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Morphological and physiological responses to water stress and potassium nutrition in *Eucalyptus grandis* Hill Ex Maiden seedlings

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In this study, the response of *Eucalyptus grandis* seedlings to varied water availability and potassium fertilization in coarse-textured soil was investigated. The experiment took place in a greenhouse in Buenos Aires, Argentina, over 56 days, with 15 replicates for each treatment. We maintained pots in the non-water-limited group at field capacity (FC), while those in the water-restricted group were maintained at 40% FC. Each water regime received one of three potassium levels (K1, K2, and K3). We measured plant height and root-collar diameter periodically. At the end of the experiment, biomass allocation among plant parts was quantified. We also evaluated physiological traits by measuring stomatal conductance and relative water content (RWC). The results show that potassium fertilization did not improve seedling performance under water stress or enhance growth in well-watered seedlings. Water restriction was the main limiting factor. Keeping soil moisture at 40% of FC reduced height growth, root-collar diameter, biomass, and leaf area. It also negatively affected physiological traits such as stomatal conductance and RWC, but did not cause seedling mortality.

Keywords: Coarse-textured Soil, Fertilization, Greenhouse, Seedlings.

Introduction

The establishment phase of forest plantations is critical for trees, as newly transplanted trees are very vulnerable to environmental stressors. These stress conditions are worse on coarse-textured soils, which are now a target for forest production (Reichert et al. 2021). Such soils have low water retention, little organic matter, and fewer nutrients (Reichert et al. 2021). In these conditions, effective nursery hardening and good soil management are essential for successful plantation establishment and productivity. Traditional hardening techniques usually involve regulating irrigation and adjusting nutrient supply.

Eucalyptus species require a substantial amount of water, which can lead to water shortages, particularly in areas with irregular rainfall (Caldato & Schumacher 2013). Water is the main factor limiting plant growth and development (Dale & Frank 2022). Under water stress, plants may change structurally and functionally. In severe cases, these changes can lead to death (Bacha et al. 2024). To cope with water stress, plants develop deeper roots, regulate stomatal conductance, adjust osmotic levels, and modify leaf size or shed leaves (Yang et al. 2021, Wu et al. 2022). Severe water deficits reduce total biomass and leaf area (Luna-Flores et al. 2015, Hernández et al. 2024), directly affecting photosynthesis. Studies on *Eucalyptus grandis* show that limited water causes loss of turgor, reduced cell expansion, stomatal closure, and lower internal CO_2 concentration, thereby reducing growth and productivity (Graciano 2005, Yang et al. 2023).

In sandy-textured soils, low cation exchange capacity leads to poor nutrient retention and frequent deficiencies in essential macronutrients such as potassium. Fertilization efficiency depends on plant water status (Graciano 2005, Delbaz et al. 2023). Adequate nutrient supply can improve plant tolerance to water stress (Battie-Laclau et al. 2014). However, more nutrients can increase water uptake and transpiration, raising questions about whether potassium fertilization can help with stress hardening during drought.

Several studies on *Eucalyptus microtheca, E. occidentalis, E. torquata*, and *E. camaldulensis* show these species have many adaptive strategies for drought and water scarcity (Chemlali et al. 2022). Plants with sufficient potassium levels are more resistant to drought and low temperatures. Potassium is crucial for osmotic regulation and maintaining cellular water balance (Legoas et al. 2023).

Climate change leads to prolonged droughts, wildfires, and more pest outbreaks, which increase forest mortality risk. These factors significantly impact forest structure, productivity, and biodiversity (Liu et al. 2021, Zhang et al. 2024). These effects have become more obvious in recent years. Fernandez et al. (2023) documented severe drought impacts on forest plantations in Argentine Mesopotamia, where widespread tree mortality occurred. These findings highlight the long-term consequences of increased drought on plantations (Battie-Laclau et al. 2014), especially since forest expansion often occurs on limited soils.

Globally, *Eucalyptus* plantations are a ma-

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jor source of raw materials for timber and non-timber products. This has led to a significant increase in planted areas over recent decades (Legoas et al. 2023). In Argentina, *Eucalyptus grandis* has the highest commercial value and occupies the largest planted area (DNDFI 2025). It adapts well to the environmental conditions of the Mesopotamian region. This species grows on various soil types, including sandy (Entisols), mixed (Mollisols), and clayey (Ultisols). Sandy soils, mainly along the Uruguay River basin, support forestry, agriculture, and mixed crop-livestock systems (Tasi 2009). These soils have very sandy surface horizons (1-2% organic matter), with denser layers of loam-sandy clay at about 60 cm depth (Tasi 2009). Given these soil conditions, any factor that improves plant water status during establishment is crucial for survival and early growth.

Considering the plasticity of *Eucalyptus* species under stress, this study aimed to evaluate the responses of *Eucalyptus grandis* seedlings to water restriction and varying potassium levels under controlled conditions. We hypothesized that potassium fertilization in sandy soils could reduce the effects of drought stress, making water-restricted and non-restricted treatments more similar.

Materials and methods

We conducted a controlled experiment in a greenhouse at the Faculty of Agronomy, University of Buenos Aires, over 56 days. The study used three-month-old *Eucalyptus grandis* seedlings from the Concordia Experimental Agricultural Station (INTA, Argentina), sourced from a clonal seed orchard (Basic Material No. 41E3066JE). Initially, seedlings were grown in 98 cm^3 containers, then transplanted into 4-L pots for the experiment.

The pots contained soil classified as Udifluvents (Tasi 2009), collected from the top 20 cm of an *E. grandis* plantation in Gualeguaychú Department, Entre Ríos Province, Argentina. Laboratory tests showed the soil was sandy: 88.72% sand, 3.28% silt, and 8.00% clay (Bouyoucos 1936). Soil pH (1: 2.5 soil: water ratio) was 4.6, indicating strongly acidic conditions. Organic carbon content was 0.96% (Walkley & Black 1934). Exchangeable base-cation concentrations were 4.24, 0.35, and 0.13 cmol(c) kg^{-1} for Ca, Mg, and Na, respectively.

