1995; Damián et al., 2018), therefore, their plasticity help explain differences in closely related taxa in variable environments (White, 1979; Xu et al., 2015). The results found the greatest plasticity of leaf functional traits of *S. superba*, followed by *Q. glauca* (Fig. 5). In line with the hypothesis (3), our study provided new empirical evidence that species with high plasticity in leaf functional traits are more likely to adopt acquisition strategies, which was consistent with previous studies on different types of species (Valladares et al., 2000a; Zhang et al., 2020). Firstly, based on life history strategies, *S. superba* and *Q. glauca* were light-loving plants that can be the first to sense changes in their environment (including N addition) and make physiological adjustments, this inference is supported by results of two previous analyses (Liu et al., 2018; Mao et al., 2018). Secondly, among the five species, *S. superba* and *Q. glauca* had greater leaf area. It is generally believed that leaf area is directly proportional to LNC and plants with larger leaf area will absorb more N for photosynthesis. In fact, many reports on higher foliar N and stronger photosynthetic capacity occurred in the situation with more access to N resources (Liang et al., 2020; Liu et al., 2018; Sun et al., 2018). Therefore, *S. superba* and *Q. glauca* are more able to respond under N addition increasing its utake and improving its photosynthetic capacity. Different species select different resource strategies under N addition, reflecting the species specificity of leaf functional traits in response to changing resource environments (Valladares et al., 2000a; Zhang et al., 2020), which in turn is expected to influence higher levels of the organization, such as community and ecosystem composition (Van Houtven et al., 2019).

Interestingly, our SEM results showed how the association of leaf functional traits affects the photosynthetic capacity of different tree species under N addition conditions. Five species affect Pn through different pathways. The N addition could directly promote Pn of *S. superba*, *S. superba*, *Q. glauca* and *C. eyrei*, indirectly affect Pn through leaf phosphorus-related nutrient traits. *E. muricata* could indirectly affect Pn through stomatal factors, but the N addition could not directly or indirectly affect Pn of *R. delavayi*. This provides species-specific linkages among nutrient availability, leaf traits and photosynthetic rate.

5. Conclusions

Our results highlighted that the effect of N addition on leaf functional traits of dominant species in subtropical evergreen broad-leaf forest was species-specific. After investigating, we found that the response to the HN treatment was greater in leaf functional traits of *Q. glauca*, *S. superba* and *C. eyrei* compared to the LN treatment. The phenotypic plasticity of leaf anatomy structure was highest under N addition, and leaf photosynthetic properties were more sensitive to N addition. Simultaneously, we further found that under the influence of N addition, *S. superba* and *Q. glauca* exhibited greater phenotypic plasticity of morphological structure, nutrient partitioning and photosynthetic physiological properties than other species, and preferred to adopt resource-acquisitive strategy. In conclusion, species with the resource-acquisitive strategy showed greater plasticity in leaf functional traits compared to species with the resource-conservative. It follows that increasing N deposition will have an impact on species composition and succession of subtropical evergreen broad-leaf forests. In response to environmental changes, species differences in leaf functional traits must be carefully considered to provide scientific basis for predicting future forest vegetation dynamics in subtropical regions.

Furthermore, the present study was based on the results of a two-years study of N addition. The sensitivity and adaptation of each tree species may change with the duration of N deposition. More carefully study should be combined with the response of plant root traits to N addition in the future.

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Highlights

- The effect of nitrogen (N) addition on leaf functional traits was species-specific.
- Leaf anatomy structure had higher plasticity under N addition.
- Leaf photosynthetic properties were more sensitive to N addition.
- Species with more plastic leaf functional traits adopt resource-acquisitive strategies under N addition.

