



Climate effect on the growth and hydraulic traits of two shrubs from the top of a Mediterranean mountain

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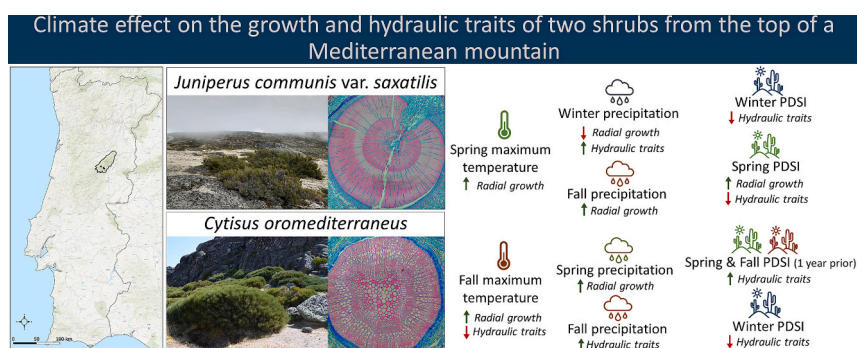
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HIGHLIGHTS

- Warming promotes growth of two high-altitude shrubs in a Mediterranean mountain.
- The gymnosperm growth is more sensitive to winter drought and spring temperature.
- The angiosperm growth is more sensitive to spring drought and fall temperature.
- Hydraulic traits in both species were positively influenced by fall precipitation.
- Climate change may alter the distribution and coexistence of these two species.

GRAPHICAL ABSTRACT



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ABSTRACT

Increasing mean global temperatures in conjunction with increases in the frequency and severity of drought events affect plant growth and physiology, particularly in more arid and mountainous ecosystems. Thus, it is imperative to understand the response of plant growth to climatic oscillations in these regions. This study used dendrochronological and wood anatomical traits of two shrub species growing over 1500 m.a.s.l. in the Serra da Estrela (Portugal), *Juniperus communis* and *Cytisus oromediterraneus*, to analyze their response to temperature and water availability parameters. Results showed an increase in shrub growth related to the increase over time of the mean minimum and maximum monthly temperature in Serra da Estrela. Warming seems to promote shrub growth because it lengthens the growing season, although *J. communis* responds mainly to spring maximum temperature while *C. oromediterraneus* is influenced by fall maximum temperature. Hydraulic traits of *J. communis* and *C. oromediterraneus* were negatively influenced by winter drought. Additionally, there were species-specific differences in response to changes in water availability. *J. communis* radial growth was significantly affected by spring drought conditions, while *C. oromediterraneus* radial growth was significantly affected by spring precipitation. *C. oromediterraneus* hydraulic traits were also significantly affected by drought conditions from the previous spring and fall. This study shed light on specific differences in the response to climate between

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two co-occurring shrub species in the top of an understudied Mediterranean mountain, which could have implications in the future distribution of woody species within this region.

1. Introduction

Mountains will be among the terrestrial systems most impacted by global warming through numerous direct and indirect effects (Fazlioglu and Wan, 2021; Lu et al., 2019; Pandey et al., 2020; Verrall and Pickering, 2020). Warming in mountain regions can either increase species richness, by allowing species from lower elevations to migrate upwards while still maintaining some habitat conditions suitable for existing species (Körner and Hiltbrunner, 2021), or reduce plant richness, as shown in Mediterranean mountains due to higher water stress (Pauli et al., 2012). Warming may also lead to shrub encroachment and a higher risk of habitat loss in alpine regions (Archer et al., 1995; Sanz-Elorza et al., 2003). Rising temperatures are expected to result in increases in the frequency, intensity and duration of drought events, particularly in semi-arid regions such as the Mediterranean where this has already been observed, further impacting plant productivity and growth (Allen et al., 2015; Anadon-Rosell et al., 2014; Peña-Gallardo et al., 2018; Trambley et al., 2020). Therefore, understanding how plant species within these regions respond to such climatic constraints might be of primary interest for the conservation of these communities.

Plant response to changes in temperature and water availability may be difficult to predict, as there are multiple, interconnected driving factors affecting different physiological processes (Allen et al., 2015; Kudo and Suzuki, 2003). In addition, these effects may elicit different responses in growth, physiology, and fitness among co-occurring species, potentially establishing climate feedback loops due to changes in species composition and cover, further altering the mosaic of Mediterranean alpine plant species communities (Anadon-Rosell et al., 2014; Fazlioglu and Wan, 2021; Gazol et al., 2017; Pandey et al., 2020). Dendrochronological techniques are an effective approach to analyze the historical relationship between secondary growth of existing plants and past climate, to predict future species response to climate change (Moullec et al., 2018; Pandey et al., 2020; Gärtner et al., 2015). While dendrochronological studies in the Mediterranean have mainly focused on tree species (Garcia-Forner et al., 2019; Vieira et al., 2020; Abrantes et al., 2013; Camarero et al., 2010), the use of shrub species has recently gained attention as they can provide information on habitats heavily threatened by warming above tree line (Francon et al., 2020; Lu et al., 2019; Garcia-Cervigón Morales et al., 2012; Myers-Smith et al., 2015).

Since temperature and water availability influence vascular tissue formation, wood anatomy characteristics such as number of conductive cells and conduit size inherently reflect plant response to climate and environmental stressors, and thus may shed light on the balance between increasing hydraulic conductance and risk of conduit embolism (Olano et al., 2012). Therefore, supplementing dendrochronological analysis with quantitative wood anatomy analysis is a useful approach to better elucidate the functional roles of vascular tissue, and the relationship between xylem formation and climatic conditions (DeSoto et al., 2011; Olano et al., 2013; von Arx et al., 2016).

The Serra da Estrela, henceforth referred to as Estrela, is the highest mountain range in mainland Portugal, reaching an elevation of 1993 m (Meireles et al., 2013). Also, the Estrela upper plateau is the only location in mainland Portugal where alpine shrublands, pasturelands and wetlands, can be found. The topography and geographic location of Estrela create distinct and unique conditions optimal to promote the formation of microclimates as well as a high rate of endemisms (Mora, 2010; Meireles et al., 2013). This granitic massif is in the transition between Mediterranean and Eurosiberian climates, a region where plant species turnover is expected to be especially high in response to climate change (Alexander et al., 2018; Anadon-Rosell et al., 2014; Bär et al., 2006; Fazlioglu and Wan, 2021; Meireles et al., 2013; Thuiller et al.,

2005). However, thus far, there is little research on the response of alpine plants to global warming in these transition areas, which hinders any effort to forecast their future and plan mitigation measures.

This study aims to analyze the growth rings and wood anatomy of the two main alpine shrubs in Estrela, the gymnosperm *Juniperus communis* var. *saxatilis* Pall. and the angiosperm *Cytisus oromediterraneus* Rivas Mart. & al., to understand the effect of temperature and precipitation on shrub secondary growth. Our main hypotheses are: i) shrub growth has increased in the last years in response to warming, and ii) shrub growth is positively influenced by increases in temperature at the start and end of the growing season (i.e., spring and fall). Then, species-specific differences in shrub response to changes in water availability will be discussed accounting for the differences between angiosperms and gymnosperms in wood traits (Choat et al., 2012; Gazol et al., 2018; DeSoto et al., 2020).

2. Materials and methods

2.1. Study species

J. communis, and *C. oromediterraneus*, the two dominant shrub species in the alpine vegetation belt of Estrela, were selected for this study because of their relevant ecological role as high-altitude large shrubs and, because they have a small distribution in Portugal that is nearly restricted to Estrela, are highly vulnerable to changes in this habitat. (Carapeto et al., 2014).

J. communis is a coniferous, dioecious shrub extremely prevalent in the high mountains of boreal, central and southern Europe and, depending on local conditions, may form either dense stands or patches in a scrubland/grassland mosaic (Jansen, 2011; European Commission, 2016; Thomas et al., 2007). Although commonly found in Estrela at elevations above 1600 m, it is one of only two occurrences in continental Portugal (Costa et al., 1998). Harsh conditions common to the alpine vegetation belt influence the phenology of this species, resulting in procumbent nanophanerophytes who are likely to have small ring widths, stem lobes and the frequent presence of growth anomalies such as false and locally absent rings (Bergmeier et al., 2014; Herrero and Zamora, 2014). *J. communis* is a light-demanding, drought and frost tolerant species (Thomas et al., 2007). Under Mediterranean conditions, this species shows bi-modal growth and can advance phenology showing a positive correlation between growth and spring temperature (Tumajer et al., 2021).

