

EFFECTS OF DROUGHT STRESS AND NITROGEN APPLICATION ON THE GROWTH AND PHYSIOLOGICAL CHARACTERISTICS OF *QUERCUS AQUIFOLIOIDES* SEEDLINGS

ZENG, J. Q.^{1,2} – LI, T.³ – LIU, F.⁴ – LI, J. T.⁴ – WU, Y. F.⁴ – LI, X.⁴

¹Research Institute of Xizang Plateau Ecology, Xizang Agriculture and Animal Husbandry University, 860000 Nyingchi, China

²Key Laboratory of Forest Ecology in Xizang Plateau, Xizang Agricultural and Animal Husbandry University/Ministry of Education, 860000 Nyingchi, China

³College of Life Sciences, Sichuan University, 610065 Chengdu, China

⁴College of Forestry and Grassland Science, Xizang Agricultural and Animal Husbandry University, 860000 Nyingchi, China

*Corresponding author

e-mail: zengjiaqin2009@126.com; phone: +86-136-5694-2960

(Received 16th Oct 2025; accepted 22nd Jan 2026)

Abstract. This study selected two-year-old *Quercus aquifolioides* seedlings and conducted a water and nitrogen control experiment using potted cultivation to investigate their growth and physiological characteristics. Results demonstrated that drought stress significantly impairs normal metabolism in *Quercus aquifolioides* seedlings, severely restricting their growth and photosynthetic capacity. Appropriate nitrogen supplementation enhances leaf osmotic regulation, maintains osmotic balance, mitigates environmental damage, and consequently improves drought resistance. A significant coupling effect exists between water and nitrogen: under moderate moisture conditions, moderate nitrogen application boosts resistance and promotes growth; however, under severe drought, increased nitrogen application inhibits plant growth. Therefore, effective drought resistance and growth promotion in *Quercus aquifolioides* seedlings require rational regulation of water and nitrogen conditions.

Keywords: *Quercus aquifolioides*, osmotic regulation, drought resistance, photosynthesis and chlorophyll content, coupling effect, The Tibetan Plateau

Background and significance

The global eco-environment changes have led to drought stress and nitrogen deposition, which are likely to get worse in the coming decades (Yu et al., 2023). The global inorganic N deposition increased from 86.6 Tg in 1984 to 93.6 Tg in 2016 (Ackerman et al., 2019). China has emerged as one of the three major hotspots for nitrogen deposition worldwide (Dentener et al., 2006). From 1980 to 2015, nitrogen deposition increased by approximately 60%, ranging from 13.50 to 20.40 kg N ha⁻¹ a⁻¹ (Yu et al., 2019). Reactive N emissions in China are projected to reach 105 Tg a⁻¹ by 2030 (Zhang et al., 2020a). Approximately 20-30 Tg N a⁻¹ is discharged into forests through anthropogenic activities after atmospheric transport and redeposition (Lamarque et al., 2013; Schwede et al., 2018). Drought and nitrogen deposition have significantly affected plant growth thus these are important factors to consider in light of global environmental concerns.

Forests are the largest carbon pool globally and play a vital role in mitigating global warming caused by greenhouse gas emissions. Nitrogen deposition increases soil carbon

storage and forest carbon sequestration by increasing net primary productivity (NPP), forest carbon allocation, litter input or by reducing soil respiration (Wang et al., 2023; Janssens and Luyssaert, 2009; Lu et al., 2021). However, as nitrogen deposition intensifies, it reaches a critical tipping point that can lead to adverse consequences for the ecosystems, including forest biodiversity decline and reduced carbon storage capacity (Arróniz-Crespo et al., 2008; Christopher et al., 2023; Granath et al., 2009; Simkin et al., 2016).

In recent years, the severity and frequency of drought events triggered by global warming have increased. In China, reduced precipitation and increased potential evapotranspiration are projected to lead to prolonged drought periods from 2021 to 2040 (Su et al., 2021). Drought stress reduces the photosynthetic rate, thereby potentially affecting the efficiency and intensity of plant nutrient absorption, particularly nitrogen (Yates et al., 2010; Chavoushi et al., 2020; Cheng et al., 2020), ultimately inhibit plant growth (Gao et al., 2020; Talbi et al., 2020; Yu et al., 2022) and alter plant morphological structure (Xu et al., 2015; Cheng et al., 2020; Wang et al., 2020a) and plant biomass allocation (Zhang et al., 2020b; Cheng et al., 2021).

While there is a growing body of research on the combined effects of drought and nitrogen deposition on plant growth and ecosystem processes, mixed results have been found. Some studies have shown that nitrogen addition can enhance plant drought resistance (Shi et al., 2020; Sun et al., 2015), whereas others have demonstrated the negative or neutral effects of nitrogen addition on plant drought resistance (Valliere et al., 2017; Pivovarovoff et al., 2017; van der Graaf et al., 2021). Consequently, the response of trees to drought and nitrogen deposition depends on various factors, including tree species, stress intensity and duration.

The Tibetan plateau, known for its role in initiating and regulating climate change in the Northern Hemisphere, is a highly sensitive region to global climate change. Forest vegetation in the Tibetan Plateau is also strongly affected by global climate change. However, most studies on the effects of stress factors on forest growth in the Tibetan plateau have focused on individual global change factors, with limited research on the interactive effects of nitrogen deposition and drought stress on tree growth and ecosystem processes.

In this study, we investigated how the seedlings of *Quercus aquifolioides*, a hard broad-leaved plant species in the Tibetan plateau, responded to nitrogen addition and drought stress with respect to seedling growth and photosynthetic characteristics. Specifically, we explored the morphological and physiological traits following a one-year treatment involving varying levels of nitrogen addition and drought intensities, as well as their combined effects. Furthermore, our objective was to gather essential data and provide theoretical support to enhance our comprehension of the alterations in forest ecosystem function and patterns resulting from global environmental changes.

Materials and methods

Plant material

The experiment was conducted under natural light in a nursery from June 2022 to September 2023 using 2-year-old *Quercus aquifolioides* seedlings. Seedlings were grown individually in polyethylene plastic pots (upper diameter of 24 cm, lower diameter of 14 cm and height of 26 cm) filled with 10 Kg of campus nursery soil, and plant 1 seedling in each basin. The soil had a PH of 8.03, and contained 18.74 g/kg organic matter,

78 mg/kg alkali-hydrolyzed nitrogen, 46.5 mg/kg available phosphorus, and 64.24 mg/kg available potassium. Plants were watered daily. A month after transplanting, 5 plants of similar size were selected for experimental manipulations.

