Plant invasion modifies isohydricity in Mediterranean tree species

Simon Haberstroh, Raquel Lobo-do-vale, Maria C Caldeira, Maren Dubbert, Matthias Cuntz, Christiane Werner

To cite this version:

Simon Haberstroh, Raquel Lobo-do-vale, Maria C Caldeira, Maren Dubbert, Matthias Cuntz, et al.. Plant invasion modifies isohydricity in Mediterranean tree species. Functional Ecology, 2022, 36 (9), pp.2384-2398. 10.1111/1365-2435.14126 . hal-04033784

HAL Id: hal-04033784
https://hal.inrae.fr/hal-04033784v1

Submitted on 17 Mar 2023

Received: 8 November 2021 | Accepted: 19 June 2022

DOI: 10.1111/1365-2435.14126

Functional Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Plant invasion modifies isohydricity in Mediterranean tree species

Simon Haberstroh[1,2] | **Raquel Lobo-do-Vale**[2] | **Maria C. Caldeira**[2] |
Maren Dubbert[1,3] | **Matthias Cuntz**[4] | **Christiane Werner**[1]

[1]Ecosystem Physiology, Faculty of Environment and Natural Resources, University Freiburg, Freiburg, Germany

[2]Forest Research Centre, School of Agriculture, University of Lisbon, Lisbon, Portugal

[3]Leibniz Centre for Agricultural Landscape Research (ZALF), Isotope Biogeochemistry and Gas Fluxes, Müncheberg, Germany

[4]Université de Lorraine, AgroParisTech, INRAE, Nancy, France

Correspondence
Simon Haberstroh
Email: simon.haberstroh@cep.uni-freiburg.de

Funding information
Agence Nationale de la Recherche, Grant/Award Number: ANR-11- LABX-0002-01; Deutsche Forschungsgemeinschaft, Grant/Award Number: CU 173/4-1, MD 1688/1-1 and WE 2681/10-1; Deutscher Akademischer Austauschdienst, Grant/Award Number: 57396941; Fundação para a Ciência e a Tecnologia, Grant/Award Number: CEECIND/02735/2018, Lisboa-01-0145-FEDER-030406-PTDC/ASP-SIL/3406/20, PTDC/ASP-SIL/3406/2017 and UID/AGR/00239/2019; Studienstiftung des Deutschen Volkes

Handling Editor: Tamir Klein

Abstract

1. Understanding of plant hydraulic strategies (i.e. the degree of iso-/anisohydricity) is crucial to predict the response of plants to changing environmental conditions such as climate-change induced extreme drought. Several abiotic factors, including evaporative demand, have been shown to seasonally modify the isohydricity of plants. However, the impact of biotic factors such as plant–plant interactions on hydraulic strategies has seldom been explored.
2. Here, we investigated adaptations and changes in hydraulic strategies of two woody species in response to seasonal abiotic conditions, experimental drought and plant invasion in a Mediterranean cork oak (*Quercus suber*) ecosystem with a combined shrub invasion (*Cistus ladanifer*) and rain exclusion experiment.
3. From the dry to wet season, *Q. suber* shifted from a partial isohydric to an anisohydric behaviour while *C. ladanifer* shifted from strict anisohydric to partial isohydric. During drought, water competition by plant invasion significantly modified the hydraulic strategy of invaded *Q. suber*, which was accompanied by lower pre-dawn leaf water potentials, sap flow density, leaf area index and trunk increment rates.
4. This altered isohydricity of invaded *Q. suber* trees was most likely caused by interspecific competition for water resources by water spending *C. ladanifer* shrubs. Both species do have the highest proportion of fine roots in the topsoil and thus, an additional water consumer, such as *C. ladanifer* can lead to more stressful conditions for *Q. suber* during times of water scarcity. Further underlying mechanisms of the altered isohydricity of *Q. suber*, such as potential allelopathic effects of *C. ladanifer* exudates on root growth of *Q. suber*, have to be investigated in the future.
5. In conclusion, we demonstrate that the degree of isohydricity of two woody Mediterranean plant species is dynamically determined by the interplay of species-specific hydraulic traits and their abiotic and biotic environment.

