

ORIGINAL PAPER

Elevated CO₂ concentration alleviates the negative effect of vapour pressure deficit and soil drought on juvenile poplar growth

Matěj Orság^{1,2*}, Gonzalo Berhongaray³, Milan Fischer^{1,2}, Karel Klem^{1,2},
Reinhart Ceulemans⁴, John S. King⁵, Marcela Hlaváčová^{1,2}, Miroslav Trnka^{1,2}

¹Global Change Research Institute CAS, Bělidla 986/4a, CZ-60300, Brno, Czech Republic

²Mendel University in Brno, Faculty of AgriSciences, Department of Agrosystems and Bioclimatology, Zemědělská 1, CZ-61300, Brno, Czech Republic

³Universidad Nacional del Litoral, Facultad de Ciencias Agrarias, ICI Agro Litoral (UNL-CONICET), P. Kreder 2805 – Esperanza, AR-3080, Argentina

⁴University of Antwerp, Department of Biology, Centre of Excellence on Plants and Ecosystems (PLECO), Universiteitsplein 1, B-2610 Wilrijk, Belgium

⁵North Carolina State University, Department of Forestry and Environmental Resources, 2820 Faucette Drive, Campus Box 8001, Raleigh, NC-27695, USA

Abstract

The growth performance of short-rotation woody coppice (SRWC) is strongly influenced by successful establishment in the initial months after planting. Future climates, expected to be warmer due to elevated atmospheric CO₂ (eCO₂), may bring about more frequent soil droughts alongside increased vapour pressure deficit (eVPD). Hence, this growth chamber experiment aimed to explore the interactive effects of eVPD, eCO₂, and soil drought on growth and physiology traits of juvenile hybrid poplars under warmer climates. Our findings with juvenile hybrid poplar J-105 revealed that eVPD resulted in reductions in leaf area (–21%), root (–20%) and stem biomass (–9%), as well as in net assimilation (–15%), stomatal conductance (–26%), and transpiration (–13%). However, these decreases were relatively minor compared to the compensating effect of eCO₂, which generally exerted a stronger influence than eVPD. While soil drought emerged as the primary growth-limiting factor in our study, elevated VPD is not expected to pose a significant additional threat to central European SRWC plantations of juvenile hybrid poplars under future conditions of ongoing climate change.

Key words: growth chamber experiment; vapour pressure deficit; stomatal control; isohydric; short rotation wood coppice

Editor: Zuzana Sitková

1. Introduction

Short rotation woody coppice (SRWC) plantations are managed ecosystems grown for the fast production of woody biomass. The establishment phase and initial growth performance of new SRWC plantations largely impact the system's overall success, and careful planning, location and prevailing climate conditions remain paramount to maximising SRWC yields. Studies have shown that commercial poplar clones exhibit a high survival rate of dormant hardwood cuttings during the establishment year (Walle et al. 2007; Al Afas et al. 2008; Trnka et al. 2008; Verlinden et al. 2015; Fischer et al.

2017) and considerable resilience to drought once the root system is established (Orság et al. 2018). However, rooting cuttings are especially vulnerable to drought stress in the initial weeks to months following planting. Previous studies have shown that soil drought is a major growth-restricting factor (Marron et al. 2003; Larchevêque et al. 2011). Even though poplar species are among the most susceptible woody plants to drought (Fichot et al. 2015), significant genotypic variability has been recorded in water deficit tolerance and patterns of response to water deficit (Monclus et al. 2006; Navarro et al. 2018). The ongoing climate change is characterised by increasing air temperatures, leading to a rise of atmos-

*Corresponding author. Matěj Orság, e-mail: orsag.m@czechglobe.cz

© 2024 Authors. This is an open access article under the CC BY 4.0 license.

pheric evaporative demand, i.e. elevated vapour pressure deficit (eVPD), through an exponential increase of saturated water vapour pressure at a rate of approximately 7%/°C according to the Clausius-Clapeyron relation (Yuan et al. 2019). The above-mentioned factors represent likely environmental conditions in the future, and the knowledge of their effects on the performance of the SRWC poplar species may be beneficial for optimising the bioenergy sector in the era of climate change.

While the role of soil water availability and eCO₂ on poplar species have been investigated extensively (Gielen & Ceulemans 2001; Taylor et al. 2001; Broeckx et al. 2013; Zenone et al. 2015; Fischer et al. 2018; Orság et al. 2018), the role of eVPD has received less attention (Kim et al. 2008; Schmidt-Walter et al. 2014; Zenone et al. 2015; Novick et al. 2016), and its full implications have not been thoroughly explored (Oren et al. 1999), and thus forming a primary focus and hypothesis of this study. Elevated VPD leads to partial stomatal closure (Oren et al. 1999), which subsequently restricts CO₂ assimilation and ecosystem productivity, similar to drought (Migliavacca et al. 2009; Novick et al. 2016; Sulman et al. 2016; Yuan et al. 2019; Lansu et al. 2020). Some studies (Novick et al. 2016; Yuan et al. 2019) indicate that progressively increasing VPD could significantly reduce the gross primary productivity of forest ecosystems, even in non-drought years (Sulman et al. 2016). However, stomatal conductance (gs) response to eVPD varies widely among species (Oren et al. 1999; Grossiord et al. 2020). Even within different varieties of poplars, owing to their broad genome, a wide range of stomatal strategies can be observed, spanning from isohydric to anisohydric (Attia et al. 2015). Isohydric species may become more susceptible to carbon starvation and growth depression under eVPD due to stomatal closure, even under conditions with ample soil moisture (Attia et al. 2015; Navarro et al. 2018). The specific impact of eVPD on poplar species grown in SRWC is also highly relevant in the context of the establishment success, as this phase and attained survival rate largely determine further productivity (Trnka et al. 2008; Fischer et al. 2017).

This study aimed to elucidate the specific effects of eVPD on the growth and physiology of a juvenile hybrid poplar J-105, a widely distributed species in central Europe while considering other interacting environmental factors. Given previous findings indicating that the clone J-105 employed in this experiment adopts an isohydric hydraulic strategy (Schmidt-Walter et al. 2014), we hypothesised that eVPD could significantly reduce physiological processes, leading to noticeable decreases in growth traits, even under conditions of sufficient soil water availability. Additionally, we anticipated that the limitations imposed by eVPD would be comparable in magnitude to those of soil drought and would not be entirely offset by the presence of eCO₂, potentially resulting in increased water use efficiency.

2. Material and methods

2.1. Plant material

Hybrid poplar J-105, also known as Max-5, is a genotype derived from the cross between *P. nigra* L. × *P. maximo-wiczii* Henry, widely cultivated across central Europe for bioenergy purposes (Trnka et al. 2008; Schmidt-Walter et al. 2014). Unrooted hardwood cuttings for this experiment were obtained from an operational SRWC plantation in Domaníněk in the Bohemian Moravian Highlands (the Czech Republic; 49.52°N, 16.24°E). On day of year (DOY) 126 of 2014, 140 most uniform hardwood cuttings measuring 20 cm in length were planted outdoors into 12 L plastic pots with drainage holes in the bottom. The pots were filled with a local luvis Cambisol arable topsoil, passed through a sieve with 1 cm openings for homogenisation. To maintain natural soil temperature and minimise desiccation risk, the pots were positioned in a shallow ditch, aligning the soil level with the surrounding terrain. Throughout the experiment, no fertilisers or pesticides were applied, and aside of natural rainfall, manual watering of 2 L per pot was administered once a week during extended periods without rainfall to prevent soil drought stress. Green shoots emerged from the cuttings within two weeks, with the mean shoot height reaching 21 cm on DOY 169 and 47 cm on DOY 196.