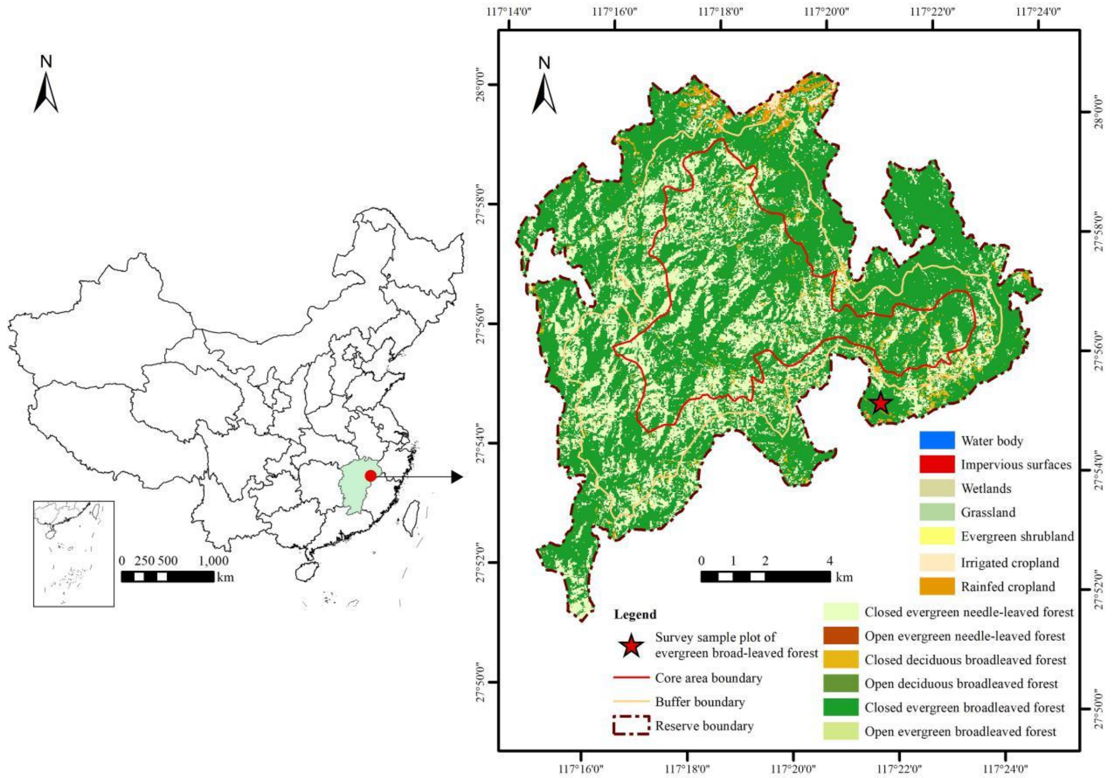

Fig. 1. Location of the study sites in Yangjifeng Nature Reserve in China.

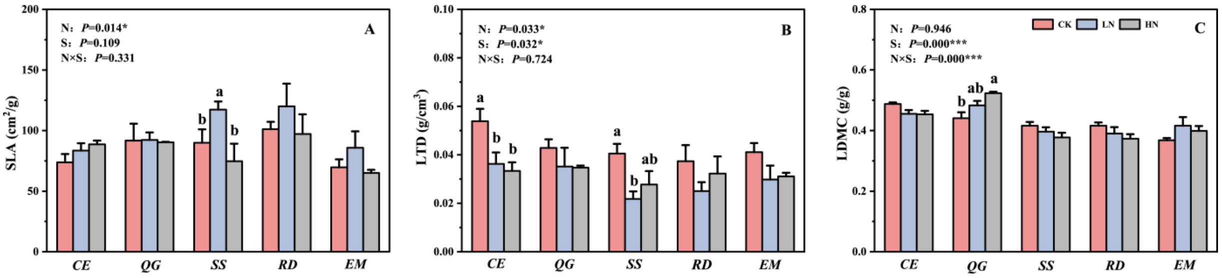

Fig. 2. The leaf morphological traits in *Castanopsis eyrei* (*CE*), *Quercus glauca* (*QG*), *Schima superba* (*SS*), *Rhododendron delavayi* (*RD*), *Eurya muricata* (*EM*) to N addition. Results of mixed linear model are given in panel (*$P < 0.05$, **$P < 0.01$; ***$P < 0.001$). N: N addition treatment, S: Species. CK = Control treatment, LN = Low N treatment, HN = High N treatment. Values are means + SE. For each group of three bars, different lowercase letters indicate significant differences among treatments ($P < 0.05$). See Table 1 for trait abbreviations.