Treatments

The experiment tested two factors: (i) potassium (K) fertilization and (ii) soil water content. We used three potassium doses, namely 0 (zero) g (K1), 0.5 g (K2), and 1.0 g (K3) of K, applied as potassium nitrate (KNO_3). All pots also received nitrogen (N) and phosphorus (P) fertilization. We applied phosphorus as triple superphosphate (0-20-0) at 2.4 g per pot (Graciano 2005). Nitrogen was supplied as urea (46-0-0), with rates based on the potassium treatment: 1.0 g urea (0.46 g N) for K1, 0.63 g urea (0.29 g N) for K2, and 0.22 g urea (0.10 g N) for K3, accounting for nitrogen from potassium nitrate.

Two soil water content levels were set: (i) field capacity (FC) for non-stress conditions and (ii) 40% of field capacity (FC40) for water-restricted conditions. We weighed pots every two days to maintain moisture levels. Non-stressed plants (FC) were watered every other day, while water-restricted plants were kept at a reduced weight to maintain a consistent soil moisture deficit. The design included 15 replicates per treatment, for a total of 90 plants.

We measured plant height with a tape measure from the stem base to the uppermost fully expanded leaf. Root collar diameter was measured with a digital caliper. We took measurements on days 7, 14, 21, 28, 35, 42, 48, and 56 of the experiment.

At the start of the experiment, 15 seedlings were randomly selected and harvested for initial biomass. We divided the plants into leaves, branches, stems, and roots. We repeated this process at the end of the experiment for the remaining seedlings. Samples were oven-dried at 70 °C until constant weight. Aboveground biomass (AGB) was estimated as the total dry mass of leaves, branches, and stems, and root biomass (RB) was assessed from dried root material. We then calculated the AGB: RB ratio.

We measured leaf area (LA) at the end of the experiment on seven randomly chosen plants per treatment. We measured all leaves from each selected plant with a LI-COR LI-3100 leaf area meter. Total leaf area per plant was the sum of all individual leaf areas.

Relative water content was assessed at the end (day 56) using seven randomly chosen plants per treatment. Fully expanded leaves were collected, and leaf discs were excised and weighed for fresh mass (FM). The discs were then saturated in deionized water, blotted dry, and reweighed for turgid mass (TM). Finally, samples were oven-dried at 70 °C for 72 hours to find dry mass (DM).

Relative water content was calculated using the following equation (eqn. 1):

$$CRA(\%) = \frac{(Pf - Ps)}{(Psat - Ps)} \cdot 100 \quad (1)$$

where *CRA* is the relative water content, *Pf* is the fresh weight, *Ps* is the dry weight, and *Psat* is the saturated weight.

Stomatal conductance (g_s) was measured in the greenhouse with a steady-state porometer (LI-1600, LI-COR Inc., Lincoln, NB, USA). We selected seven plants from each treatment for these readings. We took measurements around midday on a clear, sunny day. We used the uppermost fully expanded leaf of each plant, 56 days after the experiment began.

Statistical analysis

We performed an analysis of variance (ANOVA) to study how potassium (K) fertilization levels and soil water content affect the measured variables. Before the analysis, we checked the assumptions of normality and homogeneity of variance. When significant differences were found, the least significant difference (LSD) test at a set significance level was used to compare treatment means. We also conducted Pearson's correlation analyses to explore relationships among morphological and physiological parameters. All statistical analyses were performed in Infostat/P ver. 1.1 (Di Rienzo et al. 2020).

Results

Tab. 1 shows the significance levels for each variable analyzed. We found no interaction effects between potassium fertilization and soil water content for any growth variables. However, each factor showed significant differences. Potassium fertilization significantly influenced the aboveground biomass-to-root biomass ratio (AGB: RB) and leaf area. In contrast, soil water content affected all measured variables.

Neither water restriction during the experiment nor potassium levels significantly impacted seedling mortality. By the end of the experiment, only one seedling died, and it was subjected to the K2 treatment at field capacity (K2FC).

Tab. 1 - Significance values for growth variables, aboveground biomass (AGB), root biomass (RB), AGB/RB ratio, leaf area, relative water content, and stomatal conductance for potassium fertilization and soil water content. (ns): not significant (p>0.05).

Characteristics	Potassium Fertilization	Soil water content
Height at the end of the experiment	ns	<0.001
Collar diameter at the end of the experiment	ns	<0.001
Aboveground Biomass (AGB)	ns	<0.001
Root biomass (RB)	ns	<0.001
AGB/RB ratio	0.0182	<0.001
Leaf área	0.0013	<0.001
Relative water content	ns	<0.001
Stomatal conductance	ns	<0.001

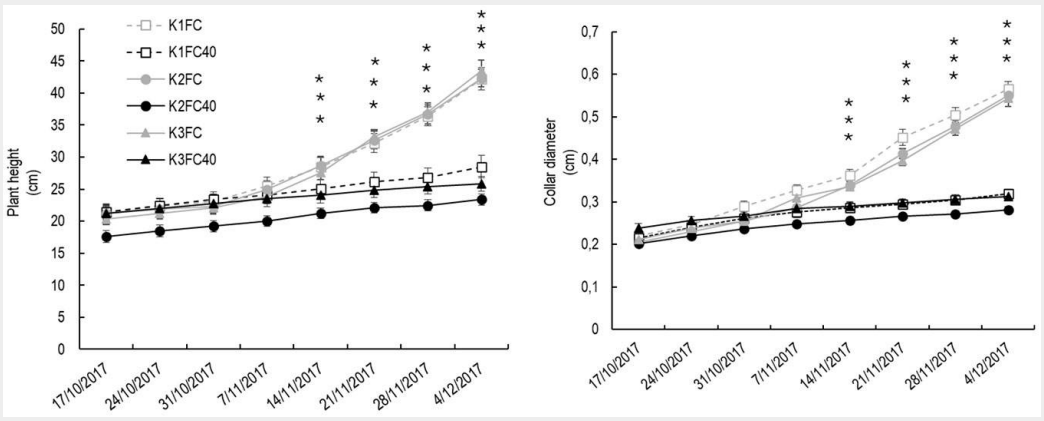


Fig. 1 - Plant height (a) and collar diameter (b) of *E. grandis* under different levels of potassium fertilization: K1: 0 g K, K2: 0.5 g K, K3: 1 g K; and soil water content levels: FC: maintenance at field capacity; FC40: maintenance at 40% of field capacity. Asterisks indicate differences between treatments with different water contents within each date after Fisher's LSD test ($p < 0.01$).