2.2. Experimental design

On Day of Year (DOY) 202, 48 of the most uniform potted poplars were selected and transported from the experimental field site in Domaníněk to four walk-in growth chambers (type FS 2700, PSI Drásov, Czech Republic) located at the Global Change Research Institute CAS (Brno, the Czech Republic). Each growth chamber could accommodate 12 pots holding 12 L of soil. The microclimate conditions within each growth chamber were fully controlled, manipulating three environmental variables: relative atmospheric humidity (to achieve desired levels of low VPD), CO₂ concentration, and watering (refer to Table 1 and Fig. A3 and Fig. A4 in Attachment for details). The daily air temperature profile was consistent across all four chambers, peaking at 32 °C and reaching a minimum of 15 °C during the night, mimicking a hot summer day representative of the top first percentile of the hottest summer days observed between 1961 and 1990 at the Domaníněk site. Although initially planned to include the effect of moderate and high air temperatures as additional factors, only four chambers were available to experiment.

Consequently, it was decided to apply elevated temperature in all chambers and other interacting factors. The elevated VPD (eVPD) treatment was implemented in two of the four chambers by adjusting the daily air temperature to reach 32 °C, resulting in a midday VPD peak-

ing at approximately 3 kPa. The remaining two chambers were subjected to the low VPD treatment, achieved by increasing relative air humidity while maintaining the same daily air temperature profile as the chambers with the eVPD treatment. Therefore, the daily VPD in the low VPD treatment never exceeded 1 kPa. For diurnal profiles of air temperature and VPD (refer to Fig. A3 in the Attachment). The arrangement of CO₂ treatments mirrored that of the VPD treatments, with two chambers containing ambient atmospheric CO₂ levels of 400 ppm and the other two chambers receiving the elevated CO₂ treatment at a concentration of 700 ppm, projected for the end of the 21st century (Meehl et al. 2007). Watering was administered according to the mean July precipitation total at the Domanínek site (67 mm per month, based on the 1961–1990 long-term average), amounting to 1.2 L per pot, manually applied once a week. The watering treatment was designed such that half of the pots within each chamber (i.e. six pots) received the full watering dose as mentioned above (100%), while the remaining six pots received a reduced dose of 0.5 L per pot each week (40%) to mimic severe drought conditions.

Light-emitting diodes served as the radiation source for the red (R; maximum emission: 640 nm), blue (B; maximum emission: 450 nm), and far-red (FR; maximum emission: 740 nm) regions, and fluorescent lamps (LT 30W T8/010UV, Narva, DE) emitted UV-A radiation (maximum emission: 370 nm), maintaining a ratio of R : G : B (1 : 0.7 : 1). The UV-A was capped at 1.3 W m⁻² (30% intensity), and the FR light intensity was adjusted to 30% to maintain a ratio of R : FR = 2. Each day began at 4:30 h with photosynthetically active radiation gradually increasing from zero to 1,200 µmol m⁻² s⁻¹ (at 8:00 h), reaching a maximum of 1,500 µmol m⁻² s⁻¹ (from 11:30 h–15:00 h), and then decreasing until sunset at 20:00 h (see Fig. A3B in Attachment). The experiment was randomised every week, i.e. pots from each growth chamber were moved to a different chamber to avoid any chamber artefact, and the environmental settings were changed accordingly.

Table 1. List of treatments and their corresponding environmental conditions: The 100% watering treatment signifies a manual irrigation dose equivalent to the mean July precipitation equivalent (based on the 1961–1990 long-term average). The CO₂ treatment comprised two levels of CO₂ concentration: ambient (400 ppm) and elevated (eCO₂) (700 ppm). The VPD treatment included low VPD and high VPD (eVPD) levels (1 kPa versus 3 kPa), representing the maximum daily VPD attained.

Watering (%)	CO ₂ (ppm)	VPD (kPa)	n
40	400	1	6
		3	6
	700	1	6
		3	6
100	400	1	6
		3	6
	700	1	6
		3	6

2.3. Physiological and biometric variables

For leaf-level gas exchange, three LI-6400 Portable Photosynthesis Systems (LICOR, Lincoln, NE, US) were utilised simultaneously to assess net assimilation (A_{net}), stomatal conductance (g_s) and transpiration. Gas exchange measurements were conducted weekly between 11:30 h and 15:00 h, under PAR intensity of 1,200 µmol m⁻² s⁻¹. Seven gas exchange campaigns were carried out between DOY 210 and 245. Due to space limitations within the chambers, pots were randomised and removed before measurements were taken in the aisle between chambers at room temperature. Measurements were performed on randomly selected healthy and fully developed leaves from the upper 1/3 part of the stem. The WUE_i was calculated as the ratio of A_{net} to g_s , while transpiration as the product of g_s and VPD. It is important to note that VPD used in this calculation was from the cuvette of the gas analyser, not the actual VPD from the growth chamber, which might introduce bias into the calculation of transpiration. The experiment concluded on DOY 245 with the harvest of all plants. Stems and coarse roots were separated from parent cuttings and dried at 70 °C until a constant dry weight was achieved. Leaf area per plant was determined by photographing leaves from each plant with a digital camera, and total leaf area (cm²) was calculated using ImageJ software (Abràmoff et al. 2004).

2.4. Statistical analysis

Statistical analyses were conducted using the R statistical software, version 4.2.2 (R Core Team 2022). Data were subjected to a multi-factorial analysis of variance (ANOVA) employing a linear model (Cleveland et al. 1992). The pot was considered the primary experimental unit, with trees in individual pots treated as independent entities due to adequate spacing, absence of shading, good ventilation, and homogeneous soil conditions. Additionally, our limited chamber availability influenced this decision. The analysis did not include the initial mass of hardwood cuttings as a covariate; instead, it was considered a random factor. Response variables encompassed stem and root biomass, leaf area, A_{net} , g_s , transpiration and WUE_i. An autocorrelation function structure was incorporated into the linear model to account for the dependence of consecutive measurements repeated throughout the season. Fisher's least significant difference post-hoc test was subsequently applied to identify statistically significant differences between treatments. Assumptions regarding the distribution of residuals from the linear models were evaluated through visual inspection of quantile-quantile probability plots and analysis of skewness and kurtosis. Since normal distribution of residuals was not consistently observed, original values were transformed using the Box-Cox procedure (Vena-

bles et al. 2002). Statistical significance was considered at $p \leq 0.05$. Furthermore, single effects of environmental factors were assessed through sensitivity analysis, where all environmental variables were maintained at their low levels (i.e. watering at 40%, CO_2 at 400 ppm, and VPD at 1 kPa). In contrast, one factor was incrementally increased at a time.