Experimental set-up

A full 2 x 2 factorial design was used to investigate the individual and joint effects of drought stress and nitrogen (N) addition on plant growth and photosynthesis. According to the measured field water capacity of local soil (FC: 28.75%), four levels of drought stress were set, namely, normal water supply (NS: 80% FC), mild drought stress (LS: 60% FC), moderate drought stress (MS: 40% FC), and severe drought stress (HS: 20% FC). For the N addition treatment, NH_4NO_3 (purity > 99%) was selected as the N source, and four doses were set: no N addition (N0: 0 kg $\text{hm}^{-2} \text{a}^{-1}$), low N addition (LN: 50 kg $\text{hm}^{-2} \text{a}^{-1}$), medium N addition (MN: 100 kg $\text{hm}^{-2} \text{a}^{-1}$), and high N addition (HN: 150 kg $\text{hm}^{-2} \text{a}^{-1}$). In total, there were 16 treatment combinations, with the combination of NS and N0 being the control (CK). Treatments were randomly assigned to each plant, with five plants per treatment. The drought stress treatment was performed using the gravimetric method by weighing each pot every three days and supplementing the transpired water. The N addition treatment was applied in the beginning of each month by spraying 20 ml NH_4NO_3 solution (prepared in water) on the seedlings, with the control plants receiving 20 ml water. The drought stress treatment and N addition treatment were conducted on different days to avoid N loss. After 15 months of drought stress and N addition, plant growth and physiological parameters were measured (see below).

Plant growth and photosynthesis measurements

At the end of the experiment, plant height and stem diameter of seedlings were measured using a tape measure and vernier caliper. Plant photosynthesis was measured on a full expanded and healthy leaf located on the similar position along the stem of each plant using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) (Zhang et al., 2019). The light intensity, CO_2 concentration, air temperature and relative humidity inside the leaf cuvette were set to 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 400 $\mu\text{mol}\cdot\text{mol}^{-1}$, 25°C and 50%, respectively. After photosynthesis measurements, leaf area was measured using the LI-3100C Area Meter (Cui et al., 2012). A sub-sample of leaf material was stored in liquid N for analyses of leaf chlorophyll content, malondialdehyde content, proline content and soluble sugar content. Then, plants were harvested and separated into leaves, stems, coarse roots (≥ 1 mm) and fine roots (1 mm), and their fresh weights were immediately weighed. All samples were dried at 70°C to constant weight, followed by dry weight determination.

Chlorophyll content was determined by the dimethyl sulfoxide (DMSO) method. Malondialdehyde content was determined by the thiobarbituric acid method (Gao, 2012). Proline content was determined by extracting ninhydrin with sulfosalicylic acid. The soluble sugar content was analyzed using the anthrone method (Li, 2003). The relative water content (RWC) of the leaf blade was measured using the saturation weighing method. Briefly, in each treatment, 3 seedlings with similar growth were selected, the mature leaves were picked from 4 directions. After wiping the dust on the surface, the leaves' average fresh mass (M_f) was immediately measured. After soaking in distilled water for 4 h, the leaves' average saturated mass (M_t) was measured. The mean dry mass (M_d) of leaves was determined, and this experiment was repeated three times. The RWC was calculated as follows (Yin et al., 2010).

$$RWC = (Mf - Md)/(Mt - Md) \times 100\%$$

Statistical analysis

All statistical analyses were performed using the software package SPSS statistics 20.0 (SPSS, Inc., Chicago, IL, USA). The data normal distribution and homogeneity of variance were investigated using Shapiro-Wilk test and Levene's test, respectively. Two-way ANOVA was used to investigate the main and interactive effects of drought stress and N addition on plant growth and photosynthesis parameters. When significant treatment effects were observed, multiple comparisons were made with Fisher's LSD method to compare the differences among different treatment combinations.

Results

Effects of drought stress and N addition on Quercus aquifolioides seedling growth

Drought stress and N deposition, both alone and in combination, significantly influenced all measured growth traits of *Quercus aquifolioides* seedlings ($P < 0.001$; Fig. 1). Specifically, drought stress reduced total biomass, leaf biomass, leaf area, stem biomass, root biomass (including fine and coarse roots), seedling height and stem basal diameter, with these negative effects becoming more pronounced as drought intensity increased. However, the sign and magnitude of N effects varied with drought intensity, N dose and the type of growth traits. Under normal soil moisture conditions (NS), there were significantly positive linear effects of N addition on all growth traits mentioned above, with the strongest effects observed at the high N levels (HN). Under light drought stress (LS), the positive N effects became weaker and peaked at low or moderate N levels, depending on the type of growth traits, whilst almost no N effects were observed at the high N level. Under moderate and severe drought stress (MS and HS), N addition at low levels still exhibited weak positive effects in fewer cases, whereas N addition at both moderate and high levels had negative effects on growth traits. For example, the strongest negative effect occurred at high N levels during severe drought, causing 24.6% decrease in total biomass. Conversely, the strongest positive effect occurred at high N levels without drought, leading to a 36.4% increase in total biomass. These results collectively suggest that low levels of N addition may mitigate the negative drought effects on seedling growth, particularly under mild and moderate drought stress, whereas high levels of N addition may inhibit seedling growth with increasing drought intensity.

In addition, drought stress and N addition significantly altered biomass allocation patterns. Overall, specific leaf area increased with increasing drought intensity. Under NS and HS conditions, specific leaf area decreased with increasing N levels, but tended to increase with increasing N levels under LS and MS conditions, particularly under MS. With respect to the root shoot ratio, N addition had little effect under NS conditions, but showed varying negative effects under LS, MS and HS conditions, depending on the N levels. The strongest negative effect was observed at moderate N levels under LS conditions.

Effects of drought stress and N addition on Quercus aquifolioides seedling photosynthesis and chlorophyll content

There were significant main and interactive effects of drought and N addition on photosynthetic parameters (Fig. 2). Overall, increasing drought intensity linearly reduced net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr), but

increased intercellular CO₂ concentrations (C_i). The magnitude and sign of increasing N addition on these photosynthetic parameters varied substantially with drought intensity. Under NS and LS conditions, increasing N addition increased P_n, G_s and Tr, but decreased C_i. In all these cases, N addition alleviated the impacts of mild drought stress to varying extent. Under MS and HS conditions, low amounts of N addition exhibited positive effects on P_n, G_s and Tr, and partially offset the effects of drought stress, whereas moderate and high amounts of N addition amplified the negative effects of drought stress. Consequently, the strongest negative effects on photosynthetic parameters were observed at high N addition under severe drought stress, while the strongest positive effects were observed at high N addition under normal soil moisture conditions.

