Type of Study:

Research Article

Title:

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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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement:

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Word Count: 9,965

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2 leaf functional traits than resource-conservative

3 species in response to nitrogen addition in subtropical

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20

21 Abstract

22 The evergreen broad-leaf forest is subtropical zonal vegetation in China, and its species

23 diversity and stability are crucial for maintaining forest ecosystem functions. The

24   region is generally affected by global changes such as high levels of nitrogen deposition.

25   Therefore, it is critical to determine the adaptation strategies of subtropical dominant

26   species under nitrogen addition. Here, we conducted two-year field experiments with

27   nitrogen addition levels as 0 kg N ha^{-1} yr^{-1} (CK), 50 kg N ha^{-1} yr^{-1} (LN) and 100 kg N

28   ha^{-1} yr^{-1} (HN). We investigated the effects of nitrogen addition on leaf functional traits

29   (including nutrition, structural and physiological characteristics) of five dominant

30   species in subtropical evergreen broad-leaf forest. Results suggested that the effect of

31   nitrogen addition on leaf functional traits was species-specific. Contrary to

32   *Rhododendron delavayi* and *Eurya muricata*, *Quercus glauca*, *Schima superba* and

33   *Castanopsis eyrei* all responded more to the HN treatment than LN treatment. The

34   phenotypic plasticity of leaf anatomy structure was highest, and the relative effect of

35   leaf photosynthetic property was highest on average for all species under N addition.

36   Among the five species, *Schima superba* was highest in terms of the index of plasticity

37   for leaf functional traits under nitrogen addition, followed by *Quercus glauca*, *Eurya*

38   *muricata*, *Castanopsis eyrei* and *Rhododendron delavayi*. The PCA analysis indicated

39   that species with high leaf plasticity adopt resource acquisitive strategy (*Schima*

40   *superba* and *Quercus glauca*), whereas species with low leaf plasticity adopt resource

41   conservative strategy (*Eurya muricata*, *Castanopsis eyrei* and *Rhododendron delavayi*).

42   In aggregate, resource-acquisitive species benefit from nitrogen addition more than

43   resource-conservative species, suggesting that *Schima superba* and *Quercus glauca*

44   will occupy the dominant position in community succession under persistently elevated

45   nitrogen deposition.

46

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

48   photosynthesis

49

1. Introduction

Nitrogen (N) is one of the crucial nutrients in the ecosystem and the primary 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 availability of N has increased in terrestrial ecosystems due to the continuous input of N, 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 a major source of carbon sinks and a participant in the N cycle, and it plays an irreplaceable role in sustaining biodiversity and ecosystem function both in regional and global levels (Song and Da, 2016; Yao et al., 2022). Clearly, the major forest plants in the community have obvious control over the structure of the community and its habitat, whereas changes in the external living environment can directly impact the major forest plants to alter its composition (Frieswyk et al., 2007; Yang et al., 2021). However, with the rapid development of industrialization, the evergreen broad-leaf

74 forest in the region generally suffers from excessive N deposition (Liu et al., 2011; Tang

75 et al., 2021). Due to the diversity of evergreen broad-leaf forest plant species, different

76 species differ in their sensitivity to N deposition (Mao et al., 2018; Yu et al., 2020).

77 Previous studies revealed that continuous N deposition may increase the competitive

78 exclusion of species in evergreen broad-leaf forest, which could reduce the importance

79 of some species or even cause their disappearance and alter the community structure of

80 the ecosystem (Liu et al., 2018; Lu et al., 2010; Mo et al., 2006). In addition, this issue

81 has received scant attention in the research literature, therefore, it is indispensable to

82 quantify how plants in subtropical evergreen broad-leaf forest can effectively use

83 resources to sustain growth and survival in a N-rich environment.

84 Plant functional traits are vital to exploring the relationship between plants and

85 environment, which are key to predicting plant growth strategies, community

86 composition and ecosystem responses to global environmental change (Li et al., 2015;

87 Liu et al., 2020). Leaf is important organ for characterizing plant responses to

88 environmental change and has a high degree of plasticity during the long-term evolution

89 of plants (Mao et al., 2018), serving as energy converter for primary producers in

90 ecosystems (Wright et al., 2005). They are prone to perceive environmental changes

91 and form adaptive mechanisms under different habitat stresses. Thus, it is very

92 convincing to reflect the influence of environmental factors through changes in leaf

93 characteristics (Sakschewski et al., 2015; Zhang et al., 2015). Leaf functional traits

94 (LFTs) are known as the predictors of individual species performance and are

95 commonly used to assess plant-environment interactions or to quantify specific

96 responses of plants to ecosystem processes (Damián et al., 2018; Jones et al., 2013).