At the start of the experiment, seedlings averaged 20.21 ± 1.88 cm in height and 0.26 ± 0.02 cm in root-collar diameter. Fig. 1 illustrates the changes in plant height and root-collar diameter over time. We detected no significant interaction between potassium dose and soil water content, nor differences among potassium treatments for these variables.

A clear separation between water regimes appeared from day 28 (14 November 2017). Seedlings at field capacity (FC) showed greater height and root-collar diameter than those at 40% of field capacity (FC40). Treatments without water limitation (K1FC: 0.57 cm, K2FC: 0.56 cm, and K3FC: 0.54 cm) had larger final root-collar diameters compared to the water-restricted treatments (K1FC40: 0.32 cm, K2FC40: 0.28 cm, and K3FC40: 0.31 cm).

These findings suggest that soil water availability, not potassium levels, was the main factor affecting early growth in *Eucalyptus grandis* seedlings.

Water restriction significantly reduced both aboveground biomass (AGB) and root biomass (RB). As shown in Fig. 2, seedlings under non-limiting water conditions (FC) had the highest biomass values, with AGB averaging 7.57 ± 0.32 g, compared to 1.66 ± 0.04 g under water-restricted conditions (FC40). Similarly, RB averaged 2.71 ± 0.13 g *versus* 0.93 ± 0.11 g under FC40.

Water-limited treatments (40% of field capacity) showed a clear reduction in biomass accumulation. This highlights soil water availability as the key factor controlling biomass production in *Eucalyptus grandis* seedlings during the study.

The AGB: RB ratio differed significantly across potassium levels. The highest ratio occurred in the K3 treatment (2.59), while the lowest was in the intermediate level, K2 (2.14). The unfertilized treatment (K1) had an intermediate ratio (2.41), not significantly different from K3 or K2 (Fig. 3).

Soil water availability also influenced the AGB: RB ratio. Seedlings at non-limiting water conditions (FC) had the highest values (2.88), while those under water restriction (FC40) had a lower ratio (1.90), indicating increased root allocation under drought conditions.

Potassium levels significantly affected leaf area (LA). The unfertilized treatment (K1) had a higher mean leaf area (602.53 cm^2) than the intermediate potassium level (K2: 410.54 cm^2). The highest level (K3: 523.46 cm^2) did not differ significantly from either K1 or K2 (Fig. 4).

Soil water availability also significantly affected leaf area. Seedlings at field capacity (FC) had a mean leaf area about four times greater (843 cm^2) than those under water restriction (FC40), which only reached 193.76 cm^2.

Significant differences in relative water content (RWC) and stomatal conductance (g_s) were found only between the two soil water content levels. Seedlings at field capacity (FC) showed higher RWC and stomatal conductance than those at 40% of field capacity (FC40 – Fig. 5).

These findings suggest that soil moisture availability strongly affected plant water status and gas exchange. In contrast, potassium levels did not significantly affect these physiological parameters in this study.

RWC was not significantly linked to any biomass components (Tab. 2). However, stomatal conductance (g_s) showed a strong positive linear relationship with several biomass variables and leaf area. This indicates a close link between gas exchange capacity and seedling growth performance.

Water restriction was the main factor driving the physiological responses of the plants, which strongly influenced their

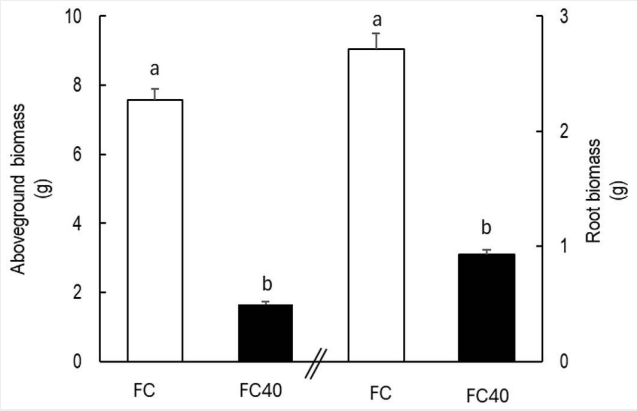


Fig. 2 - Aboveground biomass (g) and root biomass (g) at the end of the experiment in *E. grandis* seedlings under different soil water contents: FC (maintenance at field capacity) and FC40 (maintenance at 40% of field capacity). Different letters indicate significant differences among treatments after Fisher's LSD test ($p < 0.05$)

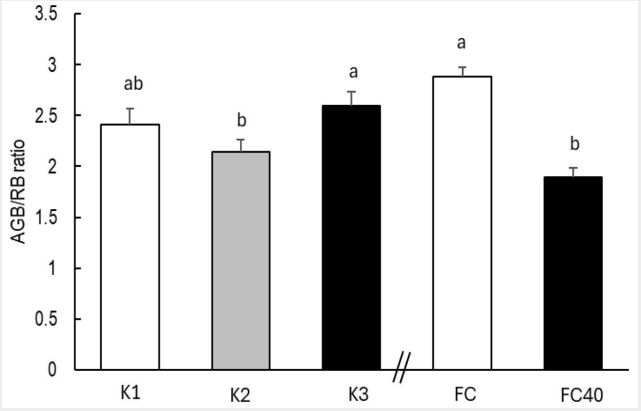


Fig. 3 - Aboveground biomass (AGB) to root biomass (RB) ratio in *E. grandis* seedlings under different levels of potassium fertilization: K1: 0 g K, K2: 0.5 g K, K3: 1 g K; and soil water content levels: FC: maintenance at field capacity; FC40: maintenance at 40% of field capacity. Different letters indicate significant differences among treatments after Fisher's LSD test ($p < 0.05$).

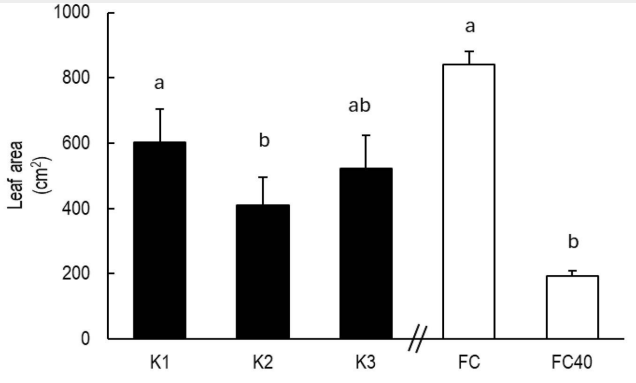


Fig. 4 - Average leaf area (cm²) of *E. grandis* seedlings under different levels of potassium fertilization: K1: 0 g K, K2: 0.5 g K, K3: 1 g K; and soil water content levels: FC: maintenance at field capacity; FC40: maintenance at 40% of field capacity. Different letters indicate significant differences for each treatment after Fisher's LSD test ($p < 0.05$).