Similarly, increasing drought stress and N addition significantly affected foliar chlorophyll contents (including Chl a and b) with few exceptions. The most obvious exception is that under MS conditions, N addition had positive effects on chlorophyll contents, with the highest effects occurring at moderate N addition.

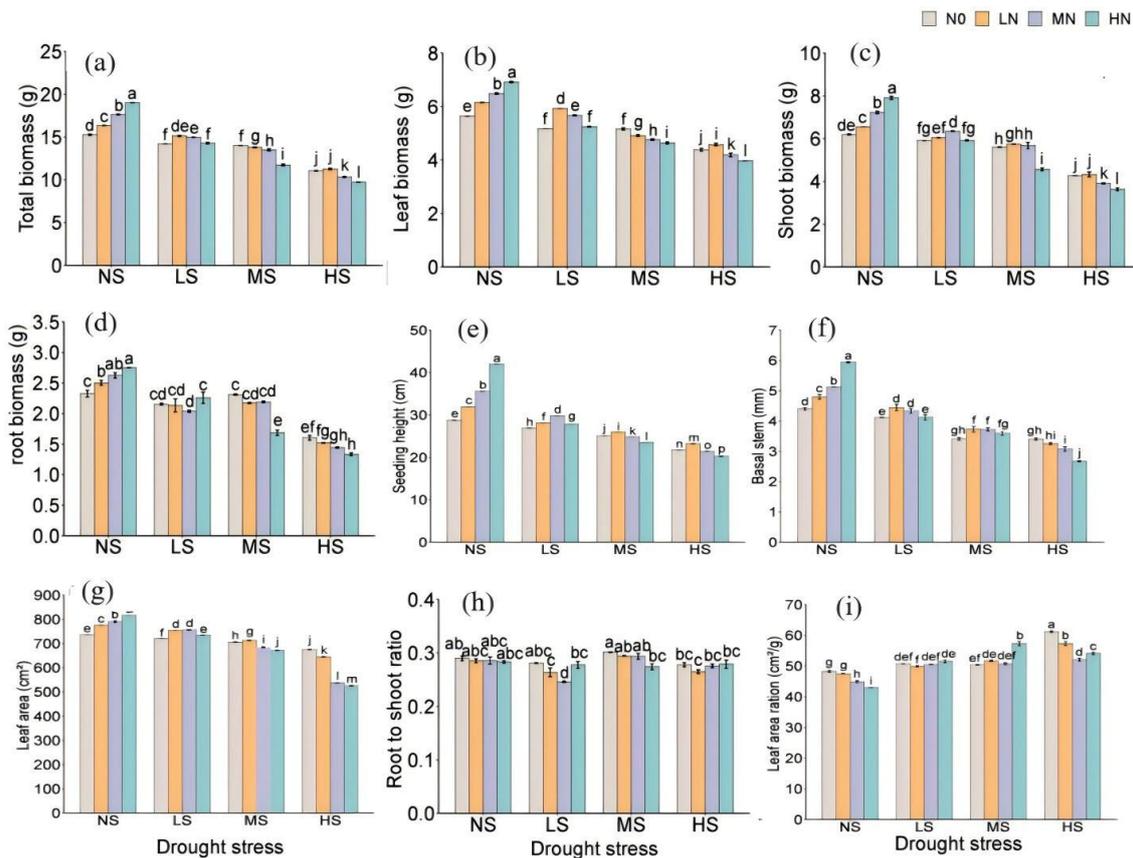


Figure 1. Effects of increasing drought intensity and N addition on growth traits of *Quercus aquifolioides* seedlings. Different lowercase letters indicate significant differences between different treatments ($P < 0.05$)

Effects of drought stress and N addition on osmotic adjustment of *Quercus aquifolioides* seedlings

Increasing drought stress linearly decreased leaf relative water content (RWC) and increased water saturation deficit (WSD), whilst the effects of increasing N addition on

RWC and SWD varied considerably with drought intensity. Under NS and LS conditions, increasing N addition (quasi-) linearly increased RWC and decreased SWD, with the strongest effects observed at high and moderate N addition under NS and LS conditions, respectively. Under MS and HS conditions, low N addition increased RWC and decreased SWD, thereby partially offset the impacts of drought stress, whereas both moderate and high N addition decreased RWC and increased SWD, thereby aggravating the impacts of drought stress (Fig. 3).

