

Article

Interactive Effect of Elevated CO₂ and Reduced Summer Precipitation on Photosynthesis Is Species-Specific: The Case Study with Soil-Planted Norway Spruce and Sessile Oak in a Mountainous Forest Plot

Kojo Kwakye Ofori-Amanfo ^{1,2}, Karel Klem ^{1,2}, Barbora Veselá ¹, Petr Holub ¹, Thomas Agyei ^{1,2}, Michal V. Marek ^{1,3}, John Grace ^{1,4} and Otmar Urban ^{1,*}

¹ Global Change Research Institute, Czech Academy of Sciences, Bělidla 986/4a, 603 00 Brno, Czech Republic; oforiamanfo.k@czechglobe.cz (K.K.O.-A.); klem.k@czechglobe.cz (K.K.); vesela.b@czechglobe.cz (B.V.); holub.p@czechglobe.cz (P.H.); agyei.t@czechglobe.cz (T.A.); marek.mv@czechglobe.cz (M.V.M.); j.grace@ed.ac.uk (J.G.)

² Mendel University in Brno, Zemědělská 1665/1, 613 00 Brno, Czech Republic

³ Slovak Technical University Bratislava, Vazovova 5, 812 43 Bratislava, Slovakia

⁴ School of GeoSciences, University of Edinburgh, Crew Bldg, Kings Bldgs, Alexander Crum Brown Rd, Edinburgh EH9 3FF, Midlothian, UK

* Correspondence: urban.o@czechglobe.cz; Tel. +420-511-192-250



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Abstract: We investigated how reduced summer precipitation modifies photosynthetic responses of two model tree species—coniferous Norway spruce and broadleaved sessile oak—to changes in atmospheric CO₂ concentration. Saplings were grown under mountainous conditions for two growing seasons at ambient (400 μmol CO₂ mol⁻¹) and elevated (700 μmol CO₂ mol⁻¹) CO₂ concentration. Half were not exposed to precipitation during the summer (June–August). After two seasons of cultivation under modified conditions, basic photosynthetic characteristics including light-saturated rate of CO₂ assimilation (A_{\max}), stomatal conductance ($G_{S\max}$), and water use efficiency (WUE) were measured under their growth CO₂ concentrations together with in vivo carboxylation rate (V_C) and electron transport rate (J) derived from CO₂-response curves at saturating light. An increase in A_{\max} under elevated CO₂ was observed in oak saplings, whereas it remained unchanged or slightly declined in Norway spruce, indicating a down-regulation of photosynthesis. Such acclimation was associated with an acclimation of both J and V_C . Both species had increased WUE under elevated CO₂ although, in well-watered oaks, WUE remained unchanged. Significant interactive effects of tree species, CO₂ concentration, and water availability on gas-exchange parameters (A_{\max} , $G_{S\max}$, WUE) were observed, while there was no effect on biochemical (V_C , J) and chlorophyll fluorescence parameters. The assimilation capacity (A_{sat} ; CO₂ assimilation rate at saturating light intensity and CO₂ concentration) was substantially reduced in spruce under the combined conditions of water deficiency and elevated CO₂, but not in oak. In addition, the stimulatory effect of elevated CO₂ on A_{\max} persisted in oak, but completely diminished in water-limited spruce saplings. Our results suggest a strong species-specific response of trees to reduced summer precipitation under future conditions of elevated CO₂ and a limited compensatory effect of elevated CO₂ on CO₂ uptake under water-limited conditions in coniferous spruce.

Keywords: chlorophyll fluorescence; climate change; electron transport rate; elevated carbon dioxide; photosynthetic acclimation; tree physiology; Rubisco carboxylation rate; water availability

1. Introduction

Forest ecosystems play dominant roles in the ecology and evolution of life on the Earth. Forests have a critical function in regulating the global climate through their effects on the biogeochemical cycles, especially the cycles of carbon and water [1]. It is well known that,

due to anthropogenic activities, an increase of CO₂ concentration ([CO₂]) in the atmosphere is occurring rapidly [2]. Emissions scenarios suggest a range of substantially increased CO₂ by the end of this century, ranging between 550 and 900 μmol CO₂ mol⁻¹. Since CO₂ serves as a substrate for photosynthesis, atmospheric [CO₂] strongly influences plant physiology and growth. Although the effects of elevated [CO₂] on trees have received great attention [3–6], investigations of combined effects with other environmental factors are still insufficient to predict future responses, as several other factors are likely to co-vary with the increase in [CO₂]. Global climate models suggest an increasing probability of changes in the seasonal pattern of precipitation and severe drought periods in future decades [1,2]. Accordingly, it is important to evaluate the sensitivity of trees grown under elevated [CO₂] to limited water availability and to understand the physiological mechanisms contributing to their adaptation capacity.

Generally, CO₂ is an activator of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) enzyme activity (carbamylation), and it is the substrate of the Calvin cycle leading to the formation of triose phosphates (carboxylation). Elevated [CO₂] thus results in stimulated carboxylation activity of Rubisco [7,8], while its oxygenase activity is reduced [9,10]. Such adjustments are mediated particularly by an increase of [CO₂] in chloroplasts and result in increased CO₂ assimilation rates in C₃ plants [6,11]. Moreover, elevated [CO₂] leads to a reduced stomatal conductance via stomatal closure and/or reduced stomata formation [12], stimulated water use efficiency and growth [13], and particularly an increased ratio between root and shoot biomass [14]. Based on this background information, it is hypothesized that plants growing under elevated [CO₂] will have higher tolerance to water-limited conditions than ambient [CO₂] plants. However, the previous findings, including those on oak and spruce [15–18], are highly variable and inconsistent. Among others, it has been shown that CO₂ treatment had a persistent stimulatory effect on photosynthesis in well-watered oaks, whereas CO₂-treated spruces showed an increased CO₂ assimilation rates only when droughted [16]. In contrary with other studies, we present a study on trees planted directly in the soil under mountainous conditions. Such research has so far received only limited attention.

Plant responses to a combined effect of elevated [CO₂] and other environmental factors could be also modulated by an occurrence of photosynthetic down-regulation under elevated [CO₂]. It is defined as a reduction in [CO₂]-enhanced photosynthesis relative to the initial [CO₂]-stimulated rate. The degree of these responses is highly variable depending on plant species, root structure, duration of CO₂ enrichment, plant age, and growth conditions [19,20]. Photosynthetic down-regulation is particularly associated with insufficient mineral supply, reduced amount and/or activity of Rubisco, and low activity of metabolic carbon sinks [21,22].