97 Leaf functional traits elucidate the mechanisms that drive the responses of plants to

98 resource addition, and plants with various would develop different coordination

99   strategies in response to environmental changes (Valladares et al., 2000b; Zhu et al.,

100   2020). Nevertheless, even if the same plants are in different habitats, their structure and

101   characteristics will also show differences, reflecting the adaptation of plants to specific

102   habitats (Mao et al., 2018). Therefore, the strategies that plants adopt to cope with

103   environmental changes may be revealed mainly from leaf functional traits (Wigley et

104   al., 2016). Research has shown that plants respond to changes in the external

105   environment not only in the adjustment of single or partial functional traits such as leaf

106   morphology, photosynthesis and nutrients, but also in the integration of traits (Jones et

107   al., 2013; Liu et al., 2020). Leaf economic spectrum (LES) provides a quantitative

108   representation of resource use and life-history trade-offs during leaf development by

109   combining a range of interrelated leaf functional traits (Valladares et al., 2000a),

110   illustrating trade-offs in plants between the acquisition and conservation of resources

111   (Gorné et al., 2022; Wright et al., 2005). For instance, specific leaf area (SLA), leaf dry

112   matter content (LDMC) and leaf nitrogen content per leaf mass (Nmass) are usually

113   good indicators for characterizing resource use of individual species within a

114   community (Reich et al., 2003). Additionally, it has been suggested that thicker leaves,

115   with greater physical toughness, tend to select for lower leaf photosynthetic rates to

116   achieve the trade-off (Gorné et al., 2022; Luo and Zhou, 2019). Consequently, the study

117   of leaf functional traits can help to predict the dynamics of plant community

118   composition and feedback in ecosystem functions.

119   The enzymes used by leaves for photosynthesis are constrained and influenced by

120   N availability (Nakaji et al., 2001). The surplus and deficit of N influence nutrient

121   uptake and transformation capacity of leaves, which in turn affects the productivity of

122   the whole community (Lu et al., 2010; Nakaji et al., 2001). A detailed analysis of leaf

123   functional traits respond to N addition at the species level is crucial to understand the

124　evolution of plant communities (Tang et al., 2021; Ye et al., 2022). There are thresholds

125　for N uptake by different plants, both redundancy and deficiency of N can alter the

126　photosynthetic carbon assimilation capacity and affect the carbon allocation pattern in

127　plants (Luo and Zhou, 2019; Mengesha, 2021). The response of leaf functional traits to

128　N addition is highly dependent on the physiological characteristics of the plant species

129　itself (Bauer et al., 2000; Elvir et al., 2006). Indeed, many forest studies have indicated

130　that different species would respond and adjust their leaf functions in different ways to

131　optimize the excess N input (Ye et al., 2022). However, it is unclear how the response

132　of dominant species in evergreen broad-leaf forest to fertilizer application is

133　accompanied by changes in functional leaf characteristics (e.g., nutritional and

134　physiological characteristics). Generally, the effects of N addition on the local

135　adaptation of individual tree species, leaf anatomy and chlorophyll fluorescence are

136　often neglected and not considered.

137　　　Numerous studies have reported that environmental variation is the main driver of

138　phenotypic plasticity, thereby influencing differences in plasticity between species or

139　traits (Arnold et al., 2022; Valladares et al., 2000b). Plasticity itself involves trade-offs

140　and differences in plasticity among species provide strong evidence for adopting

141　different resource strategies (Weih et al., 2021). Accordingly, plasticity of different trait

142　types respond differently to environmental changes, White (1979) argued that the

143　physiological characteristics of leaves are the most plastic. The experimental essays

144　have shown that deciduous tree species have high leaf plasticity and tend to adopt a

145　rapid acquisition strategy (Zhang et al., 2020). Under changes in N availability, changes

146　in resource use strategies of different species as indicated by changes in leaf functional

147　traits may lead to further changes in community composition (Van Houtven et al., 2019).

148　There is uncertainty, however, about how the plasticity of different leaf functional trait

149 types will respond to N addition and what strategies different species will adopt in

150 response to changes in N addition.

151   A random block design was used in 2017 to study the responses of dominant

152 species to N addition in a secondary evergreen broad-leaf forest in subtropical China.

153 Five dominant species (*Castanopsis eyrei*, *Quercus glauca*, *Schima superba*,

154 *Rhododendron delavayi* and *Eurya muricata*), typical of subtropical evergreen broad-

155 leaf forest in China, were selected to examine the response to different levels of N

156 addition on 25 leaf functional traits including leaf morphology, nutrient, photosynthetic

157 physiology and anatomical structure. We proposed the following three hypotheses: (1)

158 the effect of HN treatment on the leaf functional traits of different species would greater

159 than with LN treatment, (2) leaf functional traits with high plasticity would respond

160 more significantly to N addition, and (3) the effect of N addition on leaf functional traits

161 would be species-specific, and species with high plasticity of leaf functional traits

162 would adopt acquisitive strategy. Overall, the results will contribute to understanding

163 and predicting uncertainties in community composition under elevated N deposition.

164

165 **2. Material and methods**

166 **2.1. Site description**

167 Our study area was conducted at Yangjifeng Nature Reserve with an area of

168 approximately 10946 ha in Guixi (27°51′10″- 28°02′20″N, 117°11′30″- 117°28′40″E),

169 Jiangxi Province, China (Figure 1). The region has a high forest cover of up to 99.7%,

170 and it is a typical representative of subtropical evergreen broad-leaf forest in China. The

171 site has a subtropical monsoon climate with the mean annual temperature is 14.4 °C

172 and a natural nitrogen deposition rate of about 43 kg N ha^{-1} yr^{-1}. The mean annual

173 precipitation and frost-free period per year are 2114 mm and 268 days, respectively.

174   The area of natural evergreen broad-leaf forest accounts for 68% of the entire protected

175   area. The trees, shrubs, and herb species are diverse and mainly consist of species of

176   Fagaceae, Theaceae and Ericaceae. The study site is located at an altitude of 690-785

177   m asl, with topographic direction and slope of southeast and 26.3°, respectively.