3. Results

In most cases, individual environmental treatments substantially impacted response variables; however, the biometric and physiological response variables differed in response to their interactions – i.e. $\text{VPD} \times \text{CO}_2 \times \text{Watering}$ (Table 2). For example, interactions of $\text{VPD} \times \text{CO}_2 \times \text{Watering}$ influenced root biomass and leaf area. Still, they had no significant effect on stem biomass or physiological such as net assimilation (A_{net}) and stomatal conductance (g_s), transpiration, and intrinsic water use efficiency (WUE_i). Conversely, the interaction of $\text{CO}_2 \times \text{Watering}$ affected all physiological response variables, while stem biomass and leaf area were unaffected, with leaf area showing only marginal significance ($p = 0.057$). Only WUE_i was affected by the interaction of $\text{VPD} \times \text{Watering}$ ($p = 0.008$), with no significant impact observed on other response variables. Lastly, the interaction of $\text{VPD} \times \text{CO}_2$ did not influence any biometric or physiological response variable.

The individual effects of the environmental treatments on plant growth were more pronounced in the roots and leaf area than in stem biomass (Table 3). Root biomass and leaf area were significantly affected by all treatments – VPD, CO_2 , and watering ($p < 0.001$). Stem biomass was significantly influenced by the individual effects of CO_2 and watering ($p < 0.001$), with the negative effect of eVPD being marginally involved ($p = 0.065$). Regarding the relative magnitude of individual environmental effects, reduced watering had the most significant impact, leading to 39% reduction in stem biomass, a 55% reduction in root biomass, and 40% reduction in leaf area (Fig. A1 in Attachment). The effect of CO_2 increased stem and root biomass by 24 and 47%, respectively, and leaf area by 14%. Conversely, the effect of eVPD was negative, resulting in a 9% reduction in stem biomass, a 20% reduction in root biomass, and a 21% reduction in leaf area. Although the experimental treatments induced changes in roots and stem biomass, the effects were less evident in the root-shoot ratio, which ranged between 1.10 and 2.02, with an average of 1.29 in the 40% water treatment and 1.78 in the 100% water treatment.

The effects of the individual environmental treatments on A_{net} , g_s , WUE_i and transpiration (Table 4) are generally aligned with the treatment effects observed for biometric response variables (Tables 2 and 3), albeit with larger relative effects (Fig. A2 in Attachment). On aver-

age, the most substantial relative effect on physiological variables stemmed from watering, with stomatal conductance (g_s) experiencing the greatest impact, reduced by 84% under 40% watering compared to 100% watering. Transpiration was similarly affected, with an 81% reduction, followed by A_{net} , with a 76% reduction under the 40% watering treatment relative to 100% watering. Throughout the experiment, A_{net} ranged between 1.02–14.32 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with an average increase of 148% due to elevated eCO_2 , while eVPD led to a 15% reduction of A_{net} . Stomatal conductance ranged between 0.01–0.135 $\text{mol m}^{-2} \text{s}^{-1}$ across treatments, experiencing a 26% reduction under the eVPD treatment, whereas the eCO_2 treatment increased g_s by 27%. The A_{net} exhibited a strong positive correlation with both stem and root biomass (0.96 vs 0.93; $p < 0.001$), while transpiration and g_s showed weaker correlations with plant biomass (0.74; $p = 0.017$). WUE_i did not correlate with any of the biometric traits.

Individual effects of environmental treatments were also illustrated through sensitivity analysis (Fig. 1) when all environmental variables were maintained at their low levels (e.g., watering at 40%, CO_2 at 400 ppm). At the same time, one factor was increased (e.g., VPD at 3 kPa). Additionally, the relative effects of eVPD on individual biometric and physiological response variables were analysed (Fig. 2). In most cases, eVPD led to reductions in all variables.

4. Discussion

Our finding regarding juvenile hybrid poplar J-105 revealed that elevated vapour pressure deficit (eVPD) led to minor reductions in biometric variables, as well as in A_{net} , g_s and transpiration, compared to the significant impact of soil water availability and elevated CO_2 concentration (eCO_2), both of which generally exerted stronger effects. The adverse effect of eVPD on leaf area and root biomass was more than double that observed on stem biomass (9% reduction). While the negative impact of eVPD on A_{net} and WUE_i was relatively weak compared to the positive effect of eCO_2 , the effect on g_s exhibited comparable magnitudes for both opposing factors. WUE_i exhibited a significant increase due to eCO_2 , a slight decrease with eVPD, and a more pronounced decrease with full watering. The fundamental impact of watering in our experiment remained relatively consistent at both ambient and eCO_2 concentrations; however, the influence eVPD was largely mitigated by the eCO_2 under sufficient watering conditions. Nevertheless, this effect diminished when eVPD was combined with severe soil drought despite the temporary increase in WUE_i due to the elevated eCO_2 .

The relatively minor effect of elevated eVPD on the growth and physiology of young hybrid poplars observed in this study may be attributed to varying stomatal con-

Table 2. Responses of stem and root biomass, leaf area, and leaf level net assimilation (A_{net}), stomatal conductance (g_s), transpiration, and intrinsic water use efficiency (WUE_i) to contrasting conditions of vapour pressure deficit (VPD) – categorised as low (1 kPa) versus elevated (3 kPa), concentrations of CO_2 – categorised as low (400 ppm) versus elevated (700 ppm), and the amount of watering relative to the long-term mean precipitation for July – categorised as low (40%) versus adequate (100%), along with their interactions. Significant p-values are highlighted in bold.

Variable	VPD	CO_2	Watering	VPD $\times$ CO_2	VPD $\times$ Watering	$\text{CO}_2 \times$ Watering	VPD $\times$ $\text{CO}_2 \times$ Watering
Stem	0.065	< 0.001	< 0.001	0.683	0.770	0.930	0.127
Root	< 0.001	< 0.001	< 0.001	0.952	0.289	0.057	0.026
Leaf area	< 0.001	< 0.001	< 0.001	0.277	0.120	0.326	0.028
A_{net}	0.039	< 0.001	< 0.001	0.879	0.813	0.001	0.149
g_s	0.029	0.509	< 0.001	0.973	0.175	0.018	0.388
Transpiration	0.052	0.863	< 0.001	0.940	0.150	0.032	0.309
WUE_i	0.611	< 0.001	0.966	0.298	0.008	0.001	0.311

Table 3. Fisher's least significant difference post-hoc test comparison for stem and root biomass and leaf area. Values with the same letters are not significantly different ($p > 0.05$).

Watering (%)	CO_2 level (ppm)	VPD (kPa)	stem (g)		roots (g)		leaf area (cm^2)	
40	400	3	3.92	d	4.48	d	356	c
		1	3.95	d	5.32	d	434	de
	700	3	4.45	cd	4.91	d	385	e
		1	5.18	bc	8.07	c	510	cd
100	400	3	5.69	b	8.50	c	550	c
		1	7.14	a	11.46	b	774	ab
	700	3	7.94	a	15.79	a	705	b
		1	7.89	a	15.94	a	797	a

Table 4. Post hoc comparison of net assimilation (A_{net}), stomatal conductance (g_s), transpiration, and an intrinsic water use efficiency (WUE_i) based on seven gas exchange measurement campaigns conducted weekly during the period from DOY 210 to 245. Values sharing the same letters indicate no significant differences ($p > 0.05$).