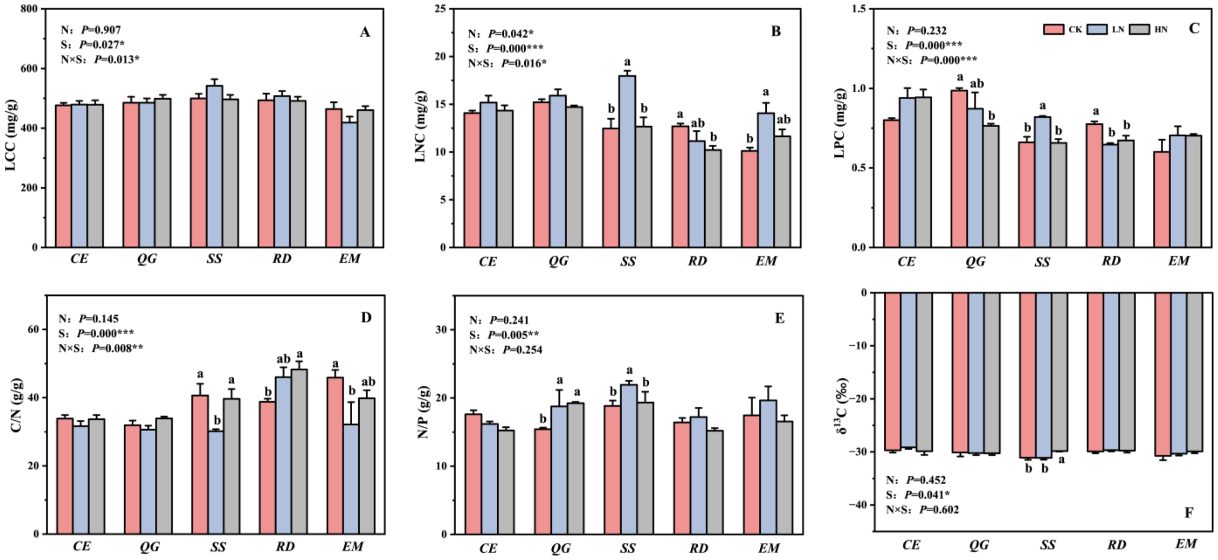

Fig. 3. The leaf nutrient traits in *Castanopsis eyrei* (*CE*), *Quercus glauca* (*QG*), *Schima superba* (*SS*), *Rhododendron delavayi* (*RD*), *Eurya muricata* (*EM*) to N addition. Results of mixed linear model are given in panel (*$P < 0.05$, **$P < 0.01$; ***$P < 0.001$). N: N addition treatment, S: Species. CK = Control treatment, LN = Low N treatment, HN = High N treatment. Values are means + SE. For each group of three bars, different lowercase letters indicate significant differences among treatments ($P < 0.05$). See Table 1 for trait abbreviations.

