Science of the Total Environment 892 (2023) 164351

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Stomatal regulation and xylem hydraulics of limber pine and Engelmann spruce in Great Basin sky-island ecosystems

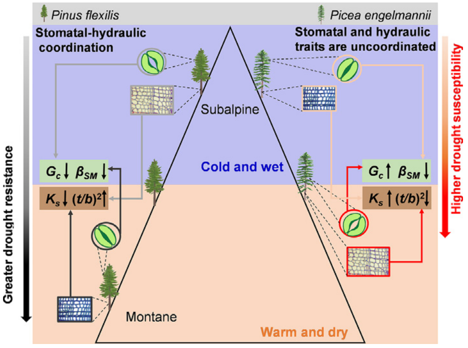
Xinsheng Liu [a,b], Emanuele Ziaco [c], Franco Biondi [b,*]

[a] Key Laboratory of Earth Surface Processes and Regional Response in the Yangtze-Huaihe River Basin, School of Geography and Tourism, Anhui Normal University, Wuhu 241000, China
[b] DendroLab, Department of Natural Resources and Environmental Science, University of Nevada, Reno, NV 89557, USA
[c] Department of Geography, Johannes Gutenberg University, Mainz, Germany

HIGHLIGHTS

- We examined stomatal regulation and xylem hydraulics of two sky-island conifers.
- *Pinus flexilis* and *Picea engelmannii* were studied at 3 Great Basin NevCAN sites.
- Stomatal sensitivity of both species to soil drying decreased at lower elevations.
- Xylem embolism resistance of *P. flexilis* increased at lower-elevation sites.
- Xylem hydraulics of *P. engelmannii* varied with elevation opposite to *P. flexilis*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Elena Paoletti

Keywords:
Sap flow
Wood anatomy
Pinus flexilis
Picea engelmannii
NevCAN
Snake Range

ABSTRACT

Integration of whole-plant stomatal regulation and xylem hydraulics is of critical importance for predicting species response to drought stress. Yet intraspecific variability of stomatal and hydraulic traits, and how these variabilities interact, remain largely unknown. We hypothesized that drought can drive less stomatal regulation but increase xylem hydraulic safety, resulting in stomatal-hydraulic coordination within a species. We estimated sensitivity of whole-tree canopy conductance to soil drying together with xylem hydraulic traits of two dominant conifers, i.e. limber pine (*Pinus flexilis*) and Engelmann spruce (*Picea engelmannii*). Our study was conducted using sub-hourly measurements over five consecutive years (2013–2017) at three instrumented sites with different elevations within the Nevada Eco-hydrological Assessment Network (NevCAN) in Great Basin sky-island ecosystems. Both conifers showed a reduction of stomatal sensitivity to soil drying at lower elevations, indicating an active stomatal acclimation to drought. While limber pine increased xylem embolism resistance in parallel with reduced stomatal sensitivity to soil drying, an opposite hydraulic adjustment was detected in Engelmann spruce. Our results provide evidence that mature trees can respond to climatic changes using coordinated shifts in stomatal regulation and xylem hydraulics, but such changes can differ within and between species in ways that need to be examined using in situ data. Deciphering intraspecific variability in whole-plant stomatal and hydraulic traits ultimately contributes to defining drought tolerance and vulnerability, particularly for tree species that inhabit a wide range of landscapes.

---

[*] Corresponding author.
E-mail address: franco.biondi@gmail.com (F. Biondi).

http://dx.doi.org/10.1016/j.scitotenv.2023.164351
Received 12 March 2023; Received in revised form 16 May 2023; Accepted 18 May 2023
Available online 23 May 2023

1. Introduction

Frequent and intense drought episodes driven by rising air temperature and shifting precipitation regimes (IPCC, 2018) have been linked to forest decline and mortality in natural ecosystems worldwide (Allen et al., 2010; Choat et al., 2018; Brodribb et al., 2020; Hammond et al., 2022; McDowell et al., 2022). These mortality events pose major challenges for forest ecosystem services and their potential to mitigate the impact of global carbon dioxide emissions (Anderegg et al., 2020). While different tree species can employ distinct survival strategies to withstand drought stress (Pivovaroff et al., 2016), a species' capacity to deal with drought depends fundamentally on active acclimation, i.e. a variety of physiological and/or structural adjustments to compensate for decline in performance under drought conditions (Choat et al., 2018; Brodribb et al., 2020). Even though potential compensating mechanisms of within-species ecophysiology may be insufficient to buffer species from mortality (Anderegg et al., 2019), understanding the extent of adaptive adjustments of physiological and hydraulic/anatomical traits can enable better predictions of species tolerance and susceptibility to future drought.

Woody plant responses to drought, and its associated mortality, involve an intricate interplay of physiological mechanisms operating at multiple scales (Choat et al., 2018; Brodribb et al., 2020; Trugman et al., 2021; Hartmann et al., 2022; McDowell et al., 2022). A hydraulic framework linking stomatal regulation of gas exchanges with the integrity of water transport systems has emerged as a promising avenue for better understanding forest tree mortality under drought conditions (McDowell et al., 2008). This approach has motivated categorizing plants as isohydric or anisohydric (Tardieu and Simonneau, 1998), but most metrics of iso/anisohydry are defined in terms of a single plant trait (e.g., Bartlett et al., 2012; Choat et al., 2012), thereby making them less predictive of whole-plant vulnerability to drought-induced mortality (Martínez-Vilalta and Garcia-Forner, 2017; Hochberg et al., 2018; Kannenberg et al., 2022).

An integrated and coordinated whole-plant function of leaves (stomatal control) and stems (mainly hydraulic traits) can deepen our understanding of tree performance during drought (Skelton et al., 2015; Fu et al., 2019; McCulloh et al., 2019). Recent field and meta-analysis studies have reported a tight coordination across species between stomatal behavior and P_{50} (xylem pressure at 50 % loss of hydraulic conductance), which is a direct measure of xylem resistance against embolism formation (Klein, 2014; Bartlett et al., 2016; Pivovaroff et al., 2018; Flo et al., 2021). While leaf and stem functional traits may have coevolved along a single trajectory and experienced convergent selection pressures, such a relationship was not evident among either angiosperms or gymnosperms in temperate rainforests (Laughlin et al., 2020). Stomatal closure and embolism resistance can in fact be decoupled when reaching a critical threshold (Martin-StPaul et al., 2017). Inconsistent or conflicting results may partly arise from large intraspecific variations in both of these traits, thereby confounding species-level mean trait values (Anderegg, 2015; Kannenberg et al., 2022). Because interspecific trait correlations are not always held at the intraspecific level (Anderegg et al., 2018; Fajardo et al., 2022), further investigations are required to determine whether and how stomatal-hydraulic relationships co-vary within a species.

The fitness and fate of a species under climate warming rely primarily on its adaptive capacity to modulate stomatal conductance to dry and hot conditions (Lin et al., 2015). Leaf drought tolerance has been normally quantified by turgor loss point, which is an indicator of stomatal closure (Brodribb et al., 2003). However, leaf turgor loss point is weakly related to stomatal closure or species drought strategy (i.e., degree of isohydry and anisohydry; Farrell et al., 2017), undermining its utility as a proxy trait for plant drought tolerance and vulnerability (but see Bartlett et al., 2012). Stomatal sensitivity to drought stress, with its explicit physiological significance and direct link to plant drought tolerance, is a better candidate for these purposes (Lin et al., 2015; Farrell et al., 2017; Flo et al., 2021). While Flo et al. (2021) uncovered a wide spectrum of stomatal sensitivity to drought stress among species, the plastic adjustment of stomata regulation under water stress is species specific and even site specific. For instance, *Pinus sylvestris* (Poyatos et al., 2007) and *Picea abies* (Peters et al., 2019) exhibited no apparent shift in sensitivity of stomata to natural atmospheric drought across their geographical or elevational ranges, whereas plasticity in stomatal regulation of *Larix decidua* was only found at the wettest site (Peters et al., 2019). In contrast, experimental warming and drought applied to mature *Pinus edulis* and *Juniperus monosperma* revealed a consistent reduction in sensitivity of stomata to atmospheric drought (Grossiord et al., 2017).

As mentioned previously, the degree of stomatal sensitivity to water stress can be constrained by hydraulic architecture across species (Bush et al., 2008; Flo et al., 2021). Hydraulic traits, including P_{50} and hydraulic transport efficiency, vary substantially between species and are crucial for understanding species' ability to cope with drought conditions (Tyree and Sperry, 1989; Choat et al., 2012). However, current knowledge of within-species variability of hydraulic traits is limited and has sometimes remained inconclusive. While some angiosperm species (e.g., beech and eucalypt) may have a higher potential to actively adjust P_{50} under increasing water stress (Wortemann et al., 2011; Schuldt et al., 2016; Stojnić et al., 2018; Pritzkow et al., 2020; but see Rosas et al., 2019; Fuchs et al., 2021; Alon et al., 2023), such plastic responses appear to be weaker in gymnosperm species (Choat et al., 2018; Brodribb et al., 2020). Limited phenotypic plasticity in embolism resistance of pine species in Europe (Martínez-Vilalta et al., 2009; Lamy et al., 2014; Rosas et al., 2019) and in North America (Maherali and DeLucia, 2000) suggests that canalization in cavitation resistance (i.e., low phenotypic plasticity or lack of genetic variability) may hamper the capacity of pine species to adapt to dry environments. Other studies instead have detected plasticity in cavitation resistance of *Pinus pinaster* (Corcuera et al., 2011) and *Pinus canariensis* (López et al., 2016), but in the latter species only at the arid limit of its distribution range. Moreover, xylem-specific hydraulic efficiency may not show adaptive adjustments with increasing aridity within a species (Martínez-Vilalta et al., 2009; Schuldt et al., 2016; Rosas et al., 2019; Fuchs et al., 2021), even though leaf-specific hydraulic conductivity could decline due to shifts in resource allocation between leaf area and sapwood area (Martínez-Vilalta et al., 2009; Schuldt et al., 2016).

Intraspecific variability of xylem anatomy-derived hydraulic efficiency and/or safety is also mixed, ranging from a high degree of plasticity of hydraulic adjustments in response to decreasing precipitation or drought stress (Liang et al., 2019; Puchi et al., 2020) to no phenotypic plasticity of hydraulic traits under precipitation exclusion experiments (Hudson et al., 2018; Petit et al., 2022). Additional investigations of synchronous adjustments in stem hydraulic traits should better elucidate the mechanism of stomatal regulation acclimation within individual species. Furthermore, the role of frost and associated freezing-thawing cycles are often overlooked (Charrier et al., 2021; McCulloh et al., 2022; but see Mayr et al., 2006; Vicente et al., 2022). This is particularly the case for species that have a wide geographical distribution that exposes them to multiple environmental stressors, which may reshape the adjustment of hydraulic traits.