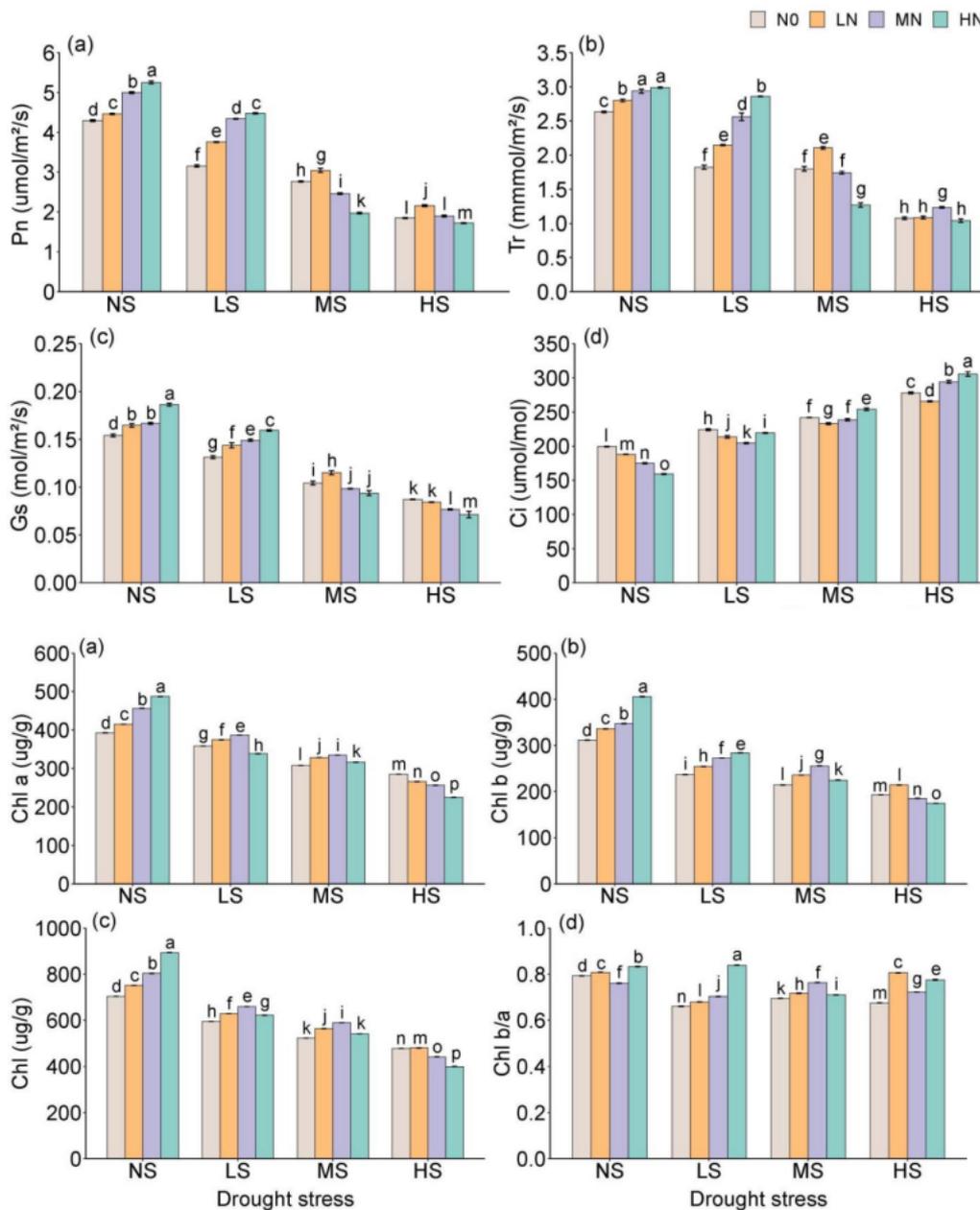


Figure 2. Effects of increasing drought intensity and N addition on photosynthetic parameters of *Quercus aquifolioides* seedlings

Likewise, increasing drought stress linearly increased foliar contents of osmolytes, including soluble sugar, proline and malondialdehyde (MDA), whilst the effects of

increasing N addition varied considerably with drought intensity. Under NS conditions, increasing N addition linearly decreased foliar contents of osmolytes. Under LS and MS conditions, foliar contents of soluble sugar, proline and MDA first decreased and then increased with increasing N addition, so did the response of MDA under HS conditions. In addition, under HS condition, foliar soluble sugar and proline contents both increased linearly with increasing N addition. Overall, the greatest negative effects on osmolytes were observed at high N addition under normal soil moisture conditions, while the strongest positive effects were observed at high N addition under severe drought conditions.

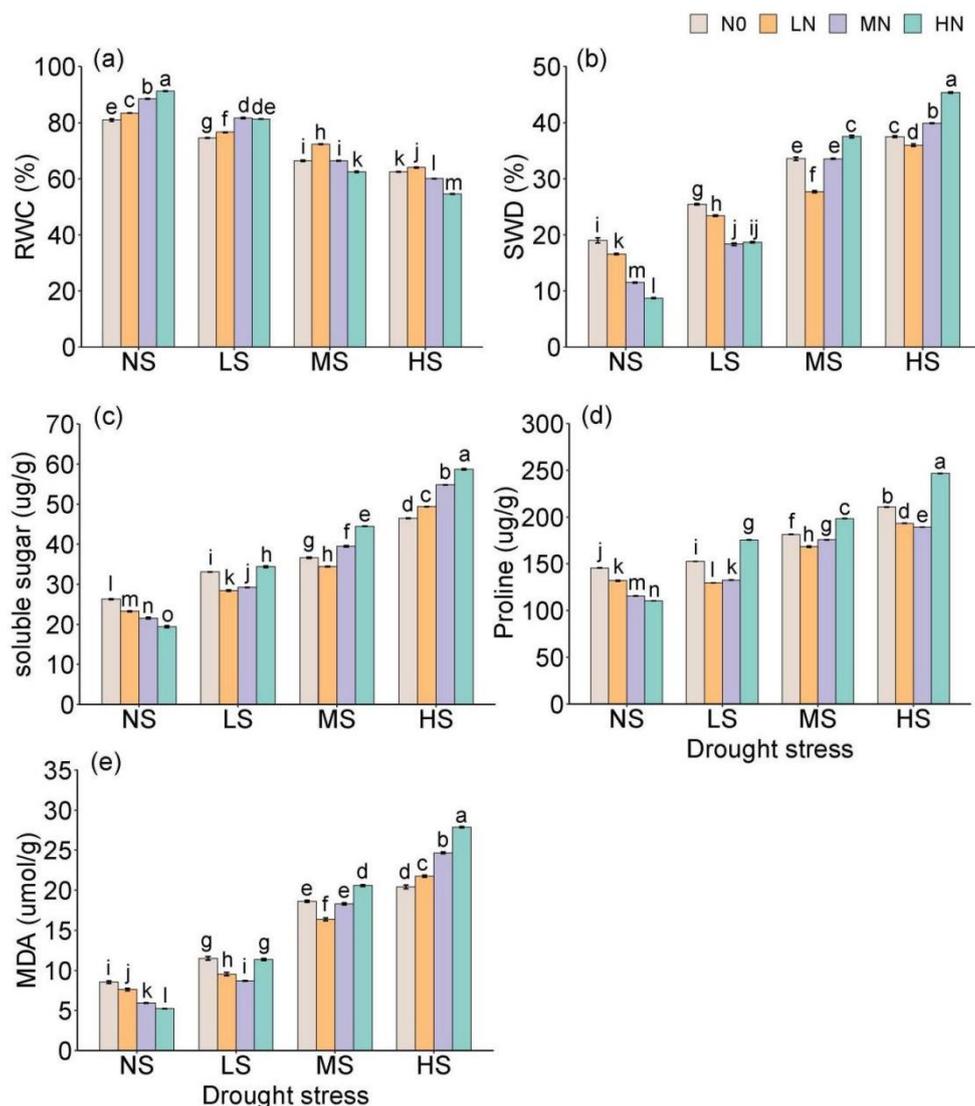


Figure 3. Responses of foliar osmolytes to increasing drought intensity and N addition in *Quercus aquifolioides* seedlings

Discussion

Effects of drought stress and N application on Quercus aquifolioides seedling growth

Soil water and nutrient availability both plays crucial roles in determining plant growth. Previous studies have shown that adequate N application under well-watered conditions

increases plant height, lateral branch number, and stem basal diameter, whereas excessive N application inhibits seedling growth (Zou et al., 2015; Dai and Zhang, 2020; Li et al., 2005). The inhibitory effects of excessive N application have been partially ascribed to soil acidification, reduced levels of base cations, and deficiencies of trace elements such as Mg, Cu, and Zn, which impede plant growth (Dai and Zhang, 2020; Li et al., 2015).