178   Evergreen broad-leaf forest average diameter at breast height (DBH) values and tree

179   heights were 6.54 cm and 13.7 m, respectively. Our previous studies showed that the

180   soil is predominantly typical red soils and the basic characteristics of the surface soils

181   (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:

182   0.33 ± 0.02 g kg^{-1}; soil total C: 74.56 ± 13.20 g kg^{-1}.

183

184   **2.2. Experimental design**

185   In July 2017, we initiated an experiment involving three N addition treatments (in

186   three replicates): 0 kg N ha^{-1} yr^{-1} (CK), 50 kg N ha^{-1} yr^{-1} (LN) and 100 kg N ha^{-1} yr^{-1}

187   (HN), excluding natural N deposition. The N addition gradient was based on both the

188   present N deposition level and its further increase in the future. Totally, nine 15×15 m

189   plots were established in a completely randomized design. Each plot was surrounded

190   by at least a 10 m wide buffer strip to the next plot. For the N addition, $CO(NH_2)_2$

191   dissolved with water, at the beginning of every three months, and a double-nozzle

192   sprayer was used to spray the forest floor manually and uniformly back and forth in all

193   sample plots from July 2017, continuous N addition for 2 years. All control plots only

194   received the same amount of water.

195

196   **2.3. Plant species and sampling**

197   Samples were collected in July 2019. Five dominant species were selected for this

198   study in subtropical evergreen broad-leaf forest. Within each plot, relative importance

199 values (RIV) were measured as the average of relative density, relative frequency and

200 relative base area at breast height of each species (Mao et al., 2018), RIV were 0.1468

201 for *C. eyrei*, 0.1218 for *S. superba*, 0.1099 for *Q. glauca*, 0.0316 for *R. delavayi* and

202 0.0245 for *E. muricata* and the five species are the most abundant tree and shrub species

203 in the evergreen broad-leaf forest in the nature reserve. Three representative individuals

204 from each species were selected in each plot, 20- 30 healthy, fully expanded and sun-

205 exposed leaves were selected from the crown of the tree from each individual. The

206 collected leaves were immediately placed in plastic bags and stored at 4°C, transported

207 to the laboratory with numbers for further analysis.

208

209 2.4. Measurements of leaf morphology and chemical

210 After photosynthetic characterization were measured, 20 collected leaves were used for

211 leaf morphological traits and element analysis. An electronic vernier caliper was used

212 to measure leaf thickness (LT, mm) by averaging three measurements (top, middle,

213 bottom), avoiding the major veins. Leaf area (LA, cm^2) was measured using Epson

214 V370 (Epson). The saturated fresh weight (Lsmass, g) was determined after submerging

215 a leaf in deionized water for 24 h. All the samples were oven-dried at 65°C for 48 h and

216 then weighed (Ldmass, g) to calculate leaf dry matter content (LDMC, g/g) and specific

217 leaf area (SLA, cm^2/g). Leaf tissue density (LTD, g/cm^3) was calculated as Ldmass/

218 (LA × LT).

219   The leaf carbon and nitrogen concentration per unit mass (LCC and LNC, mg/g)

220 were determined on the same dried leaves with the Element Analyzer (VARIO EL III

221 Element Analyzer, Elementar). The leaf phosphorus concentration per unit mass (LPC,

222 mg/g) was measured using the continuous flow analyzer (San++, Skalar) after H_2SO_4-

223 $HClO_4$ (4:1, v: v) digestion.

224   Isotope values were reported using conventional δ-notation according to the

225   following formula: $\delta^{13}C = [(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{standard}]/(^{13}C/^{12}C)_{standard}$ (Grams et

226   al., 2007).

227

228   **2.5. Measurements of leaf photosynthetic parameters**

229   We carried out gas-exchange measurements in July 2019 using a Li-6800 portable gas-

230   exchange system (Li-Cor, Lincoln, USA). In each sample plot, three standard trees were

231   selected for each five dominant tree species, six to eight leaves of each standard tree

232   with the upper and middle canopy expanded, healthy and fully matured were measured

233   from 9:00 to 11:00 a.m. on a clear and windless day. The gas exchange parameters of

234   species in the same treatment were measured on the same day. Net photosynthetic rate

235   (Pn), transpiration rate (Tr) and stomatal conductance (Gs) were obtained from the five

236   species. Photosynthetic N use efficiency (PNUE) was estimated as the ratio of Pn to

237   leaf N concentration, and photosynthetic P use efficiency (PNUE) was estimated as the

238   ratio of Pn to leaf P concentration, as recommended by Qin et al. (2021).