C. oromediterraneus is a shrubby legume endemic to France, Iberian Peninsula and North Africa; and its only occurrence in Portugal is in Estrela (Jansen, 2011). This nanophanerophyte primarily occurs in elevations above 1400 m, either growing on granitic slopes or forming dense stands in acidic soils (Fernández-Santos et al., 2004; Meireles et al., 2013). *C. oromediterraneus* occurs in the transition zone between Mediterranean and Eurosiberian climates and may be present in harsh environments, even in conditions of low water availability (Meireles et al., 2013).

2.2. Study site and sampling method

Sampling was conducted in October 2019 at the end of the growing season. Three sampling sites were selected in shrublands within the alpine vegetation belt of Estrela that were dominated by either *J. communis* or *C. oromediterraneus*. From the highest elevation to the lowest elevation, sampling was done in Covão do Boi (Upper; 1872 m), Penha do Gato (Mid; 1700 m), and Nave de Santo António (Lower; 1563 m) (Fig. 1). The three sites were also located in a western-eastern

gradient of continentality (Meireles et al., 2013), with sites in the north-western part of the Serra (Mid site) being more humid and milder than those in the south-eastern side (Lower site). Slope varied between 4 and 5 % in the Lower site to 2–3 % in the Upper and Mid sites, while slope aspect was SW in the Upper site and NW in the Mid and Lower sites. Soils in all sites are umbrisolts with a sandy loam texture. All sampled plants were selected to be of similar sizes and located in similar microtopographic conditions. In the Upper and Lower sites, samples were taken from dense shrublands surrounded by rocky outcrops and/or *Nardus stricta* grasslands, while in the Mid site the vegetation corresponded to a sparse shrubland surrounded by bare soil or *Agrostis truncatula* grasslands.

Eight individuals from each species were selected in each site for a total of 24 individuals per species. Shrub height and diameter were measured for each individual to calculate shrub volume, which was used to represent shrub size within modeling. A transverse section was taken from the basal branch of each shrub, aiming at the largest and oldest branch of the individual, for dendroecological analysis. Transverse sections were also taken 20 cm from the apical branch tip of one branch per shrub individual for wood anatomical observation. This uniform sampling distance allows for analysis while accounting for allometry and structural differences that occur from branch apices to shrub base (Rodríguez-Zaccaro et al., 2019). Additionally, as the angiosperm *C. oromediterraneus* has a more complex vascular structure, radial and tangential cuts were taken to assist in anatomical identification of the tracheary elements.

2.3. Climatic data

Climate data for each sampling site was obtained from the dataset TerraClimate (spatial resolution $\sim 4.6 \text{ km}^2$) via the open-source platform, Google Earth Engine. This interpolated dataset combines the climatological normals and high-spatial resolution of the WorldClim dataset with the high-temporal resolution and time-varying anomalies of CRU Ts4.0 and the Japanese 55-year Reanalysis (JRA55) (Abatzoglou et al., 2018). We obtained monthly data over 63 years from 1958 to 2019 for average minimum and maximum temperature ($^{\circ}\text{C}$), total

precipitation (mm), and the Palmer Drought Severity Index (PDSI). For our selected period the mean annual temperature within the alpine zone of Estrela was $8.2 \text{ }^{\circ}\text{C}$ and total annual precipitation 1745 mm (Supplementary Fig. 1).

2.4. Sample preparations and measurements

Basal branch transversal cuts were sanded in a graded series from 150 to 800 grit to facilitate growth ring visibility. Shrub Ring Widths (SRWs, mm) were measured per ring along three radial paths in each individual using a LINTAB (Rinntech) measurement table. Cross-dating methods were conducted within the statistics program COFECHA following the suggested protocol of Cook and Kairiukstis (1990) and Grissino-Mayer (2001) to ensure correct dating of growth rings.

Transversal cuts of the *J. communis* and *C. oromediterraneus* samples taken 20 cm from the apical branch tip were prepared for microscopic observation of xylem tissue following standard histologic protocols for wood samples (Prislan et al., 2014; Rossi et al., 2006) using equipment such as the Shandon HistoCentre 2 paraffin embedding station and MICROM HM 340 E rotary microtome. Microscopic images of the histologic samples were taken in sections using the Leica DM4 M imaging microscope at magnifications $\times 20$ and $\times 40$. The 'stitching' function (Preibisch et al., 2009) within image analysis software ImageJ (Abrámoff et al., 2004) was then used to stitch the sections into images of the complete radial paths for measurement. Three radial paths per ring per apical branch were measured. In *C. oromediterraneus* samples, a radial path was defined as all tracheary elements within at least two ray parenchyma radial paths and between the pith and cambial meristem.

Selected wood anatomical parameters were number of conductive cells, total conductive area (mm^2), and hydraulic conductivity (K_h) ($\text{m}^4/\text{MPa}^{-1} \times \text{s}^{-1}$). Total conductive area was calculated as the sum of vessel area (mm^2) for all conductive cells. Hydraulic conductivity is a function of inner conduit diameter to the fourth power and therefore, directly related to lumen diameter (Scholz et al., 2013; Sperry and Hacke, 2004). Diameter was calculated from the vessel area of each conduit vessel using Eq. (1) where D is the diameter and a is the area. To strengthen the role of conductive cells with larger conduits, which are either earlywood

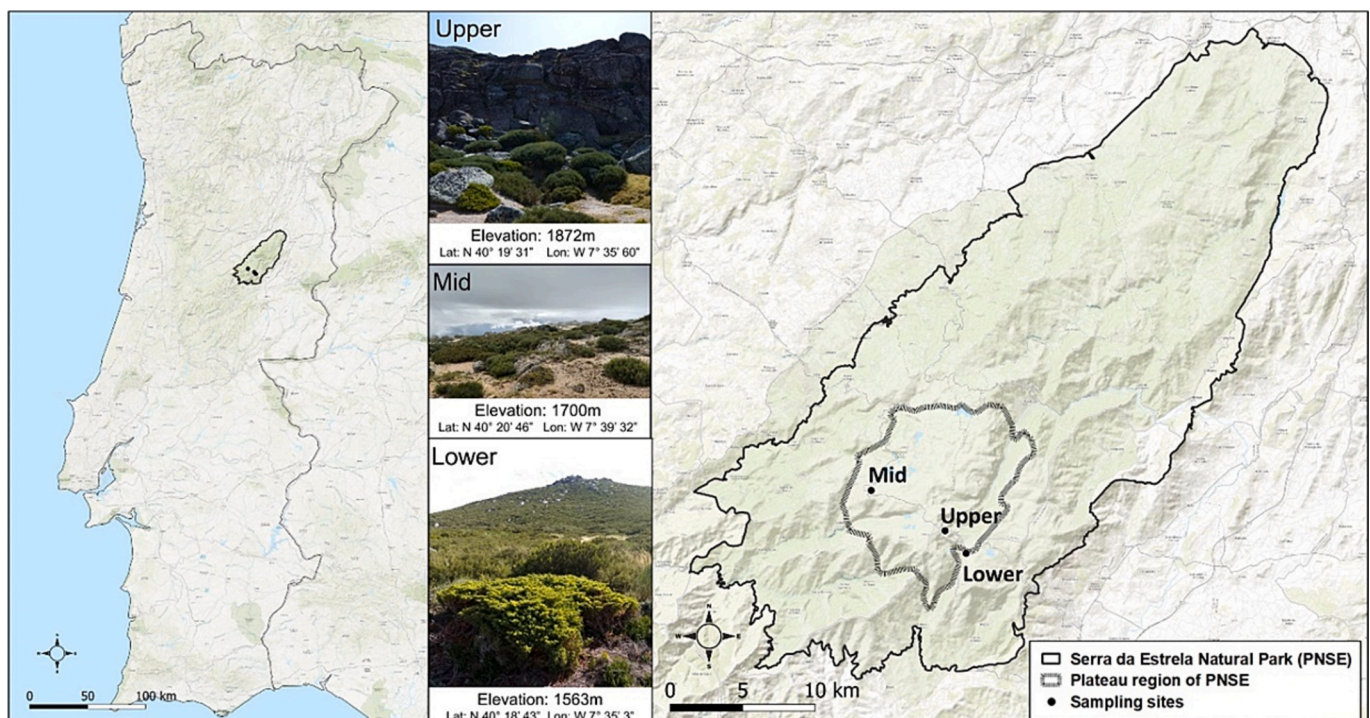


Fig. 1. Study area and Sampling sites within the alpine zone of Serra da Estrela Natural Park (PNSE), Portugal.

tracheids in *J. communis*, or vessels in *C. oromediterraneus*, the hydraulic diameter (D_H) (μm) was calculated using Eq. (2), where N is the number of conduits. Vascular tracheids were sufficiently abundant in *C. oromediterraneus* individuals to potentially affect hydraulic conductivity estimation (IAWA, 1989) thus, were included in calculations. The hydraulic diameter was then used to calculate the hydraulic conductivity (K_h) ($\text{m}^4/\text{MPa}^{-1} \times \text{s}^{-1}$; Eq. (3)) of each ring based on the Hagen-Poiseuille law: where η is the viscosity index of water (1.002×10^{-9} MPa s at 20°C). All equations were taken from Scholz et al. (2013).

