

Research paper

Responses of C:N:P stoichiometry and non-structural carbohydrate contents of *Prunus mume* seedling to nitrogen addition

Shi-yao Hu¹, Yun-yi Hu¹, Zhi-gang Liu², Zheng-quan Huang², Jia-li Liao¹, Wen-zhang Qian¹, Hong-ling Hu^{1,3} and Shun Gao^{1,3,*}

Hu, S., Hu, Y., Liu, Z., Huang, Z., Liao, J., Qian, W., Hu, H., Gao, S. 2025. Responses of C:N:P stoichiometry and non-structural carbohydrate contents of *Prunus mume* seedling to nitrogen addition. – Forestry Studies | Metsanduslikud Uurimused 83, 27–42, ISSN 1736-8723. Journal homepage: <https://mi.emu.ee/en/forestry-studies>

Abstract. *Prunus mume* (*P. mume*) is a precocious flowering fruit species valued for its use in forestry and urban greening. Nitrogen (N) is a critical factor affecting early seedling growth and nutrient acquisition. However, the organ-specific responses of carbon (C): N: phosphorus (P) stoichiometry and non-structural carbohydrate (NSC) pools to varying N supply remain unclear in this species. To address this, we examined the effects of five N levels (0, 5, 10, 20 and 40 g N plant⁻¹) on morphological characteristics, C:N:P stoichiometry, and non-structural carbohydrate contents in the leaves, stems, and roots of *P. mume* seedlings under greenhouse conditions. Results indicated that N addition had a complex impact on seedling growth. While it significantly reduced P content across three organs, it increased total N content in the roots, stems, and leaves of *P. mume* seedlings. Higher N levels (40 g N plant⁻¹) also resulted in a marked rise in the N/P ratio, especially in stems. In terms of C reserves, N addition significantly increased soluble NSC content, while starch storage significantly decreased. Overall, N addition fundamentally changed the C:N:P stoichiometric balance and C reserve allocation in *P. mume* seedlings, with the reduction in starch closely associated with the accumulation of soluble sugars. These findings provide a scientific basis for optimizing N fertilizer schedules, thereby supporting high-quality nursery production and the sustainable establishment of *P. mume* orchards in forestry and agroforestry systems.

Key words: N addition, green plum, seedling growth, ecological stoichiometry, available carbohydrates.

Authors' addresses: ¹Department of Forestry, Faculty of Forestry, Sichuan Agricultural University, Chengdu, 611130, China; ²Forestry Bureau of Mabian Yi Autonomous County, Mabian 614600, China; ³National Forestry and Grassland Administration Key Laboratory of Forest Resources Conservation and Ecological Safety on the Upper Reaches of the Yangtze River, Sichuan Agricultural University, Chengdu, 611130, China; *e-mail: shungao1220@hotmail.com

Introduction

The seedling stage represents a critical phase in the plant life cycle, underpinning physiological development and subsequent growth. Nutrient

requirements vary across developmental stages (Yang *et al.*, 2023), making appropriate fertilization essential for producing vigorous seedlings and establishing productive plantations. Nitrogen (N) is the primary growth-limiting nutri-

DOI: 10.2478/fsmu-2025-0007



©2025 by the authors. Licensee Estonian University of Life Sciences, Tartu, Estonia. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license <http://creativecommons.org/licenses/by/4.0/>.

ent in plants (LeBauer & Treseder, 2008), and soil N availability strongly influences elemental concentrations in terrestrial plants by regulating nutrient and water uptake (Sardans & Peñuelas, 2012). Fertilization must therefore be timely, balanced, and technically appropriate. Excessive N supply can be counterproductive, often delaying reproductive development, particularly in young trees with strong vegetative growth (Wang *et al.*, 2024a). Hence, controlled early fertilization following established protocols is recommended. Irrigation and nutrient management practices can profoundly affect plant physiological and ecosystem functioning. Furthermore, anthropogenic N emissions have significantly increased atmospheric N deposition globally (Kanakidou *et al.*, 2016). Both elevated deposition and top-dressing N fertilization can alter the stoichiometry of aboveground and root organs in plants (Kou *et al.*, 2018), subsequently altering the chemical composition, architecture, and functional traits in plant species.

In response to N fertilizer application, plants adjust elemental balances to modify tissue carbon (C):N:phosphorus (P) ratios across roots, stems and leaves (Ågren, 2008). C constitutes the structural framework of plant tissues, typically accounting for 39–42% of dry mass, and can limit growth and development in plants (Körner, 2003). N enhances leaf area index, prolongs photosynthesis, improves nutrient uptake, and ultimately increases productivity (Tian *et al.*, 2023; Wang *et al.*, 2024a). P availability strongly influences the transport of photosynthetic products and is recognized as a key element in plant metabolism (Dissanayaka *et al.*, 2021; Vance *et al.*, 2003). A growing body of evidence indicated that N addition differentially affects the stoichiometry of roots, stems, and leaves across plant species, and that elemental composition varies substantially among organs (Jing *et al.*, 2017; Li *et al.*, 2021). Changes in C, N and P contents as well as their stoichiometric ratios are associated with physiological activities, growth rate and overall plant performance (Ågren, 2008; Yin *et al.*, 2021). Extensive research has examined the response of N and P levels and their stoichiometric ratios to N addition in different plants (Minden & Kleyer, 2014), because plant growth and organ function depend not only on absolute nutrient supply, but also on the balance among multiple elements

(Chen & Chen, 2021; Güsewell, 2004). However, the plasticity of organ-specific stoichiometry under environmental change, and which plant organs exhibit greater homeostasis, remain unclear (Minden & Kleyer, 2014). Non-structural carbohydrates (NSC) represent the C pool available to buffer external stresses, and serve as indicators of stress resistance and environmental tolerance in plants (Dietze *et al.*, 2014; Li *et al.*, 2023). Studies have revealed a positive correlation between leaf N concentration and NSC fixation capacity (Wang *et al.*, 2019), whereas P deficiency impairs photosynthesis (Wang *et al.*, 2023). The levels of NSC and C, N, and P in plant organs reflect nutrient availability and strongly influence plant growth and development (Liu *et al.*, 2020). Thus, the individual and interactive impacts of these factors shape the survival, growth, and development strategies in plants.

Prunus mume (Siebold) Siebold & Zucc., known as Chinese plum, Qingmei, or sour plum in China, Japanese apricot in Japan, is a deciduous fruit tree species in the family Rosaceae, genus *Prunus*. Native to China, it has been cultivated for over 3,000 years throughout most of the country (Gao *et al.*, 2026; Mitani *et al.*, 2018). Its fruit has been used as a medicinal herb and an excellent natural health food in East Asian countries for more than 2,000 years (Gong *et al.*, 2021; Hu *et al.*, 2025). Beyond its traditional use as a fruit and medicinal plant, *P. mume* is increasingly recognized for its role in forestry and agroforestry systems. Its deep root system and moderate tolerance to poor soils also make it suitable for soil and water conservation in hilly areas, as well as for low value forest transformation and urban greening (Gao *et al.*, 2026; Mitani *et al.*, 2018). Nursery practices, especially fertilization, critically affect seedling morphology, nutrition, and physiology (Grossnickle & El-Kassaby, 2016; Timmer, 1997). Seedlings with adequate nutrient reserves and balanced C allocation exhibit higher post-planting survival, stress tolerance, and growth (Oliet *et al.*, 2013; Villar-Salvador *et al.*, 2015), whereas nutrient deficiencies or imbalances can impair root development, mycorrhization, and field establishment (Mason *et al.*, 2000).

Despite extensive studies on N addition effects on crop and model species, the organ-specific responses of C:N:P stoichiometry and NSC pools

in *P. mume* seedlings under varying N supply remain poorly understood. Specifically, it remains unclear whether N addition consistently increases N content across organs, whether high N exacerbates P limitation, and whether C allocation shifts between starch (storage) and soluble sugars. To address these gaps, we formulated three hypotheses: (1) N addition increases total N but decreases P in all organs, raising N:P ratios most markedly in stems, (2) moderate N supply enhances biomass and soluble NSC, while excessive N reduces starch reserves and inhibits growth, and (3) roots exhibit greater stoichiometric sensitivity than leaves due to direct soil N exposure. Herein, this study aimed to investigate the effects of N addition on the growth, elemental accumulation, and NSC dynamics of *P. mume* seedlings. These results are expected to provide theoretical and practical information for adjusting nutrient management of *P. mume* seedlings. From a forestry perspective, understanding how N availability affects C:N:P stoichiometry and NSC dynamics is essential for producing robust seedlings for field planting. These findings are intended to support evidence-based fertilization protocols for *P. mume* nurseries and young plantations, thereby improving reforestation success and long-term productivity of *P. mume* based agroforestry systems.