KEYWORDS
anisohydric, *Cistus ladanifer*, isohydric, leaf water potential, plant competition, *Quercus suber*, sap flow, shrub invasion

1 | INTRODUCTION

Plant water regulation has long been a central focus of research (Landsberg & Waring, 2016). However, there are still many open questions regarding plasticity in hydraulic strategies in response to environmental stressors, such as extreme drought (Sperry & Love, 2015). Plants have developed a broad range of water regulation strategies to maximise their carbon assimilation at minimum water cost (Anderegg et al., 2018; Cowan, 1982) while simultaneously avoiding hydraulic conductivity loss (Sperry et al., 2002) and runaway cavitation (Cochard et al., 1996; Tyree & Sperry, 1988). Yet, there is a large spectrum of responses to soil drying. Some species show conservative water use strategies with early stomatal closure at the expense of reduced carbon gain during the onset of drought. Other species have less conservative strategies, maintaining high stomatal conductance, implying an increased risk of increased hydraulic damage or failure under drought conditions (Jones & Sutherland, 1991; McDowell et al., 2008). Tardieu and Simonneau (1998) reintroduced the concept of isohydricity, initially defined by Berger-Landefeldt (1936), for these hydraulic strategies with isohydric plants exerting a strong stomatal control and maintaining comparatively constant leaf water potentials (Ψ_L) over the day. Contrarily, anisohydric plants exhibit less stomatal control and, thus, tolerate larger variations in Ψ_L (Tardieu & Simonneau, 1998).

Many studies have followed to assess the species-specific drought adaptation strategies of plants (cf. Feng et al., 2019; Hochberg et al., 2018). The simplified, response-based metric of Martínez-Vilalta et al. (2014) explores the seasonal response of the minimum Ψ_L, measured at midday (Ψ_{MD}), to declining soil water potentials (Ψ_S), reflected by pre-dawn Ψ_L (Ψ_{PD}). This approach has the advantage that the required parameters are easy to measure, yet other influential factors such as evaporative demand or leaf area, are set constant. However, this implies that the degree of isohydricity can change and be modified by abiotic and biotic factors other than soil water resources (Guo et al., 2020; Hochberg et al., 2018; Martínez-Vilalta & Garcia-Forner, 2017; Novick et al., 2019; Wu et al., 2021). Relevant abiotic factors include deep water access (Feng et al., 2019; Matheny et al., 2017), soil hydraulic properties (Javaux & Carminati, 2021), vapour pressure deficit (VPD; Guo & Ogle, 2019; Novick et al., 2019), air temperature (Guo et al., 2020) or light intensity (Guyot et al., 2012; Meinzer et al., 2016). For example, Guo et al. (2020) demonstrated that the desert shrub *Larrea tridentata* shifted from partial isohydric under drought to an extreme anisohydric behaviour under wet conditions. *Quercus douglasii* growing at a dry site behaved more anisohydric than at a wet site during the summer periods (Feng et al., 2019). These examples illustrate that plant responses are dynamic and can result from the interaction of plant physiological strategies with their local, abiotic environment (Feng et al., 2019; Hochberg et al., 2018; Kannenberg et al., 2022; Mirfenderesgi et al., 2018). Knowledge of the impact of abiotic drivers is important to predict the response of plants to recurrent extreme drought events (Sperry & Love, 2015), which are already frequently occurring in parts of the Mediterranean basin, such as the Iberian Peninsula (Moemken & Pinto, 2022) and are projected to increase in frequency and duration with ongoing climate change in future decades (Moemken et al., 2022; Spinoni et al., 2020).

