
This is the **accepted version** of the journal article:

Zou, Yuxing; Li, Baoyin; Peñuelas, Josep; [et al.]. «Response of functional traits in *Machilus pauhoi* to nitrogen addition is influenced by differences of provenances». *Forest Ecology and Management*, Vol. 513 (June 2022), art. 120207. DOI 10.1016/j.foreco.2022.120207

This version is available at <https://ddd.uab.cat/record/299928>

under the terms of the  license

1 **Response of functional traits in *Machilus pauhoi* to nitrogen addition is influenced by differences**
2 **of provenances**

3 Yuxing Zou^{1, 2}, Baoyin Li^{2, 3 *}, Josep Peñuelas^{4, 5}, Jordi Sardans^{4, 5}, Hua Yu^{1, 6}, Xiaoping Chen ^{1, 3}, Xingyu

4 Deng ^{1, 3}, Dongliang Cheng^{1, 2, 3}, Quanlin Zhong^{1, 2, 3*}

5 ¹ College of Geographical Science, Fujian Normal University, Fuzhou, Fujian Province 350007, China,

6 ² Fujian Provincial Key Laboratory of Plant Ecophysiology, Fujian Normal University, Fuzhou, Fujian

7 Province 350007, China,

8 ³ State Key Laboratory of Subtropical Mountain Ecology (Ministry of Science and Technology and

9 Fujian Province funded), Fuzhou, Fujian Province 350007, China

10 ⁴ Global Ecology Unit, CSIC, CREAF□CSIC□UAB, 08193 Bellaterra, Catalonia, Spain

11 ⁵ CREAF, 08193 Cerdanyola del Vallès, Catalonia, Spain

12 ⁶ Ocean College, Minjiang University, Fuzhou, Fujian Province 350007, China

13 * Correspondence author

14 E-mail: qlzhong@126.com (Q.Z)

15 lby938@163.com (B.L)

16 **Abstract:**

17 Comprehensive studies on the response of whole plant functional traits to nitrogen deposition can
18 provide insight into the resource acquisition strategies of plants. However, current studies on nitrogen
19 deposition have mainly focused on leaves or roots.

20 We conducted nitrogen deposition simulation experiments with *Machilus pauhoi* (Lauraceae) from
21 five provenances in a common garden experiment in the southeast of China. We measured 29 traits
22 (biomass, phenology traits, and nutrient concentrations) of leaves, stems, and roots in response to
23 nitrogen addition and selected 24 distinct important functional traits related to resource acquisition
24 strategies of the whole *M. pauhoi* seedlings.

25 We found that N (Nitrogen) addition significantly increased the biomass of *M. pauhoi* seedlings and
26 altered biomass allocation among organs. The response of the leaf, stem and root traits to nitrogen
27 addition was not always consistent among the different provenances of *M. pauhoi* seedlings. A uniform
28 variation pattern of the three organs was found in the C (Carbon) and P (Phosphorus) concentrations, as
29 well as N:P ratio. In contrast, N concentration, the C:N ratio and the phenotypic trait specific leaf area
30 (SLA) and specific root area (SRL) did not respond in the same direction at the organ level.

31 We concluded that nitrogen addition alters the biomass allocation pattern of *M. pauhoi* and the
32 resource acquisition capacity of above- and below-ground organs. N concentrations and C:N ratio may
33 play a key role in this regulatory process. Overall, N addition increased leaf mass fraction (LMF) and
34 SRL, and instead decreased SLA and root mass fraction (RMF) of *M. pauhoi*. There are also differences
35 in biomass allocation and resource acquisition patterns between the different provenances. AF and SC
36 seedlings can be preferred provenances for *M. pauhoi* due to their balanced resource acquisition strategy
37 and high phenotypic plasticity, respectively.

Our study may contribute to a more comprehensive understanding of how N deposition affects the plant as a whole and provide a theoretical basis for precise nutrient management of *M. pauhoi* seedlings and their plantations in the context of nitrogen deposition.

Keywords: leaf, stem, root, nitrogen deposition, *Machilus pauhoi*

Introduction

Nitrogen (N) is an essential nutrient that regulates plant growth and development (Güsewell, 2004; Vitousek, 2004). The optimal partitioning theory (OPT) predicts that plants allocate more biomass to the organ capturing the most limiting resource (Smith & Martin *et al.*, 2020). Plant economics spectrum (PES) suggests that the leaf, stem, and root traits are correlated with each other and consistently resource-acquisitive or resource-conservative for all resources (Reich, 2014). These two theories may be contradictory in terms of resource utilization and allocation of plants due to the difference in resource pressure faced above and below ground. Therefore, it is worth exploring which of the two theories is applicable to plant resource acquisition strategies and biomass allocation patterns in the context of nitrogen deposition.

Leaves and roots are essential resource acquisition organs of plants for leaves absorb CO₂ and produce carbohydrates (Niinemets *et al.*, 2006), while the root's primary role is to absorb nutrients (Laliberté, 2017). Both are sensitive to environmental changes and have greater phenotypic variation as indicators of changes in relevant ecological factors (Kou *et al.*, 2018). In previous studies, research on the response of plant traits to nitrogen deposition has focused on leaves (Yan *et al.*, 2015; Wang *et al.*, 2016; Zhang *et al.*, 2018a) or roots (Ostonen *et al.*, 2007; Wang *et al.*, 2018a; Zhang *et al.*, 2020a), and do not provide a complete picture of how whole plants respond to environmental changes.

N deposition increased the available N in soil that plants can absorb directly (Cao *et al.*, 2020),

which may shift the resource acquisition strategy of plants from below ground to above ground. For instance, plants can reduce belowground investment by decreasing root biomass (Li *et al.*, 2015), specific root area (SRL) (Wang *et al.*, 2018a; Zhang *et al.*, 2020a) and increasing root average diameter (AD) (Wang *et al.*, 2018a). In addition, plants can favor the resource allocation to above-ground organs by increasing specific leaf area (SLA) (Wu *et al.*, 2008a; Wang *et al.*, 2016; Valliere *et al.*, 2017), leaf nitrogen concentration (LN) (Wu *et al.*, 2008b; Wang *et al.*, 2016; Valliere *et al.*, 2017) and above-ground biomass (Poorter *et al.*, 2012; Allard *et al.*, 2013; Zhang *et al.*, 2020b). However, contrary findings have also been reported. For example, studies on *Acer mono* found that the response of SLA to nitrogen addition varied according to the provenances (Peng *et al.*, 2018). Low N additions increased SRL in the fine roots of *Pleioblastus amarus*, but medium and high N reduced it (Chen *et al.*, 2017). Nitrogen deposition can also lead to soil acidification, which can inhibit plant growth (Lu *et al.*, 2014). These contradictory findings may be related to (1) the plant characteristics; (2) the form, the total amount, and the duration of the N inputs; and (3) abiotic conditions in the environment (Bobbink *et al.*, 2010). Therefore, a comprehensive analysis of biomass allocation and resource acquisition from the perspective of a specific plant is needed to more accurately respond to nitrogen deposition (Freschet *et al.*, 2010; Carvajal *et al.*, 2019; Shen *et al.*, 2019; Wambsganss *et al.*, 2021). In addition, species with larger geographic ranges are expected to experience a wider range of environmental conditions and evolve towards adaptation to local conditions (Sykes *et al.*, 1996; Chauvin *et al.*, 2019). As a result, the same species from different provenances may have different physiological responses to N deposition (Matisons *et al.*, 2019), so it is necessary to conduct studies on different genotypes of a same species to more accurately reveal the plant response to nitrogen deposition.

In the context of increasing global nitrogen deposition, it remains to be studied how the resource

acquisition of different provenances of a given species and their biomass allocation respond in a coordinated manner. We carried out this experiment in the subtropical region of south China, which has become one of the regions with the highest nitrogen deposition rate (30-60 kg N ha⁻¹ yr⁻¹) in the world (Zhu *et al.*, 2015) due to growing agricultural and industrial activities (Liu *et al.*, 2011). At the same time, the effective phosphorus (P) in this area is low due to excessive rainfall and highly weathered soils (Du *et al.*, 2020). Different provenance *Machilus pauhoi* (Lauraceae) seedlings were selected as the experimental subject. *M. pauhoi* is a native dominant species among evergreen broad-leaved tree species in the subtropical region of China (Zhong *et al.*, 2001). It grows rapidly and is a very important tree species for afforestation in the subtropical region of China (Yu *et al.*, 2019). This tree species has long been subject to the P deficient environments influences in the subtropical region and usually has a high leaf N:P ratio (Li *et al.*, 2016a). So far, various studies have examined the aboveground and belowground biomass allocation (Zhong *et al.*, 2001), roots (Wang *et al.*, 2018b; Zou *et al.*, 2018) and leaves (Li *et al.*, 2016a; Wu *et al.*, 2016; Yu *et al.*, 2019) functional traits in *M. pauhoi*. Previous studies have found that moderate N additions can promote the growth of *M. pauhoi* (Wu *et al.*, 2016). Yu *et al.* (2018) found differences in functional traits in the leaves of *M. pauhoi* from different provenances. In the context of increasing global nitrogen deposition, it remains to be studied how the resource acquisition of different provenances of *M. pauhoi* and their biomass allocation respond in a coordinated manner.

In this study, we conducted a nitrogen deposition simulation experiment to investigate the response of leaf, stem and root of *M. pauhoi* seedlings from five different provenances to N deposition. 24 leaf, stem and root traits that have a functional significance for woody species were used to determine how nitrogen deposition affects the functional traits of *M. pauhoi* seedlings. We hypothesized that under the same environmental conditions (i.e., same light, soil water concentration, and temperature), nitrogen

addition would alter the biomass allocation and resource strategy of *M. pauhoi* seedlings due to increased soil available N (Cao *et al.*, 2020). Specifically, we predict that 1) The response of leaf, stem and root traits to N addition is not consistent; with more N other soil resources could become limiting and plants could allocate more resources to roots to increase the capacity to uptake more of these limiting sources; and 2) Resource trade-off strategies for *M. pauhoi* traits are inconsistent across different provenances. Lower latitude provenances (SC) seedlings tend to allocate biomass to the under-ground and have higher SRL and lower SLA due to more annual accumulated temperature, while higher latitude provenances (JD) seedlings tend to allocate biomass to the above-ground and have higher SLA and lower SRL. Our results can provide a theoretical basis for precise nutrient management of *M. pauhoi* seedlings and their plantations in the context of nitrogen deposition.

Materials and Methods

Seed collection

Seeds were collected in July 2014 from five different localities in the south of China: Jianou (JO) and Yanping (YP) in Fujian provenance; Anfu (AF) and Suichaun (SC) in Jiangxi prvnenance; Jiande (JD) in Zhejiang provenance (Tab 1). In our previous studies we found that the leaf functional traits of *M. pauhoi* were mainly influenced by temperature (Yu *et al.*, 2018), so in this experiment these five provenances were selected according to a temperature gradient. For each provenance 5-8 individual trees of 20-30 years old that are well grown and healthy were selected and their seeds were mixed.

Table 1. Main geographical and environmental characteristics of 5 provenances.