Materials and Methods

Study site

The study site was situated within a greenhouse at Sichuan Agricultural University, Chengdu, China (30°38' N, 103°45' E), at an elevation of 505 m above sea level. This location exhibits a mid-latitude inland subtropical humid monsoon climate, characterized by an annual average temperature of 16.4°C, and a mean annual precipitation of 1,814 mm, the majority of which occurs during summer, alongside an average relative humidity of 84%. The region experiences an annual frost-free period of 282 days and receives a total of 1,104.5 sunshine hours per year.

Materials

P. mume seeds were collected in August 2022 from Meilin Town, Mabian County, Leshan, China. The measured seed properties were: thousand-

grain weight 825.6 g, seed moisture content 8.7%; germination percentage was not systematically recorded. Agricultural soil (alluvial soil) was collected from Jingshan Village, Yongan Town, Shuangliu District, Chengdu, China. The soil was air-dried, ground, and homogenized. The measured soil physicochemical properties were: pH 8.1, organic matter 38.38 g kg⁻¹, total N 2.35 g kg⁻¹, total P 0.300 g kg⁻¹, and total K 20.15 g kg⁻¹. Available N, available P, and available K were not measured.

Seedling culture and treatment

P. mume seeds were stratified in moist sand for approximately three months and subsequently sown *via* drill sowing. Following germination, the seedlings were cultivated until they reached a height of 50 cm (approximately 30–40 days), at which point poorly growing individuals were removed. The pot experiment was conducted in a greenhouse at Sichuan Agricultural University, located in Wenjiang District, Chengdu, China. Each pot (non-woven fabric, 21 cm upper diameter × 20 cm lower diameter × 15 cm height) was filled with 10 kg of soil. On April 16, 2023, the pre-cultivated seedlings were transplanted, with one seedling per pot. Based on the pot surface area (0.0314 m²) and a typical field planting density for *P. mume* orchards ($\approx$ 833 plants ha⁻¹, corresponding to 3 m × 4 m spacing), the approximate field-equivalent N rates were: CK = 0, T1 $\approx$ 147, T2 $\approx$ 294, T3 $\approx$ 588, and T4 $\approx$ 1176 kg N ha⁻¹. The highest level (T4) was deliberately designed as supra-optimal to investigate the threshold of N, and is not intended as a practical fertilization recommendation. Consequently, four N addition levels per plant were established: CK (0 g N plant⁻¹), T1 (5 g N plant⁻¹), T2 (10 g N plant⁻¹), T3 (20 g N plant⁻¹), and T4 (40 g N plant⁻¹) (Figure 1). Each treatment comprised 10 pots, arranged in a completely randomized design. After transplanting, the seedlings were allowed a 45-day acclimatization period, after which fertilization was initiated. Fertilizer was applied in four equal split doses over a 60-day period (once every 15 days, Figure 1). For each application, the prescribed amount of urea was dissolved in 500 ml of tap water, and after thorough mixing, 25 ml of the solution was evenly poured around the seedling root zone ($\approx$ 5 cm from the stem). The entire experimental period was conducted under

a rain-out shelter to prevent N leaching from rain-fall. Daily watering was performed to maintain optimal soil moisture, and routine disease, pest, and weed control measures were carried out. At the end of the experiment, destructive sampling was performed on three randomly selected pots per treatment. From each selected seedling, roots, stems, and leaves were separately harvested. All chemical analyses were conducted on three biological replicates ($n = 3$), each derived from a single pot.

Determination of growth index and biomass

Each group was harvested for the determination of growth indices, non-structural sugar content, and elemental composition. Plant height was measured using a tape measure from the base to the apex of the stem, while the basal diameter was assessed with an electronic vernier caliper. Ten seedlings from each treatment group were dissected into roots, stems, and leaves, washed with deionized water, surface-dried, and then placed in an oven at 105 °C for 30 min followed by drying at 65 °C until constant weight was achieved. The dry mass of each organ was subsequently recorded.

Analysis of C, N, and P content

For each of the three replicate plants per treatment, roots, stems, and leaves were separately harvested, oven-dried, and ground using a grinder (FW80). The resulting composite sample of each organ per plant was passed through a 100-mesh sieve. A test solution was then prepared via concentrated $\text{H}_2\text{SO}_4\text{--HClO}_4$ digestion. Total C, N, and P contents were determined using the potassium dichromate oxidation method, the semi-micro Kjeldahl method, and the molybdenum-antimony colorimetric method, respectively (Zhi *et al.*, 2022). From the ground and sieved composite sample of each organ per plant, three technical replicates (sub-samples) were taken for C, N, P, and NSC assays. The mean value of the three technical replicates was calculated to obtain a single biological replicate value ($n = 3$ per treatment). This nested design avoids pseudoreplication, as technical replicates are not treated as independent statistical units; only biological replicates (individual pots) entered the final ANOVA.

Determination of non-structural carbohydrate content

For NSC analysis, roots, stems, and leaves from each of the three biological replicates per treatment were dried at 80 °C for 48 h, ground, and passed through a 100-mesh sieve. For each sample, 0.1 g of powder was weighed in triplicate. Thirty milliliters of 80% ethanol were added, and the mixture was boiled for 10 min. The extract was centrifuged at $21,000 \times g$ for 10 min. This extraction procedure was repeated twice. The supernatants were pooled for soluble sugar (SS) analysis, and the precipitate was used for starch (ST) analysis. Soluble sugars were determined by the anthrone-sulfuric acid colorimetric method (Liu *et al.*, 2020). Absorbance was measured at 620 nm, and concentrations were calculated from a glucose standard curve. The results are expressed as mg glucose g^{-1} dry weight (DW). Starch was determined by an enzymatic hydrolysis of the remaining pellet following standard protocols.

Statistical analysis

The experiment used a completely randomized design. For growth and biomass measurements, $n = 10$ per treatment. For chemical analyses (C, N, P, NSC), $n = 3$ per treatment, with each biological replicate derived from an individual pot containing one seedling. For each biological replicate, three technical replicates were averaged to obtain a single value. Because roots, stems, and leaves were sampled from the same plants, separate one-way ANOVAs were conducted for each organ to avoid pseudoreplication, as the primary interest was organ-specific responses to N addition rather than cross-organ comparisons. Post-hoc comparisons among treatment groups were performed using Fisher's least significant difference (LSD) test at $\alpha = 0.05$. Regression analysis and Pearson correlation were performed to examine relationships among variables. Principal component analysis (PCA) was carried out using OriginPro 2018 (OriginLab Corporation, Northampton, MA, USA) to explore multivariate patterns, variables were standardized (z-score) prior to PCA. Element accumulation per organ was calculated as organ element content $\times$ organ biomass. All statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA), Excel, and Orig-



Figure 1. Nitrogen application schedule during seedling cultivation. Four N levels were applied once every 15 days for a total of four applications.

inPro 2018. All data are expressed as mean $\pm$ standard deviation (SD).

Results

Growth responses to nitrogen addition

The variations in plant height and ground diameter across treatments were presented in Figure 2. Figure 2a showed that the initial seedling height was comparable among the five groups. After four fertilization cycles, the final plant height of the CK, T1, T2, T3 and T4 groups were 113.43 cm, 111.23 cm, 128.35 cm, 121.63 cm, and 122.40 cm, respectively. The T4 group had the highest growth rate, closely followed by the T2 group. With the exception of T1, plant height in all other treatment groups was significantly greater than that in the CK. The CK group showed greater variability in height increment compared to the other groups. Figure 2b showed that the initial ground diameter ranged primarily from 2.60 to 2.85 cm. The CK group had an initial diameter of 2.85 cm, which exceeded that of the other treatment groups. Following the fourth fertilization, the ground diameter of the CK group decreased after the fourth fertilization, relative to the other

experimental groups, whereas the ground diameter of the T2 group increased following the fourth fertilization. Overall, throughout the entire fertilization period, the lowest diameter growth rate was observed in the CK group, whereas the highest value was recorded in the T2 group.

Biomass allocation responses to nitrogen addition

Table 1 presented the biomass parameters across treatments. The root biomass, stem biomass, leaf biomass, above-ground biomass and total biomass ranged from 2.207–3.368, 6.98–8.82, 6.40–8.04, 13.38–16.86, and 15.91–19.60 g plant⁻¹, respectively. The corresponding biomass ratios (root mass ratio, stem mass ratio, and leaf mass ratio) were 0.150–0.190, 0.419–0.447, and 0.375–0.419, respectively, while the root-to-shoot ratio ranged from 0.161 to 0.245. The CK group had significantly lower biomass than the N-supplied treatments. The T4 group had lower root biomass than the other N-supplied treatments. Total biomass was highest in the T1, T2 and T3 groups and lowest in the CK and T4 groups.

