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Research

Interactions between beech and oak seedlings can modify the effects of hotter droughts and the onset of hydraulic failure

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Summary

- Mixing species with contrasting resource use strategies could reduce forest vulnerability to extreme events. Yet, how species diversity affects seedling hydraulic responses to heat and drought, including mortality risk, is largely unknown.
- Using open-top chambers, we assessed how, over several years, species interactions (monocultures vs mixtures) modulate heat and drought impacts on the hydraulic traits of juvenile European beech and pubescent oak. Using modeling, we estimated species interaction effects on timing to drought-induced mortality and the underlying mechanisms driving these impacts.
- We show that mixtures mitigate adverse heat and drought impacts for oak (less negative leaf water potential, higher stomatal conductance, and delayed stomatal closure) but enhance them for beech (lower water potential and stomatal conductance, narrower leaf safety margins, faster tree mortality). Potential underlying mechanisms include oak's larger canopy and higher transpiration, allowing for quicker exhaustion of soil water in mixtures.
- Our findings highlight that diversity has the potential to alter the effects of extreme events, which would ensure that some species persist even if others remain sensitive. Among the many processes driving diversity effects, differences in canopy size and transpiration associated with the stomatal regulation strategy seem the primary mechanisms driving mortality vulnerability in mixed seedling plantations.

Introduction

The worsening of drought events with rising air temperature alters tree water relations and causes one of the most critical environmental stresses for forests. Hotter droughts can lead to severe hydraulic impairments (e.g. Fontes *et al.*, 2018; Klein *et al.*, 2022) and more rapid and widespread tree mortality (e.g. Anderegg *et al.*, 2016; McDowell *et al.*, 2018; Hartmann *et al.*, 2022). In this context, finding mitigation strategies to lessen tree vulnerability to hot droughts has become a critical research area in plant ecology. Interactions between species have a strong potential to alleviate drought impacts and forest die-off events (e.g. Anderegg *et al.*, 2018; Grossiord, 2020). Yet, how diversity modulates the hydraulic responses of trees to hotter droughts is largely misunderstood and not accounted for in climate-vegetation models.

The interactions between functionally contrasting species can lead to facilitation processes and complementarity for resources, inducing higher water availability (e.g. Schwendenmann *et al.*, 2015; Jing *et al.*, 2021) and a potential delay in the onset of hydraulic dysfunctions during drought (Hajek *et al.*, 2022). Cohabiting tree species often exhibit distinct hydraulic strategies to deal with drought, enabling such beneficial interactions (Grossiord, 2020). For instance, mixing juvenile trees can improve the microclimate and diminish atmospheric drought impacts due to a cooler, more humid atmosphere reducing the vapor pressure deficit (VPD) (Wright *et al.*, 2021; Watson *et al.*, 2023). Many studies in various plant communities from herbaceous to forests have reported such facilitative processes (e.g. Wright *et al.*, 2015; Aguirre *et al.*, 2021; Zhang *et al.*, 2022). Similarly, complementarity between species may arise from a better sharing of belowground resources. In mixed beech and oak forests, beech (*Fagus sylvatica* L.) rooting system is not as effective in exploring deep soil layers as oak (*Quercus* spp.), suggesting that the two species partition water resources by relying on different soil depths

(Zapater *et al.*, 2011). Similar processes could be expected for these species at a younger development stage (Moreno *et al.*, 2023). Juvenile trees may further benefit from the presence of adult ones because of enhanced shading and hydraulic redistribution, improving forest regeneration (Warren *et al.*, 2008; Andivia *et al.*, 2018). However, most studies conducted on juvenile trees focused on potted seedlings where root growth is extremely limited, leading potentially to belowground competition that outweigh facilitative processes (Prieto *et al.*, 2011). Similarly, species interactions can shift from beneficial to negative due to spatial and temporal differences in resource availability (e.g. when moving from mild to extreme droughts) (Haberstroh & Werner, 2022) or during stand development (e.g. De Groote *et al.*, 2018). During hotter droughts, moisture reductions may be too intense for these complementarity mechanisms to overcome, and plasticity in functional traits during the plant development (e.g. shift in water sources and/or canopy size) might affect the occurrence of resource partitioning (Grossiord *et al.*, 2018). A crucial starting point for gaining process knowledge on how functional diversity can lessen tree vulnerability to hotter droughts is to clarify the impacts of species interactions on tree hydraulic responses to extreme events.

Drought effects on tree hydraulics have been well-studied over the past 50 yr, allowing us to gain a significant understanding of the sequence of events leading to tree decline (Choat *et al.*, 2018). When exposed to decreasing soil moisture, leaf relative water content and Ψ_{leaf} decrease, leading to stomatal closure to prevent water loss (Brodribb & Holbrook, 2003; Martin-StPaul *et al.*, 2017). As the drought intensifies, leaves lose their turgor after reaching a specific threshold of Ψ_{leaf} (i.e. the leaf turgor loss point, Ψ_{TLP}; Bartlett *et al.*, 2012). Following stomatal closure, water loss continues through the leaf cuticle and leaky stomata (i.e. the minimum stomatal conductance, g_{min}; Duursma *et al.*, 2019), thereby contributing to progressive plant dehydration. Consequently, plants with larger canopies might be subjected to earlier stomatal closure, higher global residual water loss because of the stronger evaporative demand of the crown, but also increased microclimate offset (Jucker *et al.*, 2014; Lüttschwager & Jochheim, 2020; Zhang *et al.*, 2022). Once a species-specific xylem water potential has been reached, embolism will start occurring and progressively decrease the stem hydraulic conductivity until reaching dangerous thresholds that induce hydraulic failure (e.g. the water potential leading to 50% loss of conductivity, P_{50}) and, ultimately, tree mortality (Tyree & Sperry, 1989; Cochard, 2006; Choat *et al.*, 2018). Hydraulic indicators using the Ψ_{TLP} have been widely used to provide quantitative measures of a species' capacity to tolerate drought (Meinzer *et al.*, 2009; Bartlett *et al.*, 2012; Blackman, 2018). For instance, the leaf safety margin (SM_{leaf}) is the difference between the Ψ_{TLP} (i.e. often used as a proxy for the Ψ_{leaf} at stomatal closure; Rodriguez-Dominguez *et al.*, 2016) and the minimum leaf water potential (Ψ_{min}, reflecting the midday water potential). Hence, SM_{leaf} represents the range of Ψ_{leaf} a plant can experience before stomatal closure (Fontes *et al.*, 2018). Similarly, the difference between xylem P_{50} and Ψ_{TLP}, defined as the stomatal safety margin (SM_{P50}), determines the range of Ψ_{leaf} across which plants control the risk of hydraulic failure through stomatal closure (Martin-StPaul *et al.*, 2017). Trees usually operate with narrow safety margins, regardless of the climatic conditions in which they occur (Choat *et al.*, 2012). Still, sub-Mediterranean species such as pubescent oak (*Quercus pubescens* Willd.) may present wider safety margins than temperate species such as beech, reflecting their higher drought tolerance (Fuchs *et al.*, 2021). Previous work often considered these margins as relatively static (e.g. Meinzer *et al.*, 2009; Choat *et al.*, 2012; but see Tomasella *et al.*, 2018). However, prolonged drought exposure has been shown to reduced whole-tree leaf area (e.g. DeLucia *et al.*, 2000; Markesteijn & Poorter, 2009; Martin-Stpaul *et al.*, 2013), g_{min} (e.g. James *et al.*, 2008; Duursma *et al.*, 2019), and Ψ_{TLP} (e.g. Bartlett *et al.*, 2012; Limousin *et al.*, 2022; Tordoni *et al.*, 2022), resulting in narrower SM_{leaf}, and SM_{P50} to ensure the maintenance of CO_2 assimilation (Tyree & Sperry, 1988). Hence, long-term drought acclimation of multiple leaf hydraulic traits can reduce tree evaporative demand and delay the time to hydraulic failure (THF) (e.g. Lemaire *et al.*, 2021).