To increase our knowledge of how combined long-term elevated [CO₂] and reduced water availability influence photosynthetic performance in trees, an experiment was conducted on soil-planted trees under relatively wet mountainous conditions of central Europe. During two summer months, the incoming precipitation, amounting up to 150 mm, was excluded. The specific objectives of this study were: (i) to investigate effects of elevated [CO₂] and reduced water availability on two contrasting model tree species, coniferous Norway spruce (*Picea abies*) and broadleaved sessile oak (*Quercus petraea*); (ii) to investigate changes in photochemical and gas-exchange parameters; and (iii) to evaluate how the long-term growth under elevated [CO₂] (two growing seasons) modifies the tolerance of plants to reduced water availability during summer.

2. Materials and Methods

2.1. Plants and Experimental Design

The experiment was conducted at the experimental ecological station Bílý Kříž, located in the Moravian-Silesian Beskydy Mountains, Czech Republic (49°30'77" N, 18°32'28" E, altitude 908 m a.s.l.). The climate is characterized as moderately cold and moist with an average annual air temperature of 4.9 °C, annual precipitation of 1100 mm, and the average

relative air humidity of 80% with high precipitation during the vegetation season of up to 110 mm per month.

Four-year old saplings (mean height 0.5 m) of Norway spruce (*Picea abies* (L.) Karst.) and sessile oak (*Quercus petraea* (Matt.) Liebl.) were planted into native soil (Mesozoic Godula sandstone—flysh type) in spring 2017 and then exposed to ambient ($\sim 400 \mu\text{mol CO}_2 \text{ mol}^{-1}$; hereafter AC) and elevated ($\sim 700 \mu\text{mol CO}_2 \text{ mol}^{-1}$; hereafter EC) atmospheric $[\text{CO}_2]$ for two growing seasons using the experimental domes with adjustable windows (Figure 1). Before planting, the soil was homogenized to the depth of 20 cm and thoroughly mixed (10:1 ratio) with a light peat Klasmann base substrate 422 (Klasmann-Deilmann, Geeste, Germany) using a rotary soil tiller. The domes are equipped with a set of sensors for continual monitoring of solar radiation, air and soil humidity, and soil temperatures and a feed-back regulation of internal $[\text{CO}_2]$ based on the continual monitoring of aerial $[\text{CO}_2]$ by an infra-red analyzer Li-850 (LI-COR Biosciences, Lincoln, NE, USA). See [5,23] for detailed description.

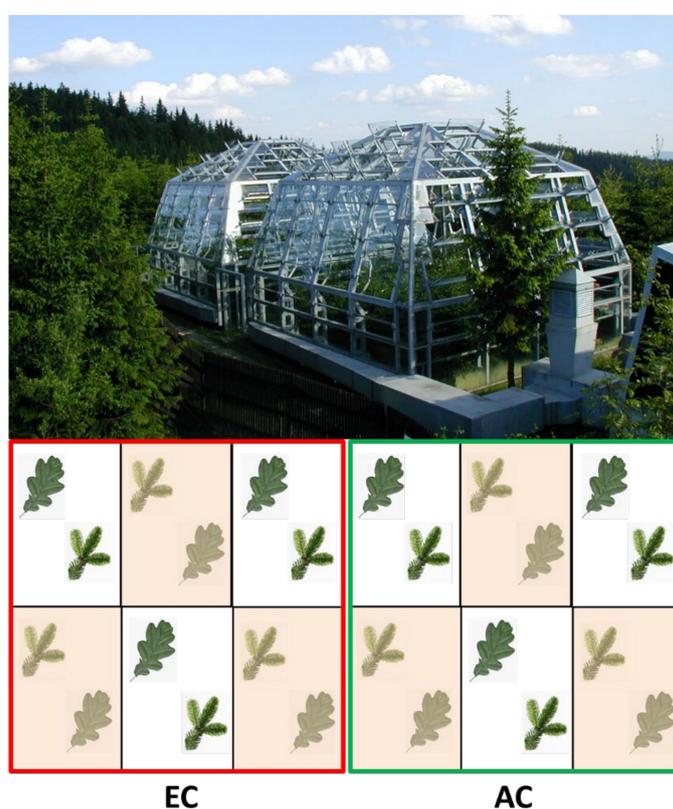


Figure 1. Photograph of cultivation domes with adjustable lamellar windows (top panel) and schematic drawing of the experiment layout (bottom panel). Each dome was divided into six blocks. Individual blocks (repetition; $n = 3$) are bounded by a thin black line. Open blocks = well-watered treatment (gular watering of 20 mm per week during vegetation period); colored blocks = water-limited treatment (no irrigation for 5 weeks before the measurements); AC = ambient CO_2 concentration ($\sim 400 \mu\text{mol CO}_2 \text{ mol}^{-1}$); EC = elevated CO_2 concentration ($\sim 700 \mu\text{mol CO}_2 \text{ mol}^{-1}$).

Each dome ($10 \times 10 \text{ m}$ in size) was split into six blocks (Figure 1). Each block (approximately 16.5 m^2) contained 12 saplings of sessile oak and 12 saplings of Norway spruce. Three random blocks (replications; $n = 3$) were regularly irrigated (WW) while the remaining 3 blocks were left without irrigation during the period of July–August (WL) in two consecutive years. WW plants received regular watering of 20 mm per week during vegetation period, while WL plants had no irrigation for 5 weeks before the measurements. To prevent soil water movement, the blocks were separated by a PVC board (thickness of 5 mm) placed in the soil up to the native bedrock (depth of ca 0.50 m). An ML3 Theta

Probe sensor (Delta-T Devices, UK) was used to measure soil water content in the depths of 5–10 cm and 30–40 cm. In topsoil layer, WL treatment led to a decrease of soil water content from 23.5 to 13%, while it remained high (22.5–23.5%) under WW treatment. However, soil water content was the same, 17.5%, under the both water treatments in the deeper soil layer.

2.2. Physiological Measurements

In total, 36 oaks and 36 spruces were measured at the end of August. Leaf- and shoot-level measurements were made on three representative saplings from each block (replication). The average from three sapling measurements per block was used for the consequent statistical analyses. Current-year spruce shoots were examined. All measurements were conducted between 10:00–15:00 CET on intact fully-expanded leaves. A Scholander type of pressure chamber was used to measure leaf water potential (Ψ) at pre-dawn (05:00) and noon (12:00).