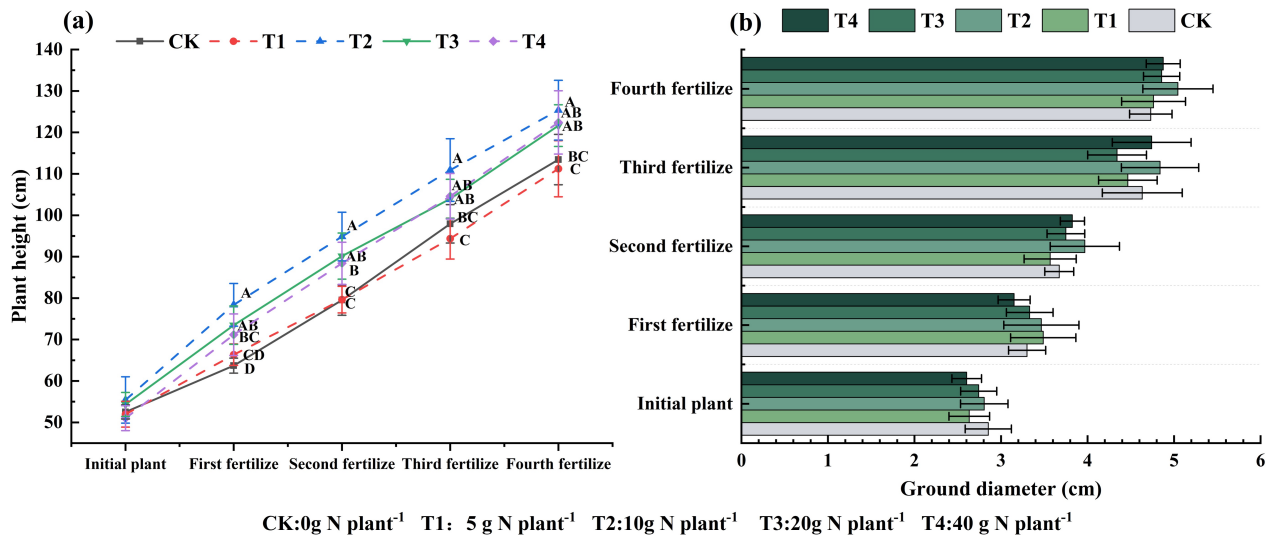


Figure 2. Effects of N addition on seedling height (a) and ground diameter (b) of *P. mume* seedlings. Data represent mean $\pm$ SD, $n = 10$ per treatment. N addition levels: CK (0), T1 (5), T2 (10), T3 (20), and T4 (40 g N plant⁻¹).

Table 1. Effects of N addition on biomass allocation in roots, stems, and leaves of *P. mume* seedlings.

Group	Root biomass (g)	Stem biomass (g)	Leaf biomass (g)	Above-ground biomass (g)	Total biomass (g)	Root-shoot ratio
CK	2.207 $\pm$ 0.166 B	6.982 $\pm$ 0.195 B	6.491 $\pm$ 0.345 B	13.467 $\pm$ 0.383 B	15.908 $\pm$ 0.398 B	0.161 $\pm$ 0.013 B
T1	3.368 $\pm$ 0.489 A	7.637 $\pm$ 0.956 AB	6.44 $\pm$ 0.599 B	14.078 $\pm$ 1.529 B	17.446 $\pm$ 1.054 AB	0.245 $\pm$ 0.06 A
T2	2.933 $\pm$ 0.429 A	8.348 $\pm$ 0.81 AB	6.965 $\pm$ 0.549 AB	15.313 $\pm$ 1.303 AB	18.246 $\pm$ 1.206 AB	0.193 $\pm$ 0.039 AB
T3	2.737 $\pm$ 0.079 AB	8.821 $\pm$ 0.166 A	8.041 $\pm$ 0.378 A	16.862 $\pm$ 0.395 A	19.599 $\pm$ 0.349 A	0.162 $\pm$ 0.007 B
T4	2.708 $\pm$ 0.059 AB	6.976 $\pm$ 1.076 C	6.401 $\pm$ 0.553 B	13.383 $\pm$ 1.624 B	16.092 $\pm$ 1.681 B	0.204 $\pm$ 0.021 AB

Data represent mean $\pm$ SD, $n = 10$. Different uppercase letters within each column denote significant differences among treatments at $p \leq 0.05$ (Fisher's LSD test).

Responses of C, N and P contents to nitrogen addition

The C, N and P contents in roots, stems and leaves under N addition conditions were shown in Figure 3. As shown in Figure 3a, C content 307.66 to 389.34 mg g⁻¹ in leaves, 371.61 to 412.87 mg g⁻¹ in stems, and 367.96 to 408.79 mg g⁻¹ in roots, respectively. Among all treatments, the T1 group exhibited the highest C content in both leaves and roots, significantly surpassing that of the T4 group. Conversely, the T4 group showed a markedly lower C content across all three organs compared to the other treatment groups. Figure 3b showed that N content ranged from 31.44 to 35.83 mg g⁻¹ in leaves, 4.83 to 8.71 mg g⁻¹ in stems, and 10.78 to 15.31 mg g⁻¹ in roots, respectively. N content was highest in T4 roots and stems (15.31 and 8.71 mg g⁻¹, respectively), representing a 42% and 80% increase over the control. Figure 3c showed that P content ranged

from 0.487 to 0.558 mg g⁻¹ in leaves, 0.300 to 0.383 mg g⁻¹ in stems, and 0.360 to 0.551 mg g⁻¹ in roots, respectively. Root P content in the control group was significantly higher than that in all four N-addition treatments. Stem P content in CK and T1 was significantly higher than in T2, T3, and T4. Overall, C, N, and P contents varied significantly among organs and across N addition levels when analyzed separately by organ.

C, N and P accumulation responses to nitrogen addition

Figure 4 showed the impact of varying N application rates on C, N, and P accumulation in *P. mume* seedlings. Figure 4a showed that the range of C accumulation was 348.81–398.71 mg plant⁻¹ under different N addition levels. From group T1 to T4, C accumulation declined significantly with increasing N supply. Only the T4 group had a lower C accumulation than that in the CK group. Figure 4b showed that N accumula-

tion ranged from 16.44 to 20.66 mg plant⁻¹. N accumulation increased significantly in all N-supplied groups (T1–T4), and the highest value (20.66 mg plant⁻¹) was observed in T4, compared to 19.01 mg plant⁻¹ in CK. As shown in Figure 4c, P accumulation ranged from 0.385 to 0.471 mg plant⁻¹. P accumulation in T4 was notably lower than in the other groups. Across root, stem, and leaf fractions, P accumulation initially increased with N supply (from T1 onward) and then declined, with the decreasing trend consistent across all three organs.

Stoichiometric ratios of C, N, P responses to nitrogen addition

Figure 5 showed the C, N, and P stoichiometric ratios across treatments. In Figure 5a, the C:N ratios in the leaves, roots, stems, and the total plant ranged from 8.57 to 12.32, 25.18 to 37.95, 42.79 to 83.28, and 16.88 to 24.03, respectively. The whole-plant C:N ratio was between the roots' and leaves' C:N ratios. The T1 group showed significantly higher C:N ratios in leaves, roots, and whole plant than the other groups. In stems, the C:N ratio in T1 group was significantly higher than in the T3, T4 and CK groups. As depicted in Figure 5b, under N addition conditions, the C:P ratios in the leaves ranged from 614.82 to 699.42, while the C:P ranges in the roots and stems were 701.44 to 1070.51 and 1017.89 to 1237.68, respectively. The C:P ratios in the seedlings fluctuated between 776.83 and 906.06, closely resembling the root C:P. Figure 5c showed that the N:P ratios in the leaves, roots, stems, and total plant ranged from 56.67 to 73.64, 22.11 to 42.56, 13.81 to 29.00, and 35.25 to 53.66, respectively. The whole-plant N:P ratio was slightly higher than the stems' N:P ratio.

NSCs content responses to nitrogen addition

The concentrations of NSCs in the roots, stems, and leaves were presented in Figure 6. As shown in Figure 6a, glucose content in the leaves, roots and stems ranged from 32.41 to 37.68 mg g⁻¹, 28.25 to 39.81 mg g⁻¹, and 21.32 to 28.55 mg g⁻¹, respectively. The highest root glucose concentration (39.81 mg g⁻¹) was in the CK group. As shown in Figure 6b, fructose content in the leaves, roots and stems ranged from 3.83 to 9.21 mg g⁻¹, 2.17–7.95 mg g⁻¹ and 2.32–4.95 mg g⁻¹, respectively. The maximum leaf fructose (9.21 mg g⁻¹) was in the T4 group. As depicted in Figure 6c,

sucrose content in the leaves, roots and stems ranged from 19.73 to 25.96 mg g⁻¹, 11.12 to 23.24 mg g⁻¹, and 8.21 to 16.93 mg g⁻¹, respectively. The highest leaf sucrose (25.96 mg g⁻¹) was in the T1 group. As illustrated in Figure 6d, starch content in the leaves, roots and stems ranged from 30.04 to 40.96 mg g⁻¹, 42.34 to 81.73 mg g⁻¹, and 46.40 to 53.42 mg g⁻¹, respectively. Starch content in roots, especially in CK and T1, was notably higher than in the other groups.