Equation 1. Calculations for vessel area of each conduit vessel. D = diameter, a = area.

$$D = \sqrt{\frac{4a}{\pi}}$$

Equation 2. Calculations for hydraulically weighted diameter. D_H = Hydraulic diameter, D = diameter, N = number of conduits.

$$D_H = \left(\frac{\sum D^4}{N} \right)^{\frac{1}{4}}$$

Equation 3. Calculations for hydraulic conductivity based on the Hagen-Poiseuille law. K_h = hydraulic conductivity, D = diameter, η = viscosity index of water (1.002×10^{-9} MPa s at 20°C).

$$K_h = \frac{\pi D^4}{128\eta}$$

2.4.1. Statistical analyses

All data analysis was conducted within the R environment (R Core Team, 2021). We selected SRW, K_h , and total conductive area as our continuous response variables, and number of conductive cells as our discrete response variable. We assumed a Gaussian distribution with an identity link for the continuous variables and a Poisson distribution with a log link for our discrete variable. To remove heteroskedasticity within the models, SRW and K_h values were log transformed while conductive area values were cube root transformed. Final model selections were determined by the lowest AIC, and the 'Anova' function (Fox and Weisberg, 2019) was used to identify significant explanatory variables.

2.4.2. Effect of site, age and size in shrub growth and hydraulic traits

To initially determine whether differences in shrub growth could be due to site, shrub size or shrub age we ran Autoregressive mixed models by species. Continuous variables were run in Linear Mixed Models (LMMs) using the 'nlme' package (Pinheiro et al., 2021) and the discrete variable in Generalized Linear Mixed Models (GLMMs) using the 'MASS' package (Venables and Ripley, 2002), both fit via a Restricted Maximum Likelihood (REML). We selected these packages as they allowed for specification of a first-order temporal autocorrelation structure within the model to remove non-high frequency variations and account for any temporal growth trends (R Core Team, 2021). Cambial age and the interaction between site and shrub volume were set as explanatory variables. Individual and replicate radial path nested within individual were set as random factors.

2.4.3. Warming in Serra da Estrela and influence of monthly temperature on shrub growth

To confirm warming within our study area, we use year as a unit of time and ran Pearson's correlations between year and mean monthly minimum and maximum temperatures. We then tested shrub response to

monthly minimum and maximum temperature by site and species to further elucidate the relationship between shrub growth and temperature. Different approaches were used for SRW and wood anatomical parameters. SRW chronologies were prewhitened within the package 'dplr' (Bunn et al., 2021) by site and species, then analyzed using the 'treeclim' package (Zang and Biondi, 2015). SRW values for each year were correlated with monthly mean minimum and maximum temperature data ranging from January of the previous year to October of the current analyzed ring year within the 'dcc' function. This allowed us to observe potential lags in shrub response to temperature. Monthly temperature values significantly correlated with SRW were then run as explanatory variables within the 'dlm' function to determine the monthly temperature variables that influence SRW.

The hydraulic traits K_h , conductive area and number of conductive cells were analyzed by site and species using the 'cor.test', 'lme', and 'glmmPQL' functions (R Core Team, 2021; Pinheiro et al., 2021; Venables and Ripley, 2002). For each parameter, a Pearson's correlation was run with monthly mean minimum and maximum temperature from January of the previous year to October of the current ring year. To determine the influence of temperature on the interannual variation of hydraulic traits, significantly correlated temperature variables were run in Autoregressive LMMs and GLMMs and set as explanatory variables, with site set as a random factor.

2.4.4. Influence of temperature at start and end of growing season on shrub growth

To test whether shrub growth is influenced by temperature at the beginning and end of the growing season, we ran Autoregressive LMMs and GLMMs by species, including minimum and maximum spring and fall temperatures. Spring was considered the average March, April, and May values, and fall was the average September, October, and November values. Individual and replicate radial path nested within individual were set as random factors.

2.4.5. Species-specific differences in shrub response to water availability

To determine species-specific differences of shrub growth in response to changes in water availability we ran Autoregressive LMMs and GLMMs by species with seasonal PDSI (Palmer Drought Severity Index) and precipitation set as explanatory variables to represent water availability. Seasonal values were calculated as the average of December, January, and February values for winter; March, April, and May values for spring; June, July, August values for summer; and September, October and November values for autumn. We used values starting at the spring of the year previous to the analyzed ring and spanning up to the fall of the analyzed ring year, thus including values for a complete hydrological year and the previous growing season to check for legacy effects of droughts. Individual and replicate radial path nested within individual were set as random factors.

3. Results

3.1. Effect of site, age and size in shrub growth and hydraulic traits

Shrubs of *J. communis* were smallest at the Upper site (6.05 m^3 , $p < 0.001$; Supplementary Tables 1 & 2), while the largest individuals were located at the Lower site (12.45 m^3 , $p < 0.001$; Supplementary Tables 1 & 2). In contrast, *C. oromediterraneus* shrub size was largest at the Upper site (3.27 m^3 , $p < 0.001$; Supplementary Tables 1 & 2), and smallest at the Lower site (1.94 m^3 , $p < 0.001$; Supplementary Tables 1 & 2).

Significant differences in shrub age were found for *J. communis* with the youngest individuals found at the Upper site (18 years, $p < 0.001$; Supplementary Table 1), and the oldest individuals at the Mid site (72 years, $p < 0.001$; Supplementary Table 1). Significant differences were also found for *C. oromediterraneus* shrub age between the Upper site with the oldest individuals (23 years, $p < 0.001$; Supplementary Table 1), and the other two sites (12 years, $p < 0.001$; Supplementary Table 1).

J. communis SRW was significantly smaller at the Mid site than at the other two elevations ($p < 0.01$; Fig. 2, Supplementary Table 1), while all the hydraulic parameters measured were significantly larger at the Upper site ($p < 0.01$; Fig. 2, Supplementary Table 1). For *C. oromediterraneus*, significant differences in SRW were found between the Mid and the Upper sites, with the smaller SRW at the highest altitude ($p < 0.001$; Fig. 2, Supplementary Table 1). No significant differences between sites were found for *C. oromediterraneus* hydraulic parameters.

LMMs including site, shrub size and age showed that the growth and hydraulic traits of *J. communis* were significantly mediated by shrub age ($p < 0.001$, Table 1), although site also had a significant effect on the number of conductive cells ($p < 0.05$, Table 1). Similarly, age also had a significant effect on *C. oromediterraneus* SRW and number of conductive cells ($p < 0.01$, Table 1). The interaction between shrub size and site also had a significant effect on *C. oromediterraneus* SRW and number of conductive cells ($p < 0.05$, Table 1). *C. oromediterraneus* K_h and conductive area were not significantly influenced by site, shrub age or shrub size.

3.2. Warming in Serra da Estrela and influence of monthly temperature on shrub growth

Year was significantly positively correlated with mean minimum temperature for all months except January and February, and with mean maximum temperatures for all months. The highest significant correlation value was found with August mean minimum temperature ($R = 0.53$; $p < 0.001$) and the lowest with February mean maximum temperature ($R = 0.19$; $p < 0.01$; Supplementary Fig. 2).

Correlation analysis, “dendro-flavoured” models, and LMMs showed significant correlations (Supplementary Figs. 3 & 4) and significantly

influencing monthly minimum and maximum temperatures (Supplementary Table 2) from both the current and previous years of the analyzed ring growth on SRW and hydraulic traits. SRW for both species was significantly affected by temperatures from winter and spring months of the previous year as well as fall temperatures during the previous and current analyzed year (Supplementary Table 2). For *J. communis*, current year July maximum temperature also had a negative significant effect on SRW at the Lower sites. Still, for *J. communis*, July maximum temperature had a negative significant effect in all hydraulic parameters at the Upper site, while prior year May maximum temperature of the current year and June minimum temperature of the previous year had a significant positive effect on the conductive area and the number of conductive cells at the Mid and Lower sites (Supplementary Table 2). Although *C. oromediterraneus* SRW appears to be mediated by fall temperatures, there was a higher variability between sites of influencing monthly parameters affecting hydraulic traits. An overall significant positive effect of the maximum temperature of prior December on the hydraulic traits was found at the Lower site (Supplementary Table 2). *C. oromediterraneus* conductive area at the Mid site was influence by March minimum and maximum temperatures of the prior year with a positive and negative influence, respectively, while K_h at the Mid site was similarly influenced by March minimum and maximum temperatures of the current year. June temperatures had an overall negative influence on hydraulic traits at the Upper site (Supplementary Table 2).

