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Acclimation to High Vapor Pressure Deficit in Warmer Air Can Reduce Tree Vulnerability to Drought-Induced Mortality

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ABSTRACT

Climate change imposes new constraints on tree survival, emphasising two key parameters: the vapour pressure deficit (VPD) and air temperature. Yet, no study has experimentally evaluated drought-induced tree mortality risk following acclimation to elevated temperatures with low or high VPD. Three tree species of contrasting temperature and drought tolerances (*Prunus mahaleb, Quercus robur*, and *Populus nigra*) underwent a growing season of acclimation to elevated temperature and/or VPD, and a lethal drought the following year until stem hydraulic failure was confirmed through micro-CT. Our mechanistic approach to assess temperature and VPD acclimation impacts on drought-induced mortality includes tracking stomatal conductance (g_{s}), minimum stomatal conductance (g_{min}), total leaf area (LA_{tot}), water potential at turgor loss point (Ψ_{TLP}), and estimating the time to hydraulic failure using modelling. Acclimation to elevated VPD and temperature accelerated stomatal closure, reduced g_{min} , and raised Ψ_{TLP} . In contrast, while high temperature reduced g_{min} , it also increased LA_{tot} and height. Consequently, hydraulic failure occurred faster in high-temperature-acclimated trees, while it was generally delayed by adding higher VPD. Our findings highlight that the balancing effects of temperature-driven leaf area expansion, which accelerate mortality, and VPD-driven acclimation in stomatal sensitivity, counteract each other, stabilising the timing of mortality.

1 | Introduction

In addition to chronic temperature rise, drought and heat wave frequencies and intensities are projected to increase everywhere in Europe in spring and summer (Spinoni et al. 2018). Heat and drought (both soil and atmospheric drought) are two interrelated components of climate change that can represent different risks for forests. Rising temperature increases leaf transpiration (e.g., Kirschbaum and McMillan 2018) resulting in atmospheric water stress on plants (e.g., Chaves 2002; Way et al. 2013; Teskey et al. 2015) because of higher evaporative demand (i.e., vapour pressure deficit, VPD, e.g., Ficklin and Novick 2017; Grossiord et al. 2020). Hence, high temperature and VPD contribute to more frequent and severe drought events, which further amplifies plant water stress and could exacerbate tree die-off events (Dai 2013; Park Williams et al. 2013; McDowell 2022). Still, whether plants acclimation to the additive effects of higher temperatures and VPD could mitigate their risk of drought-induced mortality remains ambiguous.

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Plants can acclimate to novel climatic conditions to mitigate their adverse impacts at different timescales by modifying their morphology and physiology; this is called phenotypic plasticity (Nicotra et al. 2010). Overall, much work has been conducted on plant acclimation to soil drought. Dynamic adjustments, such as the opening or closing of stomata, can be triggered within minutes and can be of crucial importance for the survival of plants, especially during critical heat peaks in the day (e.g., Schymanski et al. 2013) or extended soil droughts (e.g., (Tardieu and Simonneau 1998; Martin-StPaul et al. 2017). On relatively short time scales, plants can lower the water potential at the turgor loss point (Ψ_{TLP} ; Luebbe et al. 2016; Forner et al. 2018; Petruzzellis et al. 2022), allowing plants to maintain leaf function under moderate drought. Similarly, reduced water loss through the cuticle and leaky stomata, i.e., the minimum stomatal conductance (gmin; James et al. 2008; Qin et al. 2020) was found in plants exposed to soil drought, limiting the desiccation time and delaying the time to hydraulic failure past stomatal closure (Cochard et al. 2021). On longer time scales (seasons to decades), plants can further acclimate to changed soil moisture by modifying their evaporative surfaces (Martínez-Vilalta et al. 2009; Seidel and Menzel 2016; Mas 2023). Through these multiple adjustments, plants can maintain metabolic function while reducing their water loss and delaying critical hydraulic failure occurring once a species-specific xylem water potential (Ψ) has been reached, ultimately inducing mortality (e.g., Limousin et al. 2010; Barbeta et al. 2013).

Regarding air temperature acclimation, although prior exposure to warmer air generally confers increased tolerance to subsequent heatwaves (e.g., Ahrens et al. 2021), whether it also affects drought tolerance and the timing of drought-induced mortality is less clear. Acclimation to moderate warming under wet soil conditions generally leads to higher photosynthetic rates (e.g., Campbell et al. 2007; Kumarathunge et al. 2019) and growth (e.g., Saxe et al. 2001; Wang et al. 2023). For instance, Drake et al. (2014) found that warming of $+3.5^{\circ}$ C increased tree biomass, including total leaf area, by up to 60% in different Eucalyptus tereticornis and E. grandis provenances, with the ones originating from colder regions showing the highest gain. However, acclimation towards a higher evaporative surface in species from colder and wetter areas could enhance their water loss during drought, possibly accelerating mortality events (but see Seidel and Menzel 2016). Similarly, during favourable climatic conditions, structural overshoot of aboveground biomass has been associated with accelerated mortality (Jump et al. 2017), especially during hotter droughts (Zhang et al. 2021). Moreover, warming can increase transpiration and stomatal conductance (gs) (e.g., Marchin et al. 2023; Diao et al. 2024; Mills et al. 2024), leading to a faster desiccation time. Still, previous studies generally monitor VPD during manipulative experiments but without varying it within a given temperature treatment, making it difficult to identify the impacts of temperature on the hydraulic functions involved in the mortality process (e.g., g_s , g_{min} , and Ψ_{TLP}), as these effects are confounded with those of VPD.