No.	site	Longitude (E)	Latitude (N)	Annual precipitation (mm)	Annual average temperature (°C)
1	JO	118°09'	27°03'	1664	18.7
2	YP	118°07'	26°41'	1663	19.3
3	AF	114°14'	27°15'	1553	17.7
4	SC	114°23'	26°21'	1421	17.5

Site Description and Experimental Design

Experiments were conducted in a common garden at Forestry Technology Center of Shunchang County (117°48'E, 26°48'N), Nanping City, Fujian Province, south of China. The area is characterized by a subtropical monsoon climate with a mean annual temperature of 18.9 °C. The annual rainfall ranges from 1600 to 1900 mm, with the most precipitation occurring from February to September. In view of the fact that the various components of the seedlings are young and vulnerable to summer heat scorching and winter freezing (extreme high and low temperatures of 52°C and -3°C respectively) (Zhao *et al.*, 2018), we provided shade to the seedlings in the nursey using shade cloth. Seeds were sown in July 2014 in the test field and transplanted in March 2015. Seedlings were transplanted individually into polyvinyl chloride pots (inner diameter of 30 cm and height of 30 cm) containing red soil from Shunchang County. The transplanted seedlings were grown in a 70% shade frame and watered regularly to field capacity. After an adaptation period of 2 months, the exogenous nitrogen input experiment was carried out in the period May to November 2016 when seedlings were nearly 2 years old. The information about the seedlings and soil traits is shown in Table 2.

Table 2. Information concerning the seedlings and soil traits (Avg ± SE)

Seedling Trait	Tree height (cm)	61.33±15.76
	Basal diameter (cm)	5.58±1.35
	Specific leaf area(cm ² /g)	195.694±24.89
	Leaf carbon concentration(mg/g)	481.998±8.04
	Leaf nitrogen concentration(mg/g)	10.448±1.09
	Leaf phosphorus concentration(mg/g)	1.021±0.19
Soil Trait	Soil Total Nitrogen concentration (mg/g)	0.717±0.01
	Soil Total Carbon concentration (mg/g)	4.63±0.05
	Soil Total Phosphorus concentration (mg/g)	1.142±0.07
	Soil available phosphorus concentration (mg/L)	1.142±0.01
	pH	4.97±0.18
	Soil field capacity (cm ³ /cm ³)	0.309±0.02

Due to the high and increasing rate of nitrogen deposition in this area (50 kg ha⁻¹ yr⁻¹). At the same

time, in the previous nitrogen addition experiment, Pei *et al.* (2016) found that *M. pauhoi* seedling were suitable for growing at $100 \text{ kg ha}^{-1} \text{ yr}^{-1}$. Therefore, to easily observe the response of *M. pauhoi* seedling to nitrogen deposition in future, we set the double the local N deposition rate as our N additions amount (N_1 , $100 \text{ kg ha}^{-1} \text{ yr}^{-1}$). In addition, a control treatment was established for comparison (CT, $0 \text{ kg ha}^{-1} \text{ yr}^{-1}$). Each treatment was completed in triplicate for statistical significance. Ammonium nitrate (NH_4NO_3) was used for the addition of N, and the soil surface area was calculated based on the inner diameter of the pot. Then, the amount of N added per pot was calculated using the soil surface area (based on the inner diameter of the pot) (N_{100} treatment: 2.02 g of NH_4NO_3). NH_4NO_3 was dissolved in 10 ml of distilled water and uniformly applied with a pipette to the treatment and control pots. For each replicate we have 9 seedlings, so we have a total of 5 (provenances) $\times 2$ (N treatments) $\times 3$ (replicates) $\times 9 = 270$ seedlings.

Sampling and Chemical Analysis

At the end of the experiment in November 2016, three standard seedlings were selected and harvested from each treatment (See Figure S1 for information on standard seedlings basal diameter and plant height). The leaves, stems, and fine roots were collected separately and transported to the laboratory as soon as possible. Roots were cleaned with distilled water before scanning. Each organ from each seedling was treated as an individual sample.

Three healthy and fully expanded leaves from each tree were selected to measure leaf phenotypic traits. A Vernier caliper (0.01 mm) was used to measure the thickness of the leaves (top, middle, and bottom; avoiding important veins), and the average of these values was used as the leaf thickness (LT; mm). The leaf area (LA, cm^2) was measured using an LI-3000C portable leaf-area meter (Li-Cor, Lincoln, NE, USA). The saturated mass (Lsmass, g) was determined after submerging a leaf in deionized water

for 24 h in the dark (we dabbed dry the leaf surfaces with paper towels before measuring). The Specific leaf area (SLA, cm²/g), Leaf dry matter concentration (LDMC, g/g) and leaf tissue density (LTD, g/cm³) were calculated for selected leaf samples. For root phenotype trait, we selected 3 fine root branch (<2 mm in diameter) with intact terminal branch orders (Pregitzer *et al.*, 2002). Next, Root were scanned with an Epson V370 scanner and analyzed using WinRHIZO root-scanning software (Regent Instruments Inc., Ottawa, ON Canada). Tweezers was used to separate overlapping roots if needed. The Specific root length (SRL, cm/g), Average root diameter (AD, mm) and root tissue density (RTD, g/cm³) were calculated for selected root samples.

The dry weights of each leaf, stem and root (Lmass, Smass and Rmass, respectively) samples were determined after oven-drying at 65 °C until a constant weight was obtained. The total plant biomass (Tbio) of a seedling is the sum of the biomass of the three organs. Leaf mass fraction (LMF), stem mass fraction (SMF) and root mass fraction (RMF) were calculated. The formulae for calculating the functional traits in this paper can be found in Table 3.

For both the C, N and P concentration analyses in plant organs, the dried samples were ground into a fine powder using a sample grinder and screened with a 1-mm sieve. The C and N concentration was determined using a CHNS/O Elemental Analyzer (Vario EL III, Elementar, Langenselbold, Germany). The P concentration was determined using a Continuous Flow Analytical System (SAN ++, Skalar, Breda, Holland) after digestion and boiling in a solution of H₂SO₄-HClO₄. The formulae for calculating the functional traits in this paper can be found in Table 3.

Table 3. The definition (computational formula), abbreviation and unit for 29 functional trait in this paper

Organ	Trait	Abbreviation	Definition	Units
Leaf	Specific leaf area	SLA	Leaf area /Leaf mass	cm ² /g
	Tissue density	LTD	Lmass/(Leaf area * leaf thickness)	g/cm ³
	Dry matter content	LDMC	Leaf mass/Leaf saturated mass	g/g

	Carbon concentration	LC	Leaf carbon concentration per mass	mg/g
	Nitrogen concentration	LN	Leaf nitrogen concentration per mass	mg/g
	Phosphorus concentration	LP	Leaf phosphorus concentration per mass	mg/g
	Carbon/Nitrogen	LCN	Leaf carbon concentration / Leaf nitrogen concentration	g/g
	Nitrogen/Phosphorus	LNP	Leaf nitrogen concentration / Leaf phosphorus concentration	g/g
	Biomass	Lbio	Leaf mass	g
	Mass fraction	LMF	Leaf biomass / total biomass * 100%	%
Stem	Carbon concentration	SC	Stem carbon concentration per mass	mg/g
	Nitrogen concentration	SN	Stem nitrogen concentration per mass	mg/g
	Phosphorus concentration	SP	Stem phosphorus concentration per mass	mg/g
	Carbon/Nitrogen	SCN	Stem carbon concentration / Stem nitrogen concentration	g/g
	Nitrogen/Phosphorus	SNP	Stem nitrogen concentration / Stem phosphorus concentration	g/g
	Biomass	Sbio	Stem mass	g
	Mass fraction	SMF	Stem mass / total biomass * 100%	%
Root	Specific root length	SRL	Root length/Rmass	cm/g
	Tissue density	RTD	Rmass/Rvolume	g/cm ³
	Average diameter	AD	Root average diameter	mm
	Carbon concentration	RC	Root carbon concentration per mass	mg/g
	Nitrogen concentration	RN	Root nitrogen concentration per mass	mg/g
	Phosphorus concentration	RP	Root phosphorus concentration per mass	mg/g
	Carbon/Nitrogen	RCN	Root carbon concentration / Root nitrogen concentration	g/g
	Nitrogen/Phosphorus	RNP	Root nitrogen concentration / Root phosphorus concentration	g/g
	Biomass	Rbio	Root mass	g
	Mass fraction	RMF	Root biomass / total biomass * 100%	%
Whole	Biomass	Wbio	Whole biomass	g
	Total N concentration of aboveground/underground	TNa/u	(Leaf biomass * leaf N concentration + stem biomass * stem N concentration) / root biomass * root N concentration	mg/mg

182 Statistical Analysis

183 Before the analysis, all data were log₁₀ transformed to fit a normal distribution pattern. We calculated the

184 index of phenotypic plasticity (Valladares *et al.*, 2000) for each trait and provenances as the difference

185 between the minimum mean and the maximum mean values among the N treatment divided by the

186 maximum mean value. We calculated the relative effects (RE) (Duval *et al.*, 2011) of N addition [(means

from N addition plots – means from ambient plots)/(means from ambient plots)×100] on the function traits of different provenances *M. pauhoi* seedlings.

We performed two-way factorial ANOVA at a significance level of 0.05 for all 29 functional traits in SPSS 22 software (SPSS Inc., Chicago, IL, USA), with N deposition intensity and provenances as the independent factors and their interaction also included. Bonferroni test was used to determine whether significant ($P < 0.05/k$, where k is the number of comparisons)

Principal component analysis (PCA) were performed to investigate the effect of nitrogen on the resource acquisition strategy of 26 functional trait (except leaf, stem and root biomass because of their high similarity to LMF, SMF and RMF) of *M. pauhoi* seedlings by using “*factoextra* (Kassambara and Mundt, 2020)” package in R (version 4.1.1) (R Core Development Team, 2021). Permutational multivariate analysis of variance analysis (PERMANOVA) were performed to examine the differences in different N addition and provenances using “*adonis*” function of “*vegan* (Oksanen *et al.*, 2020)” package, and pairwise comparisons (Bonferroni correction) were performed between different provenances ($P < 0.05/k$). The data for all variables were standardized by the Z-score. Packages “*ggplot2* (Wickham, 2016)” and “*ggpubr* (Kassambara, 2020)” were used for visualization.

Results

3.1 Effect of nitrogen addition on main functional traits

3.1.1 Effect of nitrogen addition on biomass and allocation

N addition significantly increased the biomass of the organs of *M. pauhoi* (Table 4). The biomass of organs also varied significantly among different provenances (Table 4). The interaction between N addition and provenance had a significant effect on leaf biomass (Table 4). Notably, although N addition increased the biomass of each organ of the JO seedlings and leaf and stem biomass of the SC seedlings,

the effect was not significant (**Fig 1A, B, C**). The seedling total biomass of different provenances from large to small was AF > SC > JD > YP > JO (**Fig S2**).