Semiarid subalpine sky-island forests occur in isolated mountain ranges surrounded by lowland desert "seas". In these unique ecosystems, mixed evergreen conifers grow across a broad range of environmental conditions along elevation gradients, from warm-dry edges to cool-wet subalpine treelines (Debano et al., 1995). Among recently established instrumental observatories, the Nevada Climate-ecohydrological Assessment Network (NevCAN; Fig. 1a; Mensing et al., 2013) provides a rare opportunity for exploring tree responses to varying hydro-climatic conditions (e.g., Liu and Biondi, 2020, 2021). In this study, we hypothesized that drought should drive reduced stomatal sensitivity to soil drying together with increased xylem resistance against air embolism, resulting in stomatal-hydraulic coordination within conifer populations. We then asked the following questions: (i) did trees growing in warmer-drier conditions downregulate stomatal sensitivity to drought compared to trees in cooler-wetter habitats? (ii) how did xylem hydraulic traits vary along an elevation gradient within a species? (iii) were stomatal and xylem hydraulic traits coordinated at the intraspecific level? To answer these questions, multi-year sap flow measurements with sub-hourly resolution were used to approximate

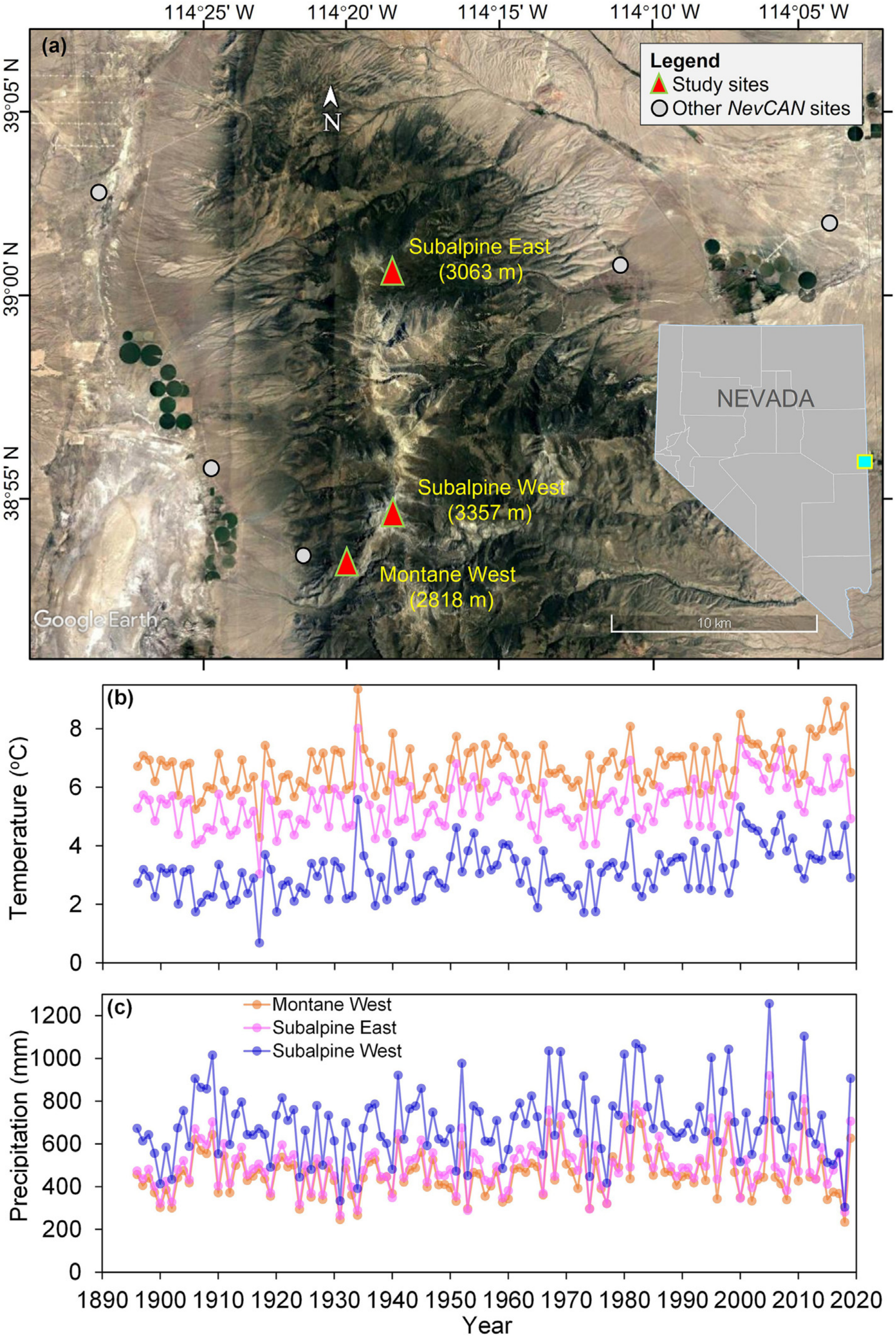


Fig. 1. Geographic location of the Nevada Climate-ecohydrological Assessment Network (NevCAN) and water-year (October–September) climate time series over the 1896–2019 period (Tables 1 and S1). (a) Satellite image of the Snake Range "sky island" (cyan square in Nevada map) showing the three study sites (red triangles). (b) Time series of mean air temperature at the three sites. (c) Time series of total precipitation at the three sites.

whole-tree canopy stomatal conductance and quantify its sensitivity to soil drying. In addition, xylem hydraulic traits of instrumented trees were derived from cell wall reinforcement, a proxy for xylem cavitation resistance, to determine hydraulic efficiency and safety.

2. Materials and methods

2.1. Study sites, tree species, and environmental conditions

Field measurements were carried out at three NevCAN sites (Montane West, Subalpine West and Subalpine East) located at different elevations on the Snake Range of eastern Nevada (Fig. 1a; Table S1). These sites represented a natural experiment across thermal and moisture conditions of limber pine (*Pinus flexilis* James) and Engelmann spruce (*Picea engelmannii* Parry) populations (Ziaco et al., 2014; Ziaco and Biondi, 2016). Limber pine and Engelmann spruce naturally occur in mixed-conifer stands on the Snake Range, although Engelmann spruce was absent at the lower elevation Montane West site, where Douglas fir (*Pseudotsuga menziesii*) and white fir (*Abies concolor*) co-occurred with limber pine. Other overstory species were bristlecone pine (*Pinus longaeva*) at the Subalpine West site, and quaking aspen (*Populus tremuloides*) at the Subalpine East site. Soil features were described in details by Johnson et al. (2014, 2016).

Study sites are characterized by a continental climate with cold winters and dry summers (Houghton et al., 1975). Standard meteorological instrumentation, with additional in-situ environmental and plant sensors, are located at each NevCAN site to monitor sub-hourly changes in soil and vegetation – see Liu and Biondi (2020, 2021) and Liu et al. (2021) for details on measured environmental and plant variables. At each site, environmental conditions, including air temperature (T_a, °C), relative humidity (RH, %), precipitation (Prec, mm), soil moisture at 10 and 20 cm depths (SM_{10} and SM_{20}, %), were measured during 2013–2017 with sub-hourly resolution. We only used soil moisture from 20 cm depth (hereafter referred as SM) for further analysis because growing-season transpiration of limber pine and Engelmann spruce relies on winter snowpack sourced from deeper soils (Liu and Biondi, 2021). Vapor pressure deficit (VPD, kPa) was computed according to Jones (1992) using 10-min values of air temperature and relative humidity.

Long-term temperature and precipitation were available from the public domain version of the Parameter-Regression at Independent-Slopes Model (PRISM) dataset (Daly et al., 2008). Water-year (October–September) mean air temperature and total precipitation were calculated from monthly records for the 4-km grid cell that contained each site. Moving from the highest elevation site (Subalpine West) to the lowest one (Montane West), long-term (1896–2019) mean annual temperature increased from 3.1 ± 0.8 to 6.7 ± 0.8 °C, whereas total annual precipitation decreased from 696 ± 168 to 462 ± 109 mm (Fig. 1b, c; Table S1).

2.2. Sap flow measurements

Two to five healthy and mature trees per species per site (Table 1) were instrumented with Granier-type thermal dissipation sensors (Granier, 1987; TDP30, Dynamax, Houston, Texas, USA) to continuously measure sap flux density (see Liu and Biondi, 2020, 2021 for additional sensor details). To examine the possible thermal effects of radiation on circumferential variation in sap flux density, one additional sensor was placed on the south side of three limber pines and one Engelmann spruce (Table 1). Given that freezing/thaw events in spring and autumn may introduce substantial noise into output signals, we focused on growing-season (June–September) sap flow data in subsequent analyses. Two increment cores were collected at breast height from each instrumented tree to estimate sapwood thickness (Table 1). Sap flux density was calculated following the empirical equation developed by Granier (1987). Corrections of raw data, and other sap flux calculations, were applied as in Liu and Biondi, 2020, 2021 (Table 1).

To better represent sap flow across the entire active sapwood (for most trees, sapwood depth was longer than probes; Table 1) and the corresponding whole-tree canopy stomatal conductance, we separated sapwood into 1–3 concentric rings at 3-cm intervals, depending on sapwood depths, and employed species-specific radial decreases in sap flow density for the inner interval(s) (Pataki et al., 2011). For Engelmann spruce, we used a semi-logarithm relationship between sap flux in the inner and outer xylem based on a published radial profile of sap-flow velocity (Mark and Crews, 1973; Table S2). For limber pine, we applied a generalized Gaussian equation for gymnosperms (Pataki et al., 2011; Table S2). Whole-tree sap flow density was finally obtained by summing sapwood-area weighted sap flow density of all concentric rings. While radial distributions of sap flow may vary during drought and non-drought periods (e.g., Ford et al., 2004; Yi et al., 2017), we assumed a constant radial profile of sap flow for each species during and across the growing seasons.

Table 1
Characteristics of instrumented trees for sap flow measurement at three sites in the Snake Range, including diameter at breast height (DBH), height, sapwood depth (D_s), and sapwood area (A_s).

Species	Site	Tree code	DBH (cm)	Height (m)	D_s (cm)	A_s (cm^2)
Pinus flexilis	Montane West (2818 m)	Mon_P1	27.0	7.5	4.12	296.24
		Mon_P2[a]	30.0	7.5	4.38	352.23
	Subalpine East (3063 m)	SubE_P1	61.0	11.0	6.09	1049.81
		SubE_P2	44.0	10.0	5.30	644.17
	Subalpine West (3357 m)	SubW_P1	17.5	4.5	3.08	139.42
		SubW_P2	25.0	6.0	3.94	260.47
		SubW_P3	35.0	8.0	4.75	451.15
		SubW_P4[a]	24.5	4.0	4.50	282.60
		SubW_P5[a,b]	19.0	3.5	2.25	118.34
Picea engelmannii	Subalpine East (3063 m)	SubE_S1	26.0	9.0	3.89	270.24
		SubE_S2	61.0	12.0	7.57	1270.04
		SubE_S3	63.0	14.0	7.86	1361.37
	Subalpine West (3357 m)	SubW_S1	54.0	12.0	6.70	995.10
		SubW_S2[a]	41.0	12.0	3.80	443.87

[a] Two pairs of sap flow sensors were placed on the stem.
[b] Sap flow values were corrected (see Liu and Biondi, 2020).

2.3. Field sampling and anatomical measurements

In the years 2013–2014, three to six trees per species per site (Table S3) were selected for wood anatomical analysis. Sampled trees had been instrumented with sap flow sensors, with the only exception being limber pine at the Subalpine West site, where four trees with sap flow sensors, and two trees without, were sampled. Wood microcores (1–2 cm long and ca.1 mm in diameter) that covered up to 17 annual rings in sapwood were extracted using a Trephor (Rossi et al., 2006) or a surgical bone-marrow needle (Dufour and Morin, 2010), with the latter tool being preferred for trees equipped with sensors. After collecting microcores at breast height, they were immersed in a 50 % ethanol solution and stored in ice to prevent tissue deterioration during transport from the field to the laboratory.

For wood cell anatomical measurements, microcores were processed following the procedure proposed by Lupi et al. (2014), which includes (i) dehydrating with ethanol (Protocol SAFECLEAR II) and then embedding them in paraffin blocks, (ii) cutting 8–10 μm thick transversal sections with a rotary microtome (Leica, Buffalo Grove, Illinois, USA) and mounting on microscope slides, (iii) removing paraffin from the slides with SAFE-CLEAR II and pure ethanol, (iv) staining with safranin (0.50 % aqueous solution), and (v) digitalizing images at 100–200× magnification with a digital camera mounted on Nikon compound microscope. Cell anatomical parameters were measured along three radial profiles within each annual ring, and further averaged by year for each tree (Ziaco et al., 2014; Ziaco and Biondi, 2016). Image analysis performed with WinCELL software (Guay, 2013) generated measures of radial cell lumen diameter (*LD*, μm), radial cell length (*CL*, μm), and cell wall thickness (*CWT*, μm) calculated as the average of left and right radial wall thickness.

2.4. Whole-tree canopy stomatal conductance

Whole-tree sap flux density was aggregated to daytime averages (06:00–20:00 local time) to minimize possible effects of stem water capacitance on the response of canopy conductance to soil moisture (Oren et al., 1998). A unit conversion from [g cm$^{-2}{}_{Asw}$ s^{-1}] to [kg m$^{-2}{}_{Asw}$ s^{-1}] was made for daytime sap flux density before further data processing. As our study sites are characterized by open stands with high wind speeds, we assumed that the forest canopy was aerodynamically well coupled to the atmosphere (i.e., large boundary layer conductance). Whole-tree canopy stomatal conductance per unit of sapwood area (G_c, mol m$^{-2}{}_{Asw}$ s^{-1}) for individual trees at each site was determined as (Flo et al., 2021):

$$G_c = \frac{(115.8 + 0.4236T_a)F_d}{VPD} \times \eta \times \frac{T_0}{(T_0 + T_a)} \times \exp^{-0.00012h} \tag{1}$$

where F_d is sap flux density, VPD is daytime vapor pressure deficit (kPa), T_a is air temperature (°C), h is the site elevation (m), η and T_0 are respectively the conversion factor and a physical constant equal to 44.6 mol m^{-3} and 273 K. This formula considered temperature effects on the psychometric constant, air density, latent heat of water vaporization and specific heat of air at constant pressure (Phillips and Oren, 1998). Canopy conductance was then transformed to traditional units using water vapor density (as a function of T_a in °C) and site-specific atmospheric pressure.