Indeed, acclimation to higher VPD will also affect plant water use by leading to higher stomatal sensitivity to VPD, i.e., a steeper decrease in g_s as VPD rises (e.g., Marchin et al. 2016; Grossiord et al. 2017), a reduction in turgor pressure (López et al. 2021; Novick 2024), Ψ_{TLP} (Tordoni et al. 2022), and g_{min} (Duursma et al. 2019), which should all help delay the development of hydraulic impairments during an intense drought. Still, other studies also showed that high VPD could lead to loss of stem hydraulic conductivity even when soil water is nonlimiting (Schönbeck et al. 2022) or critically low (Wagner et al. 2022). This is because some plants can maintain high g_s (e.g., Rasheed et al. 2015; Marchin et al. 2016) leading to important drops in Ψ beyond thresholds inducing embolism (Schönbeck et al. 2022). Finally, the few studies that have explored VPD effects on tree structure have found reduced growth and leaf area (Lendzion and Leuschner (2008); Orság et al. (2024). Yet, while higher stomatal sensitivity to VPD is usually found in more drought-tolerant species (Grossiord et al. 2020; Novick 2024), the acclimation potential between species is unknown. Moreover, the contrasting impacts of temperature and VPD on plant acclimation complicate our understanding of their impacts on mortality risks. While we have gained a further understanding of temperature and VPD impacts on plants in recent years, few work have investigated the drivers of tree mortality, the acclimation mechanisms potentially mitigating them, and their accurate depiction in climate-vegetation models. Given their longevity, trees will face prolonged exposure to changing climate conditions, making it essential to assess their acclimation capacity to mitigate risks of drought-induced mortality and maintain forest health (Erlichman et al. 2024).

This study aimed to determine how acclimation to elevated temperature and VPD affects the time to hydraulic failure during intense droughts, and through which physiological and structural changes. Rather than relying on observational data, we experimentally tested the direct mechanisms governing time to hydraulic failure during drought by combining physiological measurements and modelling. We used three European tree species selected along a gradient of temperature and drought tolerance (Prunus mahaleb, Quercus robur, and Populus nigra) that underwent an entire growing season exposed to combinations of air temperature and VPD treatment (i.e., control, moderate warming + humid air reflecting the same VPD as in the control, and high warming + dry air leading to a significant increasing temperature and VPD compared to the control). All plants were subjected to an experimental drought at the same ambient temperature and VPD conditions the following year until mortality was reached. We expected that acclimation to moderate warming would result in higher leaf area while higher warming and VPD acclimation would trigger reduced (i.e., more negative) Ψ_{TLP} and lower g_{min} . Due to the warminginduced leaf area increase, we expected a faster decrease in gs and Ψ during the drought for plants grown in moderate warming and humid air conditions, thereby advancing the timing to mortality. In contrast, the VPD-driven reduction in Ψ_{TLP} and g_{min} for plants acclimated to hot and dry air conditions should delay the mortality timing. We further expected that the species occurring in milder climates (Q. robur and P. *nigra*) would show the strongest temperature acclimation under moderate warming (i.e., increased leaf area enhancing water loss). In contrast, the one occurring in the drier and hotter conditions (P. mahaleb) would acclimate more strongly to high temperature and VPD (i.e., reduced Ψ_{TLP} and g_{min} limiting water loss). Hence, the species from milder climates may be subjected to an earlier mortality under moderate warming acclimation, while *P. mahaleb* may be able to delay it under a hotter and drier one.

2 | Materials and Methods

2.1 | Experimental Design

Saplings of three European broadleaved tree species (60-80 cm height-2 years old) were grown in three polytunnels (semicylindrical structures made of metal frames covered with transparent plastics, used to create controlled environments). The polytunnels were equipped with temperature and relative humidity (RH) control at EPFL (Lausanne, Switzerland) from May 2022 to September 2023 (Figure 1). The species were selected for their differentiated temperature and drought tolerances (see Table S1): the mahaleb cherry (Prunus mahaleb L., water potential at 50% loss of hydraulic conductivity $P_{50} = -5.57$ MPa, Cochard et al. 2008), which is native to Southern Europe and North Africa (Bourlière 1969; Ernst and Wallace Roy 1969) and grows in the driest and warmest areas among all selected species, the common oak (Quercus robur, $P_{50} = -2.83$ MPa, Urli 2014) that is native in central Europe where temperatures are milder (Ducousso and Bordacs 2003), and the black poplar (*Populus nigra*, $P_{50} = -1.45$ MPa, De Baerdemaeker et al. 2017), which is found throughout Europe, including in continental climates and wetter areas (Vanden Broeck 2003). All the species were obtained from local nurseries and are from Swiss provenances.