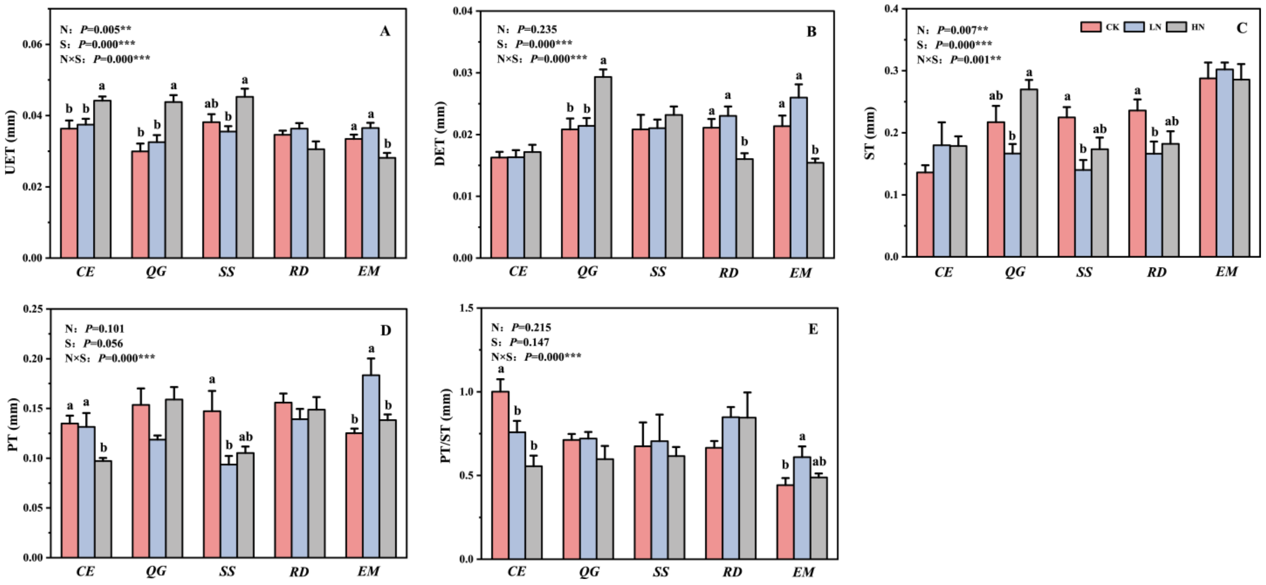

Fig. 4. The leaf anatomical structure traits in *Castanopsis eyrei* (*CE*), *Quercus glauca* (*QG*), *Schima superba* (*SS*), *Rhododendron delavayi* (*RD*), *Eurya muricata* (*EM*) to N addition. Results of mixed linear model are given in panel (*$P < 0.05$, **$P < 0.01$; ***$P < 0.001$). N: N addition treatment, S: Species. CK = Control treatment, LN = Low N treatment, HN = High N treatment. Values are means + SE. For each group of three bars, different lowercase letters indicate significant differences among treatments ($P < 0.05$). See Table 1 for trait abbreviations.

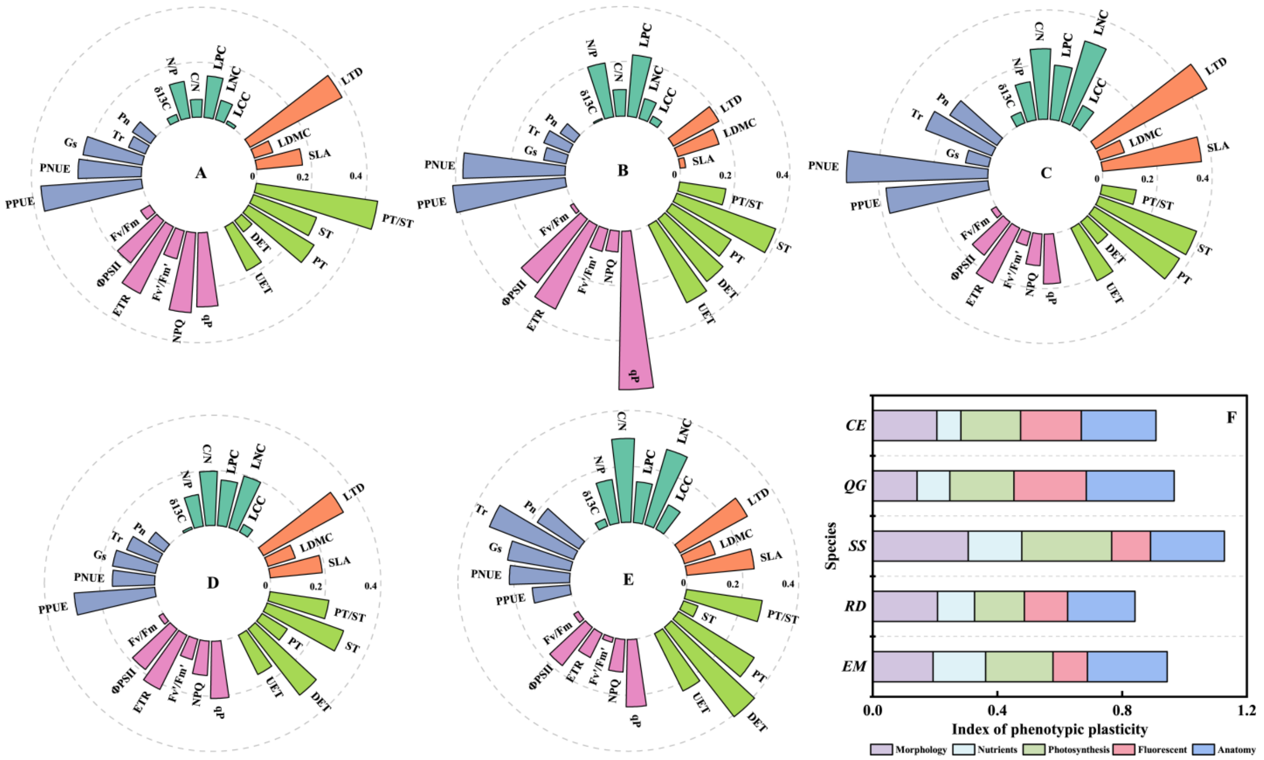

Fig. 5. Index of phenotypic plasticity for leaf functional traits of *Castanopsis eyrei* (A), *Quercus glauca* (B), *Schima superba* (*C*), *Rhododendron delavayi* (*D*), *Eurya muricata* (E) and the average phenotypic plasticity of each species (F) under N addition. See Table 1 for trait abbreviations.