2.5. Hydraulic traits

Hydraulic efficiency and safety traits per tree per year were derived from wood anatomical parameters. Mean hydraulic diameter (D_h, μm) was calculated based on all measured cells assuming circular tracheids (Sperry et al., 1994):

$$D_h = \frac{\Sigma_{i=1}^{n}\left(LD_i^5\right)}{\Sigma_{i=1}^{n}\left(LD_i^4\right)} \tag{2}$$

with LD_i being the diameter of the ith tracheid, and n the number of tracheids.

According to the Hagen-Poiseuilles law (Tyree and Ewers, 1991), theoretical specific hydraulic conductivity (K_s, kg m^{-1} s^{-1} MPa^{-1}) of each annual ring was computed as:

$$K_s = \frac{\pi\rho}{128\eta A}\Sigma_{i=1}^{n}\left(LD_i^4\right) \tag{3}$$

with ρ and η being the density (998.2 kg m^{-3}) and viscosity (1.002 × 10^{-9} MPa s) of water at 20 °C, and A the surface area of the ring.

We approximated hydraulic safety using cell wall reinforcement [$(t/b)^2$], a surrogate for xylem cavitation resistance within (Rosner et al., 2016) and across species (Hacke et al., 2001; Mayr et al., 2006; Cochard et al., 2008), in which t is the double cell wall thickness and b is the conduit wall span (Hacke et al., 2001). For each annual ring, cell wall reinforcement was determined by averaging individual values for tracheids with diameter D_h (Cochard et al., 2008; Guérin et al., 2020). To refine the estimate of hydraulic safety, P_{50} of each annual ring was calculated from its $(t/b)^2$ according to the published equation for western USA conifers (Hacke et al., 2001).

2.6. Data analysis

To prevent a biased estimation of model parameters for the G_c–SM relationship, we discarded canopy stomatal conductance values when daily rainfall > 0 mm, daily mean VPD < 0.5 kPa, and daily total solar radiation < 4.0 kW h m^{-2} (Phillips and Oren, 1998; Peters et al., 2019; Liu and Biondi, 2020, 2021; Flo et al., 2021). During each growing season (June–September) in 2013–2017, mean and maximum canopy stomatal conductance ($G_{c,\mathrm{mean}}$ and $G_{c,\mathrm{max}}$; both mol m$^{-2}_{\mathrm{Asw}}$ s^{-1}) were calculated for every instrumented trees. Canopy conductance was also averaged by species and site to investigate its sensitivity to soil drought.

A simple linear regression was fitted to canopy conductance (G_c) versus soil moisture (SM), both $\log_{10}$-transformed. We used the slope of the G_c–SM relationship, β_{SM}, to define canopy stomatal sensitivity to soil drying, which described site- and species-specific stomatal regulation traits (Liu and Biondi, 2020, 2021; Flo et al., 2021). For each species, differences in β_{SM} among sites were tested using analysis of covariance (ANCOVA), with G_c as dependent variable, soil moisture as a covariate, and site as a grouping variable.

We obtained whole-tree hydraulic traits considering that sampled sapwood could account for the majority of xylem water transport and cavitation resistance (Bouche et al., 2014). Because growing season (Jun-Sep) sap flow data were used to derive stomatal traits of that year, to avoid a mismatch between stomatal and xylem hydraulic traits at the whole-tree level, the current-year hydraulic traits were calculated per tree as ring width-weighted averages of all available (12–17) previous rings except the current-year one, whose hydraulic functions are not fully developed. Since ring width-weighted D_h, K_s and $(t/b)^2$ (i.e., whole-tree hydraulic traits) for limber pine and Engelmann spruce did not show significant differences among years (i.e., 2008–2015; $P > 0.95$ for all; post-hoc analysis with Tukey HSD test), in case of failed wood microcore sampling of 2–4 rings prior to the year of sap flow observation, we used the computed ring width-weighted averages of the most adjacent year as a surrogate for current-year whole-tree hydraulic traits.

Repeated measures analysis of variance (ANOVA) was used to assess effects of site and year on intraspecific variations in sap flow, stomatal ($G_{c,\mathrm{mean}}$, $G_{c,\mathrm{max}}$ and β_{SM}) and hydraulic [D_h, K_s, $(t/b)^2$ and P_{50}] traits along the elevation gradient. Bivariate linear models were fitted for each species to evaluate the intraspecific relationships between $\log_{10}$-tranformed stomatal and hydraulic traits. All statistical analyses were performed in SPSS (v.22; SPSS Inc., USA).

3. Results

3.1. Environmental conditions and sap flow along the elevation gradient

Long-term PRISM data showed the expected topographic effect of increasing air temperature and decreasing total precipitation with decreasing elevation (Fig. 1b, c; Table S1). In general, environmental parameters recorded by NevCAN sensors mirrored the same topographic pattern along the elevation gradient (Fig. S1). From 2013 to 2017, the growing-season (June–September) air temperature/vapor pressure deficit were consistently higher at the Montane West site (15.30 °C/1.28 kPa) than at the Subalpine East (11.75 °C/0.87 kPa) and West (10.63 °C/0.83 kPa) sites, but differences were relatively small between the Subalpine East and West sites (Fig. S1a–b). Growing-season precipitation increased with elevation between sites, and varied little between the two high-elevation sites (Fig. S1c). Soil moisture underwent substantial seasonal variability across sites and years (less so in year 2014), but it was usually higher at the lower elevations (Fig. S1d), most likely because of different soil texture (Johnson et al., 2014, 2016) or because of the interaction between vegetation, soil, and the wind- and insulation-driven snow pack accumulation and melting patterns.

For the two conifer species, sap flow varied significantly among sites and years (Table S4), with different topographic patterns (Fig. S1e–f). Throughout the five-year study period, sap flow of limber pine consistently displayed a decreasing trend towards lower elevations, with the highest and lowest sap flow at the Subalpine West and Montane West sites, respectively. By contrast, an inverse pattern of sap flow was found in Engelmann spruce, whose sap flow was higher at the Subalpine East than at the Subalpine West site, which is higher in elevation but was characterized by lower soil moisture.

3.2. Canopy stomatal conductance and its response to soil drought

Mean ($G_{c,\mathrm{mean}}$) and maximum ($G_{c,\mathrm{max}}$) canopy stomatal conductance of both species were mainly different by site (Tables S5, S6), with opposite species-specific differences (Fig. 2). At the Subalpine West site (3357 m), limber pine had a significantly higher $G_{c,\ \mathrm{mean}}$ and $G_{c,\ \mathrm{max}}$ than at the other two sites ($P < 0.05$), whereas Engelmann spruce showed significantly lower $G_{c,\ \mathrm{mean}}$ and $G_{c,\ \mathrm{max}}$ ($P < 0.05$). Across species and sites, canopy stomatal conductance relationship with declining soil moisture followed a power relationship (Table S7), which could be best fitted using a logarithmic transformation (Fig. 2; Table 2). Differences in stomatal sensitivity to soil drying among sites were found to be statistically significant for limber ($P = 0.015$) and marginally non-significant ($P = 0.062$) for spruce (Table S8).

We observed a prevailing weakened stomatal control under warm-dry climate in both species, as indicated by a lower slope of the G_c–SM relationship at lower elevations (Fig. 2; Table 2), even though the G_c–SM slope of limber pine did not differ significantly between the two lower elevations ($F = 0.14$, $P = 0.71$). These findings did not change when the analysis was restricted to 2015–2017, i.e., when soil moisture was available at all three sites (Fig. S2; Tables S7, S9).

3.3. Hydraulic and anatomical adjustments across elevations

Generally, the outermost tree rings did not show inter-annual variation in anatomical (mean hydraulic diameter, D_h) and hydraulic (theoretical specific hydraulic conductivity, K_s; cell wall reinforcement, $(t/b)^2$) traits

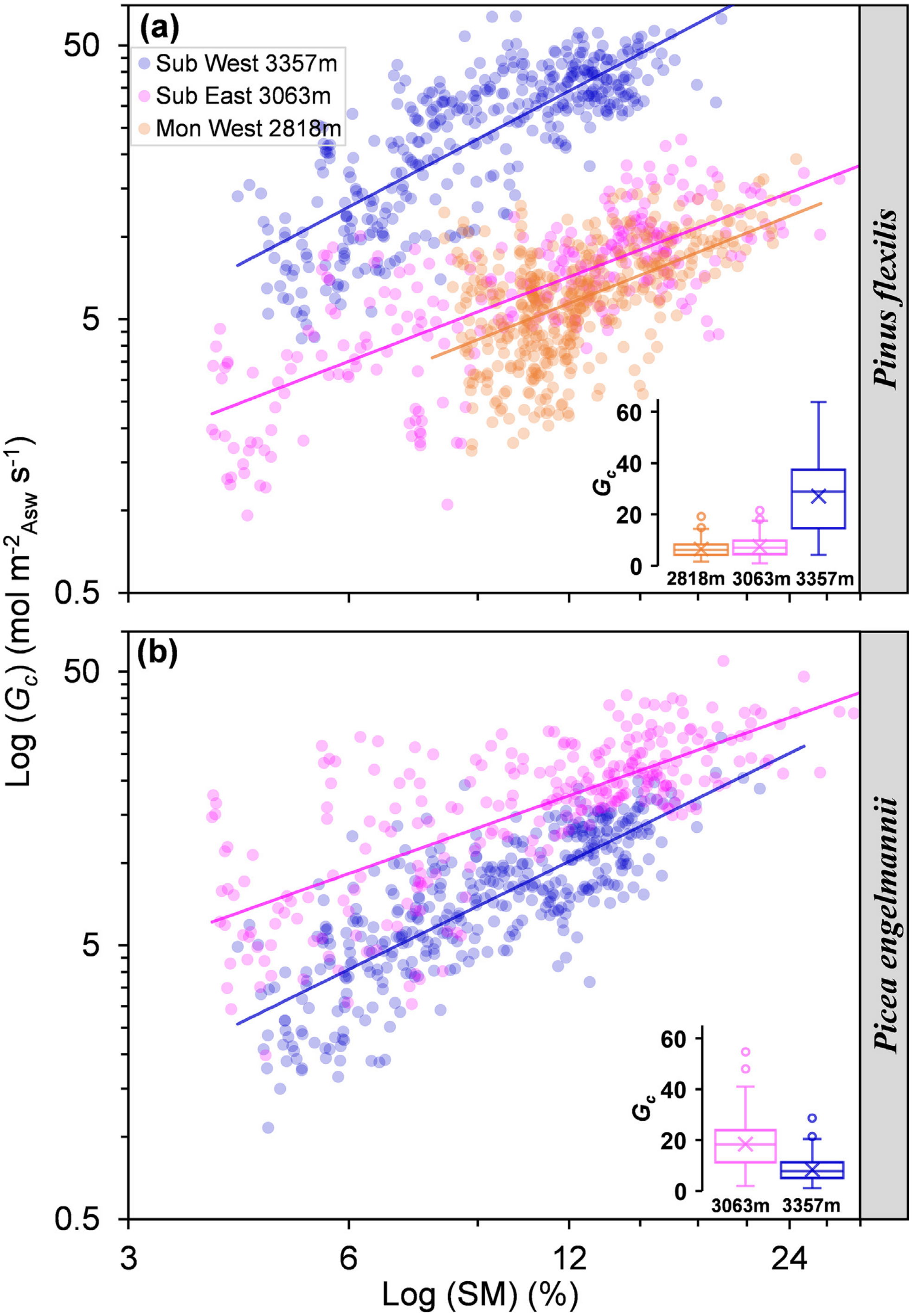


Fig. 2. Relationships between canopy stomatal conductance (G_c; log-scale) and soil moisture (SM; log-scale) for (a) *Pinus flexilis* and (b) *Picea engelmannii* at the study sites. For each site and species, data were pooled across the study period (2013–2017). Darker colors indicate greater overlap of data points; regression statistics are shown in Table 2. Boxplot insets show the distribution of G_c by species and site.

across species and sites. We observed a slight, and non-significant, decline in D_h and K_s of limber pine at the Montane West site since 2012, as well as an increase in $(t/b)^2$ of Engelmann spruce at the Subalpine West site since 2007 (Fig. S3b–d, f–h). All anatomical and hydraulic traits varied significantly among sites, and were barely influenced by years and their interaction with sites (Tables S10-S13). As a result, limber pine showed lower K_s and D_h together with higher $(t/b)^2$ with decreasing elevation, leading to a 48.6 % and 17.8 % reduction in mean K_s and D_h, and a 73.5 % increase in mean $(t/b)^2$ at the Montane West site relative to the Subalpine West site (Fig. 3a, c, e). Cell wall reinforcement was similar between the Montane West and Subalpine East sites. These site differences were reversed in Engelmann spruce, whose mean K_s and D_h increased by 91.4 % and 28.3 % from the Subalpine West (3357 m) to the Subalpine East (3063 m) sites, while $(t/b)^2$ declined by 54.5 % (Fig. 3b, d, f). Site differences in P_{50} followed those of $(t/b)^2$ for both species (insets, Fig. 3e, f). The estimated P_{50} for Engelmann spruce (−2.15 to −3.67 MPa) was close to the values obtained by direct measurements at a nearby site (Clute et al., 2021).