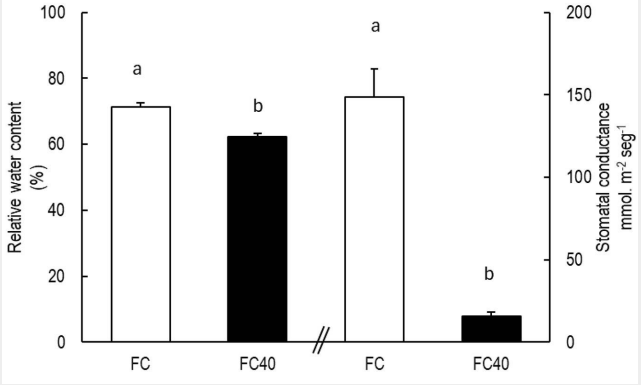


Fig. 5 - Relative water content and stomatal conductance in *E. grandis* seedlings, evaluated at 51 and 57 days after the start of the experiment, respectively, under field capacity maintenance (FC) and maintenance at 40% of field capacity (FC40). Different letters indicate significant differences for each treatment after Fisher's LSD test ($p < 0.05$).

Tab. 2 - Correlation matrix between different morphological and physiological variables. (Bram): branch biomass; (SB): stem biomass; (LB): leaf biomass; (RB): root biomass; (Btot): total biomass; (AGB): aboveground biomass; (LA): leaf area; (SLA): Specific leaf area; (LMA): leaf mass per area; (CRA): relative water content; (sc): stomatal conductance. (*): p<0.05; (**): p<0.01; (***): p<0.001.

-	Bram	SB	LB	RB	Btot	AGB	LA	SLA	LMA	CRA
SB	0.97**	1	-	-	-	-	-	-	-	-
LB	0.98**	0.98**	1	-	-	-	-	-	-	-
RB	0.95**	0.93**	0.95**	1	-	-	-	-	-	-
Btot	0.99**	0.98**	0.99**	0.98**	1	-	-	-	-	-
AGB	0.99**	0.99**	1**	0.95**	0.99**	1	-	-	-	-
LA	0.77**	0.83**	0.83**	0.71**	0.8**	0.82**	1	-	-	-
SLA	0.09	0.24	0.2	0.02	0.14	0.19	0.68**	1	-	-
LMA	-0.12	-0.25	-0.23	-0.09	-0.18	-0.22	-0.58*	-0.85**	1	-
CRA	0.24	0.26	0.32	0.27	0.29	0.3	0.2	0.1	-0.27	1
sc	0.87**	0.83**	0.82**	0.74**	0.81**	0.83**	0.82**	0.29	-0.27	0.02

Discussion

Growing *Eucalyptus* in sandy soils needs careful planning. These soils retain little water and have few nutrients, especially in the months after planting. As expected, the sandy substrate showed low organic matter and nutrient levels. This directly reduced water-holding ability. Under these conditions, seedlings rely heavily on available soil moisture for early growth.

Plants use many adaptive strategies to handle water stress. These responses can differ by species and environment. In this study, restricting water caused clear changes in *Eucalyptus grandis* seedlings. We observed growth inhibition, smaller leaf areas, and stomatal closure. These adaptations help reduce thermal stress and limit water loss through transpiration. Our results align with previous studies that report similar responses under drought (Ahrens et al. 2020, Mateus et al. 2021).

morphological development. As shown in Fig. 6, reductions in leaf area and leaf biomass under water-restricted conditions occurred proportionally, leading to a linear decrease in stomatal conductance. Correlation analyses of the evaluated parameters further supported this pattern (Tab. 2).

Strong positive linear relationships were found between leaf, branch, stem, and root biomass and total plant biomass (Tab. 2). Leaf biomass was also significantly associated with leaf area (Fig. 6). Root biomass showed a similar trend, but the correlation strength was lower than for aboveground biomass components (Tab. 2), indicating a weaker, though still positive, relationship.

Effect of potassium fertilization

Potassium (K) fertilization is common at the nursery stage or during transplanting. It aims to boost plant tolerance to water deficits (De Souza Mateus et al. 2022). However, in this study, K addition did not significantly affect height growth, root-collar diameter, or biomass in coarse-textured

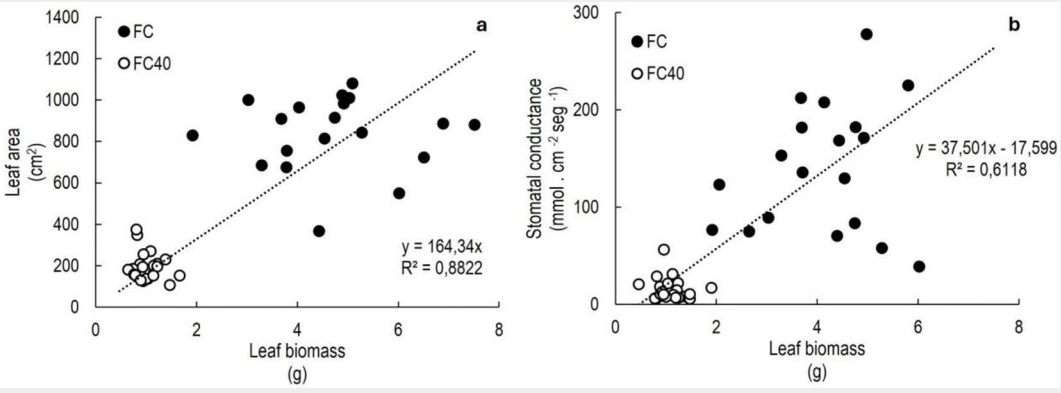


Fig. 6 - Relationship between leaf biomass and leaf area (a) and leaf biomass and stomatal conductance (b) in *E. grandis* seedlings, evaluated at 51 days after the start of the experiment, under field capacity maintenance (FC – filled symbols) and maintenance at 40% of field capacity (FC40 – open symbols).

soil (Tab. 1). In water-restricted treatments, low soil water availability was the main limit on biomass production (Tab. 1, Fig. 1). Similarly, Susiluoto & Berninger (2007) found no increase in root biomass under drought conditions.