Correlation analysis, PCA, and linear fitting

Figure 7a showed the correlation and cluster analysis of morphological indicators and C, N, and P accumulation under the N addition. Positive correlations were observed between biomass and root length, root length and plant height, plant height and ground diameter, root-shoot ratio and ground diameter, C and P accumulation. In contrast, N accumulation had significant negative correlations with biomass, C accumulation, and P accumulation. Cluster analysis showed that N accumulation contrasts with both C and P accumulation, whereas C accumulation aligned with P accumulation. Biomass with root length, and seedling height with ground diameter, formed smaller clusters. These findings indicated that increasing N addition leads to significant differences in element accumulation among treatments relative to CK, yet the relationships between C, N, P accumulation and morphological indicators remained non-significant. As shown in Figure 7b, PCA was performed to explore multivariate relationships among C, N, P, soluble sugars (glucose, fructose, sucrose), starch, and biomass across all organs and treatments. All variables were standardized (z-score) prior to analysis. Each point in the PCA biplot represents an individual biological replicate, with organs distinguished by colors and treatments by symbols. The first principal component (PC1) explained 46.2% of the total variance and was characterized by positive loadings for soluble sugars and negative loadings for starch and biomass, suggesting a trade-off between immediate metabolic soluble sugars and storage starch. The second principal component (PC2) explained 22.7% of the variance and was primarily associated with N and P contents. In the ordination space, roots from T3 and T4 treatments showed more pronounced

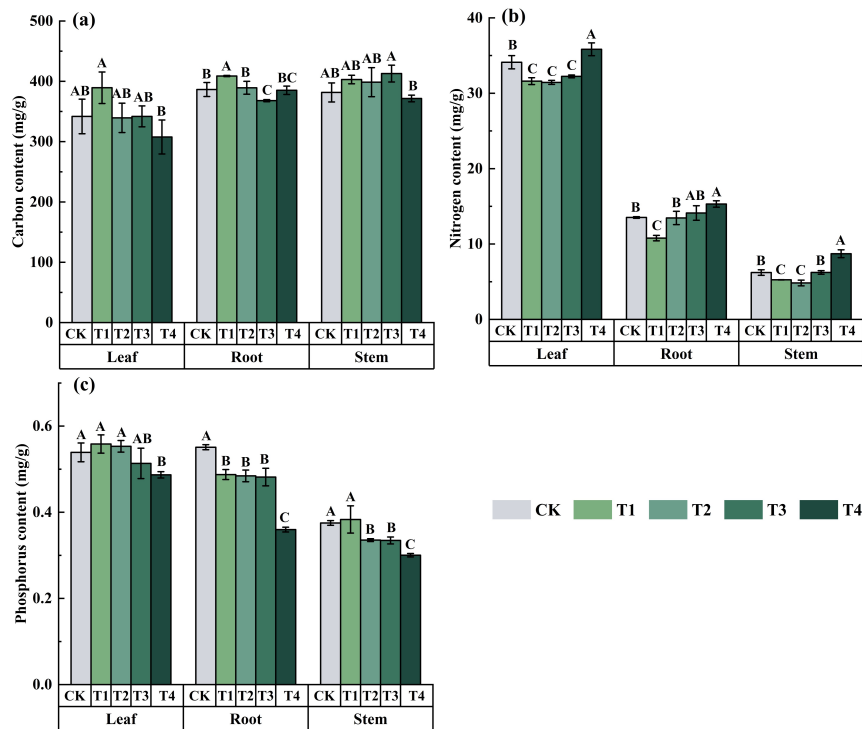


Figure 3. Effects of N addition on carbon (a), nitrogen (b), and phosphorus (c) contents in the leaves, stems, and roots of *P. mume* seedlings. Data represent mean $\pm$ SD, $n = 3$ biological replicates per treatment. DW, dry weight. Different uppercase letters above bars denote significant differences among treatments within the same organ at $p \leq 0.05$ (Fisher's LSD test).

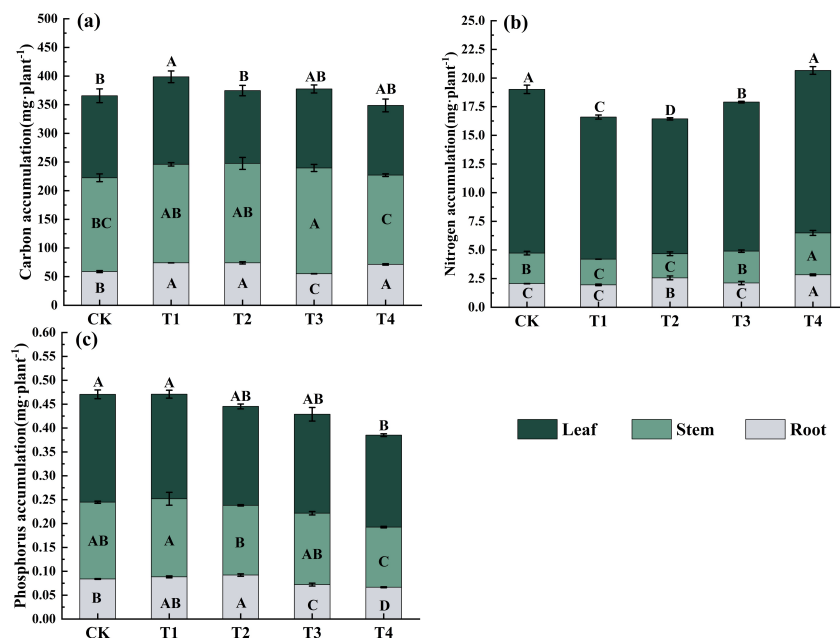


Figure 4. Effects of N addition on carbon (a), nitrogen (b), and phosphorus (c) accumulation in the leaves, stems, and roots of *P. mume* seedlings. Accumulation was calculated as organ element content $\times$ organ biomass. Data represent mean $\pm$ SD, $n = 3$ biological replicates per treatment. Different uppercase letters denote significant differences among treatments within the same organ at $p \leq 0.05$ (Fisher's LSD test).

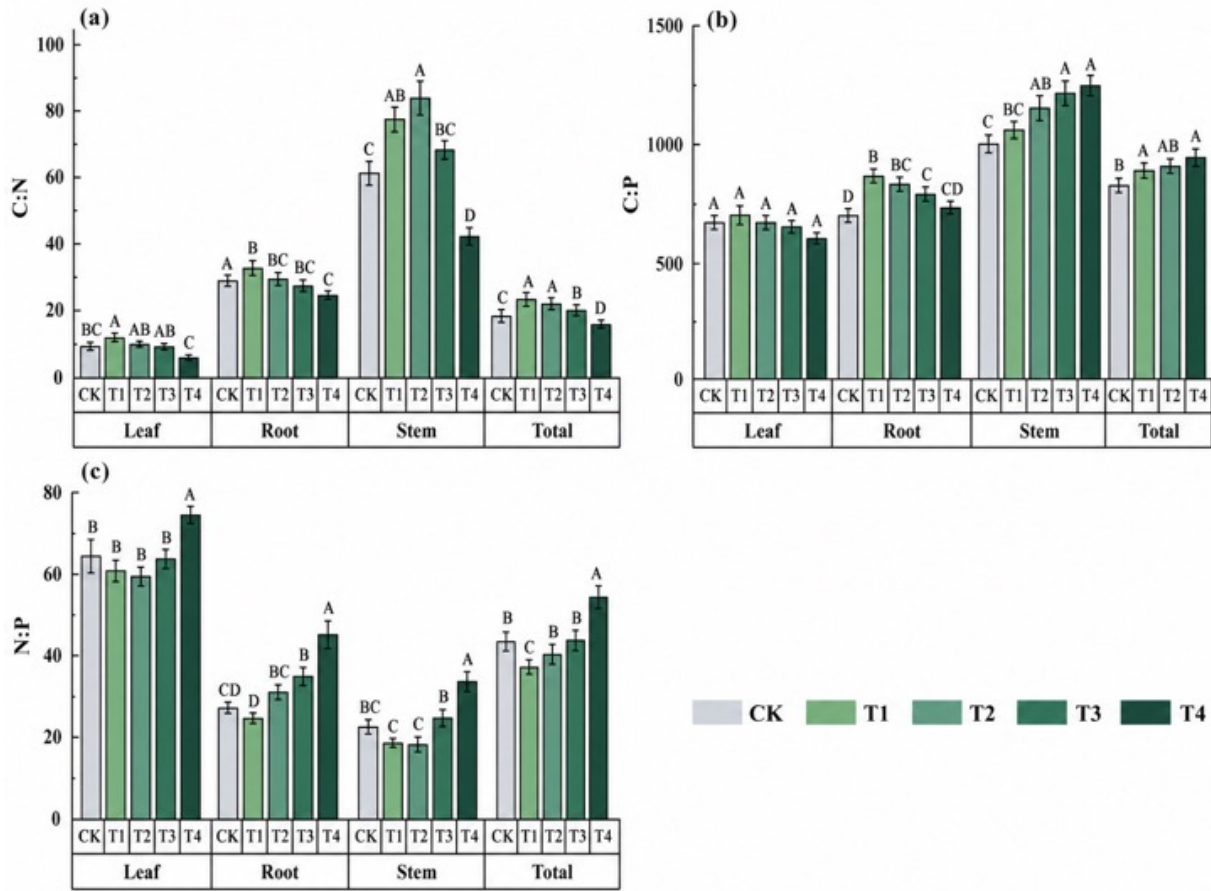


Figure 5. Effects of N addition on C:N (a), C:P (b), and N:P (c) stoichiometric ratios in the leaves, stems, and roots of *P. mume* seedlings. Data represent mean $\pm$ SD, $n = 3$ biological replicates per treatment. Different uppercase letters denote significant differences among treatments within the same organ at $p \leq 0.05$ (Fisher's LSD test).