3.3. Influence of temperature at start and end of growing season on shrub growth

Maximum spring temperature had a significant positive effect on

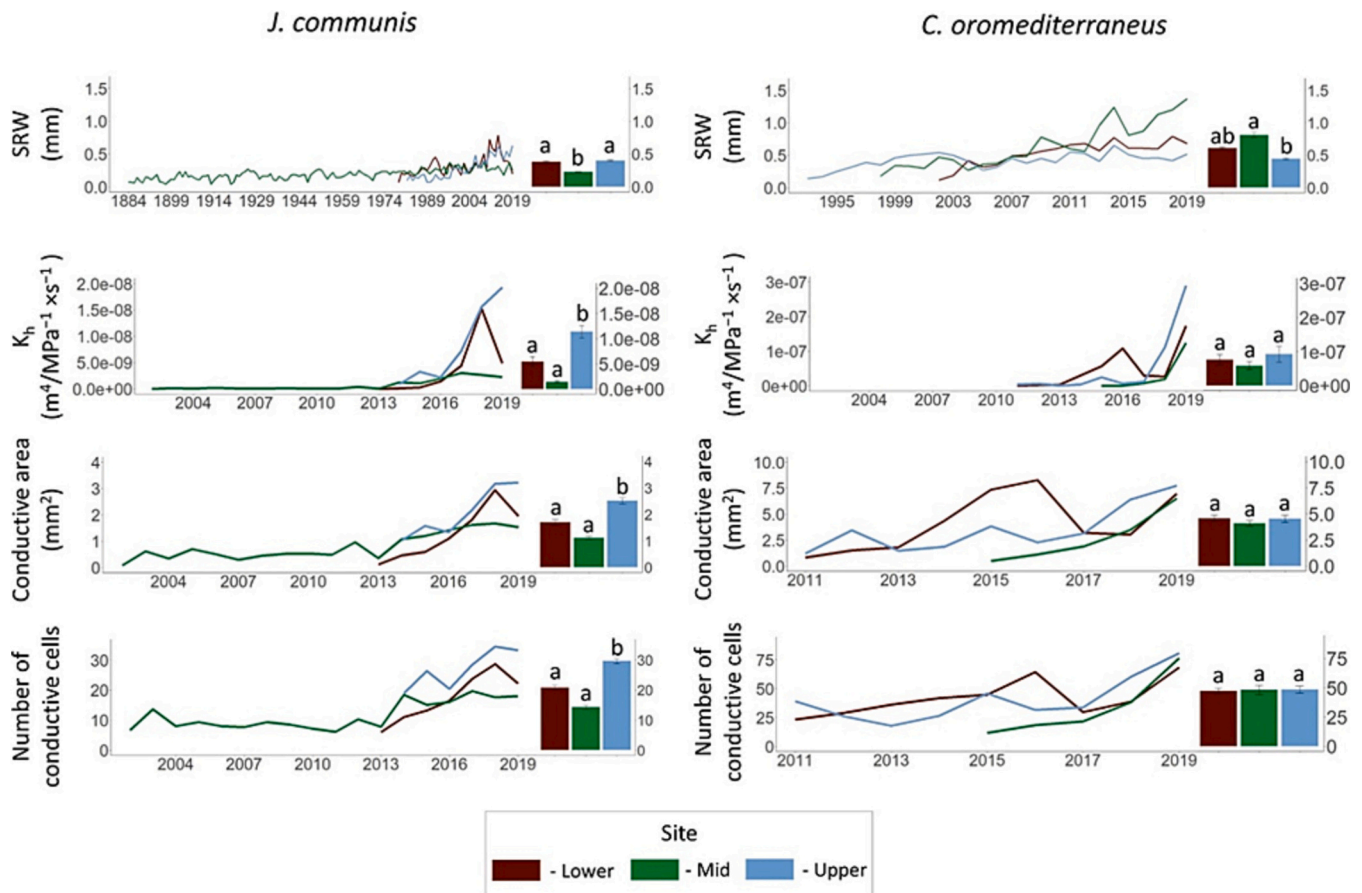


Fig. 2. Shrub parameters means and standard error by year and site for *J. communis* and *C. oromediterraneus*. SRW = Shrub Ring Width, K_h = hydraulic conductivity. Sites that are not significantly different are denoted with the same letter.

Table 1

Summary of the linear mixed-effects models of the influence of site, shrub size, and shrub age on variation of shrub growth parameters. Significant factors shown in bold. (+) indicates a positive effect and (–) indicates a negative effect on response variable. SRW = Shrub Ring Width, Kh = hydraulic conductivity, df = degrees of freedom, X2 = Chi squared, P = p-value, σ = standard deviation, R2m = marginal R2, R2c = conditional R.

<i>J. communis</i>	Fixed factors	df	SRW (mm)		Kh (m ⁴ /MPa ⁻¹ × s ⁻¹)		Conductive area (mm ²)		Number of conductive cells	
			χ ²	P	χ ²	P	χ ²	P	χ ²	P
	Shrub age	1	23.50	< 0.001 (–)	34.08	< 0.001 (–)	18.44	< 0.001 (–)	90.11	< 0.001 (–)
	Site	2	2.21	0.332	4.94	0.085	4.63	0.099	7.84	< 0.05
	Shrub size	1	1.87	0.171	0.19	0.661	0.23	0.629	0.29	0.589
	Site x Shrub size	2	0.88	0.645	1.19	0.552	1.99	0.371	1.17	0.558
	Random factors									
	ID (ring(radial path))									
	σ radial path / ring / individual		0.26 / 0.49 / 0.25		0.83 / 1.95 / 0.00		0.10 / 0.24 / 0.05		0.09 / 0.36 / 1.81e-05	
	No. of radial paths / rings / individuals		2193 / 731 / 24		390 / 130 / 24		390 / 130 / 24		390 / 130 / 24	
	R ² m / R ² c		0.87 / NA		0.99 / NA					
<i>C. oromediterraneus</i>	Fixed factors	df	χ ²	P	χ ²	P	χ ²	P	χ ²	P
	Shrub age	1	7.31	< 0.01 (–)	2.91	0.088	0.63	0.426	9.46	< 0.01 (–)
	Site	2	9.69	< 0.01	1.39	0.498	0.29	0.866	3.52	0.172
	Shrub size	1	1.17	0.280	0.77	0.382	0.16	0.688	3.08	0.079
	Site x Shrub size	2	6.25	< 0.05	2.49	0.288	1.71	0.426	8.73	< 0.05
	Random factors									
	ID (ring(radial path))									
	σ radial path / ring / individual		0.23 / 0.48 / 0.23		1.43 / 2.27 / 0.43		0.20 / 0.44 / 0.07		0.30 / 0.48 / 6.13e-05	
	No. of radial paths / rings / individuals		1117 / 377 / 24		243 / 81 / 24		243 / 81 / 24		243 / 81 / 24	
	R ² m / R ² c		0.90 / NA		0.53 / NA					

J. communis SRW ($p < 0.001$; Table 2), which was also significantly influenced by shrub age ($p < 0.001$). Neither spring nor fall minimum and maximum temperatures significantly influenced *J. communis* hydraulic traits. Fall maximum temperature had a significant effect on all *C. oromediterraneus* variables (Table 2), with a positive effect on SRW and a negative effect on hydraulic traits. SRW in *C. oromediterraneus* was also significantly influenced by shrub age ($p < 0.05$), site ($p < 0.01$), and the interaction of site and shrub size ($p < 0.05$). The number of conductive cells in *C. oromediterraneus* was also significantly influenced

by shrub age ($p < 0.05$) and the interaction between site and shrub size ($p < 0.05$; Table 2).

3.4. Response to water availability

Seasonal precipitation and PDSI were included in the models to check for significant effects on shrub growth and hydraulic traits. Both spring PDSI ($p < 0.001$) and fall precipitation ($p < 0.05$) had a significant positive effect on *J. communis* SRW while winter precipitation from

Table 2

Spring and fall temperature variables influencing shrub growth. Significant factors shown in bold. (+) indicates a positive effect and (–) indicates a negative effect on response variable. SRW = Shrub Ring Width, Kh = hydraulic conductivity, df = degrees of freedom, X² = Chi squared, P = p-value, tmx = mean maximum temperature, σ = standard deviation, R²m = marginal R², R²c = conditional R².