Watering (%)	CO_2 (ppm)	VPD (kPa)	A_{net} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		g_s ($\text{mol m}^{-2} \text{s}^{-1}$)		transpiration ($\text{mmol m}^{-2} \text{s}^{-1}$)		WUE_i ($\mu\text{mol mmol}^{-1} \text{m}^{-2} \text{s}^{-1}$)	
40	400	3	1.02	d	0.010	d	0.20	c	95.4	c
		1	1.44	d	0.013	cd	0.23	c	111.1	b
	700	3	4.23	c	0.019	c	0.31	c	229.1	a
		1	4.35	c	0.020	cd	0.29	c	224.8	a
100	400	3	9.08	b	0.097	ab	1.59	ab	97.0	c
		1	9.49	b	0.135	a	1.80	a	72.4	d
	700	3	11.23	ab	0.065	b	1.00	b	177.6	ab
		1	14.32	a	0.132	a	1.52	a	105.2	b

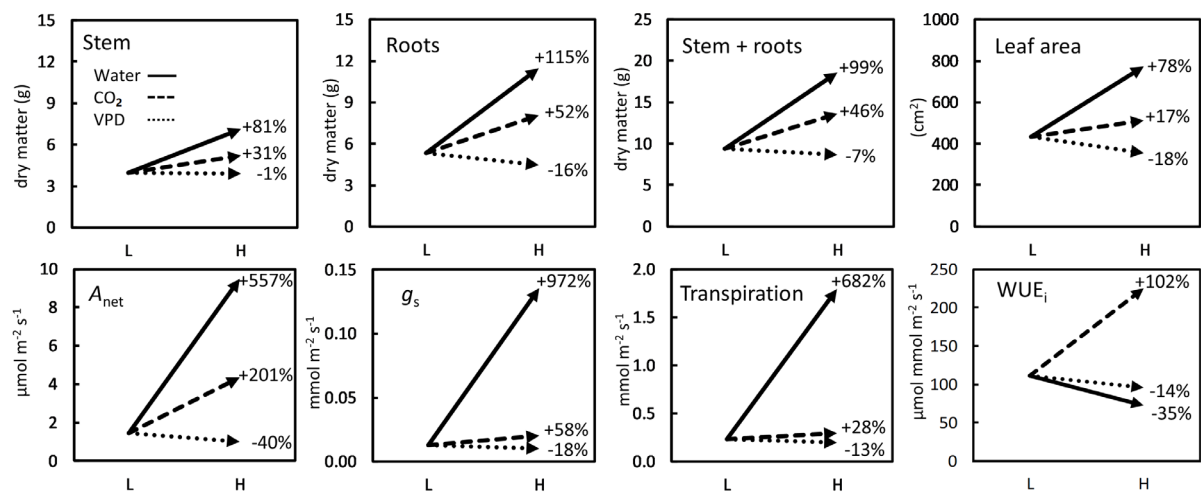


Fig. 1. Sensitivity analysis depicts the individual effects of each environmental factor at its high level (H), with watering at 100%, CO_2 at 700 ppm, and VPD at 3 kPa, while maintaining other factors at low levels (L), with watering at 40%, CO_2 at 400 ppm, and VPD at 1 kPa. The analysed response variables encompass stem and root biomass, leaf area, as well as leaf-level net assimilation (A_{net}), stomatal conductance (g_s), transpiration, and intrinsic water use efficiency (WUE_i).

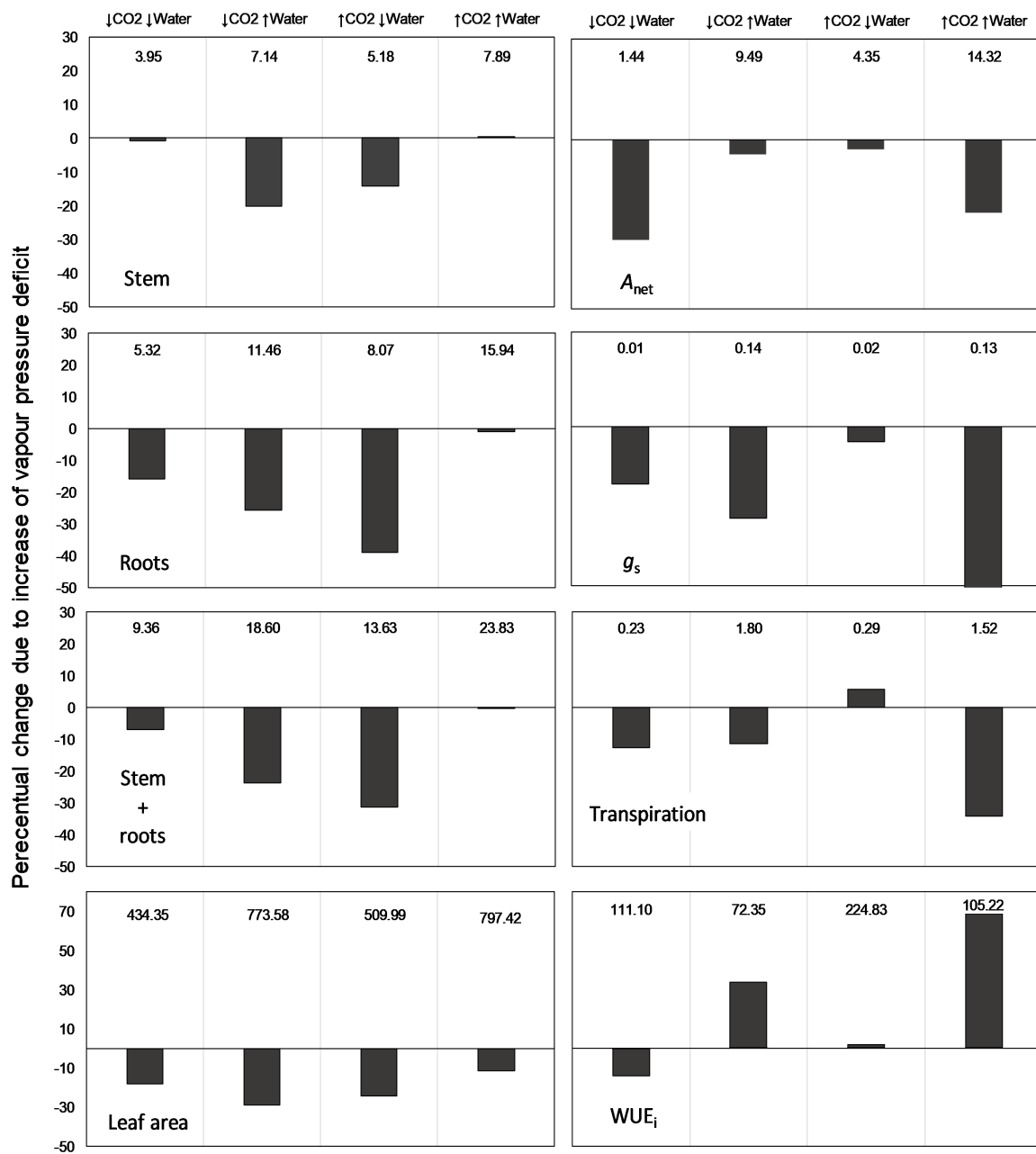


Fig. 2. Depicts the percent change of individual variables in response to contrasting treatments resulting from increased vapour pressure deficit (VPD) from 1 to 3 kPa. The analysed variables comprise stem and root biomass, leaf area, leaf-level net assimilation (A_{net}), stomatal conductance (g_s), transpiration and intrinsic water use efficiency (WUE_i). The numerical values at the top of each plot represent the mean values of the analysed variables for the treatment with low VPD (1 kPa). Arrows in the figure header pointing upwards and downwards indicate the levels of environmental factors, high versus low, i.e. CO₂ concentration at 700 ppm versus 400 ppm, VPD at 3 kPa versus 1 kPa, etc.