Nitrogen addition significantly increased and decreased LMF and RMF, respectively (Table 4). LMF, SMF and RMF differed significantly among different provenances, and the interaction of N and different provenances had no significant effect on each organ mass fraction (Table 4). Leaf had the lowest fraction (18.5% ~ 26.5%) in biomass in five provenances (**Fig 1D**). Root biomass fraction (44% ~ 48.3%) is higher than stem (30.1% ~ 34.5%) in JO, YP, and JD, but lower in SC (**Fig 1E, F**). N addition increased the leaf biomass fraction and decreased root biomass fraction in JO, YP, AF, JD seedlings, and reduced the leaf biomass fraction, and increased the root biomass fraction in SC (Table 4).

Fig 1 Leaf, stem and fine root biomass and mass fraction of different provenance of *M. pauhoi* seedling. See Table 3 for trait abbreviations.

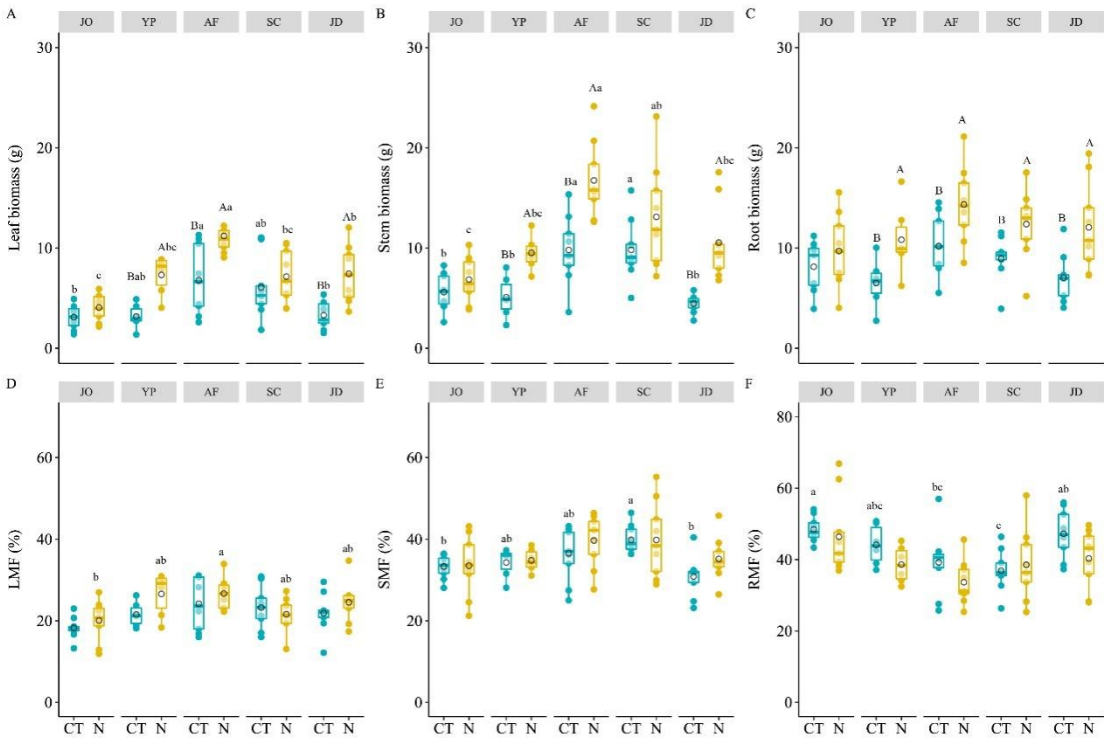


Table 4. Two-way ANOVA analysis of the effect of nitrogen addition and provenance on the functional trait of *M. pauhoi* seedling. See Table 3 for trait abbreviations.

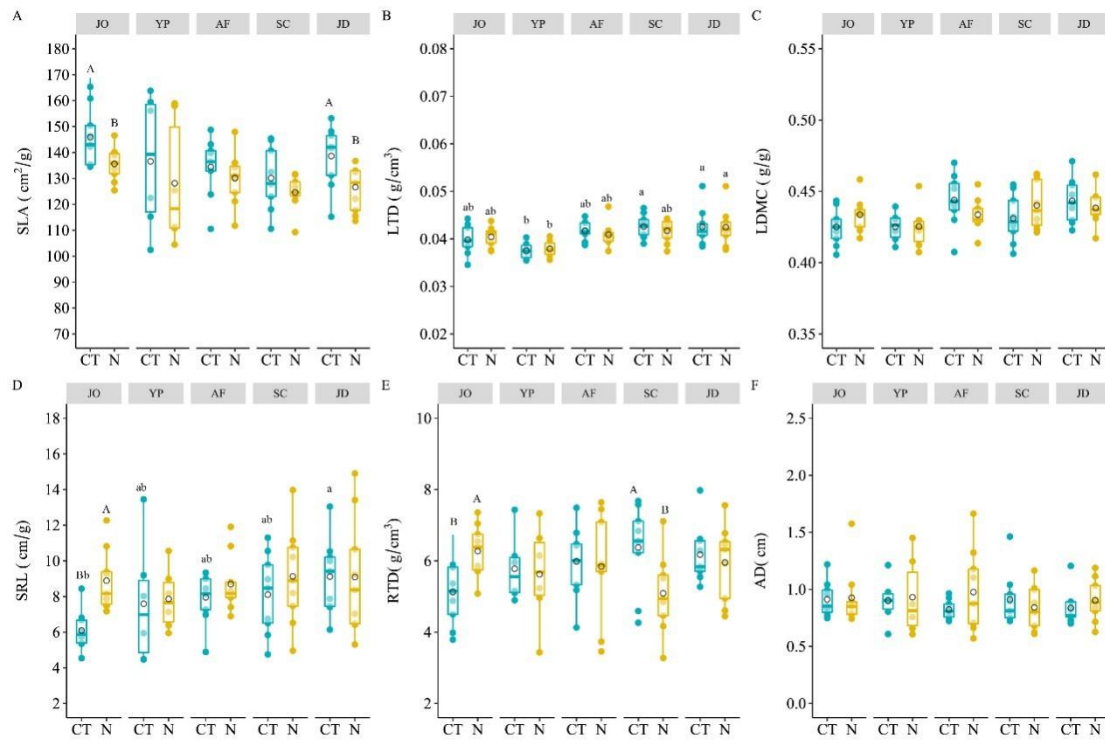
Trait		N		Pr		N * Pr	
		F	P	F	P	F	P
Leaf	SLA	7.559	0.007	2.356	0.061	0.267	0.898

	LDMC	0.028	0.867	3.045	0.022	1.522	0.205
	LTD	0.067	0.797	6.716	<0.001	0.251	0.908
	LN	0.316	0.576	1.299	0.278	2.180	0.079
	LC	27.835	<0.001	2.189	0.078	1.519	0.205
	LCN	0.242	0.624	1.181	0.326	3.001	0.024
	LP	6.554	0.013	1.488	0.215	2.918	0.027
	LNP	3.627	0.061	0.605	0.660	0.244	0.912
Stem	SN	4.601	0.035	3.195	0.018	1.744	0.149
	SC	1.967	0.165	2.362	0.061	1.933	0.114
	SCN	7.205	0.009	3.602	0.01	3.243	0.017
	SP	13.259	0.001	1.041	0.392	2.032	0.099
	SNP	3.249	0.076	0.258	0.904	0.088	0.986
Root	SRL	3.776	0.056	1.457	0.224	1.060	0.382
	RTD	0.293	0.590	0.381	0.822	2.863	0.029
	AD	0.592	0.444	0.165	0.956	0.570	0.685
	RN	6.302	0.014	0.561	0.692	0.888	0.476
	RC	0.075	0.785	0.983	0.422	0.606	0.660
	RCN	4.335	0.041	0.483	0.748	1.024	0.401
	RP	13.752	<0.001	0.722	0.580	1.203	0.317
	RNP	26.475	<0.001	1.483	0.216	0.651	0.628
Biomass	Lbio	34.348	<0.001	13.829	<0.001	2.579	0.044
	Rbio	26.921	<0.001	3.484	0.012	0.739	0.568
	Sbio	41.300	<0.001	16.826	<0.001	2.400	0.058
	LMF	4.04	0.048	4.47	0.003	1.02	0.403
	RMF	4.867	0.03	6.286	<0.001	0.935	0.449
	SMF	1.606	0.209	4.868	0.002	0.502	0.734
Whole	Tbio	51.507	<0.001	14.764	<0.001	2.385	0.060
	TNa/u	0.006	0.938	2.624	0.041	1.546	0.198

3.1.2 Effect of N addition on the leaf and fine root phenotype traits

N addition significantly reduced SLA of *M. pauhoi* seedlings but had no significant effect on other leaf and root phenotypic traits (Table 4, Fig 2). There were significant differences in LDMC and LTD in leaves of seedlings from different provenances, while there were no differences between root morphologies (Table 4). The interaction of N and provenance significantly affected the root RTD (Table 2).

Fig 2 Leaf and fine root phenotype trait of different provenance of *M. pauhoi* seedling. See Table 3 for trait abbreviations.

