

EFFECTS OF NITROGEN AND PHOSPHORUS ADDITION ON *CASTANOPSIS SCLEROPHYLLA* SEEDLINGS GROWTH AND WATER PHYSIOLOGY IN NORTH SUBTROPICAL, CHIZHOU CHINA

CHENG HUANG^{1,3#}, ZHAOCHENG WANG^{2,3*}, FAN YANG^{4*}, YUHUA MA⁵, HAN SHENG⁶, JUNFENG YUAN⁷, LUIS CARLOS RAMOS AGUILA⁷, XU LI⁷ AND MUHAMMAD SADIQ KHAN^{7,8*}

¹Hubei Key Laboratory of Biologic Resources Protection and Utilization, Hubei Minzu University, Enshi, 445000, China

²Huzhou Vocational and Technical College, Huzhou, 313000, China

³School of Forestry and Landscape Architecture, Anhui Agricultural University, Hefei, 230036, China

⁴Cultural and Creative College, Anhui Finance & Trade Vocational College, Hefei, 230601, China

⁵College of Civil and Architecture Engineering, Chuzhou University, Chuzhou, 239099, China

⁶School of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou, 510642, China

⁷Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510560, China

⁸Institute of Tropical Forestry and Forest Products (INTROP), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Corresponding author's email: yangfan880921@163.com; ksadiq259@gmail.com

Abstract

Nitrogen (N) and phosphorus (P) are key elements required for plant growth and development and are the most common limiting nutrients that constrain net primary productivity in terrestrial ecosystems. As a result of anthropogenic activities, N deposition has increased in subtropical regions of China; however, the impact of forest P associated with N is relatively scarce. Four treatments, including control, were set up in the forest of *Castanopsis sclerophylla* (seedlings) to evaluate the effects of long-term N and P applications on plant growth and water physiological characteristics. In the forest's understory, N, P, and combined N+P additions were applied to similar growth conditions in the 50-year-old secondary forest. The results show that the mid-day water potential of *C. sclerophylla* seedlings under the N+P addition treatments was significantly higher ($p<0.05$) than in the P and N treatments, and found significant ($p<0.05$) variation in seasonal growth. In the dry season, the conduit density of young *C. sclerophylla* seedlings under P inputs and control was significantly higher ($p<0.05$) than combined N+P addition. N addition, it possibly signifies the drought resistance of *C. sclerophylla* seedlings in seasons. Remarkably, without adding nutrients, the *C. sclerophylla* seedlings showed the strongest resistance to drought conditions in all seasons. Therefore, under the influence of global climate change in the future, drought and N deposition will negatively affect the understory regeneration of *C. sclerophylla* in subtropical secondary forests. The outcomes of this study direct the forest manager and silviculturist regarding the adverse situation ahead regarding the *C. sclerophylla* regrowth in subtropical forests and help prioritize plant species for future drought conditions.

Key words: *Castanopsis sclerophylla*; Water use efficiency; Leaf traits; Secondary Forest; Seedling

Introduction

Nitrogen (N) and phosphorus (P) interact closely in the biochemical responses that control plant ecophysiological functions, and fluctuations in the availability of elements may trigger changes in the uptake and availability of the other growth elements (Li *et al.*, 2024; Wang *et al.*, 2024). N and P uptake from the soil or assimilation within the plant relies primarily on a negative water potential gradient from the soil to the leaves to complete water and nutrient transport (Salazar-Tortosa *et al.*, 2018). N and P availability have been shown to alter water relations by affecting plant hydraulic structure (Li *et al.*, 2021). Therefore, plant hydraulic structure, water transport capacity, and nutrient status interactions can lead to drought stress or reduced plant survivability under global change.

Since the Industrial Revolution, anthropogenic activities have forced the input of unprecedented amounts of available N into many ecosystems (Gallo-way *et al.*, 2004).

The overall N deposition rate in China increased to 21.1 kg ha⁻¹ year⁻¹, and N deposition reached 36.0 kg ha⁻¹ year⁻¹ in the subtropical region in the southeast (Zhu *et al.*, 2021), making it a high N deposition region. The intensified N deposition further accelerated the soil P cycle and caused P limitation (Marklein *et al.*, 2012). In the case of long soil development and high weathering, P often complexes with iron or aluminium oxides to form a closed storage state and cannot be used by plants. The effective soil P content is low, so P is often considered a limiting factor in subtropical forest ecosystems (Vitousek *et al.*, 2010). Appropriate P addition can significantly improve soil P availability (Zheng *et al.*, 2016), increase soil total P, and soluble organic carbon content, as well as increase microbial abundance and soil enzyme activities in the soil (Feng *et al.*, 2021; Li *et al.*, 2024); in P-deficient areas, P application can significantly increase P availability, enhance the ability of vegetation to take up P, and reduce soil N:P, thus alleviating the limiting effect of P on plant and microbial growth (Cleveland *et al.*,

2002). In addition, plant water physiology can be influenced by changes in nutrient availability and, thus, plant physiological and ecological traits, such as changes in conduit density and size, stomatal size and density in xylem tissues, and hydraulic characteristics in leaves. Several studies have shown that N addition leads to more efficient xylem hydraulic conductivity but more susceptibility to cavitation, as increased N availability may lead to larger conduit diameters, thinner conduit walls, and easier penetration of gases into the conduit through the striatal membrane (Zhang *et al.*, 2021).

In subtropical forest ecosystems, studies on N and P interactions have mainly focused on the effects of N deposition on plant stoichiometry (e.g., C: N: P) and carbon economic traits (e.g., biomass production, photosynthesis) (Wang *et al.*, 2017; Hu *et al.*, 2019). As a key element and limiting factor for plant growth, water may also occur in respond to N and P additions regarding its hydraulic structure and water transport. Yet, the response mechanism remains unclear, requiring an in-depth study of the potential impacts of N deposition and P supply in subtropical forests to clarify their interactions on plant water relations. Thus, in this study, the growth, leaf, and branch hydraulic structures of naturally regenerated annual *Castanopsis sclerophylla* seedlings were measured in a nine-year nutrient (N, P) addition fixed sample plot in a natural secondary *C. sclerophylla* forest in the mountains of southern Anhui Province, using the dominant species of subtropical broad-leaved evergreen forest community, *C. sclerophylla* seedling (Zhang *et al.*, 2007). We hypothesize that: (1) nutrient addition affects the hydrophysiological properties of stems and leaves of *C. sclerophylla* seedlings and relates to the type of nutrient addition; (2) nutrient addition stimulates the growth of *C. sclerophylla* seedlings but reduces its drought resistance.

Material and Methods

Study area: The experimental site was situated within the Rending Mountain Forest Farm, Shimentai County, Chizhou City, Anhui Province, China (117°26'24 "N, 30°15'37 "W), at an elevation of 120 m. This region experiences a subtropical monsoon climate, characterized by high temperatures during summer, with an average peak of 38.8°C in previous years and an annual mean temperature of 16.1°C. The average yearly precipitation is 1,626 mm, accompanied by an annual average of 1,704.4 hours of sunshine and a frost-free period lasting 234 days. The soil was predominantly sandy-yellow-red loam. The vegetation comprised a broad-leaved evergreen forest, with dominant species including *C. sclerophylla*, and *Quercus acutissima* (Zhang *et al.*, 2007; Wang *et al.*, 2024). The area primarily consisted of secondary forests of *C. sclerophylla*, covering approximately 500 acres, largely regenerated naturally following logging activities in the 1960s and subsequent forest management practices over the past five decades.