2.2.1. Gas-Exchange Measurements

Basic photosynthetic characteristics—CO₂ assimilation rate (A), intercellular [CO₂] (C_i), stomatal conductance (G_s), and transpiration rate (E)—were measured using an open gas-exchange system Li-6400 (LI-COR Biosciences, USA) equipped with an LED light source 6400-02B. The A – C_i response curves were determined at saturating light intensity (1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and starting at growth [CO₂], i.e., at 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ for AC and 700 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ for EC plants. In this way, instantaneous light-saturated rates of CO₂ assimilation (A_{max}) and stomatal conductance ($G_{s\text{max}}$) at growth [CO₂] were determined. Then, the following set of [CO₂] in the assimilation chamber was applied: 1500, 700, 400, 250, 150, 90, and 50 $\mu\text{mol CO}_2 \text{ mol}^{-1}$. Plants were acclimated approximately 10 min to each [CO₂]. Such measuring protocol enabled us to derive CO₂ assimilation capacity (A_{sat}), CO₂ assimilation rate at saturating light intensity and saturating CO₂ concentration (1500 $\mu\text{mol CO}_2 \text{ mol}^{-1}$), as well as the in vivo Rubisco carboxylation rate (V_C) and electron transport (J) at 25 °C. See [24] for the calculation details. Moreover, water use efficiency was calculated under saturating light conditions (WUE, defined as the ratio between A_{max} and E_{max}). During all measurements, the leaf temperature was kept at 25 °C (± 0.5 °C) using the Peltier thermo-electric cooler mounted on the assimilation chamber. Relative air humidity was maintained during the measurement at 50% ($\pm 1\%$), thus keeping vapor pressure deficit (VPD) at 1.1–1.5 kPa.

2.2.2. Chlorophyll Fluorescence

The efficiency of primary photochemical reactions was determined by chlorophyll fluorescence (Chl-F) following the protocols described in details by Urban et al. [25]. In brief, the emission of the Chl-F signal at the red band (near 690 nm) was measured by a fluorometer PAM-2500 (H. Walz, Effeltrich, Germany). Night-time Chl-F measurement was carried out to estimate maximum quantum yield of photosystem (PS) II photochemistry (F_v/F_m). Leaves adapted to saturating light intensity (1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were used to assess an actual quantum yield of PSII photochemistry (Φ_{PSII}) and thermal energy dissipation (D) according to Demmig-Adams et al. [26]. All measurements were done on intact leaves in situ at growth [CO₂].

2.3. Statistical Data Processing

Three-way analysis of variance (ANOVA) was used to analyze effects of tree species (TS), CO₂ concentration ([CO₂]), water availability (WA), and their interactions (\times) on physiological parameters. Tukey's HSD post-hoc test was used to analyze significant differences between means at $p \leq 0.05$. Statistical analyses were conducted using the software STATISTICA 12 (StatSoft, Tulsa, CA, USA). The bar graphs representing means with standard deviations were developed with the software SigmaPlot 11.0 (Systat Software, San Jose, CA, USA).

3. Results

3.1. Growth Conditions

Pre-dawn and noontime leaf water potentials were significantly influenced by tree species (TS) and water availability (WA), but CO₂ treatment had significant effect only on $\Psi_{\text{pre-dawn}}$ (Table 1). No interactive effects of these factors on Ψ values were observed. Exclusion of precipitation led to mild decreases in leaf Ψ at pre-dawn and particularly noon hours. However, the Ψ_{noon} values between -1.0 and -1.3 MPa measured in this experiment for WL plants are not very severe. On average, the noon Ψ decrease amounted up to 22% in oak and up to 25% in spruce. Although there were mostly no significant differences between AC and EC counterparts, lower (more negative) Ψ values were observed in EC plants (Figure 2).

Table 1. Summary of significance levels (p -values of the three-way ANOVA) for the factors of trees species (TS), CO₂ concentration ([CO₂]), water availability (WA), and their interactions (\times) on physiological parameters ($\Psi_{\text{pre-dawn}}$ —pre-dawn leaf water potential, Ψ_{noon} —noon leaf water potential, A_{max} —light-saturated CO₂ assimilation rate, G_{Smax} —light-saturated stomatal conductance, E_{max} —light-saturated transpiration rate, and WUE—water use efficiency, V_C —in vivo Rubisco carboxylation rate, J —in vivo electron transport rate, Φ_{PSII} —actual quantum yield of PSII photochemistry under saturating light intensity, D —thermal energy dissipation under saturating light intensity). Bold p -values indicate significant effect at $p \leq 0.05$.

Factors	$\Psi_{\text{pre-dawn}}$	Ψ_{noon}	V_C	J	A_{sat}	A_{max}	G_{Smax}	E_{max}	WUE	Φ_{PSII}	D
TS	<0.001	0.036	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.134	<0.001
[CO ₂]	0.001	0.217	<0.001	0.003	0.691	0.008	0.573	0.921	<0.001	0.574	0.840
WA	0.002	0.015	0.211	0.022	0.017	0.066	0.970	0.910	0.008	0.363	0.279
TS \times [CO ₂]	0.086	0.701	0.827	0.265	<0.001	0.128	0.228	0.071	0.016	0.006	<0.001
TS \times WA	0.374	0.217	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.696	0.047	0.002
[CO ₂] \times WA	0.374	0.956	0.245	0.191	0.016	0.452	0.002	0.010	0.040	0.566	0.973
TS \times [CO ₂] \times WA	0.086	0.783	0.156	0.910	0.010	0.137	0.006	0.016	0.005	0.615	0.234