During hot droughts, elevated temperature increases the vapor pressure deficit (VPD; Grossiord *et al.*, 2020), exacerbating hydraulic dysfunctions (e.g. Liu *et al.*, 2020; Jagadish *et al.*, 2021). High VPD increases leaf-level transpiration, which accelerates soil moisture reductions (Teskey *et al.*, 2015). Higher air temperature further instantaneously amplifies g_{min} (Riederer & Müller, 2008), which can increase hydraulic conductivity losses (Schönbeck *et al.*, 2022), especially if high temperature is combined with low soil moisture (Cochard, 2021). Furthermore, exposure to prolonged warming can produce larger and thinner leaves (e.g. Hudson *et al.*, 2011; Wu *et al.*, 2020), lower the stomatal sensitivity to VPD (e.g. Ameye *et al.*, 2012; Teskey *et al.*, 2015; Drake *et al.*, 2018), decrease g_{min} (e.g. Duursma *et al.*, 2019; Schönbeck *et al.*, 2022) and reduce Ψ_{TLP} (via osmoregulation, e.g. Loik & Harte, 1997; Tordoni *et al.*, 2022), thereby possibly limiting the adverse impacts of hotter droughts. Moreover, high VPD decreases steady-state stomatal aperture and g_s (Buckley *et al.*, 2011), which could also ease drought impacts on hydraulic functions (Fontes *et al.*, 2018). Overall, significant uncertainties remain on how tree species deal with an extended combination of high temperatures, VPD, and low soil moisture (Brodribb *et al.*, 2020). Nonetheless, whether interactions between tree species with different hydraulic strategies and long-term acclimation to these extreme conditions could slow the events leading to tree mortality has never been addressed experimentally.

The main objective of this study was to investigate how species interactions alter the hydraulic responses and timing to hydraulic failure during hot droughts. We studied pubescent oak and European beech, two widely distributed and cohabiting European tree species. Pubescent oak grows in warm sub-Mediterranean to temperate regions and is more tolerant to drought and heat than European beech, a temperate species growing in rather moist and cool environments (Didion-Gency *et al.*, 2022). We exposed oak and beech seedlings planted in intra- or interspecific combinations for 4 yr to chronic air warming and soil drought acting alone or together in open-top chambers. We expected chronic drought to reduce g_s and Ψ_{leaf} and to lead to the development of smaller and thicker leaves with lower g_{min} and Ψ_{TLP}. These responses should result in narrower SM_{leaf} and SM_{P50} and faster hydraulic failure compared with the control when subsequently exposed to comparable drought conditions. We expected that under multiyear heating, the trees could produce larger and thinner leaves with less sensitive stomata, allowing higher g_s and Ψ_{leaf} but with lower g_{min} and $\Psi_{TLP,}$ leading to narrower hydraulic safety margins and faster onset of hydraulic failure compared with the control. The combination of drought and heat should exacerbate the effects observed under drought alone. The individual tree response to climatic treatments should be amplified for beech compared with oak, an already more drought tolerant species. Besides, interspecific interactions are expected to mitigate the degree of soil moisture or atmospheric drought stress (through improved water resource partitioning and reduced VPD), leading to lower adverse impacts of the climatic treatments than intra-specific interactions.

Materials and Methods

Site description

The study was conducted at the model ecosystem facility Modoek located at the Swiss Federal Research Institute WSL in Birmensdorf (47°21′48″N, 8°27′23″E, 545 m above sea level). Sixteen hexagonal glass-walled open-top chambers of 6 m^2, 3 m height, and 1.5 m deep were filled with a 1 m-deep layer of gravel for fast drainage, covered with a fleece layer to avoid root proliferation past the soil layer, and topped by a 50-cm layer of artificial acidic sandy forest soil (Ökohum, DE; pH 6.3) that allows fast drainage. A shallow soil depth was selected to promote soil drying in the treatments and promote aboveground growth more rapidly. Nevertheless, this design also reduces the potential role of belowground complementarity in water uptake depth between species. The glass walls and roofs reduced photosynthetic active radiation (PAR) inside the chambers by *c.* 50% compared to the outside (but still reached up to 1700 $\mu mol\ m^{-2}\ s^{-1}$ PAR during sunny days). Each chamber was divided into eight compartments (i.e. 0.75 m^2 each), irrigated from April to November every 2 d and every 2 wk during winter (Fig. 1c,d). Air temperature and humidity at 50 cm and 2 m above the ground were monitored inside each chamber every 10 min (Atmos 14; Meter Group Inc., Pullman, WA, USA). Soil temperature and moisture were measured in four compartments at 25 cm depth every 10 min (5TM Decagon Devices; Meter Group Inc., Pullman, WA, USA). In October 2018, 2-yr-old tree seedlings of European beech (*Fagus sylvatica* L.) and pubescent oak (*Quercus pubescens* Willd.) were planted in two species combinations: monocultures (i.e. four trees of the same species) and mixtures (i.e. two trees of each species). Each chamber included all the possible species combinations. Grossiord *et al.* (2022) provide more details on the experimental design.

In April 2019, we started applying four climatic treatments in the chambers: (1) control (C), with ambient air temperature and soil moisture maintained at field capacity (i.e. *c.* 10% considering the sandy soil composition); (2) heating (H), where the air temperature inside the chambers was maintained at *c.* 5°C above the temperature of the control and soil moisture at field capacity; (3) drought (D), where the soil moisture was reduced by *c.* 45% relative to the control and ambient air temperature; and (4) hot drought (HD), where both treatments were applied simultaneously (Fig. 1a,b). Our study aimed to understand the physiological mechanisms under chronic warming and reduced soil moisture rather than predict the response of trees to periodic extreme events. The selected conditions have been chosen at our facility to match a possible future shift in mean air temperature leading also to constantly drier soils (Lyon *et al.*, 2022). Each combination of climatic treatment ($n=4$ chambers), species ($n=2$), and species combinations ($n=2$) was repeated six times. One tree per species was randomly selected in each species combination for repetitive measurements leading to 96 trees in total (i.e. 48 per species). Leaf-level hydraulic traits were measured in all trees once per year at the end of the growing season in September (i.e. before the first sign of senescence) for 3 yr from 2020 (i.e. 1 yr after the treatments started) to 2022.

Leaf-level stomatal conductance and water potential

We measured the leaf-level light-saturated stomatal conductance (g_s, mol $m^{-2}\ s^{-1}$) on one leaf from the highest part of the crown of each selected tree. Gas exchange measurements were conducted between 9:00 h and 15:00 h (local time) using two LI-6800 infrared gas exchange analyzers (LI-6800; Li-Cor Biosciences, Lincoln, NE, USA). The relative humidity was set to 50% (to match the average daily environmental conditions inside the chambers), the CO_2 concentration to 400 ppm, the photosynthetic photon flux density (PPFD) to 1500 $\mu mol\ m^{-2}\ s^{-1}$ (to ensure saturating light conditions), and the air temperature inside the cuvette to 20°C in the nonheated chambers and 25°C in the heated ones (to fit the mean midday air temperature during the measurements). On the same day as stomatal conductance measurements, one leaf per tree was collected before sunrise ($\Psi_{predawn}$) and at midday (Ψ_{midday}) to measure the leaf water potential (MPa) with a Scholander-type pressure chamber (M1505D; PMS Instruments, Albany, OR, USA).