Plot design: In August 2011, we established twelve 15 m × 15 m plots (with four treatments and three replicates each) within a 50-year-old *C. sclerophylla* secondary forest. To prevent nutrient infiltration interference from runoff between plots, a buffer zone of more than 10 m was

maintained between them, delineated by signs and pull ropes. A randomized block design was employed for the nutrient addition experiment across the 12 plots, with four treatment levels and three plots designated for each treatment. The four nutrient addition treatments included control (CK), N, P, and N+P group (Fig. 1). N and P were administered via ammonium nitrate (NH₄NO₃) and calcium superphosphate [Ca(H₂PO₄)₂], respectively. These fertilizers were dissolved in 20 L of water and sprayed evenly throughout the forest using an artificial sprayer, while control plots received an equal amount of water spray.

Plant growth measurement and water use efficiency:

Leaf water potential was determined using a pressure chamber hydrometer (1505D, PMS Instruments), and the early morning and midday water potential was measured at 5:00 and 12:00 on each measurement day. The early morning water potential was required to be conducted before sunrise due to the influence of sunrise time in winter and summer. Healthy five seedlings with the same growth were selected for each treatment (CK, N, P, and N+P addition) in the middle of the wet season in August 2020 and the dry season in January 2021 in sunny weather. Branches with leaves in the upper middle of the south-facing crown of the sample trees of about 50 cm were cut with high pruning shears. Then leaves were quickly picked, and the *C. sclerophylla* seedlings' leaves were inserted upside down into the lid of the pressure chamber to ensure that the lid was clamped to the leaf petiole. Then, the cylinder valve was opened, and the control valve was facing towards it. Open the valve of the cylinder, make the control valve face to the pressure, slowly open the measuring valve, make the pressurization rate up to 0.1 bar s⁻¹, and carefully observe the base of the petiole that extends out of the lid of the pressure chamber, and close the valve immediately when a water film appears on the petiole, and record the readings. Two branches were cut down for each sample tree, and from each branch, two leaves were picked to determine the water potential in the sample plot as soon as possible to ensure that the data could reflect the real water situation.

Anatomical observation of leaf and branch structures:

Five samples of healthy trees with essentially uniform growth conditions were selected for each treatment, and in mid-sunny weather in August 2020 during the wet season and January 2021 during the dry season, foliage-bearing branches with leaves of about 50 cm from the south-facing upper-middle part of the crowns of the sample trees were cut, and the cut ends of the branches were placed in plastic buckets with water, put on a black plastic bag, and brought back to the laboratory immediately (Clearwater *et al.*, 2021). Under each treatment, five different *C. sclerophylla* seedlings were selected, fresh mid-upper branches with a diameter of 8-10 mm were intercepted from the top, and each branch was cut into small segments of 3 cm in length. The temporary sections were made to be placed under a 40x optical microscope for observation, and photographs were taken for three fields of view (Zhang *et al.*, 2021). The number of conduits and conduit area in the field of view was calculated using Image J software, and the percentage of conduit tissue and conduit density were calculated (Yang *et al.*, 2009).

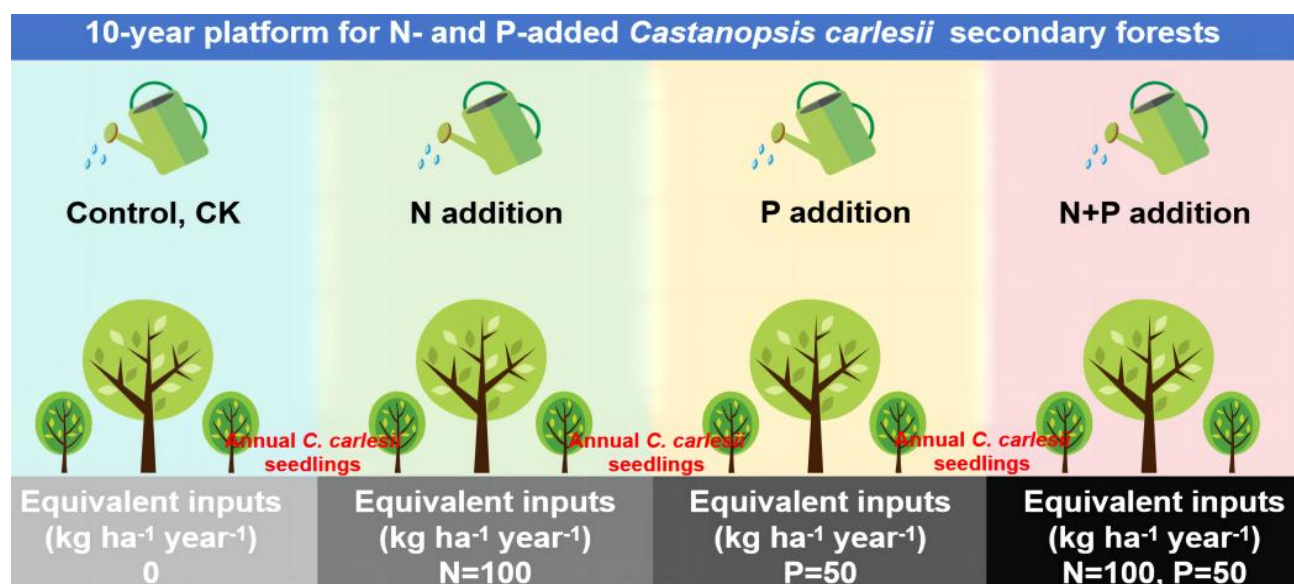


Fig. 1. Nutrition added a design and implementation plan.

Statistical analysis

Data analysis and graphing were done using Excel 2016 with R 4.0.5. and applied for ANOVA (One-way ANOVA) and multiple comparison test (LSD test) to know the significance differences between treatments, with the level of significant difference being $p < 0.05$; correlation analysis between variables (Pearson correlation analysis (Pearson correlation) and plotting were done in R 4.0.5.

Results

Effects of N and P addition on the *C. sclerophylla* seedling's growth: In contrast, the P-added treatments showed no significant difference from the control ($p > 0.05$). In the addition treatment, the highest growth (D^2H) of 81.06 cm^3 , followed by the N addition treatment with 74.54 cm^3 , is observed.