The plants underwent different VPD and temperature treatments during the 2022 growing season (July-September) and before the drought in 2023 (from the beginning of June to the beginning of July, when the drought started). In the control treatment, saplings were grown in ambient air temperature $[26 \pm 4^{\circ}C \ (\pm SD)$ on average during the daytime in Jul-Sep 2022] and humid air (low VPD, 1.2 ± 0.6 kPa). In the moderate warming-humid air (MW-H) treatment, saplings have grown in $+2^{\circ}$ C warmer air temperature on average (28 ± 5°C) and humid air (low VPD as in the control, 1.2 ± 0.7 kPa). The high warming-dry air (HW-D) treatment consisted of +4°C warmer air $(30 \pm 4^{\circ}C)$ and higher VPD $(1.9 \pm 0.8 \text{ kPa})$. With this design, we aimed at reaching a similar VPD in the MW-H and control treatments (i.e., only temperature varies). In contrast, in the HW-D treatment, both temperature and VPD increased. VPD manipulation was done through misting systems installed in each tunnel to maintain a stable and set RH in the tunnels. Temperature regulation was done passively inside the tunnels by an automatic opening/closing system of the side walls. The sprinklers for RH control were evenly distributed over the trees, and the trees were placed at the same distance from the side openings to ensure equal conditions for all trees. There was no standalone high VPD treatment, as the experimental setup allowed air humidification but not dehumidification.

Ten individuals per treatment and species were planted in individual 3.5 L pots (n = 3 species $\times 3$ treatments $\times 10$ replicates; 90 plants in total). A soil with high sand content was used to ensure good drainage and quickly establish drought conditions the following year. The trees were watered to field



FIGURE 1 | Experimental set-up. (a) Species of interest ordered by increasing temperature and drought tolerance. (b) Picture of one of the three automated polytunnels used to modulate temperature and VPD. (c) Mean daytime temperature (T) and VPD for each of the three treatments (control, moderate warming and humid air—MW-H, high warming and dry air—HW-D) during the 2022 summer (i.e., the acclimation phase). The treatments were active from about 11 AM to 7 PM. The panels on the right show mean values for temperature and VPD per treatment with standard deviations. [Color figure can be viewed at wileyonlinelibrary.com]

capacity during the first year to avoid soil moisture stress before the drought planned the following year. Three measurement campaigns took place during the first-year growing season to track the physiological acclimation of trees, and a fourth campaign was done in July 2023, right before the drought.

In early July 2023 (after the leaves had flushed and were fully mature), seedlings were subjected to a soil drought by withholding irrigation. All trees were subjected to the same air conditions (VPD and temperature, see Supporting Information S1: Figure S1) during the drought to determine the impact of the potential acclimation taking place during the first year (i.e., without accounting for differences in drought stress during hot/ high VPD conditions leading to a faster drying out of the soil). Eight days after the drought started, all leaves were thoroughly dried, and 3 weeks later, stem xylem embolism was close to 100% for most trees (see below). During the drought period, physiological measurements were tracked daily, which were used to calibrate the SurEau model (see below) and estimate the time to stomatal closure (TSC) and time to hydraulic failure (THF) as performance parameters. Tree mortality was assessed again in May 2024 (year 3), similarly to (Hammond et al. 2019). No new growth was observed after 1 month of re-watering (by June 2024) in all trees. A scheme of the experimental design timeline is available in Supporting Information S1: Figure S2.

2.2 | Canopy Leaf Area and Growth

In June 2023, right before the drought, ten fully expanded mature leaves were photographed for each tree next to a scale, and ImageJ (1.54e) was used to extract the mean individual leaf area (LA_{leaf}, cm²). The whole-canopy leaf area (LA_{total}, cm²) was then computed by multiplying LA_{leaf} by the total count of leaves for each individual (Supporting Information S1: Figure S3). The leaf width (W_{leaf}) was also extracted from the images as it was used in the modelling part of the study (Supporting Information S1: Table S2, see below). At the start of each year (2022 and 2023), we measured stem diameter at about 15-20 cm above the ground [using a digital caliper (Toolland-Velleman group, Belgium)] and tree height (Supporting Information S1: Table S2 and Figure S4). Root biomass was not assessed during the experiment, as pot size constraints limited belowground growth, resulting in all pots being fully occupied by roots at the end of the experiment.

2.3 | Photosynthetic Assimilation and Stomatal Conductance

Three times during the first growing season (acclimation phase), measurements of the net light-saturated photosynthesis (A_{net} , μ mol m⁻² s⁻¹) and stomatal conductance (g_s , mmol m⁻² s⁻¹) were conducted on two leaves per plant with two LiCor LI-6800 (LiCor Inc., Lincoln, USA) equipped with a 2 cm² fluorescence leaf chamber (Supporting Information S1: Figure S5). Each leaf was clipped in the cuvette, and the conditions inside the chamber were set to ambient air temperature and relative humidity. The measurements were done at saturated light intensity (1500 μ mol m⁻² s⁻¹), CO₂ concentration of 400 ppm, and flow at 500 μ mol s⁻¹. While 1500 μ mol m⁻² s⁻¹ is generally above the ambient light

conditions, using this standard light value during gas exchange measurements ensured cross-comparison with other studies and between the measurements. All the measurements took place between 9 AM and 12 PM local time to ensure capturing the moment with the highest activity.

During the experimental drought in 2023, one LI-600 porometer (LiCor Inc., Lincoln, USA) was used daily or every second day to monitor instantaneous g_s for at least the first 8 days after the irrigation was stopped. After 8 days, the leaves of all species were completely dry, and the measurements stopped. We used a porometer instead of the LI-6800 during the drought to increase the measurement speed and allow measurements of all plants within an hour (around 10 AM each day).