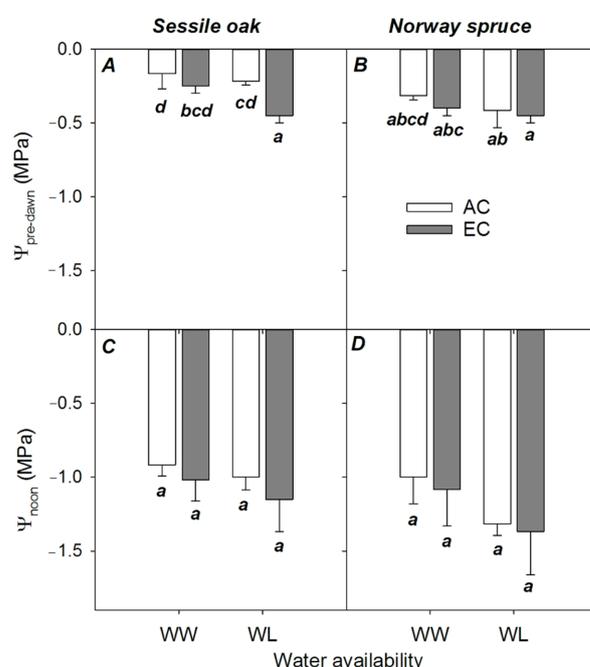


Figure 2. Effect of CO₂ concentration and water availability on pre-dawn leaf water potential ($\Psi_{\text{pre-dawn}}$) and noon leaf water potential (Ψ_{noon}) in sessile oak (left) and Norway spruce (right). Means (columns) and standard deviations (error bars) are presented ($n = 3$). Different letters indicate significant differences ($p \leq 0.05$) between means based on Tukey's ANOVA post-hoc test. AC—ambient CO₂ concentration ($\sim 400 \mu\text{mol CO}_2 \text{ mol}^{-1}$), EC—elevated CO₂ concentration ($\sim 700 \mu\text{mol CO}_2 \text{ mol}^{-1}$), WW—well-watered (regular watering of 20 mm per week during vegetation period), WL—water-limited (no irrigation for 5 weeks before the measurements).

3.2. Gas-Exchange Parameters

3.2.1. Assimilation Capacity (A_{sat}) and Light-Saturated CO_2 Assimilation Rate (A_{max})

Assimilation capacity (A_{sat}) was significantly influenced by tree species (TS) and water availability (WA), but not by $[\text{CO}_2]$ (Table 1). Generally, we found higher assimilation capacity in broadleaved oak compared to coniferous spruce (Figure 3A,B). WL treatment led to an increase of A_{sat} in oak saplings treated under both $[\text{CO}_2]$ conditions. However, the assimilation capacity was substantially reduced in spruce saplings under the combined conditions of WL and EC. Strong interactive effect of all factors investigated ($\text{TS} \times [\text{CO}_2] \times \text{WA}$) on A_{sat} was also found (Table 1).

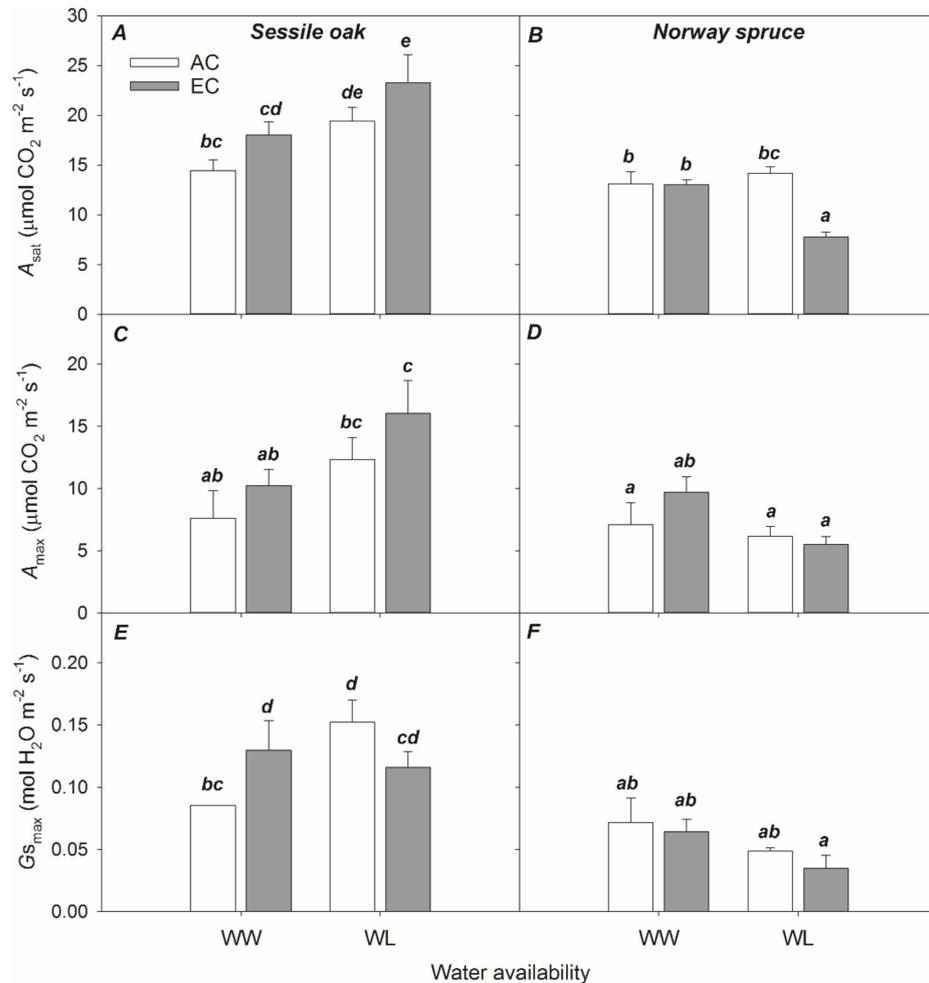


Figure 3. Effect of CO_2 concentration and water availability on CO_2 assimilation capacity (A_{sat} ; (A,B)), light-saturated CO_2 assimilation rate at growth $[\text{CO}_2]$ (A_{max} ; (C,D)) and light-saturated stomatal conductance (G_{smax} ; (E,F)) in sessile oak (left) and Norway spruce (right). The measurements were done during the second growing season of cultivation under manipulated conditions: AC—ambient CO_2 concentration ($\sim 400 \mu\text{mol CO}_2 \text{ mol}^{-1}$), EC—elevated CO_2 concentration ($\sim 700 \mu\text{mol CO}_2 \text{ mol}^{-1}$), WW—well-watered (regular watering of 20 mm per week during vegetation period), WL—water-limited (no irrigation for 5 weeks before the measurements). Means (columns) and standard deviations (error bars) are presented ($n = 3$). Different letters indicate significant differences ($p \leq 0.05$) between means based on Tukey's ANOVA post-hoc test.