Effects of N and P addition on plant hydraulic drip: Applying N, P, and N+P showed varied effects on Huber values. Specifically, the N treatment exhibited a moderate increase in Huber values in the control and N+P groups in both wet and dry seasons. At the same time, the P treatment showed a minimal effect. However, the combined N+P treatment notably enhanced Huber values, indicating a synergistic influence on plant growth (Fig. 2a). The responses of stem sapwood-specific hydraulic conductivity to the treatments differ significantly. Nitrogen treatment triggers a moderate conductivity development, showing improved water passage within the stem. Phosphorus treatment showed a more substantial enhancement in conductivity in the dry season compared to the wet season, signifying a distinct effect on sap flow properties. Remarkably, the highest significant conductivity values were observed under the control treatment, indicating no response from adding N, P, and N+P. (Fig. 2b). The treatments employed distinction effects on K_{leaf} . Nitrogen application resulted in a slight increase in K_{leaf} , indicating improved leaf water transport efficiency. Phosphorus treatment showed a more significant difference in K_{leaf}

compared to N+P treatment, while the means of P and N remained overlapped with the highest values in treatment; however, the effect is minimal seasonally in the means, suggesting a pronounced influence on leaf hydraulic properties under N+P. The combined N+P treatment exhibited the lowest mean K_{leaf} values, demonstrating a non-cooperative effect on leaf water conductance (Fig. 2c). Adding P treatments significantly impacted dry and wet seasons and supplemented positive turgor pressure ($p < 0.05$). Seasonally, N treatment caused a slight increase in turgor pressure but remained insignificant in balancing cellular water balance. However, surprisingly, in the control treatment, a more considerable improvement in turgor pressure compared to N, N+P treatments, demonstrating improved flow in the xylem (Fig. 2d). Overall, the results highlight the variation effects of N, P, and combined N+P treatments on plant water uptake properties, with the combined treatment generally exerting the most noticeable impact on growth-related parameters.

Effects of N and P addition on leaf traits of the *C. sclerophylla* seedling: Seasonally, N treatment showed significant growth ($p < 0.05$) in leaf thickness compared to the control and P, N+P treatments, demonstrating improved leaf structural development. Phosphorus treatment contributed somewhat to increased leaf thickness, but no significant difference was noticed in wet and dry seasons (Fig. 3a). N and P treatments result in uncertain increases in upper epidermis thickness, representing modifications in leaf surface structure. However, it was non-significant in the wet and dry seasons. The combined N+P treatment resulted in a similar increase in upper epidermis thickness to the control treatment, showing effects on epidermal layers under $p > 0.05$ (Fig. 3b).

Adding N resulted in a notable increase in palisade and sponge tissue thickness, indicating enhanced photosynthetic capacity compared to P and N+P in wet and dry seasons. Phosphorus treatment also increased palisade and sponge tissue thickness, suggesting improved leaf function; however, there was no significant variation in wet and dry seasons (Fig. 3c, d). In addition, applying N treatment resulted in an

insignificant increase in lower epidermis thickness compared to the control, indicating structural modifications in wet and dry seasons. Phosphorus treatment showed a similar trend, but to a lesser extent. The combined N+P treatment exhibited the most distinct increase in lower epidermis thickness, suggesting mutual effects on epidermal growth (Fig. 3e). N treatment showed a moderate rise in spongy and palisade mesophyll tissues %, signifying improved photosynthetic activity and cellular density under all treatments. P treatment contributed to increased tissue development, and seasonal variation signified their growth. Similarly, the combined N+P treatment significantly increased spongy and palisade mesophyll tissues, suggesting synergetic effects on tissue association (Fig. 3f). Indeed, the results revealed that N, P, and combined N+P treatments apply varied effects on leaf morphology and tissue growth and development, with the combined treatment usually showing the most noticeable influences on physical and functional characteristics.

Effects of N and P additions on hydraulic traits in young *C. sclerophylla* seedlings: The response of the water potential of young *C. sclerophylla* seedlings to nutrient addition during the dry and wet seasons is shown (Fig. 4). The changes in the early morning water potential during the wet and dry seasons under different treatments maintained the same trend, i.e., the control young *C.*

sclerophylla seedlings' early morning water potential was significantly higher than that of the N-addition treatment, and CK, P-addition and N-addition treatments were considerably higher than that of the N- and P-addition treatments ($p<0.05$) and showed significant seasonal differences under different treatments ($p<0.05$). The mid-day water potential of young *C. sclerophylla* seedlings in the dry and wet seasons under different treatments maintained the same trend, i.e., the midday water potential of young *C. sclerophylla* seedlings in the N- and P-added treatments, the control was significantly higher than that in the P-added treatment, and the N-added treatment ($p<0.05$), and significant seasonal differences were observed under different treatments ($p<0.05$).

The results of the correlation analysis indicate that the water potential of the leaves is closely related to the anatomical structure of the leaves. Among them, stem hydraulic conductivity, leaf thickness, palisade tissue thickness, sponge tissue thickness, and stomatal density are compared with leaf (Fig. 5). The predawn water potential was significantly positively correlated ($p<0.05$), while the Huber values, VD (transverse), and Vd were significantly negatively correlated with the early morning water potential of the leaves ($p<0.05$). VD (longitudinal) and VD (transverse) were extremely significantly negatively correlated with the midday water potential ($p<0.01$).