Many studies reported that increased root biomass helps plants extract water from the soil when moisture is low (Graciano 2005, Li et al. 2024). However, Sá et al. (2014) found that fertilization did not promote growth in water-stressed seedlings. Also, Maseda & Fernández (2016) noted that root biomass in different *Eucalyptus* provenances decreased with increasing drought intensity. Graciano (2005) found that applying nitrogen (N) and phosphorus (P) to sandy soils resulted in limited nutrient uptake when water was scarce.

Plant nutrient uptake depends on plant demand, soil nutrient availability, and soil physicochemical properties (Holz et al. 2024). In sandy soils, nutrient availability can be low because hydraulic conductivity drops sharply when soil moisture is low. This limits nutrient movement to the roots (Wankmüller et al. 2024). Additionally, limited nitrogen and phosphorus can further reduce the effectiveness of potassium fertilization (Graciano 2005, Sá et al. 2014).

In contrast to our findings (Tab. 1), other studies reported positive effects of potassium fertilization under drought. Gazola et al. (2019) found that K fertilization partially reduced water-deficiency effects on *Eucalyptus grandis* seedlings. Teixeira et al. (2006, 2008) studied *E. grandis, E. urophylla, E. camaldulensis*, and a hybrid (*E. urophylla* × *E. grandis*). They found that adding potassium significantly boosted height growth and dry matter production across different K doses and soil water potentials.

Furthermore, Teixeira et al. (2008) noted that K application improved photosynthesis by reducing stomatal conductance, limiting water loss through transpiration. They found K-deficient plants had lower photosynthetic rates and higher stomatal conductance, reducing water-use efficiency. Santos et al. (2021) showed that responses to potassium fertilization varied by *Eucalyptus* genotype. Under severe drought, the *E. urophylla* × *E. camaldulensis* clone maintained biomass through K regulation, while the *E. urophylla* clone showed reduced K content and biomass.

Similarly, De Souza Mateus et al. (2022) found that potassium improved osmotic adjustment and drought avoidance in *Eucalyptus* seedlings experiencing short-term water stress. This improved leaf development, gas exchange, mesophyll conductance to CO_2, and water use efficiency. K-supplied plants acclimated better to osmotic stress than K-deficient plants, which had lower CO_2 assimilation and dry matter accumulation.

In this study, potassium dose affected both the AGB: RB ratio and leaf area, though no clear trend emerged (Tab. 1, Fig. 3). Graciano (2005) reported a reduction in the AGB: RB ratio in *E. grandis* grown in sandy soils with N and P under water stress. In contrast, Teixeira et al. (2008) observed leaf area increased with K doses up to 100 mg dm^{-3}, with increases over 200%.

Because *Eucalyptus* is the most widely planted tree genus and often grows in nutrient-poor, low-water soils, researchers have explored alternative nutritional strategies. Sodium (Na) can substitute for potassium in some physiological functions, affecting plant water relations. Recent studies (Mateus et al. 2021) showed that Na can partially replace K in *Eucalyptus* seedlings, with optimal rates increasing with species drought tolerance, including *Eucalyptus saligna, E. urophylla,* and *E. camaldulensis*.

Effect of soil water content

Eucalyptus productivity is very sensitive to water availability (Cavalli et al. 2022, Legoas et al. 2023). Drought conditions significantly reduce growth, biomass production, and harvestable yield. These declines mainly result from reduced transpiration, which limits carbon assimilation by reducing photosynthesis (Whitehead & Beadle 2004, Yang et al. 2023). Water stress also negatively affects dry matter production by restricting water flow through the plant-atmosphere continuum, which constrains photosynthesis (Whitehead & Beadle 2004).

In this study, reductions in height and root-collar diameter were noticeable by day 21 in water-stressed plants (FC40 – Fig. 1a, Fig. 1b). Limited soil water has been shown to reduce stem growth (Hussain et al. 2020, Ishimwe & Deligöz 2024). Drought reduced root-collar diameter and seedling height by over 20% in various tree species (Ravanbakhsh et al. 2023, Yang et al. 2023). This is linked to reduced cell expansion and limited cell division under water-deficit conditions (Yang et al. 2021). Hernández et al. (2024) found that water scarcity significantly affected height growth and leaf area in *Eucalyptus globulus* seedlings. However, our results differ from Maseda & Fernández (2016), who found no significant effect of irrigation on leaf area in *E. globulus* and *E. camaldulensis* seedlings in greenhouses.

Lozano et al. (2020) and Ishimwe & Deligöz (2024) showed that water stress mainly affects leaves and roots, the organs most involved in water uptake and loss. In coarse soils with low water retention, root growth is crucial for water acquisition. Under drought, plants can improve water absorption by changing root length, structure, and internal features (Hussain et al. 2020, Wu et al. 2022). In our study, plants under FC40 showed marked reductions in various traits, including root biomass (down 65.79%) and aboveground biomass (down 78.12%), compared to FC treatments, leading to a lower stem-to-root ratio in water-stressed plants (1.90) than in well-watered ones (2.88 – Tab. 1, Fig. 2, Fig. 3).

Da Silva et al. (2004) noted that potassium has limited effects on transpiration under severe water stress but is more influential under milder stress. This suggests that while potassium helps regulate stomatal behavior, seedlings' acclimation to water stress is critical for this mechanism to work in the field.

Consistent with earlier findings (Oliveira et al. 2022), seedlings in this study responded to water stress with changes in both their structure and processes. Total biomass (AGB + RB) under FC40 was about four times lower than under the FC treatment (Fig. 2). The drop was more significant in aboveground biomass (4.5-fold decrease) than in root biomass (2.9-fold decrease – Fig. 2). This aligns with Ishimwe & Deligöz (2024), who reported large decreases in both biomass types in *Laurus nobilis* under water restrictions in greenhouses.

Teixeira et al. (2006) studied seedlings of *E. grandis, E. urophylla, E. camaldulensis*, and a hybrid (*E. urophylla* × *E. grandis*) under various potassium doses and soil moisture levels. They found that while water stress did not significantly affect height growth, it clearly reduced aboveground dry matter for all species evaluated. Although the differences were not significant, plants under non-water-limited conditions had the highest stomatal conductance. As shown in Fig. 5, plants significantly reduced stomatal conductance under FC40, indicating that *E. grandis* controlled water loss by closing stomata during moderate water stress. Similar trends in *E. camaldulensis* (Ezzine et al. 2023) suggest that *Eucalyptus* improves water-use efficiency by regulating stomatal conductance and using water conservatively during drought (Yang et al. 2023).