<i>J. communis</i>	Fixed factors	df	SRW (mm)		Kh (m ⁴ /MPa ⁻¹ × s ⁻¹)		Conductive area (mm ²)		Number of conductive cells	
			χ ²	P	χ ²	P	χ ²	P	χ ²	P
	Shrub age	1	20.84	< 0.001 (–)	34.08	< 0.005 (–)	18.44	< 0.001 (–)	90.11	< 0.001 (–)
	Site	2	2.26	0.323	4.94	0.085	4.63	0.100	7.84	< 0.05
	Shrub size	1	1.78	0.183	0.19	0.661	0.23	0.629	0.29	0.589
	Site x Shrub size	2	0.83	0.661	1.19	0.552	1.99	0.371	1.17	0.558
	Spring tmx	1	33.25	< 0.001 (+)						
	Random factors									
	ID (ring(radial path))									
	σ radial path / ring / individual		0.26 / 0.48 / 0.26		0.83 / 1.95 / 0.00		0.10 / 0.24 / 0.05		0.09 / 0.36 / 1.81 e-05	
	No. of radial paths / rings / individuals		2193 / 731 / 24		390 / 130 / 24		390 / 130 / 24		390 / 130 / 24	
	R ² m / R ² c		0.88 / NA		0.99 / NA					
<i>C. oromediterraneus</i>	Fixed factors	df	χ ²	P	χ ²	P	χ ²	P	χ ²	P
	Shrub age	1	4.91	< 0.05 (–)	0.77	0.381	0.01	0.914	5.34	< 0.05 (–)
	Site	2	10.61	< 0.01	7.96	< 0.05	4.74	0.094	5.51	0.063
	Shrub size	1	1.15	0.283	0.49	0.484	0.03	0.871	3.14	0.076
	Site x Shrub size	2	6.39	< 0.05	1.98	0.372	1.15	0.562	8.84	< 0.05
	Fall tmx	1	15.73	< 0.001 (+)	20.36	< 0.001 (–)	20.08	< 0.001 (–)	18.81	< 0.001 (–)
	Random factors									
	ID (ring(radial path))									
	σ radial path / ring / individual		0.23 / 0.47 / 0.23		1.44 / 1.97 / 0.43		0.20 / 0.39 / 0.09		0.29 / 0.42 / 3.50e-05	
	No. of radial paths / rings / individuals		1117 / 377 / 24		243 / 81 / 24		243 / 81 / 24		243 / 81 / 24	
	R ² m / R ² c		0.91 / NA		0.81 / NA					

the previous year had a significant negative effect on *J. communis* SRW ($p > 0.05$; Table 3). Winter PDSI from the previous year had a significant negative effect on the three hydraulic traits: K_h ($p < 0.001$), conductive area ($p < 0.001$) and number of conductive cells ($p < 0.01$, Table 3). *C. oromediterraneus* SRW was significantly positively affected by spring precipitation both from the previous year ($p > 0.001$) and current ring year ($p > 0.001$). Fall PDSI from the previous year had a positive effect on *C. oromediterraneus* hydraulic conductivity ($p > 0.001$) *C. oromediterraneus* number of conductive cells was positively affected by fall precipitation ($p > 0.001$) and negatively affected by fall PDSI ($p > 0.001$, Table 3). Similar to *J. communis*, winter PDSI from the previous year had a significant negative effect on the three hydraulic traits in *C. oromediterraneus*: K_h ($p < 0.001$), conductive area ($p < 0.001$) and number of conductive cells ($p < 0.01$, Table 3).

4. Discussion

Our results indicate an increase in temperatures over most of the year within the alpine zone of Serra da Estrela over the last decades, supporting findings of warming in the Iberian Peninsula (Espírito Santo et al., 2014; González-Hidalgo et al., 2022). An ontogenetic effect can be seen for *J. communis* since there were age-related differences in shrub growth for all variables, reflecting changes in xylem structure associated with increasing age (Rodríguez-Zaccaro et al., 2019). For *C. oromediterraneus*, SRW and number of conductive cells were influenced also by shrub age as well as size, although this was site-specific. This highlights the influence of local conditions of the alpine zone of Estrela on *C. oromediterraneus* growth. Previous studies have shown that the relative allocation to conductive tissue for other alpine species may

be dependent on local microclimatic conditions (Körner and Hiltbrunner, 2021; Myers-Smith et al., 2015; Scherrer and Körner, 2011; Verrall and Pickering, 2020).

Both species were also sensitive to changes in monthly minimum and maximum temperatures, although this was species and site-specific, reflecting the growth plasticity of high elevation shrubs as well as highlighting the importance of local microclimates on alpine shrub morphology (Myers-Smith et al., 2015). A significant site- and species-specific effect of previous year monthly temperatures was also found, revealing legacy effects of climate on the growth of woody species. Minimum temperature in the previous autumn and winter might reveal differences on the timing of a shrub individual’s quiescent stage before the year of ring formation, or freezing events causing frost damage and delaying the start of growth (Körner and Hiltbrunner, 2021).

A clearer effect on both shrub species growth and wood anatomy was found for seasonal temperature at the putative start and end of the growing season. Interestingly, *J. communis* appears to be sensitive to spring maximum temperature while *C. oromediterraneus* is influenced by fall maximum temperature. A higher temperature in early spring can lead to an earlier reactivation of cambial activity, with consequent increase in the length of growing season, and ultimately larger radial growth (Begum et al., 2013; Lenz et al., 2013; Olano et al., 2012; Tumajer et al., 2021). However, *J. communis* growth is also controlled by winter temperatures, that directly influence water availability and evapotranspiration, as an increase in July minimum temperature had a negative effect on *J. communis* SRW in the Lower site. This negative effect of increased July minimum temperature might reflect a synergistic effect of increasing temperature and summer drought that leads to the suppression of growth during summer (Tumajer et al., 2021).

Table 3

Seasonal water availability variables influencing shrub growth. Significant factors shown in bold. (+) indicates a positive effect and (–) indicates a negative effect on response variable. SRW = Shrub Ring Width, K_h = hydraulic conductivity, PDSI = Palmer Drought Severity Index, pr = total precipitation, df = degrees of freedom, X^2 = Chi squared, P = p-value, R^2m = marginal R^2 , R^2c = conditional R^2 .

<i>J. communis</i>	Fixed factors	df	SRW (mm)		K_h ($m^4/MPa^{-1} \times s^{-1}$)		Conductive area (mm^2)		Number of conductive cells		
			χ^2	P	χ^2	P	χ^2	P	χ^2	P	
	Shrub age	1	0.83	0.361	16.84	< 0.001 (–)	7.91	< 0.01 (–)	75.61	< 0.001 (–)	
	Site	2	2.40	0.301	3.75	0.153	4.11	0.128	7.10	< 0.05	
	Shrub size	1	0.96	0.326	0.19	0.665	0.16	0.693	0.27	0.606	
	Site x Shrub size	2	3.93	0.141	1.52	0.468	1.82	0.402	1.30	0.523	
	Winter PDSI 1 yr prior	1			17.41	< 0.001 (–)	20.10	< 0.001 (–)	8.98	< 0.01 (–)	
	Winter pr 1 yr prior	1	4.05	< 0.05 (–)					4.93	< 0.05 (+)	
	Spring PDSI	1	20.03	< 0.001 (+)	7.82	< 0.01 (–)					
	Fall pr	1	6.28	< 0.05 (+)							
	<i>Random factors</i>										
	ID (ring(radial path))										
	σ radial path / ring / individual		0.25 / 0.57 / 0.41		0.75 / 1.79 / 0.26		0.10 / 0.22 / 0.07		0.09 / 0.34 / 1.76e-05		
	No. of radial paths / rings / individuals		2193 / 731 / 24		390 / 130 / 24		390 / 130 / 24		390 / 130 / 24		
	R^2m / R^2c		0.52 / NA		0.95 / NA						
<hr/>											
<i>C. oromediterraneus</i>	Fixed factors	df	χ^2	P	χ^2	P	χ^2	P	χ^2	P	
	Shrub age	1	5.97	< 0.05 (–)	0.28	0.598	1.93	0.165	1.31	0.253	
	Site	2	8.18	< 0.01	2.04	0.361	0.32	0.853	4.07	0.131	
	Shrub size	1	1.12	0.290	0.50	0.480	0.10	0.749	3.62	0.570	
	Site x Shrub size	2	6.54	< 0.05	2.65	0.266	2.35	0.308	10.24	< 0.01	
	Spring PDSI 1 yr prior	1					8.32	< 0.01 (+)			
	Spring pr 1 yr prior	1	30.46	< 0.001 (+)							
	Fall PDSI 1 yr prior	1			17.50	< 0.001 (+)					
	Winter PDSI 1 yr prior	1			34.95	< 0.001 (–)	24.10	< 0.001 (–)	10.09	< 0.01 (–)	
	Spring pr	1	8.39	< 0.001 (+)							
	Fall PDSI	1							15.84	< 0.001 (–)	
	Fall pr	1							20.66	< 0.001 (+)	
	<i>Random factors</i>										
	ID (ring(radial path))										
	σ radial path / ring / individual		0.23 / 0.46 / 0.23		1.43 / 1.78 / 0.58		0.20 / 0.38 / 0.09		0.30 / 0.39 / 5.69e-05		
	No. of radial paths / rings / individuals		1117 / 377 / 24		243 / 81 / 24		243 / 81 / 24		243 / 81 / 24		
	R^2m / R^2c		0.91 / NA		0.84 / NA						