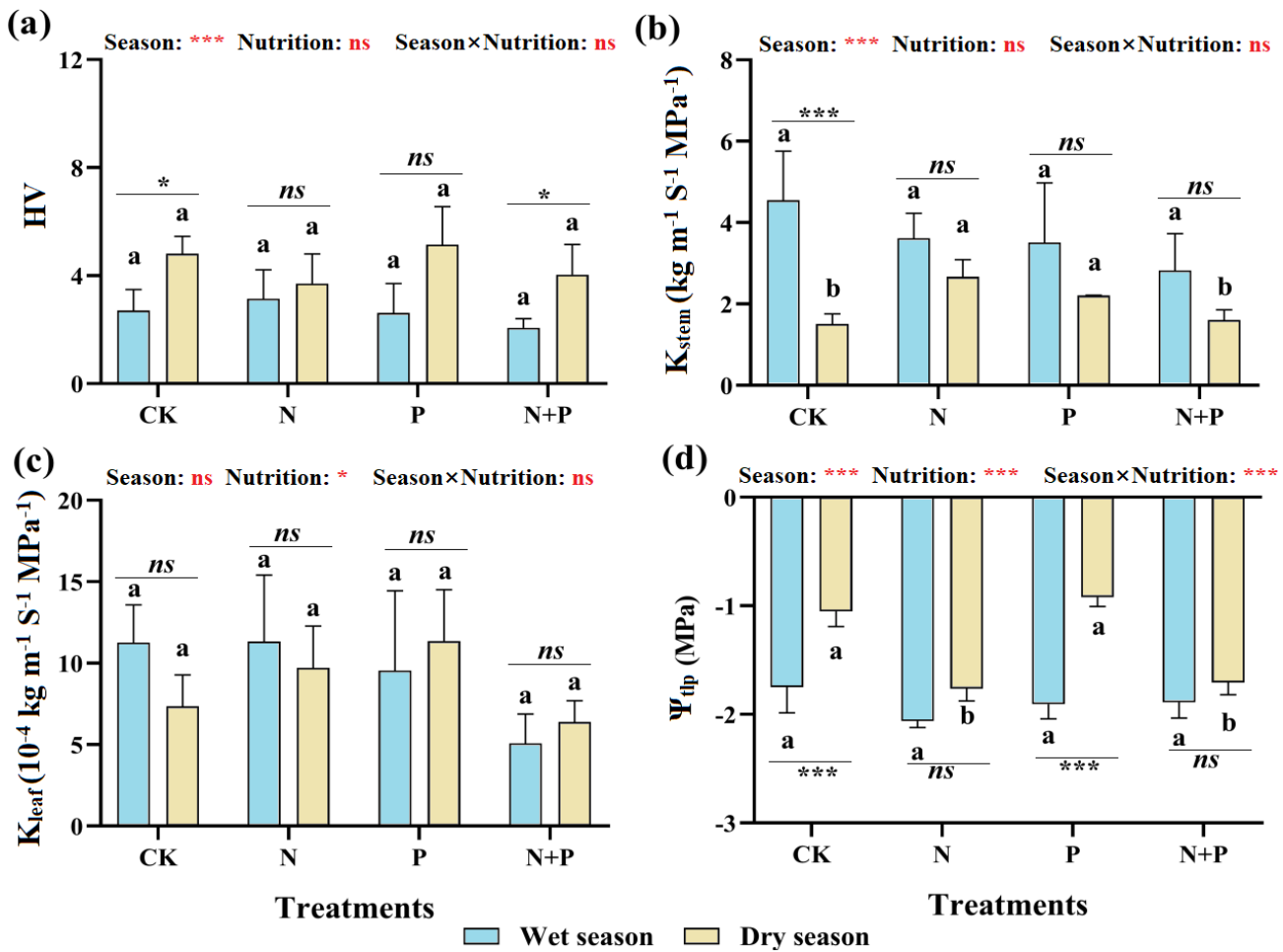


Fig. 2. Effects of N, P addition on Huber values (a, HV), stem hydraulic conductivity (b, K_{stem}), K_{leaf}, turgor loss point (d, Ψ_{tp}) of *C. sclerophylla* seedlings.
Note: ns, *, **, and *** denote no significance, $p<0.05$, $p<0.01$ and $p<0.001$, respectively. Different lowercase letters indicate significant differences between different treatments.

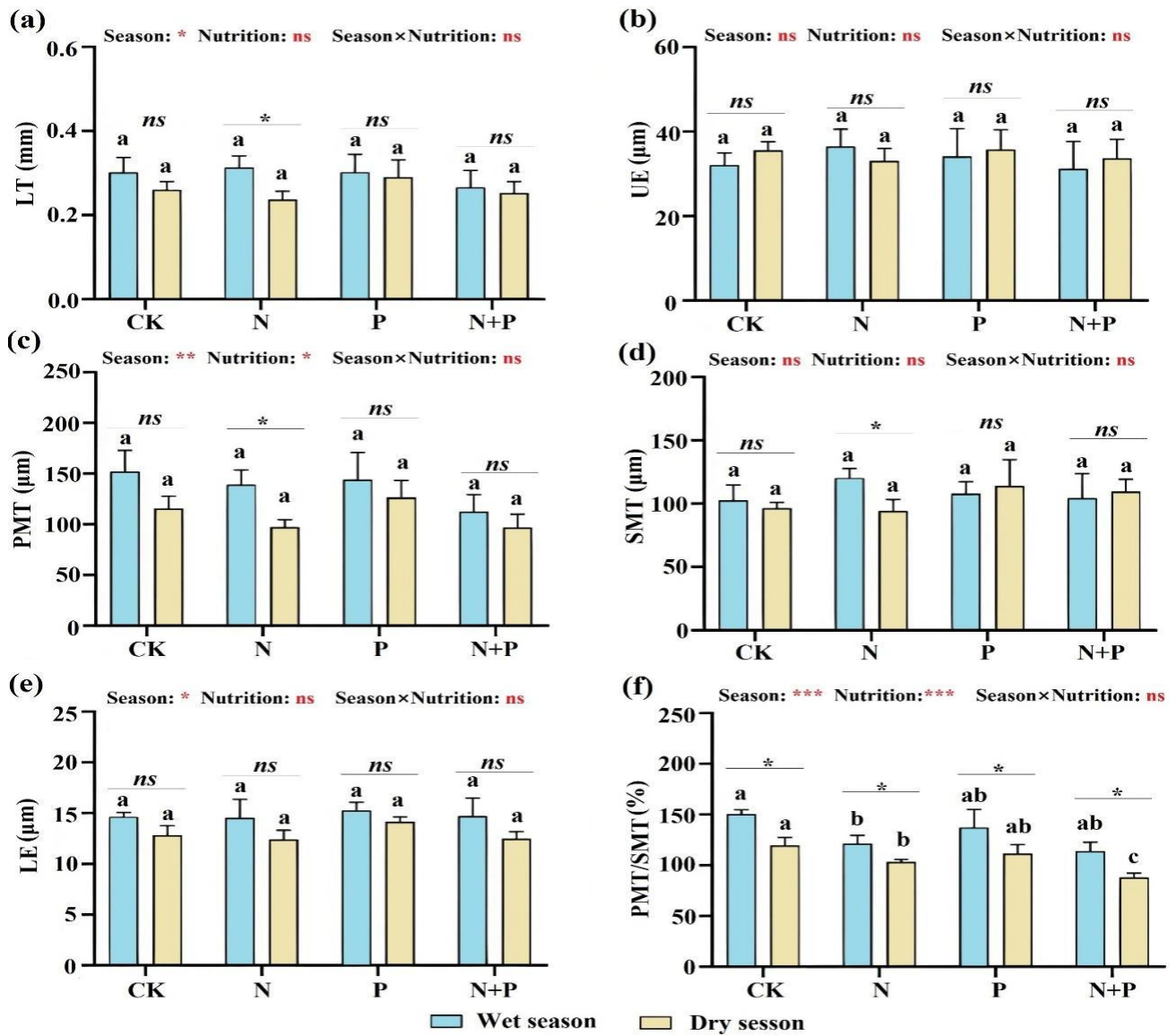


Fig. 3. Effects of N, P addition on leaf thickness (a, LT), upper epidermis thickness (b, UP), palisade tissue thickness (c, PMT) and sponge tissue thickness (d, SMT), lower epidermis thickness (e, LP), and palisade tissue thickness and sponge tissue thickness ratios (PMT/SMT) of *C. sclerophylla* seedlings.

Note: ns, *, **, and *** denote no significance, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Different lowercase letters indicate significant differences between different treatments.

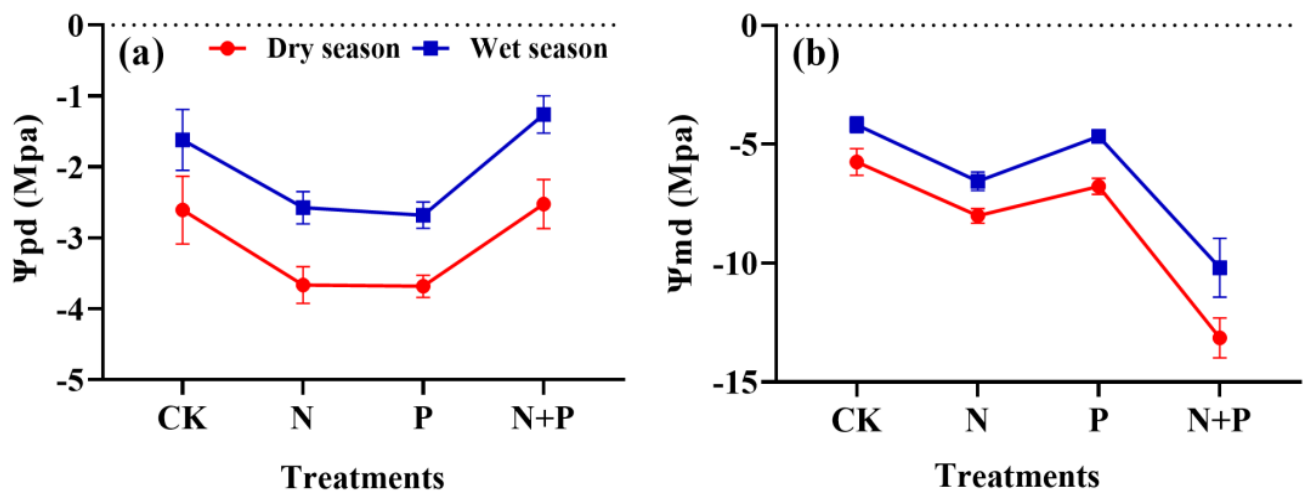


Fig. 4. Effects of N, P addition on leaf predawn water potential (a, Ψ_{pd}) and midday water potential (b, Ψ_{md}) in the leaf of *C. sclerophylla* seedlings. Note: ns, *, **, and *** denote no significance, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Different lowercase letters indicate significant differences between different treatments.