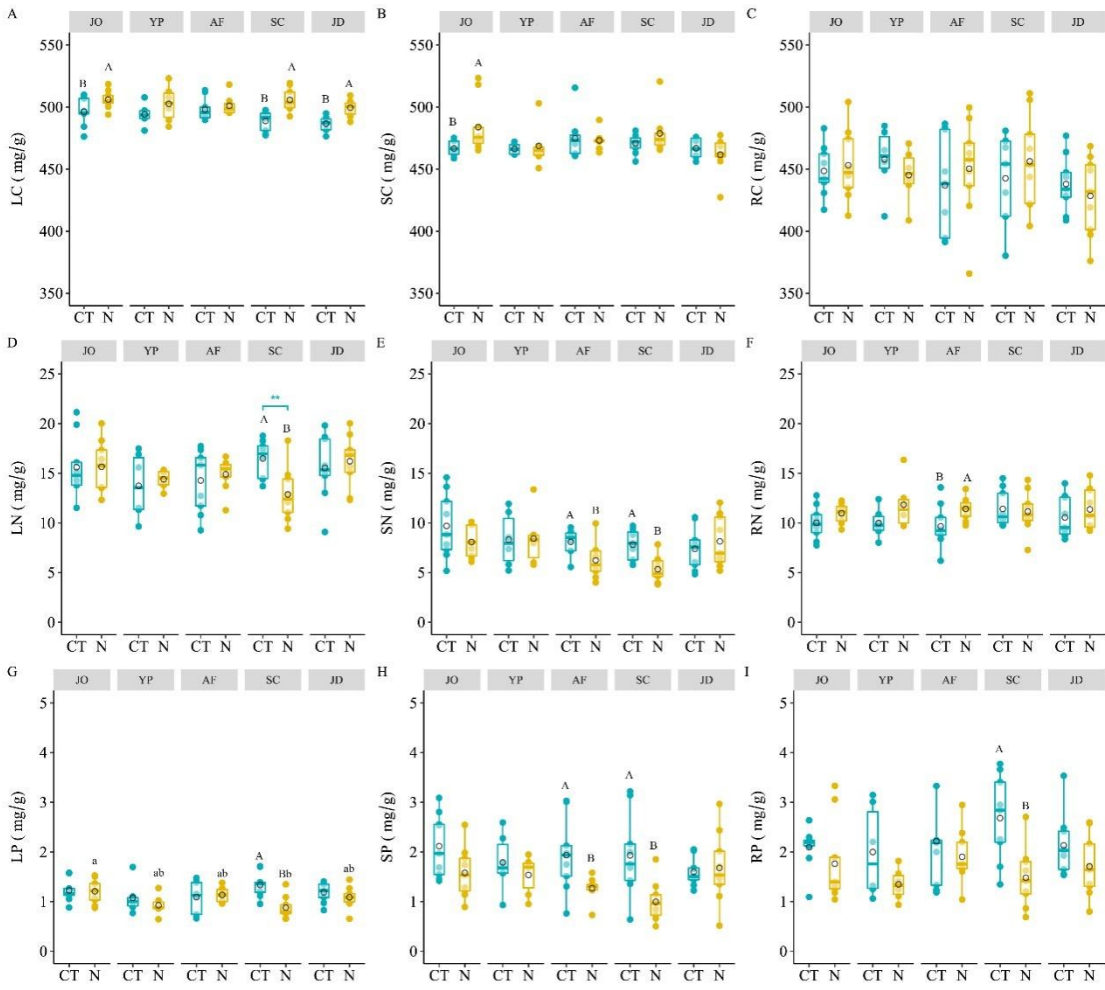


3.1.3 Effect of N addition on the leaf, stem and fine root nutrient traits

N addition significantly increased LC, SC: N, RN and RN:P and decreased SN, LP, SP, RP and RC:N of *M. pauhoi* seedlings (Fig 3, Fig S3). The stem N concentration and C:N ratio of seedlings from different provenance were significantly different. No differences were found in leaf and root nutrient traits between the different provenances. Nitrogen addition and provenance had significant effects on LC:N, SC:N and LP. The effect of N addition on leaf, stem and root N and P concentrations of *M. pauhoi* seedlings was not consistent among different provenances. For example, N addition increased the LN of YP, AF and JD seedlings, but decreased the LN of JO, SC seedlings. The leaves and stem have a higher C concentration and a lower P concentration and C:N ratio than the roots (Fig 3, Fig S3). Meanwhile, leaf and root have a higher N concentration and N:P ratio than stems (Fig 3, Fig S3).

There was no significant effect of N addition on TNa/u, while there were significant differences between the TNa/u of the different provenances, with YP and JD seedlings showing an increase in TNa/u after N addition, while JO, AF and SC seedling showed a decrease (Fig S4).

Fig 3 Leaf, stem and root nutrient traits of different provenance of *M. pauhoi* seedling. See Table 3 for trait abbreviations.



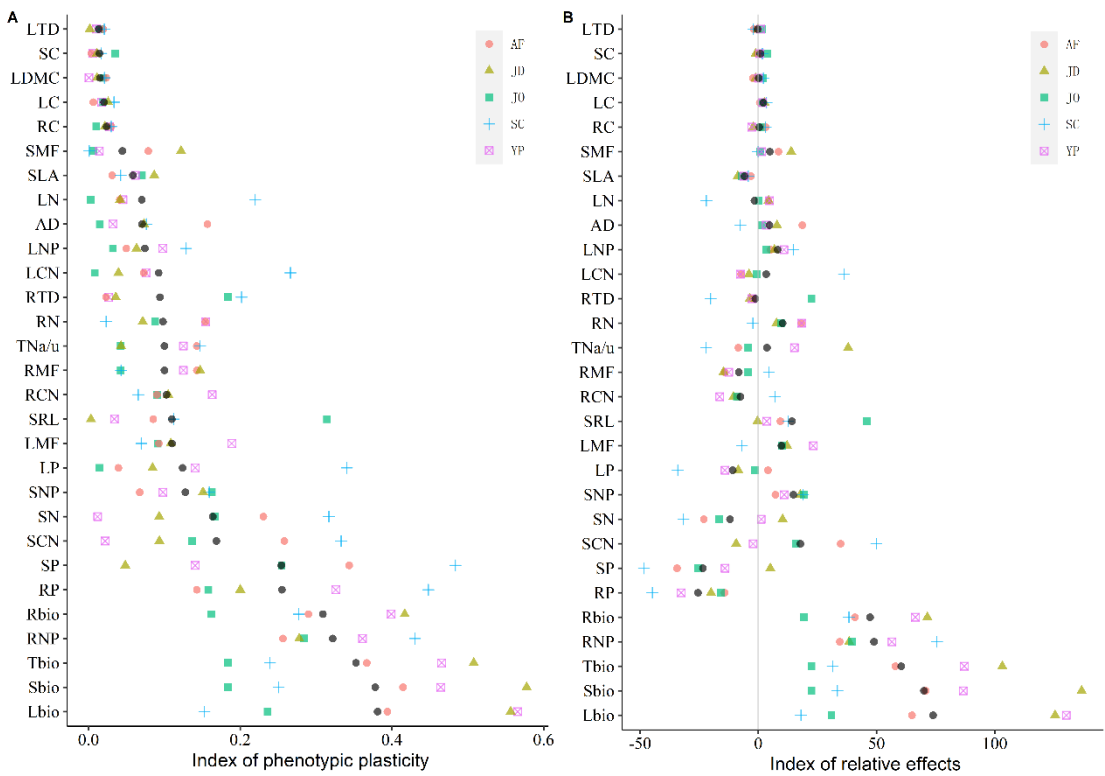
3.2 Phenotypic plasticity and relative effects of functional traits of *M. pauhoi* from different provenances

At the seedlings level, phenotypic plasticity was highest for biomass-related traits (e.g. Rbio, Tbio, Sbio and Lbio) (Fig 4A). At the organ level, roots had the highest phenotypic plasticity, followed by stems, and leaves had the lowest phenotypic plasticity. Nutrient-related traits (e.g. LC, LN, SN, LP, RP, and RNP) were more plastic than phenotype-related traits (e.g. LTD, LDMC, SLA, AD, and SRL). The plasticity of nutrient concentrations from low to high is C, N, and P, respectively. For the different provenances level, SC seedlings had the highest phenotypic plasticity (0.171), while JO seedlings had the lowest phenotypic plasticity (0.104).

The relative effects of LTD, LDMC, LC, SC, RC, LN and AD were stable in seedlings, while SLA,

RTD, RMF, RCN, LP, SN, SP and RP had negative relative effects on N addition (Fig 4B). The rest, such as SMF, LNP, RN, TNa/u, SRL, LMF, SNP, etc., have positive relative effects on N addition. At the seedlings level, relative effects were highest for biomass-related traits (e.g. Rbio, Tbio, Sbio and Lbio) and lowest for P related traits (e.g. RP, SP, and LP). For the different provenances level, JD seedlings had the highest relative effects (17.8), while SC seedlings had the lowest relative effects (3.6).

Fig 4. Index of phenotypic plasticity and relative effects for leaf and root traits of *M. pauhoi* seedlings with different provenances. Black dots are mean values The mean values of phenotypic plasticity of all traits for different provenances were SC (0.171), YP (0.145), AF (0.140), JD (0.139), and JO (0.104) in descending order. The mean values of relative effects of all traits for different provenances were JD (17.8), YP (14.2), AF (9.4), JO (6.5), and SC (3.6) in descending order. See Table 3 for trait abbreviations.

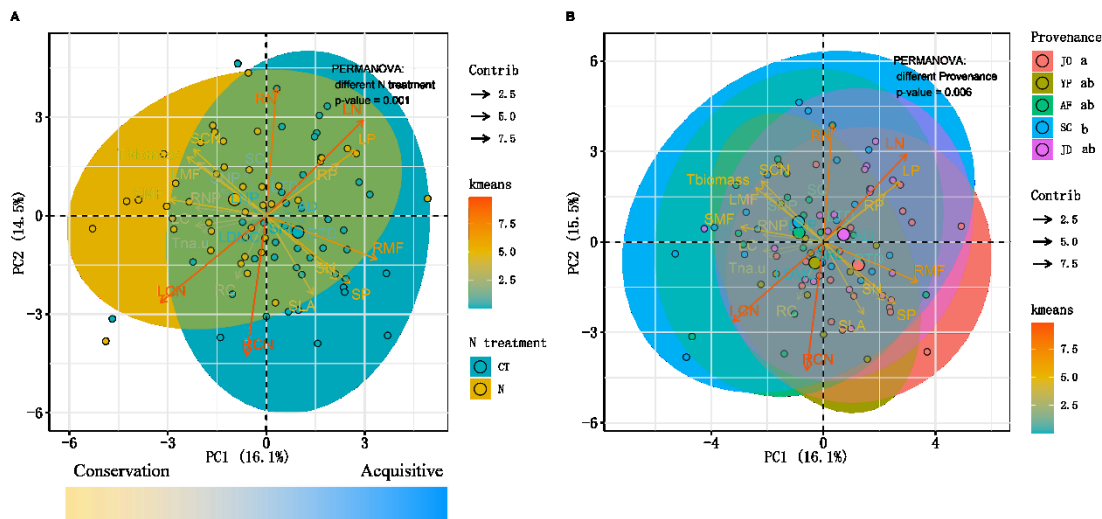


3.3 Influence of N addition and provenances on the resource acquisition strategy of *M. pauhoi*

The results of the whole plant PCA analysis for the 26 functional traits are shown in Fig 5. The first PCA axis accounted for 16.1% of overall variation and was related in one extreme (negative values) with high values of traits (LC, LCN, LDMC, RNP and LNP) and representative of the conservation resource

strategy. At the opposite extreme (positive values) were *M. pauhoi* with high values of traits (LN, LP, RP) and indicative of the resource acquisition strategy. The second principal component, which explained 14.5% of the overall variance and was related in one extreme (positive values) with high values of traits (RN, SRL, SC, SCN, LMF and SMF). At the opposite extreme (negative values) were *M. pauhoi* with high values of traits (SLA, RC, RCN, SN, SP and RMF). Among the functional traits of the whole plant, leaf and root N concentration and C:N ratio were the highest contributing indicators. *M. pauhoi* seedlings may regulate the response of whole seedling functional traits to N deposition through these two key nutrient traits. PERMANOVA analysis showed a significant difference between CT and N addition treatment ($P=0.002$, Fig 5); and there was a significant difference among different provenances ($P=0.025$).

Fig 5 Biplot of the principal component analysis (PCA) using traits of leaves, stems and roots at the N treatment level (A) and provenances level (B). PC1 represents a resource strategy gradient, from high-LN, high-LP, high AD, low LDMC, low-LNP, that are characterized by resource acquisition strategy to low-LN, low LP, low AD, high LDMC, high LNP, which can be related to conservation resource strategy. The large dot is the center of the category. Different letters after the provenances in the graph on the right indicate significant differences between the different provenances. See Table 3 for trait abbreviations.