Our study demonstrates that the joint effects of increasing drought stress and N addition on *Quercus aquifolioides* seedling growth are complex and dependent on the severity of drought stress and the levels of N addition. Drought stress had a significantly negative impact on seedling growth, with biomass accumulation, height, and basal diameter being adversely affected. The negative effects of drought intensified with increasing drought severity. Under well-watered conditions, N application, particularly at high levels, enhanced seedling growth. Moderate N levels promoted growth under mild water stress, but high N levels hindered growth. In moderate and severe drought conditions, N addition, especially at excessive levels, had the most pronounced negative effects on seedling growth. These findings are consistent with previous studies, although the magnitude and sign of drought stress and N application on plant growth have been shown to vary across species and ecosystems (Bonnink et al., 2010; Alvarez-Clare et al., 2013; Wu et al., 2023).

The study shows that soil water and nitrogen have the main effects on plant biomass. The biomass of each part of *Q. aquifolioides* seedlings showed significant differences under varying drought stress and N concentrations. Under normal water supply and mild drought stress, the biomass, leaf area, and leaf area ratio increased with increasing N concentrations; however, the R/S was relatively small. The total seedling biomass and leaf area decreased with drought severity and increased N concentrations. The aboveground biomass was less than the underground biomass, consistent with the results of Guiz et al. (2018), Wang et al. (2020b), Sun et al. (2021), and Zhang et al. (2018), Liu et al. (2024).

Effects of drought stress and N application on the photosynthetic indices of Quercus aquifolioides seedlings

N is an essential element for chlorophyll formation, which is vital for photosynthesis. Meanwhile, most land plants' photosynthesis is often limited by the availability of N in the soil. N deposition, which increases the concentration of N in leaves, have been found to have a direct and positive correlation with plant photosynthetic rate (Magill et al., 2000). At certain concentrations, N deposition can enhance the photosynthetic rate by increasing Rubisco concentration and activity, as well as chlorophyll content (Nakji et al., 2001; Jiao et al., 2011). However, it is important to note that high levels of N treatment have been shown to inhibit plant growth in a South Asian tropical forest, and the net photosynthetic rate demonstrated an initial increase followed by a decrease with increasing N concentrations (Li et al., 2015).

In the present study, N application was positively correlated with P_n , T_r , G_s , and chlorophyll content, but negatively correlated with C_i under both normal soil moisture conditions and mild drought conditions, which agrees with previous findings (Magill et al., 2000; Jiao et al., 2011; Li et al., 2020). This negative correlation suggests that the high carboxylation activity of leaves, resulting from the increased photosynthetic rate leads to a decrease in C_i . As drought stress intensity increased, the growth-promoting effect of N addition on P_n , T_r , G_s , and C_i weakened and eventually turned into an inhibitory effect. The photosynthetic capacity of the seedlings decreased with increasing drought conditions, indicating potential irreversible damage to the photosynthetic organs under drought conditions, which consequently led to a reduction in the photosynthetic rate. However,

regardless of the levels of N addition, N supplementation was able to offset or even override the negative effects of mild drought stress. Conversely, high levels of N addition exacerbated the negative drought effects under moderate and severe drought conditions. These results suggest that the mitigating effects of N addition on drought stress are dependent on both the intensity of drought stress and the level of N addition,

Effects of drought stress and N application on osmotic adjustment of Quercus aquifolioides seedlings

In water-deficient soil conditions, the relative water content (RWC) and water saturation deficit (WSD) of plant leaves can serve as indicators of the extent of water deficit and drought resistance in plants (Gindaba et al., 2004). In this study, increasing drought stress intensity resulted in a linear decrease in leaf RWC and an increase in leaf WSD. This indicates that plants experience progressively greater water shortage as the intensity of drought stress increases. Under well-watered and mild drought conditions, N addition had a linear and positive effect on leaf RWC. However, under moderate and severe drought stress conditions, only low levels of N addition led to an increase in leaf RWC, while moderate and high levels of N addition resulted in a decrease in leaf RWC. Moreover, environmental stress often induces oxidative stress in plants, which in turn leads to lipid peroxidation and malondialdehyde (MDA) accumulation (Jaffel et al., 2011; Batish et al., 2006; Chen et al., 2019; Wang et al., 2018; Ke et al., 2016; Ding et al., 2021). Indeed, MDA has long been used as a marker of oxidative lipid injury caused by biotic and abiotic stress (Jaffel et al., 2011; Batish et al., 2006). The present study demonstrated a linear decrease in MDA content with increasing drought stress intensity, especially at high levels of N addition. These findings suggest that N supplementation can alleviate mild water stress, likely by promoting photosynthesis and improving water use efficiency. However, under moderate and severe drought stress conditions, N addition, especially at excessive levels, can exacerbate the negative impacts of drought stress.

Plants adapt to drought stress by regulating osmotic processes, increasing intracellular solute concentration, and reducing osmotic potential. This adjustment helps to decrease water potential and enables them to continue absorbing water from the surrounding medium, even when it has a lower water potential. By doing so, plants maintain turgor pressure and support normal metabolic functions to mitigate the impact of drought stress (Wang et al., 2023). Free proline and soluble sugar are the primary substances involved in osmotic regulation in plants during drought stress. Under osmotic stress, plants often increase the production of osmolytes to maintain osmotic balance and protect against cell damage. Fertilization has been shown to enhance the osmotic regulation capacity under drought conditions (Li et al., 1991; Chen et al., 1996). In this study, as drought intensity increased, there was a linear increase in the contents of soluble sugars and proline, especially at excessive levels of N addition. This indicates that N application can promote the accumulation of proline and soluble sugars, leading to a reduction in cell osmotic potential, minimizing water loss, and alleviating cell damage during osmotic stress caused by drought.