2.4 | Pressure Volume Curves and Leaf Water Potential at Predawn and Midday

Pressure-volume curves were determined in late summer 2022 (Supporting Information S1: Figure S6) and early summer 2023 before the drought using the bench-dehydration method (Koide 2000). Before dawn, a leaf from the top of the crown was cut off and immediately sealed in a plastic bag (Whirlpak) that was previously exhaled in. Predawn water potential (Ψ_{PD} , MPa) was measured directly using a Scholander-type pressure chamber (PMS Instrument Company, Model 1505D). The same leaf was immediately weighed using a fine-precision scale (Mettler-Toledo), placed in a plastic bag, and allowed to dry progressively in the open plastic bag on a lab bench. The procedure of measuring Ψ , weighing, and drying was repeated with increasing drying time intervals (from 10 s to 1 h) for P. mahaleb and Q. robur until achieving Ψ of about -4 MPa or until Ψ reached a plateau. For P. nigra, the procedure was repeated continuously without letting the leaves dry on the bench due to the rapid water loss and a corresponding drop in Ψ . Subsequently, the leaves were individually put in a paper bag and dried in an oven at 60°C for 24 h to determine the dry mass. Leaf water potential at turgor loss point (Ψ_{TLP} , MPa) was calculated after Koide (2000). In addition, two parameters were computed to parameterise the SurEau model: the modulus of elasticity (ε), calculated as the slope of the pressure-volume curve in its early stages, where leaf volume changes significantly with pressure but turgor remains relatively high, and the osmotic potential at full turgor (Ψ_0), reflecting the leaf's pressure potential when fully hydrated.

Leaf Ψ_{PD} and midday (Ψ_{MD}) were measured three times during the acclimation phase (together with the gas exchange measurements, Supporting Information S1: Figure S7). During the drought in 2023, Ψ_{PD} and Ψ_{MD} were measured on all plants the same day as g_s measurements (every 1–2 days for a period of 8 days) using the same Scholander pressure chamber. Each tree was measured as long as its leaves remained measurable (i.e., not overly dry). If no value could be obtained for a given tree indicated by the absence of water outflow into the leaf stem during the pressure bomb measurement, even at pressures exceeding 8 MPa—a default value of 10 MPa was assigned to represent complete leaf dryness. This extreme value allowed us to adjust the species—and treatment—weighted averages downward in cases of leaf desiccation.

2.5 | Minimum Stomatal Conductance

Minimum stomatal conductance (g_{min}) (Kerstiens 1996) was measured once during the summer of 2022 (Supporting Information S1: Figure S6) and in the early summer of 2023 before the drought, as described in Pearcy et al. (2000). One leaf per individual was cut before dawn when stomata were assumed to be still closed. The cut petiole was immediately sealed with melted candle wax, and the leaf area was scanned using a flatbed scanner, followed by analysis using ImageJ 1.54e. The leaves were stuck to a lab tape run between two lab stands, standing in a dark room with stable temperature (23°C) and relative humidity (68%). Every 15-20 min, the leaves were taken and weighed using a fine-precision scale (Mettler-Toledo, Switzerland-Model MS104TS/00). This procedure was repeated eight times. $g_{min} \pmod{m^{-2} s^{-1}}$ was calculated as cuticular transpiration per mole fraction VPD, assuming the leaf internal air to be fully saturated (Pearcy et al. 2000).

2.6 | Stem Percentage Loss of Conductive Area

To ensure stem embolism was occurring, three trees per treatment and species were scanned during the third week of drought. They were transported to the Interdisciplinary Platform for X-ray microcomputed tomography (µCT) (PIXE, EPFL) and stored in a cool room in the absence of direct light (to avoid transpirational water loss) until they were scanned following the same method as Schönbeck et al. (2022). More specifically, each tree was first scanned while still in its pot, then scanned again after being cut and flushed to empty all vessels, making them fully visible in the images by highlighting density differences. For the initial µCT scan, the tree was secured in a custom-built plant holder, with its branches wrapped in cling film to prevent movement that could compromise image quality. A 1 cm section of the stem, located approximately 30 cm above the base, was marked with tape before scanning. After the first scan, this section was cut and flushed with air at 1 bar pressure for 1.5 min, then rescanned to obtain a fully embolized stem cross-section, serving as a reference to visualise all vessels in the sapwood. Image analysis was done with the Avizo software (2023.2) using the same approach as Mekarni et al. (2024). The percentage loss of conductive area (PLC, %) was calculated as the total embolized area in the intact stem divided by the total vessel area in the flushed stem (Supporting Information S1: Figure S8).

2.7 | SurEau Model

The soil-plant hydraulic model SurEau (Martin-StPaul et al. 2017; Cochard et al. 2021; Ruffault et al. 2022) was applied to understand the impact of the trait acclimation to the different treatments on the risk of hydraulic failure and time to stomatal closure. In brief, SurEau simulates water fluxes and water potential through a plant hydraulic scheme, including different symplasmic and apoplasmic resistances. The model computes leaf stomatal and cuticular transpiration as the product between leaf-to-air VPD and stomatal and cuticular conductance, divided by the atmospheric pressure. Then,

stomatal and cuticular fluxes are used to calculate the water potential in the different plant compartments (the symplasm and the apoplasm of leaves, stems, and roots), while accounting for (1) the symplasmic capacitance, (2) water released by cavitation, and (3) the potential hydraulic conductance losses due to xylem embolism (if any). The soil water potential and the soil hydraulic conductance are also computed from soil water content at each time step using water retention curves. A peculiarity of the SurEau model is the prediction of what occurs beyond the point of stomatal closure, under extreme water stress, when g_{min} leads to plant dehydration and hydraulic failure (defined as 98% conductivity loss in the present study).