Leaf biomass and leaf area are closely linked ($r = 0.83$, $p = 0.01$), reflecting canopy size and function. Leaf area in water-deficient plants was 4.6 times lower than in non-water-limited plants (FC – Fig. 6). Graciano (2005) and Oliveira et al. (2022) noted that reduced leaf area reduces transpiration. This helps the plant absorb water from low-moisture soils, maintaining the turgor needed for physiological activity (Lambers et al. 2008).

Binks et al. (2016) noted drought-related adaptive responses, such as changes in leaf thickness. Leaf mass is vital for plant growth and indicates ecological strategies (Westoby et al. 2002). In this study, leaf mass per area did not differ significantly between water regimes, with values of 6.39 ± 2.26 mg cm^{-2} for FC40 and 5.80 ± 2.49 mg cm^{-2} for FC (data not shown). However, a significant inverse relationship emerged between leaf mass and leaf area ($R = -0.58$; $p = 0.0496$ – Tab. 2), suggesting that as leaf area decreased under water stress, leaf mass per unit area increased.

Poorter et al. (2009) found that leaves under low water availability expand less, leading to smaller, denser cells and thicker

walls. This reduces the overall surface area for transpiration, lowering water demand during drought and acting as an effective acclimation strategy.

Plants also develop physiological defenses to protect their photosynthesis from drought damage (Sharma et al. 2020, Yang et al. 2020). Oliveira et al. 2022, Wu et al. 2022, and Ishimwe & Deligöz 2024 noted that under mild stress, plants can usually regulate stomatal opening and transpiration, adjust leaf water potential, and recover when water improves. Sometimes, photosynthesis may even rise temporarily. However, moderate to severe drought stress typically leads to notable drops in net photosynthetic rate, stomatal conductance, and transpiration.

Soil water content was crucial for stomatal regulation in this study, consistent with Hernández et al. (2024). Plants under the FC40 treatment showed a significant drop in both relative water content (RWC) and stomatal conductance (g_s) by the end of the experiment (Fig. 5, Tab. 1). These results indicate that *Eucalyptus grandis* responded to moderate water stress by closing stomata, thus reducing transpiration. However, this did not prevent a decline in leaf RWC (from 71.2% to 62%), though it was sufficient to avoid plant mortality.

As noted by Hernández et al. 2024, seedlings in low soil water showed a marked decrease in stomatal conductance. Their reported values were lower than those in this study, likely due to more severe water restrictions. These findings highlight the sensitivity of stomatal regulation in *Eucalyptus* species and emphasize the importance of soil water availability as a key factor in physiological performance during early seedling development.

Conclusion

Under sandy soil conditions with low natural fertility, potassium fertilization did not help *Eucalyptus grandis* seedlings under water stress. Similarly, adding nitrogen and phosphorus with increasing potassium levels did not improve growth or physiological performance in seedlings with enough water.

In contrast, soil water availability was the main factor for seedling growth. Reducing water to 40% of field capacity significantly decreased height, root-collar diameter, total biomass, and leaf area. It also reduced key physiological metrics such as stomatal conductance and relative water content but did not cause seedling deaths.

These findings emphasize the need for adequate soil moisture during early *Eucalyptus grandis* growth, especially in coarse-textured soils. Water availability is more crucial than potassium fertilization for early seedling performance in these conditions.

List of abbreviations

AGB (aboveground biomass); CE (stomatal conductance); CRA (relative water content); EEA (agricultural experimental station); FC (field capacity); FC40 (maintenance at 40% of field capacity); INTA (National Institute of Agricultural Technology); K (potassium); LA (leaf area); N (nitrogen); P (phosphorus); RB (root biomass).

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Authors' contributions:

LAU: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. PLF: Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Writing - original draft, Writing - review & editing. AML: Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Writing - original draft, Writing - review & editing.

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References

Ahrens CW, Andrew ME, Mazanec RA, Ruthrof KX, Challis A, Hardy G, Byrne M, Tissue D, Rymer P (2020). Plant functional traits differ in adaptability and are predicted to be differentially affected by climate change. Ecology and Evolution 10: 232-248. - doi: 10.1002/ece3.5890

Bacha AL, Santos RTDS, Braga AF, Rodrigues J, Carrega WC, Bergua EC, Grey TL, Da Costa PL (2024). *Eucalyptus urograndis* physiological and hormonal changes under drought conditions in response to trinexapac-ethyl. Environmental and Experimental Botany 219: 105628. - doi: 10.1016/j.envexpbot.2023.105628

Battie-Laclau P, Laclau JP, Domec JC, Christina M, Bouillet JP, De Cassia Piccolo M, De Moraes Gonçalves JL, Moreira RM, Krusche AV, Bouvet JM, Nouvellon Y (2014). Effects of potassium and sodium supply on drought-adaptive mechanisms in Eucalyptus grandis plantations. New Phytologist 203: 401-413. - doi: 10.1111/nph.12810

Binks O, Meir P, Rowland L, Costa ACL, Vasconcelos SS, Oliveira A, Ferreira L, Mencuccini M (2016). Plasticity in leaf-Â level water relations of tropical rainforest trees in response to experimental drought. New Phytologist 211 (2): 477-488. - doi: 10.1111/nph.13927

Bouyoucos GJ (1936). Directions for making mechanical analysis of soils by the hydrometer method. Soil Science 4: 225-230. - doi: 10.1097/00010694-193609000-00007

Caldato SL, Schumacher MV (2013). O uso de água pelas plantações florestais - Uma revisão [Water use by forest plantations - A review]. Ciência Florestal 23 (3): 507-516. [in Portuguese] - doi: 10.5902/1980509810562

Cavalli JP, Frank De Araújo E, Reichert JM (2022). *Eucalyptus* growth responses to soil water storage capacity in Arenosols and Acrisols soils: wood and biomass stock modelling. Sustainability 14(19): 12215. - doi: 10.3390/su141912215