Interestingly, the effect of spring temperature on *J. communis* wood anatomical and hydraulic traits was site-dependent and correlated with individual monthly values and not with seasonal means. There was a positive effect of March and May maximum temperatures on *J. communis* hydraulic traits in the Mid site. Since this site had the shallowest soil of the three studied locations, this effect might be explained by an increase in water availability at the beginning of the growing season, due to earlier snow melt as a result of higher temperature, which inherently influences cambial activity and hydraulic conductance (Inouye, 2020; Scherrer and Körner, 2011). This fine-scale response contrasts with previous studies showing a small variability in hydraulic traits for temperate populations of *J. communis* (Unterholzner et al., 2020), and supports that hydraulic plasticity might be larger in rear edge populations, particularly at the southern dryer margins, like those studied in Estrela (Unterholzner et al., 2020; Wortemann et al., 2011).

On the other hand, *C. oromediterraneus* growth was positively influenced not by spring temperature but by fall maximum temperature, highlighting the different physiology of these two shrubs. Differences between gymnosperms and angiosperms in growth and phenology and in their responses to climate change have been reported for trees (Carnicer et al., 2013; Marcati et al., 2016). Although temperature is the main driver of the start of cambial activity in conifers, day length appears to exert an important control in triggering cambial activity in angiosperms (Savage and Chuine, 2021), which could explain the result obtained for *C. oromediterraneus*. In spite of the positive correlation between SRW and fall maximum temperature, *C. oromediterraneus* K_h , conductive area, and number of conductive cells were negatively influenced by fall maximum temperature. This opposing response between radial growth and hydraulic conductance variables in *C. oromediterraneus* may be reflecting the transition to latewood formation in fall, which decreases the development of conductive tissue, but not necessarily non-vascular tissue, which contributes to overall growth.

Water availability parameters greatly influenced shrub growth, with species-specific differences in seasonal sensitivity. Precipitation in the winter before the growing season had a negative effect on *J. communis* SRW but a positive effect on number of conductive cells. *Juniperus thurifera* L. from cold sites in Spain also showed a negative correlation between tree-ring width and winter precipitation (DeSoto et al., 2014). In winter, higher moisture conditions associated with low temperatures in the mountains will increase the deposition of snow. A thicker snowpack can take more time to melt in the following spring, implying a delayed start of the growing season reflected in a smaller SRW. PDSI values in the previous winter had a significant negative influence on all hydraulic traits in both species, that is, the more intense the drought conditions during the winter before the growing season the higher the values for the hydraulic trait parameters of the analyzed ring year. A drier winter results in the formation of a smaller snowpack, and, thus, a faster snowmelt in the following spring, implying an earlier water availability and reflected in a positive effect on the hydraulic traits of both shrubs. Fall PDSI in the year before the growing season had a positive effect on *C. oromediterraneus* K_h , which could suggest that a higher water availability in the prior fall will increase the water reserves available for the next growing season. The larger vessels formed in the beginning of the growing season are responsible for most of the hydraulic conductivity, and the diameter of the vessels is largely dependent on the turgor pressure during the maturation process of the vessels (Rodríguez-Zaccaro and Groover, 2019). *Cytisus oromediterraneus* radial growth appears to be highly linked with spring precipitation as both spring conditions the year prior and in the current ring year had a positive effect on SRW. Finally, *J. communis* SRW and *C. oromediterraneus* number of conductive cells were positively influenced by fall precipitation, which may be explained by a release from summer drought stress, and confirms the bimodal growth of alpine shrubs in this mountain range (Dobbert et al., 2022).

5. Conclusion

To sum up, this work shows an increase in shrub growth in the last years parallel to the increase in temperature in Serra da Estrela and identifies the main climatic factors affecting shrub growth. Maximum temperature at the start and end of the growing season are the main drivers of the growth of *C. oromediterraneus* and *J. communis* in Serra da Estrela. However, species-specific differences in the response to these changes imply that their growth and fitness will be affected differently by climate change and mediated by winter drought intensity. Also, the response to water availability and extreme drought conditions are species-specific and might contribute to local differences in the growth, fitness, and survival of both species. Ultimately, understanding their response to the current changes in climatic conditions in Serra da Estrela is essential to predict their future distribution since this location corresponds to their westernmost end in the Iberian Peninsula.

CRedit authorship contribution statement

D. Rudley: Methodology, Investigation, Formal analysis, Writing – original draft, Visualization. **L. DeSoto:** Methodology, Formal analysis, Writing – review & editing. **S. Rodríguez-Echeverría:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration. **C. Nabais:** Conceptualization, Methodology, Writing – review & editing, Supervision.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.165911>.

References

- Abatzoglou, J.T., Dobrowski, S.Z., Parks, S.A., Hegewisch, K.C., 2018. TerraClimate, a high-resolution global dataset of monthly climate and climatic water balance from 1958-2015. *Sci. Data* 5, 1–12. <https://doi.org/10.1038/sdata.2017.191>.
- Abámoff, M.D., Magalhães, P.J., Ram, S.J., 2004. Image processing with ImageJ. *Biophoton. Int.* 11 (7), 36–42.
- Abrantes, J., Campelo, F., García-González, I., Nabais, C., 2013. Environmental Control of Vessel Traits in *Quercus Ilex* under Mediterranean Climate: Relating Xylem Anatomy to Function. *Trees* 27 (3), 655–662. <https://doi.org/10.1007/s00468-012-0820-6>.
- Alexander, J.M., Chalmandrier, L., Lenoir, J., Burgess, T.I., Essl, F., Haider, S., Kueffer, C., McDougall, K., Milbau, A., Nuñez, M.A., Pauchard, A., Rabitsch, W., Rew, L.J., Sanders, N.J., Pellissier, L., 2018. Lags in the response of mountain plant