Table 2

Estimated parameters of the simple linear model [$\log(y) = \beta \log(x) + \alpha$] relating canopy stomatal conductance and soil moisture by species and sites (Fig. 2). Differences in canopy stomatal conductance sensitivity to soil drought (i.e., slopes; parameter β) among sites were tested using ANCOVA.

Species	Sites	Slope (β)	Intercept (α)	R^2	P
Pinus flexilis	2818 m	1.0597	−0.3871	0.36	<0.001
	3063 m	1.0234	−0.2513	0.58	<0.001
	3357 m	1.4080	0.0131	0.62	<0.001
	F-test	14.024			
	P-value	<0.001			
Picea engelmannii	3063 m	0.9467	0.2245	0.54	<0.001
	3357 m	1.3114	−0.4093	0.64	<0.001
	F-test	26.555			
	P-value	< 0.001			

3.4. Coordination between stomatal and hydraulic traits

At the intraspecific level, there were positive correlations between D_h (K_s) and $G_{c,\mathrm{mean}}$ ($G_{c,\max}$) (all $r > 0.87$, $P < 0.01$; Figs. 4a, c, S4), suggesting coordination between whole-tree hydraulic efficiency-related traits and stomatal conductance for both species. However, coordination between hydraulic safety and stomatal regulation (i.e., stomatal sensitivity to soil drought) differed between species (Fig. 4b, d). Stem xylem cell wall reinforcement was negatively correlated with stomatal sensitivity to soil drought in limber pine ($r = -0.81$, $P < 0.01$; Fig. 4b), suggesting that limber pines from relatively colder and wetter habitats, with more vulnerable xylem, were characterized by greater stomatal regulation than pines from relatively warmer and drier habitats. Hydraulic safety and stomatal regulation of Engelmann spruce, on the other hand, seemed unable to adjust in a coordinated manner ($r = 0.81$, $P < 0.01$; Fig. 4d), as shown by vulnerability of xylem embolism and loose stomatal regulation in spruces from the relatively lower elevation site, which however had higher soil moisture.

4. Discussion

4.1. Acclimation of stomatal sensitivity in response to drought stress

A plant's persistence and survival when exposed to increased drought severities in its current habitat largely depends upon its adaptive capacity to optimize water use while maximizing photosynthesis through active adjustment in stomatal behavior (Lin et al., 2015; Lu et al., 2020). While stomatal sensitivity to atmospheric and soil drought is highly variable across species (Oren et al., 1999; Grossiord et al., 2020; Flo et al., 2021), we investigated how individuals of the same species adjusted stomatal behavior in response to soil moisture. When comparing sites, in every year we observed reduced stomatal sensitivity to soil drying at lower elevations for both species (Fig. 2; Tables 2, S8). Although soil moisture varied from year to year (Fig. S1d), stomatal sensitivity to soil drying at a certain elevation did not differ significantly among years for both species (Table S8), suggesting that pine and spruce stomatal acclimation to local conditions was unrelated to site differences in soil moisture. The higher sap flow of Engelmann spruce at lower elevation was related to its higher hydraulic efficiency (K_s in Fig. 3d) and maximum stomatal conductance (Fig. 2b; Table S6). The greater soil moisture at lower elevation can induce a higher sap flow sensitivity to vapor pressure deficit (Liu and Biondi, 2020), thus leading to a higher transpiration rate of Engelmann spruce despite a small difference in vapor pressure deficit between the two high-elevation sites (Fig. S1b).

Limber pine did not show higher sap flow at lower elevations with higher soil moisture (Fig. S1), which can be explained by its lower K_s (Fig. 3c) and maximum stomatal conductance (Fig. 2a; Table S6). In addition, the higher stomatal conductance sensitivity of low-elevation limber pine to soil drought compared to co-occurring Engelmann spruce (1.02–1.06 vs. 0.95; Table 2, Fig. 2) can induce a rapid downregulation of transpiration rates, thereby outpacing the positive influence of high soil moisture on sap flow sensitivity to vapor pressure deficit. Partial stomatal closure due to high vapor pressure deficit, especially at the driest site, might occur, and also contribute to the low transpiration rates of limber pine at lower elevations. The tight coordination between hydraulic efficiency traits and stomatal conductance (Fig. 4a, c) thus allows for trees to approach their potential maximum transpiration rates (Manzoni et al., 2013). Our findings are consistent with plasticity in stomatal sensitivity to vapor pressure deficit under experimental warming and drought (Grossiord et al., 2017), suggesting an active within-species acclimation in stomatal regulation that could switch from isohydric behaviors at colder and wetter habitats to relatively anisohydric behaviors at warmer and drier habitats. These findings also corroborate the spatial and temporal plasticity in stomatal regulation along a spectrum of plant species spanning isohydry to anisohydry that is strongly influenced by varying environmental conditions (Ogle et al., 2012; Feng et al., 2019; Guo et al., 2020).

Reduced stomatal sensitivity, as shown by lower sap flow decline, can lead to a more negative leaf turgor loss point and delayed stomatal closure (Maréchaux et al., 2018), thus enabling trees to sustain relatively high carbon acquisition rates (Will et al., 2013; Grossiord et al., 2017). A relatively anisohydric stomatal behavior in lower-elevation habitats also likely conferred trees the ability to recover faster from episodes of water stress (Nolan et al., 2017; Liu and Biondi, 2021). Keeping stomata open is a vital mechanism for preventing needles from overheating via evaporative cooling (Drake et al., 2018; Blasini et al., 2022). Adaptive plasticity in stomatal regulation is presumably related to osmotic adjustments (Bartlett et al., 2012), foliar abscisic acid content (Nolan et al., 2017), and/or stomatal density and diameter (Guérin et al., 2018), but parallel reinforcements in anatomical and hydraulic properties to avoid the induction of xylem embolism were also present in limber pine (Figs. 3, 4). This suggests physiological and structural integration between exposure to drought (determined by stomatal control and other functional traits) and capacity to cope with drought (i.e., embolism resistance; Sanchez-Martinez et al., 2020). Engelmann spruce, on the other hand, appeared to prioritize carbon gain over hydraulic safety.

4.2. Species-specific hydraulic adjustment

Contrary to our initial hypothesis, increasing drought stress had various effects on xylem hydraulic adjustments depending on the species (Fig. 3). Although limber pine was characterized by an increase in xylem embolism resistance (but also a decrease in xylem conductivity) at the lower elevations, the ability to adjust hydraulic properties was site-specific, partially supporting results obtained for other pine species (Maherali and DeLucia, 2000; Martínez-Vilalta et al., 2009; Corcuera et al., 2011; Lamy et al., 2014; López et al., 2016; Rosas et al., 2019). Moreover, our data revealed an opposite hydraulic adjustment in Engelmann spruce, since spruces at the lower elevation developed wide conduits with vulnerable xylem.

At high elevations, although stomata closure may be normally present during winter due to frozen soil and repeated freeze-thaw events, low rates of cuticular water loss may lead to winter desiccation (Körner, 2003; Wieser and Tausz, 2007) and greater xylem embolism than during growing season drought (Sperry et al., 1994; Mayr et al., 2006). A three-fold lower cuticular conductance in pine (*Pinus cembra*) than in coexisting spruce (*Picea abies*) at alpine treelines was considered responsible for freezing-induced embolism in spruce compared to pine (Anfodillo et al., 2002; Mayr et al., 2003, 2006). A similar species difference in cuticular conductance has been found in high-elevation limber pine and Engelmann spruce (Hadley and Smith, 1990). Spruce is more capable than pine of restoring xylem conductivity (i.e., embolism refilling) before the onset of the growing season by either foliar and/or branch water uptake from melting snow or through aquaporin activity (Mayr et al., 2014, 2020 and references therein). Because adjustments in xylem structure act as a prerequisite mechanism to avoid freezing-induced embolism (Hacke and Sperry, 2001), conifers develop narrow-diameter (<30 μm) tracheids in cold subalpine habitats to enhance cell mechanical resistance against frost drought and freeze-thaw cycles (Pittermann and Sperry, 2003, 2006). Lower minimum air temperatures at the higher elevation most likely influenced wood

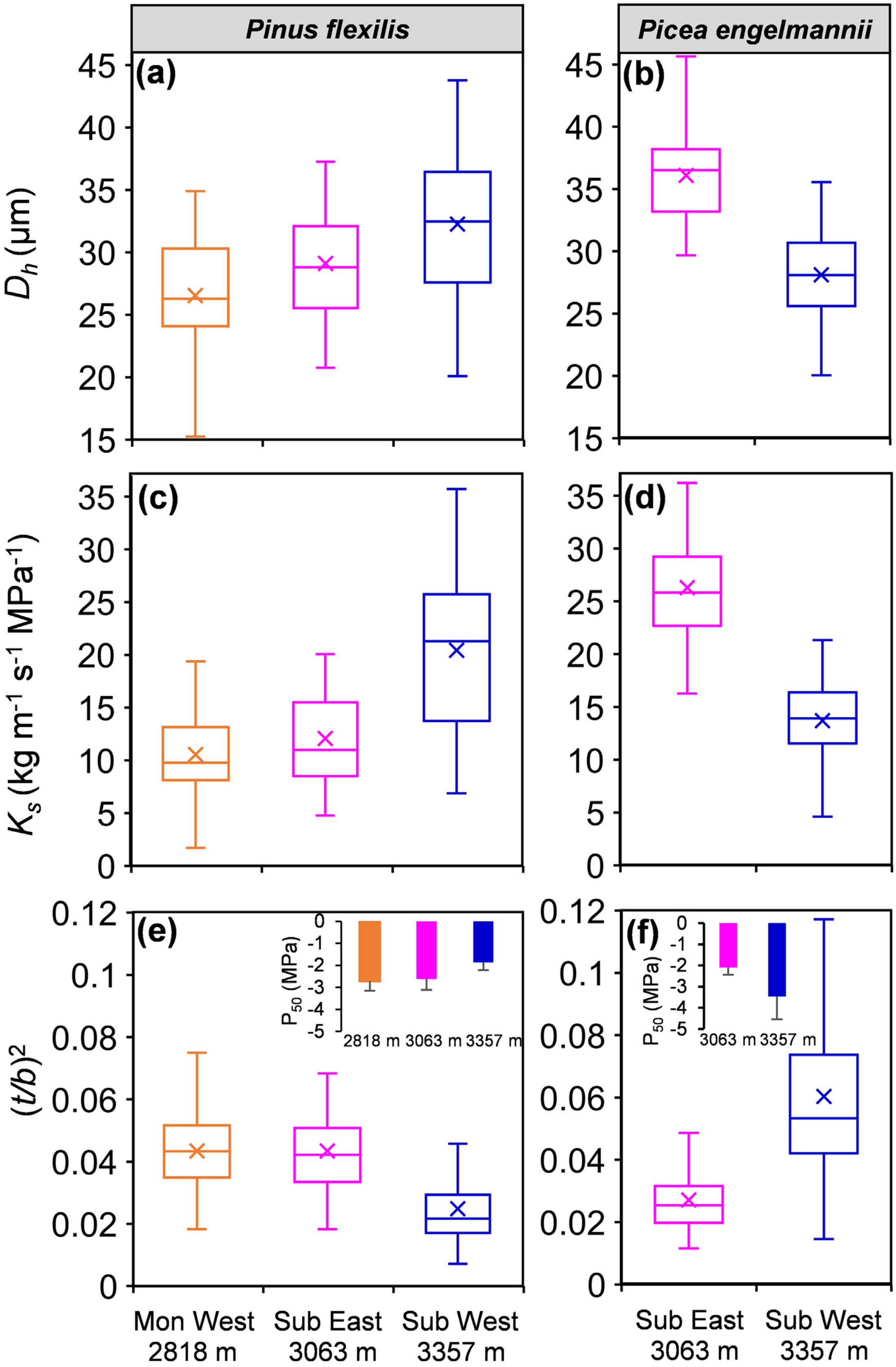


Fig. 3. Boxplots of (a, b) mean hydraulic diameter (D_h), (c, d) theoretical specific hydraulic conductivity (K_s) and (e, f) cell wall reinforcement [$(t/b)^2$] calculated during a 17-yr period (1998–2014) for *Pinus flexilis* and *Picea engelmannii* at the study sites. Insets show the estimated xylem pressure at 50 % loss of hydraulic conductance (P_{50}) by species and site.