Generally, tree species and $[\text{CO}_2]$ had significant effects on A_{max} , while the effect of water availability was statistically non-significant (Table 1). A higher stimulatory effect of EC on A_{max} was found in broadleaved oak compared to coniferous spruce. EC conditions tended to increase A_{max} values in oak saplings across all water treatments; however, this effect fell short of being statistically significant (Figure 3C). Noticeably, the stimulatory

effect of EC on A_{\max} completely diminished in WL-treated spruce saplings (Figure 3D). Analysis of variance revealed significant TS \times WA on A_{\max} , while no significant interactive effect of $[\text{CO}_2]$ and WA was evident (Table 1).

3.2.2. Light-Saturated Stomatal Conductance ($G_{S_{\max}}$) and Water Use Efficiency (WUE)

Generally, the only significant effect on $G_{S_{\max}}$ and E_{\max} (light-saturated rate of transpiration) was the tree species (TS); rather surprisingly, $[\text{CO}_2]$ and water availability themselves had no effect (Table 1). There were, however, some interactions. While $G_{S_{\max}}$ decreased under WL conditions in spruce saplings at both $[\text{CO}_2]$ and oak saplings at EC, an opposite response to WL was observed in broadleaved oak saplings under AC conditions. EC reduced $G_{S_{\max}}$ in spruce irrespective of water availability, but it was true for oak only under WL conditions (Figure 3E,F). The effect of EC was, however, not statistically significant at any water availability. Since the $G_{S_{\max}}$ and E_{\max} are tightly interconnected, the same findings were noted for E_{\max} (Figure 4A,B). WUE, defined as A_{\max}/E_{\max} ratio, was significantly influenced by all factors investigated—tree species, $[\text{CO}_2]$, and water availability (Table 1). Moreover, significant interactive effects of tree species and $[\text{CO}_2]$, $[\text{CO}_2]$ and water availability, as well as tree species, $[\text{CO}_2]$, and water availability on WUE were found. High $[\text{CO}_2]$ substantially increased WUE in all saplings under both water regimes, with the exception of the well-watered broadleaved oak (Figure 4C,D).

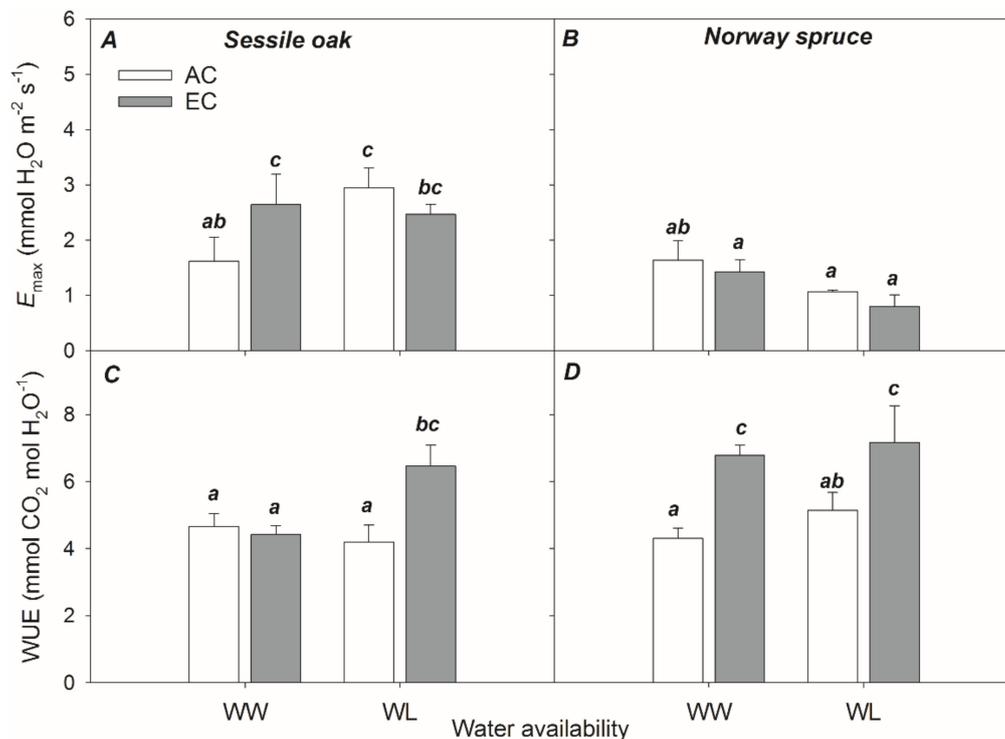


Figure 4. Effect of CO_2 concentration and water availability on transpiration rate (E_{\max} ; (A,B)) and water use efficiency (WUE; (C,D)) at saturating light intensity in sessile oak (left) and Norway spruce (right) saplings. The measurements were made during the second growing season of cultivation under manipulated conditions: AC—ambient CO_2 concentration ($\sim 400 \mu\text{mol CO}_2 \text{ mol}^{-1}$), EC—elevated CO_2 concentration ($\sim 700 \mu\text{mol CO}_2 \text{ mol}^{-1}$), WW—well-watered (regular watering of 20 mm per week during vegetation period), WL—water-limited (no irrigation for 5 weeks before the measurements). Means (columns) and standard deviations (error bars) are presented ($n = 3$). Different letters indicate significant differences ($p \leq 0.05$) between means based on Tukey's ANOVA post-hoc test.

3.2.3. Biochemical Parameters Derived from A–Ci Response Curves

In vivo rates of Rubisco carboxylation (V_C) and electron transport (J) were derived from A–Ci response curves measured at saturating light intensity and a temperature of

25 °C. Analysis of variance revealed significant effects of tree species and [CO₂] on both parameters, while water availability has significant effect only on *J* (Table 1). Moreover, significant interactive effects of tree species and water availability on *V_C* and *J* were found. In contrast, no interactive effects of [CO₂] with other investigated factors were observed (Table 1).

WL treatment increased *V_C* and *J* values under both [CO₂] conditions in sessile oak saplings, but not in Norway spruce (Figure 5). EC treatment tended to reduce *V_C* and *J* in oak as well as spruce saplings under both water regimes. These changes, however, are not statistically significant ($p > 0.05$). The *J/V_C* ratio for EC-WW (4.24) and AC-WL (6.42) were the lowest and highest, respectively, for sessile oak saplings, whereas EC-WL (3.98) and AC-WW (4.73) were the lowest and highest, respectively, for Norway spruce. Values of *V_C* and *J* were linearly correlated in both tree species irrespective of [CO₂] and water availability (Figure 6), suggesting a relatively constant balance between carboxylation and regeneration of RuBP.