shifts toward higher soluble sugar and N content, whereas stems and leaves exhibited less separation across treatments. This descriptive pattern suggests that roots are more responsive to N addition in terms of C-nutrient reallocation, which is consistent with the organ-specific ANOVA results showing significant N-induced changes in root NSC and stoichiometry. However, the PCA does not provide formal statistical evidence for organ differences; rather, it serves as a complementary visualization of the multivariate trends. Figure 7c showed the linear correlations between N content and C, P, and NSC. Negative correlations between N content and C content as well as starch were revealed, respectively. In contrast, N content and P content showed a positive correlation ($K = 0.006$). Furthermore, positive correlations were observed between N content and glucose, fructose, sucrose, with K values of 0.292, 0.075, and 0.366, respectively.

Discussion

Fertilization is a cornerstone agronomic practice for cultivating robust seedlings and ensuring their subsequent growth and development (Jiang *et al.*, 2024; Yang *et al.*, 2023). Consequently, identifying an appropriate N addition regime is of paramount importance for the successful expansion of *P. mume* cultivation areas. In this study, *P. mume* seedlings exhibited viable growth across a gradient of N supply levels, however, significant changes were observed in morphological indicators, C, N, P and NSC concentrations, accumulation, distribution, and stoichiometric ratios across roots, stems, and leaves. Furthermore, correlation analysis, PCA, and linear fitting collectively revealed multiple significant positive and negative relationships among growth indicators, C:N:P ratios, and NSC contents in response to differential N addition (Figure 7). These multifaceted re-

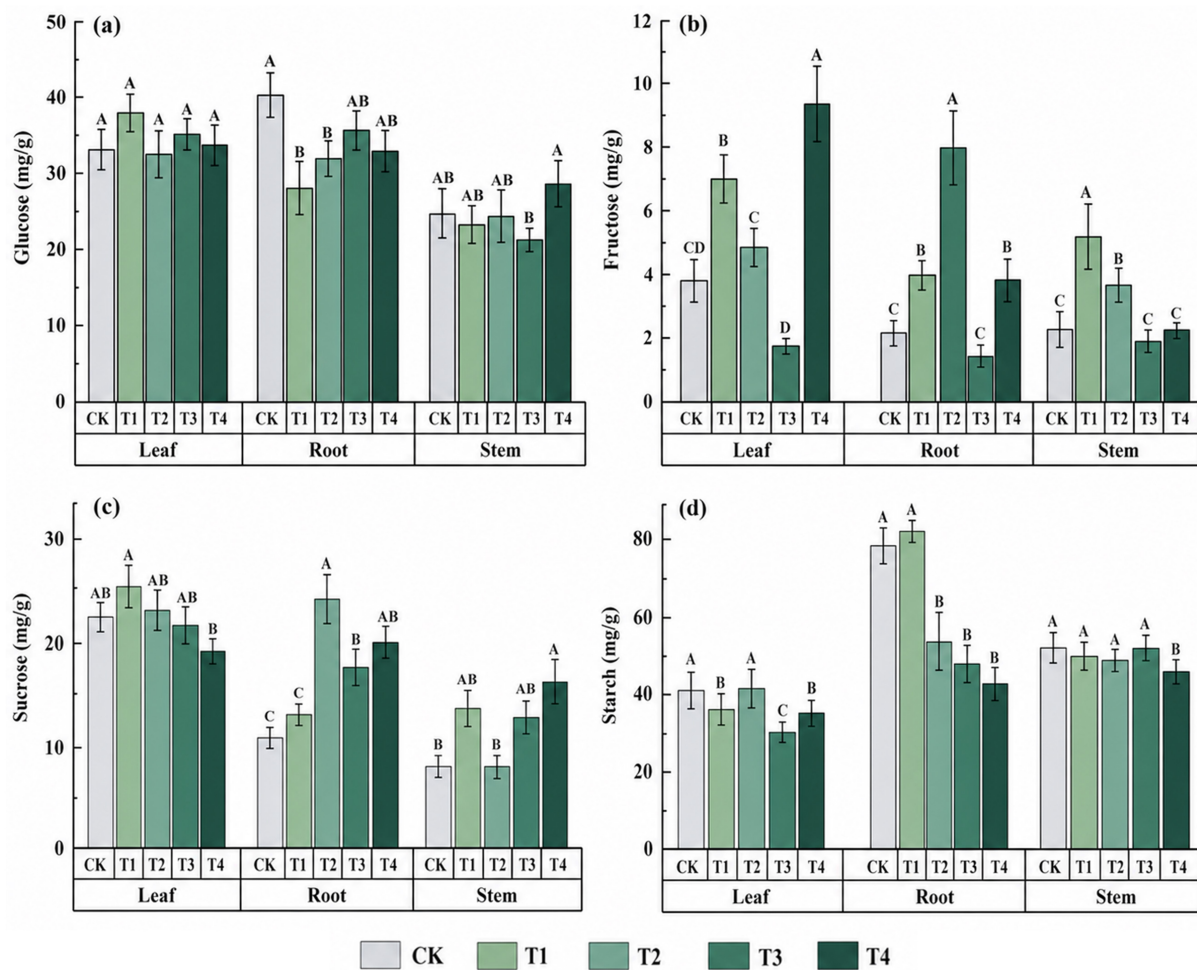


Figure 6. Effects of N addition on glucose (a), fructose (b), sucrose (c), and starch (d) contents in the leaves, stems, and roots of *P. mume* seedlings. Data represent mean $\pm$ SD, $n = 3$ biological replicates per treatment. Different uppercase letters denote significant differences among treatments within the same organ at $p \leq 0.05$ (Fisher's LSD test).

sponses highlight that both deficiency and excess in N supply can disrupt internal nutrient homeostasis, with implications for stress resilience and post-transplant establishment. These findings provide both theoretical foundations and practical guidelines for refining nutrient management strategies during the nursery stage of *P. mume* cultivation. Moreover, they provide a basis for optimizing field transplantation protocols and tailoring N application schedules to specific soil conditions, ultimately contributing to enhanced orchard productivity and sustainable cultivation practices.

Morphological indicators are fundamental in seedling research, as they directly reflect growth status and provide actionable insights for optimizing cultivation practices. Changes in these indicators can be effectively employed to assess

the supply-demand balance between seedlings and N fertilizer. In the present study, plant height reached a maximum at a low N addition level and then decreased when N supply either increased or decreased, demonstrating a dual (unimodal) response to N fertilizer. A similar trend was observed for several biomass parameters, with peak values occurring at either T1 (5 g N plant⁻¹) or T3 (20 g N plant⁻¹). The root-to-shoot ratio peaked under low N addition but also showed a moderate increase at the high N level. In contrast, ground diameter showed no significant change across N gradients, whereas root length increased steadily with rising N supply (Table 1 and Figure 2). The inhibitory effect observed under the highest N addition (40 g N plant⁻¹) may be attributed to multiple factors, including altered soil pH, shifts in soil microbial community com-