The model can be parameterised with ecophysiological traits measured empirically and can be run using different environmental conditions. Here, we used the detailed version coded in C (Cochard et al. 2021), which works at a time step of 0.01 s. The model was parameterised with species-specific plant traits in each treatment, which are key plant traits known to influence the time to hydraulic failure (Ruffault et al. 2022). These traits include soft traits: (1) height and diameter of the main stem (used to compute the wood volume and area, and thus, the water storage in the plant), which influence the timing of stomatal conductance reaching g_{min} , (2) LA_{tot}, which influences tree transpiration, and (3) leaf width W_{leaf} , which influences the boundary layer conductance. W_{leaf} was measured manually from the same photos used for LA measurements on one leaf per tree, and the dataset was completed by predicting W_{leaf} for the other nine leaves based on the known leaf areas. Predictions were made using a species- and treatment-specific linear regression model derived from the measured data. This estimate is acceptable, as W_{leaf} is only used to calculate the leaf boundary layer in the model (whereas LAtot is a much more sensitive parameter in the model). In addition, we included hard traits: (4) g_{min} and the maximum g_s , which defines a possible range for tree transpiration when combined to VPD, and (5) the pressurevolume curve parameters measured per species and treatment, which influence the symplasm capacitance and are used to compute turgor-mediated stomatal closure (Martin-StPaul et al. 2017) (see Table S2). The P_{50} and the slope of the curve at P₅₀ were taken from literature and assumed constant at the species level as it was impossible to measure it on site, and in agreement with the low plasticity previously found for this trait (Matzner et al. 2001: Torres-Ruiz et al. 2013). The vulnerability curve was then based on the Pammenter and Van der Willigen (1998) model, a sigmoid that used these two P_{50} parameters. The model was run for the different trait combinations corresponding to each individual within species and treatment with the actual atmospheric conditions during the drought, and an initial soil water content at saturation. The simulations stopped when the plants reached total hydraulic failure. Two integrative metrics were computed by the model: (1) the time to stomatal closure (TSC), and (2) the time to hydraulic failure (THF), which we define as the survival time during drought. First, we compared the treatment effects on TSC and THF. Then, to isolate the influence of the soft traits from hard traits, we performed the same test with either constant soft traits (height, stem diameter, W_{leaf}, and LA_{tot} set constant, matching the mean of Control trees values) or with constants hard traits (g_{min} , Ψ_0 , ϵ , Ψ_{MD} , and g_s , matching Control trees mean values).

A variance-based sensitivity analysis was further carried out to assess the impact of each parameter on the THF (Supporting Information S1: Figure S9) and showed that the default values taken from the literature used for the variables that couldn't be measured had a low weight in explaining the variance of THF and TSC. The datasets were prepared with the library "sensobol" using R v.4.0.4 (R Core Team 2021), and the corresponding method is described in Puy et al. (2022). We obtained and analyzed the Total Sobol's indices, which decompose the total variance of the model's output into components that account for both the input variables and their interactions. Each tested parameter (28 in total) was varied by $\pm 10\%$ around mean values of the Control for each species, and the model was run 31 108 times (= 28 × 1111, where 1111 is the sample size of the base matrix used in the Sensobol R library) for each of the three species.

2.8 | Statistical Analyses

To assess the impact of temperature and VPD changes on the measured or modelled parameters (g_s , Ψ_{TLP} , Ψ_{PD} , Ψ_{MD} , g_{min} , LA_{tot}, PLC, modelled THF and TSC) we used linear mixed-effects models for each species and year (acclimation phase and drought period) separately. The temperature (26°C, 28°C, 30°C) and the VPD (1.2 kPa, 1.9 kPa) were fixed effects, and trees repetition was considered a random effect. The interaction between temperature and VPD could not be assessed because the study design does not include all the VPD combinations for each temperature level. Post hoc analyses were performed with Tukey's HSD test. All statistical analyses were performed with R v.4.0.4 (R Core Team 2021).

3 | Results

3.1 | Acclimation Responses to Temperature and VPD

During the first year (in 2022), high VPD and warming (HW-D treatment) led to increased A_{net} and g_s (apart from g_s in *Q. robur*) compared to the control for all species (Supporting Information S1: Figure S5). In contrast, moderate warming with low VPD (MW-H treatment) only increased A_{net} , except for *P. mahaleb*. HW-D also reduced g_{min} for *P. mahaleb*, while MW-H did not affect this trait (Supporting Information S1: Figure S6). Ψ_{TLP} , Ψ_{PD} , and Ψ_{MD} were unaffected by the treatments during the first year (Supporting Information S1: Figures S6 and S7).

We found that MW-H increased tree height and stem diameter for *P. mahaleb*, while only diameter was enhanced with HW-D (Supporting Information S1: Figure S4). The other species exhibited minimal changes in height or stem diameter, except *Q. robur*, which showed increased stem diameter in MW-H conditions, and *P. nigra*, which demonstrated greater height in the HW-D treatment.

At the start of 2023, higher temperature increased LA_{tot} for *P*. *mahaleb*, which was equally increased for the MW-H and HW-D treatments compared to control trees (+130% on average) (Figure 2, Supporting Information S1: Table S3). In

contrast, the HW-D treatment impacted both Ψ_{TLP} and g_{min} , which were either increased by 24% (i.e., less negative) or reduced by 61%, respectively, compared to the control.