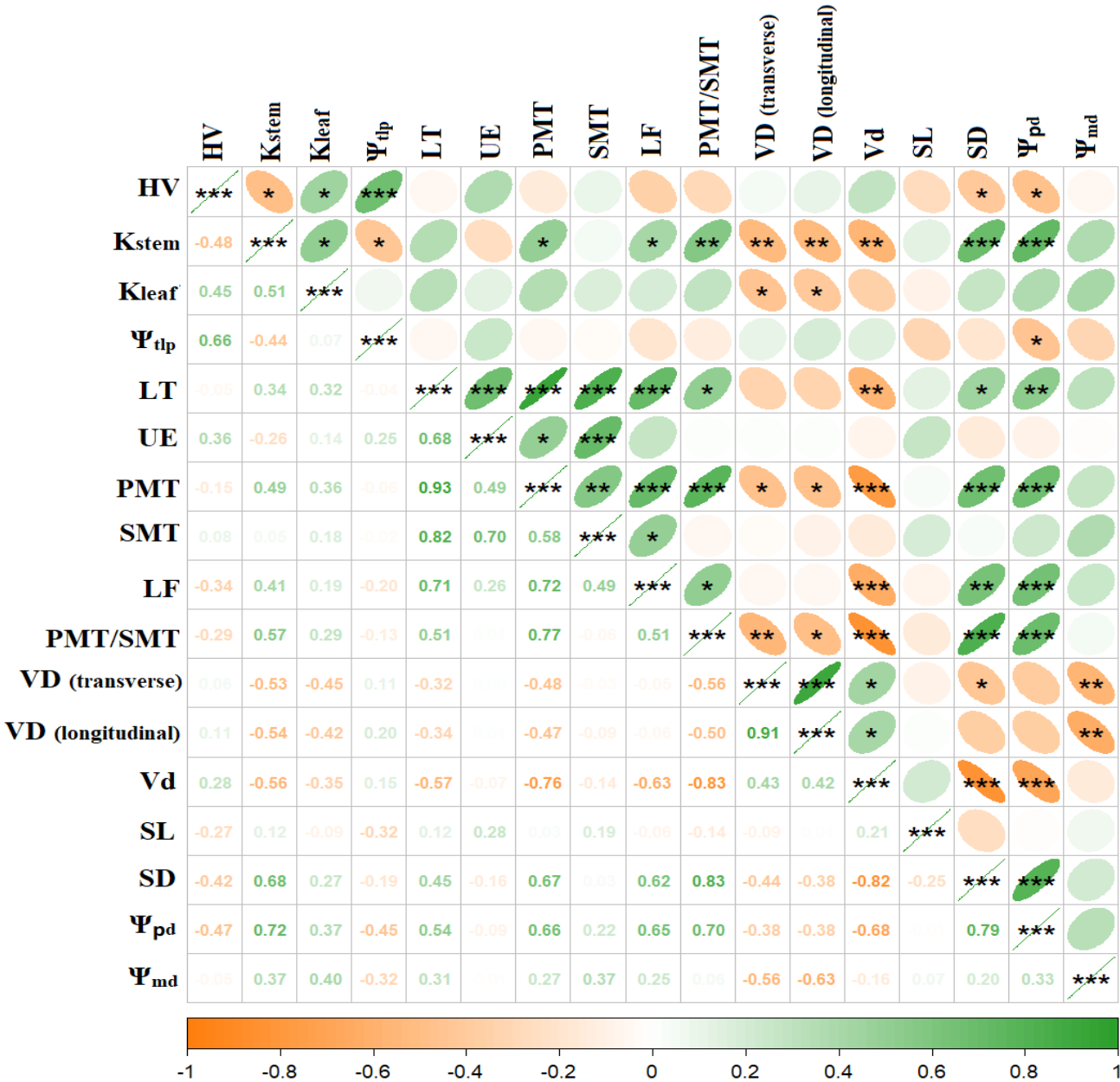


Fig. 5. Correlation between *Castanopsis sclerophylla* seedlings growth and water physiology in the north subtropical.

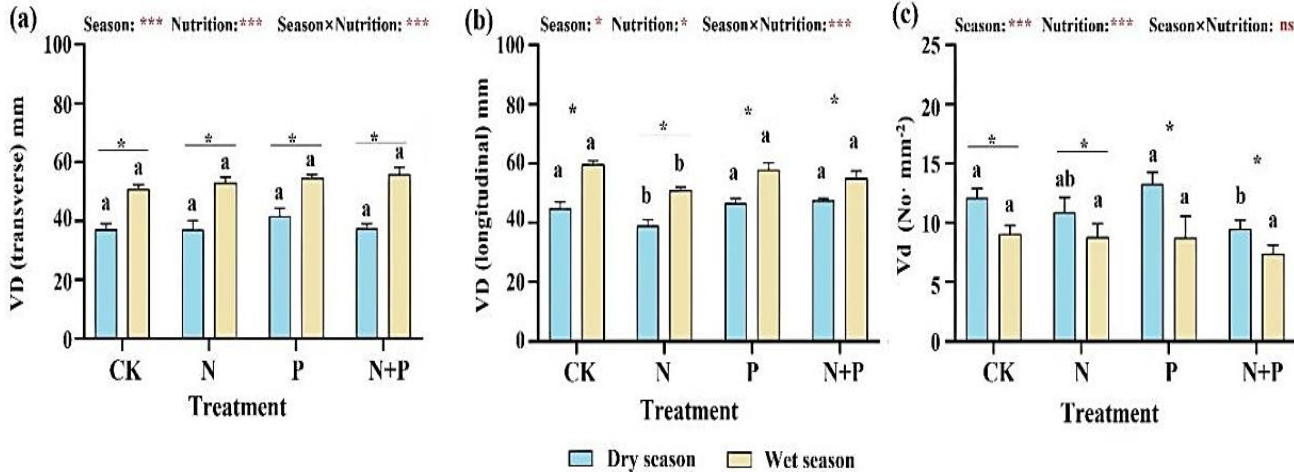


Fig. 6. Effects of N, P addition on vessel characteristics (VD, vessel diameter; Vd, vessel density) in different organs of *C. sclerophylla* seedlings. Note: ns, *, **, and *** denote no significance, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Different lowercase letters indicate significant differences between different treatments.