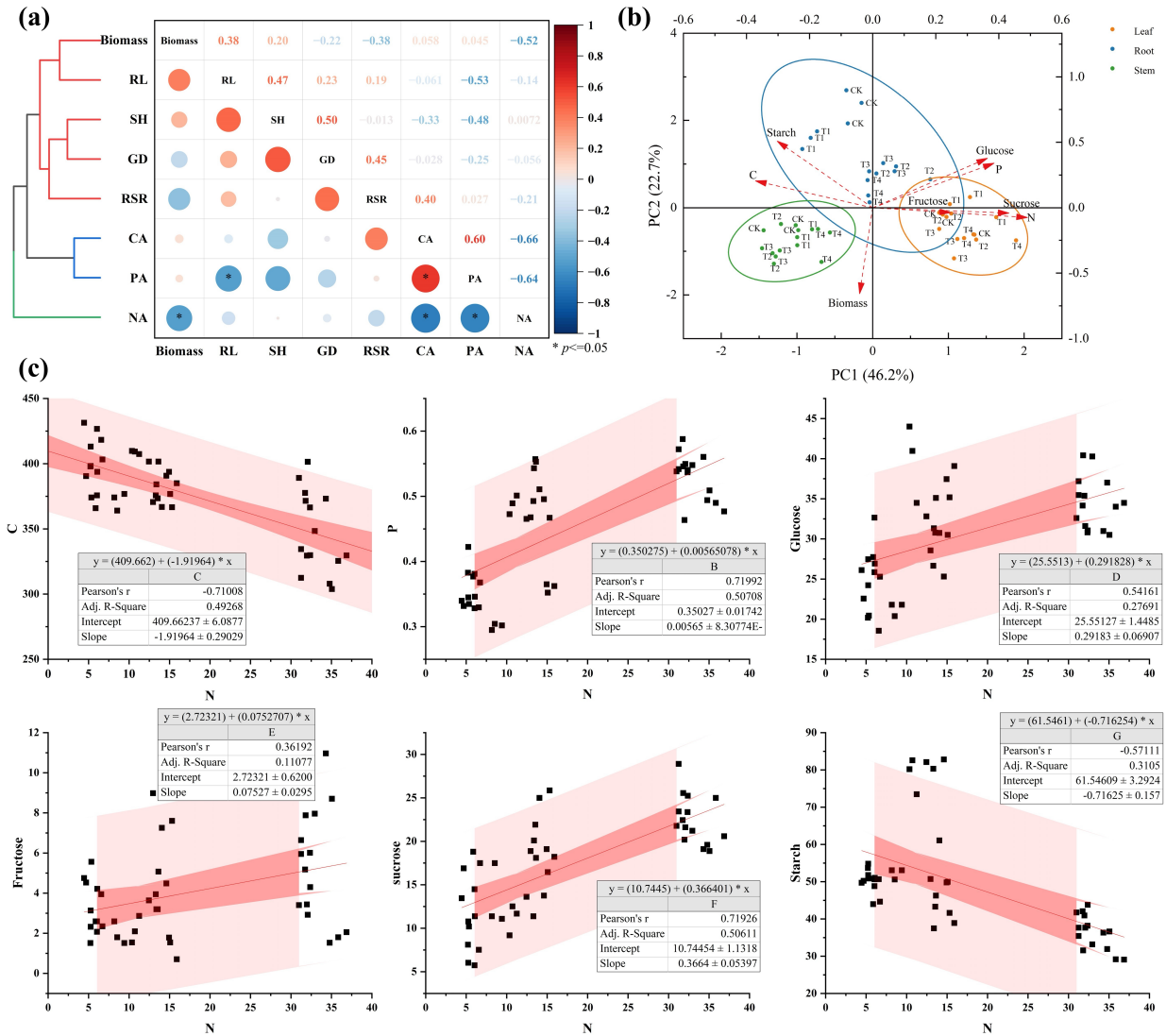


Figure 7. Multivariate analyses of growth, stoichiometric, and NSC variables. (a) Pearson correlation matrix among morphological indicators and element accumulation. Blue indicates negative correlation ($r = -1.0$), red indicates positive correlation ($r = 1.0$); * $p < 0.05$, ** $p < 0.01$. (b) Principal component analysis (PCA) biplot of C, N, P, soluble sugars (glucose, fructose, sucrose), starch, and biomass across organs and treatments. Variables were standardized (z-score); PC1 and PC2 explain 46.2% and 22.7% of variance, respectively. Points represent individual biological replicates ($n = 3$ per treatment), with organs color-coded and treatments shape-coded. (c) Linear regression between N content and C content, P content, and NSC components; regression coefficients (K) and significance levels are shown.

position and activity, reduced nitrogen use efficiency (NUE), and intensified competitive partitioning between aboveground and belowground organs (Jia *et al.*, 2024). Comparable unimodal responses have been documented in other woody species. For instance, *Cunninghamia lanceolata* (Lamb.) Hook. seedlings also exhibit biomass promotion under low to moderate N but inhibition under excessive N (Xu & Timmer, 1999). Importantly, the deliberate inclusion of our highest dose (T4, 40 g N plant⁻¹) as a supra-optimal treatment was specifically designed to capture the declining phase of the response curve. This level was never intended to replicate realistic field fertilization, but rather to define the upper threshold beyond which N becomes phytotoxic. The observed growth suppression in T4, together with the marked reduction in root starch reserves (Figure 6d), confirms that this level exceeds the physiological optimum for *P. mume* seedlings and provides a mechanistic rationale for avoiding excessive N application in nursery practice. Unlike certain coniferous species in which high N supply suppresses root elongation, we found a steady increase in *P. mume* root length with rising N addition. This interspecific difference may reflect the higher N demand of fruit trees or alternatively, a compensatory foraging response under confined pot conditions. The observed root length increase could also result from accelerated fine-root growth and a greater root node length at lower N levels. Overall, the growth suppression under high N in this experiment likely results from diminished NUE, osmotic imbalance, and the accumulation of metabolic by-products that exert toxic effects on seedling physiology. Taken together, these morphological and allocation patterns indicated that moderate N (10–20 g N plant⁻¹) constitutes the optimal dosage for promoting biomass accumulation in *P. mume* seedlings.

Nutrient availability is a primary determinant of seedling performance, and stoichiometric analysis offers a powerful lens for examining the internal balance among elements and its modulation by N supply. Across a gradient of N addition, the concentrations of C, N, and P contents and their stoichiometric ratios in leaves, stems, and roots, collectively reflect the plant's resource allocation strategies, growth strategy, and stress resistance (Scharler *et al.*, 2015). Our results showed substantial organ-specific shifts in C, N, and P con-

tents under varying N addition. With increasing N supply, N content rose markedly in all three organs, whereas P content decreased significantly. Notably, N content in the CK group was equal to or even higher than that in the three lowest N-treated groups, indicating that the relationship between N addition and tissue N accumulation is not strictly linear, particularly at low doses. Elevated N application was associated with a clear reduction in C content in both roots and leaves (Figure 3). These patterns are consistent with previous studies in other woody species. For instance, N addition significantly increased N content in the roots, stems, and leaves of *Pinus tabulaeformis* Carrière while decreasing P content (Jing *et al.*, 2017). Similarly, trends were reported for *Pinus massoniana* Lamb. seedlings, in which fertilization elevated N content relative to controls (Wu *et al.*, 2019). In our experiment, total P accumulation decreased substantially with escalating N fertilizer, whereas N accumulation showed a significant upward trend. Among all treatments, the T4 group (40 g N plant⁻¹) had the highest N accumulation but the lowest P accumulation across all organs (Figure 4). Figure 7 illustrated significant negative correlations between N accumulation and both C accumulation and P accumulation, as well as between P accumulation and biomass and root length. These findings suggested that N- and P-related stoichiometric changes under N addition are closely linked to morphological adjustments in plant parts.

The sensitivity of C:N:P stoichiometry to N addition was also evident. Our results indicated that the C:N ratio in seedling roots, stems, and leaves decreased significantly with increasing N supply, while the N:P ratio increased markedly. Comparable findings have been reported for *Pleioblastus amarus* (Keng) Keng f. and *Pinus koraiensis* Siebold & Zucc. (Li *et al.*, 2021; Yang *et al.*, 2019). Additionally, N application coincided with a pronounced increase in the C:P ratio in roots and stems. It is widely accepted that C:N and C:P ratios reflect C sequestration capacity and nutrient-use efficiency, whereas the N:P ratio serves as an effective indicator of plant response to environmental change and growth limitation. According to the established threshold, N:P values between 14 and 16 suggest co-limitation by N and P, while values exceeding 16 points to P limitation (Güsewell, 2004). In this study, only

stems under low N addition fell within the 14–16 range, all other plant parts had N:P ratios above 16 (Figure 5). This suggests that under low N conditions, stems are co-limited by N and P, possibly reflecting lower sensitivity to N availability or a higher N demand compared to roots and leaves. Indeed, roots have been reported to respond particularly strongly to N addition (Yin *et al.*, 2021), and our root N:P data are consistent with that pattern. The fact that both roots and leaves showed N:P ratios exceeding 16, coupled with the significant increases in C:P and N:P ratios and the sharp decline in P content under high N, supports the interpretation that excessive N supply may exacerbate P scarcity or reduce P utilization efficiency.