trol strategies, particularly the response of g_s across the gradient of VPD. This response has been documented to range from minimal, referred as anisohydric (Hall et al. 1996; Allen et al. 1999; Navarro et al. 2018), to moderate (Zenone et al. 2015), to strong stomatal control, indicative of isohydric behaviour, resulting in a sharp decline of g_s under eVPD (Kim et al. 2008; Schmidt-Walter et al. 2014; Navarro et al. 2018). To delve deeper into the response of g_s to eVPD, stomatal sensitivity was exam-

ined using the function $g_s = g_{s,ref} - m \ln(VPD)$, where $g_{s,ref}$ represents g_s at VPD of 1 kPa (Oren et al. 1999). The ratio of m to $g_{s,ref}$ serves as a crucial hydraulic parameter, indicating whether a species is isohydric or anisohydric. In our study, considering the g_s under low (1 kPa) and eVPD (3 kPa) in ambient CO₂ and 100% watered treatment, which were 0.135 and 0.096 mol m⁻² s⁻¹, respectively, the ratio of m to $g_{s,ref}$ was calculated as 0.26 mol m⁻² s⁻¹ ln(kPa)⁻¹. This value contrasts with the typical ratio of m

to $g_{s, \text{ref}}$ reported for isohydric species, which is $0.60 \text{ mol m}^{-2} \text{ s}^{-1} \ln(\text{kPa})^{-1}$ (Oren et al. 1999), indicating that J-105 in the juvenile phase exhibits weak stomatal control, suggesting a low degree of isohydricity. The concept an/isohydric strategy has been scrutinised by Schmidt-Walter et al. (2014), who investigated the identical clone J-105 as in our study and concluded that it generally exhibits isohydric behavior. Furthermore, they demonstrated that the degree of isohydricity is not necessarily static but may vary throughout the season. A higher value of $0.45 \text{ mol m}^{-2} \text{ s}^{-1} \ln(\text{kPa})^{-1}$ was reported by (Zenone et al. 2015) for a multi genotype short rotation woody crop plantation of *P. trichocarpa* × *P. deltoides*, while values of 0.45 and $0.64 \text{ mol m}^{-2} \text{ s}^{-1} \ln(\text{kPa})^{-1}$ were determined using porometry and sap flow, respectively, in another study by Kim et al. (2008). Interestingly, under $e\text{CO}_2$ and well-watered conditions in our study, the ratio of m to $g_{s, \text{ref}}$ increased to $0.46 \text{ mol m}^{-2} \text{ s}^{-1} \ln(\text{kPa})$, indicating a shift towards a more isohydric hydraulic strategy in a CO_2 -enriched atmosphere.

5. Conclusion

While concerns have been raised regarding the global impact of elevated VPD on the gross primary productivity of forest ecosystems, our study suggests that the effects of elevated VPD on juvenile hybrid poplar J-105 are not as pronounced. Instead, our findings highlight the fundamental importance of soil water availability, which had a greater impact than both elevated VPD and elevated CO_2 levels in our experiment. In future climates where elevated CO_2 levels coincide with elevated VPD, the detrimental effects of eVPD on poplars may be completely offset by the predominant growth-promoting effect of $e\text{CO}_2$. However, this mitigating effect diminishes when elevated VPD is combined with severe soil drought. Additionally, the study observed that the J-105 clone exhibited weak stomatal control under current CO_2 levels but may transition towards a more isohydric hydraulic strategy in a future CO_2 -enriched world.

Acknowledgements

This research has been supported by the Ministry of Education, Youth and Sports of the Czech Republic, grant AdAgriF – Advanced methods of greenhouse gases emission reduction and sequestration in agriculture and forest landscape for climate change mitigation (CZ.02.01.01/00/22_008/0004635) and by the Technology Agency of the Czech Republic (grant no. SS02030040, PERUN). We sincerely thank Dr. Joanna Horemans (University of Antwerp) and Dr. Eric J. Ward (US Geological Survey) for detailed statistical advice.

References

- Abràmoff, M. D., Magalhães, P. J., Ram, S. J., 2004: Image processing with ImageJ. *Biophotonics International*, 11:36–42.
- Al Afas, N., Marron, N., Van Dongen, S., Laureysens, I., Ceulemans, R., 2008: Dynamics of biomass production in a poplar coppice culture over three rotations (11 years). *Forest Ecology and Management*, 255:1883–1891.
- Allen, S. J., Hall, R. L., Rosier, P. T., 1999: Transpiration by two poplar varieties grown as coppice for biomass production. *Tree Physiology*, 19:493–501.
- Attia, Z., Domec, J.-C., Oren, R., Way, D. A., Moshelion, M., 2015: Growth and physiological responses of isohydric and anisohydric poplars to drought. *Journal of Experimental Botany*, 66:4373–4381.
- Broeckx, L. S., Verlinden, M. S., Berhongaray, G., Zona, D., Fichot, R., Ceulemans, R., 2013: The effect of a dry spring on seasonal carbon allocation and vegetation dynamics in a poplar bioenergy plantation. *GCB Bioenergy*, 6:473–487.
- Cleveland, W. S., Grosse, E., Shyu, W. M., Chambers, J. M., Hastie, T. J., 1992: Statistical models in S. Local regression models. New York, Wadsworth & Brooks/Cole, Chapter 8, 624 p.
- Fichot, R., Brignolas, F., Cochard, H., Ceulemans, R., 2015: Vulnerability to drought-induced cavitation in poplars: synthesis and future opportunities. *Plant, Cell & Environment*, 38:1233–1251.
- Fischer, M., Kelley, A., Ward, E., Boone, J., Ashley, E., Domec, J.-C. et al., 2017: A critical analysis of species selection and high vs. low-input silviculture on establishment success and early productivity of model short-rotation wood-energy cropping systems. *Biomass and Bioenergy*, 98:214–227.
- Fischer, M., Zenone, T., Trnka, M., Orság, M., Montagnani, L., Ward, E. J. et al., 2018: Water requirements of short rotation poplar coppice: Experimental and modelling analyses across Europe. *Agricultural and Forest Meteorology*, 250-251:343–360.
- Gielen, B., Ceulemans, R., 2001: The likely impact of rising atmospheric CO_2 on natural and managed *Populus*: a literature review. *Environmental Pollution*, 115:335–358.
- Grossiord, C., Buckley, T. N., Cernusak, L. A., Novick, K. A., Poulter, B., Siegwolf, R. T. W. et al., 2020: Plant responses to rising vapor pressure deficit. *New Phytologist*, 226:1550–1566.
- Hall, R., Allen, S., Rosier, P., Smith, D., Hodnett, M., Roberts, J. et al., 1996: Hydrological effects of short rotation energy coppice. Final report to ETSU. Wallingford, Institute of Hydrology, 201 p.
- Kim, H.-S., Oren, R., Hinckley, T. M., 2008: Actual and potential transpiration and carbon assimilation in an irrigated poplar plantation. *Tree Physiology*, 28:559–577.