Discussion

Responses of main functional traits of plants to N addition

One of the mechanisms by which plants adapt to exogenous resource imbalances is the allocation of new biomass to the organs involved in acquiring the most scarce resources (Freschet *et al.*, 2018; Ma *et al.*, 2021). In some cases, the increase in soil nitrogen indicates that plants do not need to build more roots for resource capture (especially when N is the most limiting factor) except in other contexts when P or water are more limiting (Ostonen *et al.*, 2011). Consistently with OPT theory (Smith & Martin *et al.*, 2020), as N-availability increases, *M. pauhoi* will reduce root biomass (Hendricks *et al.*, 2006; Wang *et al.*, 2013; Chen *et al.*, 2017) and transfer more biomass to aboveground parts to produce more photosynthetic products (Wang *et al.*, 2016; Valliere *et al.*, 2017). However, our study does not fully support the PES, as leaf, stem and root traits do not respond consistently to N addition (Reich, 2014). The results of organ response to N addition varied by trait, with roots having higher phenotypic plasticity than leaves and being more sensitive to environmental changes. Nutrient traits and biomass allocation were more susceptible to N addition than phenotypic traits (Kramer-Walter and Laughlin, 2017). Therefore, our first hypothesis is only partially supported.

In our study, under 70% shade, N addition changed the biomass allocation of each organ of *M. pauhoi* seedlings by increased LMF and decreased RMF. At the same time, N addition decreased SLA but increased SRL of *M. pauhoi* seedling from different provenance. There are two ways in which different plants achieve similar resource acquisition capacity in both above and below ground parts: A) more leaf biomass with lower SLA, and less root biomass with higher SRL; B) more root biomass with lower SRL, and less leaf biomass with higher SLA (Freschet *et al.*, 2015). *M. pauhoi* may increase its total light capture area by increasing the number of leaves. We consider this as a way to balance resource

acquisition and biomass allocation in *M. pauhoi* seedlings after nitrogen addition under the low availability phosphorus in the test soils (Fort *et al.*, 2012; Fortunel *et al.*, 2012; Collins *et al.*, 2016; Carvajal *et al.*, 2019; Wambsganss *et al.*, 2021).

The SLA represents the light resources captured on the LA per unit of dry leaf matter, which is closely related to the light interception efficiency of plants (Reich, 2014). The SRL represents the length of roots potentially able to explore soil per unit mass invested (Ostonen *et al.*, 2007). N addition decreased SLA and increased SRL of *M. pauhoi* seedling from different provenance but had no significant effect on LTD, RTD, LDMC and AD, indicating the insensitivity of plant tissue traits to external environmental changes (Davidson *et al.*, 2004; Zhang *et al.*, 2006; Högberg, 2007; Li and Bao, 2015).

M. pauhoi reduced the N concentration of leaves and stems and increased the N concentration of roots under N addition. RN has been increased with N addition, which is consistent with the meta-analysis result by (Li *et al.*, 2015) and the N controls experimental in temperate forest ecosystems by Hendricks *et al.* (2000), but inconsistent with *Pleioblastus amarus* in the subtropical forest by Chen *et al.* (2017). This increase of RN may be attributed to nitrogen addition improving the soil available N concentration, thus more N became absorption and stock in the root (Matson *et al.*, 1999; Hyvönen *et al.*, 2008). At the same time, plants and microbes may invest an element that is in excess into the acquisition of a limiting factor until their growth is equally limited by both elements (Bloom *et al.*, 1985; Zhang *et al.*, 2018b).

Nitrogen addition decreased P concentration and increased N:P in leaves, stems and roots, an indication lack of P may occur in *M. pauhoi* seedling growth (Güsewell, 2004; Powers *et al.*, 2005; Fan *et al.*, 2019). Lack in phosphorus nutrients can be explained by the following three reasons: 1) the “dilution effect” (Jarrell and Beverly, 1981; Firn *et al.*, 2019), because significantly increased *M. pauhoi* seedling biomass outpaces nutrient accumulation in whole plant biomass. 2) P deficiency is widespread

in the subtropics (Du *et al.*, 2020) and the soils we use are indeed low in their natural P-availability (3.76 mg/kg) (Wu *et al.*, 2015). At the same time, N addition may exacerbate this phenomenon by causes soil acidification and combines phosphorus with metal ions (e.g., Al^{3+} and Fe^{3+}), reducing the available phosphorus in the soil and affecting plant growth due to the reduced amount of phosphorus available for uptake (Bünemann *et al.*, 2010; Kou *et al.*, 2018). The decrease in the soil available P may have reduced the leaf P concentration, which, on account of the P availability, was insufficient to balance the increasing P requirements under N addition (Jonard *et al.*, 2015; Huang *et al.*, 2016; Li *et al.*, 2016b). 3) The mycorrhizal activity may have been inhibited by the N addition (Nilsson and Wallander, 2003), even though nitrogen enrichment has been known to increase the phosphatase activity and soil P availability (Olander and Vitousek, 2000; Deng *et al.*, 2017). Consequently, the availability of the phosphorous required for the seedlings would be limited (Wallander *et al.*, 2001). In addition, in the case of nitrogen addition, plants and microorganisms develop more strategies to allocate more nitrogen to take up more phosphorus, for example by enhancing strong phosphatase activity (Treseder and Vitousek, 2001; Zhang *et al.*, 2018b), or by altering symbiotic mycorrhizae (Lilleskov *et al.*, 2002). However, in the case of long-term nitrogen addition, this supplementary phosphorus is not sufficient to compensate for the phosphorus deficiency, and therefore phosphorus limitation gradually increases (Sardans and Peñuelas, 2012; Peñuelas *et al.*, 2013; Peñuelas *et al.*, 2020). So a moderate addition of P may be able to alleviate the P limitation of the plants in future N addition trials.

Effect of provenances on the trade-off strategy among traits in *M. pauhoi*

Species with larger geographic ranges are expected to experience a wider range of environmental conditions and evolve towards adaptation to local conditions (Sykes *et al.*, 1996; Chauvin *et al.*, 2019). The undergrowth seedlings of *M. pauhoi* can respond to geographic changes by adjusting leaf

functional traits (Yu *et al.*, 2018). Analysing the ratio of total N concentration above ground (leaves and stems) to below ground (roots) helps us to synthesise how *M. pauhoi* allocate N to responded to N deposition. Overall, TNa/u were greater than 1 for all provenances, thus suggesting that *M. pauhoi* seedlings have a greater N allocation to above-ground components than below. The response of TNa/u to N addition was not consistent across different provenances. N addition increased the TNa/u of YP and JD seedlings, probably due to the increased demand for above-ground light access resulting from shade, which allocated more N to the above-ground (Schall *et al.*, 2012; Deng *et al.*, 2020). N addition reduced TNa/u in JO, AF and SC seedlings, probably due to the fact that JO, AF and SC seedlings allocated more N to the ground after N addition to alleviate phosphorus limitation through a exchange nitrogen for phosphorus strategy (Olander and Vitousek, 2000; Deng *et al.*, 2017). This may go some way to explaining the higher ability of these three provenances to exchange nitrogen for phosphorus and the fact that nutrient resources below ground are more limiting to their growth than light. Future research should focus on the ability of different provenances of *M. pauhoi* to exchange nitrogen for phosphorus as a means of verification. Differences among seedlings from different provenances may be related to the environmental conditions of that provenances site (e.g. climate and soil traits) (Yu *et al.*, 2018; Ren *et al.*, 2020). However, due to the lack of data on soil conditions at the provenances site, we were not able to analyses this further. Therefore, environmental conditions at the provenances site should be included in future multi-provenances studies.

Phenotypic plasticity is an important mechanism for species distribution and maintenance of local adaptation (Valladares *et al.*, 2014). Within a species, response to environmental conditions are vary among genotypes (Ren *et al.*, 2020). Plasticity occurs when a given genotype produces different phenotypes in response to external factors, which may increase its short-term success when confronted

with varying environments (Morel-Journel *et al.*, 2020). Our second hypothesis that resource trade-off strategies for *M. pauhoi* traits are inconsistent across different provenances was supported. The SC seedlings had the highest phenotypic plasticity and favored a conservative (low LN, low LP) resource strategy to obtain belowground resources (high SRL) and had a high total biomass. In contrast, JO seedlings had the lowest phenotypic plasticity, favored access to aboveground resources (high SLA), and was an access resource strategy (high LN, high LP), and had a low total biomass. Among all the provenances, the AF seedlings had the highest biomass, perhaps because this source did not adopt an obvious above- or belowground, conservative or acquisitive resource acquisition trait, but rather achieved a better balance between resource acquisition and biomass allocation. Therefore, we believe that AF and SC provenances can be the preferred provenances for *M. pauhoi* management. At the same time, since our experiments were conducted on 2-year-old seedlings, the exact effect of nitrogen deposition on mature plantation forests deserves further study in follow-up.

Conclusions

To fully understand the response of plant functional traits and resource acquisition strategies to nitrogen deposition, we need to understand how nitrogen deposition affects different organ traits and their interrelationships. Here, nitrogen addition simulation experiments were used to test the effects on leaves, stems and roots traits and resource acquisition strategies of *M. pauhoi* seedlings were tested. After investigating, we found that N addition increased biomass of *M. pauhoi* seedlings and changed the biomass distribution and main functional traits of leaves, stems and roots. The response of different functional traits to nitrogen addition was influenced by the provenances. The phenotypic plasticity of biomass was highest. Phenotypic plasticity varied among provenances, organs, nutrient traits and phenotypic traits. After N addition, *M. pauhoi* seedlings regulate the resource acquisition strategy of

leaves and roots by regulating the N and C:N ratios to achieve a balance with biomass allocation. Under the experimental premise of 70% shade, N deposition accelerated investment in aboveground growth and light capture by increasing plant height and LMF for YP and JD seedlings, and allocated more N to the above-ground. However, different findings were found for the AF, SC and JO seedlings. Despite the increase in height, these three provenances increased their N investment in the below-ground fraction, perhaps to exchange N for P. The JO source had the lowest biomass of the five provenances, which may account for its lowest plasticity, while the SC source, which had the best plasticity, and the AF source, which taught a balanced resource allocation strategy, could be the primary choice for *M. pauhoi* extension in the context of nitrogen deposition. Our results can provide a reference basis for carrying out *M. pauhoi* cultivation and subtropical seedling cultivation, and specific provenances should be considered for future afforestation in the context of nitrogen deposition.

Acknowledgments

We would like to thank Jiajing Chen, Zhihao Zhang, Mengke Sun, Hanzhao Zeng and Ting Yang for their assistance in collecting plant and soil materials used for experiments. We are sincerely grateful to the anonymous reviewers for their valuable comments to improve the manuscript.

Authors' contributions

Conceptualization: Yuxing Zou, Quanlin Zhong.

Data curation: Yuxing Zou, Xingyu Deng, Xiaoping Chen.

Formal analysis: Yuxing Zou, Hua Yu, Xiaoping Chen.

Funding acquisition: Quanlin Zhong, Baoyin Li, Hua Yu, Dongliang Cheng.

Investigation: Yuxing Zou, Xingyu Deng, Xiaoping Chen, Josep Penuelas, Jordi Sardans

Methodology: Yuxing Zou, Hua Yu, Xiaoping Chen.

Project administration: Quanlin Zhong, Yuxing Zou, Xingyu Deng.

Software: Yuxing Zou, Xiaoping Chen.

Supervision: Quanlin Zhong, Dongliang Cheng, Baoyin Li, Josep Penuelas

Validation: Quanlin Zhong.

Visualization: Yuxing Zou, Quanlin Zhong.