Conclusions

Drought stress and N application have significant effects on the growth and physiological characteristics of 2-year-old *Quercus aquifolioides* seedlings. Drought stress negatively impacts seedling photosynthesis and growth, with the magnitude increasing as drought intensity rises. However, under well-watered and mild drought

stress conditions, N supplementation enhances seedling photosynthesis and growth. On the other hand, under moderate and severe drought stress, especially severe drought stress, excessive N addition suppresses seedling photosynthesis and growth. This study demonstrates that N supplementation can alleviate and even counteract the negative effects of mild drought stress on *Q. aquifolioides* seedling photosynthesis and growth. However, under moderate and severe drought stress, particularly with excessive N levels, N supplementation can worsen the negative impacts of drought stress. Therefore, careful management of water and N conditions is essential for improving drought resistance and growth in *Q. aquifolioides* seedlings. By optimizing these factors, it is possible to enhance the seedlings' ability to withstand drought and promote their overall development.

Author contributions. Jiaqin Zeng designed the experiment, performed and supervised the project, and analyzed the data, and wrote; Feng Liu, Jiangtao Li, Yuefei Wu, Xiang Li experiment carried, Tao Li reviewed. All authors have read and agreed to the published version of the manuscript.

Funding. Forestry Discipline Innovation Team Construction Project of Tibet Agriculture and Animal Husbandry University (Tibetan Financial Forecast 2020-001), National Natural Science Foundation of China (31850123), Graduate Education Innovation and Discipline Construction Project of Tibet Agriculture and Animal Husbandry University (Tibetan Financial Forecast No. 2022-1).

REFERENCES

- [1] Ackerman, D., Millet, D. B., Chen, X. (2019): Global estimates of inorganic nitrogen deposition across four decades. – *Global Biogeochemical Cycles* 33(1): 100-107. <https://doi.org/10.1029/2018GB005990>.
- [2] Alvarez-Clares, S., Mack, M. C., Brooks, M. (2013): A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest. – *Ecology* 94(7): 1540-1551. <https://doi.org/10.1890/12-2128.1>.
- [3] Arróniz-Crespo, M., Leake, J. R., Horton, P., Phoenix, G. K. (2008): Bryophyte physiological responses to, and recovery from, long-term nitrogen deposition and phosphorus fertilisation in acidic grassland. – *New Phytol* 180: 864-874. <https://doi.org/10.1111/j.1469-8137.2008.02617.x>.
- [4] Batish, D. R., Singh, H. P., Setia, N., et al. (2006): 2-Benzoxazolinone (BOA) induced oxidative stress, lipid peroxidation and changes in some antioxidant enzyme activities in mung bean (*Phaseolus aureus*). – *Plant Physiology and Biochemistry* 44: 819-827. <https://doi.org/10.1016/j.plaphy.2006.10.014>.
- [5] Bonnink, R., Hicks, K., Galloway, J., et al. (2010): Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. – *Ecol Appl* 20(1): 30-59. <https://doi.org/10.1890/08-1140.1>.
- [6] Chavoushi, M., Najafi, F., Salimi, A., Angaji, S. A. (2020): Effect of salicylic acid and sodium nitroprusside on growth parameters, photosynthetic pigments and secondary metabolites of safflower under drought stress. – *Sci Hort* 259: 108823. <https://doi.org/10.1016/j.scienta.2019.108823>.
- [7] Chen, J. J., Ren, Y. H., Chen, P. Y., et al. (1996): Effects of nitrogen nutrition on the growth of different drought resistant wheat varieties under drought conditions. – *Journal of Crops* 22(4): 483-489.
- [8] Chen, L. F., Liu, Y., Li, X. M., et al. (2019): Research progress in plant drought resistance. – *Jilin Agriculture* (2): 78-79. <https://doi.org/10.14025/j.cnki.jlny.2019.02.034>.
- [9] Cheng, H. Y., Wei, M., Wang, S., Wu, B. D., Wang, C. Y. (2020): Atmospheric N deposition alleviates the unfavorable effects of drought on wheat growth. – *Braz J Bot* 43: 229-238. <https://doi.org/10.1007/s40415-020-00598-4>.

