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Relation between drought-exposed photosynthetic apparatus and tree water deficit derived from stem diameter variations in Norway spruce seedlings

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Abstract

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Ten five-year-old seedlings of Norway spruce (*Picea abies* L. Karst) from the Western Carpathians were subjected to drought (30 days without irrigation) in laboratory conditions (D trees). The control group (C trees) were irrigated regularly. Parameters such as stem diameter variations (*SDV*), soil water potential (Ψ_S), gas exchange and chlorophyll *a* fluorescence were measured. Stem growth of D trees was significantly reduced below $\Psi_S = -0.3$ MPa, and completely stopped below $\Psi_S = -1.1$ MPa. Tree water deficit (*TWD*, calculated from *SDV*) of D trees started to increase substantially below the threshold $\Psi_S = -0.9$ MPa, and closely correlated with Ψ_S. Photosynthetic traits of D trees reacted synchronously with *TWD* during drought, and recovered after rehydration. Gas exchange and most of the chlorophyll *a* fluorescence parameters were tightly positively related to *TWD*. Growth and *TWD* parameters, as well as those of gas exchange and most of the chlorophyll *a* fluorescence parameters differed against C trees during drought. Thus, the parameters derived from *SDV* may serve as indicators of the functionality of photosynthetic apparatus.

Keywords

chlorophyll *a* fluorescence, dendrometers, photosynthesis, *Picea abies*, water scarcity

Introduction

Due to the increasing severity of drought, the decline of Norway spruce forests was observed many years ago (Mäkinen et al., 2001). Norway spruce exhibits relatively high sensitivity to water stress (Ge et al., 2013; Oberhuber and Mennel, 2010), and is considered to be an isohydric species because the trees close their stomata immediately during the start of a drought event (Klein, 2014; Rötzer et al., 2017). This reaction prevents the trees from high water losses and disruptions of the water balance but may lead to carbon starvation (e.g., Klein, 2014; Rötzer et al., 2017).

Climate models predict an increase in annual air temperature for Central Europe, and a seasonal shift in the distribution of precipitation, principally from summer to late winter and spring (Christensen and Christensen, 2007), which will lead to a higher frequency and severity of drought periods in the near future (Orlowsky and Seneviratne, 2012). As a consequence, it is predicted that there will be an overall decline in tree vitality and an increase in tree mortality. Such conditions may result in a massive dieback of spruce ecosystems (Hlásny et al., 2021; Vanická et al., 2020), caused by the combined effects of biotic and abiotic stresses (Altmanet al., 2017; Schurman et al., 2018). For example, the summer of 2018 is considered the hottest and driest in the last 500 years in Europe (Salomón et al., 2022; Schuldt et al., 2020), and

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its significant negative impact on spruce growth at lower suboptimal altitudes has been reported (Krejza et al., 2021; Neuwirth et al., 2021). Thus, developing a better understanding of Norway spruce sensitivity to drought stress and mechanisms governing drought response is important.

Physiological processes reflect the damage to the individual tree early before it is visibly distinguishable, either on the tree or at the ecosystem level. Therefore, studying physiological processes can provide reliable information about the vitality of the studied individuals (Konôpková et al., 2018). One of the most responsive processes in changing the environment is photosynthetic performance. Under drought stress, CO_2 assimilation decreases, leading