Chemlali I, Yahia K, Belaïd S, Rabeh S, Romdhane C, Messoudi N, Saadaqui E (2022). Anatomical, physiological, biochemical and molecular responses of *Eucalyptus* spp. under water deficit conditions and characteristics of Tunisian arid species: an overview. Notulae Scientia Biologicae 14 (3): 11218. - doi: 10.55779/nsb14311218

Dale A, Frank S (2022). Water availability determines tree growth and physiological response to biotic and abiotic stress in a temperate North American urban forest. Forests 13 (7): 1012. - doi: 10.3390/f13071012

Da Silva MR, Klar AE, Passos JR (2004). Efeitos do manejo hídrico e da aplicação de potássio nas características morfofisiológicas de mudas de *Eucalyptus grandis* (Hill ex. Maiden) [Effects of water management and potassium application on the morphophysiological characteristics of *Eucalyptus grandis* (Hill ex. Maiden) seedlings]. Irriga, Botucatu 9 (1): 31-40. - doi: 10.15809/irriga.2004v9n1p31-40

Delbaz R, Ebrahimian H, Abbasi F, Ghameshlou AN, Liaghat A, Ranazadeh D (2023). A global meta-analysis on surface and drip fertigation for annual crops under different fertilization levels, Agricultural Water Management 289: 108504. - doi: 10.1016/j.agwat.2023.108504

De Souza Mateus N, Oliveira Ferreira EV, Florentino AL, Vicente Ferraz A, Domec JC, Jordan-Meille L, Bendassolli JA, Moraes Gonçalves JL, Lavres J (2022). Potassium supply modulates *Eucalyptus* leaf water-status under PEG-induced osmotic stress: integrating leaf gas exchange, carbon and nitrogen isotopic composition and plant growth. Tree Physiology 42 (1): 59-70. - doi: 10.1093/treephys/tpab095

Di Rienzo JA, Casanoves F, Balzarini MG, González L, Tablada M, Robledo CW (2020). InfoStat: Software Estadístico, versión 2020. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba. [online] URL: http://www.infostat.com.ar

DNDFI (2025). Tablero de Plantaciones Forestales [Forest Plantation Board]. Website. [in Spanish] [online] URL: http://www.magyp.gob.ar/sitio/areas/desarrollo-foresto-industrial/inventarios/tablero.php

Ezzine H, Metougui ML, Boukcim H, Abbas Y (2023). Physiological responses of three field-grown species (*Ceratonia siliqua, Eucalyptus camaldulensis*, and *Moringa oleifera*) to water deficits in a Mediterranean semi-arid climate. Scientific Reports 13: 4536. - doi: 10.1038/s41598-023-31664-y

Fernandez ME, Gatica G, Bulfe N, Phar N, Gyenge J (2023). Mortalidad de pinos en Corrientes: cuando los pronósticos del cambio climático dejan de serlo y se convierten en actualidad [Pine tree mortality in Corrientes: when climate change predictions cease to be predictions and become reality]. In "Ciencia y Tecnología Forestal en Argentina" (Lupi AM, Area C; Escobar P eds). Ciudad Autónoma de Buenos Aires, Consejo Nacional Investigaciones Científicas Técnicas, Buenos Aires, Argentina, pp. 235-241 [in Spanish] [online] URL: http://hdl.handle.net/20.500.12123/17760