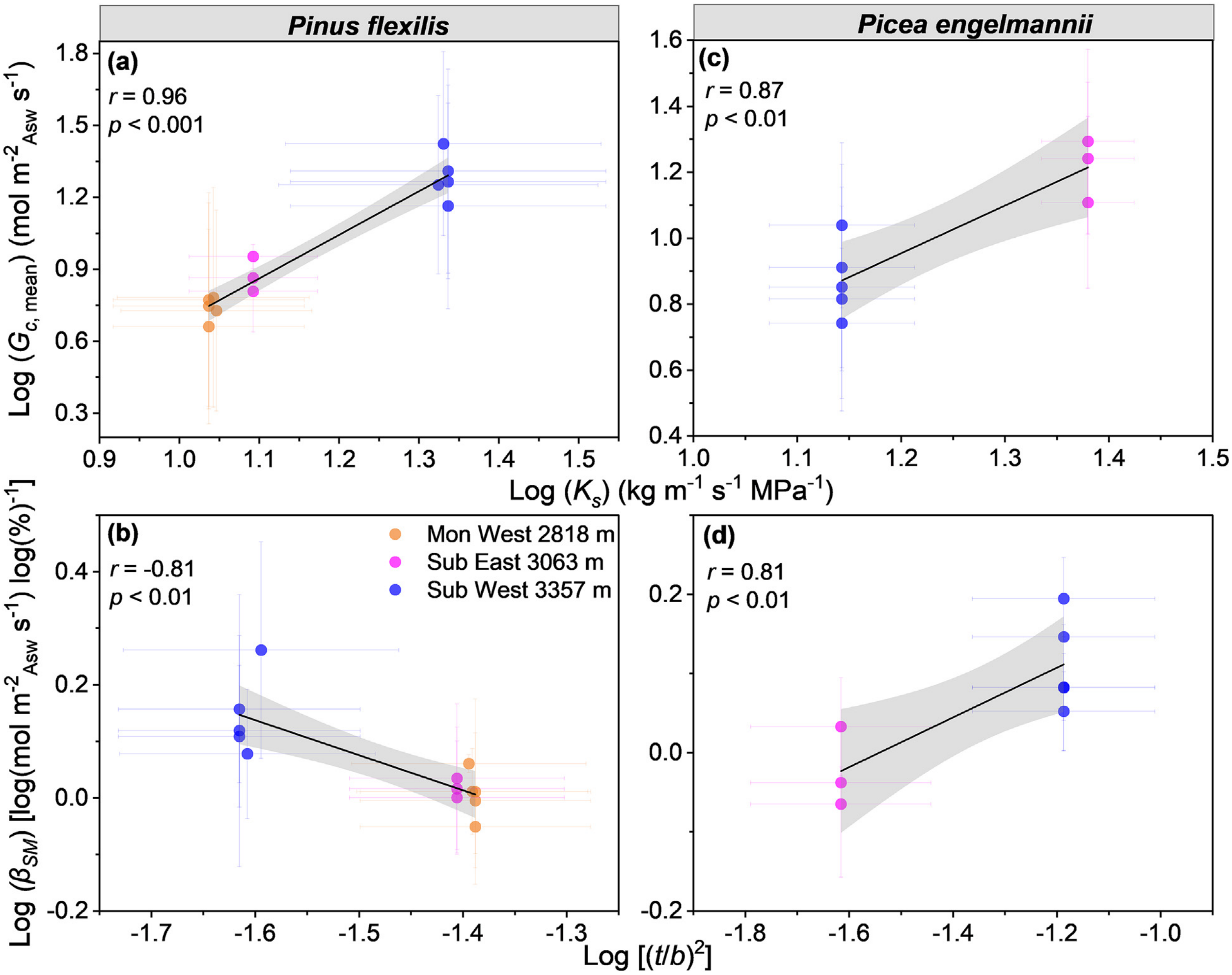


Fig. 4. Hydraulic traits (x axes; log-scale) versus stomatal traits (y axes; log-scale) for *Pinus flexilis* and *Picea engelmannii* at the study sites. Solid dots identify annual mean values, gray bars represent one standard deviation, and gray shaded areas mark 95 % confidence intervals. Plot axes have different scales between species.
(a, c) Ring width-weighted theoretical specific hydraulic conductivity (K_s) is linked with mean canopy conductance ($G_{c,\ \mathrm{mean}}$) similarly for both species.
(b, d) Ring width-weighted cell wall reinforcement [$(t/b)^2$] is linked with canopy stomatal conductance sensitivity to soil drought (β_{SM}) differently in pine and in spruce.

anatomy and hydraulics of Engelmann spruce more than those of limber pine. As plant survival capacity depends on cuticular conductance beyond stomatal closure (Blackman et al., 2016), future research should explore its plasticity and interaction with stomatal and hydraulic traits.

When freezing is rare or absent, natural selection tends to produce larger vascular conduits to enhance xylem conductivity rather than safety in conifers (Cocozza et al., 2016; Wang et al., 2018). The hydraulic adjustment observed in lower elevation Engelmann spruces agrees well with the hypothesis that the initial exposure to frost would affect the vulnerability to drought (Charrier et al., 2021). Drought and associated low turgor pressure negatively affect cell enlargement, producing tracheids with narrow lumens and thick cell walls (Ziaco and Biondi, 2016). Such anatomical adjustments in response to increasing water stress can facilitate xylem hydraulic safety against drought-induced embolism (Puchi et al., 2020), thereby allowing trees to maintain high stomatal openness (i.e., being relatively anisohydric). However, we cannot always expect this positive feedback to be the case (Grossiord et al., 2017; Hudson et al., 2018; Petit et al., 2022), especially when trees are exposed to amplified drought conditions. Constructing a more drought-resistant xylem comes at the expense of increased carbon investment in xylem (greater wood density), lower stem efficiency and capacitance (Fu et al., 2019), and high variability in non-structural carbohydrate storage (Jiang et al., 2021), some of which will challenge the ability to recover from hydraulic impairment via embolism reversal (Sala et al., 2012; Meinzer and McCulloh, 2013; Klein et al., 2018). These evolutionary and physiological tradeoffs naturally lead to the notion that high hydraulic safety is not necessarily required when growing in xeric environments (Gleason et al., 2016), possibly explaining the substantial but similar degree of adjustment of hydraulic traits in limber pine regardless of site-specific conditions.

4.3. Stomatal-hydraulic coordination occurred in limber pine but not in Engelmann spruce

Coordinated intraspecific trait variations in xylem hydraulic safety and stomatal sensitivity to drought occurred in limber pine but not in Engelmann spruce (Figs. 4b, d, 5). Acclimation to reduced water availability through coordinated adjustments in osmotic potential and xylem vulnerability has been already reported for grapevines (Sorek et al., 2021). Coordinated shifts in stomatal behavior and xylem hydraulics can guarantee the maintenance of an invariant hydraulic safety margin between stomatal closure and embolism formation (cf. Choat et al., 2012; Martin-StPaul et al., 2017), so that limber pine can respond to water stress by maximizing carbon gain while preventing xylem cavitation despite an increase in tree water deficit (Ziaco and Biondi, 2016). Engelmann spruce seems to maximize gas exchange at the risk of operating with low hydraulic safety, so that its hydraulic safety margin may approach zero and thus will be vulnerable to increasing drought severities. Such divergent drought resistance and vulnerability between sky-island coniferous species (Fig. 5)

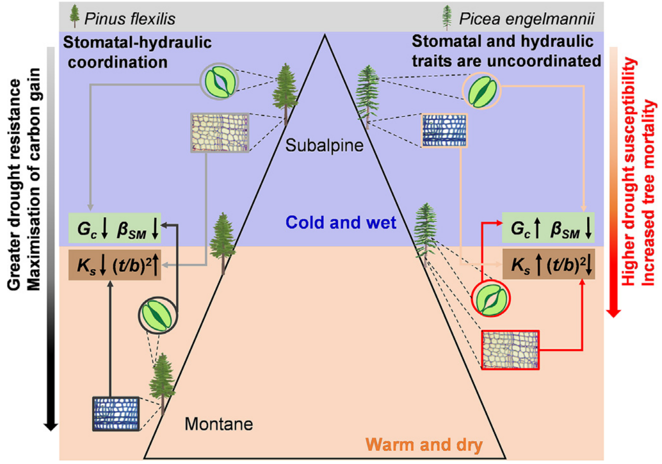


Fig. 5. Conceptual scheme for divergent coordination between stomatal regulation (G_c and β_{SM} are canopy stomatal conductance and its sensitivity to soil drought, respectively) and hydraulic traits (K_s and $(t/b)^2$ are theoretical specific hydraulic conductivity and safety, respectively) in *Pinus flexilis* and *Picea engelmannii* at different elevations. Short black arrows indicate the changing directions of stomatal regulation and hydraulic traits for each species.

pinpoint a physiological basis for understanding the higher mortality rate for Engelmann spruce than for limber pine in western US subalpine forests (Andrus et al., 2021).

Examining trait coordination across the whole plant improves our ability to understand drought survival strategies and predict plant functioning under changing environmental conditions (Fu et al., 2019; McCulloh et al., 2019). While previous studies found that stomatal behavior and xylem hydraulic traits were coordinated at the interspecific level (Pivovaroff et al., 2018; Flo et al., 2021), our findings suggest that intraspecific differences in acclimation could obscure broad-scale parallel coevolution between key plant traits (Anderegg et al., 2018). Our results underscore the importance of deciphering plastic adjustments in stomatal and hydraulic traits using in situ data. Understanding trait coordination helps with determining plant drought tolerance and vulnerability under future climates, particularly for mature tree species that inhabit a wide range of landscapes (Charrier et al., 2021; McCulloh et al., 2022).

Natural ecosystems are complex, and interactions with a number of factors, both biotic and abiotic, can have an impact on tree physiology (e.g., McCulloh et al., 2022). In addition, field observations, even when conducted with exceptional duration at sub-hourly resolution as was done in our study, cannot prove causal relationships (Biondi, 2014). As an example, we cannot exclude the possibility that stomatal sensitivity depends more on vapor pressure deficit, as Grossiord et al. (2017) reported, because the method we used for calculating whole-tree canopy stomatal conductance (G_c) from sap flow measurements does not allow examining its sensitivity to vapor pressure deficit. It should also be noted that we focused on the environmental factors that have been found to be most relevant in the ecosystems and for the species under consideration, but other species and/or ecosystems may present different responses. Overall, further research is needed to clarify the mechanistic pathways that underlie the observed within-species patterns of stomatal-hydraulic coordination.

5. Conclusion

Intraspecific covariation between stomatal regulation and xylem hydraulics is central to understanding the ability of a species to withstand increasing drought severities. Our combined analysis of sap flow measurements and wood anatomy revealed that *Pinus flexilis* and *Picea engelmannii* exhibit a consistent acclimation of reduced stomatal sensitivity to soil drought but species-specific adjustments in xylem hydraulics. As a result of distinct within-species trait coordination, *Pinus flexilis* is capable of surviving in relatively drier conditions, whereas *Picea engelmannii* is potentially vulnerable to increasing water stress because it maximizes gas exchange at the cost of hydraulic damage. The stomatal-hydraulic coordination we uncovered complements current tree ecophysiological frameworks, and advances our understanding of conifers' adaptive capacity to survive under changing environmental conditions.