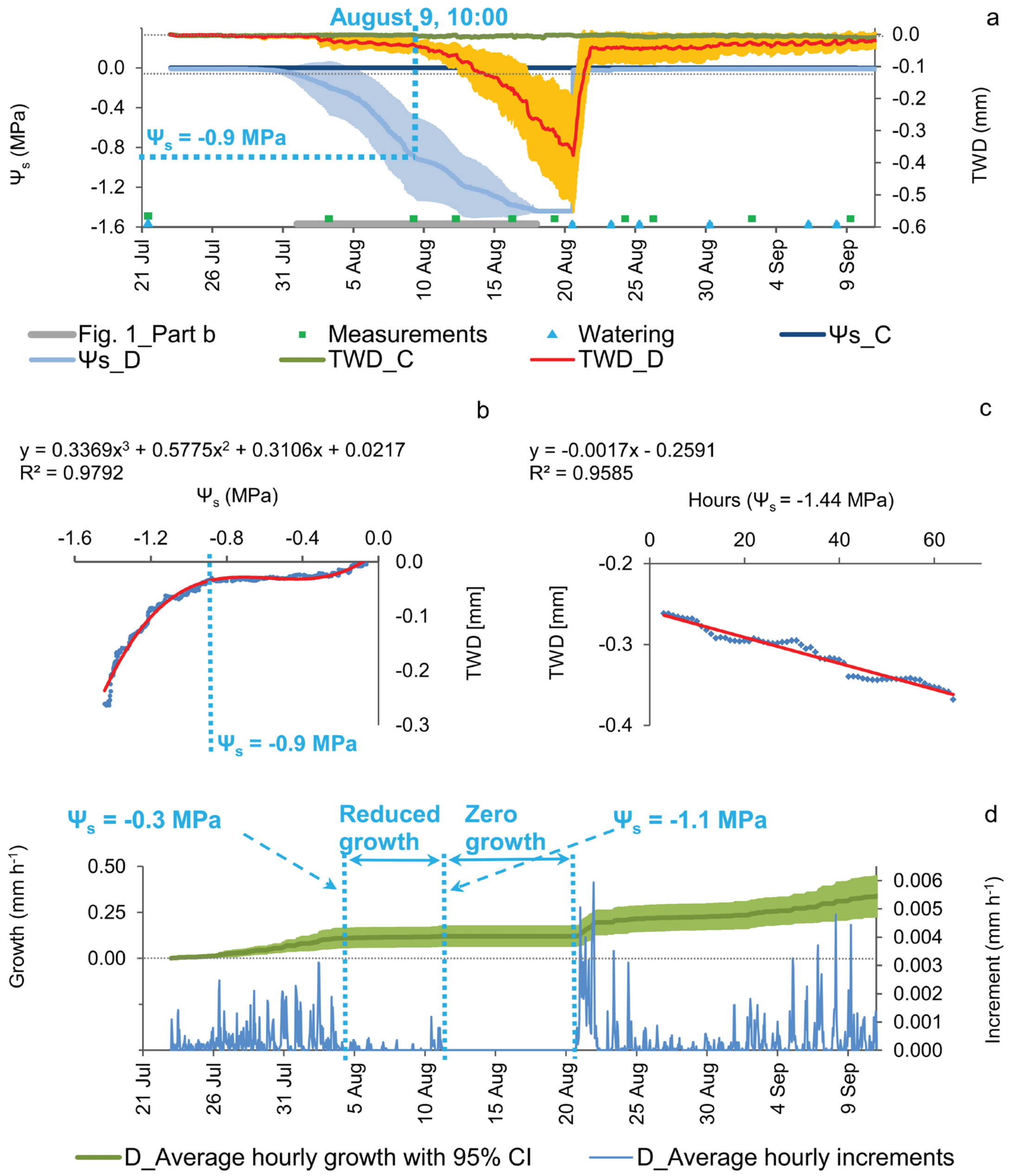

Fig. 1. (a) Average soil water potential (Ψ_S) of drought-treated (D, light blue) and control (C, dark blue) trees, and average tree water deficit (*TWD*) of D (red) and C (green) trees. Vertical lines represent 95% confidence intervals. Small green rectangles indicate dates of physiological measurements on D trees (referenced in Fig. 2, 3, and 4). Small blue triangles indicate watering dates for D trees. The horizontal grey line marks the period of continuous Ψ_S decline in D trees, from August 1 at 00:00 to August 17 at 22:00, for which the relationship between Ψ_S and *TWD* is shown in part (b). (b) Relationship between the average Ψ_S and average *TWD* of D trees during the time window indicated in part (a) (blue circles). A third-order polynomial is fitted to these values (red line). The dashed cyan line represents $\Psi_S = -0.9$ MPa, reached on August 9 at 10:00 am. (c) Relationship between time (in hours during which the average $\Psi_S = -1.44$ MPa) and the average *TWD* of D trees from 22:00 on August 17 to 13:00 on August 20. A linear regression is fitted to the data (red line). R² denotes the coefficient of determination, indicating the goodness of fit. (d) Average growth (green) and increment (blue) values of D trees. Vertical lines represent 95% confidence intervals.

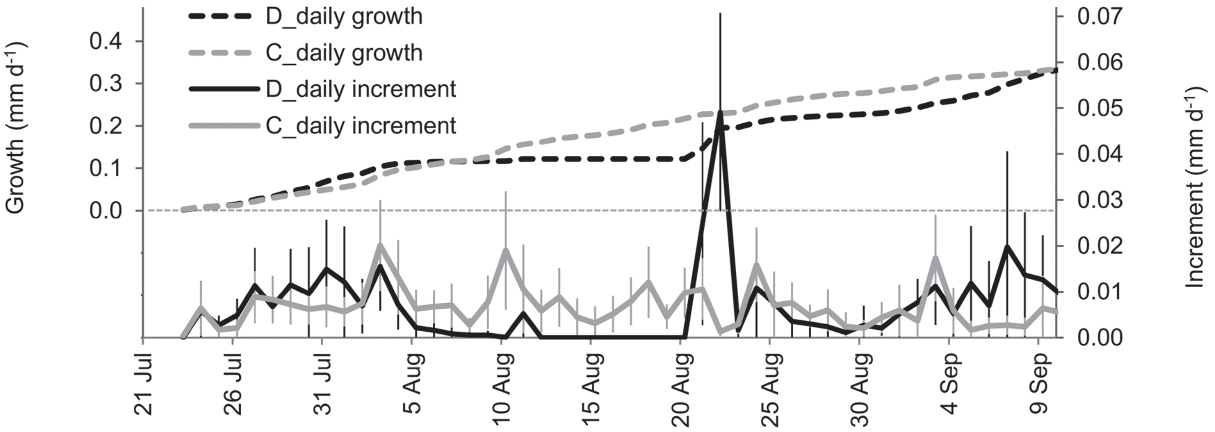

Fig. 2. Average daily growth (dashed black line) and increment (solid black line) of D trees, and average daily growth (dashed grey line) and increment (solid grey line) of C trees. Vertical lines represent 95% confidence intervals of the increments.

to the decline of transpiration and stomatal closure, followed by other photosynthetic responses that are directly connected to the severity and length of the drought. Severe drought affects photosynthesis negatively from the reduction of CO_2 fixation, Rubisco activity reduction, and photorespiration to damages of several photosystem II (PSII) components, where PSII represents the most sensitive structure of photosynthetic apparatus. Chlorophyll *a* fluorescence kinetics reflects the performance or damage of PSII and its components under drought conditions (Brestic et al., 2012; Tang, 2002).

Likewise, stem size variations monitored using high-resolution dendrometers, are considered to be an effective tool for providing information on tree production, stress status, and physiological processes in trees (Krejza et al., 2021; Offenthaler et al., 2001; Salomón et al., 2022). The obtained complex signals represent tree stem irreversible growth and reversible fluctuations associated with the water balance of stem tissues. Longer-lasting contractions of stem diameter, generally referred to as tree water deficit (*TWD*), arise as a consequence of more severe and prolonged drought (Zweifel et al., 2016).

Although several studies are focused on the relationships between stem shrinkage and photosynthetic traits, mainly aimed at agricultural crops (Ohashi et al., 2006), only a few are connected to tree species widely spread in Europe (Lindfors et al., 2015). Here, we evaluate the effect of drought on these parameters and their mutual relations in 5-year-old spruce seedlings in a manipulative experiment. We hypothesized that, concurrently, with more severe and more prolonged drought: (1) diameter growth was reduced and *TWD* increased, (2) drought was reflected in gas exchange and chlorophyll *a* fluorescence, and (3) *TWD* was closely linked to gas exchange and chlorophyll *a* fluorescence.

Materials and methods

Plant material and experimental design

We studied five-year-old seedlings of Norway spruce (*Picea abies* (L.) H. Karst) representing the natural distribution of Norway spruce in the Western Carpathians. The origin of individuals was distributed along an altitudinal gradient from 650 to 1,500 m asl. The long-term mean annual air temperature ranged from 3.4 to 6.9 °C, and annual precipitation ranged from 808 to 1,155 mm. The seeds originated from the gene bank of the Forests of the Slovak Republic, State Enterprise (OZ Semenoles Liptovský Hrádok, Slovakia) and were sown in an experimental research plot of the Mlyňany Arboretum of the Slovak Academy of Sciences (western-central Slovakia). After 2 years, the seedlings were transplanted into containers with a height of 14 cm and a diameter of 10 cm, with 4.5 litres of soil volume with a purified substrate of Baltic peat moss (*Sphagnum balticum*, Russow).

Five-year-old seedlings were transported to the Institute of Forest Ecology laboratory three weeks before the experiment to acclimate to the lab conditions. The experiment was performed from 21 July to 10 September 2022. Seedlings were equally divided by the origin of natural distribution into two groups, drought treated (D) and control (C, irrigated). The initial seedling diameter and height of 10 drought-treated trees were 11.5 mm (standard deviation (sd) = 1.6 mm) and 70.1 cm (sd = 7.9 cm), respectively. The same parameters of 10 control (irrigated) treated trees were 11.6 mm (sd = 1.3 mm) and 74.5 cm (sd = 8.3 cm).

We simulated daylight using halogen lamps with gradually increasing light intensity from 150 to 400 µmol–2 s^{-1} for 14 hours (from 06:00 to 20:00). The absence of light created night conditions lasting 10 hours (from 20:00 to 06:00). The average daily air temperature was 22.8 °C and relative air humidity 60%. Seedlings were exposed to drought without watering from 21 July to 19 August. From 20 August, seedlings were regularly irrigated until the end of the experiment.