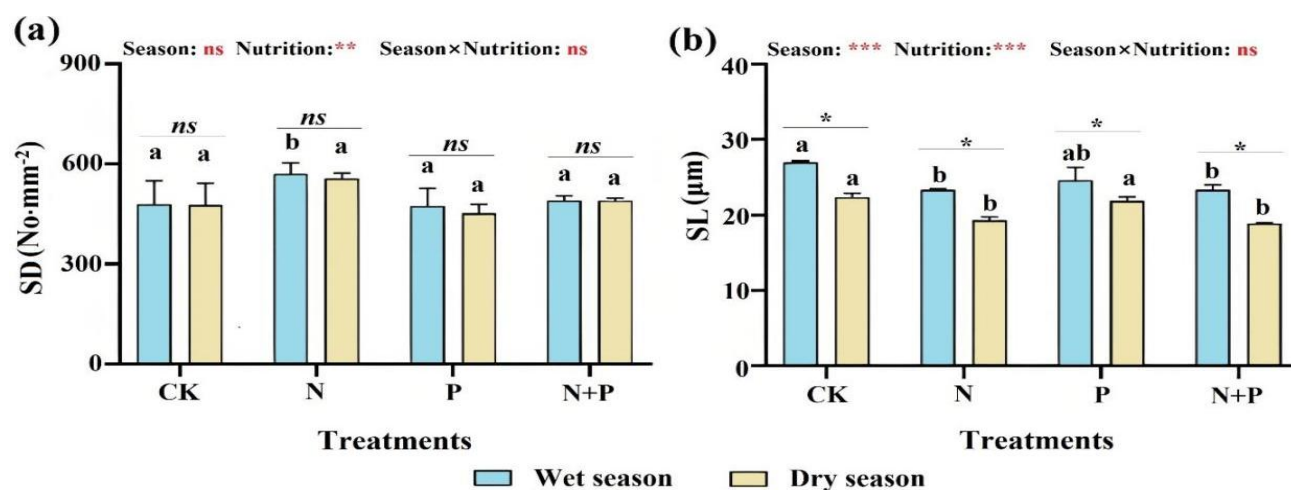


Fig. 7. Leaf stomatal density (a, SD) and stomatal length (b, SL) of *C. sclerophylla* seedlings to N and P addition during the growing and dry season.

Note: ns, *, **, and *** denote no significance, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Different lowercase letters indicate significant differences between different treatments.

Effects of N and P additions on branch conduit characteristics in young *C. sclerophylla* seedlings:

Analyses showed that there was no significant difference ($p > 0.05$) in conduit diameter (transverse) of young *C. sclerophylla* seedlings under different treatments, both in dry and wet seasons, but observed significant seasonal differences ($p < 0.05$) were observed under different treatments (Fig. 6). Specifically, under N and P addition treatments, the conduit (xylem) diameter (transverse) of young *C. sclerophylla* trees in the dry season was 37.57 ± 1.46 mm, while that in the wet season was 55.68 ± 2.61 mm; under N addition treatment, the conduit diameter (transverse) of young *C. sclerophylla* trees in the dry season was 37.07 ± 3.18 mm, while that in the wet season was 52.84 ± 2.06 mm; and under P addition treatment, the diameter of young *C. sclerophylla* trees in the dry season was 37.07 ± 3.18 mm, while that in the wet season was 52.84 ± 2.06 mm. Conduit diameter (transverse) of young *C. sclerophylla* trees in the dry season was 41.69 ± 2.72 mm compared to 54.58 ± 0.98 mm in the wet season, and in the control, conduit diameter (transverse) of young *C. sclerophylla* trees in the dry season was 37.10 ± 1.97 mm compared to 50.62 ± 1.66 mm in the wet season (Fig. 6a).

The conduit diameter (longitudinal) of young *C. sclerophylla* trees under different treatments in dry and wet seasons maintained the same trend, i.e., N, P-added treatments, P-added treatment and control were significantly higher than N-added treatments ($p < 0.05$), and significant seasonal differences ($p < 0.05$) were observed under different treatments (Fig. 6b). Specifically, under N- and P-added treatments, the conduit diameter (longitudinal) of young *C. sclerophylla* trees in the dry season was 47.64 ± 0.60 mm, compared with 54.97 ± 2.51 mm in the wet season; under N-added treatment, the conduit diameter (longitudinal) in the dry season was 39.06 ± 2.10 mm, compared with 50.93 ± 0.92 mm in the wet season; and under P-added treatment, the diameter in the dry season was 39.06 ± 2.10 mm, conduit diameter (longitudinal) of young *C. sclerophylla* trees in the dry season was 46.67 ± 1.53 mm compared to 57.79 ± 2.53 mm in the wet season, and in the control, conduit diameter (longitudinal) of young *C. sclerophylla* trees in the dry

season was 44.86 ± 2.08 mm compared to 59.74 ± 1.16 mm in the wet season.

Under the dry season, the conduit density of young *C. sclerophylla* trees under P-addition treatment and control was significantly higher than that of N and P-addition treatments ($p < 0.05$). Still, however, the difference with the N-added treatment group was insignificant ($p > 0.05$). In contrast, the conduit density of young *C. sclerophylla* trees under different treatments during the wet season showed no significant difference ($p > 0.05$). Significant seasonal differences ($p < 0.05$) were observed under different treatments (Fig. 6c). Specifically, under N and P addition treatments, the conduit density of young *C. sclerophylla* trees in the dry season was 9.48 ± 0.77 , while that in the wet season was 7.37 ± 0.76 ; under N addition treatment, the conduit density of young *C. sclerophylla* trees in the dry season was 10.88 ± 1.26 , while that in the wet season was 8.75 ± 1.18 ; under P addition treatment, the conduit density of young *C. sclerophylla* trees in the dry season was 13.28 ± 0.99 compared to 8.71 ± 1.84 in the wet season; in control, the conduit density of young *C. sclerophylla* trees in the dry season was 12.13 ± 0.76 compared to 9.04 ± 0.75 in the wet season.

Comprehensive evaluation of drought tolerance in young *C. sclerophylla* seedlings under N and P additive treatments:

The results of variance analysis indicated that, compared with the control, the addition of N significantly increased the stomatal density of the leaves of *C. sclerophylla* seedlings in the wet season. Under the same treatment, there was no significant difference in the stomatal density of the leaves in different seasons (Fig. 7a). However, the addition of N, P, and N+P significantly affected the stomatal length of the leaves of *C. sclerophylla* seedlings ($p < 0.001$). Compared with the control, the stomatal length of the leaves decreased to varying degrees after the addition of N and P (Fig. 7b). Among them, the stomatal density of leaves under the N addition and N+P addition treatments was significantly lower than that of the control ($p < 0.05$). This indicates that the addition of N will increase the risk of water evaporation and loss to a certain extent.