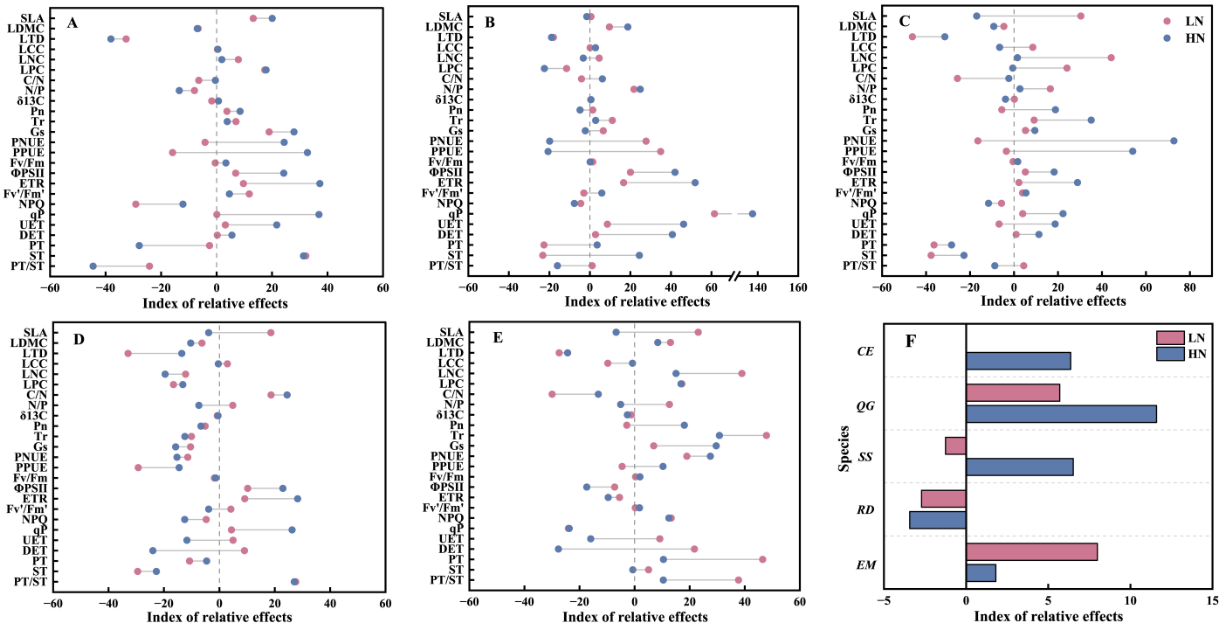

Fig. 6. Index of relative effects of *Castanopsis eyrei* (A), *Quercus glauca* (B), *Schima superba* (C), *Rhododendron delavayi* (D), *Eurya muricata* (E) and the total relative effects of each species (F) under different N addition. See Table 1 for trait abbreviations.