Diameter variations, extraction of Tree water deficit (*TWD*), and Soil water potential (Ψ_S) measurements

Variations of stem diameters were monitored continuously for 20 min intervals using a non-invasive high-resolution PDS40 SDI sensor (*EMS Brno*, Czech Republic), which was mounted at ca. 15 cm height of the tree stem. We

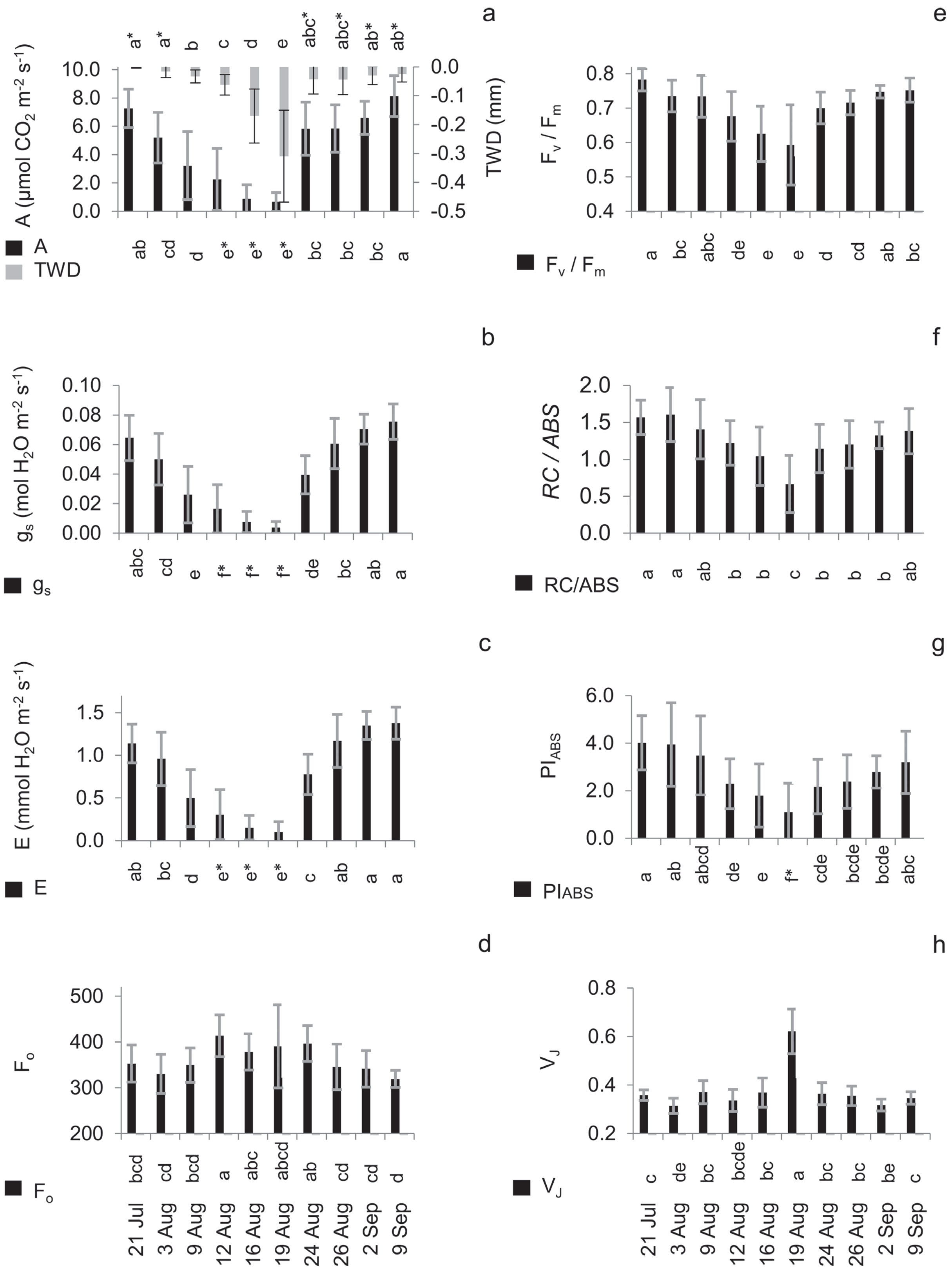

Fig. 3. (a) Average tree water deficit of D trees (*TWD*, grey) and average photosynthetic rate (*A*, black), (b) average stomatal conductance (g_S), (c) average transpiration rate (*E*), (d) average basal fluorescence (F_0), (e) average maximum quantum yield of PSII photochemistry (F_v/F_m), (f) average number of active reaction centers (*RC/ABS*), (g) average photosynthetic performance index (PI_{ABS}), and (h) average relative variable fluorescence at 2 ms (V_J). Vertical lines represent 95% confidence intervals. Letters a, b, etc., denote homogeneous groups at $P < 0.05$, sorted in descending order of mean values. Asterisks (*) indicate means that are statistically equal to zero ($P < 0.05$).