N is indispensable for photosynthesis, which drives the synthesis and accumulation of various nutrients, particularly NSCs. Soluble NSCs serve immediate functions and act as rapid C sinks, whereas starch functions as a long-term C sink (Dietze *et al.*, 2014). Consequently, N addition can directly or indirectly affect NSC content in *P. mume* seedlings. Our results showed that high N application was associated with a marked reduction in starch content, especially in roots (Figure 6). This aligns with previous studies on *Quercus mongolica* Fisch. ex Ledeb. and *Acer pseudoplatanus* L. (Mackie-Dawson *et al.*, 1994; Wang *et al.*, 2024b). The underlying mechanisms remain unclear, but possible explanations include shifts in C allocation priorities, altered source–sink relationships, or accelerated starch turnover. In contrast, N addition significantly increased fructose content in all three organs, and increased sucrose content in stems and roots (Figure 6), implying a redistribution of C among NSC fractions under varying N supply. Whether the increase in soluble NSCs reflects enhanced photosynthetic output, reduced conversion to starch, or accelerated starch turnover cannot be determined from our current data. Notably, we found positive correlations between N and P contents and soluble NSCs, while soluble NSC showed a negative correlation with C content, indicating coordinated shifts in nutrient and C status, although causal links remain speculative. This pattern reflects the possibility that C availability constrains seedling growth (Wiley & Helliker, 2012). Resolving these relationships would require direct measurements of C fluxes, photosynthetic rates, and activities of

enzymes involved in NSC metabolism. Furthermore, prior research indicated that the growth-promoting effect of N addition on seedlings diminishes with sustained high N supply, as excessive N impairs multiple aspects of seedling performance. This further explains why morphological indicators did not improve under high N in our experiment. The apparent shift from starch to soluble NSCs under high N supply has potential ecological implications. Starch reserves are important for stress tolerance and resprouting capacity following disturbance (Chapin *et al.*, 1990; Villar-Salvador *et al.*, 2015). The observed reduction in starch, particularly in roots, may compromise seedling resilience to transplant shock, drought, or other adverse conditions, although we did not directly measure stress tolerance or post-planting survival. Whether such compositional changes affect subsequent establishment in the field remains an open question that warrants further investigation.

From a practical standpoint, our results offer preliminary guidance for nursery management of *P. mume* seedlings intended for afforestation or agroforestry systems. A unimodal growth response was observed, with moderate nitrogen supply (10–20 g N plant⁻¹) associated with enhanced biomass accumulation, whereas supra-optimal N (≥ 40 g N plant⁻¹) conferred no additional growth gains and coincided with a substantial depletion of root starch reserves. This starch depletion, coupled with elevated N:P ratios (> 16) indicative of progressive P limitation under high N, suggests that excessive fertilization may compromise seedling quality and stress tolerance. However, these interpretations should be viewed with caution. We did not quantify C assimilation rates, dark respiration, or the enzymatic activities governing starch–sugar interconversion, nor did we assess soil microbial activity, pH shifts, or rhizosphere processes – all of which could mediate plant responses to N fertilization. Importantly, our study was conducted under controlled greenhouse conditions using a single soil type, and the pot environment inherently restricts root development and may alter nutrient dynamics compared to open-field situations. Consequently, the generalizability of our findings is limited, and the tentative recommendations (10–20 g N plant⁻¹) require rigorous validation under operational nursery and field condi-

tions before practical implementation. Long-term field trials across diverse soil types and climatic regimes, incorporating comprehensive physiological and edaphic measurements are essential to confirm these thresholds, establish mechanistic causality, and provide a robust basis for refining nutrient management in *P. mume* nursery production.

Conclusion

This study comprehensively analyzed the effects of five N levels (0–40 g N plant⁻¹) on morphological traits, C:N:P stoichiometry, and NSC pools in *P. mume* seedlings, with particular attention to organ-specific responses in roots, stems and leaves. Moderate N supply (10–20 g N plant⁻¹) maximized total biomass (18.2–19.6 g plant⁻¹), whereas supra-optimal N (40 g N plant⁻¹) reduced root starch by up to 48% without further growth gains. Across all organs, N addition consistently increased N content and N:P ratios, but decreased P content. In T3 and T4, leaf and root N:P ratios exceeded 16, indicating progressive P limitation. Soluble sugars (glucose, fructose, sucrose) increased with N supply, while starch content declined, particularly in roots, suggesting a reallocation of C away from long-term storage toward immediate metabolic demands. Correlation and cluster analyses further revealed that root length and total biomass clustered together at low dissimilarity, indicating coordinated growth between above- and below-ground organs under low to moderate N additions. For nursery production of *P. mume* seedlings intended for afforestation or agroforestry, we tentatively suggest that N application within 10–20 g N plant⁻¹ may be beneficial, provided that this is validated under actual nursery and field conditions before practical implementation. Routine soil P monitoring is also recommended to avoid nutritional imbalance. High N levels (≥ 40 g N plant⁻¹) appeared detrimental in this experimental setting and should be avoided. These findings are necessarily constrained by the controlled greenhouse conditions using a single soil type, field trials and longer-term observations are essential to confirm their applicability. Moreover, direct measurements of C flux, soil processes, and key physiological parameters are required to establish causal

relationships. Nevertheless, the consistent stoichiometric patterns observed across the N gradient provide a useful framework for improving seedling quality of *P. mume* seedlings and for predicting growth responses in cultivation systems.

Acknowledgements. This work was financially supported by the National Key Research and Development Program of China (No. 2023YFD1600400). We are grateful to all of the group members and workers for their assistance during seed collection, seedling cultivation and harvesting.

References

- Ågren, G. I. (2008). Stoichiometry and nutrition of plant growth in natural communities. *Annual Review of Ecology, Evolution, and Systematics*, 39, 153–170. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173515>
- Chapin, F. S., Schulze, E.-D., & Mooney, H. A. (1990). The ecology and economics of storage in plants. *Annual Review of Ecology, Evolution, and Systematics*, 21, 423–447. <https://doi.org/10.1146/annurev.es.21.11019.0.002231>
- Chen, X., & Chen, H. Y. H. (2021). Plant mixture balances terrestrial ecosystem C:N:P stoichiometry. *Nature Communications*, 12, 4562. <https://doi.org/10.1038/s41467-021-24889-w>
- Dietze, M. C., Sala, A., Carbone, M. S., Czimczik, C. I., Mantooth, J. A., Richardson, A. D., & Vargas, R. (2014). Nonstructural carbon in woody plants. *Annual Review of Plant Biology*, 65(1), 667–687. <https://doi.org/10.1146/annurev-arplant-050213-040054>
- Dissanayaka, D. M. S. B., Ghahremani, M., Siebers, M., Wasaki, J., & Plaxton, W. C. (2021). Recent insights into the metabolic adaptations of phosphorus-deprived plants. *Journal of Experimental Botany*, 72(2), 199–223. <https://doi.org/10.1093/jxb/eraa482>
- Gao, S., Hu, Y., Qian, W., Xiao, Y., Liao, J., & Chen, J. (2026). Sourness signature during *Prunus mume* fruit development: Integrative insight from metabolomics, organic acid profiles, E-tongue, and computational binding simulation. *Food Chemistry*, 514, 149111. <https://doi.org/10.1016/j.foodchem.2026.149111>
- Gong, X.-P., Tang, Y., Song, Y.-Y., Du, G., & Li, J. (2021). Comprehensive review of phytochemical constituents, pharmacological properties, and clinical applications of *Prunus mume*. *Frontiers in Pharmacology*, 12, 679378. <https://doi.org/10.3389/fphar.2021.679378>
- Grossnickle, S. C., & El-Kassaby, Y. A. (2016). Bareroot versus container stocktypes: A performance comparison. *New Forests*, 47, 1–51. <https://doi.org/10.1007/s11056-015-9476-6>