- Lansu, E. M., van Heerwaarden, C., Stegehuis, A. I., Teuling, A. J., 2020: Atmospheric aridity and apparent soil moisture drought in European forest during heat waves. *Geophysical Research Letters*, 47:e2020GL087091.
- Larchevêque, M., Maurel, M., Desrochers, A., Larocque, G. R., 2011: How does drought tolerance compare between two improved hybrids of balsam poplar and an unimproved native species? *Tree Physiology*, 31:240–249.
- Marron, N., Dreyer, E., Boudouresque, E., Delay, D., Petit, J.-M., Delmotte, F. M. et al., 2003: Impact of successive drought and re-watering cycles on growth and specific leaf area of two *Populus × canadensis* (Moench) clones, ‘Dorskamp’ and ‘Luisa_Avanzo’. *Tree Physiology*, 23:1225–1235.
- Migliavacca, M., Meroni, M., Manca, G., Matteucci, G., Montagnani, L., Grassi, G. et al., 2009: Seasonal and interannual patterns of carbon and water fluxes of a poplar plantation under peculiar eco-climatic conditions. *Agricultural and Forest Meteorology*, 149:1460–1476.
- Meehl, G. A., 2007: Global Climate Projections. In *Climate Change 2007. The Physical Science Basis Contribution of Working Group I to the Fourth Assessment Report of the IPCC*:747–846.
- Monclus, R., Dreyer, E., Villar, M., Delmotte, F. M., Delay, D., Petit, J.-M. et al., 2006: Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoids × Populus nigra*. *New Phytologist*, 169:765–777.
- Navarro, A., Portillo-Estrada, M., Arriga, N., Vanbever, S. P., Ceulemans, R., 2018: Genotypic variation in transpiration of coppiced poplar during the third rotation of a short-rotation bio-energy culture. *GCB Bioenergy*, 10:592–607.
- Novick, K. A., Ficklin, D. L., Stoy, P. C., Williams, C. A., Bohrer, G., Oishi, A. C. et al., 2016: The increasing importance of atmospheric demand for ecosystem water and carbon fluxes. *Nature Climate Change*, 6:1023–1027.
- Oren, R., Sperry, J., Katul, G., Pataki, D., Ewers, B., Phillips, N. et al., 1999: Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant, Cell & Environment*, 22:1515–1526.
- Orság, M., Fischer, M., Tripathi, A. M., Žalud, Z., Trnka, M., 2018: Sensitivity of short rotation poplar coppice biomass productivity to the throughfall reduction – Estimating future drought impacts. *Biomass and Bioenergy*, 109:182–189.
- Schmidt-Walter, P., Richter, F., Herbst, M., Schuldt, B., Lamersdorf, N. P., 2014: Transpiration and water use strategies of a young and a full-grown short rotation coppice differing in canopy cover and leaf area. *Agricultural and Forest Meteorology*, 195:165–178.
- Sulman, B. N., Roman, D. T., Yi, K., Wang, L., Phillips, R. P., Novick, K. A., 2016: High atmospheric demand for water can limit forest carbon uptake and transpiration as severely as dry soil. *Geophysical Research Letters*, 43:9686–9695.
- Taylor, G., Ceulemans, R., Ferris, R., Gardner, S., Shao, B., 2001: Increased leaf area expansion of hybrid poplar in elevated CO₂. From controlled environments to open-top chambers and to FACE. *Environmental Pollution*, 115:463–472.
- Trnka, M., Trnka, M., Fialová, J., Koutecký, V., Fajman, M., Žalud, Z. et al., 2008: Biomass production and survival rates of selected poplar clones grown under a short-rotation system on arable land. *Plant Soil and Environment*, 54:78–88.
- Venables, W. N., Ripley, B. D., Venables, W., Ripley, B., 2002: Random and mixed effects. In: *Modern applied statistics with S. Statistics and Computing*. New York, Springer, p. 271–300.
- Verlinden, M. S., Broeckx, L. S., Ceulemans, R., 2015: First vs. second rotation of a poplar short rotation coppice: Above-ground biomass productivity and shoot dynamics. *Biomass and Bioenergy*, 73:174–185.
- Walle, I. V., Van Camp, N., Van de Castele, L., Verheyen, K., Lemeur, R., 2007: Short-rotation forestry of birch, maple, poplar and willow in Flanders (Belgium) I – Biomass production after 4 years of tree growth. *Biomass and Bioenergy*, 31:267–275.
- Yuan, W., Zheng, Y., Piao, S., Ciais, P., Lombardozzi, D., Wang, Y. et al., 2019: Increased atmospheric vapor pressure deficit reduces global vegetation growth. *Science Advances*, 5:eaax1396.
- Zenone, T., Fischer, M., Arriga, N., Broeckx, L. S., Verlinden, M. S., Vanbever, S. et al., 2015: Biophysical drivers of the carbon dioxide, water vapor, and energy exchanges of a short-rotation poplar coppice. *Agricultural and Forest Meteorology*, 209:22–35.

Other sources

R Core Team, 2022: R: A language and environment for statistical computing. In: *R Foundation for Statistical Computing*, Vienna, Austria.

Attachment

Following two plots depict variability of biometric and physiological traits of juvenile potted hybrid poplars grown under increased air temperature (32 °C) with multiple interacting environmental factors: high vs. low VPD, ambient vs. elevated CO₂ concentration, and adequate vs. reduced watering.