Minimum conductance and specific leaf area

Minimum conductance (g_{min}, mmol $m^{-2}\ s^{-1}$) was measured as described in Sack *et al.* (2003). 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 (CanoScan LiDE 300; Canon, Uxbridge, UK), followed by analysis with FIJI from IMAGEJ (Schindelin *et al.*, 2019). The leaves were stuck to a laboratory tape run between two laboratory stands, standing in a small dark climatic chamber with stable air temperature (22 ± 2°C) and humidity (55 ± 12%). Every 15 min, the leaves were taken from the climatic chamber and stored in a closed black plastic bag while waiting to be weighed using a high-precision scale (MS104; Mettler Toledo, Bussigny). This procedure was repeated eight times. g_{min} was obtained from the slope of the linear relationship between leaf

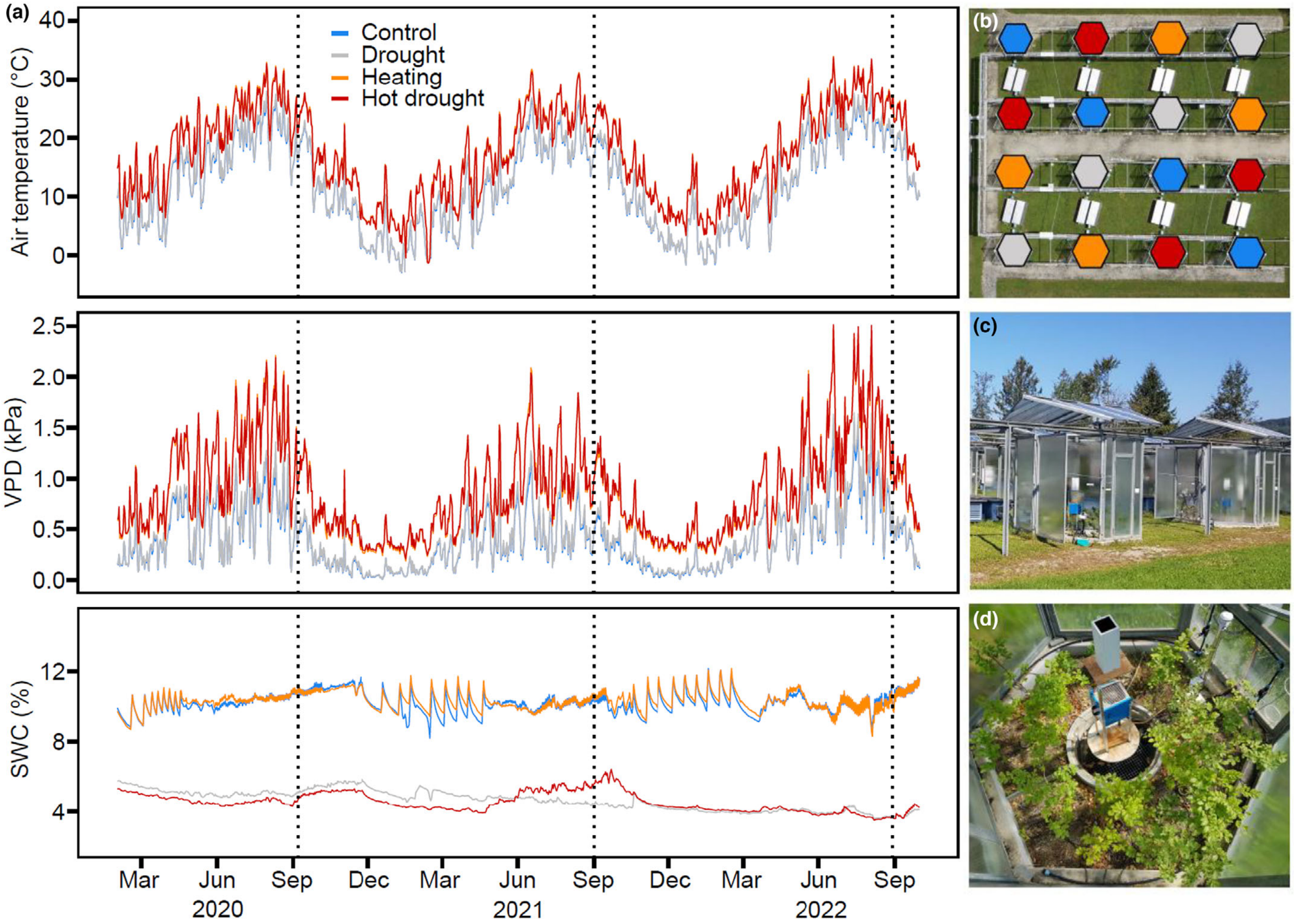

Fig. 1 Mean daily air temperature, vapor pressure deficit (VPD), and soil water content (SWC) (a) under control (blue), drought (gray), heating (orange), and hot drought (red) conditions in the open-top chambers ($n = 4$ chambers per treatment). Dashed vertical lines indicate the measurement campaigns. Aerial picture of the 16 open-top chambers with the four treatments (b), picture showing the side of the open-top chambers (c), and aerial picture from a heated chamber including a central heating system and eight compartments with different European beech and pubescent oak combinations (d).

mass and its drying time, corresponding to the cuticular transpiration per mole fraction VPD, assuming the leaf's internal air to be fully saturated (Pearcy *et al*., 2000).

Five fully expanded mature leaves were collected for each tree and scanned using a flatbed scanner (CanoScan LiDE 300; Canon), followed by analysis with ImageJ to extract the mean individual leaf area (LA_{leaf}, cm^2). Then, the leaves were dried at 60°C for 24 h and weighed using a high precision scale (MS104; Mettler Toledo) to calculate the specific leaf area (SLA, $cm^2\ g^{-1}$). In June 2022, we further estimated the whole-canopy leaf area (LA_{total}, cm^2) for modeling purposes (see below and Supporting Information Notes S1).

Pressure–volume curves

Pressure–volume curves were determined using the bench-dehydration method (Koide *et al*., 2000). One fully expanded mature leaf per tree was cut the evening preceding the measurement. The petiole was recut under water, and the leaf was stored with the petiole submerged in water in the darkness for the night, ensuring that full hydration was reached before the start of the measurements the following morning. Leaf water potential and weight were measured using a Scholander-type pressure bomb (M1505D; PMS Instruments) and a high-precision scale (MS104; Mettler Toledo). Different levels of water potential were reached by letting the leaves dry progressively in an open plastic bag on a lab bench. For oak, the procedure of measuring water potential, weighing, and drying was repeated with increasing drying time intervals (from 10 s to 1 h) until achieving water potentials of *c*. −4 MPa or until water potential reached a plateau. For beech, the procedure was repeated continuously without letting the leaves dry on the bench due to the rapid water loss and the corresponding drop in leaf water potential. After the measurements, the leaf was dried for 24 h at 60°C to determine the dry mass. The pressure–volume curves were constructed by plotting the reciprocal of the water potential vs the relative water deficit. Leaf water potential at turgor loss point (Ψ_{TLP}, MPa), the osmotic potential at full turgor (Ψ_o, MPa), modulus of elasticity (ε, MPa), and relative water content (RWC, %) were calculated following Koide *et al.* (2000).