239   After remaining in complete darkness overnight, we measured minimal and

240   maximal fluorescence yield (Fo and Fm) in dark-adapted leaves, respectively. We

241   recorded the steady-state yield of fluorescence (Fs) and maximal light-adapted

242   fluorescence yield (Fm') for the leaves irradiated by an actinic light for 20 min

243   continuously. The minimal fluorescence yield (Fo') in light-adapted state was measured

244   after 5s of far-infrared illumination, which was performed after the actinic light had

245   been turned off. The variable fluorescence (Fv) is calculated from the difference

246   between Fm and Fo. The chlorophyll fluorescence parameters were calculated using the

247   following formulas (Kramer et al., 2004):

248    $$Fv/Fm = (Fm - Fo)/Fm \qquad (1)$$

249        $Fv'/Fm' = (Fm' - Fo')/Fm'$ (2)

250        $\Phi PSII = (Fm' - Fs)/Fm'$ (3)

251        $NPQ = Fm/Fm' - 1$ (4)

252        $qP = (Fm' - Fs)/(Fm' - Fo')$ (5)

253        $ETR = \Phi PSII \times PPFD \times 0.5 \times 0.85$ (6)

254   where Fv/Fm represents the maximal photochemical efficiency of photosystem II

255 (PSII), Fv'/Fm' is excitation energy capture efficiency of PSII reaction centers, ΦPSII

256 represents actual photochemical efficiency of PSII, qP and NPQ represent

257 photochemical and non-photochemical quenching, respectively, ETR (electron transfer

258 rate), where the coefficient 0.85 was the assumed average leaf absorbance and the

259 coefficient 0.5 is the assumed proportion of absorbed photon allocated to PSII.

260

261 **2.6. Measurements of leaf anatomical structure**

262 The healthy and mature leaf sections (5mm×4mm) were collected from the middle of

263 the leaf near the vein, and fixed in an FAA solution (38% formaldehyde, glacial acetic

264 acid, 70% alcohol, 5:5:90, v/v/v) for 24 h at 4°C. Following that, the leaf sections were

265 dehydrated with graded ethanol series. We stained the sections in 0.05% toluidine blue

266 solution for 3 minutes after slicing them with a microtome (Falcioni et al., 2017).

267 Photographs were observed under a light photographic microscope (ZEISS Imager A1).

268 We used the software provided in Axio Vision Release 4.5 SP1 to measure leaf up-

269 epidermal thickness (UET), down-epidermal thickness (DET), palisade tissue thickness

270 (PT) and spongy tissue thickness (ST) for averaged over multiple measurements. The

271 palisade and spongy parenchyma ratio are as follows: PT/ST.

272

273 **2.7. Data processing and analysis**

274 In order to investigate the effects of N addition, species, and their interaction on leaf

275 functional traits, linear mixed effects modelling was employed. N addition, species and

276 their interactions were considered fixed effects, while random effects included different

277 individuals. We used the lme function in the lmerTest package to analyze linear mixed

278 effects models in R version 4.2.2.

279   One-way analysis of variance followed by multiple least significant difference

280 comparisons was used to compare the 25 leaf functional traits for different N addition

281 treatments in the evergreen broad-leaf forest. An independent T-test was used to

282 compare the indicators above between five dominant species. All the results reported

283 were significant at $P < 0.05$. The data are presented as the mean ± standard error (SE).

284 The statistical analysis was performed in SPSS 22.0 software, and graphs were created

285 with Origin 2022 software. An index of phenotypic plasticity was calculated as the

286 difference between the minimum and maximum values divided by the maximum value

287 (Cheplick, 1995). The relative effect (RE) of leaf functional traits on N addition were

288 calculated as the difference between the mean value of the treatment sample and the

289 mean value of the control sample divided by the value of the control sample (Duval et

290 al., 2011), RE_{LN} is the relative effect under low N treatment and RE_{HN} is the relative

291 effect under high N treatment.

292   Principal component analysis (PCA) was performed to investigate the effect of N

293 addition on the resource acquisitive strategy of 25 leaf functional traits of each species

294 by using the "factoextra" package in R (version 4.2.2). Permutational multivariate

295 analysis of variance analysis (PERMANOVA) was performed on the traits of

296 different species using the "adonis" function of the "vegan (Oksanen et al., 2019)"

297 package. The results are visualized through packages "ggplot2" and "ggpubr".

298 Structural equation modeling (SEM) was carried out to analyze the effects on the

299 photosynthetic capacity of different species through pathways between leaf

300 functional traits under the influence of N addition. SEM analyses were performed in

301 R version 4.2.2 (R Development Core Team).

302

303 ## 3. Results

304 ### 3.1. Leaf morphological and nutrient traits

305 With the pooled data of the evergreen broad-leaf forest by N addition experiments,

306 mixed linear model analysis showed that SLA and LTD significantly responded to the

307 N addition treatment ($P < 0.05$), LTD and LDMC significantly varied among all study

308 species ($P < 0.05$), LDMC was significantly affected by the interaction of N addition

309 and species (Fig. 2). When analyzing by species, relative to CK, the SLA of *S. superba*

310 was significantly increased under LN treatment (Fig. 2A). We found that LTD

311 significantly decreased in *C. eyrei and S. superba* under N addition (Fig. 2B). Relative

312 to CK, LDMC in *Q. glauca* was increased in response to HN treatment (Fig. 2C).

313 The N addition significantly affected the LNC ($P < 0.05$), all nutrient traits in this

314 study were significantly different between species, and the interaction effect between

315 N addition and species was significant on LCC, LNC, LPC and C/N. In terms of the

316 responses of individual species to N addition treatments, LNC in *S. superba* and *E.*

317 *muricata* were elevated in response to LN, but *R. delavayi* had significantly lower LNC

318 in HN treatment (Fig. 3B). N addition significantly decreased LPC in *C. glauca* and *R.*

319 *delavayi*, relative to CK, the LPC of *S. superba* was increased by 24.1% in LN treatment

320 (Fig. 3C). C/N was significantly decreased in *S. superba* and *E. muricata* under LN

321 treatment (Fig. 3D), but increased N/P and $\delta^{13}C$ in *S. superba* (Fig. 3E, F).