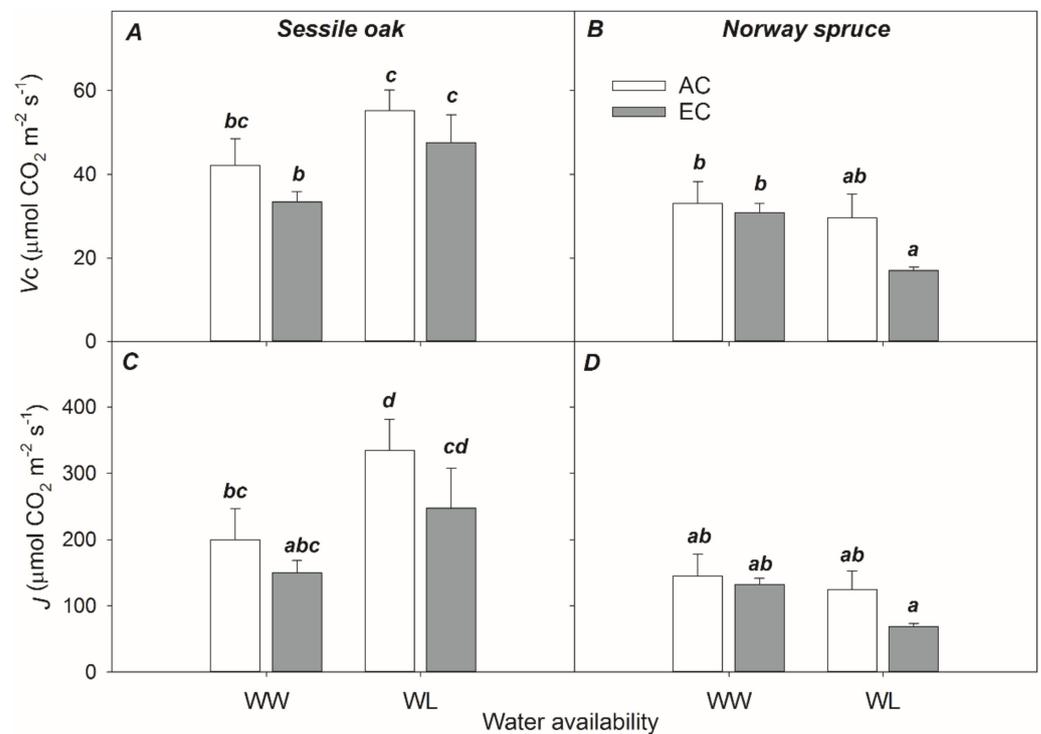


Figure 5. Effect of CO₂ concentration and water availability on in vivo rates of Rubisco carboxylation (*V_C*; (A,B)) and electron transport (*J*; (C,D)) in sessile oak (left) and Norway spruce (right) saplings. Both parameters were derived from *A*–*C_i* response curves estimated at saturating light intensity and temperature of 25 °C. The measurements were made during the second growing season of cultivation under manipulated conditions: AC—ambient CO₂ concentration (~400 μmol CO₂ mol⁻¹), EC—elevated CO₂ concentration (~700 μmol CO₂ mol⁻¹), WW—well-watered (regular watering of 20 mm per week during vegetation period), WL—water-limited (no irrigation for 5 weeks before the measurements). Means (columns) and standard deviations (error bars) are presented ($n = 3$). Different letters indicate significant differences ($p \leq 0.05$) between means based on Tukey's ANOVA post-hoc test.

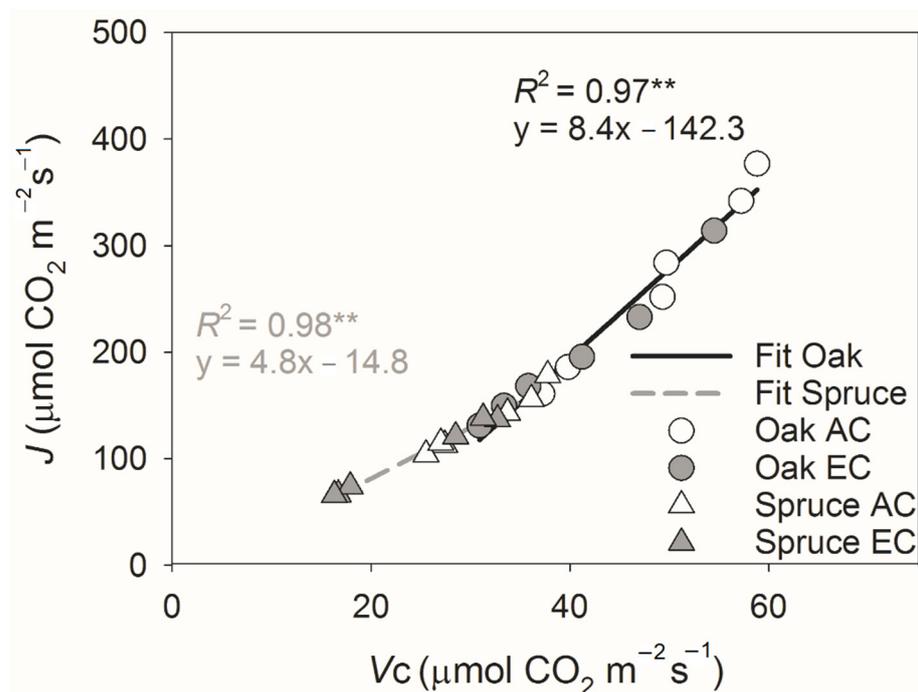


Figure 6. Relationship between in vivo rates of Rubisco carboxylation (V_C) and electron transport (J) at 25 °C in sessile oak and Norway spruce. The solid and dashed lines represent the linear regressions (Fit) of oak and spruce data, respectively. Coefficient of determination (R^2) and significance level (**; $p < 0.01$) are shown.

3.3. Chl-F Parameters

Maximum quantum yield of PSII photochemistry (F_v/F_m) ranged between 0.790 and 0.810 (data not shown). The F_v/F_m ratio was not influenced by any investigated factor and/or their interactions. Under saturating light conditions, species-specific effects of $[CO_2]$ and water availability on Φ_{PSII} and D were observed (see statistically significant interactions $TS \times [CO_2]$ and $TS \times WA$ in Table 1). While Φ_{PSII} values increased with increasing $[CO_2]$ and reduced water availability in oak, opposite trends were found in spruce (Figure 7A,B). These differences were, however, statistically not significant ($p > 0.05$). In contrast to Φ_{PSII} changes, EC-induced differences in D were mostly statistically significant ($p \leq 0.05$; Figure 7C,D). Significant interactive effect of $TS \times [CO_2] \times WA$ on investigated fluorescence parameters was not observed.