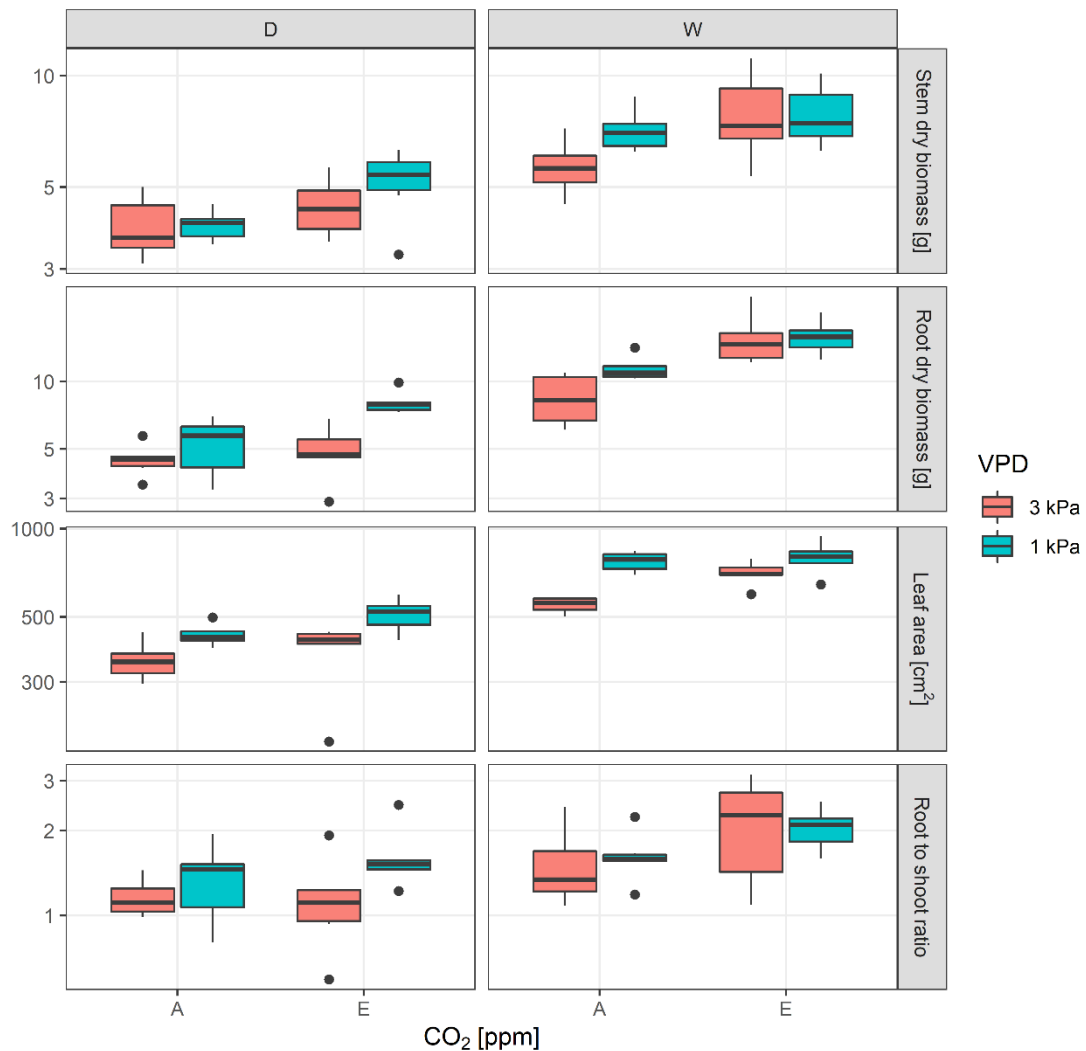


Fig. A1. Box plots of stem biomass, root biomass, leaf area and root to shoot ratio of poplar plants – determined by destructive sampling at the end of 42-days experiment – for the different interacting environmental treatments. The left and right vertical panel represent the soil water availability treatments (D, 40% watered, left, W, 100% watered, right). Each figure panel is split according to CO₂ concentration (A, 400 ppm – left, E, 700 ppm, right) and color-coded according to VPD levels (L, low VPD = 1 kPa – red; H, VPD = 3 kPa – blue). The black dots represent the outliers detected according to 1.5 times IQR method. Note the logarithmic y-axis scale.

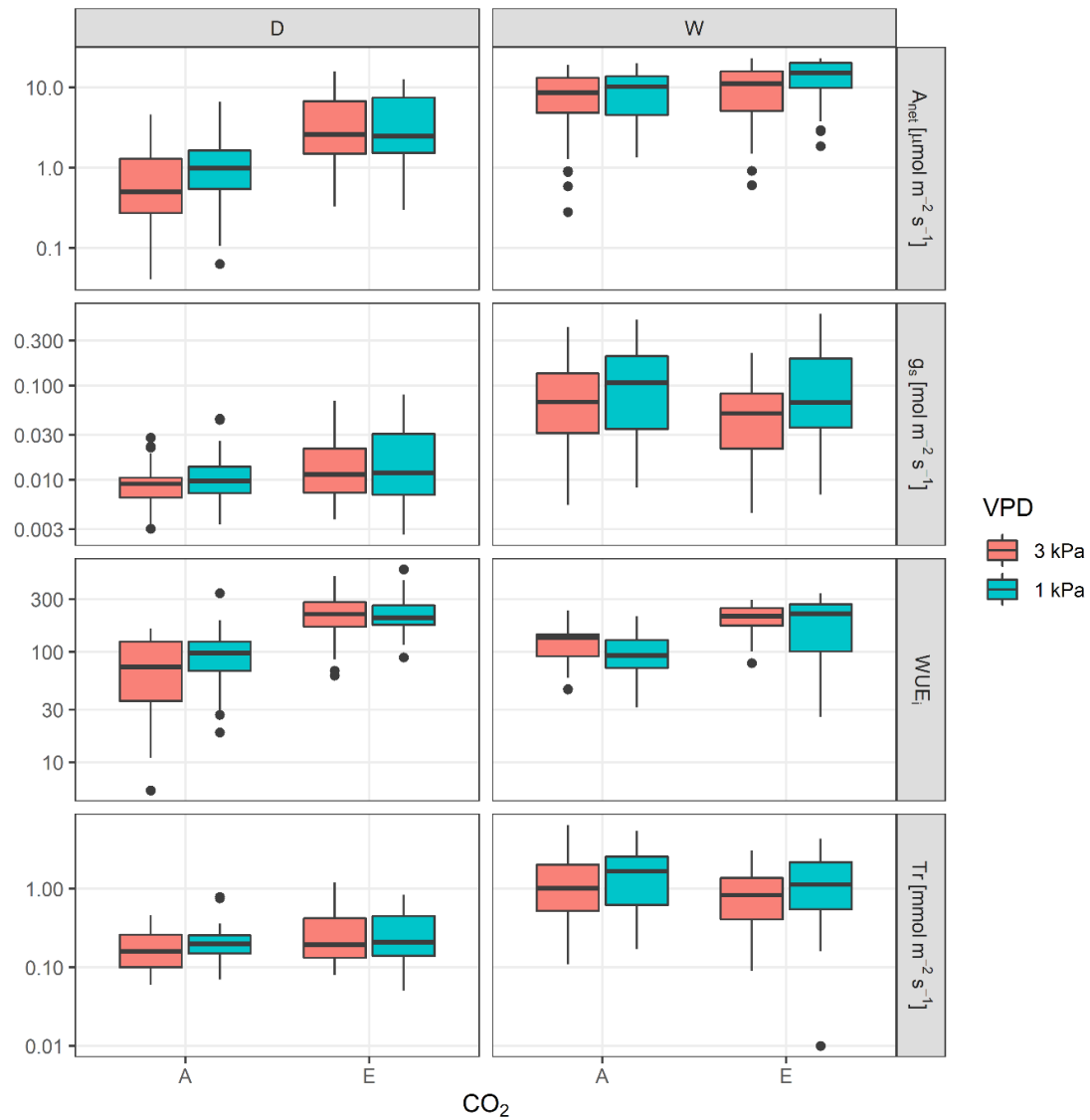


Fig. A2. Box plots depicting the rate of net assimilation (A_{net}), stomatal conductance (g_s), intrinsic water use efficiency (WUE_i) and transpiration rate (Tr) of young poplars under different interacting environmental treatments. The box plotted values represent medians of seven weekly measurements. The left and right vertical panel represent the soil water availability treatments (D, 40% watered, left, W, 100% watered, right). Each figure panel is split according to CO_2 concentration (A, 400 ppm – left, E, 700 ppm, right) and color-coded according to VPD levels (L, VPD = 1 kPa – red, H, VPD = 3 kPa – blue). The black dots represent the outliers detected according to 1.5 times IQR method. Note the logarithmic y-axis scale. VPD: maximum vapour pressure deficit.