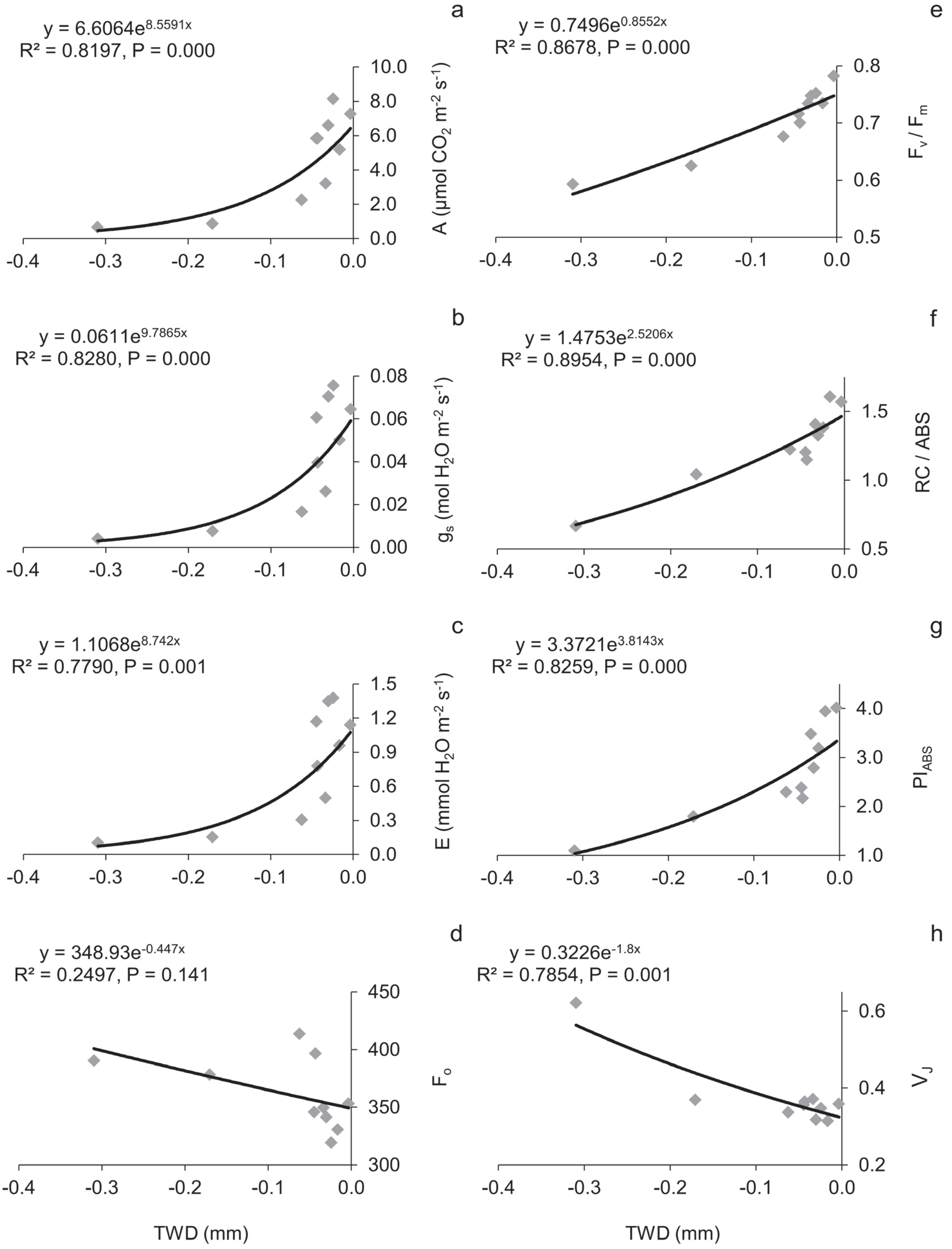

Fig. 4. (a–h, grey points): Relationships between average tree water deficit (*TWD*) and (a) average photosynthetic rate (*A*), (b) average stomatal conductance (g_S), (c) average transpiration rate (*E*), (d) average basal fluorescence (F_0), (e) average maximum quantum yield of PSII photochemistry (F_v/F_m), (f) average number of active reaction centers (*RC/ABS*), (g) average photosynthetic performance index (PI_{ABS}), and (h) average relative variable fluorescence at 2 ms (V_J). (a–h, black lines): Exponential functions representing the relationships between average *TWD* and the above gas exchange and fluorescence parameters. R² indicates the coefficient of determination, and P represents statistical significance.

used one-hour averages of tree stem diameter for calculations. We used the zero-growth concept (e.g., Zweifel et al., 2016, 2021) for the calculation of water-related and growth components of stem radius variation. This method assumes that diameter variations below the preceding maximum from the dendrometer time series are considered as *TWD*. Growth increases when the stem diameter exceeds its previous maximum in the seasonal time series of stem diameters. Differences between consecutive values were used to create chronologies of increments. We used hourly growth and increment values for Fig. 1d. The daily values of growth and increment (Fig. 2) discussed in the text were calculated from values at 06:00 (i.e. morning). The calculations started two days after the installation (21 July) when the instruments sat properly on the stems of the seedlings. For comparison with other parameters (Fig. 3, 4, S1), we use the value from 23 July as the first value (for 21 July). The values of *TWD* at 08:00 were used for comparison with gas exchange and fluorescence parameters in the below-mentioned Figures and analyses.

Soil water potential measurements (Ψ_S [MPa]) were continuously monitored at 1 h intervals in each pot by calibrated gypsum block (*Delmhorst Inc.*, USA) installed at 10 cm depths and stored in data loggers (*EMS Brno*, Czech Republic). The soil water potential values were in the range of 0 up to –1.44 MPa, which were at the lower measurable limit of the equipment.

Gas exchange measurements

We used a Li-6400XT open gasometric system with a chamber fitted with a 6400–02B LED light source (*LI-COR Inc.*, Lincoln, NE, USA) for gas exchange and water–use efficiency measurements. The reference CO_2 concentration was 400 µmol mol^{-1}, photosynthetic active radiation was maintained at 1,500 µmol m^{-2} s^{-1} and the system temperature was 24 °C inside the chamber. Values of the CO_2 photosynthetic rate (A), transpiration rate (E), and stomatal conductance to water vapour (g_S) were recorded immediately after the adaptation of leaves inside the chamber when the values of CO_2 assimilation rate persisted steady (1–2 min).

Fast kinetics of chlorophyll *a* fluorescence measurements

We used the portable fluorimeter *Handy PEA* (*Hansatech Instruments, Ltd.*, United Kingdom) for the measurements of the PSII performance. We adapted needles to dark conditions using the leaf clips for 30 minutes. After dark adaptation, needles were illuminated by the saturation pulse with high radiation intensity (3,500 µmol m^{-2} s^{-1}) to enhance chlorophyll *a* fluorescence for 1 s. Every 10 µs, chlorophyll *a* fluorescence was detected to attain a polyphasic fluorescence curve with an OJIP shape (Strasser et al., 2004). We determined the basic fluorescence parameters in dark-adapted leaves: the basal fluorescence (F_0), measured 50 µs after the enlightenment of the saturation pulse, the maximal fluorescence (F_m), the variable fluorescence (F_v), and the maximum quantum yield of the photochemistry of PSII (F_v/F_m), calculated as the ratio between the variable chlorophyll *a* fluorescence (F_v) and the maximum of the chlorophyll *a* fluorescence (F_m). We also quantified the performance index of photochemical activity based on the absorption (PI_{ABS}) and the number of active reaction centres per antenna in PSII, based on the absorption (*RC/ABS*), as well as the relative variable fluorescence at 2 ms (V_J, J-step of fluorescence OJIP curve).

Statistical analysis

Statistical analysis was performed using Statistica® statistical software (Statsoft, Tulsa, OK, USA) and Excel (MS Office). Homogeneous groups of individual variables (Fig. 3) were determined based on paired t-tests at $P < 0.05$. Statistical significance of their differences from zero ($P < 0.05$) was tested by t-tests. For comparison between different treatments and dates (Table 1) we used ANOVA and post hoc comparison by Tukey HSD tests.

Coefficients of determination (R^2) represent the goodness of fit of the analysed data sets to the individual functions used (Fig. 1b – the 3rd order polynomial, Fig. 1c – linear function, Fig. 4 – exponential functions, Fig. S1 – linear functions).

Table 1. Statistical differences in leaf gas exchange parameters and the parameters of fast kinetics of chlorophyll *a* fluorescence measured in needles of Norway spruce seedlings. The data presented are the means ± SD (n = 10). Different letters indicate significant differences among the variants (C – control and D – drought) and the period of the experiment after Tukey HSD tests ($p < 0.05$).