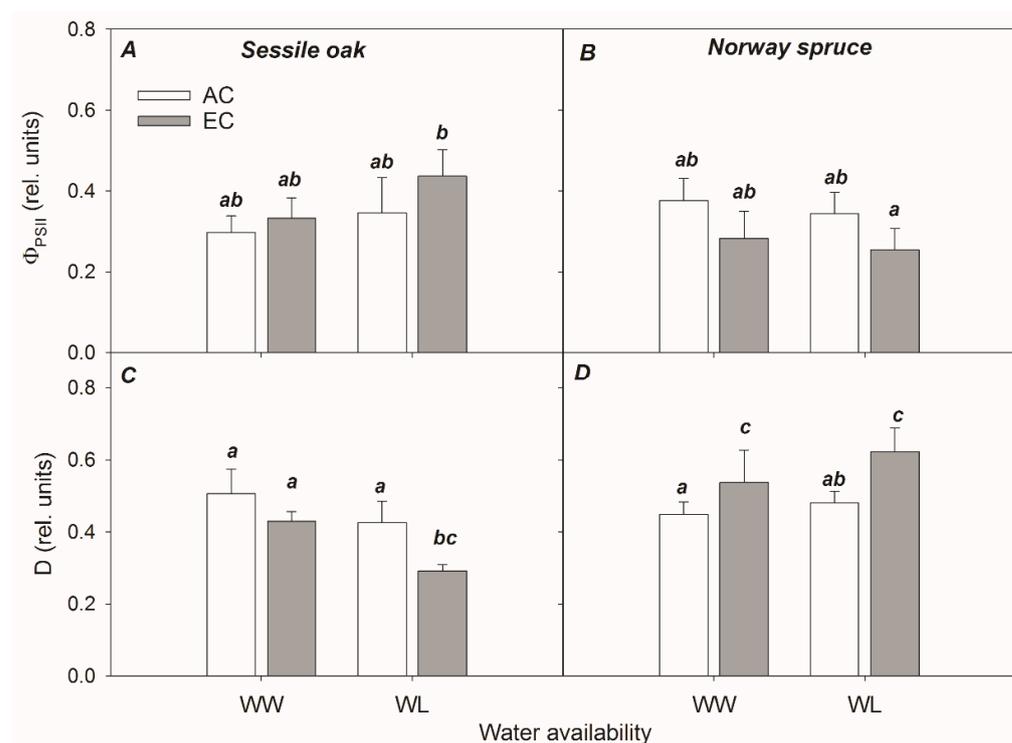


Figure 7. Effect of CO₂ concentration and water availability on actual quantum yield of PSII photochemistry (Φ_{PSII} ; upper panels) and thermal energy dissipation (D; bottom panels) under saturating light intensity in sessile oak (left) and Norway spruce (right). Means (columns) and standard deviations (error bars) are presented ($n = 3$). Different letters indicate significant differences ($p \leq 0.05$) between means based on Tukey's ANOVA post-hoc test. AC—ambient CO₂ concentration ($\sim 400 \mu\text{mol CO}_2 \text{ mol}^{-1}$), EC—elevated CO₂ concentration ($\sim 700 \mu\text{mol CO}_2 \text{ mol}^{-1}$), WW—well-watered (regular watering of 20 mm per week during vegetation period), WL—water-limited (no irrigation for 5 weeks before the measurements).

4. Discussion

4.1. Evaluation of Experimental Conditions

The experiment was carried out on a mountainous site receiving usually a large amount of precipitation (100–150 mm) during the summer months (July–August). The regular watering (20 mm per week) was designed to ensure a common total amount of natural precipitations, while water-limited treatment allowed no moisture into the soil for two months. Such experimental design agrees with expectations of increased frequency, duration, and severity of summer drought periods in the central European region [27]. Applied water regimes led to the desiccation of topsoil layers (5–10 cm), while the soil water content in deeper soil layers (30–40 cm) was unaffected by exclusion of precipitation. Although the experimental conditions led to a reduction of leaf water potentials in both tree species studied (Figure 2), changes in water potentials indicate a rather mild drought stress in both tree species investigated and particularly in oak. Species-specific differences are likely caused by the different architecture of the root system typical for these two species. We assume that the gradual desiccation of topsoil layer influenced particularly shallow-rooting spruce, while deep-rooting oak saplings remained less affected by the exclusion of precipitation. This assumption is supported by higher (less negative) $\Psi_{\text{pre-dawn}}$ values found in oak than spruce saplings (Table 1).

4.2. Species Specificity in Response to Elevated CO₂ and Water Availability

Elevated [CO₂] induces many adaptive responses in plants, including reduced stomatal conductance, increased WUE, and stimulated root growth as well as accumulation of osmolytes and phyto-hormones [28–31]. Since these responses can increase resistance

of plants to drought, enhanced resistance of future plants grown under EC conditions is likely. To test this hypothesis, tree species with distinct drought sensitivity and carbon sink capacity were selected. While oak species are known as drought-tolerant species [17,32], spruce is significantly more drought-sensitive [15,16,33]. Higher sensitivity of spruce to water-limited conditions was indeed evident in the current study by the more pronounced reduction of leaf water potential (Figure 2), CO₂ assimilation rate, transpiration, and increased WUE (Figures 3 and 4). In general, measured values of physiological parameters are not so much reduced as observed in other studies on related tree species [15–18,34]. Exclusion of summer precipitation resulted even in slight increases of V_C and J values in oak saplings. The stimulatory effect of mild stress conditions was observed also in other plant species [33,35].

Our results further show significant effect of tree species (Table 1) on most of the gas-exchange, chlorophyll fluorescence, and biochemical parameters investigated. In accordance with previous studies [6,25,28,35–37], we have found higher CO₂ assimilation rate and capacity in well-watered broadleaved than coniferous trees under ambient [CO₂] (Figure 3). Such findings are in agreement with higher values of V_C , J , and G_{Smax} in broadleaved saplings (Figures 3 and 5) and document higher strength of carbon sinks in oak as compared to spruce.