For *Q. robur*, warming also increased LA_{tot} (+89%) but only in the MW-H treatment and not the HW-D one (Figure 2, Supporting Information S1: Table S3). While no significant treatment effects were found for Ψ_{TLP} , warming reduced g_{min} (-35% in the MW-H treatment compared to the control), and the impact was exacerbated in the HW-D treatment (-60%).

P. nigra responded to increased temperature by increasing LA_{tot} by 120% compared to the control in MW-H conditions, while it was only increased by 25% in the HW-D. Moreover, warming led to lower g_{min} (about -40% on average in both warming treatments), while no treatment effects were found for Ψ_{TLP} .

3.2 | Physiological Responses During the Drought

At the start of the drought, g_s was significantly higher in the HW-D treatment (and MW-H for *Q. robur*) than the control for all species, while Ψ_{MD} was similar across treatments. During the drought, a rapid (within 2–4 days) drop in g_s , Ψ_{MD} , and Ψ_{PD} (see Supporting Information S1: Figure S7 for Ψ_{PD}) was observed for all species (Figure 3).

For *P. mahaleb*, g_s started to drop rapidly once Ψ_{MD} passed the Ψ_{TLP} (Figure 3). This timing was accelerated by acclimation to warming and happened slightly first for HW-D acclimated trees (after 2 days), then for MW-H acclimated trees (between 2 and 3 days), and finally for control trees (shortly before the fourth day of the drought). The same trend was observed for Ψ_{PD} (Supporting Information S1: Figure S7).

For *Q. robur*, temperature and VPD acclimation had little effect on the physiological responses during the drought. We found that g_s dropped about 4 days after the drought started for all treatments, corresponding approximately to the time when Ψ_{MD} crossed the Ψ_{TLP} (between 4 and 5 days). Still, throughout the drought, g_s was significantly higher in the HW-D treatment, while it was similar to the control for the MW-H trees.

For *P. nigra*, g_s started dropping earlier in both warming treatments (after 2 days) compared to the control (about 4 days after the drought started). Interestingly, while the drop in g_s matched the time of Ψ_{MD} crossing the Ψ_{TLP} for the MW-H and control treatments, g_s started dropping earlier in the HW-D treatment.

3.3 | Impact of Temperature and VPD Acclimation on the Modelled Time to Stomatal Closure and Hydraulic Failure

For all species, we found that temperature and/or VPD affected the modelled TSC and THF (Figure 4, Supporting Information S1: Table S4) when all traits were acclimated, except for *P. nigra* whose THF was constant. For *P. mahaleb*, while acclimation to



FIGURE 2 | Mean (\pm SE, *n* = 5) water potential at turgor loss point (Ψ_{TLP}), minimal stomatal conductance (g_{min}), and total leaf area measured in July 2023 after the acclimation phase and before the start of the drought for each treatment (control, moderate warming and humid air—MW-H, high warming and dry air—HW-D). Letters denote significant differences between treatments for each species and measurement. [Color figure can be viewed at wileyonlinelibrary.com]



FIGURE 3 | Mean (\pm SE, n = 5) stomatal conductance (g_s) and leaf water potential at midday (Ψ_{MD}) during the terminal drought phase for each treatment (control, moderate warming and humid air—MW-H, high warming and dry air—HW-D). Dashed horizontal lines in the bottom plots show the mean turgor loss point (Ψ_{TLP}), and dotted vertical lines represent the day at which Ψ_{MD} crossed the Ψ_{TLP} for each species and treatment. T₀ stands for the start of the drought. Embedded images show stem cross-sections with fully embolized xylem vessels for each species obtained during the third week of drought. Stars denote significant differences from the control for a given date (* < 0.05, ** < 0.01, *** < 0.001). [Color figure can be viewed at wileyonlinelibrary.com]

higher temperature and VPD advanced the TSC (both MW-H and HW-D treatments), lower VPD advanced the THF (only in the MW-H treatment), and high VPD acclimation mitigated the THF with hydraulic failure occurring similarly after 8 days in the control and HW-D conditions. Similarly, for *Q. robur*, while MW-H had no impact on TSC and THF, high temperature and VPD mitigated the TSC (similar under HW-D and control treatments) and delayed the THF by 4 days compared to the

control. Finally, *P. nigra* showed no significant impact of temperature or VPD on the THF, even if acclimation to a higher temperature significantly reduced the TSC.

The model accounting for hard traits acclimation only (using the averaged $LA_{tot} - W_{leaf} - height - stem diameter of control$ trees for all treatments) showed significant delays in TSC andTHF for*P. mahaleb*in the warmer treatments (+39% for HW-Dand +32% for MW-H in THF compared to when all traits areconsidered acclimated). Similarly, for*Q. robur*, trees acclimatedto high temperature (MW-H) also slightly delayed their THF(+15% compared to the full acclimation scenario). Finally, in*P. nigra*, acclimation to lower VPD delayed the THF (+34% MW-H), suggesting that acclimation of hard traits to lower VPDmarginally improved trees resistance to drought.

When accounting only for the soft traits acclimation (using the averaged g_{min} , Ψ_{TLP} , Ψ_{MD} , g_s of control trees for all treatments), we observed that THF was reduced for all species compared to the hard traits acclimation scenario, mainly in the HW-D treatment (between -14% and -62%, with the greatest reductions for *P. mahaleb*). In contrast, the TSC was not strongly affected by the acclimation of soft traits compared to acclimated hard traits effects. These findings suggest that acclimation of soft traits to elevated temperature and VPD accelerates the mortality speed.