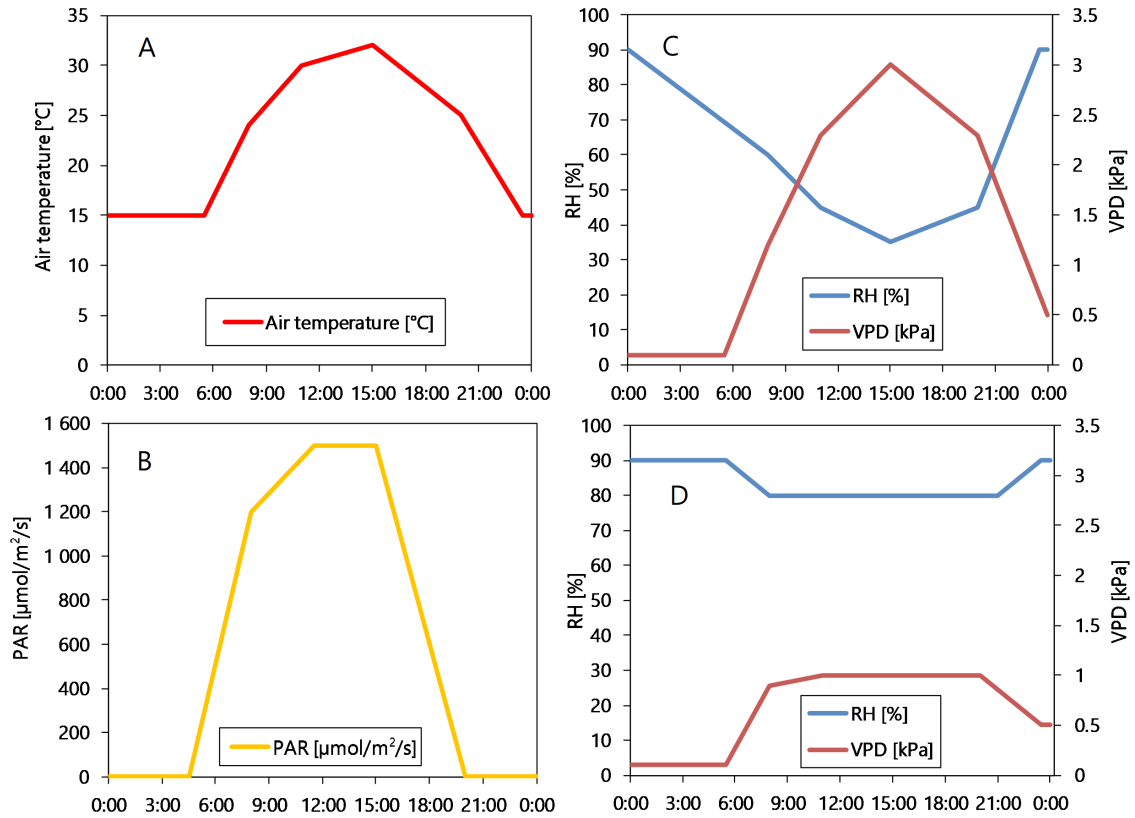


Fig. A3. Daily patterns of air temperature (A), photosynthetically active radiation (PAR) (B). The daily course of air temperature as well as PAR was identical for all four experimental chambers. Panels C and D depict the daily patterns of relative air humidity to achieve the target maximum daily vapour pressure deficit, in conjunction with the aforementioned air temperature within the chambers. Panel C illustrates the treatment with high VPD (eVPD), peaking a daily maximum VPD of 3 kPa, whereas panel D illustrates the treatment with low VPD, which never surpasses 1 kPa.

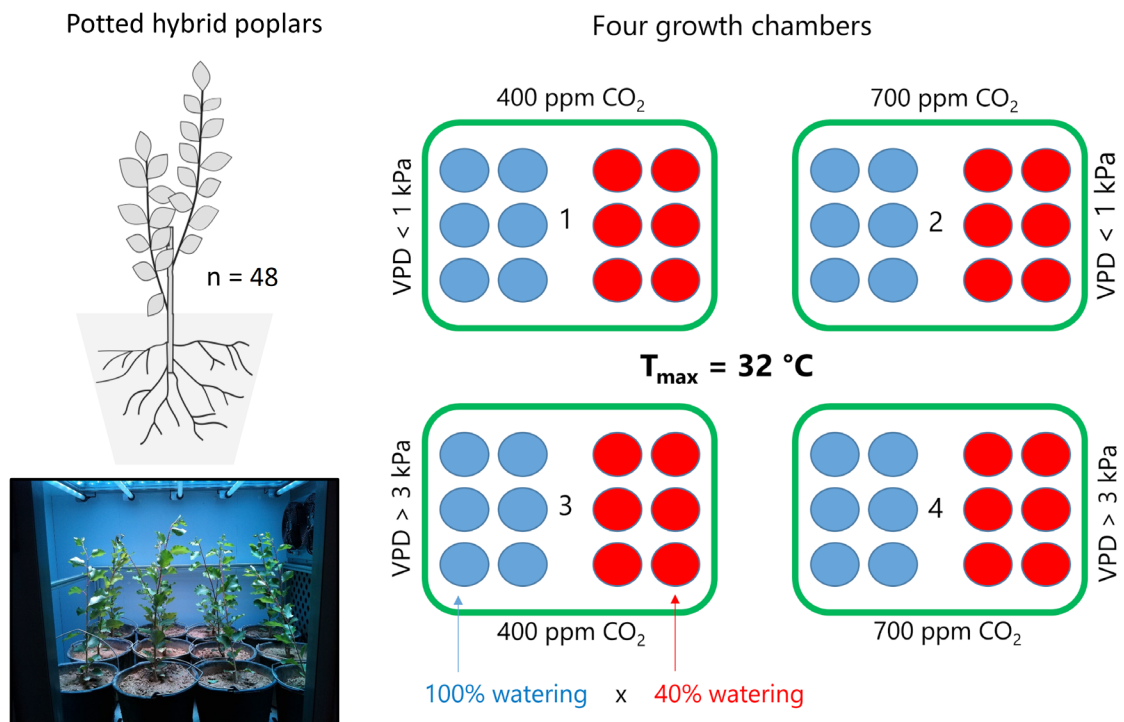


Fig. A4. Experimental design diagram.