Period of the experiment	Variant	A (µmol CO_2 m^{-2} s^{-1})	g_S (mol H_2O m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	F_0	F_v/F_m	*RC/ABS*	PI_{ABS}	V_J
1st day of the experiment	C	7.74 ± 2.9^{a}	0.067 ± 0.03^{a}	1.16 ± 0.3^{a}	350 ± 39^{ab}	0.798 ± 0.02^{ab}	1.64 ± 0.4^{a}	4.5 ± 1.7^{a}	0.33 ± 0.1^{a}
	D	7.27 ± 2.1^{a}	0.065 ± 0.02^{a}	1.14 ± 0.4^{a}	353 ± 62^{ab}	0.783 ± 0.05^{ab}	1.57 ± 0.4^{a}	4.0 ± 1.8^{a}	0.36 ± 0.0^{a}
29 days without irrigation	C	5.81 ± 2.3^{a}	0.062 ± 0.02^{a}	1.19 ± 0.4^{a}	272 ± 39^{a}	0.824 ± 0.01^{a}	1.37 ± 0.4^{a}	3.7 ± 1.7^{ab}	0.43 ± 0.1^{a}
	D	0.66 ± 1.1^{c}	0.004 ± 0.01^{b}	0.10 ± 0.2^{b}	390 ± 138^{b}	0.593 ± 0.19^{c}	0.67 ± 0.6^{b}	1.1 ± 1.9^{b}	0.62 ± 0.1^{b}
7 days of recovery	C	4.09 ± 1.8^{b}	0.043 ± 0.02^{a}	0.84 ± 0.3^{a}	369 ± 53^{ab}	0.680 ± 0.15^{bc}	1.31 ± 0.5^{a}	2.6 ± 1.6^{ab}	0.38 ± 0.1^{a}
	D	5.84 ± 2.6^{ab}	0.061 ± 0.03^{a}	1.17 ± 0.5^{a}	346 ± 76^{ab}	0.716 ± 0.06^{abc}	1.20 ± 0.5^{ab}	2.4 ± 1.8^{ab}	0.36 ± 0.1^{a}

Results

Dynamics of Soil water potential and Tree water deficit (*TWD*), Growth

The average Ψ_S of D trees started to decrease continuously after 28 July (Fig. 1a) until it reached the lowest limit value of –1.44 MPa (this was the lowest measurable limit of the equipment) on 17 August at 24:00, and remained at this value until 20 August (13:00). Subsequently, after irrigation, within 1 h it returned sharply to values close to 0 (–0.02 MPa). The average Ψ_S of C trees remained during whole observation close to zero with low variability.

The decrease in Ψ_S of D trees was accompanied by an obvious contraction (decrease of *TWD* values) of tree trunks, which was especially accelerated after 9 August, when Ψ_S at 10:00 dropped ca. below –0.9 MPa (Fig. 1a, b). Until this time, the contraction of the stems did not exceed the values of *TWD* = –0.036 mm, but until then contraction was maintained below this level, approximately from the beginning of August after the decrease of Ψ_S approximately below the level of –0.15 MPa (Fig. 1b). Here, we have to take into account that this is an average value, and the measuring device was limited by a value of –1.44 MPa. After the first sensors reached this value, they remained there, even though the water potential probably continued to decrease. Also, when all of the sensors reached –1.44 MPa on 17 August, there was probably a further decrease and, therefore, the values of *TWD* compared to the real values of Ψ_S in Fig. 1b would not decrease so sharply. The average velocity of shrinkage was 0.0011 mm h^{-1} during this period. Following that, all of the sensors reached –1.44 MPa, *TWD* values correlated well and decreased linearly with time during which (64 hours) they were under this level (Fig. 1c). The average velocity of shrinkage was 0.0017 mm h^{-1} during this period. The maximum contraction of the trunks (*TWD* = –0.3769 mm, which represented 3.3% of the initial diameter) was reached on 20 August at 14:00. It was followed by a sharp expansion (swelling) of the trunks by an average value of –0.043 mm on 21 August at 20:00. Based on confidence intervals, the value statistically equal to zero was reached, for the first time, on 21 August at 05:00 (16 hours after irrigation), with an average *TWD* = –0.159 mm, which represented the average swelling velocity of 0.0145 mm h^{-1}. The subsequent course was not statistically different from zero until the end of the monitored period on 10 September. The average *TWD* of C trees remained during the whole observation close to zero with low variability (Fig. 1a).

After 4 August, we observed significantly reduced growth (Fig. 1d, 2) of D trees, when daily increments decreased and started to be statistically non-significant after ΨS dropped below –0.3 MPa. The average daily increments were zero from 12 August (Ψ_S = –1.1) until 20 August. Daily increments of D trees became statistically positive again on 21 August (Ψ_S = –0.02 MPa). We found positive correlations between Ψ_S and stem diameter increments of D trees, on both an hourly (r = 0.253, p = 0.000) and a daily (r = 0.474, p = 0.000) basis. For the period from 4 August (6:00) to 22 August (6:00), correlations were higher for both hourly (r = 0.478, p = 0.000) and daily increments (r = 0.673, p = 0.002). Both D and C trees reached almost the same growth at the end of the experiment but with different dynamics throughout observation (Fig. 2). The daily increments of C trees reached more-less balanced values throughout the observation. The daily increments of D trees became zero during drought and then accelerated sharply (with high variability) after watering, as well as towards the end of the observation.

In Fig. 3a we can see the values of *TWD* for individual dates of measurements of gas exchange and chlorophyll fluorescence of D trees. These were statistically significantly different from zero on 9, 12, 16, and 19 August, with the most significant decrease against the other dates on 16 and 19 August.

The relatively high variability persisted in *TWD* data following watering on 20 August. This was due to the fact, that two trees did not reach their initial diameter, and permanently stayed slightly shrunk. Also, logically, we did not record growth on them after drought treatment.

Dynamics of Gas exchange and Fast kinetics of chlorophyll *a* fluorescence

As we can see in Fig. 3a, b, c, values of *A*, g_S, and *E* of D trees continually decrease after 21 July until 19 August, when on 19 August they were only 9.0, 6.2, and 9.0% of the 21 July values, respectively. Their values were not statistically different from zero on 12, 16 and 19 August.

After the resumption of irrigation, there was a relatively sharp recovery of gas exchange parameters on 24 August to the level approximately before 12 August, and eventually (A, E) before 9 August. The values of *A*, g_S, and E reached 80.3, 61.2, and 68.1%, respectively, of their initial values on 21 July. Subsequently, the recovery continued until, on 9 September, we recorded the highest values during the entire experiment, statistically identical to the values from 21 July.

The values of F_0 did not show such an expressive drought-related trend (Fig. 3d), although they were slightly elevated on 12, 16, and 19 August, as well as on 24 August, after irrigation was restored. The responses of other parameters (F_v/F_m, PI_{ABS}, and *RC/ABS*) were similar to those of the gas exchange parameters (Fig. 3e, f, g), although decreases were observed 4–5 days later in the case of gas-exchange parameters. With increasing drought, there was a decrease in their gas-exchange parameter values to 75.8, 42.5, and 27.3% of the values from July 21. However, the water deficit reduced the *Fv/Fm* ratio close to the disturbance limit of 0.725. The average value of PI_{ABS} up to 19 August was not statistically different from zero. After the resumption of irrigation, we recorded the recovery of these parameters.

The average value of V_J was significantly highest on 19 August (Fig. 3h), during the most pronounced drought, with approximately the same and significantly lower values during measurements on the other dates.

Comparison with control treatment (Table 1) confirmed the emergence of statistically significant differences in most parameters between C and D trees during the drought period and subsequent recovery of the D group after the resumption of irrigation. During the experiment a significant decrease in net photosynthesis (*A*) was observed in the seedlings in the C group, however other gas exchange parameters (g_S, *E*) and parameters of photosynthetic biochemistry stayed relatively homogenous. Moreover, the D group after recovery reached slightly higher values of gas exchange parameters as well as chlorophyll fluorescence parameters compared to the C group. The findings indicated that photosynthetic capacity, which was negatively affected by drought stress enhanced after recovery and surpassed the values of gas exchange in the C group.