Writing - original draft: Yuxing Zou.

Writing - review & editing: Yuxing Zou, Quanlin Zhong, Josep Peñuelas, Jordi Sardans.

Funding

This research was funded by the National Natural Science Foundation of China (31971643, 31370589, 31470501), the National Key Research and Development Program of China (2017YFC0505400), the Central Finance Forestry Science and Technology Promotion Project (2015-12), the Major S&T Project of Fujian Province (2019N5009), the Fujian Natural Science Fund for Distinguished Young Scholars (2018J07003), and the Natural Science Foundation of Fujian Province of China (2018J01479). JP and JS acknowledge financial support from the Spanish Government grants PID2019-110521GB-I00 and PID2020115770RB-I, the Fundación Ramón Areces grant ELEMENTAL-CLIMATE, and the Catalan Government grant SGR 2017-1005.

Conflicts of interest/Competing interests

The authors declare that they have no conflicts of interest.

References

- Allard, V., Martre, P., Le Gouis, J., 2013. Genetic variability in biomass allocation to roots in wheat is mainly related to crop tillering dynamics and nitrogen status. *Eur J Agron* 46, 68-76. <https://doi.org/10.1016/j.eja.2012.12.004>
- Bloom, A.J., Chapin III, F.S., Mooney, H.A., 1985. Resource limitation in plants-an economic analogy. *Annu Rev Ecol Evol Syst* 16, 363-392.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol Appl* 20, 30-59. <https://doi.org/10.1890/08-1140.1>
- Bünemann, E.K., Oberson, A., Frossard, E., 2010. Phosphorus in action: biological processes in soil phosphorus cycling. Springer Science & Business Media.
- Cao, J., Pang, S., Wang, Q., Williams, M.A., Jia, X., Dun, S., Yang, J., Zhang, Y., Wang, J., Lü, X., 2020. Plant–bacteria–soil response to frequency of simulated nitrogen deposition has implications for global ecosystem change. *Functional Ecology* 34, 723-734. <https://doi.org/10.1111/1365-2435.13484>
- Carvajal, D.E., Loayza, A.P., Rios, R.S., Delpiano, C.A., Squeo, F.A., 2019. A hyper-arid environment shapes an inverse pattern of the fast–slow plant economics spectrum for above-, but not below-ground resource acquisition strategies. *J Ecol* 107, 1079-1092. <https://doi.org/10.1111/1365-2745.13092>
- Chauvin, T., Cochard, H., Segura, V., Rozenberg, P., 2019. Native-source climate determines the Douglas-fir potential of adaptation to drought. *For Ecol Manag* 444, 9-20. <https://doi.org/10.1016/j.foreco.2019.03.054>

- Chen, G.-t., Tu, L.-h., Peng, Y., Hu, H.-l., Hu, T.-x., Xu, Z.-f., Liu, L., Tang, Y., 2017. Effect of nitrogen additions on root morphology and chemistry in a subtropical bamboo forest. *Plant Soil* 412, 441-451. <https://doi.org/10.1007/s11104-016-3074-z>
- Collins, C.G., Wright, S.J., Wurzbarger, N., 2016. Root and leaf traits reflect distinct resource acquisition strategies in tropical lianas and trees. *Oecologia* 180, 1037-1047. <https://doi.org/10.1007/s00442-015-3410-7>
- Davidson, E.A., Reis de Carvalho, C.J., Vieira, I.C.G., Figueiredo, R.d.O., Moutinho, P., Yoko Ishida, F., Primo dos Santos, M.T., Benito Guerrero, J., Kalif, K., Tuma Sabá, R., 2004. Nitrogen and phosphorus limitation of biomass growth in a tropical secondary forest. *Ecol Appl* 14, 150-163. <https://doi.org/10.1890/01-6006>
- Deng, Q., Hui, D., Dennis, S., Reddy, K.C., 2017. Responses of terrestrial ecosystem phosphorus cycling to nitrogen addition: A meta-analysis. *Glob Ecol Biogeogr* 26, 713-728. <https://doi.org/10.1111/geb.12576>
- Deng, X., Xiao, W., Shi, Z., Zeng, L., Lei, L., 2020. Combined effects of drought and shading on growth and non-structural carbohydrates in *Pinus massoniana* Lamb. seedlings. *Forests* 11, 18. <https://doi.org/10.3390/f11010018>
- Du, E., Terrer, C., Pellegrini, A.F.A., Ahlström, A., van Lissa, C.J., Zhao, X., Xia, N., Wu, X., Jackson, R.B., 2020. Global patterns of terrestrial nitrogen and phosphorus limitation. *Nature Geoscience* 13, 221-226. <https://doi.org/10.1038/s41561-019-0530-4>
- Duval, B.D., Dijkstra, P., Natali, S.M., Megonigal, J.P., Ketterer, M.E., Drake, B.G., Lerdau, M.T., Gordon, G., Anbar, A.D., Hungate, B.A., 2011. Plant– soil distribution of potentially toxic elements in response to elevated atmospheric CO₂. *Environ Sci Technol* 45, 2570-2574. <https://doi.org/10.1021/es102250u>
- Fan, Y., Zhong, X., Lin, F., Liu, C., Yang, L., Wang, M., Chen, G., Chen, Y., Yang, Y., 2019. Responses of soil phosphorus fractions after nitrogen addition in a subtropical forest ecosystem: Insights from decreased Fe and Al oxides and increased plant roots. *Geoderma* 337, 246-255. <https://doi.org/10.1016/j.geoderma.2018.09.028>
- Firn, J., McGree, J.M., Harvey, E., Flores-Moreno, H., Schütz, M., Buckley, Y.M., Borer, E.T., Seabloom, E.W., La Pierre, K.J., MacDougall, A.M., Prober, S.M., Stevens, C.J., Sullivan, L.L., Porter, E., Ladouceur, E., Allen, C., Moromizato, K.H., Morgan, J.W., Harpole, W.S., Hautier, Y., Eisenhauer, N., Wright, J.P., Adler, P.B., Arnillas, C.A., Bakker, J.D., Biederman, L., Broadbent, A.A.D., Brown, C.S., Bugalho, M.N., Caldeira, M.C., Cleland, E.E., Ebeling, A., Fay, P.A., Hagenah, N., Kleinhesselink, A.R., Mitchell, R., Moore, J.L., Nogueira, C., Peri, P.L., Roscher, C., Smith, M.D., Wragg, P.D., Risch, A.C., 2019. Leaf nutrients, not specific leaf area, are consistent indicators of elevated nutrient inputs. *Nature Ecology & Evolution* 3, 400-406. <https://doi.org/10.1038/s41559-018-0790-1>
- Fort, F., Jouany, C., Cruz, P., 2012. Root and leaf functional trait relations in Poaceae species: implications of differing resource-acquisition strategies. *Journal of Plant Ecology* 6, 211-219. <https://doi.org/10.1093/jpe/rts034>
- Fortunel, C., Fine, P.V.A., Baraloto, C., 2012. Leaf, stem and root tissue strategies across 758 Neotropical tree species. *Funct Ecol* 26, 1153-1161. <https://doi.org/10.1111/j.1365-2435.2012.02020.x>
- Freschet, G.T., Cornelissen, J.H., Van Logtestijn, R.S., Aerts, R., 2010. Evidence of the ‘plant economics spectrum’ in a subarctic flora. *J Ecol* 98, 362-373. <https://doi.org/10.1111/j.1365-2745.2009.01615.x>
- Freschet, G.T., Kichenin, E., Wardle, D.A., 2015. Explaining within-community variation in plant biomass allocation: a balance between organ biomass and morphology above vs below ground? *J Veg Sci* 26, 431-440. <https://doi.org/10.1111/jvs.12259>
- Freschet, G.T., Violle, C., Bourget, M.Y., Scherer-Lorenzen, M., Fort, F., 2018. Allocation, morphology, physiology, architecture: the multiple facets of plant above- and below-ground responses to resource

stress. *New Phytol* 219, 1338-1352. <https://doi.org/10.1111/nph.15225>

Güsewell, S., 2004. N : P ratios in terrestrial plants: variation and functional significance. *New Phytol* 164, 243-266. <https://doi.org/10.1111/j.1469-8137.2004.01192.x>

Hendricks, J.J., Aber, J.D., Nadelhoffer, K.J., Hallett, R.D., 2000. Nitrogen Controls on Fine Root Substrate Quality in Temperate Forest Ecosystems. *Ecosystems* 3, 57-69. <https://doi.org/10.1007/s100210000010>

Hendricks, J.J., Hendrick, R.L., Wilson, C.A., Mitchell, R.J., Pecot, S.D., Guo, D., 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. *J Ecol* 94, 40-57. <https://doi.org/10.1111/j.1365-2745.2005.01067.x>

Högberg, P., 2007. Nitrogen impacts on forest carbon. *Nature* 447, 781-782. <https://doi.org/10.1038/447781a>

Huang, Z., Liu, B., Davis, M., Sardans, J., Peñuelas, J., Billings, S., 2016. Long-term nitrogen deposition linked to reduced water use efficiency in forests with low phosphorus availability. *New Phytol* 210, 431-442. <https://doi.org/10.1111/nph.13785>

Hyvönen, R., Persson, T., Andersson, S., Olsson, B., Ågren, G.I., Linder, S., 2008. Impact of long-term nitrogen addition on carbon stocks in trees and soils in northern Europe. *Biogeochemistry* 89, 121-137. <https://doi.org/10.1007/s10533-007-9121-3>

Jarrell, W.M., Beverly, R.B., 1981. The Dilution Effect in Plant Nutrition Studies. In: Brady, N.C. (Ed.), *Adv Agron*. Academic Press, pp. 197-224.

Jonard, M., Fürst, A., Verstraeten, A., Thimonier, A., Timmermann, V., Potočić, N., Waldner, P., Benham, S., Hansen, K., Merilä, P., Ponette, Q., de la Cruz, A.C., Roskams, P., Nicolas, M., Croisé, L., Ingerslev, M., Matteucci, G., Decinti, B., Bascietto, M., Rautio, P., 2015. Tree mineral nutrition is deteriorating in Europe. *Global Change Biology* 21, 418-430. <https://doi.org/10.1111/gcb.12657>

Kassambara, A., 2020. ggpubr: 'ggplot2' Based Publication Ready Plots. <https://CRAN.R-project.org/package=ggpubr>

Kassambara, A., Mundt, F., 2020. factoextra: Extract and Visualize the Results of Multivariate Data Analyses.