- [10] Cheng, H. Y., Wu, B. D., Wang, S., Wei, M., Wang, C. Y. (2021): Nitrogen application and osmotic stress antagonistically affect wheat seed germination and seedling growth. – Int J Phytoremediat 23: 1289-1300. <https://doi.org/10.1080/15226514.2021.1895715>.
- [11] Cui, X. M., Liu, X. B., Li, Z. H., et al. (2012): Effects of salicylic acid on growth and photosynthetic physiology of *Melilotoides ruthenica* at branching stage under different water stress. – Journal of Grass Industry 21(6): 82-93. <http://cyxb.magtech.com.cn/EN/Y2012/V21/I6/82>.
- [12] Dai, M., Zhang, Y. M. (2020): Effects of coupling of water and nitrogen on the accumulation of soil nitrogen in root and yield of rice. – Research of Soil and Water Conservation 27(3): 168-173. <https://doi.org/10.13869/j.cnki.rswc.2020.03.025>.
- [13] Dentener, F., Drevet, J., Lamarque, J. F., et al. (2006): Nitrogen and sulfur deposition on regional and global scales: a multimodel evaluation. – Global Biogeochemical Cycles 20: GB4003. <https://doi.org/10.1029/2005GB002672>.
- [14] Ding, H., Cheng, B., Zhang, G. C., et al. (2021): Effects of nitrogen fertilizer application on physiological characteristics of peanut leaves under drought stress. – Journal of Peanut Science 50(2): 64-68. <https://doi.org/10.14001/j.issn.1002-4093.2021.02.011>.
- [15] Gao, J. F. (2012): Plant Physiology Experiment Guidance. – Higher Education Press, Beijing.
- [16] Gao, S. S., Wang, Y. L., Yu, S., Huang, Y. Q., Liu, H. C., Chen, W., He, X. Y. (2020): Effects of drought stress on growth, physiology and secondary metabolites of two Adonis species in Northeast China. – Sci Hortic 259: 108795. <https://doi.org/10.1016/j.scienta.2019.108795>.
- [17] Gindaba, J., Rozanov, A., Negash, L. (2004): Response of seeding of two Eucalyptus and three deciduous tree species from Ethiopia to severe water stress. – Forest Ecology and Management 201: 119-129. <https://doi.org/10.1016/j.foreco.2004.07.009>.
- [18] Granath, G., Wiedermann, M. M., Strengbom, J. (2009): Physiological responses to nitrogen and sulphur addition and raised temperature in *Sphagnum balticum*. – Oecologia 161: 481-490. <https://doi.org/10.1007/s00442-009-1406-x>.
- [19] Guiz, J., Ebeling, A., Eisenhauer, N., et al. (2018): Interspecific competition alters leaf stoichiometry in 20 grassland species. – Oikos 127: 903-914. <https://doi.org/10.1111/oik.04907>.
- [20] Jaffel, K., Sai, S., Bouraoui, N. K., et al. (2011): Influence of salt stress on growth, lipid peroxidation and antioxidative enzyme activity in borage (*Borago officinalis* L.). – Plant Biosyst. 145: 362-369. <https://doi.org/10.1080/11263504.2011.558689>.
- [21] Janssens, I. A., Luyssaert, S. (2009): Nitrogen's carbon bonus. – Nature Geoscience 2: 318-319. <https://doi.org/10.1038/ngeo505>.
- [22] Jiao, J. Y., Yin, C. Y., Chen, K. (2011): Effects of soil water and nitrogen supply on photosynthetic characteristics of *Jatropha curcas* seedlings. – Plant Ecology 35(1): 91-99. <https://doi.org/10.3724/SP.J.1258.2011.00091>.
- [23] Ke, J., Li, J., Li, Y. J. (2016): Physiological responses of *Lycium ruthenicum* seedlings on exogenous salicylic acid under the drought stress. – Plant Physiology 52(4): 497-504. <https://doi.org/10.13592/j.cnki.ppj.2015.0643>.
- [24] Lamarque, J. F., Dentener, F., McConnell, J., et al. (2013): Multi-model mean nitrogen and sulfur deposition from the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP): evaluation historical and projected changes. – Atmospheric Chemistry and Physics 13(16): 7997-8018. <https://doi.org/10.5194/acp-13-7997-2013>.
- [25] Li, D. J., Mo, J. M., Fang, Y. T., et al. (2005): Effects of simulated nitrogen deposition on biomass production and allocation in *Schima Superba* and *Cryptocarya Concinna* seedlings in subtropical China. – Acta Phytocologica Sinica 29(4): 543-549. <https://doi.org/10.17521/cjpe.2005.0073>.
- [26] Li, H. S. (2003): Experimental Guidance on Plant Physiology. – Higher Education Press, Beijing.

- [27] Li, X., Zhang, Y. Q., Wang, D. Y., et al. (2015): Effects of coupling water and nitrogen on root physio-ecological indices and yield of Adzuki bean. – Chinese Journal of Eco-Agriculture 23(12): 1511-1519. <https://doi.org/10.13930/j.cnki.cjea.150489>.
- [28] Li, Y., Chen, P. Y., Chen, J. J. (1991): Comparative study on response of different early resistant varieties to nitrogen nutrition under water stress. – Northwest Botanical Journal 11(4): 309-315.
- [29] Li, Z. Y., Jiang, H., Wang, Y. N., et al. (2020): Effects of nitrogen and water stress on growth and physiology of *Coreopsis tinctoria* seedlings. – Xinjiang Agricultural Science 57(1): 127-138. <https://doi.org/10.6048/j.issn.1001-4330.2020.01.015>.
- [30] Liu, C., Liu, J. J., Wang, J., Ding, X. Y. (2024): Effects of short-term nitrogen additions on biomass and soil phytochemical cycling in alpine grasslands of Tianshan, China. – Plants 13(8): 1103. <https://doi.org/10.3390/plants13081103>.
- [31] Lu, X. K., Vitousek, P. M., Mao, Q. G., et al. (2021): Nitrogen deposition accelerates soil carbon sequestration in tropical forests. – PNAS 118(16): 1-7. <https://doi.org/10.1073/pnas.2020790118>.
- [32] Magill, A. H., Aber, J. D., Berntson, G. M., et al. (2000): Long-term nitrogen additions and nitrogen saturation in two temperate forests. – Ecosystems 3(3): 238-253. <https://doi.org/10.1007/s100210000023>.
- [33] Nakji, T., Fukani, M., Dokiya, Y., et al. (2001): Effects of high nitrogen load on growth, photosynthesis and nutrient status of *Cryptomeria japonica* and *Pinus densiflora* seedlings. – Trees 15(8): 453-461. <https://doi.org/10.1007/s00468-001-0130-x>.
- [34] Pivovarov, A. L., Santiago, L. S., Vourlitis, G. L., Grantz, D. A., Allen, M. F. (2016): Plant hydraulic responses to long-term dry season nitrogen deposition alter drought tolerance in a Mediterranean-type ecosystem. – Oecologia 181: 721-731. <https://doi.org/10.1007/s00442-016-3609-2>.
- [35] Schwede, D. B., Simpson, D., Tan, J. N., et al. (2018): Spatial variation of modelled total, dry and wet nitrogen deposition to forests at global scale. – Environ Pollut 243: 1287-1301. <https://doi.org/10.1016/j.envpol.2018.09.084>.
- [36] Shi, W., Lin, L., Shao, S., He, A., Ying, Y. (2020): Effects of simulated nitrogen deposition on *Phyllostachys edulis* (Carr.) seedlings under different watering conditions: Is seedling drought tolerance related to nitrogen metabolism? – Plant and Soil. 448: 539-552. <https://doi.org/10.1007/s11104-020-04445-z>.
- [37] Simkin, S. M., Allen, E. B., Bowman, W. D., et al. (2016): Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the United States. – Proc Natl Acad Sci USA 113: 4086-4091. <https://doi.org/10.1073/pnas.1515241113>.
- [38] Su, B. D., Huang, J. L., Mondal, S. K., Zhai, J., et al. (2021): Insight from CMIP6 SSP-RCP scenarios for future drought characteristics in China. – Atmospheric Research 250: 105375. <https://doi.org/10.1016/j.atmosres.2020.105375>.
- [39] Sun, M. W., Xu, Y. Q., Wang, G., et al. (2021): Responses of the rhizosphere effect and photosynthetic physiology of two ecotypes of *Leymus chinensis* to drought stress in Songnen grassland. – Chinese Journal of Grassland 43(5): 8-16. <https://doi.org/10.16742/j.zgcdxb.20200218>.
- [40] Sun, Y. Y., Tang, B., Yin, C. Y., et al. (2015): Effect of water and nitrogen coupling on growth of *Betula platyphylla* seedlings and its physiological mechanism. – Application and Environmental Biology 21(4): 710-716. <https://doi.org/10.3724/SP.J.1145.2015.02018>.
- [41] Talbi, S., Rojas, J. A., Sahrawy, M., et al. (2020): Effect of drought on growth, photosynthesis and total antioxidant capacity of the Saharan plant *Oudeneya africana*. – Environ Exp Bot. 176: 104099. <https://doi.org/10.1016/j.envexpbot.2020.104099>.
- [42] Valliere, J. M., Irvine, I. C., Santiago, L., Allen, E. B. (2017): High N, dry: experimental nitrogen deposition exacerbates native shrub loss and nonnative plant invasion during extreme drought. – Glob Change Biol 23: 4333-4345. <https://doi.org/10.1111/gcb.13694>.
- [43] Van der Graaf, S. C., Janssen, T. A. J., Erisman, J. W., Schaap, M. (2021): Nitrogen deposition shows no consistent negative nor positive effect on the response of forest