Yet, the effect of biotic factors, such as plant–plant interactions, plant invasion and water competition (Lu et al., 2020), has rarely been considered (Craine & Dybzinski, 2013; Kannenberg & Phillips, 2017). However, there is strong evidence that competition for water is able to modify the hydraulic strategy of plants (e.g. Caldeira et al., 2015; Magh et al., 2020; Rascher et al., 2011). For instance, *Fagus sylvatica* trees were not able to recover to maximum transpiration after a drying–wetting cycle when co-occurring with *Abies alba* (Magh et al., 2020). Grossiord et al. (2015) reported negative impacts of plant–plant interactions on water fluxes for *Pinus sylvestris*, but positive effects for *Quercus faginea* and *Pinus nigra*. Thus, plant–plant interactions can buffer or amplify drought stress for co-existing plants (Grossiord, 2020; Haberstroh & Werner, 2022). Therefore, it is likely that plant competition or facilitation trigger modifications of the plants' hydraulic strategy in comparable ways to abiotic factors.

Thus, the main objectives of this study are to (a) investigate seasonal shifts in isohydricity caused by varying abiotic conditions, such as VPD and soil water resources and (b) explore modifications in plant hydraulic strategies (e.g. isohydric vs. anisohydric) in response to plant invasion and experimental drought. To this end, we established a shrub invasion and rain exclusion experiment in a savanna-type Mediterranean ecosystem including two co-occurring species with different drought adaptation strategies. The first investigated species, *Quercus suber* L. (cork oak), is characterised as a mostly isohydric, water-saving tree, which maintains relatively high Ψ_L (David et al., 2007). The dimorphic rooting system consists of a dense shallow network in 0.10–0.40 m depth and taproots, which can reach deep water reserves (David et al., 2013). Despite common groundwater access (Dubbert et al., 2019), a tight coupling between soil water content and stomatal conductance was reported (David et al., 2007). *Cistus ladanifer* L. (gum rockrose) is a shallow-rooted, native Mediterranean shrub (Rivest et al., 2011) with invasive characteristics (Acácio et al., 2009; Haberstroh et al., 2021), which can endure low Ψ_L and has a high water consumption (Caldeira et al., 2015; Correia et al., 1987; Correia & Ascensao, 2016). Low Ψ_L and high stomatal conductance under moderate drought (Quero et al., 2011) indicate a mostly anisohydric behaviour of this species.

We hypothesise (1) that seasonal abiotic conditions dynamically determine the degree of isohydricity in these species and (2) that biotic and abiotic effects, such as shrub invasion and experimental drought can strongly modify the hydraulic strategy of the dominant species in the cork oak system.

2 | MATERIALS AND METHODS

2.1 | Experimental site

The study was conducted in a savanna-type cork oak ecosystem in south-eastern Portugal (38°47′N, 7°22′W) between October 2017 and March 2020. Field work in the Tapada Real de Vila Viçosa was carried out with permission of the Fundação da Casa de Bragança. The Mediterranean climate (Cs; Kottek et al., 2006) is dominated by frequent winter rainfalls and a typical summer drought with low precipitation. The long-term average air temperature is 16.8°C with a long-term average precipitation of 515 mm (1981–2010; Haberstroh et al., 2021).

On site, we investigated different combinations of shrub invasion and rain exclusion treatments including two woody Mediterranean species: the tree *Q. suber* and the shrub *Cistus ladanifer*. Experimental plots (180 m^2 each) were classified into four treatments: (a) *Q. suber* trees receiving ambient precipitation (Control), (b) *Q. suber* trees with a rain exclusion (RE), (c) *Q. suber* trees with *C. ladanifer* in the understorey receiving ambient precipitation (Invasion) and (d) *Q. suber* trees with *C. ladanifer* in the understorey with a rain exclusion (Invasion + RE; see Figure S1). In each experimental plot, three trees and three shrubs (when present), were randomly selected for measurements. The four experimental treatments were replicated in three spatially separated blocks (=12 experimental plots), which resulted in a total number of 36 trees (nine per treatment) and 18 shrubs (nine per treatment).