- communities to climate change. In: *Global Change Biology*, 24(2). Blackwell Publishing Ltd., pp. 563–579. <https://doi.org/10.1111/gcb.13976>
- Allen, C.D., Breshears, D.D., McDowell, N.G., 2015. On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. *Ecosphere* 6 (8), 1–55. <https://doi.org/10.1890/ES15-00203.1>
- Anadón-Rosell, A., Rixen, C., Cherubini, P., Wipf, S., Hagedorn, F., Dawes, M.A., 2014. Growth and phenology of three dwarf shrub species in a six-year soil warming experiment at the alpine treeline. *PLoS One* 9 (6). <https://doi.org/10.1371/journal.pone.0100577>
- Archer, S., Schimel, D.S., Holland, E.A., 1995. Mechanisms of shrubland expansion: land use, climate or CO₂? *Clim. Chang.* 29 (1), 91–99. <https://doi.org/10.1007/BF01091640>
- von Arx, G., Crivellaro, A., Prendin, A.L., Čufar, K., Carrer, M., 2016. Quantitative wood anatomy—practical guidelines. *Front. Plant Sci.* 7 (JUNE2016), 1–13. <https://doi.org/10.3389/fpls.2016.00781>
- Bär, A., Bräuning, A., Löffler, J., 2006. Dendroecology of dwarf shrubs in the high mountains of Norway – a methodological approach. *Dendrochronologia* 24 (1), 17–27. <https://doi.org/10.1016/j.dendro.2006.05.001>
- Begum, S., Nakaba, S., Yamagishi, Y., Oribe, Y., Funada, R., 2013. Regulation of cambial activity in relation to environmental conditions: understanding the role of temperature in wood formation of trees. *Physiol. Plant.* 147 (1), 46–54. <https://doi.org/10.1111/j.1399-3054.2012.01663.x>
- Bergmeier, E., Zimowski, M., Leuschner, H.H., Holger, G., 2014. Age and diversity of Mediterranean dwarf shrublands: a dendrochronological approach along an altitudinal gradient on Crete. *J. Veg. Sci.* 25, 122–134. <https://doi.org/10.1111/jvs.12067>
- Bunn, A., Korpela, M., Biondi, F., Campelo, F., Mérian, P., Qeadan, F., Zang, C., 2021. dplR: Dendrochronology Program Library in R. R package version 1.7.2. <https://CRAN.R-project.org/package=dplR>
- Camarero, J.J., Olano, J.M., Parras, A., 2010. Plastic Bimodal Xylogenesis in Conifers from Continental Mediterranean Climates. *New Phytol.* 185, 471–480. <https://doi.org/10.1111/j.1469-8137.2009.03073.x>
- Carapeto, A., Porto, M., Araújo, P.V., Lourenço, J., Almeida, J.D., Clamote, F., Portela-Pereira, E., Pereira, A.J., Silva, A., Caperta, A., et al., 2014. Flora-on: Interactive Flora of Portugal. Sociedade Portuguesa de Botânica. Online. <http://www.flora-on.pt/>
- Carnicer, J., Barbeta, A., Sperlich, D., Coll, M., Penuelas, J., 2013. Contrasting trait syndromes in angiosperms and conifers are associated with different responses of tree growth to temperature on a large scale. *Front. Plant Sci.* 4 (10) <https://doi.org/10.3389/fpls.2013.00409>
- Choat, B., Jansen, S., Brodrribb, T.J., et al. Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. *Nature* 491 (7426), 752–755. <https://doi.org/10.1038/nature11688>
- Cook, E.R., Kairiukstis, L.A., 1990. Methods of dendrochronology. In: *Applications in the Environmental Sciences*. International Institute for Applied Systems Analysis. Kluwer Academic Publishers, Dordrecht. https://doi.org/10.1007/978-94-015-7879-0_394
- Costa, J.C., Aguiar, C., Capelo, J., Lousa, M., Neto, C., 1998. *Biogeografia de Portugal Continental*. Querceteca 0, 5–56.
- DeSoto, L., de la Cruz, M., Ponti, P., 2011. Intra-annual patterns of tracheid size in the Mediterranean tree *Juniperus thurifera* as an indicator of seasonal water stress. *Can. J. For. Res.* 41 (6), 1280–1294.
- DeSoto, L., Cailleret, M., Kramer, K., Sterck, F., Jansen, S., Robert, E., Martínez-Vilalta, J., et al., 2020. Low growth resilience to drought is related to future mortality risk in trees. *Nat. Commun.* 11, 545.
- DeSoto, L., Varino, F., Andrade, J.P., Gouveia, C.M., Campelo, F., Trigo, R.M., Nabais, C., 2014. Different growth sensitivity to climate of the conifer *Juniperus thurifera* on both sides of the Mediterranean Sea. *Int. J. Biometeorol.* 58, 2095–2109. <https://doi.org/10.1007/s00484-014-0811-y>
- Dobbert, S., Albrecht, E.C., Pape, R., et al., 2022. Alpine shrub growth follows bimodal seasonal patterns across biomes – unexpected environmental controls. *Commun. Biol.* 5, 793. <https://doi.org/10.1038/s42003-022-03741-x>
- Espírito Santo, F., de Lima, M.I.P., Ramos, A.M., Trigo, R.M., 2014. Trends in seasonal surface air temperature in mainland Portugal, since 1941. *Int. J. Climatol.* 34 (6), 1814–1837. <https://doi.org/10.1002/joc.3803>
- European Commission, Directorate-General for Environment, Tsiripidis, I., Piernik, A., Janssen, J., et al., 2016. European red list of habitats. <https://doi.org/10.2779/091372>. Part 2, Terrestrial and freshwater habitats, Publications Office of the European Union. ISBN:978-92-79-61588-7.
- Fazlioglu, F., Wan, J.S.H., 2021. Warming matters: alpine plant responses to experimental warming. *Clim. Chang.* 164 (56), 1–17.
- Fernández-Santos, B., Martínez, C., García, J.A., Puerto, A., 2004. Postfire regeneration in *Cytisus oromediterraneus*: sources of variation and morphology of the below-ground parts. *Acta Oecol.* 26 (2), 149–156. <https://doi.org/10.1016/j.actao.2004.03.011>
- Fox, J., Weisberg, S., 2019. An {R} Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. URL: <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Francon, L., Corona, C., Till-Bottraud, I., Carlson, B.A., Stoffel, M., 2020. Some (do not) like it hot: shrub growth is hampered by heat and drought at the alpine treeline in recent decades. *Am. J. Bot.* 107 (4), 607–617. <https://doi.org/10.1002/ajb2.1459>
- García-Cervigón Morales, A.I., Olano Mendoza, J.M., Eugenio Gozalbo, M., Camarero Martínez, J.J., 2012. Arboreal and prostrate conifers coexisting in Mediterranean high mountains differ in their climatic responses. *Dendrochronologia* 30 (4), 279–286. <https://doi.org/10.1016/j.dendro.2012.02.004>
- García-Fórner, Vieira, J., Nabais, C., Carvalho, A., Martínez-Vilalta, J., Campelo, F., 2019. Climatic and physiological regulation of the bimodal xylem formation pattern in *Pinus pinaster* saplings. *Tree Physiol.* 39, 2008–2018. <https://doi.org/10.1093/treephys/tpz099>
- Gärtner, H., Cherubini, P., Fonti, P., von Arx, G., Schneider, L., Nievergelt, D., Verstege, A., Bast, A., Schweingruber, F.H., Büntgen, U., 2015. A technical perspective in modern tree-ring research - how to overcome dendroecological and wood anatomical challenges. *J. Vis. Exp.* 2015 (97) <https://doi.org/10.3791/52337>
- Gazol, A., Camarero, J.J., Anderegg, W.R.L., Vicente-Serrano, S.M., 2017. Impacts of droughts on the growth resilience of northern hemisphere forests. *Glob. Ecol. Biogeogr.* 26 (2), 166–176. <https://doi.org/10.1111/geb.12526>
- Gazol, A., Camarero, J.J., Vicente-Serrano, S.M., Sánchez-Salguero, R., Gutiérrez, E., de Luis, M., Sangüesa-Barreda, G., Novak, K., Rozas, Galván, J.D., 2018. Forest resilience to drought varies across biomes. *Glob. Chang. Biol.* 24 (5), 2143–2158. <https://doi.org/10.1111/gcb.14082>
- González-Hidalgo, J.C., Beguería, S., Peña-Angulo, D., Sandonis, L., 2022. Variability of maximum and minimum monthly mean air temperatures over mainland Spain and their relationship with low-variability atmospheric patterns for period 1916–2015. *Int. J. Climatol.* 42 (3), 1723–1741. <https://doi.org/10.1002/joc.7331>
- Grissino-Mayer, H.D., 2001. Assessing crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree Ring Res.* 57, 205–221.
- Herrero, A., Zamora, R., 2014. Plant responses to extreme climatic events: a field test of resilience capacity at the southern range edge. *PLoS One* 9 (1), e87842. <https://doi.org/10.1371/journal.pone.0087842>
- Inouye, D.W., 2020. Effects of climate change on alpine plants and their pollinators. In: *Annals of the New York Academy of Sciences*, vol. 1469(1). Blackwell Publishing Inc., pp. 26–37. <https://doi.org/10.1111/nyas.14104>
- International Association of Wood Anatomists (IAWA), 1989. *IAWA List of microscopic features for hardwood identification*. IAWA Bulletin n.s. 10 (3), 219–332 [4th printing 2007].
- Jansen, J., 2011. *Managing Natura 2000 in a changing world. The example of Serra da Estrela (Portugal)*. PhD thesis. Radboud University Nijmegen.
- Körner, C., Hiltbrunner, E., 2021. Why is the alpine flora comparatively robust against climatic warming? *Diversity* 13 (8), 383. <https://doi.org/10.3390/D13080383>
- Kudo, G., Suzuki, S., 2003. Warming effects on growth, production, and vegetation structure of alpine shrubs: a five-year experiment in northern Japan. *Oecologia* 135 (2), 280–287. <https://doi.org/10.1007/s00442-003-1179-6>
- Lenz, A., Hoch, G., Körner, C., 2013. Early season temperature controls cambial activity and total tree ring width at the alpine treeline. *Plant Ecol. Divers.* 6 (3–4), 365–375. <https://doi.org/10.1080/17550874.2012.711864>
- Lu, X., Liang, E., Wang, Y., Babst, F., Leavitt, S.W., Julio Camarero, J., 2019. Past the climate optimum: recruitment is declining at the world's highest juniper shrublands on the Tibetan Plateau. *Ecology* 100 (2), 1–9. <https://doi.org/10.1002/ecy.2557>
- Marcati, C.R., Machado, S.R., Podadera, D.S., de Lara, N.O.T., Bosio, F., Wiedenhoef, A. C., 2016. Cambial activity in dry and rainy season on branches from woody species growing in Brazilian Cerrado. In: *Flora: Morphology, Distribution, Functional Ecology of Plants*, 223, pp. 1–10. <https://doi.org/10.1016/j.flora.2016.04.008>
- Meireles, C., Mendes, P., Vila-Viçosa, C., Cano-Carmona, E., Pinto-Gomes, C., 2013. Geobotanical aspects of *Cytisus oromediterraneus* and *Genista cinerascens* in Serra da Estrela (Portugal). *Plant Sociol.* 50 (1), 23–31. <https://doi.org/10.7338/pls2013501/03>
- Mora, C., 2010. A synthetic map of the climates of the Serra da Estrela (Portugal). *J. Maps* 6 (1), 591–608. <https://doi.org/10.4113/jom.2010.1112>
- Moullec, M.L., Buchwal, A., Van Der Wal, R., Sandal, L., Hansen, B.B., 2018. Annual ring growth of a widespread high arctic shrub reflects past fluctuations in community-level plant biomass. *J. Ecol.* 107 (1), 436–451. <https://doi.org/10.1111/1365-2745.13036>
- Myers-Smith, I.H., Hallinger, M., Blok, D., Sass-Klaassen, U., Rayback, S.A., Weijers, S., Trant, A.J., Tape, K.D., Naito, A.T., Wilking, M., 2015. Methods for measuring arctic and alpine shrub growth: a review. *Earth Sci. Rev.* 140, 1–13. <https://doi.org/10.1016/j.earscirev.2014.10.004>
- Olano, J.M., Eugenio, M., García-Cervigón, A.I., Folch, M., Rozas, V., 2012. Quantitative tracheid anatomy reveals a complex environmental control of wood structure in continental mediterranean climate. *Int. J. Plant Sci.* 173 (2), 137–149. <https://doi.org/10.1086/663165>
- Olano, J.M., Almería, I., Eugenio, M., von Arx, G., 2013. Under pressure: how a Mediterranean high-mountain forb coordinates growth and hydraulic xylem anatomy in response to temperature and water constraints. *Funct. Ecol.* 27 (6), 1295–1303. <https://doi.org/10.1111/1365-2435.12144>
- Pandey, J., Sigdel, S.R., Lu, X., Salerno, F., Liang, E., Camarero, J.J., 2020. Early growing-season precipitation drives radial growth of alpine juniper shrubs in the Central Himalayas. *Geografiska annaler: series a, physical geography* 102(3):317, 330. <https://doi.org/10.1080/04353676.2020.1761097>
- Pauli, H., Gottfried, M., Dullinger, S., Grabherr, G., et al., 2012. Recent plant diversity changes on Europe's mountain summits. *Science* 336 (6079), 353–355. <https://doi.org/10.1126/science.1219033>
- Peña-Gallardo, M., Vicente-Serrano, S.M., Camarero, J.J., Gazol, A., Sánchez-Salguero, R., Domínguez-Castro, F., el Kenawy, A., Beguería-Portugés, S., Gutiérrez, E., Galván, J.D., 2018. Drought sensitiveness on forest growth in peninsular Spain and the Balearic Islands. *Forests* 9 (9). <https://doi.org/10.3390/f9090524>
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2021. *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1–152. URL: <https://CRAN.R-project.org/package=nlme>