Discussion

Trees absorb water from the soil through the root system. Water is transported along xylem conduits to various tissues and organs in the plant body, driven by potential tissue differences (Simonin *et al.*, 2015). Plant water use efficiency is a comprehensive measure of the plant body's carbon and water balance. Droughts exacerbate the decline in soil moisture in mountainous regions, which affects the ability of the plant body to transport water in the branches and leaves, thus limiting the stomatal conductance and photosynthetic rate (Scoffoni *et al.*, 2016; Wu *et al.*, 2022b). Limited soil moisture supply can cause cavitation to bodies' plants' xylem water conductance system, exacerbating the risk of drought-related mortality in plant bodies (Anderegg *et al.*, 2015). Hydraulic disorders are an important factor in causing tree mortality in natural forests, and 70% of species globally have narrow hydraulic safety thresholds and will be at higher risk of mortality under future climate extremes (Choat *et al.*, 2012). In mountainous areas more prone to drought stress, it is necessary to pay continuous attention to the changes in water uptake and transport capacity of the plant body (Xu *et al.*, 2012; Cohu *et al.*, 2014; Garcia-Valdes *et al.*, 2021); combined with the changes in global nutrient cycling, it was found that there were significant seasonal differences in the four treatments in terms of leaf water potentials, midday and dawn water potentials, and the dry season was greater than the wet season. The dawn water potential of the *C. sclerophylla* leaves in the wet season was expressed as follows: N+P addition > P addition > N addition and control, and the midday water potential showed a similar pattern. Regarding water conductivity, the leaf-specific water conductivity of *C. sclerophylla* seedlings showed P added > N added and CK > N, which was added in the wet season. In contrast, the sapwood water conductivity showed P added, N added, and CK > N+P addition. In terms of expansion pressure loss point, *C. sclerophylla* seedlings showed N, P-added and N-added > P-added and CK in the wet season, which indicated that N and P-added treatments were more susceptible to water stress in the wet season, and drought tolerance of *C. sclerophylla* seedlings was found to be greater in control and P-added than in N+P-added and N-added during the wet season through 2 different assignments, which was in line with the above results. It might be related to its conservative growth strategy (Guo *et al.*, 2017). Under nutrient-sufficient conditions, the plant showed lower plasticity. Although it is growing well, it will be more susceptible to water stress under extreme climate change conditions.

Thicker leaf epidermis can reduce plant water loss (Shi *et al.*, 2020). In this experimental area, due to the influence of subtropical monsoon climate, the dry period of *C. sclerophylla* seedlings is usually accompanied by a significant reduction in rainfall, and this change in epidermal thickness is conducive to the adaptation of dry *C. sclerophylla* seedlings to the arid habitat (Wu *et al.*, 2020; Wu *et al.*, 2022a; Wu *et al.*, 2022b). Therefore, we speculate that the drought resistance of dry *C. sclerophylla* is higher than *C. sclerophylla* seedlings in the wet season.

Changes in stomatal conductance, stomatal size, and stomatal density can not only affect plants' photosynthetic rate but also change plants' water loss. For example, smaller stomata are favorable for plants to respond to changes in external environmental conditions promptly, especially in the case of soil moisture deficiency, and a decrease in stomatal size is conducive to reducing water loss (Haworth *et al.*, 2021). However, an increase in stomatal density is conducive to both reducing water loss and maintaining photosynthetic stability, and in general, the greater the stomatal density of a plant, the lower the resistance to stomatal transport and the higher the photosynthetic rate and photosynthetic capacity (Wu *et al.*, 2020). Most plants reduce water loss by reducing stomatal size when faced with a water deficit since smaller stomata respond rapidly to drought and can save the plant from cavitation embolism (Beerling *et al.*, 2009). It has been shown that the *Syzygium rehderianum* effectively reduced water loss under drought conditions by reducing stomatal size, which, combined with its decreased stomatal conductance, significantly reduced transpiration rate (Wu *et al.*, 2018). However, its stomatal density did not change considerably under drought conditions (Wu *et al.*, 2018). In contrast, it has been found that *M. xylostella* adopted a more conservative strategy to adapt to drought conditions, i.e., reducing its stomatal density rather than changing its stomatal size, suggesting that *M. xylostella*'s reduction of water loss also reduced its photosynthetic rate (Hepworth *et al.*, 2015), an adaptive strategy that would be detrimental to *M. xylostella*'s growth but an adjustment mechanism that facilitates the plant's assimilation of CO₂ under conditions of water deficit.

Conclusions

Applying N and P significantly reduced mid-day water potential in wet and dry seasons, with N having a greater influence than P. N application reduced mid-day water potential more than P and control treatments, associated with fluctuations in photosynthesis. Seasonal differences in conducting tissue's structural properties are intensely subjective, with more noticeable effects observed in the wet season. Both N and P treatments enhanced the conducting tissue structure. Nitrogen addition improved leaf thickness, tissue cellular density, and water transport efficacy, emphasizing its role in plant water uptake. P application boosted photosynthetic capacity and tissue growth, mainly in dry seasons. Collectively, N+P treatments confirmed combined effects on plant growth-related parameters. Nutrient contributions significantly impacted the water transport and hydraulic structure in the subtropical forest, highlighting the complex interaction between nutrient-plant physiology.