The percentage loss of conductive area (PLC) after 10 days of drought ranged between 44% and 115% (Supporting Information S1: Figure S8). The few measurements above 100% are due to small variations in stem location scanned after being flushed. Almost no treatment effects were found on the PLC, except for *Q. robur* whose HW-D acclimated trees were less embolized (65% on average) than the control and MW-H ones (90% on average), but suggesting that trees reached the point of hydraulic failure quickly afterwards.

4 | Discussion

Our findings revealed that acclimation to rising temperature (moderate vs. high) and VPD (low vs. high) can have contrasting impacts on plant hydraulic and structural characteristics, significantly altering their mortality risk during subsequent soil droughts. Acclimation to moderate warming per se (i.e., at a similar VPD as the control) primarily influenced soft traits, resulting in increased total leaf area (LAtot), height, and stem diameter, which, in turn, elevated the risk of hydraulic failure during drought because of more rapid water use. This phenomenon is referred to as structural overshoot (e.g., Jump et al. 2017), when favourable climate drives excessive tree growth, leading to water demand later exceeding its availability. Indeed, within the tested temperature range, growing at a chronic +2°C-4°C in irrigated conditions, independently of VPD levels, stimulated photosynthesis (Anet) in all species during the acclimation phase in 2022 (Supporting Information S1: Figure S5), leading to higher LA_{tot} (Figure 2) and growth (Supporting Information S1: Figure S4) before the drought. These effects are consistent with the literature indicating overall positive effects of warming on tree growth for several species (Saxe et al. 2001; Campbell et al. 2007; Wang

et al. 2023), although it was unclear whether different levels of VPD could intensify or prevent this temperature-induced trend. As initially expected, warming-enhanced Anet and LAtot were strongest for Q. robur and P. nigra, the two species originating from the coldest climates, similarly as in Drake et al. (2014), and potentially suggesting higher risks for those species when water stress will occur. Still, this effect was reduced at the highest level of warming and elevated VPD in Q. robur and P. nigra, indicating that concurrent temperature and VPD rise may mitigate the investment into a larger canopy. Interestingly, our treatments did not impact the minimum stomatal conductance (g_{min}) and the turgor loss point (Ψ_{TLP}) the first year, but moderate and high warming significantly reduced gmin for the following growing season (year 2). Schönbeck et al. (2022) and Mas (2023) reported no change in g_{min} and Ψ_{TLP} for temperate European species exposed to +5°C for 1-3 years. In our study, the combination of high temperature and elevated VPD intensified g_{min} reduction for all species-indicating a crucial enhancement in drought resilience through improved water conservation - but also increased Ψ_{TLP} in *P. mahaleb* (Figure 2). These findings contrast with responses to soil drought where Ψ_{TLP} is usually found to decrease (e.g., Tordoni et al. 2022, but see Mas 2023). While no work has reported VPD impacts alone on Ψ_{TLP} , we believe that increasing Ψ_{TLP} may reflect a more secure behaviour for facing hot drought, as turgor loss leads to stomatal closure (e.g., Rodriguez-Dominguez et al. 2016) or even leaf fall-out that could allow saving water and preserving vital organs. Higher VPD combined with temperature rise also prevented LA_{tot} to increase in Q. robur and P. nigra (which occurred in the MW-H treatment) and increased leaf width and growth in P. nigra (Supporting Information S1: Tables S2 and S3, Figure S4). To our knowledge, the effects of VPD per se on leaf size have not been studied yet. Still, in P. nigra it seems that trees increased their leaf width in response to moderate warming and low VPD (potentially to enhance transpiration and maintain evaporative cooling) while high temperature and VPD mitigated this phenomenon, potentially because hydraulic stress generally triggers narrower leaves to minimise water loss (Wright et al. 2006, 2017). Independently of the acclimation responses, all species rapidly

(within a few days) suffered from mortality during the drought. For *P. mahaleb* and *P. nigra*, the drop in g_s (and Ψ_{MD} for *P.* mahaleb) happened slightly faster in trees acclimated to warming, independently of VPD levels (Figure 3). These results were further confirmed by the model where TSC occurred earlier in warmer treatments for both species (Figure 4). The relationship between the rate of physiological decline during drought and the timing of mortality can be complex: a rapid physiological decline, such as a sharp drop in Ψ and g_s , may represent a more efficient survival strategy. This could involve preemptive water conservation or even anticipated leaf desiccation to safeguard more critical organs and enhance the tree's overall chances of survival (Tyree 1993; Johnson et al. 2016; Wolfe et al. 2016). In other cases, rapid physiological decline or drop in Ψ can also represent higher vulnerability to droughtinduced embolism in all organs (Li et al. 2020), leading to early resource depletion and threatening mortality. Nevertheless, faster TSC did not appear as an active acclimation process aiming at protecting the tree, as it did not delay the point of hydraulic failure for those species.