CRediT authorship contribution statement

Xinsheng Liu: Conceptualization; Methodology; Formal analysis; Resources; Visualization; Writing – original draft.
Emanuele Ziaco: Investigation; Field and laboratory work; Validation; Resources; Writing – review & editing.
Franco Biondi: Funding acquisition; Project administration; Supervision; Field work; Data curation; Validation; Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

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

Acknowledgements

We thank S. Strachan for NevCAN maintenance and three anonymous reviewers for their comments and suggestions. X. Liu was funded by the National Natural Science Foundation of China (41961008). FB was funded, in part, by the United States National Science Foundation under grant AGS-P2C2-1903561, and, in part, by the Experiment Station of the College of Agriculture, Biotechnology and Natural Resources at the University of Nevada, Reno, USA. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the funding agencies and supporting institutions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.164351.

References

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H., et al., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manag. 259, 660–684. https://doi.org/10.1016/j.foreco.2009.09.001.

Alon, A., Cohen, S., Burlett, R., Hochberg, U., Lukyanov, V., Rog, I., Klein, T., Cochard, H., Delzon, S., David-Schwartz, R., 2023. Acclimation limits for embolism resistance and osmotic adjustment accompany the geographical dry edge of Mediterranean species. Funct. Ecol. https://doi.org/10.1111/1365-2435.14289.

Anderegg, W.R.L., 2015. Spatial and temporal variation in plant hydraulic traits and their relevance for climate change impacts on vegetation. New Phytol. 205, 1008–1014. https://doi.org/10.1111/nph.12907.

Anderegg, L.D.L., Berner, L.T., Badgley, G., Sethi, M.L., Law, B.E., HilleRisLambers, J., 2018. Within-species patterns challenge our understanding of the leaf economics spectrum. Ecol. Lett. 21, 734–744. https://doi.org/10.1111/ele.12945.

Anderegg, W.R.L., Anderegg, L.D.L., Kerr, K.L., Trugman, A.T., 2019. Widespread drought-induced tree mortality at dry range edges indicates that climate stress exceeds species' compensating mechanisms. Glob. Chang. Biol. 25, 3793–3802. https://doi.org/10.1111/gcb.14771.

Anderegg, W.R.L., Trugman, A.T., Badgley, G., Anderson, C.M., Bartuska, A., Ciais, P., Cullenward, D., Field, C.B., Freeman, J., Goetz, S.J., et al., 2020. Climate-driven risks to the climate mitigation potential of forests. Science 368, 6497. https://doi.org/10.1126/science.aaz7005.

Andrus, R.A., Chai, R.K., Harvey, B.J., Rodman, K.C., Veblen, T.T., 2021. Increasing rates of subalpine tree mortality linked to warmer and drier summers. J. Ecol. 109, 2203–2218. https://doi.org/10.1111/1365-2745.13634.

Anfodillo, T., Pasqua Di Bisceglie, D., Urso, T., 2002. Minimum cuticular conductance and cuticle features of *Picea abies* and *Pinus cembra* needles along an altitudinal gradient in the dolomites. Tree Physiol. 22, 479–487. https://doi.org/10.1093/treephys/22.7.479.

Bartlett, M.K., Scoffoni, C., Sack, L., 2012. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecol. Lett. 15, 393–405. https://doi.org/10.1111/j.1461-0248.2012.01751.x.

Bartlett, M.K., Klein, T., Jansen, S., Choat, B., Sack, L., 2016. The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. Proc. Natl. Acad. Sci. U. S. A. 113, 13098–13103. https://doi.org/10.1073/pnas.1604088113.

Biondi, F., 2014. Paleoecology grand challenge. Front. Ecol. Evol. Paleoecol. 2, 50. https://doi.org/10.3389/fevo.2014.00050 (3 pp.).

Blackman, C.J., Pfautsch, S., Choat, B., Delzon, S., Gleason, S.M., Duursma, R.A., 2016. Toward an index of desiccation time to tree mortality under drought. Plant Cell Environ. 39, 2342–2345. https://doi.org/10.1111/pce.12758.

Blasini, D.E., Koepke, D.F., Bush, S.E., Allan, G.J., Gehring, C.A., Whitham, T.G., Day, T.A., Hultine, K.R., 2022. Tradeoffs between leaf cooling and hydraulic safety in a dominant arid land riparian tree species. Plant Cell Environ. 45, 1664–1681. https://doi.org/10.1111/pce.14292.

Bouche, P.S., Larter, M., Domec, J.C., Burlett, R., Gasson, P., Jansen, S., Delzon, S., 2014. A broad survey of hydraulic and mechanical safety in the xylem of conifers. J. Exp. Bot. 65, 4419–4431. https://doi.org/10.1093/jxb/eru218.

Brodribb, T.J., Holbrook, N.M., Edwards, E.J., Gutiérrez, M.V., 2003. Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. Plant Cell Environ. 26, 443–450. https://doi.org/10.1046/j.1365-3040.2003.00975.x.

Brodribb, T.J., Powers, J., Cochard, H., Choat, B., 2020. Hanging by a thread? Forests and drought. Science 368, 261–266. https://doi.org/10.1126/science.aat7631.

Bush, S.E., Pataki, D.E., Hultine, K.R., West, A.G., Sperry, J.S., Ehleringer, J.R., 2008. Wood anatomy constrains stomatal responses to atmospheric vapor pressure deficit in irrigated, urban trees. Oecologia 156, 13–20. https://doi.org/10.1007/s00442-008-0966-5.

Charrier, G., Nicolas, Martin-StPaul, Damesin, C., Delpirre, N., Hänninen, H., Torres-Ruiz, J.M., Davi, H., 2021. Interaction of drought and frost in tree ecophysiology: rethinking the timing of risks. Ann. For. Sci. 78, 40. https://doi.org/10.1007/s13595-021-01052-5.

Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Feild, T.S., Gleason, S.M., Hacke, U.G., et al., 2012. Global convergence in the vulnerability of forests to drought. Nature 491, 752–755. https://doi.org/10.1038/nature11688.

Choat, B., Brodribb, T.J., Brodersen, C.R., Duursma, R.A., López, R., Medlyn, B.E., 2018. Triggers of tree mortality under drought. Nature 558, 531–539. https://doi.org/10.1038/s41586-018-0240-x.

Clute, T., Martin, J., Looker, N., Hu, J., 2021. Hydraulic traits of co-existing conifers do not correlate with local hydroclimate condition: a case study in the northern Rocky Mountains, U.S.A. Oecologia 197, 1049–1062. https://doi.org/10.1007/s00442-020-04772-z.

Cochard, H., Barigah, S.T., Kleinhentz, M., Eshel, A., 2008. Is xylem cavitation resistance a relevant criterion for screening drought resistance among *Prunus* species? J. Plant Physiol. 165, 976–982. https://doi.org/10.1016/j.jplph.2007.07.020.

Cocozza, C., Palombo, C., Tognetti, R., Porta, N.L., Anichini, M., Giovannelli, A., Emiliani, G., 2016. Monitoring intra-annual dynamics of wood formation with microcores and dendrometers in *Picea abies* at two different altitudes. Tree Physiol. 36, 832–846. https://doi.org/10.1093/treephys/tpw009.

Corcuera, L., Cochard, H., Gil-Pelegrin, E., Notivol, E., 2011. Phenotypic plasticity in mesic populations of *Pinus pinaster* improves resistance to xylem embolism (P50) under severe drought. Trees 25, 1033–1042. https://doi.org/10.1007/s00468-011-0578-2.

Daly, C., Halbleib, M., Smith, J.I., Gibson, W.P., Doggett, M.K., Taylor, G.H., Curtis, J., Pasteris, P.P., 2008. Physiographically sensitive mapping of temperature and precipitation across the conterminous United States. Int. J. Climatol. 28, 2031–2064. https://doi.org/10.1002/joc.1688.

DeBano, L.H., Ffolliott, P.H., Ortega-Rubio, A., Gottfried, G.J., Hamre, R.H., Carleton, B., 1995. Biodiversity and Management of the Madrean Archipelago: the Sky Islands of Southwestern United States and Northwestern Mexico. Rocky Mountain Forest and Range Experiment Station, General Technical Report. USDA Forest Service, General Technical Report, RM-GTR-264, Tucson, AZ.

Drake, J.E., Tjoelker, M.G., Värhammar, A., Medlyn, B.E., Reich, P.B., Leigh, A., Pfautsch, S., Blackman, C.J., López, R., Aspinwall, M.J., et al., 2018. Trees tolerate an extreme heatwave via sustained transpirational cooling and increased leaf thermal tolerance. Glob. Chang. Biol. 24, 2390–2402. https://doi.org/10.1111/gcb.14037.

Dufour, B., Morin, H., 2010. Tracheid production phenology of *Picea mariana* and its relationship with climatic fluctuations and bud development using multivariate analysis. Tree Physiol. 30, 853–865. https://doi.org/10.1093/treephys/tpq046.

Fajardo, A., Piper, F.I., García-Cervigón, A.I., 2022. The intraspecific relationship between wood density, vessel diameter and other traits across environmental gradients. Funct. Ecol. 36, 1585–1598. https://doi.org/10.1111/1365-2435.14069.

Farrell, C., Szota, C., Arndt, S.K., 2017. Does the turgor loss point characterize drought response in dryland plants? Plant Cell Environ. 40, 1500–1511. https://doi.org/10.1111/pce.12948.

Feng, X., Ackerly, D.D., Dawson, T.E., Manzoni, S., McLaughlin, B., Skelton, R.P., Vico, G., Weitz, A.P., Thompson, S.E., 2019. Beyond isohydricity: the role of environmental variability in determining plant drought responses. Plant Cell Environ. 42, 1104–1111. https://doi.org/10.1111/pce.13486.

Flo, V., Martínez-Vilalta, J., Mencuccini, M., Granda, V., Anderegg, W.R.L., Poyatos, R., 2021. Climate and functional traits jointly mediate tree water-use strategies. New Phytol. 231, 617–630. https://doi.org/10.1111/nph.17404.

Ford, C.R., Goranson, C.E., Mitcvhell, R.J., Will, R.E., Teskey, R.O., 2004. Diurnal and seasonal variability in the radial distribution of sap flow: predicting total stem flow in *Pinus taeda* trees. Tree Physiol. 24, 951–960. https://doi.org/10.1093/treephys/26.9.1217.

Fu, X., Meinzer, F.C., Woodruff, D.R., Liu, Y., Smith, D.D., McCulloh, K.A., Howard, A.R., 2019. Coordination and trade-offs between leaf and stem hydraulic traits and stomatal regulation along a spectrum of isohydry to anisohydry. Plant Cell Environ. 42, 2245–2258. https://doi.org/10.1111/pce.13543.

Fuchs, S., Leuschner, C., Link, R.M., Schuldt, B., 2021. Hydraulic variability of three temperate broadleaf tree species along a water availability gradient in central Europe. New Phytol. 231, 1387–1400. https://doi.org/10.1111/nph.17448.

Gleason, S.M., Westoby, M., Jansen, S., Choat, B., Hacke, U.G., Pratt, R.B., Bhaskar, R., Brodribb, T.J., Bucci, S.J., Cao, K.-F., et al., 2016. Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. New Phytol. 209, 123–136. https://doi.org/10.1111/nph.13646.

Granier, A., 1987. Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. Tree Physiol. 3, 309–320. https://doi.org/10.1093/treephys/3.4.309.

Grossiord, C., Sevanto, S., Borrego, I., Chan, A.M., Collins, A.D., Dickman, L.T., Hudson, P.J., McBranch, N., Michaletz, S.T., Pockman, W.T., et al., 2017. Tree water dynamics in a drying and warming world. Plant Cell Environ. 40, 1861–1873. https://doi.org/10.1111/pce.12991.

Grossiord, C., Buckley, T.N., Cernusak, L.A., Novick, K.A., Poulter, B., Siegwolf, R.T.W., Sperry, J.S., McDowell, N.G., 2020. Plant responses to rising vapor pressure deficit. New Phytol. 226, 1550–1566. https://doi.org/10.1111/nph.16485.