Analysis of variance revealed the significant interactive effects of tree species and water availability on almost all parameters investigated (Table 1), while the significant interactive effects of tree species and [CO₂] were found only for A_{sat} , WUE, and both fluorescence parameters. Similarly, $TS \times [CO_2] \times WA$ had significant effects only on the assimilation capacity and parameters associated with WUE. Generally, well-watered oak saplings had higher stimulation of A_{max} by EC as compared to spruce saplings.

Our experiment showed that photosynthesis of *Q. petraea* was stimulated by elevated [CO₂] (an increase of the A_{max}), but the stimulatory effect of elevated [CO₂] diminished in *P. abies* under the WL conditions. Others have also shown that photosynthesis of sessile oak could be stimulated when subjected to mild drought (simulated or natural) and CO₂ [38]. Nevertheless, most of physiological processes associated with photosynthetic carbon uptake tend to decline under severe drought conditions irrespective of [CO₂] treatment [18,34]. As expected, water use efficiency improved too, especially in spruce saplings. However, enhancement of WUE in oak saplings was observable only under the combined effect of WL and elevated [CO₂]. Such variability could be explained by a stronger CO₂ effect on stomatal closure in broadleaved than in coniferous tree species [28,39].

4.2.1. Fluorescence Parameters

Primary photochemical reactions measured as a quantum yield of PSII photochemistry (Φ_{PSII}) and thermal energy dissipation (D) show substantial species-specific response to a combined treatment of EC and WL. While Φ_{PSII} tended to increase in oak under combined EC and WL conditions, substantial decrease was found in spruce (Figure 7). Such findings are consistent with species-specific responses in G_{Smax} . Higher availability of intercellular [CO₂] and faster A_{max} in oak represents higher sink strength for primary photochemical products ATP and NADPH, consequently leading to a higher rate of electron transport measured as Φ_{PSII} [40]. In contrast, reduced carbon sink capacity in spruce saplings resulted in increased demands for alternative pathways of absorbed radiation energy, including non-radiative thermal dissipation (D). Such phenomena have been observed in many stressed plant species having closed stomata [12] and/or having reduced rate of photosynthetic CO₂ uptake [41]. Reduced efficiency of photochemical reactions may, vice versa, lead to a deepening of photosynthetic down-regulation as reported in plants exposed to combined heat and drought stress [42] or elevated [CO₂] when the stomata remain closed [5,43].

4.2.2. Gas-Exchange Parameters

We have found evidence of substantial photosynthetic down-regulation in spruce saplings exposed to combined impact of EC and WL, but not in oak which probably has access to water in the lower layers of the soil. Down-regulation was associated with decline in both CO₂ assimilation capacity (A_{sat}) as well as light-saturated rate of CO₂ assimilation (A_{max} ; Figure 3). These findings are in agreement with reduced values of J and V_C (Figure 5) reflecting the reduction of RuBP- and Rubisco-limited rate of photosynthesis, respectively [44]. Such phenomena are often observable in sink-limited coniferous trees, while EC usually stimulates CO₂ uptake in deciduous trees over long time periods [13,45]. Here we have shown, in addition, that down-regulation of photosynthesis is accelerated by insufficient water availability. Such interactive effects have received only limited attention so far [4,46]. Reduced J is in accordance with reduced Φ_{PSII} value as described above. Suppressed V_C in spruce saplings has been earlier linked to a decline in the total amount of Rubisco enzyme, and/or an increase of the proportion of inactive and active Rubisco forms [44]. Generally, biochemical adjustment, including phosphorus redistribution, nitrogen dilution, and/or inhibition of photosynthetic genes that are associated with photosynthetic down-regulation, are induced by an accumulation of non-structural assimilates in leaves [13,29].

Species-specific responses of trees to combined effects of elevated [CO₂] and limited water availability could arise from their biological and environmental diversity [39], different feedback mechanisms, such as their ability to export assimilates from leaves to roots and avoiding photosynthetic down-regulation [47]. Moreover, plant metabolic activities can play a key role in the modulation of photosynthetic down-regulation through an accumulation of certain compounds under stress conditions. Drought, among others, triggers increases in contents of some monosaccharides, disaccharides, and their derivatives. These sugars serve as osmolytes, anti-oxidative protectants, and provide energy during drought stress to minimize negative impacts of drought on plants [31]. Accordingly, we assume that an over-accumulation of these assimilates protecting plants against stress conditions may consequently lead to a transient down-regulation of photosynthesis under elevated [CO₂].

On the other hand, long-term exposure to elevated [CO₂] is known to stimulate root growth [30,48], rooting depth, and proportion of fine roots [49]. Development of the root system could lead to a better acquisition of water and mineral nutrients having a positive effect on stomatal conductance and photosynthesis [50,51]. Accordingly, such morphological adjustment results in a positive effect of elevated [CO₂] on photosynthesis in arid regions [4,52], and/or periods of insufficient water availability [13]. In accordance with these findings, we observed an increase of WUE under elevated [CO₂] in deep-rooting oak only under WL conditions, while it remained unchanged in well-watered oaks (Figure 4). In contrast, [CO₂]-enhanced WUE was found in shallow-rooting spruce irrespective of water regime. Similar species-specific responses were reported by Picon et al. [17] in drought-tolerant *Q. petraea* and drought-avoiding *Pinus pinaster*.

5. Conclusions

Limited research has explored responses of mountainous trees to future climate conditions. Accordingly, we have investigated species-specific responses of two widespread tree species of the temperate zone—Norway spruce and sessile oak—exposed to a combined impact of elevated [CO₂] and excluded summer precipitation. The experimental setup resulted in a desiccation of the topsoil layers, but not deeper ones. Limited water availability influenced particularly shallow-rooting spruce, while deep-rooting oak saplings remained less affected as documented by higher $\Psi_{\text{pre-dawn}}$ values. In general, changes in leaf water potential revealed rather mild drought stress. Under water-limited conditions, down-regulation of photosynthesis by elevated [CO₂] was observed in drought-sensitive coniferous spruce, but it remained stimulated in drought-tolerant deciduous oak. Down-regulation was associated with reduction of RuBP- as well as Rubisco-limited rate of photosynthesis. We conclude that elevated [CO₂] increases resistance to reduced summer

precipitation in oak, but not in spruce. Our results thus suggest a strong species-specific response of trees and a limited compensatory effect of elevated [CO₂] against even mild drought conditions in coniferous spruce.

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