322

323 ### 3.2. Leaf photosynthetic performance

324   The mixed linear model indicated that N addition significantly affected the Pn and

325   PPUE, all photosynthetic characteristics significantly varied among all study species,

326   the interaction of N addition and species affected all indicators except Tr (Table 2).

327   Relative to the CK, Pn and Tr were significantly increased in *S. superba* and *E. muricata*

328   under HN treatment. Gs of *C. eyrei* and *E. muricata* were increased in response to HN,

329   in contrast, *R. delavayi* had significantly lower Gs in HN treatment. The analysis

330   showed that PNUE of *Q. glauca* and *S. superba* were significantly increased by 27.9%

331   and 72.8% in LN and HN treatment, respectively. PPUE of *C. eyrei* and *S. superba* was

332   increased in HN treatment, while the PPUE of *R. delavayi* was decreased by 29.4% in

333   LN treatment.

334

335   **3.3. Leaf chlorophyll fluorescence Parameters**

336   We found significant effects of N addition and species, and also significant

337   interactions of them, on ΦPSII, ETR and qP (Table 3). The Fv/Fm of the five species

338   did not show significant differences among all N addition treatment. For *C. eyrei*, the

339   Fv'/Fm' increased slightly (11.5%) treated by LN compared with the CK treatment.

340   ΦPSII and ETR significantly increased in *Q. glauca, S. superba* and *R. delavayi* treated

341   by HN, while for *C. eyrei*, ETR was significantly increased by 37.2%. NPQ in *C. eyrei*

342   was decreased by 29.0% in response to LN treatment, and qP of *Q. glauca* was

343   increased by 16.8% in HN treatment. N addition had no effect on chlorophyll

344   fluorescence parameters of *E. muricata*.

345

346   **3.4. Leaf anatomical structure**

347   N addition, species and their interaction had significant effects on UET and ST (P

348   < 0.01), DET, UET and ST were varied by species, the interaction of N addition and

349 species influenced all five indicators (Fig. 4, Fig S2). UET of *C. eyrei* and *Q. glauca*

350 declined under HN treatment, but *E. muricata* is the opposite (Fig. 4A). Compared with

351 the CK, the DET of *R. delavayi* and *E. muricata* decreased but that of *Q. glauca*

352 increased in response HN (Fig. 4B). Compared to CK treatment, ST of *S. superba* and

353 *R. delavayi* decreased by 37.7% and 29.5%, respectively (Fig. 4C). With increasing N

354 addition, the values of PT in *C. eyrei* and *S. superba* were decreased (Fig. 4D). LN

355 treatment greatly increased PT and PT/ST in *E. muricata* (Fig. 4D, E).

356

357 **3.5. Phenotypic plasticity and relative effects of different species**

358 We divided the 25 leaf functional traits into five trait categories, at the trait level,

359 the leaf anatomical structure traits had the highest average plasticity (0.246) but the leaf

360 nutrient traits had the lowest average plasticity (0.128) on average for all species (Fig.

361 5F). Leaf anatomical structure had the least interspecific variation, while the leaf

362 morphological traits were the opposite. Overall, the average plasticity index ranking of

363 the five species: *S. superba* > *Q. glauca* > *E. muricata* > *C. eyrei* > *R. delavayi*. In this

364 order, the highest plasticity indicators for different species were PNUE, qP, DET,

365 PT/ST and LTD. The highest plasticity was observed for leaf morphological traits of *S.*

366 *superba* and leaf anatomical structure traits of the other four species.

367 Relative to RE_{LN}, RE_{HN} for leaf functional traits of *S. superba*, *Q. glauca* and *C.*

368 *eyrei* was greater than other species, whereas the opposite results were observed in *E.*

369 *muricata* and *R. delavayi* (Fig. 6F). Moreover, the leaf functional traits of *R. delavayi*

370 had negative relative effects on N addition and the consistent results with *S. superba*

371 under LN treatment. The RE_{LN} of the leaf functional traits was the highest in *E. muricata*

372 (7.99) and RE_{HN} of the leaf functional traits was the highest in *Q. glauca* (11.59). For

373 all five species, the maximum values of RE_{LN} or RE_{HN} were found in leaf

374 photosynthesis and chlorophyll fluorescence variables, indicating that leaf

375 photosynthesis was more responsive than other leaf traits to N addition.

376

377 **3.6. Effect of N addition on the resource trade-off strategy of different species**

378 Based on the autocorrelation among the photosynthetic physiological indicators,

379 PCA analysis was first performed to screen out the indicators with significant

380 contributions as Pn and $\Phi PSII$ (Fig S1). The results of PCA of leaf functional traits

381 (Fig. 7) reveal the resource trade-off strategies of the different species. PC1 explained

382 21.4% of the variation in leaf functional traits, and showed strong positive loadings on

383 LNC, LPC, Pn and SLA, indicating the resource acquisitive strategy. By contrast, while

384 traits representing the resource conservative strategy of LTD, LDMC and C/N showed

385 strong negative loadings. Considering species, *S. superba* and *Q. glauca* were occupy

386 the acquisitive side, while *E. muricata*, *C. eyrei* and *R. delavayi* distributed on the

387 conservative side. PERMANOVA analysis showed a non-significant difference

388 between N addition treatment ($P = 0.458$, Fig. 7A), whereas there was a significant

389 difference among different species ($P = 0.001$, Fig. 7B).