Guay, R., 2013. WinCELL 2013 for Wood Cell Analysis. Regent Instruments Canada Inc., Ontario.

Guérin, M., Martin-Benito, D., von Arx, G., Andreu-Hayles, L., Griffin, K.L., Hamdan, R., McDowell, N.G., Muscarella, R., Pockman, W., Gentine, P., 2018. Interannual variations in needle and sapwood traits of *Pinus edulis* branches under an experimental drought. Ecol. Evol. 8, 1655–1672. https://doi.org/10.1002/ece3.3743.

Guérin, M., von Arx, G., Martin-Benito, D., Andreu-Hayles, L., Griffin, K.L., McDowell, N.G., Pockman, W., Gentine, P., 2020. Distinct xylem responses to acute vs prolonged drought in pine trees. Tree Physiol. 40, 605–620. https://doi.org/10.1093/treephys/tpz144.

Guo, J.S., Hultine, K.R., Koch, G.W., Kropp, H., Ogle, K., 2020. Temporal shifts in iso/anisohydry revealed from daily observations of plant water potential in a dominant desert shrub. New Phytol. 225, 713–726. https://doi.org/10.1111/nph.16196.

Hacke, U.G., Sperry, J.S., 2001. Functional and ecological xylem anatomy. Perspect. Plant Ecol. Evol. Syst. 4, 97–115. https://doi.org/10.1078/1433-8319-00017.

Hacke, U.G., Sperry, J.S., Pockman, W.T., Davis, S.D., McCulloh, K.A., 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. Oecologia 126, 457–461. https://doi.org/10.1007/s004420100628.

Hadley, J.L., Smith, W.K., 1990. Influence of leaf surface wax and leaf area to water content ratio on cuticular transpiration in western conifers, U.S.A. Can. J. For. Res. 20, 1306–1311. https://doi.org/10.1139/x90-173.

Hammond, W.M., Williams, A.P., Abatzoglou, J.T., Adams, H.D., Klein, T., López, R., Sáenz-Romero, C., Hartmann, H., Breshears, D.D., Allen, C.D., 2022. Global field observations of tree die-off reveal hotter-drought fingerprint for Earth's forests. Nat. Commun. 13, art1761. https://doi.org/10.1038/s41467-022-29289-2.

Hartmann, H., Bastos, A., Das, A.J., Esquivel-Muelbert, A., Hammond, W.M., Martínez-Vilalta, J., McDowell, N.G., Powers, J.S., Pugh, T.A.M., Ruthrof, K.X., et al., 2022. Climate change risks to global forest health: emergence of unexpected events of elevated tree mortality worldwide. Annu. Rev. Plant Biol. 73, 673–702. https://doi.org/10.1146/annurev-arplant-102820-012804.

Hochberg, U., Rockwell, F.E., Holbrook, N.M., Cochard, H., 2018. Iso/anisohydry: a plant–environment interaction rather than a simple hydraulic trait. Trends Plant Sci. 23, 112–120. https://doi.org/10.1016/j.tplants.2017.11.002.

Houghton, J.G., Sakamoto, C.M., Gifford, R.O., 1975. Nevada's Weather and Climate. Special Publication 2: Nevada Bureau of Mines and Geology, University of Nevada, Reno, NV.

Hudson, P.J., Limousin, J.M., Krofcheck, D.J., Boutz, A.L., Pangle, N.G., McDowell, N.G., Pockman, W.T., 2018. Impacts of long-term precipitation manipulation on hydraulic architecture and xylem anatomy of piñon and juniper in Southwest USA. Plant Cell Environ. 41, 421–435. https://doi.org/10.1111/pce.13109.

IPCC, 2018. In: Masson-Delmotte, V., Zhai, P., Pörtner, H., Roberts, D., Skea, J., Shukla, P.R., Pirani, A., Moufouma-Okia, W., Péan, C., Pidcock, R., et al. (Eds.), Global Warming of 1.5 °C: An IPCC Special Report on the Impacts of Global Warming of 1.5 °C Above Pre-industrial Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of Strengthening the Global Response to the Threat of Climate Change, Sustainable Development, and Efforts to Eradicate Poverty. World Meteorological Organization, Geneva, Switzerland.

Jiang, P., Meinzer, F.C., Fu, X., Kou, L., Dai, X., Wang, H., 2021. Trade-offs between xylem water and carbohydrate storage among 24 coexisting subtropical understory shrub species spanning a spectrum of isohydry. Tree Physiol. 41, 403–415. https://doi.org/10.1093/treephys/tpaa138.

Johnson, B.G., Verburg, P.S.J., Arnone III, J.A., 2014. Effects of climate and vegetation on soil nutrients and chemistry in the Great Basin studied along a latitudinal-elevational climate gradient. Plant Soil 382, 151–163. https://doi.org/10.1007/s11104-014-2144-3.

Johnson, B.G., Verburg, P.S.J., Arnone III, J.A., 2016. Plant species effects on soil nutrients and chemistry in arid ecological zones. Oecologia 182, 299–317. https://doi.org/10.1007/s00442-016-3655-9.

Jones, H.G., 1992. Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology. 2nd Ed. Cambridge University Press, Cambridge, UK.

Kannenberg, S.A., Guo, J.S., Novick, K.A., Anderegg, W.R.L., Feng, X., Kennedy, D., Konings, A.G., Martínez-Vilalta, J., Matheny, A.M., 2022. Opportunities, challenges and pitfalls in characterizing plant water-use strategies. Funct. Ecol. 36, 24–37. https://doi.org/10.1111/1365-2435.13945.

Klein, T., 2014. The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. Funct. Ecol. 28, 1313–1320. https://doi.org/10.1111/1365-2435.12289.

Klein, T., Zeppel, M.J.B., Anderegg, W.R.L., Bloemen, J., de Kauwe, M.G., Hudson, P., Ruehr, N.K., Powell, T.L., von Arx, G., Nardini, A., 2018. Xylem embolism refilling and resilience against drought-induced mortality in woody plants: processes and trade-offs. Ecol. Res. 33, 839–855. https://doi.org/10.1007/s11284-018-1588-y.

Körner, C., 2003. Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. Springer, Berlin, Germany.

Lamy, J.B., Delzon, S., Bouche, P.S., Alia, R., Vendramin, G.G., Cochard, H., Plomion, C., 2014. Limited genetic variability and phenotypic plasticity detected for cavitation resistance in a Mediterranean pine. New Phytol. 201, 874–886. https://doi.org/10.1111/nph.12556.

Laughlin, D.C., Delzon, S., Clearwater, M.J., Bellingham, P.J., McGlone, M.S., Richardson, S.J., 2020. Climatic limits of temperate rainforest tree species are explained by xylem embolism resistance among angiosperms but not among conifers. New Phytol. 226, 727–740. https://doi.org/10.1111/nph.16448.

Liang, X., He, P., Liu, H., Zhu, S., Uyehara, I.K., Hou, H., Wu, G., Zhang, H., You, Z., Xiao, Y., et al., 2019. Precipitation has dominant influences on the variation of plant hydraulics of the native *Castanopsis fargesii* (Fagaceae) in subtropical China. Agric. For. Meteorol. 271, 83–91. https://doi.org/10.1016/j.agrformet.2019.02.043.

Lin, Y.S., Medlyn, B.E., Duursma, R.A., Prentice, I.C., Wang, H., Baig, S., Eamus, D., de Dios, V.R., Mitchell, P., Ellsworth, D.S., et al., 2015. Optimal stomatal behaviour around the world. Nat. Clim. Chang. 5, 459–464. https://doi.org/10.1038/nclimate2550.

Liu, X., Biondi, F., 2020. Transpiration drivers of high-elevation five-needle pines (*Pinus longaeva* and *Pinus flexilis*) in sky-island ecosystems of the North American Great Basin. Sci. Total Environ. 739, 139861. https://doi.org/10.1016/j.scitotenv.2020.139861.

Liu, X., Biondi, F., 2021. Inter-specific transpiration differences between aspen, spruce, and pine in a sky-island ecosystem of the North American Great Basin. For. Ecol. Manag. 491, 119157. https://doi.org/10.1016/j.foreco.2021.119157.

Liu, X., Ziaco, E., Biondi, F., 2021. Water-use efficiency of co-occurring sky-island pine species in the North American Great Basin. Front. Plant Sci. 12, 787297. https://doi.org/10.3389/fpls.2021.787297.

López, R., Cano, F.J., Choat, B., Cochard, H., Gil, L., 2016. Plasticity in vulnerability to cavitation of *Pinus canariensis* occurs only at the driest end of an aridity gradient. Front. Plant Sci. 7, 1–10. https://doi.org/10.3389/fpls.2016.00769.

Lu, Y., Duursma, R.A., Farrior, C.E., Medlyn, B.E., Feng, X., 2020. Optimal stomatal drought response shaped by competition for water and hydraulic risk can explain plant trait covariation. New Phytol. 225, 1206–1217. https://doi.org/10.1111/nph.16207.

Lupi, C., Rossi, S., Vieira, J., Morin, H., Deslauriers, A., 2014. Assessment of xylem phenology: a first attempt to verify its accuracy and precision. Tree Physiol. 34, 87–93. https://doi.org/10.1093/treephys/tpt1.

Maherali, H., DeLucia, E.H., 2000. Xylem conductivity and vulnerability to cavitation of ponderosa pine growing in contrasting climates. Tree Physiol. 20, 859–867. https://doi.org/10.1093/treephys/20.13.859.

Manzoni, S., Vico, G., Katul, G., Palmroth, S., Jackson, R.B., Porporato, A., 2013. Hydraulic limits on maximum plant transpiration and the emergence of the safety-efficiency trade-off. New Phytol. 198, 169–178. https://doi.org/10.1111/nph.12126.

Maréchaux, I., Bonal, D., Bartlett, M.K., Burban, B., Coste, S., Courtois, E.A., Dulormne, M., Goret, J.-Y., Mira, E., Mirabel, A., et al., 2018. Dry-season decline in tree sapflux is correlated with leaf turgor loss point in a tropical rainforest. Funct. Ecol. 32, 2285–2297. https://doi.org/10.1111/1365-2435.13188.

Mark, W.R., Crews, D.L., 1973. Heat-pulse velocity and bordered pit condition in living Engelmann spruce and Lodgepole pine trees. For. Sci. 19, 291–296. https://doi.org/10.1093/forestscience/19.4.291.

Martínez-Vilalta, J., Garcia-Forner, N., 2017. Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. Plant Cell Environ. 40, 962–976. https://doi.org/10.1111/pce.12846.

Martínez-Vilalta, J., Cochard, H., Mencuccini, M., Sterck, F., Herrero, A., Korhonen, J.F.J., Llorens, P., Nikinmaa, E., Nolè, A., Poyatos, R., et al., 2009. Hydraulic adjustment of Scots pine across Europe. New Phytol. 184, 353–364. https://doi.org/10.1111/j.1469-8137.2009.02954.x.

Martin-StPaul, N., Delzon, S., Cochard, H., 2017. Plant resistance to drought depends on timely stomatal closure. Ecol. Lett. 20, 1437–1447. https://doi.org/10.1111/ele.12851.

Mayr, S., Schwienbacher, F., Bauer, H., 2003. Winter at the alpine timberline. Why does embolism occur in Norway spruce but not in stone pine? Plant Physiol. 131, 780–792. https://doi.org/10.1104/pp.011452.

Mayr, S., Hacke, U., Schmid, P., Schwienbacher, F., Gruber, A., 2006. Frost drought in conifers at the alpine timberline: xylem dysfunction and adaptations. Ecology 87, 3175–3185. https://doi.org/10.1890/0012-9658(2006)87[3175:FDICAT]2.0.CO;2.

Mayr, S., Schmid, P., Laur, J., Rosner, S., Charra-Vaskou, K., Dämon, B., Hacke, U.G., 2014. Uptake of water via branches helps timberline conifers refill embolized xylem in late winter. Plant Physiol. 164, 1731–1740. https://doi.org/10.1104/pp.114.236646.

Mayr, S., Schmid, P., Beikircher, B., Feng, F., Badel, E., 2020. Die hard: timberline conifers survive annual winter embolism. New Phytol. 226, 13–20. https://doi.org/10.1111/nph.16304.

McCulloh, K.A., Domec, J., Johnson, D.M., Smith, D.D., Meinzer, F.C., 2019. A dynamic yet vulnerable pipeline: integration and coordination of hydraulic traits across whole plants. Plant Cell Environ. 42, 2789–2807. https://doi.org/10.1111/pce.13607.