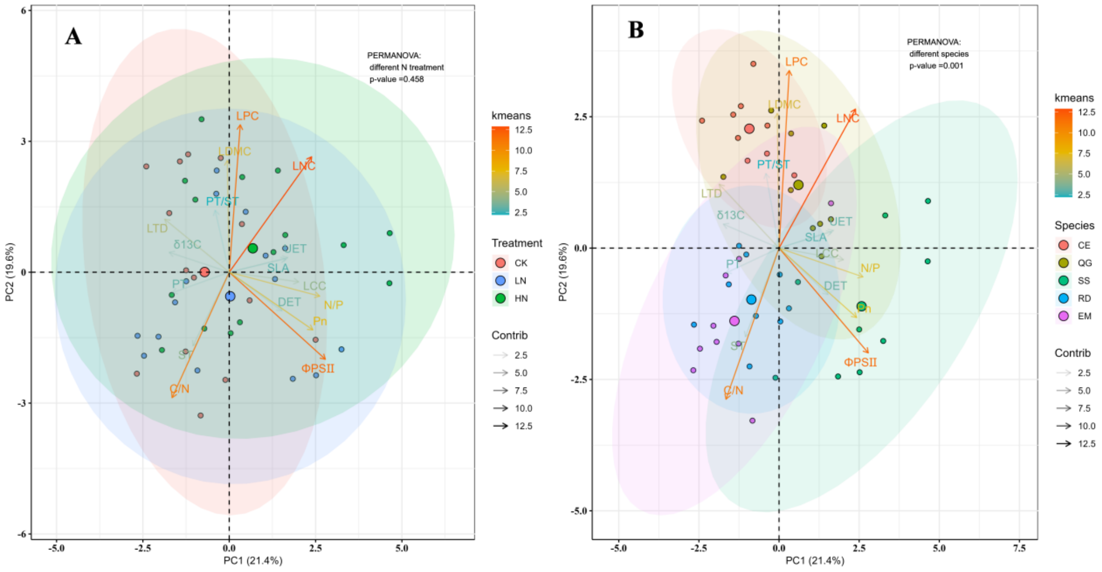

Fig. 7. Biplot of the principal component analysis (PCA) using leaf functional traits at the N treatment level (A) and species level (B). The large dot is the centerof the category. See Table 1 for trait abbreviations.

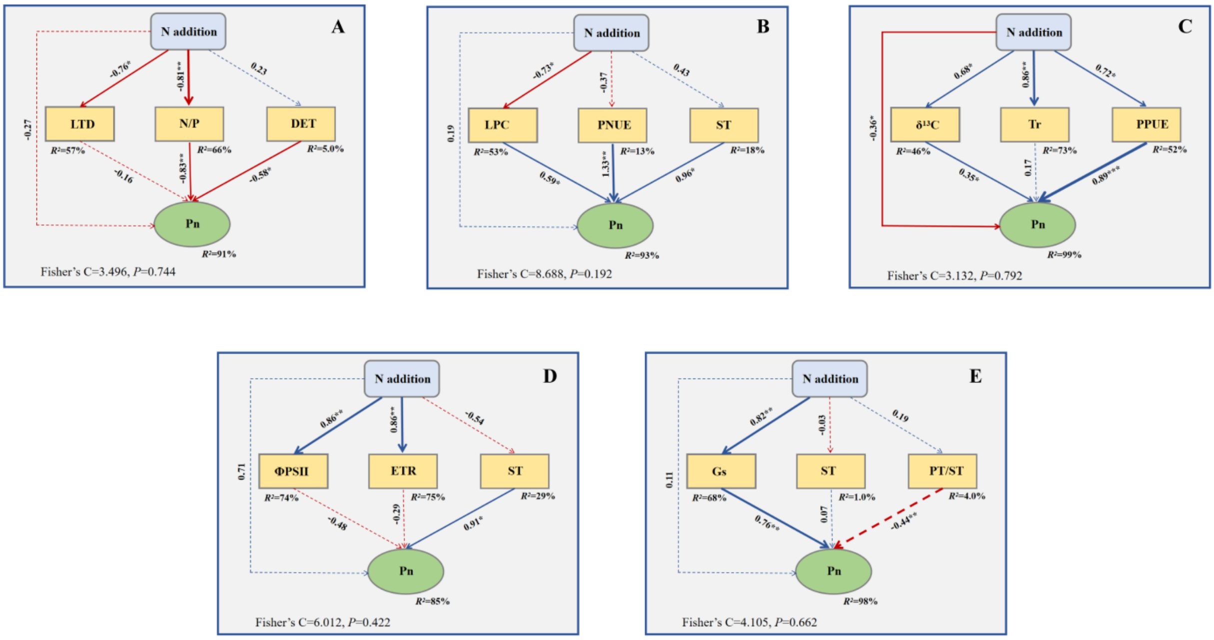

Fig. 8. Structure equation model (SEM) illustrate how N addition affects Pn in *Castanopsis eyrei* (A), *Quercus glauca* (B), *Schima superba* (C), *Rhododendron delavayi* (D) and *Eurya muricata* (E) through leaf functional traits. Arrows indicate the hypothesized direction of causation. The dotted lines indicate no significant relationships ($P > 0.05$), and the solid lines indicate significant relationships ($P < 0.05$). Blue and red arrows represent the significantly positive and negative relationships, respectively. Numbers next to solid arrows are standardized path coefficients. R square represents the explain rate of each factor in the model. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. See Table 1 for trait abbreviations.