We calculated the stomatal safety margin (SM_{P50}, MPa) as the difference between the Ψ_{TLP} and the water potential at 50% of xylem conductivity loss (P_{50}, MPa) (Martin-StPaul *et al.*, 2017). Because of the destructiveness of P_{50} measurements, we extracted the P_{50} of each species from a database (Choat *et al.*, 2012) with a value of −3.2 and −3.3 MPa for beech and oak, respectively. Although we acknowledge that using the same P_{50} values for all trees does not account for the potential acclimation of this trait to the climatic treatments, previous work has shown limited P_{50} plasticity to drought in our species (e.g. Matzner *et al.*, 2001; Torres-Ruiz *et al.*, 2013). However, Tomasella *et al.* (2018) observed a decrease of up to 0.4 MPa for beech under repetitive drought. Hence, care must be taken with result interpretation. We further calculated the leaf safety margin (SM_{leaf}, MPa) for each tree and year as the difference between Ψ_{midday} (proxy of Ψ_{min}) and Ψ_{TLP} (Fontes *et al.*, 2018).

Timing to hydraulic failure and mechanisms of species interactions

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 species interactions and trait plasticity to the different treatments on the overall plant performances. 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. Then, stomatal and cuticular fluxes are used to compute 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 (Ψ_{soil}) 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 explicit representation of what occurs beyond the point of stomatal closure, under extreme water stress, when g_{min} leads to plant dehydration and hydraulic failure (i.e. 100% conductivity loss). The model can be parameterized with ecophysiological traits measured empirically and can be run using different environmental conditions and species composition to estimate their overall effect on plant performances (time with open stomata or time until total hydraulic failure). Here, we used the detailed version coded in C (Cochard *et al.*, 2021) which works at a time step of 0.01 s and was adapted to allow two individuals to compete for the same stock of water (Moreno *et al.*, 2023). In this case, the model allows two trees to absorb water in the same soil volume. Technically, two codes corresponding to two trees with their own set of traits were run in parallel, withdrawing the water from the same volume.

We aimed to test the effect of trait plasticity in the different treatments (due to species interactions and climatic treatments) on the risk of hydraulic failure at the seedling stage. The model was parameterized with species-specific plant traits in each treatment for the year 2022 (due to LA_{total} missing in previous years). We used key measured plant traits that are known to influence the time to hydraulic failure (Ruffault *et al.*, 2022) and that were affected by the treatments. 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 and bark), which influence g_{min}, (2) LA_{total} (Notes S1), which influences tree transpiration, and (3) LA_{leaf}, which influences the boundary layer conductance. In addition, we included hard traits: (4) g_{min} and the maximum stomatal conductance, which defines tree transpiration, and (5) the pressure volume curve parameters, which influence the symplasm capacitance and are used to compute turgor-mediated stomatal closure (Martin-StPaul *et al.*, 2017) (see later Table S4 for a detailed description of the parameters). P_{50} was assumed constant at the species level in agreement with the low plasticity previously found for this trait (Matzner *et al.*, 2001; Torres-Ruiz *et al.*, 2013). However, it is important to acknowledge that P_{50} acclimation to drought was also reported in adult beech trees (Tomasella *et al.*, 2018), which could alter the simulated responses in the model. To assess the impact of P_{50} acclimation on our results, four scenarios were run: (1) no acclimation of P_{50} to D and HD in both species (i.e. −3.2 and −3.3 MPa for beech and oak, respectively in all treatments), (2) acclimation of P_{50} to D and HD in all species (i.e. −3.6 and −3.7 MPa for beech and oak, respectively; only for the D and HD treatments), (3) acclimation of P_{50} to D and HD only for beech, and (4) acclimation of P_{50} to D and HD only for oak. Acclimation was estimated to be maximum 0.4 MPa based on published observations for *F. sylvatica* (Tomasella *et al.*, 2018). In all treatments, the root area was assumed proportional to the leaf area. The model was run for the different trait combinations with constant atmospheric conditions (air temperature at 20°C, 0.7 kPa VPD, and PPFD at $500\ \mu mol\ m^{-2}\ s^{-1}$) and an initial soil water content at saturation. Hence, the model does not account for potential mitigating impacts of the microclimate in mixtures. Then, the model was run until the plants reach total hydraulic failure (100% embolism in branches). Two integrative metrics of plant performance during drought were estimated: (1) the time to stomatal closure (TSC), which indicates the time over which the plant can be productive, and (2) the time to hydraulic failure (THF), which indicates the survival time during drought. First, we compared monocultures and mixtures (i.e. the trees in mixtures share the same water pool) accounting for the measured trait plasticity (i.e. in soft and hard traits). Then, to isolate the influence of soft traits (related to tree size and leaf area) from hard traits, we performed the same test with soft traits set constant on the monocultures only to simplify the simulations.

Statistical analysis

All analyses were performed using the R v.4.2.2 statistical software (R Development Core Team, Vienna, Austria, 2022). The effects of the climatic treatments and species combinations on $\Psi_{predawn}$, Ψ_{midday}, g_s, g_{min}, Ψ_{TLP}, SLA, LA_{leaf}, SM_{leaf}, and SM_{P50}

were determined through linear mixed-effects models for each species using the package LMER. The interactive effects of heating (yes/no), drought (yes/no), and species combination (monocultures/mixtures) were used as fixed effects. The year in which the measurements were done (i.e. 2020, 2021, and 2022) and the individual open-top chambers were treated as random effects as no significant differences were found between them. The effects of climatic treatments and species combinations on TSC and THF were determined through a simple linear model for each species. To reveal significant differences between treatments for each measurement, *post hoc* analyses were performed with a Tukey's HSD test, with FDR correction for multiple testing. Linear and non-linear regressions were used to test the relationships between g_s, $\Psi_{predawn}$, g_{min}, SLA, SM_{leaf}, SM_{P50}, soil water content, and VPD. The significance of these relationships and the differences between species combinations were revealed using ANOVA. Before performing each model, the homogeneity of variances and the normality of residuals were assessed, and data were log-transformed if necessary.

Results

Species interaction effects on leaf hydraulic traits under heating and drought

Drought (D and HD) significantly reduced predawn leaf water potential ($\Psi_{predawn}$) and light-saturated stomatal conductance (g_s) for both species and reduced midday leaf water potential (Ψ_{midday}) for oak (Fig. 2; Table S1). For both species, heat (H) significantly reduced $\Psi_{predawn}$ but had no significant impact on Ψ_{midday} and g_s. Indeed, while we found a significant increase of g_s with soil moisture for both species, we found a positive relationship with VPD only for beech, suggesting a lower sensitivity to atmospheric drought in oak (Fig. S1). For beech, species interactions modulated the responses of Ψ_{midday} to D and HD, with an increase in Ψ_{midday} in monocultures whereas no change was observed in mixtures (Fig. S2; Tables S1, S2). On the contrary, for oak, lower reduction in Ψ_{midday} and g_s were found in monocultures compared with mixtures under D and HD (Fig. S2; Tables S1, S3).

We found a significant reduction of the minimal stomatal conductance (g_{min}), the mean leaf area (LA_{leaf}), and the specific leaf area (SLA) under D and HD for both species. By contrast, no effect of heat was observed on these traits (Fig. 2; Table S1). When mixed, both species had significantly lower LA_{leaf} and SLA (for beech only), independently of the climatic treatment. We did not find a significant impact of species interactions on g_{min} (Fig. 2; Table S1).

We observed a significant increase in the water potential at the turgor loss point (Ψ_{TLP}) under D and HD for both species, independently of species combinations (Fig. 2; Table S1). Under D and HD, the stomatal safety margin (SM_{P50}) significantly increased only for beech, and the leaf safety margin (SM_{leaf}) decreased in both species. No significant impact of H and species combinations on Ψ_{TLP}, SM_{leaf}, or SM_{P50} was detected (Fig. S3; Table S1).