Rainfall was excluded with PVC tubes (0.125 m in diameter) mounted 0.1–0.4 m above-ground to secure water run-off, covering 30% of the plot ground area starting in November 2017 (Figure S1). In April 2019, this area was extended to 45% to simulate more severe drought conditions. Care was taken that the ground area covered by tree crowns and beyond was sheltered by the rain exclusion to avoid tree water uptake from outside of the rainout shelter. Non-invaded plots were cleared of shrubs in 2011 and populated by a sparse grass layer (Caldeira et al., 2015). Trees were adult (50–60 years) with a diameter at breast height of 28.0 ± 4.3 cm and a crown area of 29.6 ± 1.5 m^2 in October 2017. Tree density was similar between treatments with 259 ± 19 trees/ha in the Control, 222 ± 56 trees/ha in the Rain Exclusion treatment, 259 ± 19 trees/ha in the Invasion treatment and 296 ± 81 trees/ha in the Invasion + Rain Exclusion treatment. Shrub density in all experimental plots was set to 11,000 shrubs/ha in March 2018. The poorly developed, acidic soils were on average 0.40 m deep, classified as haplic Leptosols and contained a high gravel content of the bedrock material schist (Haberstroh et al., 2018). Information about other soil parameters and rooting depths can be found in Appendix S1, Table S1 and Figure S2.

2.2 | Meteorological conditions

Meteorological conditions were monitored with three permanent meteorological stations (one per block) located two meters above-ground including air temperature, relative humidity sensors (RHT2, AT2, Delta-T Devices Ltd.; S-THB-M002, Onset) and rain gauges (ARG100, Campbell Scientific; RG1, Delta-T Devices Ltd.). Data were stored on independent data loggers (HOBO USB Micro Station Data Logger, Onset; ECH2O Em50, METER Group, Inc. USA). Vapour pressure deficit (VPD) and daily maximum values for VPD (VPD_{max}) were calculated with half-hourly values of air temperature and relative humidity.

2.3 | Volumetric soil water content

Volumetric soil water content (θ) was measured with ECH2O 5TM probes (METER Group, Inc.) in three depths (0.05, 0.20 and 0.40 m). Per plot, we installed two profiles of θ sensors within the range of tree crowns. The raw data were stored half hourly and converted to θ in m^3/m^3 with sensor-specific calibrations. Calibrations were conducted in the laboratory before field installation and supplemented by frequent field soil coring. Data gaps caused by logger or sensor failure were gap filled with linear regressions, using close-by sensors at the same depth.

2.4 | Leaf area measurements

Leaf area index (LAI) was measured with the LAI-2000 plant canopy analyser (LICOR Inc.) for trees and shrubs after the growing period in early summer and after the natural drought period in autumn to capture maximum and minimum values. Measurements were taken at dusk and dawn with the 45° view cap to minimise the impact of the operator on the measuring sensor (LI-COR., 1992). For trees, we measured one point in an open site, followed by 10 measurements under the canopy. Shrub LAI was measured in three transects per experimental plot ($n = 15$ per transect, $n = 45$ per plot).

2.5 | Sap flow measurements

Tree sap flow was measured with thermal dissipation probes (Granier, 1985) with the sensor SFS2 (Type M-M12, UP GmbH) and calculated according to Granier (1985). For the determination of the maximum nocturnal temperature difference of the two measurement needles, we applied a 3-day average moving window (Haberstroh et al., 2020). For shrub sap flow, we used custom-made sap flow gauges similar to the SGA13 (Dynamax Inc.). Sap flow analysis followed the stem heat balance method (Sakuratani, 1984). Data were stored half-hourly on independent data loggers (CR300/CR800/CR1000, Campbell Scientific). Data gaps were filled with linear regressions using data from shrubs and trees growing in close proximity according to the best fit ($R^2 = 0.60–0.97$). Daily sap flow density (J_s) sums were computed in mol cm^{-2} sapwood day^{-1}. Sap flow density was separated into five seasonal phases for the analysis of the abiotic controls on sap flow. Wet phases (hereafter 'wet') were characterised by increasing sap flow rates, while dry-down phases (hereafter 'dry') were defined by decreasing sap flow (Haberstroh et al., 2021). The exact start and end dates for each phase can be found in Table 1.