390 The results of structural equation modeling revealed that N addition affected the

391 Pn of five species through different pathways (Fig. 8). N addition could indirectly

392 decline the Pn by lowering the allocation of foliar N/P in *C. eyrei* (Fig. 8A). For *Q.*

393 *glauca*, N addition had an indirect positive effect on the Pn its negative effect on the

394 LPC (Fig. 8B). The N addition could either directly reduce Pn or indirectly increase it

395 by increasing $\delta^{13}C$ and PPUE in *S. superba* (Fig. 8C). N addition indirectly increased

396 Gs and promoted Pn in *E. muricata* (Fig. 8E).

397

398 **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). Leaf traits that are easily measured 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

424 light energy utilization and enhancing the Pn (Table 2). These results are consistent

425 with those of previous studies on woody plants (He et al., 2018; Zhu et al., 2020).

426   In terrestrial ecosystems, the concentration of foliar elements (C, N, P) and their

427 ratios are habitually linked to the balance between plant metabolism and nutrient supply

428 (Mao et al., 2018; Ye et al., 2022). In this study, we found empirical evidence of

429 interspecific differences in leaf nutrients changes among five different species as a

430 response to N addition. Tessier and Raynal (2003) reported that the elements associated

431 with the limitation of plant growth and development could gradually change with the

432 addition of N. Under N addition, LNC and LPC were significantly higher in *S. superba*,

433 but the effect on *R. delavayi* was the opposite (Figure 3B, C). The possible reason for

434 this difference in response was that the effects of N addition on N and P uptake might

435 vary depending on specific species (Liu et al., 2020; Ye et al., 2022). Also, ratios of

436 C:N represent the ability of plants in assimilating C when simultaneously absorbing N,

437 and were considered to be effective to reflect the growth status of plants (Vrede et al.,

438 2004). We observed that LNC increased and C: N decreased significantly in *S. superba*

439 and *E. muricata*, and LCC of both were not changed under N addition. However, the

440 LNC and C: N of *R. delavayi* had the opposite variation from the above. Part of this

441 result could be attributed to the significant effect of N addition on LNC, and it also

442 suggested that assimilated C allocation did not change, and N uptake and C assimilation

443 in the above three plants under N addition might be decoupled (Vrede et al., 2004; Ye

444 et al., 2022). The Growth Rate Hypothesis (GRH) suggests that the plant growth rate

445 was significantly and negatively correlated with C:N (Elser et al., 2007), consistent with

446 these earlier studies, it showed that the N addition is beneficial for *S. superba* and *E.*

447 *muricata* to improve the N utilization and move towards the trend of higher growth. N:

448 P ratio in leaves is widely recognized as a reliable indicator to characterize nutrient

449   limitation (Elser et al., 2007; Liu et al., 2020). Previous studies suggested that N:P ratios

450   greater than 16 and P concentrations lower than 1.0 mg/g reflected P limitation, which

451   was consistent with our study (N/P: 17.67 ± 0.39). This is consistent with the majority

452   of studies concluded that P rather than N is the main factor limiting plant growth in

453   subtropical China (Liu et al., 2018; Vrede et al., 2004). Besides, our results showed that

454   N addition significantly increased the N:P of *Q. glauca* and *S. superba*, however, the

455   LNP of *S. superba* increased while the LNP of *Q. glauca* decreased under N addition,

456   suggesting that different plants have different levels of investment on P uptake and also

457   distinct mechanisms of P uptake, allocation and utilization under N addition (Tessier

458   and Raynal, 2003). The elevated LPC of *Q. glauca* indicated N addition increased the

459   P utilization efficiency. $\delta^{13}C$ has been used for intrinsic water use efficiency and to

460   determine the long-term physiological regulation of plants in response to environmental

461   changes (Liang et al., 2020). With reference to past studies, we attempted to relate $\delta^{13}C$

462   with both instantaneous water efficiency (WUEi) (Zhu et al., 2019) and the long-term

463   water efficiency (long-WUE) (Liang et al., 2020). Nonetheless, our experiment showed

464   that N addition did not significantly change the $\delta^{13}C$, WUEi and long-WUE values of

465   different species, only the $\delta^{13}C$ of the *S. superba* increased under HN treatment (Fig.

466   3F). Thus, it is surmisable that the experimental area has a relatively sufficient water

467   supply, and indicating a minor impact of N addition on plant carbon-water relations.

468   Increasing N effectiveness did not cause plants to compete for water resources, but

469   instead to compete for other resources (e.g., light resources), which agree with N

470   addition experiment conducted on dominant woody species in an evergreen broad-leaf

471   forest (Liu et al., 2020).