Impact of species interactions on the relationships between hydraulic traits

With decreasing $\Psi_{predawn}$, g_s exponentially decreased for both species (Fig. 3). While no modulating effect of the species combination was observed on this relationship for beech, oak exhibited a steeper relationship (i.e. earlier stomatal closure) in monocultures compared with mixtures (Fig. 3).

With increasing g_{min}, g_s significantly increased, and SLA decreased for both species, independently of the species combination (Fig. 4).

We found a significant negative correlation between SM_{P50} and SM_{leaf} for both species (Fig. 5). The species combination affected this relationship for beech ($P = 0.01$) with a steeper decrease (i.e. narrower SM_{leaf} for the same SM_{P50}) in mixtures than monocultures (Fig. 5).

Timing to hydraulic failure and mechanisms of species interactions

During a simulated drying cycle and under standardized climatic conditions, the time to stomatal closure (TSC) was consistently very close to hydraulic failure (THF) across all climatic treatments and for both species (6-d difference on average for beech and oak). Time to hydraulic failure was significantly longer under D and HD than the control for oak and beech in monocultures (+31 d and +321 d on average for oak and beech, respectively; Fig. 6). With the inclusion of P_{50} acclimation in the model, THF slightly varied from +1 d in the monoculture to −2 d in the mixture on average for both species in D and HD, suggesting that potential acclimation of P50 to drought is negligible in the THF simulation (Fig. S4). Moreover, the inclusion of soft traits in the simulation increased THF by 35% and 89% on average for the monoculture of oak and beech, respectively, in HD (Fig. S5), suggesting that THF was mainly explained by the smaller LA_{total} (Fig. S6) rather than the lower g_{min}, and higher Ψ_{TLP} (Fig. 2) in the D and HD treatments compared with the control for both species. Interspecific interactions significantly reduced THF for beech in the D and HD treatments (by 84% and 95%, respectively), leading to similar values as oak. For oak, THF in mixtures was not significantly affected by species interactions in all climatic treatments (Fig. 6).

Discussion

The broad screening of leaf hydraulic traits conducted in this study over multiple years allows us to shed light on how species interactions affect the events leading to drought-induced tree decline. As widely observed in temperate forests (e.g. Arend *et al.*, 2013; Bolte *et al.*, 2016), drought and heat increased hydraulic stress by altering multiple water-related traits, starting with decreasing the leaf water potential at predawn ($\Psi_{predawn}$) for both species (Fig. 2). However, we found more negative $\Psi_{predawn}$ for beech than oak, independently of the species combination (Fig. 3), suggesting that beech experienced stronger soil moisture limitation under the same climate manipulation. A lower tolerance to high VPD (e.g. Schönbeck *et al.*, 2022) as highlighted by

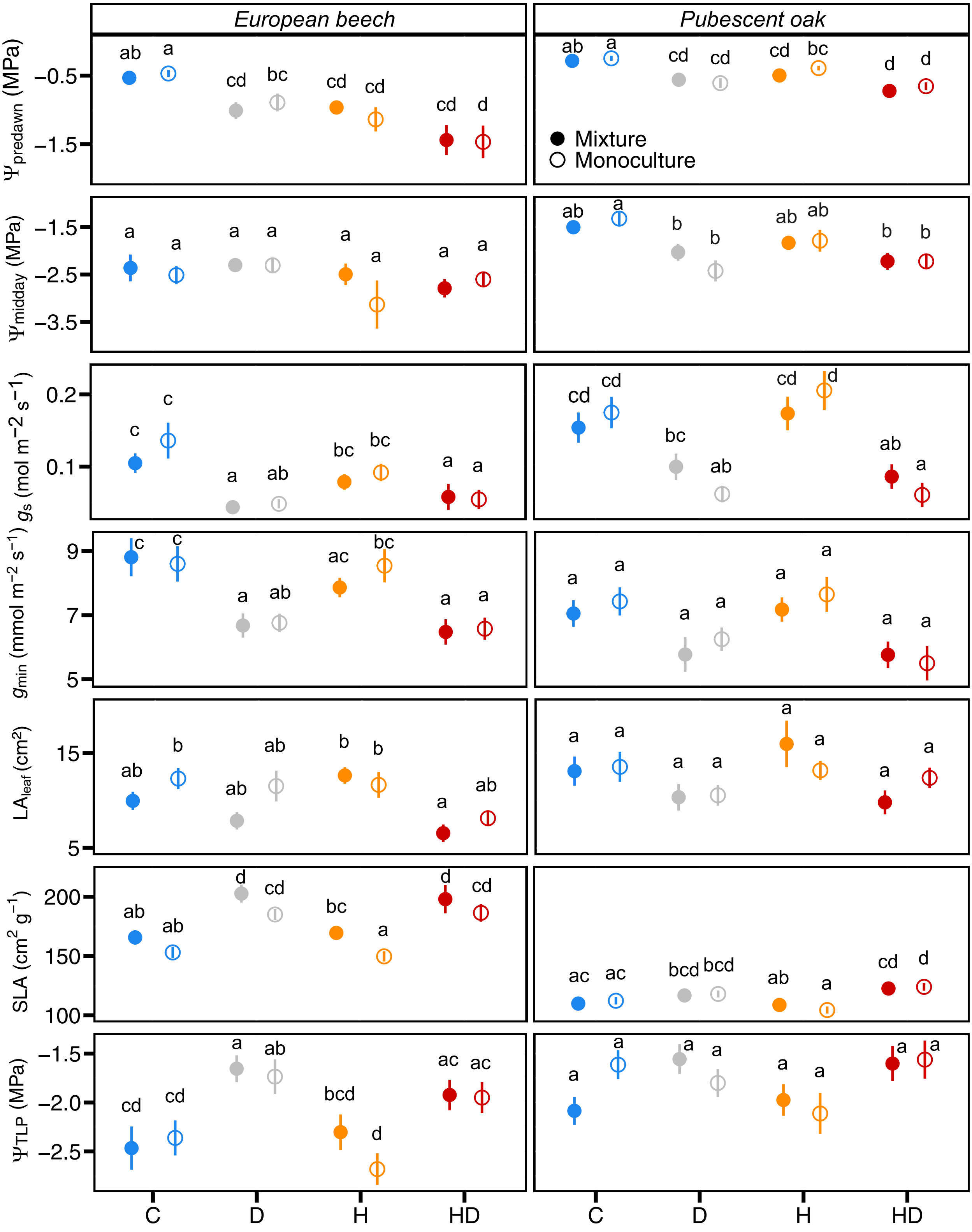

Fig. 2 Leaf water potential at predawn ($\Psi_{predawn}$) and midday (Ψ_{midday}), light-saturated stomatal conductance (g_s), minimal stomatal conductance (g_{min}), mean individual leaf area (LA$_{leaf}$), specific leaf area (SLA), and water potential at turgor loss point (Ψ_{TLP}) for all years (i.e. 2020, 2021, 2022, mean ± SE, $n = 18$ trees) for European beech and pubescent oak in control (C, blue), drought (D, gray), heating (H, orange), and hot drought conditions (HD, red) in monocultures (empty circles) and mixtures (full circles). The letters indicate significant differences between climatic treatments and species combinations.