Links of Tree water deficit (*TWD*) to Gas exchange and Fluorescence parameters

As is shown in Fig. 4, we observed close positive relationships, especially between decreasing *TWD* values and the parameters of fluorescence F_v/F_m, *RC/ABS*, PI_{ABS} (Fig. 4e, f, g), as well as photosynthetic rate *A* (Fig. 4a), stomatal conductance g_S (Fig. 4b) and transpiration rate *E* (Fig. 4c). The relationships between TWD values and fluorescence parameters F_0 and V_J were negative (Fig. 4d, h) and also markedly weaker in the case of F_0. While the relationship was tight for V_J, it depended on only one value of increased V_j on August 19 during the most severe drought and lowest *TWD* (Fig. 3h). The relationships of *TWD* with *A*, g_S , and *E* (Fig. 3a, b, c) were well described by the exponential function just used. The relationships principally with F_0, F_v/F_m, *RC/ABS* and V_J were close to linear.

Since these results mainly depended on two extreme values during the most pronounced drought on August 16 and 19, we also used the non-parametric Spearman rank correlation method (Table 2) as well as the natural logarithm of the absolute values of *TWD* (Fig. S1). As is shown in Fig. S1 and Table 2, we noted close positive relationships (Fig. S1), especially between decreasing *TWD* values (be aware that higher values of the natural logarithm of absolute values represent a higher water deficit (more negative *TWD* values), in contrast, more negative values represent a smaller one) and the parameters of fluorescence F_v/F_m, *RC/ABS*, PI_{ABS} (Fig. S1e, f, g), as well as photosynthetic rate A (Fig. S1a), stomatal conductance g_S (Fig. S1b), and transpiration rate E (Fig. S1c). The relationships between *TWD* values and fluorescence parameters F_0 and V_J were negative (Fig. S1d, h), and also weaker. Spearman rank correlations (Table 2) gave us almost the same message.

Table 2. Values of Spearman rank correlations (p-significance level) between *TWD* and average photosynthetic rate (*A*), average stomatal conductance (g_S), average transpiration rate (*E*), average basal fluorescence (F_0), the average maximum quantum yield of the photochemistry of PSII (F_v/F_m), average number of active reaction centres (*RC/ABS*), average photosynthetic performance index (PI_{ABS}), average relative variable fluorescence at 2 ms (V_J) of D trees

Variables	Spearman R TWD	p-level
A	0.782	0.008
g_S	0.794	0.006
E	0.721	0.019
F_0	–0.636	0.048
F_v/F_m	0.952	0.000
RC/ABS	0.903	0.000
PI_{ABS}	0.927	0.000
V_j	–0.527	0.117

Discussion

Understanding the relationships between soil water dynamics and tree water use seems to be essential for current as well as future forest management (Klein et al., 2014), due to the expectation of more frequent and severe droughts in many parts of Europe in the future. The stem size variations that were registered by dendrometers reflect a complex mixture of various environmental variables. Studying their relationship to the physiology of trees can help us to expand the possibilities of their interpretation and use in monitoring physiology, stress load, and health status.

Dynamics of Diameter variations and Soil water potential

The stem size variations are, generally, linked to environmental factors, including drought stress. They are associated with two basic components: (i) seasonal growth of stem tissues (phloem and mainly xylem) via production and expansion of new cells produced by the cambium to build the transport system, store substances, and ensure mechanical support (e.g., Chan et al., 2016; Rossi et al., 2016; Steppe et al., 2015), and (ii) variations in the water balance of stem tissues. An imbalance in transpiration, transportation, and water uptake by roots of trees is connected with the depletion and recovery of tree water storage compartments. They serve as a water reservoir that buffer fluctuations in water uptake and transpiration, and are reflected in reversible stem size variations (e.g., Balducci et al., 2019; Betsch et al.; 2011, Köcher et al., 2012). These variations are connected with sap flow rates and related variations of water content, water potential, and its gradients (e.g., Steppe et al., 2006; Wei et al., 1999) of xylem conduits (e.g., Irvine and Grace, 1997; Offenthaler et al., 2001; Zweifel et al., 2014) or phloem elastic tissues (Čermák et al., 2007; Herzog et al., 1995). Eventually, the stem size variations are attributed, at least partially, to seasonal changes in osmotic concentration (Mencuccini et al., 2013) or hygroscopic changes in the bark related to humidity (Oberhuber et al., 2020). Therefore, they provide a sensitive measure of tree water status (Zweifel and Hasler, 2001). It is highlighted during drought periods and the high transpiration demands of the atmosphere. Thus, they are closely connected through a chain of relationships with basic physiological processes, such as stomatal conductance, transpiration, photosynthesis, etc.

Mild circadian variations of *TWD*, that we recorded (Fig. 1a), partially contrasted with other reports (e.g., Oberhuber et al., 2015; Zweifel et al., 2005). It could be the result of low daily dynamics in humidity and vapour pressure deficit. Trees were planted in pots and isolated from their surroundings. Thus they were not able to replenish water storage from the surroundings during the night, e.g., with the possible participation of mycorrhiza (Simard, 20188; Piovesan and Biondi, 2021).

The close relation between *TWD* and Ψ_S (Fig. 1a, b, c) was most probably mediated through the decrease of water potential of stems' hydraulic pathways, as a consequence of increasing soil drought. Decreasing *TWD* is known to reflect decreasing water potential within the storage tissues of the stem. This can result in stem shrinkage over several weeks, even during the wood growth period (Zweifel et al., 2006; Zweifel et al., 2016). When the soil is not fully hydrated, soil and stem water potentials are lower, and water storage pools are no longer replenished (Steppe et al., 2015). Stem shrinkage is reversed only after water is absorbed by roots or stored in tissues with higher water potential, which eliminates the water deficit in the stem (Nalevanková et al., 2018).

If there is enough soil water available, trees are rehydrated during one night, which also occurs when more prolonged cumulated morning contractions (morning *TWD*) are present. We also observed similar facts in the present study. It took 16 hours for the stems to be saturated with water again to values that were statistically equal to zero after being watered on 20 August, and the time delay could potentially be affected by the laboratory conditions. The needles and the bark of the seedlings were not moistened, as in the case of precipitation events in natural conditions. Small rain events and fog may induce the necessary relaxation of low water potentials within trees, and increase their water status (Goldsmith et al., 2017; Simonin et al., 2009; Zweifel et al., 2001).