Kou, L., Chen, W., Jiang, L., Dai, X., Fu, X., Wang, H., Li, S., 2018. Simulated nitrogen deposition affects stoichiometry of multiple elements in resource-acquiring plant organs in a seasonally dry subtropical forest. *Sci Total Environ* 624, 611-620. <https://doi.org/10.1016/j.scitotenv.2017.12.080>

Kramer-Walter, K.R., Laughlin, D.C., 2017. Root nutrient concentration and biomass allocation are more plastic than morphological traits in response to nutrient limitation. *Plant Soil* 416, 539-550. <https://doi.org/10.1007/s11104-017-3234-9>

Laliberté, E., 2017. Below-ground frontiers in trait-based plant ecology. *New Phytol* 213, 1597-1603. <https://doi.org/10.1111/nph.14247>

Li, F.L., Bao, W.K., 2015. New insights into leaf and fine-root trait relationships: implications of resource acquisition among 23 xerophytic woody species. *Ecology and Evolution* 5, 5344-5351. <https://doi.org/10.1002/ece3.1794>

Li, M., Jin, B.J., Zhong, Q.L., Ma, Y.Z., Lu, H.D., Guo, B.Q., Zheng, Y., Cheng, D.L., 2016a. Effect of nitrogen and phosphorus fertilization on leaf N and P stoichiometric characteristics of *Machilus pauhoi* seedlings. *Chinese Journal of Applied and Environmental Biology*, 0285-0291. <https://doi.org/10.3724/SP.J.1145.2015.06022>

Li, W., Jin, C., Guan, D., Wang, Q., Wang, A., Yuan, F., Wu, J., 2015. The effects of simulated nitrogen deposition on plant root traits: A meta-analysis. *Soil Biology and Biochemistry* 82, 112-118. <https://doi.org/10.1016/j.soilbio.2015.01.001>

Li, Y., Niu, S., Yu, G., 2016b. Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: a meta-analysis. *Global Change Biology* 22, 934-943.

<https://doi.org/10.1111/gcb.13125>

- Lilleskov, E.A., Fahey, T.J., Horton, T.R., Lovett, G.M., 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology* 83, 104-115. [https://doi.org/10.1890/0012-9658\(2002\)083\[0104:BEFCCO\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0104:BEFCCO]2.0.CO;2)
- Liu, X., Duan, L., Mo, J., Du, E., Shen, J., Lu, X., Zhang, Y., Zhou, X., He, C., Zhang, F., 2011. Nitrogen deposition and its ecological impact in China: an overview. *Environ Pollut* 159, 2251-2264. <https://doi.org/10.1016/j.envpol.2010.08.002>
- Lu, X., Mao, Q., Gilliam, F.S., Luo, Y., Mo, J., 2014. Nitrogen deposition contributes to soil acidification in tropical ecosystems. *Global Change Biology* 20, 3790-3801. <https://doi.org/10.1111/gcb.12665>
- Ma, H., Mo, L., Crowther, T.W., Maynard, D.S., van den Hoogen, J., Stocker, B.D., Terrer, C., Zohner, C.M., 2021. The global distribution and environmental drivers of aboveground versus belowground plant biomass. *Nature Ecology & Evolution* 5, 1110-1122. [10.1038/s41559-021-01485-1](https://doi.org/10.1038/s41559-021-01485-1)
- Matisons, R., Krišāns, O., Kārkliņa, A., Adamovičs, A., Jansons, Ā., Gärtner, H., 2019. Plasticity and climatic sensitivity of wood anatomy contribute to performance of eastern Baltic provenances of Scots pine. *For Ecol Manag* 452, 117568. <https://doi.org/10.1016/j.foreco.2019.117568>
- Matson, P.A., McDowell, W.H., Townsend, A.R., Vitousek, P.M., 1999. The globalization of N deposition: ecosystem consequences in tropical environments. *Biogeochemistry* 46, 67-83. <https://doi.org/10.1023/A:1006152112852>
- Morel-Journel, T., Thuillier, V., Pennekamp, F., Laurent, E., Legrand, D., Chaine, A.S., Schtickzelle, N., 2020. A multidimensional approach to the expression of phenotypic plasticity. *Funct Ecol* 34, 2338-2349. <https://doi.org/10.1111/1365-2435.13667>
- Niinemets, Ü., Portsmuth, A., Tobias, M., 2006. Leaf size modifies support biomass distribution among stems, petioles and mid-ribs in temperate plants. *New Phytol* 171, 91-104. <https://doi.org/10.1111/j.1469-8137.2006.01741.x>
- Nilsson, L.O., Wallander, H., 2003. Production of external mycelium by ectomycorrhizal fungi in a norway spruce forest was reduced in response to nitrogen fertilization. *New Phytol* 158, 409-416. <https://doi.org/10.1046/j.1469-8137.2003.00728.x>
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2020. *vegan: Community Ecology Package*.
- Olander, L.P., Vitousek, P.M., 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry* 49, 175-191. <https://doi.org/10.1023/A:1006316117817>
- Ostonen, I., Helmisaari, H.-S., Borken, W., Tedersoo, L., Kukumägi, M., Bahram, M., Lindroos, A.-J., Nöjd, P., Uri, V., Merilä, P., Asi, E., Lõhmus, K., 2011. Fine root foraging strategies in Norway spruce forests across a European climate gradient. *Global Change Biology* 17, 3620-3632. <https://doi.org/10.1111/j.1365-2486.2011.02501.x>
- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M., Lõhmus, K., Majdi, H., Metcalfe, D., Olsthoorn, A., Pronk, A., 2007. Specific root length as an indicator of environmental change. *Plant Biosyst* 141, 426-442. <https://doi.org/10.1080/11263500701626069>
- Pei, P., Zhong, Q., Cheng, D., Xu, C., Zhang, Z., 2016. The effect of nitrogen and phosphorus foliage spray on the growth of young free growing *Machilus pauhoi* plantations. *Chinese Journal of Applied Environmental Biology* 22, 0831-0838. <https://doi.org/10.3724/SP.J.1145.2016.04048>
- Peng, S.Z., He, N.P., Hou, J.H., 2018. Effect of nitrogen addition on leaf nitrogen and phosphorus stoichiometric characteristics of different provenance *Acer mono* seedlings. *Acta Ecologica Sinica* 38, 254-262. <https://doi.org/10.5846/stxb201612152596>
- Peñuelas, J., Janssens, I.A., Ciais, P., Obersteiner, M., Sardans, J., 2020. Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity,

food security, and human health. *Global Change Biology* 26, 1962-1985. <https://doi.org/10.1111/gcb.14981>

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., Janssens, I.A., 2013. Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat Commun* 4, 2934. <https://doi.org/10.1038/ncomms3934>

Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P., Mommer, L., 2012. Biomass allocation to leaves, stems and roots: meta - analyses of interspecific variation and environmental control. *New Phytol* 193, 30-50. <https://doi.org/10.1111/j.1469-8137.2011.03952.x>

Powers, J.S., Treseder, K.K., Lerdau, M.T., 2005. Fine roots, arbuscular mycorrhizal hyphae and soil nutrients in four neotropical rain forests: patterns across large geographic distances. *New Phytol* 165, 913-921. <https://doi.org/10.1111/j.1469-8137.2004.01279.x>

Pregitzer, K.S., DeForest, J.L., Burton, A.J., Allen, M.F., Ruess, R.W., Hendrick, R.L., 2002. Fine root architecture of nine North American trees. *Ecol Monogr* 72, 293-309. [https://doi.org/10.1890/0012-9615\(2002\)072\[0293:FRAONN\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2002)072[0293:FRAONN]2.0.CO;2)

R Core Development Team, 2021. R: A language and environment for statistical computing. 1.

Reich, P.B., 2014. The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto. *J Ecol* 102, 275-301. <https://doi.org/10.1111/1365-2745.12211>

Ren, L., Guo, X., Liu, S., Yu, T., Guo, W., Wang, R., Ye, S., Lambertini, C., Brix, H., Eller, F., 2020. Intraspecific variation in *Phragmites australis*: Clinal adaption of functional traits and phenotypic plasticity vary with latitude of origin. *J Ecol* 108, 2531-2543. <https://doi.org/10.1111/1365-2745.13401>

Sardans, J., Peñuelas, J., 2012. The Role of Plants in the Effects of Global Change on Nutrient Availability and Stoichiometry in the Plant-Soil System *Plant Physiol* 160, 1741-1761. <https://doi.org/10.1104/pp.112.208785>

Schall, P., Lödige, C., Beck, M., Ammer, C.J.F.E., Management, 2012. Biomass allocation to roots and shoots is more sensitive to shade and drought in European beech than in Norway spruce seedlings. *For Ecol Manag* 266, 246-253. <https://doi.org/10.1016/j.foreco.2011.11.017>

Shen, Y., Gilbert, G.S., Li, W., Lu, H., Fang, M., Yu, S., 2019. Linking aboveground traits to root traits and local environment: implications of the plant economics spectrum. *Frontiers in plant science* 10, 1412. <https://doi.org/10.3389/fpls.2019.01412>

Smith - Martin, C.M., Xu, X., Medvigy, D., Schnitzer, S.A., Powers, J.S., 2020. Allometric scaling laws linking biomass and rooting depth vary across ontogeny and functional groups in tropical dry forest lianas and trees. *New Phytol* 226, 714-726. <https://doi.org/10.1111/nph.16275>

Sykes, M.T., Prentice, I.C., Cramer, W., 1996. A Bioclimatic Model for the Potential Distributions of North European Tree Species Under Present and Future Climates. *J Biogeogr* 23, 203-233. <https://www.jstor.org/stable/2845812>

Treseder, K.K., Vitousek, P.M., 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology* 82, 946-954. [https://doi.org/10.1890/0012-9658\(2001\)082\[0946:EOSNAO\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[0946:EOSNAO]2.0.CO;2)

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garzón, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D.E., Nicotra, A.B., Poorter, H., Zavala, M.A., 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol Lett* 17, 1351-1364. <https://doi.org/10.1111/ele.12348>

Valladares, F., Wright, S.J., Lasso, E., Kitajima, K., Pearcy, R.W., 2000. Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. *Ecology* 81, 1925-1936. [https://doi.org/10.1890/0012-9658\(2000\)081\[1925:PPRTLO\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[1925:PPRTLO]2.0.CO;2)

Valliere, J.M., Irvine, I.C., Santiago, L., Allen, E.B., 2017. High N, dry: experimental nitrogen deposition

exacerbates native shrub loss and nonnative plant invasion during extreme drought. *Global Change Biology* 23, 4333-4345.

Vitousek, P.M., 2004. *Nutrient Cycling and Limitation: Hawai'i as a Model System*. Princeton University Press.

Wallander, H., Nilsson, L.O., Hagerberg, D., Bååth, E., 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytol* 151, 753-760. <https://doi.org/10.1046/j.0028-646x.2001.00199.x>

Wambsganss, J., Freschet, G.T., Beyer, F., Goldmann, K., Prada-Salcedo, L.D., Scherer-Lorenzen, M., Bauhus, J., 2021. Tree species mixing causes a shift in fine-root soil exploitation strategies across European forests. *Funct Ecol* 35, 1886-1902. <https://doi.org/10.1111/1365-2435.13856>

Wang, C., Xiao, H., Liu, J., Zhou, J., Du, D., 2016. Insights into the Effects of Simulated Nitrogen Deposition on Leaf Functional Traits of *Rhus Typhina*. *Pol J Environ Stud* 25, 1279-1284. <https://doi.org/10.15244/pjoes/61788>

Wang, G., Fahey, T.J., Xue, S., liu, F., 2013. Root morphology and architecture respond to N addition in *Pinus tabuliformis*, west China. *Oecologia* 171, 583-590. <https://doi.org/10.1007/s00442-012-2441-6>

Wang, W., Wang, Y., Hoch, G., Wang, Z., Gu, J., 2018a. Linkage of root morphology to anatomy with increasing nitrogen availability in six temperate tree species. *Plant Soil* 425, 189-200. <https://doi.org/10.1007/s11104-018-3563-3>

Wang, Y., Zhong, Q.l., Xu, C.b., Zhang, Z.r., liang, C.D., 2018b. Effect of adding a combination of nitrogen and phosphorus on fine root morphology and soil microbes of *Machilus pauhoi* seedling. *Acta Ecologica Sinica* 38, 2271-2278. <https://doi.org/10.5846/stxb201704240735>

Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. <https://ggplot2.tidyverse.org>

Wu, F., Bao, W., Li, F., Wu, N., 2008a. Effects of water stress and nitrogen supply on leaf gas exchange and fluorescence parameters of *Sophora davidii* seedlings. *Photosynthetica* 46, 40-48.