Gazola RN, Buzetti S, Teixeira Filho MCM, Gazola

RPD, Celestrino TS, Silva AC, Silva PHM (2019). Potassium fertilization of *Eucalyptus* in an Entisol in low-elevation Cerrado. Revista Brasileira de Ciência do Solo 43: 20180085. - doi: 10.1590/18069657rbcs20180085
Graciano C (2005). Productividad temprana de *Eucalyptus* con diferentes disponibilidades de nutrientes y agua [Early productivity of *Eucalyptus* under different nutrient and water availability]. PhD thesis, Universidad Nacional de la Plata, BA, Argentina, pp. 165. [in Spanish] - doi: 10.35537/10915/4474
Hernández M, Mutke S, Montes F, Pita P (2024). Early testing for improving growth under water shortage in *Eucalyptus globulus* Labill. Forest Systems 33 (1): e01. - doi: 10.5424/fs/2024331-20868
Holz M, Zarebanadkouki M, Benard P, Hoffmann M, Dubbert M (2024). Root and rhizosphere traits for enhanced water and nutrients uptake efficiency in dynamic environments. Frontiers in Plant Science 15: 1383373. - doi: 10.3389/fpls.2024.1383373
Hussain MS, Nawaz MF, Tanvir MA, Hira N (2020). Comparative effect of drought on *Eucalyptus camaldulensis* and *Tamarix aphylla* at early stages of growth. Sarhad Journal of Agriculture 36 (2): 559-566. - doi: 10.17582/journal.sja/2020/36.2.559.566
Ishimwe C, Deligöz A (2024). Drought effects on growth, biochemical changes and leaf gas exchange in laurel (*Laurus nobilis* L.). Central European Forestry Journal 70: 122-132. - doi: 10.2478/forj-2024-0004
Lambers H, Chapin IIIFS, Pons TL (2008). Photosynthesis, respiration and long-distance transport. In: "Plant Physiological Ecology (2nd edn)". Springer Science and Business Media, New York, USA, pp. 11-99. - doi: 10.1007/978-0-387-78341-3_2
Legoas C, Tomazello-Filho M, Laclau JP, Chaix G (2023). Potassium fertilization enhances xylem plasticity and growth recovery of *Eucalyptus grandis* trees in response to drastic changes in water availability. Forest Ecology and Management 528: 120656. - doi: 10.1016/j.foreco.2022.120656
Li B, Wang X, Li Z (2024). Plants extend roots deeper rather than increase root biomass triggered by critical age and soil water depletion. Science of the Total Environment 914: 169689. - doi: 10.1016/j.scitotenv.2023.169689
Liu H, Ye Q, Gleason SM, He P, Yin D (2021). Weak tradeoff between xylem hydraulic efficiency and safety: climatic seasonality matters. New Phytologist Foundation 229: 1440-1452. - doi: 10.1111/nph.16940
Lozano YM, Aguilar-Trigueros CA, Flaig IC, Rillig MC (2020). Root trait responses to drought are more heterogeneous than leaf trait responses. Functional Ecology 34: 2224-2235. - doi: 10.1111/1365-2435.13656
Luna-Flores W, Estrada-Medina H, Morales-Maldonado E, Alvarez-Rivera O (2015). Plant stress by water deficit: a review. Chilean Journal of Agricultural Animal Sciences 31: 61-69. [ISSN 0719-3890]
Maseda P, Fernández R (2016). Growth potential limits drought morphological plasticity in seedlings from six *Eucalyptus* provenances. Tree Physiology 36: 243-251. - doi: 10.1093/treephys/tpv137
Mateus N, Florentino A, Santos E, Ferraz A, Goncalves J, Lavres J (2021). Partial substitution of K by Na alleviates drought stress and increases water use efficiency in *Eucalyptus* species seedlings. Frontiers in Plant Science 12: 632342. - doi: 10.3389/fpls.2021.632342
Oliveira TWG, Rubilar R, Sanquetta CR, Dalla Corte AP, Bozo D, Medina A, Mardones O, Emhart V, Quiroga JJ, Valenzuela H (2022). Changes in leaf physiology for *Eucalyptus* genotypes with increase in integral water stress and its consequence for initial growth in central Chile. Scientia Forestalis 50: e3802. - doi: 10.18671/scifor.v50.09
Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytologist 182 (3): 565-588. - doi: 10.1111/j.1469-8137.2009.02830.x
Ravanbakhsh M, Babakhani B, Ghasemnezhad M, Serpooshan F, Bigloui MH (2023). *Acer velutinum* Bioss. (velvet maple) seedlings are more tolerant to water deficit than *Alnus subcordata* C. A. Mey. (Caucasian alder) seedlings. Acta Botanica Croatica 82: 60-70. - doi: 10.37427/botcro-2022-029
Reichert JM, Prevedello J, Gubiani PI, Vogelmann ES, Reinert DJ, Consensa COB, Soares JC, Srinivasan R (2021). *Eucalyptus* tree stockings affect on water balance and use efficiency in subtropical sandy soil. Forest Ecology and Management 497: 119473. - doi: 10.1016/j.foreco.2021.119473
Santos EF, Mateus NS, Rosário MO, Garcez TB, Mazzafera P, Lavres J (2021). Enhancing potassium content in leaves and stems improves drought tolerance of *Eucalyptus* clones. Physiologia Plantarum 172 (2): 552-563. - doi: 10.1111/ppl.13228
Sá AF, Valeri SV, Cruz MC, Barbosa JC, Rezende GM, Teixeira MP (2014). Effects of potassium application and soil moisture on the growth of *Corymbia citriodora* plants. Cerne 20 (4): 645-651. - doi: 10.1590/01047760201420041422
Sharma A, Kumar V, Shahzad B, Ramakrishnan M, Sidhu G, Bali A, Handa N, Kapoor D, Yadav P, Khanna K (2020). Photosynthetic response of plants under different abiotic stresses: a review. Journal of Plant Growth Regulation 39: 509-531. - doi: 10.1007/s00344-019-10018-x
Susiluoto S, Berninger F (2007). Interacciones entre las respuestas morfológicas y fisiológicas a la sequía en *Eucalyptus microtheca* [Interactions between morphological and physiological responses to drought in *Eucalyptus microtheca*]. Silva Fennica 41 (2): 221-233. [in Spanish] - doi: 10.14214/sf.292
Tasi H (2009). Aplicación de las cartas de suelos de Entre Ríos, Argentina para evaluar índices de productividad específicos para los principales cultivos agrícolas [Application of soil maps of Entre Ríos, Argentina to evaluate specific productivity indices for the main agricultural crops]. PhD thesis. Instituto Universitario de Xeoloxía, Universidade da Coruña, España, pp. 622. [in Spanish] [online] URL: http://hdl.handle.net/2183/5679
Teixeira PC, Gonçalves JL, Arthur Junior JC (2006). Crescimento e partição da matéria seca de mudas de eucalipto em função da adubação potássica e da água do solo [Growth and dry matter partitioning of *Eucalyptus* seedlings as a function of potassium fertilization and soil water]. Ceres, Viçosa 53 (510): 672-676. [in Portuguese]
Teixeira PC, Gonçalves JL, Arthur Junior JC, Dezordi C (2008). *Eucalyptus* sp. seedling response to potassium fertilization and soil water. Ciência Florestal, Santa Maria 18 (1): 47. - doi: 10.5902/19805098510
Walkley A, Black IA (1934). An examination of the method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science 37: 29-38. - doi: 10.1097/00010694-193401000-00003
Wankmüller FJP, Delval L, Lehmann P, Baur MJ, Cecere A, Wolf S, Or D, Javaux M, Carminati A (2024). Global influence of soil texture on ecosystem water limitation. Nature 635: 631-638. - doi: 10.1038/s41586-024-08089-2
Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ (2002). Plant ecological strategies: some leading dimensions of variation between species. Annual Review of Ecology and Systematics 33: 125-159. - doi: 10.1146/annurev.ecolsys.33.010802.150452
Whitehead D, Beadle CL (2004). Physiological regulation of productivity and water use in Eucalyptus: a review. Forest Ecology and Management 193 (1-2): 113-140. - doi: 10.1016/j.foreco.2004.01.026
Wu J, Wang J, Hui W, Zhao F, Wang P, Su C, Gong W (2022). Physiology of plant responses to water stress and related genes: a review. Forests 13 (2): 324. - doi: 10.3390/f13020324
Yang L, Kong J, Gao Y, Chen Z, Lin Y, Zeng S, Su Y, Li J, He Q, Qiu Q (2023). A simulated drier climate reduces growth and alters functional traits of Eucalyptus trees: a three-year experiment in South China. Forest Ecology and Management (1): 121435. - doi: 10.1016/j.foreco.2023.121435
Yang X, Lu M, Wang Y, Wang Y, Liu Z, Chen S (2021). Response mechanism of plants to drought stress. Horticulturae 7(3): 50. - doi: 10.3390/horticulturae7030050
Yang X, Li Y, Chen H, Huang J, Zhang Y, Qi M, Liu Y, Li T (2020). Photosynthetic response mechanism of soil salinity-induced cross-tolerance to subsequent drought stress in tomato plants. Plants 9: 363. - doi: 10.3390/plants9030363
Zhang B, Wang S, Slater L (2024). Anthropogenic climate change doubled the frequency of compound drought and heatwaves in low-income regions. Communications Earth and Environment 5: 715. - doi: 10.1038/s43247-024-01894-7