According to Čermák et al. (2007), water stored in the xylem and phloem tissues can meet the transpirational requirements of certain trees for as long as a week. According to Oberhuber et al. (2015), spruce seedlings have been shown to use 10–65% of water reserves daily, depending on transpirational demands during sunny and cloudy days, respectively. Daily, mature sub-alpine spruce stems have been observed to lose between 0.2% and 0.5% of their volume as a result of bark dehydration, corresponding to about 2 to 5 litres of water, which contributed directly to transpiration (Zweifel and Hasler, 2001). In our case, we recorded a shrinkage that represented up to 3.3% of the initial diameter, which may result from the fact that we were studying seedlings. We can assume that bark, phloem, cambial zone, and sapwood represent a smaller part of the total stem diameter in mature trees than in seedlings. It not only represents a relatively larger water reservoir in seedlings than in mature trees, but also a relatively larger stem compartment affected by changes in water potential. Ježík et al. (2015) found that morning *TWD* was correlated with Ψ_S in both the irrigated ($R^2 = 0.53$) and to natural-drought exposed ($R^2 = 0.60$) groups of 25-year-old spruce trees. The sap flow rate was limited below $\Psi_S = –0.3$ MPa under conditions of higher potential evapotranspiration. In this context, (Lu et al. 1996) indicate a critical threshold value of the water potential for spruce that can cause xylem cavitation, a value close to –2.5 MPa. Zweifel and Hasler (2001) give a value of –2.3 ± 0.3 MPa as the threshold for the beginning of the use of xylem water reserves after the previous use of needle and bark reserves (including phloem and cambium). Brinkmann et al. (2016) found an exponential relationship between *TWD* and decreasing soil moisture, which led to a decrease in sap flow. This suggests a threshold response of *TWD* to decreasing soil moisture.

In our case, we noted a significant decrease in *TWD* after the average Ψ_S dropped below –0.9 MPa (Fig. 1a, b). However, Zweifel et al. (2021) give a value of Ψ_S= –0.9 MPa as the threshold for radial growth. Those authors associated high growth rates with high Ψ_S values between –0.006 to –0.065 MPa. Cabon et al. (2020) report a value of internal water potential of –1.1 MPa as the threshold for wood formation through its influence on turgor pressure, and strong inhibition of cambial activity below this threshold at all elevations along a steep elevational gradient. Peters et al. (2021) state the low probability of growth between –0.2 and –0.6 MPa and almost no growth below –0.6 MPa. It is less negative than the value limiting photosynthesis reported by Muller et al. (2011). In our case, we recorded reduced growth already after 4 August (Ψ_S= –0.3 MPa). Zero growth values were recorded after 11 August (Ψ_S = –1.1 MPa), which is close to the threshold values of Muller et al. (2011). On average, we recorded a sharp decrease in *TWD* already below a value of Ψ_S = –0.9 MPa, which indicates that, already at this value, the growth of the majority of trees was mostly halted. In this context, Zweifel et al. (2016) state, that there is very little or limited growth activity during periods of shrinkage. The turgor threshold for growth cannot be exceeded once a tree stem starts to shrink. Zweifel et al. (2021) point out that, especially during the night, stem growth consistently occurred even at lowered Ψ_S , suggesting that growth is possible under moderate soil dryness if vapour pressure deficit is low enough at the same time. Trees grow mainly at night (Zweifel et al., 2021), when they are presumed to be well replenished with water, to exceed the turgor threshold for cell division and expansion (Cabon et al., 2020; Peters et al., 2021). The highest increment regarding both treatments was recorded in D trees after irrigation (Fig. 1d, 2). In this context, Hsiao and Bradford (2015) state that cell growth is much more sensitive to drought than cell division. During the duration of the water deficit, cell elongation is delayed. Still, cell division does not stop completely, at least at the beginning of the drought period, when drought is not as severe. Growth (cell elongation) is realized after the increase of low pressure in the cambium.

Dynamics of Gas exchange and Fast kinetics of chlorophyll *a* fluorescence, their links to Tree Water Deficit (*TWD*)

Despite the fact that fluorescence emanates from only the top few layers of chlorenchyma, whereas gas exchange is integrated across the thickness of the assimilation organ, simultaneous measurements have emerged as a powerful tool for investigating the relationship between light use efficiency, CO_2 fixation, and photoinhibition (Brestic et al., 2012). Progressive drought significantly reduced stomatal activity, slowed down photosynthesis and so shifted towards carbon starvation. Stomatal closure minimizes hydraulic failure during drought, causing photosynthetic C uptake to decline to low levels. This promotes carbon starvation as carbohydrate demand continues for maintenance of metabolism and defence. The plant either starves outright or succumbs to attack by insects or pathogens, whichever occurs first.

Our results confirmed that gas-exchange parameters responded faster to the drought than fluorescence parameters of fast kinetics. Gas-exchange parameters are the first to react to even minor changes and fluctuations in the environment. Slower changing values of fluorescence parameters indicated the drought-reduced capacity of primary photosynthetic processes and quite a severe drought deficit. Although the basal fluorescence F_0, for which any significant increase already reflects serious damage to photosystems, was not so affected, the F_v/F_m (quantum efficiency) was reduced to the disturbance level. As reported in many other studies (Brestic et al., 2012; Bussotti et al., 2020), F_v/F_m values are kept high and start to decline below 70% of relative water content in assimilatory organs (Brestic et al., 2012; Bussotti et al., 2020; Strasser et al., 2004). We also noted significant changes at the PI_{ABS} level (performance parameter). The PI_{ABS} has been identified as a very reliable and sensitive parameter to indicate the onset and progression of drought stress (Ceusters et al., 2019). It is a multifactor parameter involving the density of fully active reaction centres, the efficiency of electron movement by trapped excitons into the electron transport chain beyond the Q_A, and the probability that an absorbed photon will be trapped by reaction centres (Strasser et al., 2004). Moreover, it reflects the functionality of both photosystems II and I.

The fluorescence parameters, including F_vF_m, RC/ABS, PI_{ABS}, and *TWD* values, demonstrated a stronger relationship. It indicates that chlorophyll fluorescence may be the more reliable and more detailed measurement of photosynthesis and its metabolism, than measurements of gas-exchange parameters, for the detection of types of stresses, such as drought.

Isohydric species, such as Norway spruce, use a conservation strategy with rapid stomata closing to maintain leaf water homeostasis during the drought period (Brodribb and McAdam, 2013). CO_2 assimilation in spruce species can be influenced more by stomatal closure. Stomatal response is the first event under mild water deficit to regulate transpiration, and it is more closely linked to soil moisture content than to leaf water status (Medrano, 2002; Yordanov et al., 2000). Stomata respond to non-hydraulic chemical signals, and part of diurnal changes in stomatal function may result from metabolic processes with a circadian rhythm (Chaves, 2002).

Even though the seedling represents the most vulnerable stage of development of forest trees, the recovery process of physiological processes at this stage in spruce seems to be faster, compared to adult spruce individuals. This can be used as essential information to reflect how resources and time demanding the recovery period can be (Hesse et al., 2023). It seems to be in connection with smaller tree sizes, their architecture – the canopy-to-root ratio, the anatomy of the wood and the thickness of the trunk (Kannenberg et al., 2019; Rosati et al., 2024) and overall faster growth and metabolic processes of young trees. Compared to the most important deciduous tree (beech), a limited capacity to restore internal water reserves was observed in spruce. This limitation may show pronounced legacy effects in trunk water reserves (Knüver et al., 2022).