FIGURE 4 | Mean (\pm SE, *n* = 10) time to hydraulic failure (THF) and time to stomatal closure (TSC) computed for each individual with the model SurEau, averaged for each species and treatment (control, moderate warming and humid air—MW-H, high warming and dry air—HW-D): (a) considering the acclimation of all traits (soft traits: height, total leaf area, stem diameter, leaf width; and hard traits: g_{min} , Ψ_{TLP} , Ψ_{MD} , g_s), (b) with hard traits acclimated only (fixed soft traits corresponding to the averages of the control), and (c) with soft traits acclimated only (fixed hard traits corresponding to the graphs denote significant differences between treatments for each species and acclimation scenario. [Color figure can be viewed at wileyonlinelibrary.com]

In contrast, for Q. robur, high temperature and VPD acclimation delayed the drop in g_s while no changes were found under moderate warming at low VPD and control trees. Still, for both P. mahaleb and Q. robur, the model predicted earlier THF for MW-H than HW-D trees. For *Q. robur*, the higher mortality delay (+5 days) was further consistent with the lower PLC observed in the HW-D treatment (Supporting Information S1: Figure S8). Indeed, this species developed a sparser canopy under higher temperature and VPD (same as in the control), allowing it, together with lower gmin, to delay the water loss despite higher gs. In contrast, for P. mahaleb, the mortality delay may have been more strongly driven by lower g_{min} as LAtot was similar between the two warmer treatments (Figure 2). This finding contrasts with results from (Mas et al. 2024) showing that THF was mainly explained by the smaller LA_{tot} rather than lower g_{min} and higher Ψ_{TLP} , and highlights the strong species-specific acclimation responses that may be involved in the mortality process.

Hence, our findings suggest that high VPD acclimation in high temperature partially delays drought-induced tree mortality, especially in the most drought-tolerant species (*P. mahaleb* and *Q. robur*). In *P. mahaleb*, the acclimation of hard traits (g_{min} and Ψ_{TLP}) to increased temperature and VPD compensated for the acclimation of soft traits, leading to a similar THF in control and HW-D treatments, despite increased LA_{tot}. As initially hypothesise, *P. mahaleb*—being the most drought tolerant species—showed the most important acclimation of hard traits. *Q. robur* also benefited from high temperature and VPD acclimation but shifted both hard and soft traits (lower g_{min} and LA_{tot}), leading to the largest delay in hydraulic failure compared

to the control. Finally, as expected, *P. nigra* showed strong acclimation to temperature, but despite lower g_{min} and slightly lower LA_{tot} in HW-D than MW-H conditions, THF was similar across all treatments. This species was also the least drought-tolerant ($P_{50} = -1.45$ MPa) and had the fastest mortality overall, suggesting that small physiological and structural shifts were insufficient to delay tree death.

Future work should test if similar responses may be observed in natural forests and adult trees where the process of tree decline may occur over an extended time (weeks to months, e.g., Arend et al. 2021). While understanding the importance of temperature and VPD acclimation in the field has been hampered by a lack of experimental platforms, recent developments with artificial tree misting in adult trees could open new avenues for future research on this topic (Novick 2024). Moreover, while this study uncovers critical mechanisms that enhance our understanding of trees' medium-term responses to drought stress, it is essential to emphasise that the pot conditions examined here differ largely from the natural soil conditions in which forest trees typically evolve in. Any below-ground acclimation was blocked by the restricting pot size, as all trees had spread their roots throughout the entire pot volume at the end of the experiment. Thus, a bigger leaf area could not be compensated by a deeper/wider root system. The fact that plants were in pots may also explain the relatively similar THF for all species despite their different theoretical drought tolerances. Furthermore, it is important to note that a warming range of 2°C-4°C and VPD, peaking at 3.2 MPa and 34°C, is relatively mild compared to conditions during extreme events. In contrast, extreme heat events, such as heatwaves, can drive mortality

through heat stress (e.g., Still et al. 2023), in which case acclimation may not be sufficient to delay tree mortality.

Overall, our study provides mechanistic insights into how acclimation to high temperature and VPD shapes tree mortality vulnerability. We found that such acclimation promotes faster stomatal closure, reduces g_{min} , and raises the Ψ_{TLP} , making trees more conservative in their water use. In contrast, moderate warming at low VPD primarily affects soft traits, such as increased LAtot, height, and stem diameter, which can hasten hydraulic failure. From an ecosystem management perspective, our findings suggest that artificially lowering VPD through misting may disturb and restrict natural acclimation processes, potentially leaving the trees unprepared for intense soil water stress. In the longer term, our results suggest that trees don't get more resistant to drought through acclimation, but they rather attempt homeostasis by adjusting leaf area and physiological traits to moderate the risk of structural overshoot. On the other hand, O. robur displayed a unique pattern in our study, by showing both modest rise in leaf area coupled with significant improvement in water conservation traits. While this study advances our mechanistic understanding of how temperature and VPD jointly influence time to hydraulic failure during drought, the long-term consequences of this acclimation (here observed after only 1 year) require further validation in natural settings, where other processes alter mortality (e.g., rooting depth acclimation, competition between species, insects, and parasites). Interestingly, the trees demonstrated both favourable acclimation mechanisms and unfavourable ones, which roughly balanced each other out. This suggests that the drought-induced mortality vulnerability of today's trees and tomorrow's trees may remain comparable for the studied species, despite possible growth stimulation from rising temperatures. Finally, while consistent vulnerability is preferable to rising vulnerability, the growing intensity and frequency of extreme events inevitably signal a rise in tree mortality.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Dryad at https://zenodo.org/communities/dryad/records?q=&l=list& p=1&s=10&sort=newest. Data used in this manuscript will be available from the Dryad Digital Repository after acceptance. Data supporting the findings of this study are also available from the corresponding author, LM. Supporting Information is available for this paper.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.