McCulloh, K.A., Augustine, S.P., Goke, A., Jordan, R., Krieg, C.P., O'Keefe, K., Smith, D.D., 2022. At least it's a dry cold: the global distribution of freeze–thaw and drought stress and the traits that may impart poly-tolerance in conifers. Tree Physiol. 43, 1–15. https://doi.org/10.1093/treephys/tpac102.

McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G., et al., 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol. 178, 719–739. https://doi.org/10.1111/j.1469-8137.2008.02436.x.

McDowell, N.G., Sapes, G., Pivovaroff, A., Adams, H.D., Allen, C.D., Anderegg, W.R.L., Arend, M., Breshears, D.D., Brodribb, T., Choat, B., et al., 2022. Mechanisms of woody plant mortality under rising drought, CO_2, and vapor pressure deficit. Nat. Rev. Earth Environ. 3, 294–308. https://doi.org/10.1038/s43017-022-00272-1.

Meinzer, F.C., McCulloh, K.A., 2013. Xylem recovery from drought-induced embolism: where is the hydraulic point of no return? Tree Physiol. 33, 331–334. https://doi.org/10.1093/treephys/tpt022.

Mensing, S., Strachan, S., Arnone, J., Fenstermaker, L., Biondi, F., Devitt, D., Johnson, B., Bird, B., Fritzinger, E., 2013. A network for observing Great Basin climate change. EOS Trans. Am. Geophys. Union 94, 105–106. https://doi.org/10.1002/2013EO110001.

Nolan, R.H., Tarin, T., Santini, N.S., McAdam, S.A.M., Ruman, R., Eamus, D., 2017. Differences in osmotic adjustment, foliar abscisic acid dynamics, and stomatal regulation between an isohydric and anisohydric woody angiosperm during drought. Plant Cell Environ. 40, 3122–3134. https://doi.org/10.1111/pce.13077.

Ogle, K., Lucas, R.W., Bentley, L.P., Cable, J.M., Barron-Gafford, G.A., Griffith, A., Ignace, D., Jenerette, G.D., Tyler, A., Huxman, T.E., et al., 2012. Differential daytime and night-time stomatal behavior in plants from North American deserts. New Phytol. 194, 464–476. https://doi.org/10.1111/j.1469-8137.2012.04068.x.

Oren, R., Philips, N., Katul, G., Ewers, B.E., Pataki, D.E., 1998. Scaling xylem sap flux and soil water balance and calculating variance: a method for partitioning water flux in forests. Ann. For. Sci. 55, 191–216. https://doi.org/10.1051/forest:19980112.

Oren, R., Sperry, J.S., Katul, G.G., Pataki, D.E., Ewers, B.E., Phillips, N., Schäfer, K.V.R., 1999. Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. Plant Cell Environ. 22, 1515–1526. https://doi.org/10.1046/j.1365-3040.1999.00513.x.

Pataki, D.E., McCarthy, H.R., Litvak, E., Pincetl, S., 2011. Transpiration of urban forests in the Los Angeles metropolitan area. Ecol. Appl. 21, 661–677. https://doi.org/10.1890/09-1717.1.

Peters, R.L., Speich, M., Pappas, C., Kahmen, A., von Arx, G., Pannatier, E.G., Steppe, K., Treydte, K., Stritih, A., Fonti, P., 2019. Contrasting stomatal sensitivity to temperature and soil drought in mature alpine conifers. Plant Cell Environ. 42, 1674–1689. https://doi.org/10.1111/pce.13500.

Petit, G., Zambonini, D., Hesse, B.D., Häberle, K., 2022. No xylem phenotypic plasticity in mature *Picea abies* and *Fagus sylvatica* trees after 5 years of throughfall precipitation exclusion. Glob. Chang. Biol. 28, 4668–4683. https://doi.org/10.1111/gcb.16232.

Phillips, N., Oren, R., 1998. A comparison of daily representations of canopy conductance based on two conditional time-averaging methods and the dependence of daily conductance on environmental factors. Ann. For. Sci. 55, 217–235. https://doi.org/10.1051/forest:19980113.

Pittermann, J., Sperry, J., 2003. Tracheid diameter is the key trait determining the extent of freezing-induced embolism in conifers. Tree Physiol. 23, 907–914. https://doi.org/10.1093/treephys/23.13.907.

Pittermann, J., Sperry, J.S., 2006. Analysis of freeze–thaw embolism in conifers. The interaction between cavitation pressure and tracheid size. Plant Physiol. 140, 374–382. https://doi.org/10.1104/pp.105.067900.

Pivovaroff, A.L., Pasquini, C., DeGuzman, M.E., Alstad, K.P., Stemke, J.S., Santiago, L.S., 2016. Multiple strategies for drought survival among woody plant species. Funct. Ecol. 30, 517–526. https://doi.org/10.1111/1365-2435.12518.

Pivovaroff, A.L., Cook, V.M.W., Santiago, L.S., 2018. Stomatal behaviour and stem xylem traits are coordinated for woody plant species under exceptional drought conditions. Plant Cell Environ. 41, 2617–2626. https://doi.org/10.1111/pce.13367.

Poyatos, R., Martínez-Vilalta, J., Čermák, J., Ceulemans, R., Granier, A., Irvine, J., Köstner, B., Lagergren, F., Meiresonne, L., Nadezhdina, N., et al., 2007. Plasticity in hydraulic architecture of Scots pine across Eurasia. Oecologia 153, 245–259. https://doi.org/10.1007/s00442-007-0740-0.

Pritzkow, C., Williamson, V., Szota, C., Trouvé, R., Arndt, S.K., 2020. Phenotypic plasticity and genetic adaptation of functional traits infuences infra-specific variation in hydraulic efficiency and safety. Tree Physiol. 40, 215–229. https://doi.org/10.1093/treephys/tpz121.

Puchi, P.F., Castagneri, D., Rossi, S., Carrer, M., 2020. Wood anatomical traits in black spruce reveal latent water constraints on the boreal forest. Glob. Chang. Biol. 26, 1767–1777. https://doi.org/10.1111/gcb.14906.

Rosas, T., Mencuccini, M., Barba, J., Cochard, H., Saura-Mas, S., Martínez-Vilalta, J., 2019. Adjustments and coordination of hydraulic, leaf and stem traits along a water availability gradient. New Phytol. 223, 632–646. https://doi.org/10.1111/nph.15684.

Rosner, S., Světlík, J., Andreassen, K., Børja, I., Dalsgaard, L., Evans, R., Luss, S., Tveito, O.E., Solberg, S., 2016. Novel hydraulic vulnerability proxies for a boreal conifer species reveal that opportunists may have lower survival prospects under extreme climatic events. Front. Plant Sci. 7, 831. https://doi.org/10.3389/fpls.2016.00831.

Rossi, S., Anfodillo, T., Menardi, R., 2006. Trephor: a new tool for sampling microcores from tree stems. IAWA J. 27, 89–97. https://doi.org/10.1163/22941932-90000139.

Sala, A., Woodruff, D.R., Meinzer, F.C., 2012. Carbon dynamics in trees: feast or famine? Tree Physiol. 32, 764–775. https://doi.org/10.1093/treephys/tpr143.

Sanchez-Martinez, P., Martínez-Vilalta, J., Dexter, K.G., Segovia, R.A., Mencuccini, M., 2020. Adaptation and coordinated evolution of plant hydraulic traits. Ecol. Lett. 23, 1599–1610. https://doi.org/10.1111/ele.13584.

Schuldt, B., Knutzen, F., Delzon, S., Jansen, S., Müller-Haubold, H., Burlett, R., Clough, Y., Leuschner, C., 2016. How adaptable is the hydraulic system of European beech in the face of climate change-related precipitation reduction? New Phytol. 210, 443–458. https://doi.org/10.1111/nph.13798.

Skelton, R.P., West, A.G., Dawson, T.E., 2015. Predicting plant vulnerability to drought in biodiverse regions using functional traits. Proc. Natl. Acad. Sci. U. S. A. 112, 5744–5749. https://doi.org/10.1073/pnas.1503376112.

Sorek, Y., Greenstein, S., Netzer, Y., Shtein, I., Jansen, S., Hochberg, U., 2021. An increase in xylem embolism resistance of grapevine leaves during the growing season is coordinated with stomatal regulation, turgor loss point and intervessel pit membranes. New Phytol. 229, 1955–1969. https://doi.org/10.1111/nph.17025.

Sperry, J.S., Nichols, K.L., Sullivan, J.E.M., Eastlack, S.E., 1994. Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of Northern Utah and Interior Alaska. Ecology 75, 1736–1752. https://doi.org/10.2307/1939633.

Stojnić, S., Suchocka, M., Benito-Garzón, M., Torres-Ruiz, J.M., Cochard, H., Bolte, A., Cocozza, C., Cvjetković, B., de Luis, M., Martinez-Vilalta, J., et al., 2018. Variation in xylem vulnerability to embolism in European beech from geographically marginal populations. Tree Physiol. 38, 173–185. https://doi.org/10.1093/treephys/tpx128.

Tardieu, F., Simonneau, T., 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. J. Exp. Bot. 49, 419–432. https://doi.org/10.1093/jxb/49.Special_Issue.419.

Trugman, A.T., Anderegg, L.D.L., Anderegg, W.R.L., Das, A.J., Stephenson, N.L., 2021. Why is tree drought mortality so hard to predict? Trends Ecol. Evol. 36, 520–532. https://doi.org/10.1016/j.tree.2021.02.001.

Tyree, M.T., Ewers, F.W., 1991. The hydraulic architecture of trees and other woody plants. New Phytol. 119, 345–360. https://doi.org/10.1111/j.1469-8137.1991.tb00035.x.

Tyree, M.T., Sperry, J.S., 1989. Vulnerability of xylem to cavitation and embolism. Ann. Rev. Plant Phys. Mol. Biol. 40, 19–36. https://doi.org/10.1146/annurev.pp.40.060189.000315.

Vicente, E., Didino-Gency, M., Morcillo, L., Morin, X., Vilagrosa, A., Grossiord, C., 2022. Aridity and cold temperatures drive divergent adjustments of European beech xylem anatomy, hydraulics and leaf physiological traits. Tree Physiol. 42, 1720–1735. https://doi.org/10.1093/treephys/tpac029.

Wang, A.Y., Han, S.J., Zhang, J.H., Wang, M., Yin, X.H., Fang, L.D., Yang, D., Hao, G.Y., 2018. The interaction between nonstructural carbohydrate reserves and xylem hydraulics in Korean pine trees across an altitudinal gradient. Tree Physiol. 38, 1792–1804. https://doi.org/10.1093/treephys/tpy119.

Wieser, G., Tausz, M., 2007. Trees at Their Upper Limit: Treelife Limitation at the Alpine Timberline. Springer, Dordrecht, the Netherlands.

Will, R.E., Wilson, S.M., Zou, C.B., Hennessey, T.C., 2013. Increased vapor pressure deficit due to higher temperature leads to greater transpiration and faster mortality during drought for tree seedlings common to the forest-grassland ecotone. New Phytol. 200, 366–374. https://doi.org/10.1111/nph.12321.

Wortemann, R., Herbette, S., Barigah, T.S., Fumanal, B., Alia, R., Ducousso, A., Gomory, D., Roeckel-Drevet, P., Cochard, H., 2011. Genotypic variability and phenotypic plasticity of cavitation resistance in *Fagus sylvatica* L. across Europe. Tree Physiol. 31, 1175–1182. https://doi.org/10.1093/treephys/tpr101.

Yi, K., Dragoni, D., Phillips, R.P., Roman, D.T., Novick, K.A., 2017. Dynamics of stem water uptake among isohydric and anisohydric species experiencing a severe drought. Tree Physiol. 37, 1379–1392. https://doi.org/10.1093/treephys/tpw126.

Ziaco, E., Biondi, F., 2016. Tree growth, cambial phenology, and wood anatomy of limber pine at a Great Basin (USA) mountain observatory. Trees 30, 1507–1521. https://doi.org/10.1007/s00468-016-1384-7.

Ziaco, E., Biondi, F., Rossi, S., Deslauriers, A., 2014. Climatic influences on wood anatomy and tree-ring features of Great Basin conifers at a new mountain observatory. Appl. Plant Sci. 2, 1400054. https://doi.org/10.3732/apps.1400054.