Wu, F.Z., Bao, W.K., Li, F.L., Wu, N., 2008b. Effects of water stress and nitrogen supply on leaf gas exchange and fluorescence parameters of *Sophora davidii* seedlings. *Photosynthetica* 46, 40-48. <https://doi.org/10.1007/s11099-008-0008-x>

Wu, Y.h., Wang, L.h., Cheng, D.l., Xu, C.b., Zhang, Z.r., Li, J., Zhong, Q.l., 2016. Effect of water and nitrogen treatment on photosynthetic characteristics and the biomass allocation of annual cutting seedling of *Machilus pauhoi*. *Journal of Anhui Agricultural University* 43, 202-208. <https://doi.org/10.13610/j.cnki.1672-352x.20160311.024>

Wu, Z., Haack, S.E., Lin, W., Li, B., Wu, L., Fang, C., Zhang, Z., 2015. Soil Microbial Community Structure and Metabolic Activity of *Pinus elliottii* Plantations across Different Stand Ages in a Subtropical Area. *PLoS ONE* 10, e0135354. [10.1371/journal.pone.0135354](https://doi.org/10.1371/journal.pone.0135354)

Yan, Z., Kim, N., Han, W., Guo, Y., Han, T., Du, E., Fang, J., 2015. Effects of nitrogen and phosphorus supply on growth rate, leaf stoichiometry, and nutrient resorption of *Arabidopsis thaliana*. *Plant Soil* 388, 147-155. <https://doi.org/10.1007/s11104-014-2316-1>

Yu, H., Cheng, D., Li, B., Xu, C., Zhang, Z., Zhong, Y., Zhong, Q., 2019. Short-Term Nitrogen Addition Does Not Significantly Alter the Effects of Seasonal Drought on Leaf Functional Traits in *Machilus pauhoi* Kanehira Seedlings. *Forests* 10, 78. <https://doi.org/10.3390/f10020078>

Yu, H., Zhong, Q.l., Huang, Y.b., Cheng, D.l., Pei, P., Zhang, Z.r., Xu, C.b., Zheng, W.t., 2018. Relationships between leaf functional traits of *Machilus pauhoi* understory seedlings from different provenances and geographical environmental factors. *Chinese Journal of Applied Ecology* 29, 449-458. <https://doi.org/10.13287/j.1001-9332.201802.008>

Zhang, H., Li, W., Adams, H.D., Wang, A., Wu, J., Jin, C., Guan, D., Yuan, F., 2018a. Responses of woody plant functional traits to nitrogen addition: a meta-analysis of leaf economics, gas exchange, and

hydraulic traits. *Frontiers in plant science* 9, 683. <https://doi.org/10.3389/fpls.2018.00683>

Zhang, S., Dang, Q.-L., Yü, X., 2006. Nutrient and [CO₂] elevation had synergistic effects on biomass production but not on biomass allocation of white birch seedlings. *For Ecol Manag* 234, 238-244. <https://doi.org/10.1016/j.foreco.2006.07.017>

Zhang, X., Xing, Y., Wang, Q., Yan, G., Wang, M., Liu, G., Wang, H., Huang, B., Zhang, J., 2020a. Effects of long-term nitrogen addition and decreased precipitation on the fine root morphology and anatomy of the main tree species in a temperate forest. *For Ecol Manag* 455, 117664. <https://doi.org/10.1016/j.foreco.2019.117664>

Zhang, X., Yang, Y., Zhang, C., Niu, S., Yang, H., Yu, G., Wang, H., Blagodatskaya, E., Kuzyakov, Y., Tian, D., Tang, Y., Liu, S., Sun, X., 2018b. Contrasting responses of phosphatase kinetic parameters to nitrogen and phosphorus additions in forest soils. *Funct Ecol* 32, 106-116. <https://doi.org/10.1111/1365-2435.12936>

Zhang, Z., Tariq, A., Zeng, F., Graciano, C., Zhang, B., 2020b. Nitrogen application mitigates drought-induced metabolic changes in *Alhagi sparsifolia* seedlings by regulating nutrient and biomass allocation patterns. *Plant Physiol Biochem* 155, 828-841. <https://doi.org/10.1016/j.plaphy.2020.08.036>

Zhao, C., Chen, J., Du, P., Yuan, H., 2018. Characteristics of Climate Change and Extreme Weather from 1951 to 2011 in China. *Int J Environ Res Public Health* 15, 2540. <https://doi.org/10.3390/ijerph15112540>

Zhong, q.l., Zhang, z.y., Zhang, c.h., Zhou, h.l., qiang, H.z., 2001. The Dynamic Analysis of the Biomass and the Structure of *Machilus Pauhoi*. *Acta Agriculturae Universitatis Jiang xiensis* 23, 533-536.

Zhu, J., He, N., Wang, Q., Yuan, G., Wen, D., Yu, G., Jia, Y., 2015. The composition, spatial patterns, and influencing factors of atmospheric wet nitrogen deposition in Chinese terrestrial ecosystems. *Sci Total Environ* 511, 777-785. <https://doi.org/10.1016/j.scitotenv.2014.12.038>

Zou, Y.X., Zhong, Q.L., You, Y.L., Yu, H., Zheng, W.T., Chen, J.J., Cheng, D.L., 2018. Short-term effects of nitrogen and water treatments on fine root order morphology of *Machilus pauhoi* seedlings. *Chinese Journal of Applied Ecology* 29, 2323–2329. <https://doi.org/10.13287/j.1001-9332.201807.014>

Supporting information

Fig S1 Basal diameter and tree height at harvest of seedlings of different provenances *M. pauhoi* seedling

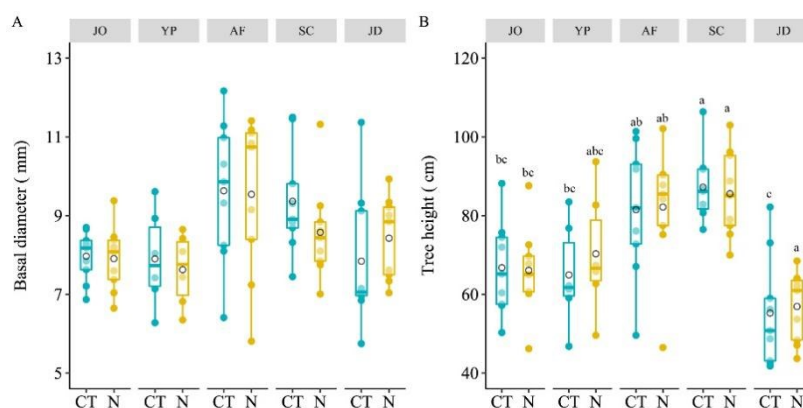


Fig S2 Total plant biomass of different provenances *M. pauhoi* seedling

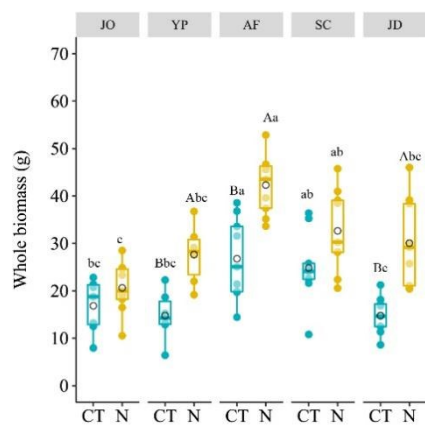


Fig S3 Leaf, stem and root stoichiometric ratio of different provenances *M. pauhoi* seedling. See

Table 3 for trait abbreviations.

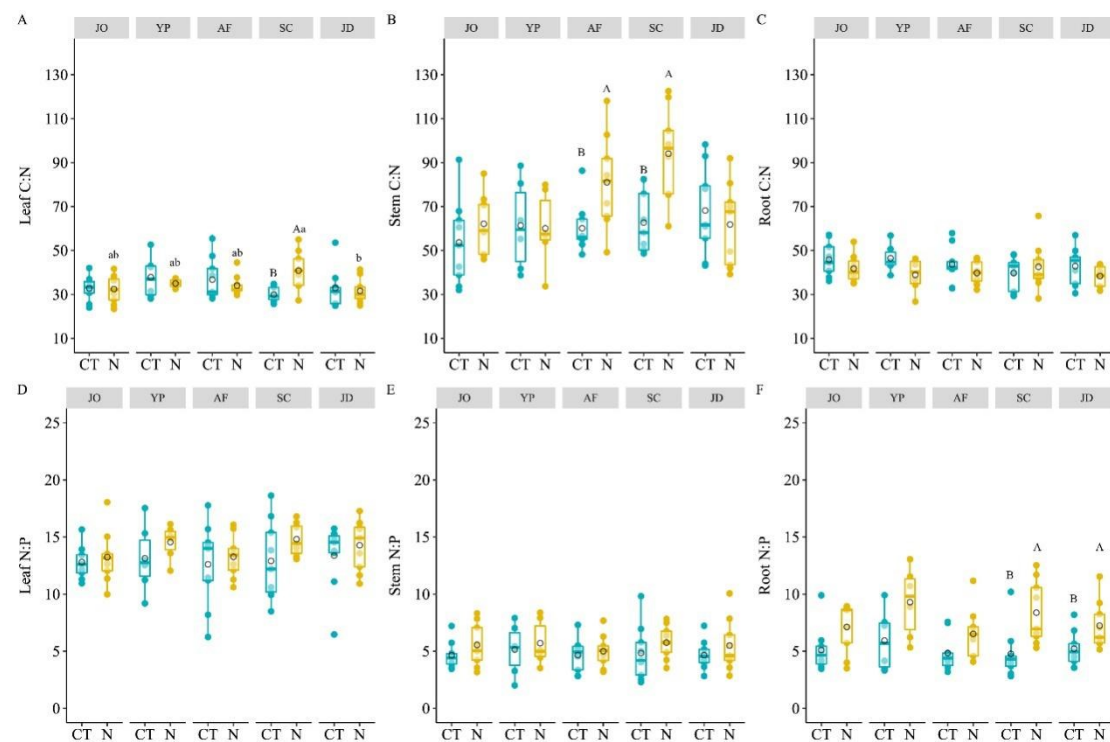


Fig S4 N absorption efficiency of shoot:root ratio of different provenances *M. pauhoi* seedling. See

Table 3 for trait abbreviations.