472

473   **4.1.2 Effect of N addition on leaf photosynthesis traits**

474 The N in leaves is mainly distributed in the photosynthetic protein complex and

475 chloroplast, which can affect the intensity of photosynthesis and related parameters

476 (Kong et al., 2016; Nakaji et al., 2001). As expected, the effect of N addition on plant

477 photosynthesis varied according to the intrinsic characteristics of the species. Pn is an

478 important indicator of photosynthetic productivity and strength has been reported in

479 many previous studies (Mao et al., 2018; Zhang et al., 2015). Our results showed that

480 N addition, species and their interaction had significant effects on Pn, which suggested

481 that the mechanism of the effect of N addition on photosynthesis can be more clearly

482 characterized by the high or low Pn. The variation of Gs determined the rate at which

483 O_2, CO_2 and water vapor moved in and out of plant cells (Mengesha, 2021), a meta-

484 analysis showed that N addition might affect leaf photosynthesis by affecting leaf

485 stomatal conductance (Liang et al., 2020). In this experiment, HN treatment

486 significantly increased Pn, Tr and Gs in *E. muricata*, but Pn and Tr of *S. superba* were

487 significantly higher at HN treatment, and Gs of *S. superba* did not change (Table 2).

488 The former is consistent with the results of many studies that have observed how N

489 addition may have promoted stomatal opening, and accelerated transport of

490 photosynthetic CO_2, thus exhibiting high stomatal conductance and leading to an

491 increase in Pn (Sun et al., 2018). The latter result has not previously been described,

492 which may be because the increase in Pn due to an increase in available N in the leaves

493 as a result of N addition. Such as the HN treated trees had used the surplus N to

494 synthesize more Rubisco, and thus chloroplast activity was elevated. It has been

495 demonstrated that stomatal factors dominate when stomata open to elevate

496 photosynthesis and transpiration (Mengesha, 2021). Non-stomatal factors were thought

497 to act in conjunction with mesophyll conductance and leaf biochemistry, which respond

498 to photosynthesis from the perspective of biochemical processes (Qin et al., 2021; Ye

499 et al., 2022). We found *E. muricata* was mainly regulated by stomatal factors, on the

500 contrary, the variation in *S. superba* was more consistent with the influence of non-

501 stomatal factors under N addition. The partitioning of N in Rubisco and cell wall

502 affected PNUE, N allocation to cell membrane proteins, water-soluble proteins and free

503 amino acid content could also be factors that affected the high PNUE (Qin et al., 2021).

504 Many studies have confirmed that plant leaves with higher PNUE and PPUE tend to be

505 more tolerant to changes in the external environment (Byeon et al., 2021; Novriyanti et

506 al., 2012), which enables the leaves to make full use of their internal N and P throughout

507 the growth period, providing power for photosynthesis (Liang et al., 2020). It is

508 generally accepted that increased N availability in plants under N addition resulted in a

509 slight increase in leaf N and P concentration of species, thereby increasing the PNUE

510 and PPUE of the leaves (Liu et al., 2018; Mao et al., 2018). Consistent with this view,

511 our study revealed N addition significantly increased the PNUE and PPUE of *S. superba*,

512 *Q. glauca* and *C. eyrei*, respectively. We hypothesize that the maximum Pn of *S.*

513 *superba* at N addition, at least in part, consequence of the increase of PNUE and PPUE.

514 Further certified that N addition increased nutrients availability and had better uptake

515 of N for some tree species leaves. This outcome is contrary to that of Chen et al. (2015)

516 who found forests in Dinghushan, a subtropical area in southern China, where plant

517 photosynthesis has been demonstrated to respond moderately even negatively to N

518 addition.

519 Chlorophyll fluorescence during photosynthesis can be considered as an excellent

520 and effective parameter for characterizing plant adaptations (Melo et al., 2017; Stirbet

521 et al., 2018). However, its response to external environmental changes depends on the

522 genotype of the plant itself (Larcher, 1995; Lovelock et al., 1994). ΦPSII characterizes

523 the actual photochemical quantum yield, higher ΦPSII indicates the higher ability of

524 leaves to convert photon energy into chemical energy (Larcher, 1995; Porcar-Castell et

525 al., 2008). ETR characterizes the fast and slow electron transport rate in leaves, increase

526 in ETR means more fast electron transport and greater proportion of captured light

527 energy is transferred to photosynthesis (Melo et al., 2017). Our study suggested that

528 ΦPSII and ETR of *Q. glauca*, *S. superba* and *R. delavayi* were significantly increased

529 under HN treatment, indicating that HN treatment promotes the light energy conversion

530 efficiency of the above three dominant plants. Through accelerating electron capture

531 and delivery capability to further stimulate the potential active center of PSII thus to

532 enhanced photochemical efficiency of the reaction center, which is consistent with Qin

533 et al. (2021).

534 Our results partly supported hypothesis (1) that the effects of HN treatment on the

535 leaf functional traits of different species would be greater than with LN treatment (Fig.

536 6F). The positive relative effect of HN treatment on leaf functional traits was greater in

537 *Q. glauca*, *S. superba* and *C. eyrei*, indicating a higher threshold of N availability for

538 these species. It can rapidly convert excess N into energy substances that promote

539 growth and development when the availability of N increases (Elvir et al., 2006; Liu et

540 al., 2020). Moreover, the functional traits of some species (e.g., *R. delavayi*) may be

541 saturated at the background of N addition, therefore the response to N addition was not

542 significantly increased or even negative. This difference is not only a result of the rate

543 of uptake, but also depends on the difference in the ability of the plant species to absorb

544 N during growth (Chen et al., 2015; Weih et al., 2021). Sun et al. (2018) reported that

545 the enhanced photosynthetic capacity of leaves is usually associated with high N

546 availability, explaining the relative effect of leaf photosynthetic traits were greater than