Acknowledgments

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References

- Anderegg, W.R.L., A. Flint, C.Y. Huang, L. Flint, J.A. Berry, F.W. Davis, J.S. Sperry and C.B. Field. 2015. Tree mortality predicted from drought-induced vascular damage. *Nat. Geosci.*, 8(5): 367-371.
- Beerling, D.J. and P.J. Franks. 2009. Evolution of stomatal function in 'lower' land plants. *New Phytol.*, 183(4): 921-925.
- Choat, B., S. Jansen, T.J. Brodribb, H. Cochard, S. Delzon, R. Bhaskar, S.J. Bucci, T.S. Feild, S.M. Gleason, U.G. Hacke, A.L. Jacobsen, F. Lens, H. Maherali, J. Martínez-Vilalta, S. Mayr, M. Mencuccini, P.J. Mitchell, A. Nardini, J. Pittermann, R.B. Pratt, J.S. Sperry, M. Westoby, I.J. Wright and A.E. Zanne. 2012. Global convergence in the vulnerability of forests to drought. *Nature*, 491(7426): 752-755.
- Clearwater, M.J. and F.C. Meinzer. 2001. Relationships between hydraulic architecture and leaf photosynthetic capacity in nitrogen-fertilized *Eucalyptus grandis* trees. *Tree Physiol.*, 21(10): 683-690.
- Cleveland, C.C., A.R. Townsend and S.K. Schmidt. 2002. Phosphorus limitation of microbial processes in moist tropical forests: Evidence from short-term laboratory incubations and field studies. *Ecosystems*, 5(7): 0680-0691.
- Cohu, C.M., O. Muller, W.W. Adams and B. Demmig-Adams. 2014. Leaf anatomical and photosynthetic acclimation to cool temperature and high light in two winter versus two summer annuals. *Physiol. Plant.*, 152(1): 164-173.
- Feng, J. and B. Zhu. 2021. Global patterns and associated drivers of priming effect in response to nutrient addition. *Soil Biol. Biochem.*, 153: 108118.
- García-Valdes, R., J. Vayreda Retana and J. Martínez-Vilalta. 2021. Low forest productivity associated with increasing drought-tolerant species is compensated by an increase in drought-tolerance richness. *Glob. Chang. Biol.*, 27(10): 2113-2127.
- Guo, C., L. Ma, S. Yuan and R. Wang. 2017. Morphological, physiological and anatomical traits of plant functional types in temperate grasslands along a large-scale aridity gradient in northeastern China. *Sci. Rep.*, 7(1): 40900.
- Haworth, M., G. Marino, F. Loreto and M. Centritto. 2021. Integrating stomatal physiology and morphology: evolution of stomatal control and development of future crops. *Oecologia*, 197(4): 867-883.
- Hepworth, C., T. Doheny-Adams, L. Hunt, D.D. Cameron and J.E. Gray. 2015. Manipulating stomatal density enhances drought tolerance without deleterious effect on nutrient uptake. *New Phytol.*, 208(2): 336-341.
- Hu, M. and S. Wan. 2019. Effects of fire and nitrogen addition on photosynthesis and growth of three dominant understory plant species in a temperate forest. *J. Plant Ecol.*, 12(4): 759-768.
- Li, X., T. Wu, G. Wu, L.C.R. Aguila, X. Liu, Y. Liu, Y. Cheng, F. Jiang, Z. Lie and J. Liu. 2024. Increasing stand age increases N deficiency but alleviates relative P limitations in *Castanopsis hystrix* plantations in Southern China. *Land Degrad. Dev.*, 35(6): 2173-2183.
- Li, Y., Z. Wang, H. Liu, C. Zhang, S. Fu and X. Fang. 2021. Responses in growth and anatomical traits of two subtropical tree species to nitrogen addition, drought, and their interactions. *Front. Plant Sci.*, 12(1606): 709510.
- Marklein, A.R. and B.Z. Houlton. 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol.*, 193(3): 696-704.
- Salazar-Tortosa, D., J. Castro, P. Villar-Salvador, B. Viñeola, L. Matías, A. Michelsen, R. Rubio de Casas and J.I. Querejeta. 2018. The "isohydric trap": A proposed feedback between water shortage, stomatal regulation, and nutrient acquisition drives differential growth and survival of European pines under climatic dryness. *Glob. Chang. Biol.*, 24(9): 4069-4083.
- Scoffoni, C., D.S. Chatelet, J. Pasquet-Kok, M. Rawls, M.J. Donoghue, E.J. Edwards and L. Sack. 2016. Hydraulic basis for the evolution of photosynthetic productivity. *Nat. Plants*, 2(6): 16072.
- Shi, W., L. Lin, S. Shao, A. He and Y. Ying. 2020. Effects of simulated nitrogen deposition on *Phyllostachys edulis* (Carr.) seedlings under different watering conditions: is seedling drought tolerance related to nitrogen metabolism? *Plant Soil*, 448(1-2): 539-552.
- Shi, Y., J. Zhang, K. Jiang, M. Cui and Y. Li. 2011. Development and characterization of polymorphic microsatellite markers in *Castanopsis sclerophylla* (Fagaceae). *Amer. J. Bot.*, 98(2): 19-21.
- Simonin, A., N. Montalbetti, G. Gyimesi, J. Pujol-Giménez and M.A. Hediger. 2015. The hydroxyl side chain of a highly conserved serine residue is required for cat-ion selectivity and substrate transport in the glial glutamate transporter GLT-1/SLC1A2. *J. Biol. Chem.*, 290(51): 30464-30474.
- Vitousek, P.M., S. Porder, B.Z. Houlton and O.A. Chadwick. 2010. Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen-phosphorus interactions. *Ecol. Appl.*, 20(1): 5-15.
- Wang, M., W. Zhang, N. Li, Y. Liu, X. Zheng and G. Hao. 2017. Photosynthesis and growth responses of *Fraxinus mandshurica* Rupr. seedlings to a gradient of simulated nitrogen deposition. *Ann. Forest Sci.*, 75(1): 1.
- Wang, Z., X. Li, M. Arif, J. Shamshad, A. Wu, W. Zhan, B. Ahmad, N. Tan, K.M. Al-Anazi, M.A. Farah and M.S. Khan. 2024. Analyzing the impact of phosphorous and nitrogen on *Castanopsis sclerophylla* early growth stages. 36(11): 103517.
- Wu, G., H. Liu, L. Hua, Q. Luo, Y. Lin, P. He, S. Feng, J. Liu and Q. Ye. 2018. Differential responses of stomata and photosynthesis to elevated temperature in two co-occurring subtropical forest tree species. *Front. Plant Sci.*, 9: 467.
- Wu, T., D.T. Tissue, G. Zhou, J. Yan, M. Zheng, X. Li, Y. Song, Y. Li, X. Tang, S. Liu, G. Chu, Z. Meng, Q. Ye and J. Liu. 2022a. Direct and indirect effects of long-term field warming methods on the physical environment and biological responses in a sub-tropical forest. *Front. Forest. Glob. Chang.*, 5: 877025.
- Wu, T., D.T. Tissue, X. Li, S. Liu, G. Chu, G. Zhou, Y. Li, M. Zheng, Z. Meng and J. Liu. 2020. Long-term effects of 7-year warming experiment in the field on leaf hydraulic and economic traits of subtropical tree species. *Glob. Chang. Biol.*, 26: 7144-7157.
- Wu, T., N. Tan, D.T. Tissue, J. Huang, H. Duan, W. Su, Y. Song, X. Liu, Y. Liu, X. Li, Z. Lie, S. Yang, S. Zhou, J. Yan, X. Tang, S. Liu, G. Chu, X. He and J. Liu. 2022b. Physiological traits and response strategies of four subtropical tree species exposed to drought. *Environ. Exp. Bot.*, 203: 105046.
- Xu, C., A. Salih, O. Ghannoum and D.T. Tissue. 2012. Leaf structural characteristics are less important than leaf chemical properties in determining the response of leaf mass per area and photosynthesis of *Eucalyptus saligna* to industrial-age changes in CO₂ and temperature. *J. Exp. Bot.*, 63(16): 5829-5841.

- Yang, B., S.G. Pallardy, T.P. Meyers, L. Gu, P.J. Hanson, S.D. Wullschlegel, M. Heuer, K.P. Hosman, J.S. Riggs and D.W. Sluss. 2009. Environmental controls on water use efficiency during severe drought in an Ozark Forest in Missouri, USA. *Glob. Chang. Biol.*, 16(8): 2252-2271.
- Zhang, H., F. Yuan, J. Wu, C. Jin, A.L. Pivovarov, J. Tian, W. Li, D. Guan, A. Wang and N.G. McDowell. 2021. Responses of functional traits to seven-year nitrogen addition in two tree species: coordination of hydraulics, gas exchange and carbon reserves. *Tree Physiol.*, 41(2): 190-205.
- Zhang, X., G. Xu, D. Shen, Y. Gu, H. Gao, X. Luo and X. Chen. 2007. Maintenance and natural regeneration of *Castanopsis sclerophylla* populations on islands of Qiandao Lake Region, China. *Acta Ecologica Sinica*, 27(2): 424-430. (in Chinese)
- Zheng, M., D. Li, X. Lu, X. Zhu, W. Zhang, J. Huang, S. Fu, X. Lu and J. Mo. 2016. Effects of phosphorus addition with and without nitrogen addition on biological nitrogen fixation in tropical legume and non-legume tree plantations. *Biogeochemistry*, 131: 65-76.
- Zhu, X., J. Shen, Y. Li, X. Liu, W. Xu, F. Zhou, J. Wang, S. Reis and J. Wu. 2021. Nitrogen emission and deposition budget in an agricultural catchment in subtropical central China. *Environ. Pollut.*, 289: 117870.