Generally, we may summarise that stem diameter variations were strongly linked to gas exchange (A, g_s, E) and fast kinetics of chlorophyll *a* fluorescence (principally F_v/F_m, RC/ABS, PI_{ABS}) parameters. While the first mild deceleration of gas exchange was noted after growth slowed, we recorded a significant decline after growth stopped and stems contracted, as reflected in *TWD*. In this context, Muller et al. (2011) and Körner (2015) state that growth processes are generally more sensitive to atmospheric drought than carbon assimilation. Under well-watered conditions, tight relationships linking C availability and growth illustrate the source limitation of growth in sink organs such as roots, leaves (at night), flowers, and fruits. Under water deficit, these relationships are modified, suggesting that other mechanisms, possibly involving cell wall rheology or water fluxes to growing cells, override the role of C and take the lead on growth. According to Zweifel et al. (2021) stem growth, controlled by turgor, depends mainly on nocturnal conditions, while assimilation depends on daytime conditions. More pronounced *TWD* did not lead only to suppression of gas exchange parameters towards zero but also significantly affected fluorescence parameters (principally, F_v/F_m, RC/ABS, PI_{ABS}). After rehydration of D seedlings, they showed a recovery in photosynthetic capacity close to the level of C seedlings. They showed the enhancement of photosynthetic activity in some parameters that can be associated with the stomata reopening and on photochemical biochemistry level in the synthesis of D1 protein (as a core PSII protein). This led to the restoration of the electron transport chain in PSII after 7 days of the recovery process. However, the reversibility of impairment of the photosynthetic apparatus after dehydration depends primarily on the drought duration, intensity, and rehydration period after the drought stress (Gomes et al., 2012; Wang et al., 2018). Photosynthesis recovery after drought stress involves an intricate interplay between various factors. The ability to adjust and enhance this process is crucial for Norway spruce survival and productivity.

Conclusions

In the present study, stem diameter variations, and especially tree water deficit derived from them, were closely related to gas exchange and chlorophyll *a* fluorescence in young Norway spruce seedlings exposed to drought. Monitored parameters reacted synchronously during drought, started to differ against control, and recovered after rehydration. It can be stated that tree water deficit has the potential to serve as a tool for the approximation of gas exchange and chlorophyll *a* fluorescence parameters and thus reflect the overall condition and stress load of photosynthetic apparatus.

Author contributions

Conceptualisation, M.J., P.F.J., H.H. and G.J., methodology, M.J., P.F.J., H.H. and G.J., software, M.J. and P.F.J., formal analysis, M.A.S., A.R. and G.J., investigation, M.J., H.H, M.A.S., P.F.J., Ľ.D. and G.J., data curation, M.J., P.F.J., writing—original draft preparation, M.J., H.H. and G.J., visualization, M.J., P.F.J., A.R., project administration, G.J. and Ľ.D., funding acquisition, G.J. and Ľ.D. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The data presented in this study are available on request from the corresponding author.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

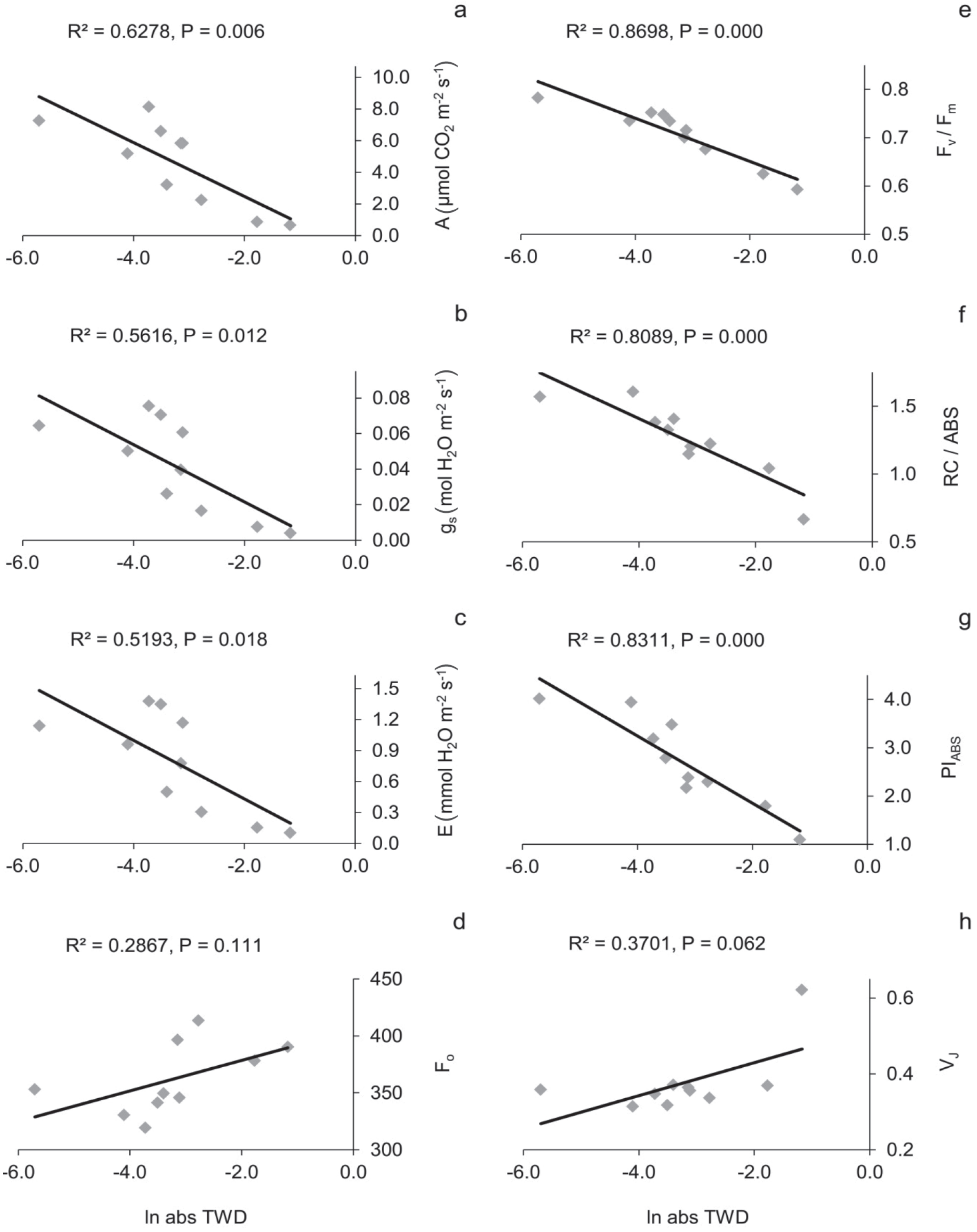

Fig. S1. (a–h, grey points) Relationships between a natural logarithm of absolute values of the average tree water deficit (ln abs *TWD*) and (a) average photosynthetic rate (*A*), (b) average stomatal conductance (g_S), (c) average transpiration rate (*E*), (d) average basal fluorescence (F_0), (e) average maximum quantum yield of the photochemistry of PSII (F_v/F_m), (f) average number of active reaction centers (*RC/ABS*), (g) average photosynthetic performance index (PI_{ABS}), (h) average relative variable fluorescence at 2 ms (V_J) of D trees. (a–h, black line) Linear function expressing the relationships between a natural logarithm of absolute values of the average *TWD* and the above parameters of gas exchange and fluorescence (R^2– coefficient of determination expressing the fit of functions to the data, P – significance level, be aware that higher values of the natural logarithm of absolute values represent a higher water deficit (more negative *TWD* values), while more negative values represent a smaller one).