547 other leaf functional traits among the five species in this study (Fig. 6), and indicating

548 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

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

574 (White, 1979; Xu et al., 2015). The results found the greatest plasticity of leaf functional

575 traits of *S. superba*, followed by *Q. glauca* (Fig. 5). In line with the hypothesis (3), our

576 study provided new empirical evidence that species with high plasticity in leaf

577 functional traits are more likely to adopt acquisition strategies, which was consistent

578 with previous studies on different types of species (Valladares et al., 2000a; Zhang et

579 al., 2020). Firstly, based on life history strategies, *S. superba* and *Q. glauca* were light-

580 loving plants that can be the first to sense changes in their environment (including N

581 addition) and make physiological adjustments, this inference is supported by results of

582 two previous analyses (Liu et al., 2018; Mao et al., 2018). Secondly, among the five

583 species, *S. superba* and *Q. glauca* had greater leaf area. It is generally believed that leaf

584 area is directly proportional to LNC and plants with larger leaf area will absorb more N

585 for photosynthesis. Actually, many reports on higher foliar N and stronger

586 photosynthetic capacity occurred in the situation with more access to N resources

587 (Liang et al., 2020; Liu et al., 2018; Sun et al., 2018). Therefore, *S. superba* and *Q.*

588 *glauca* are more able to respond under N addition increasing its uptake and improving

589 its photosynthetic capacity. Different species select different resource strategies under

590 N addition, reflecting the species specificity of leaf functional traits in response to

591 changing resource environments (Valladares et al., 2000a; Zhang et al., 2020), which

592 in turn is expected to influence higher levels of the organization, such as community

593 and ecosystem composition (Van Houtven et al., 2019).

594 Interestingly, our SEM results showed how the association of leaf functional traits

595 affects the photosynthetic capacity of different tree species under N addition conditions.

596 Five species affect Pn through different pathways. The N addition could directly

597 promote Pn of *S. superba*, *S. superba*, *Q. glauca* and *C. eyrei*, indirectly affect Pn

598 through leaf phosphorus-related nutrient traits. *E. muricata* could indirectly affect Pn

599 through stomatal factors, but the N addition could not directly or indirectly affect Pn of

600 *R. delavayi*. This provides species-specific linkages among nutrient availability, leaf

601 traits and photosynthetic rate.

602

603 **4.3 Prediction under the context of elevated N deposition**

604   We predict that subtropical forest communities dominated by resource-acquisitive

605 species (*S. superba* and *Q. glauca*) will undergo an ongoing transformation as a result

606 of long-term elevated atmospheric nitrogen deposition. The results Liu et al. (2018)

607 contradict this assumption, which suggests that canopy N addition treatments affect the

608 N metabolism of woody plants, leading to a continuous transformation of subtropical

609 forests into small tree and shrub communities. One reason may be that the dominant

610 species and N deposition pattern are quite different with our experiment. Additionally,

611 resource-acquisitive species (*S. superba* and *Q. glauca*) have a higher degree of

612 phenotypic plasticity than resource-conservative species, which enables them to occupy

613 a broader ecological niche (Richards et al., 2005). It was found that acquisitive species

614 with higher LNC tended to have a faster photosynthetic rate and larger SLA in

615 conditions of high N deposition (Heberling and Fridley, 2016). It is therefore possible

616 that N addition would increase photosynthetic efficiency and resource acquisition

617 capabilities, thereby allowing plants to cope with high nitrogen levels more effectively.

618 Several decades later, resource-acquisitive species gradually occupy the main position,

619 while resource-conservative species gradually decrease. In addition, other studies have

620 demonstrated that N enrichment is also beneficial to tall species, since taller ones have

621 an advantage in obtaining light resources, thereby resulting in the turnover of species

622 and changing the composition of the community. It is likely that short-statured species

623 will gradually become more scarce as competition for light and nutrients increases

624 (Hautier et al., 2009; Kaarlejärvi et al., 2017).

625

626 **5. Conclusions**

627 Our results highlighted that the effect of N addition on leaf functional traits of dominant

628 species in subtropical evergreen broad-leaf forest was species-specific. After

629 investigating, we found that the response to the HN treatment was greater in leaf

630 functional traits of *Q. glauca*, *S. superba* and *C. eyrei* compared to the LN treatment.

631 The phenotypic plasticity of leaf anatomy structure was highest under N addition, and

632 leaf photosynthetic properties were more sensitive to N addition. Simultaneously, we

633 further found that *S. superba* and *Q. glauca* exhibited greater phenotypic plasticity in

634 terms of morphological structure, nutrient partitioning, and photosynthetic

635 physiological properties than other species under the influence of N addition and

636 preferred to adopt resource-acquisitive strategy. In conclusion, species with the

637 resource-acquisitive strategy showed greater plasticity in leaf functional traits than

638 species with the resource-conservative strategy. Therefore, species with resource-

639 acquisitive strategy were "more favored" and will dominate the future community

640 composition in the context of N deposition. Overall, our research has important

641 implications for the conservation and management of subtropical forest ecosystems, as

642 well as for predicting species composition and succession under the context of N

643 deposition.

644 Furthermore, the present study was based on the results of a two-years study of N

645 addition. The sensitivity and adaptation of each tree species may change with the

646 duration of N deposition. More carefully study should be combined with the response

647 of plant root traits to N addition in the future.

648

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