- Preibisch, S., Saalfeld, S., Tomancak, P., 2009. Globally optimal stitching of tiled 3D microscopic image acquisitions. *Bioinformatics* 25 (11), 1463–1465. <https://doi.org/10.1093/bioinformatics/btp184>.
- Prislan, P., Gričar, J., Čufar, K., 2014. Wood Sample Preparation for Microscopic Analysis. *March*.
- R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>.
- Rodriguez-Zaccaro, F., Groover, A., 2019. Wood and water: how trees modify wood development to cope with drought. *Plants People Planet* 1, 346–355. <https://doi.org/10.1002/ppp3.29>.
- Rodriguez-Zaccaro, F.D., Valdovinos-Ayala, J., Percolla, M.I., Venturas, M.D., Pratt, R.B., Jacobsen, A.L., 2019. Wood structure and function change with maturity: age of the vascular cambium is associated with xylem changes in current-year growth. *Plant Cell Environ.* 42 (6), 1816–1831. <https://doi.org/10.1111/pce.13528>.
- Rossi, S., Anfodillo, T., Menardi, R., 2006. Trephor: a new tool for sampling microcores from tree stems. *IAWA J.* 27 (1), 89–97. <https://doi.org/10.1163/22941932-90000139>.
- Sanz-Elorza, M., Dana, E.D., González, A., Sobrino, E., 2003. Changes in the high-mountain vegetation of the Central Iberian Peninsula as a probable sign of global warming. *Ann. Bot.* 92 (2), 273–280. <https://doi.org/10.1093/aob/mcg130>.
- Savage, J.A., Chuine, I., 2021. Coordination of spring vascular and organ phenology in deciduous angiosperms growing in seasonally cold climates. *New Phytol.* 230, 1700–1715. <https://doi.org/10.1111/nph.17289>.
- Scherrer, D., Körner, C., 2011. Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming. *J. Biogeogr.* 38 (2), 406–416. <https://doi.org/10.1111/j.1365-2699.2010.02407.x>.
- Scholz, A., Klepsch, M., Karimi, Z., Jansen, S., 2013. How to quantify conduits in wood? *Front. Plant Sci.* 4 (3), 1–11. <https://doi.org/10.3389/fpls.2013.00056>.
- Sperry, J.S., Hacke, U.G., 2004. Analysis of circular bordered pit function I. Angiosperm vessels with homogenous pit membranes. *Am. J. Bot.* 91 (3), 369–385. <https://doi.org/10.3732/ajb.91.3.369>.
- Thomas, P.A., El-Barghathi, M., Polwart, A., 2007. Biological Flora of the British Isles: *Juniperus communis* L. *J. Ecol.* 95 (6), 1404–1440. <https://doi.org/10.1111/j.1365-2745.2007.01308.x>.
- Thuiller, W., Lavorel, S., Aratújo, M.B., Sykes, M.T., Prentice, I.C., 2005. Climate change threats to plant diversity in Europe. *Proc. Natl. Acad. Sci. U. S. A.* 102 (23), 8245–8250. <https://doi.org/10.1073/pnas.0409902102>.
- Trambley, Y., Koutroulis, A., Samaniego, L., Polcher, J., et al., 2020. Challenges for drought assessment in the Mediterranean region under future climate scenarios. *Earth Sci. Rev.* 210 <https://doi.org/10.1016/j.earscirev.2020.103348>.
- Tumajer, J., Buras, A., Camarero, J.J., Carrer, M., Shetti, R., Wilmking, M., Altman, J., Sangüesa-Barreda, G., Lehejček, J., 2021. Growing faster, longer or both? Modelling plastic response of *Juniperus communis* growth phenology to climate change. *Glob. Ecol. Biogeogr.* 30 (11), 2229–2244. <https://doi.org/10.1111/geb.13377>.
- Unterholzner, L., Carrer, M., Bär, A., Beikircher, B., Dämon, B., Losso, A., Prendin, A.L., Mayr, S., 2020. *Juniperus communis* populations exhibit low variability in hydraulic safety and efficiency. *Tree Physiol.* 40 (12), 1668–1679. <https://doi.org/10.1093/treephys/tpaa103>.
- Venables, W.N., Ripley, B.D., 2002. *Modern Applied Statistics with S, Fourth edition*. Springer, New York. ISBN 0-387-95457-0.
- Verrall, B., Pickering, C.M., 2020. Alpine vegetation in the context of climate change: A global review of past research and future directions. In: *Science of the Total Environment*, 748. Elsevier B.V. <https://doi.org/10.1016/j.scitotenv.2020.141344>.
- Vieira, J., Carvalho, A., Campelo, F., 2020. Tree Growth Under Climate Change: Evidence From Xylogenesis Timings and Kinetics Study Site and Experimental Design, 11(2), pp. 1–11. <https://doi.org/10.3389/fpls.2020.00090>.
- Wortemann, R., Herbette, S., Barigah, T.S., Fumanal, B., Alia, R., Ducouso, A., Gomory, D., Roedel-Drevet, P., Cochar, H., 2011. Genotypic variability and phenotypic plasticity of cavitation resistance in *Fagus sylvatica* L. across Europe. *Tree Physiol.* 31 (11), 1175–1182. <https://doi.org/10.1093/treephys/tpr101>.
- Zang, C., Biondi, F., 2015. Treeclim: an R package for the numerical calibration of proxy-climate relationships. *Ecography* 38 (4), 431–436. <https://doi.org/10.1111/ecog.01335>.