Table 1

The definition, abbreviation and unit for 25 leaf functional traits used in this study.

Leaf Traits	Abbreviation	Units
Specific leaf area	SLA	cm^2/g
Dry matter content	LDMC	g/g
Tissue density	LTD	g/cm^3
Leaf carbon concentration	LCC	mg/g
Leaf nitrogen concentration	LNC	mg/g
Leaf phosphorus concentration	LPC	mg/g
Carbon / Nitrogen	C/N	g/g
Nitrogen / Phosphorus	N/P	g/g
Carbon isotope ratio	$\delta^{13}C$	‰
Net photoynthtic rat	Pn	$\mu mol \cdot m^{-2} \cdot s^{-1}$
Transpiration rate	Tr	$mmol \cdot m^{-2} \cdot s^{-1}$
Stomatal conductance	Gs	$mmol \cdot m^{-2} \cdot s^{-1}$
Photosynthetic nitrogen use efficiency	PNUE	$\mu mol \cdot mol^{-1} \cdot s^{-1}$
Photosynthetic phosphorus use efficiency	PPUE	$\mu mol \cdot mol^{-1} \cdot s^{-1}$
Maximum quantum yield of PSII	Fv/Fm	
Effective PSII quantum yield	ΦPSII	
Electron transfer rate	ETR	
Excitation energy capture efficiency of PSII reaction centers	Fv'/Fm'	
Non-photochemical quenching of maximum fluorescence	NPQ	
Photochemical quenching	qP	

Upper epidermal thickness	UET	mm
Lower epidermal thickness	DET	mm
Palisade tissue thickness	PT	mm
Spongy tissue thickness	ST	mm
Palisade tissue thickness / Spongy tissue thickness	PT/ST	mm

Table 2

Effect of N addition on leaf photosynthesis performance in different species.

Species	**Treatment**	**Pn (μmol·m^{-2}·s^{-1})**	**Tr (mmol·m^{-2}·s^{-1})**	**Gs (mmol·m^{-2}·s^{-1})**	**PNUE (μmol·mol^{-1}·s^{-1})**	**PPUE (μmol·mol^{-1}·s^{-1})**
C. eyrei	CK	3.35 ± 0.14	1.44 ± 0.10	23.31 ± 1.54 b	2.38 ± 0.12	41.76 ± 1.54 b
	LN	3.47 ± 0.16	1.54 ± 0.07	27.72 ± 1.41 ab	2.28 ± 0.09	35.13 ± 2.08 b
	HN	3.63 ± 0.17	1.50 ± 0.16	29.82 ± 1.30 a	2.96 ± 0.27	55.42 ± 4.27 a
Q. glauca	CK	4.88± 0.16	1.75 ± 0.12	40.24 ± 1.29	3.84 ± 0.15 b	63.06 ± 3.03 ab
	LN	4.94 ± 0.18	1.95 ± 0.12	42.87 ± 1.34	4.91 ± 0.32 a	85.06 ± 6.38 a
	HN	4.64 ± 0.13	1.80 ± 0.09	39.34 ± 1.17	3.08 ± 0.21 b	50.01 ± 4.20 b
S. superba	CK	5.25 ± 0.18 b	2.11 ± 0.10 b	53.14 ± 1.51	3.31 ± 0.13 b	62.77 ± 5.46 b
	LN	4.96 ± 0.16 b	2.31 ± 0.09 b	56.93 ± 1.90	2.77 ± 0.12 b	60.54 ± 2.33 b
	HN	6.23± 0.12 a	2.86 ± 0.10 a	59.24 ± 1.42	5.72 ± 0.55 a	96.63 ± 2.91 a
R. delavayi	CK	4.36 ± 0.15	1.86 ± 0.09	52.97 ± 1.59 a	3.27 ± 0.40	62.41 ± 2.55 a
	LN	4.13 ± 0.13	1.66 ± 0.07	47.45 ± 1.31 ab	2.90 ± 0.16	44.05 ± 2.19 b
	HN	4.07 ± 0.14	1.62 ± 0.11	44.60 ± 1.58 b	2.77 ± 0.11	53.26 ± 2.09 ab
E. muricata	CK	3.78 ± 0.11 b	1.34 ± 0.08 b	33.19 ± 1.35 b	3.04 ± 0.28	57.68 ± 1.56
	LN	3.68 ± 0.15 b	1.98 ± 0.07 a	35.49 ± 1.40 b	3.62 ± 0.21	55.05 ± 3.80
	HN	4.47 ± 0.16 a	1.75 ± 0.11 a	43.02 ± 1.56 a	3.88 ± 0.27	63.62 ± 2.13
P-value	N	0.026*	0.084	0.481	0.134	0.024*
	S	0.000***	0.004**	0.000***	0.000***	0.005**
	N×S	0.022*	0.060	0.003**	0.000***	0.000***