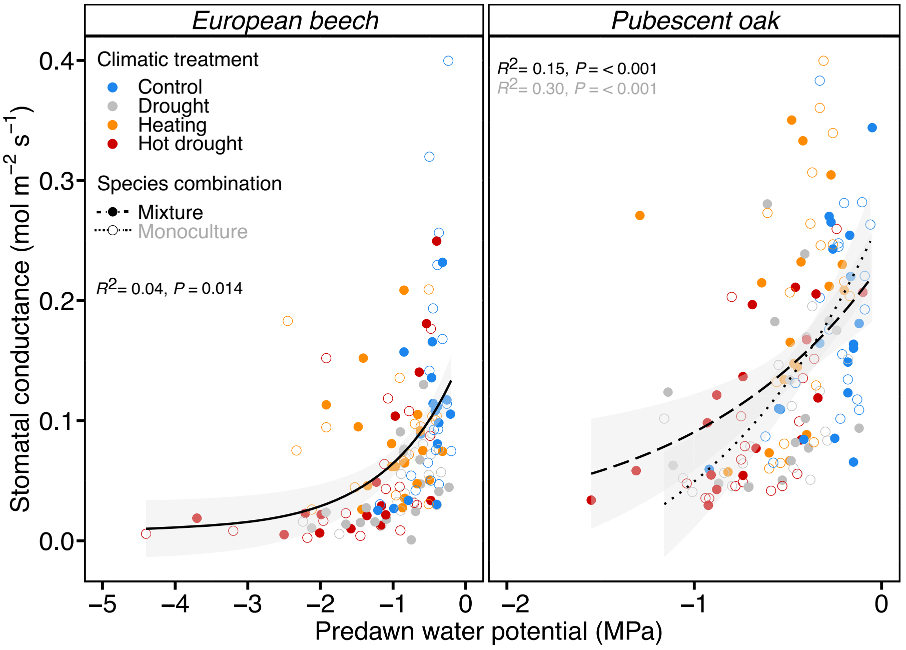

Fig. 3 Relationships between the light-saturated stomatal conductance (g_s) and the leaf water potential at predawn ($\Psi_{predawn}$) for European beech and pubescent oak in control (blue), drought (gray), heating (orange), and hot drought conditions (red) in monocultures (empty circles) and mixtures (full circles). Lines (±CI 95%) represent exponential relationships, with dotted and solid lines standing for significant ones, using non-linear regressions followed by ANOVA, within a given species combination or across all species combinations, respectively. R^2 and P-value are given in the upper left corner, when significant.

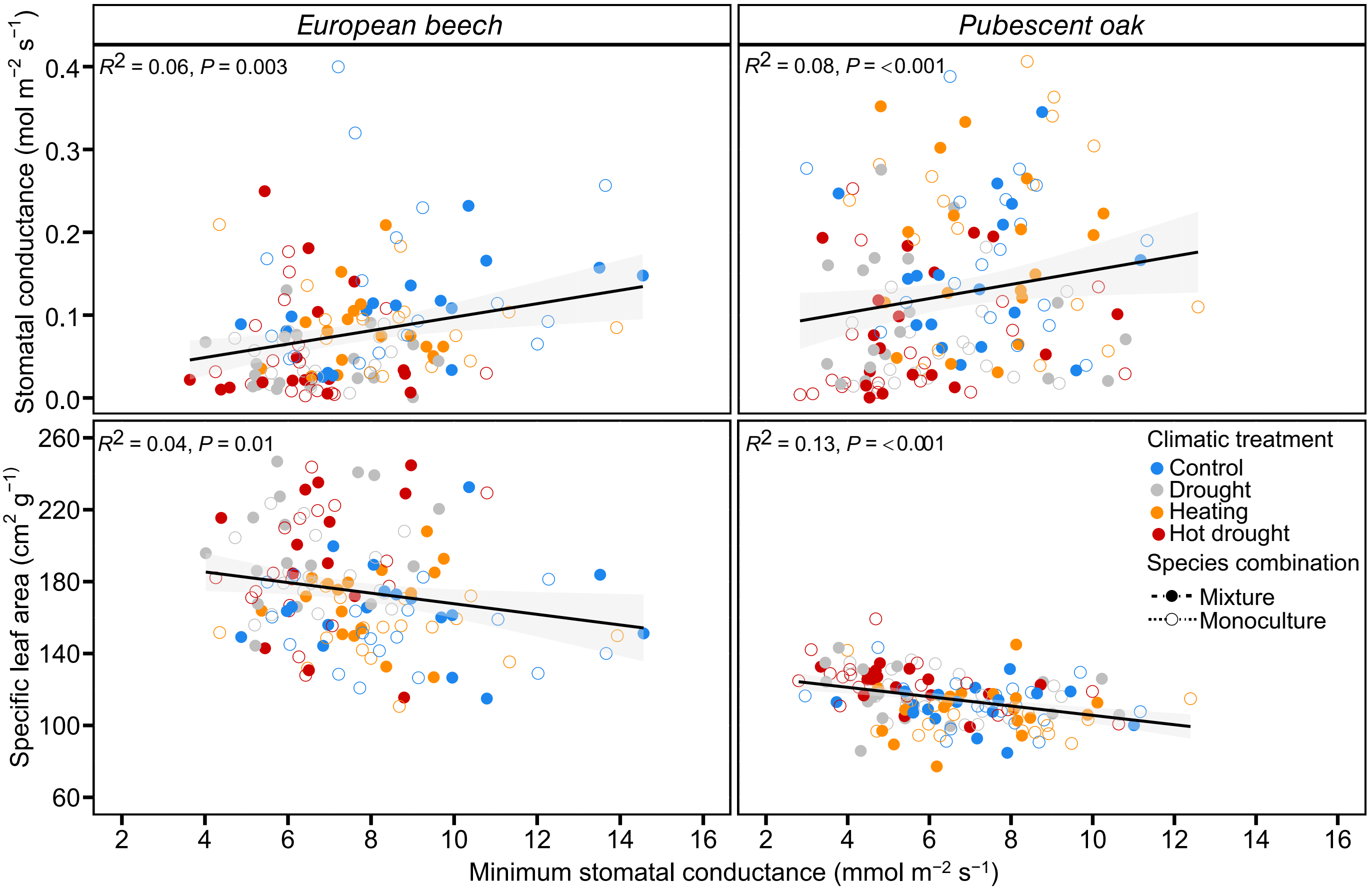

Fig. 4 Relationships between the light-saturated stomatal conductance (g_s), the specific leaf area (SLA), and the minimal stomatal conductance (g_{min}) for European beech and pubescent oak in control (blue), drought (gray), heating (orange), and hot drought conditions (red) in monocultures (empty circles) and mixtures (plain circles). Lines represent linear regressions (±CI 95%) with solid lines standing for significant relationships across all species combinations, using ANOVA. R^2 and P-value are given on the upper left corner, when significant.