- Güsewell, S. (2004). N:P ratios in terrestrial plants: Variation and functional significance. *New Phytologist*, 164(2), 243–266. <https://doi.org/10.1111/j.1469-8137.2004.01192.x>
- Hu, Y., Liao, J., Qian, W., Fan, S., Xiao, X., Yang, Y., Guo, J., & Gao, S. (2025). Metabolomics, E-tongue and HS-SPME-GC-MS reveal the smoking process of *Prunus mume*: Changes in flavor and chemical compositions. *Food Chemistry*, 484, 144401. <https://doi.org/10.1016/j.foodchem.2025.144401>
- Jia, Z., Zhang, J., Jiang, W., Wei, M., Zhao, L., & Li, G. (2024). Different nitrogen concentrations affect strawberry seedlings nitrogen form preferences through nitrogen assimilation and metabolic pathways. *Scientia Horticulturae*, 332, 113236. <https://doi.org/10.1016/j.scienta.2024.113236>
- Jiang, M., Dong, C., Bian, W., Zhang, W., & Wang, Y. (2024). Effects of different fertilization practices on maize yield, soil nutrients, soil moisture, and water use efficiency in northern China based on a meta-analysis. *Scientific Reports*, 14, 6480. <https://doi.org/10.1038/s41598-024-57031-z>
- Jing, H., Zhou, H., Wang, G., Xue, S., Liu, G., & Duan, M. (2017). Nitrogen addition changes the stoichiometry and growth rate of different organs in *Pinus tabulaeformis* seedlings. *Frontiers in Plant Science*, 8, 1922. <https://doi.org/10.3389/fpls.2017.01922>
- Kanakidou, M., Myriokefalitakis, S., Daskalakis, N., Fanourgakis, G., Nenes, A., Baker, A. R., Tsigaridis, K., & Mihalopoulos, N. (2016). Past, present, and future atmospheric nitrogen deposition. *Journal of the Atmospheric Sciences*, 73(5), 2039–2047. <https://doi.org/10.1175/JAS-D-15-0278.1>
- Körner, C. (2003). Carbon limitation in trees. *Journal of Ecology*, 91(1), 4–17. <https://doi.org/10.1046/j.1365-2745.2003.00742.x>
- 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. *Science of the Total Environment*, 624, 611–620. <https://doi.org/10.1016/j.scitotenv.2017.12.080>
- LeBauer, D. S., & Treseder, K. K. (2008). Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, 89(2), 371–379. <https://doi.org/10.1890/06-2057.1>
- Li, Q., Liu, X., Sun, X., Zhao, M., Liu, L., Wang, N., Gao, Q., Fan, P., Du, N., Wang, H., & Wang, R. (2023). Effects of drought hardening on the carbohydrate dynamics of *Quercus acutissima* seedlings under successional drought. *Frontiers in Plant Science*, 14, 1184584. <https://doi.org/10.3389/fpls.2023.1184584>
- Li, Z., Qiu, X., Sun, Y., Liu, S., Hu, H., Xie, J., Chen, G., Xiao, Y., Tang, Y., & Tu, L. (2021). C:N:P stoichiometry responses to 10 years of nitrogen addition differ across soil components and plant organs in a subtropical *Pleuroblastus amarus* forest. *Science of the Total Environment*, 796, 148925. <https://doi.org/10.1016/j.scitotenv.2021.148925>
- Liu, Q., Huang, Z., Wang, Z., Chen, Y., Wen, Z., Liu, B., & Tigabu, M. (2020). Responses of leaf morphology, NSCs contents and C:N:P stoichiometry of *Cunninghamia lanceolata* and *Schima superba* to shading. *BMC Plant Biology*, 20, 354. <https://doi.org/10.1186/s12870-020-02556-4>
- Mackie-Dawson, L. A., Pratt, S. M., & Millard, P. (1994). Root growth and nitrogen uptake in sycamore (*Acer pseudoplatanus* L.) seedlings in relation to nitrogen supply. *Plant and Soil*, 158(2), 233–238. <https://doi.org/10.1007/bf00009498>
- Mason, P. A., Ingleby, K., Munro, R. C., Wilson, J., & Ibrahim, K. (2000). The effect of reduced phosphorus concentration on mycorrhizal development and growth of *Eucalyptus globulus* Labill. seedlings inoculated with 10 different fungi. *Forest Ecology and Management*, 128(3), 249–258. [https://doi.org/10.1016/S0378-1127\(99\)00153-X](https://doi.org/10.1016/S0378-1127(99)00153-X)
- Minden, V., & Kleyer, M. (2014). Internal and external regulation of plant organ stoichiometry. *Plant Biology*, 16(5), 897–907. <https://doi.org/10.1111/plb.12155>
- Mitani, T., Ota, K., Inaba, N., Kishida, K., & Koyama, H. A. (2018). Antimicrobial activity of the phenolic compounds of *Prunus mume* against enterobacteria. *Biological and Pharmaceutical Bulletin*, 41(2), 208–212. <https://doi.org/10.1248/bpb.b17-00711>
- Oliet, J. A., Puértolas, J., Planelles, R., & Jacobs, D. F. (2013). Nutrient loading of forest tree seedlings to promote stress resistance and field performance: A Mediterranean perspective. *New Forests*, 44(5), 649–669. <https://doi.org/10.1007/s11056-013-9382-8>
- 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 Physiology*, 160(4), 1741–1761. <https://doi.org/10.1104/pp.112.208785>
- Scharler, U. M., Ulanowicz, R. E., Fogel, M. L., Wooller, M. J., Jacobson-Meyers, M. E., Lovelock, C. E., Feller, I. C., Frischer, M., Lee, R., McKee, K., Romero, I. C., Schmit, J. P., & Shearer, C. (2015). Variable nutrient stoichiometry (carbon: nitrogen: phosphorus) across trophic levels determines community and ecosystem properties in an oligotrophic mangrove system. *Oecologia*, 179(3), 863–876. <https://doi.org/10.1007/s00442-015-3379-2>
- Tian, T., Cao, H., Farag, M. A., Fan, S., Liu, L., Yang, W., Wang, Y., Zou, L., Cheng, K.-W., Wang, M., Ze, X., Simal-Gandara, J., Yang, C., & Qin, Z. (2023). Current and potential trends in the bioactive properties and health benefits of *Prunus mume* Sieb. Et Zucc: A comprehensive review for value maximization. *Critical Reviews in Food Science and Nutrition*, 63(24), 7091–7107. <https://doi.org/10.1080/10408398.2022.2042186>
- Timmer, V. R. (1997). Exponential nutrient loading: A new fertilization technique to improve seedling performance on competitive sites. *New Forests*, 13, 275–295. <https://doi.org/10.1023/A:1006502830067>
- Vance, C. P., Uhde-Stone, C., & Allan, D. L. (2003). Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource. *New Phytologist*, 157(3), 423–447. <https://doi.org/10.1046/j.1469-8137.2003.00695.x>

- Villar-Salvador, P., Uscola, M., & Jacobs, D. F. (2015). The role of stored carbohydrates and nitrogen in the growth and stress tolerance of planted forest trees. *New Forests*, 46, 813–839. <https://doi.org/10.1007/s11056-015-9499-z>
- Wang, B., Gong, J., Zhang, Z., Yang, B., Liu, M., Zhu, C., Shi, J., Zhang, W., & Yue, K. (2019). Nitrogen addition alters photosynthetic carbon fixation, allocation of photoassimilates, and carbon partitioning of *Leymus chinensis* in a temperate grassland of Inner Mongolia. *Agricultural and Forest Meteorology*, 279, 107743. <https://doi.org/10.1016/j.agrformet.2019.107743>
- Wang, M., Wang, Y., Ren, H., Yang, Y., Gu, T., He, J., & Gao, S. (2023). Effects of nitrogen on growth parameters and photosynthetic characteristics of *P. chinense* Schneid. seedlings in three soil types. *Journal of Elementology*, 28(2), 279–294. <https://doi.org/10.5601/jelem.2022.27.3.2328>
- Wang, Q., Li, S., Li, J., & Huang, D. (2024a). The utilization and roles of nitrogen in plants. *Forests*, 15(7), 1191. <https://doi.org/10.3390/f15071191>
- Wang, R., Yun, L., Mao, Y., Yan, T., Wei, W., You, W., & Zhang, H. (2024b). Nitrogen deposition alters drought-induced changes in biomass and nonstructural carbohydrates allocation patterns of *Quercus mongolica* seedlings. *Scientia Horticulturae*, 325, 112573. <https://doi.org/10.1016/j.scienta.2023.112573>
- Wiley, E., & Helliker, B. (2012). A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytologist*, 195(2), 285–289. <https://doi.org/10.1111/j.1469-8137.2012.04180.x>
- Wu, T., Lin, W., Li, Y., Lie, Z., Huang, W., & Liu, J. (2019). Nitrogen addition method affects growth and nitrogen accumulation in seedlings of four subtropical tree species: *Shima superba* Gardner & Champ., *Pinus massoniana* Lamb., *Acacia mangium* Willd., and *Ormospira pinnata* Lour. *Annals of Forest Science*, 76, 23. <https://doi.org/10.1007/s13595-019-0806-2>
- Xu, X., & Timmer, V. R. (1999). Growth and nitrogen nutrition of Chinese fir seedlings exposed to nutrient loading and fertilization. *Plant and Soil*, 216, 83–91. <https://doi.org/10.1023/A:1004733714217>
- Yang, D., Song, L., & Jin, G. (2019). The soil C:N:P stoichiometry is more sensitive than the leaf C:N:P stoichiometry to nitrogen addition: A four-year nitrogen addition experiment in a *Pinus koraiensis* plantation. *Plant and Soil*, 442(1–2), 183–198. <https://doi.org/10.1007/s11104-019-04165-z>
- Yang, Y., Hu, Y.-Y., Qian, W.-Z., Wang, Y. J., Ren, H.-Y., Gao, S., & Cao, G.-X. (2023). Early growth characterization and antioxidant responses of *Phellodendron chinense* seedling in response to four soil types at three growth stages. *Forests*, 14(9), 1746. <https://doi.org/10.3390/f14091746>
- Yin, H., Zheng, H., Zhang, B., Tariq, A., Lv, G., Zeng, F., & Graciano, C. (2021). Stoichiometry of C:N:P in the roots of *Alhagi sparsifolia* is more sensitive to soil nutrients than aboveground organs. *Frontiers in Plant Science*, 12, 698961. <https://doi.org/10.3389/fpls.2021.698961>
- Zhi, X., Yang, Y., Zou, J., Ma, N., Liu, T., Wang, H., Hu, Y., & Gao, S. (2022). Responses of the growth and nutrient stoichiometry in *Ricinus communis* seedlings on four soil types. *Journal of Elementology*, 27(2), 223–238. <https://doi.org/10.5601/jelem.2022.27.2.2224>

Submitted March 21, 2025, accepted December 29, 2025