Note: Mean ± standard error (n = 3). CK = Control treatment, LN = Low N treatment, HN = High N treatment. Different lowercase letters indicate significant differences among treatments ($P < 0.05$). The P values were expressed as follows: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. See Table 1 for trait abbreviations.

Table 3

Effect of N addition on leaf Chlorophyll fluorescence parameters in different species.

Species	Treatment	Fv/Fm	Fv'/Fm'	ΦPSII	ETR	NPQ	qP
C. eyrei	CK	0.78 ± 0.06	0.52 ± 0.02 b	0.25 ± 0.01	21.21 ± 1.17 b	2.07 ± 0.12 a	0.50 ± 0.06
	LN	0.78 ± 0.07	0.58 ± 0.03 a	0.27 ± 0.03	23.26 ± 1.15 b	1.47 ± 0.05 b	0.50 ± 0.08
	HN	0.81 ± 0.07	0.54 ± 0.01 ab	0.31 ± 0.01	29.11 ± 1.63 a	1.82 ± 0.17 ab	0.69 ± 0.09
Q. glauca	CK	0.79 ± 0.05	0.56 ± 0.02	0.29 ± 0.02 b	23.47 ± 1.94 b	1.73 ± 0.13	0.42 ± 0.06 b
	LN	0.81 ± 0.01	0.54 ± 0.02	0.35 ± 0.02 ab	27.37 ± 1.46 b	1.65 ± 0.17	0.68 ± 0.15 ab
	HN	0.79 ± 0.06	0.59 ± 0.04	0.42 ± 0.04 a	35.66 ± 1.49 a	1.59 ± 0.15	1.00 ± 0.21 a
S. superba	CK	0.80 ± 0.04	0.59 ± 0.01	0.52 ± 0.05 b	35.76 ± 1.23 b	2.04 ± 0.13	0.95 ± 0.11
	LN	0.82 ± 0.02	0.61 ± 0.03	0.55 ± 0.02 ab	36.54 ± 2.00 b	1.93 ± 0.18	0.99 ± 0.12
	HN	0.81 ± 0.01	0.62 ± 0.05	0.62 ± 0.06 a	46.06 ± 1.33 a	1.81 ± 0.15	1.17 ± 0.14
R. delavayi	CK	0.80 ± 0.09	0.59 ± 0.01	0.32 ± 0.01 b	26.55 ± 1.00 b	1.97 ± 0.10	0.50 ± 0.06
	LN	0.79 ± 0.06	0.62 ± 0.02	0.35 ± 0.03 ab	28.97 ± 0.94 b	1.88 ± 0.24	0.52 ± 0.07
	HN	0.78 ± 0.05	0.57 ± 0.03	0.39 ± 0.04 a	34.04 ± 1.54 a	1.72 ± 0.13	0.63 ± 0.09
E. muricata	CK	0.79 ± 0.07	0.53 ± 0.01	0.36 ± 0.03	27.17 ± 1.66	1.55 ± 0.15	0.76 ± 0.14
	LN	0.79 ± 0.09	0.53 ± 0.02	0.34 ± 0.01	25.68 ± 2.20	1.76 ± 0.19	0.58 ± 0.07
	HN	0.80 ± 0.04	0.54 ± 0.04	0.30 ± 0.02	24.56 ± 1.48	1.75 ± 0.18	0.58 ± 0.09
P-value	N	0.056	0.359	0.001**	0.000***	0.029*	0.000***
	S	0.684	0.015*	0.000***	0.000***	0.120	0.000***
	N×S	0.137	0.403	0.025*	0.007**	0.038*	0.006*

Note: Mean ± standard error (n = 3). CK = Control treatment, LN = Low N treatment, HN = High N treatment. Different lowercase letters indicate significant differences among treatments ($P < 0.05$). The P values were expressed as follows: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. See Table 1 for trait abbreviations.