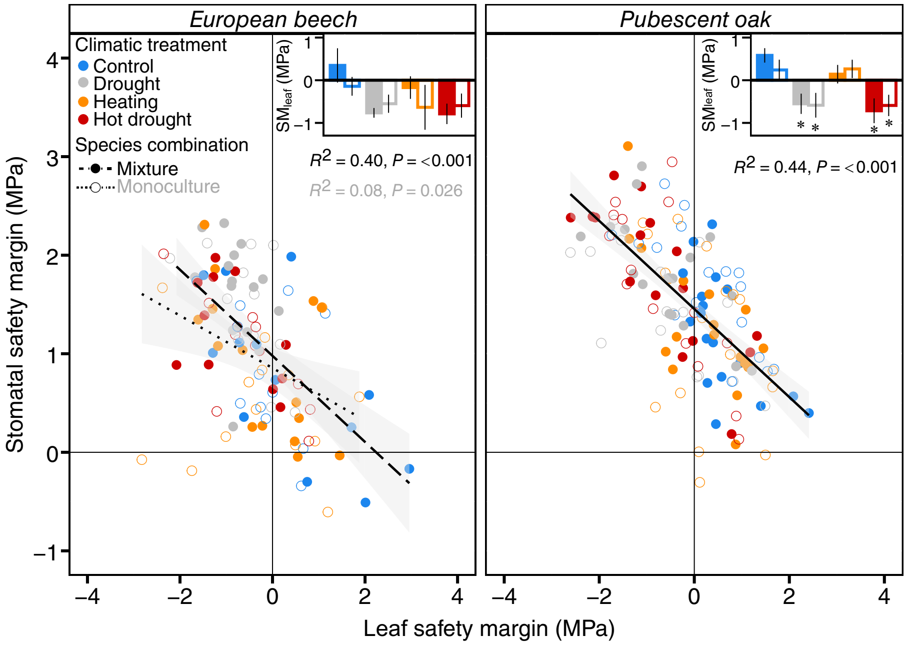

Fig. 5 Relationships between the stomatal safety margin ($\Psi_{TLP} - P_{50}$; SM_{P50}) and the leaf safety margin ($\Psi_{midday} - \Psi_{TLP}$; SM_{leaf}) for European beech and pubescent oak in control (blue), drought (gray), heating (orange), and hot drought conditions (red) in monocultures (empty circles) and mixtures (full circles). Lines represent linear regressions (±CI 95%) with dotted and solid lines standing for significant relationships using ANOVA, within a given species combination or across all species combinations, respectively. R^2 and P-value are given on the upper right corner, when significant. The captions in the upper right corners represent the mean SM_{leaf} for each climatic treatment and species combination for all years (i.e. 2020, 2021, 2022, mean ± SE, $n = 18$ trees). The stars indicate significant difference with the control, using linear mixed-effects models followed by a Tukey type *post-hoc* test (*, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \geq 0.001$).

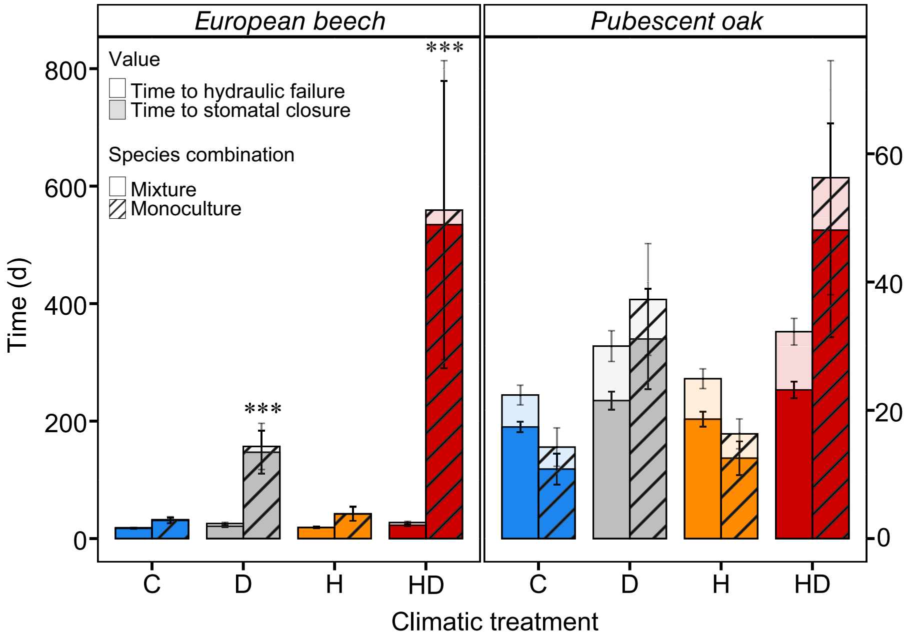

Fig. 6 Time to hydraulic failure and stomatal closure (mean ± SE) modeled with SurEau for European beech and pubescent oak using constant P_{50} and the traits measured (Supporting Information Table S4) in control (C; blue), drought (D; gray), heating (H; orange), and hot drought conditions (HD; red) in monocultures (dashed bar) and mixtures (empty bar), simulated under a constant climate (i.e. mean air temperature at 20°C, VPD at 0.7, PPFD at 500 μmol m^{-2} s^{-1}, and no rain) starting with soil water at saturation. The interspecific competition was accounted for in the simulations. The stars indicate significant differences in time to hydraulic failure between species combinations within each climatic treatment and for each species (*, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \geq 0.001$).

the significant reduction in stomatal conductance (g_s) with increasing VPD (Fig. S1), associated with a lower drought resistance (e.g. Klein, 2014; Meyer *et al.*, 2020) could explain this response. With decreasing $\Psi_{predawn}$, both species similarly reduced g_s to limit water loss. Still, the mixture delayed stomatal closure for oak, allowing a higher g_s and less negative leaf water potential at midday (Ψ_{midday}) (Figs 2, 3). By contrast, for beech, the significant interaction between drought and species combination (Table S1) indicates a larger reduction in Ψ_{midday} in mixtures than monocultures in response to D and HD, and hence, exacerbated soil moisture stress (Fig. S2). These findings point to mixtures diminishing or enhancing water stress for oak and beech, respectively, potentially due to the more efficient water uptake and use of oak compared with beech. Alternatively, an improved microclimate in mixtures could benefit oak seedlings through reduced VPD (Aguirre *et al.*, 2021; Wright *et al.*, 2021), although, we found no indication of lower g_s sensitivity to VPD in this species (Fig. S1). Additional microclimate measurements would be needed to confirm this mechanism. Moreover, as previously observed (Bussotti *et al.*, 1995; Grossoni *et al.*, 1998; Cavender-Bares *et al.*, 2007; Watson *et al.*, 2023), both species reduced their minimum stomatal conductance (g_{min}), individual and whole-tree leaf area (LA_{leaf} and LA_{total}) (Fig. S5), and specific leaf area (SLA) under D and HD, thereby showing a long-term acclimation strategy to limit water loss. However, a more substantial reduction in LA_{leaf} (for both species) and increasing SLA (for beech only) under D and HD was found in mixtures compared to monocultures, indicating smaller and thinner leaves (Fig. 2; Table S1). These findings contradict LA_{leaf} reductions in monocultures vs mixtures observed in grasslands (Watson *et al.*, 2023), which could be related to differences between grasses and trees or the relative young age of our seedlings. Nevertheless, these anatomical adjustments were insufficient for beech to overcome the higher competitiveness of oak.