- productivity to drought across European FLUXNET forest sites. – Environmental Research Communications 3: 125003. <https://doi.org/10.1088/2515-7620/ac2b7d>.
- [44] Wang, S., Wei, M., Wu, B. D., et al. (2020b): Does N deposition mitigate the adverse impacts of drought stress on plant seed germination and seedling growth? – Acta Oecologica 109: 103650. <https://doi.org/10.1016/j.actao.2020.103650>.
- [45] Wang, S., Wei, M., Cheng, H. Y., et al. (2020a): Indigenous plant species and invasive alien species tend to diverge functionally under heavy metal pollution and drought stress. – Ecotox Environ Safe 205: 111160. <https://doi.org/10.1016/j.ecoenv.2020.111160>.
- [46] Wang, X., Hu, H. L., Hu, T. X., et al. (2018): Effects of drought stress on osmotic regulation and active oxygen metabolism in young Phoebe zhennan trees and mitigation effects of nitrogen application. – Plant Ecology 42(2): 240-251. <https://doi.org/10.17521/cjpe.2017.0225>.
- [47] Wang, X., Wu, G. Y., Li, D. Y., Song, X. H. (2023): Moderate nitrogen deposition alleviates drought stress of *Bretschneidera sinensis*. – Forests 14: 137-152. <https://doi.org/10.3390/f14010137>.
- [48] Wu, X. P., Liu, S. R., Luan, J. W., Wang, Y., et al. (2023): Nitrogen addition alleviates drought effects on water status and growth of Moso bamboo (*Phyllostachys edulis*). – Forest Ecology and Management 530: 120768. <https://doi.org/10.1016/j.foreco.2023.120768>.
- [49] Xu, W., Cui, K. H., Xu, A. H., Nie, L. X., et al. (2015): Drought stress condition increases root to shoot ratio via alteration of carbohydrate partitioning and enzymatic activity in rice seedlings. – Acta Physiol Plant 37: 9. <https://doi.org/10.1007/s11738-014-1760-0>.
- [50] Yates, M. J., Verboom, G. A., Rebelo, A. G., Cramer, M. D. (2010): Ecophysiological significance of leaf size variation in proteaceae from the cape floristic region. – Funct Ecol 24: 485-492. <https://doi.org/10.1111/j.1365-2435.2009.01678.x>.
- [51] Yin, L., Hu, T. X., Liu, Y. A. (2010): The effects of drought stress on photosynthetic characteristics and growth of *Jatropha curcas* seedlings under different nitrogen levels. – Chinese Journal of Applied Ecology 21(3): 569-576. <https://doi.org/10.13287/j.1001-9332.2010.0107>.
- [52] Yu, G., Jia, Y., He, N., Zhu, J., Chen, Z., Wang, Q., et al. (2019): Stabilization of atmospheric nitrogen deposition in China over the past decade. – Nat Geosci 12(6): 424-429. <https://doi.org/10.1038/s41561-019-0352-4>.
- [53] Yu, Y., Cheng, H., Wu, B., Wang, C. (2023): Combined effects of drought stress and different forms of nitrogen deposition as response mechanism to environmental change. – International Journal of Environmental Science and Technology 20: 6697-6712. <https://doi.org/10.1007/s13762-022-04408-0>.
- [54] Yu, Y. L., Cheng, H. Y., Wang, S., Wei, M., Wang, C. Y., Du, D. L. (2022): Drought may be beneficial to the competitive advantage of *Amaranthus spinosus*. – Journal of Plant Ecology 15: 494-508. <https://doi.org/10.1093/jpe/rtac001>.
- [55] Zhang, C. M., Shi, S. L., Wu, F. (2018): Effects of drought stress on root and physiological responses of different drought-tolerant alfalfa varieties. – Scientia Agricultura Sinica 51(5): 868-882. <https://doi.org/10.3864/j.issn.0578-1752.2018.05.006>.
- [56] Zhang, H. W., Chen, B., Wen, X. Y., et al. (2019): Effects of exogenous silicon on growth, leaf photosynthesis and physiological indexes of tobacco seedlings under drought stress. – Biotechnology Bulletin 35(1): 17-26. <https://doi.org/10.13560/j.cnki.biotech.bull.1985.2018-0691>.
- [57] Zhang, S. K., Shao, L., Sun, Z. Y., Huang, Y., Liu, N. (2020a): An atmospheric pollutant (inorganic nitrogen) alters the response of evergreen broad-leaved tree species to extreme drought. – Ecotox Environ Safe 187: 109750. <https://doi.org/10.1016/j.ecoenv.2019.109750>.
- [58] Zhang, Y. J., Shang, Y. S., Wang, P. C., et al. (2020b): Effects of super absorbent polymers on growth and physiological characteristics of *So phoradavidii* vs. *Panjiang* seedling under drought stress. – Acta Prataculturae Sinica 29(7): 90-98. <https://doi.org/10.11686/cyxb.2019510>.

- [59] Zou, X. Y., Li, B. L., Song, L. Q., et al. (2015): Effect of nitrogen application and water stress at flowering stage on yield performance parameters and nitrogen use efficiency in rapeseed with different nitrogen use efficiency. – Acta Agriculturae Boreali-Sinica 30(02): 220-226. <https://doi.org/10.7668/hbxb.2015.02.037>.