These morphological shifts probably lead to a higher sensitivity to drought and heat in beech in mixtures compared to monocultures. These findings are corroborated by the steeper response of stomatal safety margins (SM_{P50}) to leaf safety margins (SM_{leaf}) in beech mixtures (Fig. 5). However, SM_{P50} calculations were based on a constant P_{50}, which excludes potential acclimation of this trait to the treatments. Yet, previous study reported lower P_{50} under drought in beech (Tomasella *et al.*, 2018) and if similar acclimation would occur in our study, SM_{P50} could increase further under drought (Fig. S7), leading to an even steeper relationship. Future work would be needed to determine how acclimation of P_{50} could alter this threshold in our study. Nevertheless, the observed trade-off highlights that beech in mixtures close their stomates earlier to extend the point of critical hydraulic failure. However, by reducing SM_{leaf}, beech also limits carbon uptake that could deplete carbohydrate reserves, especially under hot droughts (Grossiord *et al.*, 2022), and minimize allocation of carbon resources belowground (e.g. Hagedorn *et al.*, 2016). Numerous studies observed a decrease of Ψ_{TLP} under experimental drought (e.g. Serrano *et al.*, 2005; Deligoz & Gur, 2015; Binks *et al.*, 2016), extending the water potential range over which the leaf can remain turgid and functional. However, contrary to previously reported, Ψ_{TLP} increased under drought for both species in our experiment (Fig. 2), resulting in earlier stomatal closure as the soil progressively dries out. One potential explanation for higher Ψ_{TLP} could be that the smaller and thinner leaves and possible depletion of carbohydrates under prolonged hot drought may prevent osmoregulation processes from taking place (Sevanto *et al.*, 2014). In addition, as suggested by Juenger & Verslues (2022), increasing Ψ_{TLP} could lead to slower soil water depletion, which could prove favorable under prolonged drought where water conservation and increased water use efficiency would be more valuable. From our knowledge, few works have monitored Ψ_{TLP} under chronic drought over multiple years (e.g. Tomasella *et al.*, 2018; Hesse *et al.*, 2023). Hence, our current understanding of Ψ_{TLP} acclimation remains limited and would need to be addressed more extensively in future studies.

An earlier stomatal closure (TSC) for beech under chronic drought when mixed with oak due to higher Ψ_{TLP} (Fig. 5) could reflect a more conservative strategy to delay hydraulic dysfunctions in mixtures. However, this acclimation response did not significantly broaden hydraulic safety margins and appeared insufficient to delay hydraulic failure. Indeed, we found earlier hydraulic failure (THF) for beech mixtures compared with monocultures by up to 95% under hot droughts with a standardized drying cycle and regardless of the potential acclimation of P_{50} (Figs 6, S4). Time to hydraulic failure was primarily driven by the total leaf area of the tree (Figs S5, S6), especially for oak that exhausted water resources more rapidly in mixtures. Consequently, oak's onset of hydraulic failure was not impacted by the presence of beech, whereas beech extensively shortened its TSC and THF due to increasing competition with oak compared with monocultures. Several studies highlighted the role of canopy size in drought-induced mortality with lower leaf area reducing water loss and local water stress (e.g. Greenwood *et al.*, 2017; Anderegg *et al.*, 2019; Rosas *et al.*, 2019; Trugman *et al.*, 2019). In our study, tree leaf area played a stronger role for THF than treatment acclimation in leaf hydraulic traits (i.e. g_{min} and Ψ_{TLP}) for both species. For example, this led to delayed mortality in beech compared with oak in monocultures under drought conditions (Fig. 6). However, when mixed with oak, the lower evaporative demand in beech due to wider SM_{leaf} supports higher g_s in oak without decreasing soil moisture availability (as shown by the higher Ψ_{midday}). As observed here, previous work highlighted that mixtures often do not benefit the most vulnerable species to drought (i.e. beech) because the stronger competitors (i.e. oak) can exhaust water resources more rapidly or efficiently (e.g. Forrester *et al.*, 2016; Didion-Gency *et al.*, 2021). Here, we show that this mechanism would also increase the probability of drought-induced mortality for less competitive species. However, it is important to consider that our model simulations did not include facilitative effects associated with microclimate amelioration (i.e. the simulations were run under similar climate in mixtures and monocultures), a process that would be particularly important in natural ecosystems (Wright *et al.*, 2015; Aguirre *et al.*, 2021). Hence, future work should further investigate how VPD may change within the different species combinations and how it could modify the THF in natural systems.

Overall, our results on tree seedlings are consistent with previous work that showed a higher growth resilience of oak in natural mixed stands with beech (e.g. Jourdan *et al.*, 2020). Our model simulations further suggest that lower drought stress in oak could be driven by belowground competition reduction because of the lesser ability of beech to rapidly take up water resources (i.e. smaller leaf area and g_s in mixtures). However, our experiment was conducted on trees at an early development stage (i.e. 6 yr old), and interactions between trees could take multiple years to establish (Domisch *et al.*, 2015). Indeed, potential aboveground facilitation processes as microclimate feedbacks could be more dominant in mature forests (Zhang *et al.*, 2022). Studies in old-growth forests have also reported lower g_s sensitivity to soil moisture and competitive dominance of beech when mixed with oak (Jonard *et al.*, 2011; Jacobs *et al.*, 2022), suggesting that the initial detrimental interaction observed here at the seedling stage could become beneficial as trees grow older. Moreover, although reduced drought stress in mixtures could be due to water partitioning by the deeper roots in mature stands (Früchtenicht *et al.*, 2018), it is very unlikely that it occurred in our study as the trees were restricted to a 50-cm soil depth. Instead, belowground competition for water may have been predominant in our study. Work in more diverse forests using experimental drought or temperature manipulation would be needed to unravel how interaction processes may shift with tree ontogeny and tree diversity in natural conditions.

Conclusions

Our results show for the first time that species interactions affect the sequence of events leading to tree seedling decline and the risk of drought-induced mortality. Chronic and prolonged (i.e. > 4 yr) hot and dry conditions led to tighter stomatal control (lower Ψ_{predawn}, lower g_s, and higher turgor loss), smaller leaf area, and shorter leaf safety margins in juvenile beech. Similarly, oak acclimated to hot and dry conditions mainly by reducing leaf area and shortening the time to stomatal closure. Nevertheless, these physiological adjustments to chronic heat and drought proved insignificant compared to differences in canopy size and transpiration rates between species during a fatal drought. Indeed, larger canopies and more rapid exhaustion of soil moisture for oak increased the simulated mortality risk of beech. Hence, this work highlights that leaf area (individual and total) and water use as proxy of plant functional strategy are important drivers of tree competitiveness and species mortality risk in mixed forests. Overall, this study pointed out the greater sensitivity of beech seedlings to projected climate scenarios mainly when mixed with more competitive species such as oak, while oak seedlings seemed to be more resilient to these scenarios in mixtures.

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Competing interests

None declared.

Author contributions

EM and CG conceived and designed the study. EM, JD, MD-G, LM, AV, and CG collected the data. HC and NM-S conducted the model simulations. EM analyzed the data and led the writing of the manuscript. EM, JD, MD-G, LM, AV, FV, HC, NM-S, and CG critically contributed to the manuscript and gave final approval for publication.

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Data availability

Data used in this manuscript will be available from the Dryad Digital Repository, doi: 10.5061/dryad.1ns1rn918.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Relationship between the light-saturated stomatal conductance and soil water content and vapor pressure deficit for beech and oak.

Fig. S2 Mean leaf water potential at midday and light-saturated stomatal conductance in drought vs nondrought treatments for each species combination.

Fig. S3 Mean leaf and stomatal safety margins for beech and oak trees in each climatic treatment and species combination.

Fig. S4 Delta time to hydraulic failure under the same climate and variable P_{50} acclimation to D and HD for beech and oak in each climatic treatment.

Fig. S5 Mean time to hydraulic failure and stomatal closure under the same climate and constant soft trait for beech and oak in each climatic treatment.

Fig. S6 Mean total leaf area of the canopy for beech and oak trees in each climatic treatment and all species combinations.

Fig. S7 Mean stomatal safety margins for beech and oak trees in each climatic treatment and species combination with P_{50} acclimated to D and HD.

Notes S1 Total leaf area of the tree measurement description.

Table S1 Statistical output from the mixed-effect linear models on beech and oak.

Table S2 Mean beech leaf hydraulic traits per treatment and year.

Table S3 Mean oak leaf hydraulic traits per treatment and year.

Table S4 Pubescent oak and European beech hard and soft traits per treatment per year.

See also the Commentary on this article by Wright, **241**: 955–957.