2.6 | Abiotic controls on sap flow

To elucidate the influence of the abiotic factors VPD_{max} and θ on sap flow density, we conducted nonlinear mixed effect models (Sánchez-Costa et al., 2015) with the R package SAEMIX (version 2.3; Comets et al., 2017) in R (R Core Team, 2021). In doing so, tree/shrub number was defined as random effect, with abiotic predictor (θ, VPD_{max}) as fixed effect to explain the variations in sap flow. Each abiotic predictor and phase was analysed separately. To compare species and treatments, we defined respective covariates in the model and repeated the analysis. For all analysed periods, we excluded days where precipitation occurred. For VPD_{max} as predictor, we defined a logarithmic relationship:

$$J_s = b \bullet \ln(VPD_{max}) + a, \tag{1}$$

where J_s is the sap flow density, a equals J_s at 1 kPa (VPD_{max}) and b is the sensitivity of J_s to VPD_{max}. For this analysis, we only considered days with $\theta > 0.15\,m^3/m^3$ to avoid co-limitation by θ (Sánchez-Costa et al., 2015). $0.15\,m^3/m^3$ is a reasonable value for dry soils, as pF curves for soil samples from the field site indicated that the permanent wilting point is reached between 0.09 and $0.14\,m^3/m^3$ in 0–0.40 m depth.

The relationship of J_s and θ was fitted with a sigmoidal curve:

$$J_s = \frac{J_{s,max}}{1 + \exp[(\theta_{50} - \theta)/\theta_{scal}]}, \tag{2}$$

In this equation, $J_{s,max}$ is the horizontal asymptote of J_s and θ_{50} represents θ where J_s equals 50% of $J_{s,max}$. θ_{scal} is a measure of the steepness of the curve and describes the differences of θ and θ_{50}, where about 75% of $J_{s,max}$ is reached. θ measurements in 0.05, 0.20 and 0.40 m were averaged for this model to capture the variations in θ over the whole soil profile. Both equations were adopted from Sánchez-Costa et al. (2015), and data were separated into five phases according to the sap flow patterns (Table 1).

TABLE 1 Definition of wet and dry phases between October 2017 and March 2020

Phase	Start date	End date
Wet-2018	1 October 2017	14 May 2018
Dry-2018	15 May 2018	13 October 2018
Wet-2019	14 October 2018	8 April 2019
Dry-2019	9 April 2019	20 September 2019
Wet-2020	21 September 2019	22 March 2020

2.7 | Leaf water potential

Leaf water potential was measured at pre-dawn (Ψ_{PD}) between 2 and 6 am and midday (Ψ_{MD}) between 12 pm and 3 pm with a Scholander-type pressure chamber (PMS 1000, PMS Instruments). For each plant measurement, we randomly selected two leaves (*Q. suber*) or terminal shoots (*C. ladanifer*). In case of strong discrepancies, we conducted another measurement. Leaves/shoots were cut and measured immediately. Ψ_{MD} was measured on sunny days with a clear sky to avoid a bias in the data due to varying environmental conditions. Leaves were taken from the south-facing side of the sunlit crown (trees) or from high sunlit shoots (shrubs). For Ψ_{PD} measurements, we varied the crown position to avoid an overcutting of the south side, as plants equilibrate soil water over night (Richter, 1997). Ψ_{PD} measurements were conducted 17 times, Ψ_{MD} measurements 14 times in the study period. The differences in the number of Ψ_{PD} and Ψ_{MD} was due to cloudy skies for Ψ_{MD} measurements, which would have impaired the comparability between measurement dates. For defining the species-specific hydraulic strategy along the iso-/anisohydric spectrum, we adopted the definition of Martínez-Vilalta et al. (2014), where the relationship of the minimum leaf water potential (Ψ_{min}) and soil water potential (Ψ_S) is analysed. As proxies for Ψ_{min} and Ψ_S, we used Ψ_{MD} and Ψ_{PD} respectively:

$$\Psi_{MD} = \sigma\,\Psi_{PD} + \Lambda, \tag{3}$$

where σ is the slope and Λ is the intercept of the regression, which illustrates the value of Ψ_{MD} at $\Psi_{PD} = 0$. A value of σ close to 0 indicates a strict isohydric strategy; a value close to 1 strict anisohydricity. Values of $0 < \sigma < 1$ are defined as partial isohydric. Values above 1 can be interpreted as extreme anisohydric (Martínez-Vilalta et al., 2014). It must be emphasised that this definition mainly describes the reaction of Ψ_L to changes in soil water resources and sets other parameters, such as VPD, photosynthetically activa photon flux density (PPFD) or leaf area as constant (Martínez-Vilalta et al., 2014). Therefore, changes in these parameters will be reflected in alterations of σ and Λ of Equation 3.

2.8 | Data analysis

For the evaluation of significant effects of invasion and rain exclusion on θ and sap flow density, we applied linear mixed effect (lme) models (R package NLME, R Core Team, 2021). In these models, invasion (IN) and rain exclusion (RE) were set as fixed factors and allowed to interact, which enabled statements about the effect of invasion, independent of the rain exclusion and vice versa, but also about significant differences between the four experimental treatments.

Thus, if we refer to significant differences between the four treatments, we use the notations Control (IN: no/RE: no), Rain Exclusion (IN: no/RE: yes), Invasion (IN: yes/RE: no) and Invasion + Rain Exclusion (IN: yes/RE: yes). If we refer to a significant influence of the invasion, independent of the rain exclusion, or the influence of the rain exclusion, independent of the invasion, we use the simple terms 'invasion' and 'rain exclusion'.

To capture significant differences in LAI, Ψ_{PD} and Ψ_{MD} between treatments, we additionally added the measurement date or season as fixed effect. To retrieve the parameters (σ and Λ) of the relationship of Ψ_{MD} and Ψ_{PD} (Equation 3), we conducted lme models for each species and season with Ψ_{MD} as dependent variable and Ψ_{PD}, invasion, rain exclusion and their interaction as fixed factors. Tree/shrub identity nested in block number were set as random effects in all of these lme models. Significant differences between treatments, measurement periods, species, as well as the intercepts (Λ) of the relationship of Ψ_{MD} and Ψ_{PD} (Equation 3) were assessed with post hoc tests with a Tukey adjustment (function 'emmeans', R package EMMEANS). The slopes (σ) of the relationship of Ψ_{MD} and Ψ_{PD} (Equation 3) were tested for statistical differences between the treatments with the function 'emtrends' (estimated marginal means of linear trends) with a Tukey adjustment (R package EMMEANS).

In the case of autocorrelation, we introduced a continuous covariate (corAR1). Model assumptions were validated visually and by the Shapiro–Wilk's test (normal distribution of residuals) and Levene's test (homogeneity of variance). If assumptions were not met, we applied a square root or log-transformation to the data. To reduce the model's complexity and capture species differences, we repeated all models with species as additional fixed and treatment (invasion, rain exclusion) as random effects.

3 | RESULTS

3.1 | Environmental conditions

Meteorological conditions between hydrological years (October–September) 2018 and 2019 were similar with regard to air temperature (2018: 15.9°C; 2019: 16.4°C) and vapour pressure deficit (VPD; Figure 1a). However, precipitation conditions varied widely between hydrological years (Figure 1b): in 2018, precipitation was above the long-term average (515 mm, 1981–2010) for ambient (713 mm) but reduced to 520 mm for the rain exclusion treatments. Contrastingly, 2019 was a dry year with 387 mm precipitation under ambient conditions and an extremely dry year under rain exclusion conditions with 257 mm of precipitation.

The effect of the rain exclusion (Figure 1e,f) was clearly visible in the volumetric soil water content (θ), which was particularly reduced in the wet periods compared to the Control ($p < 0.001$) in all depths (Figure 1c–f). Invasion effects were mostly prominent in 0.20 m depth

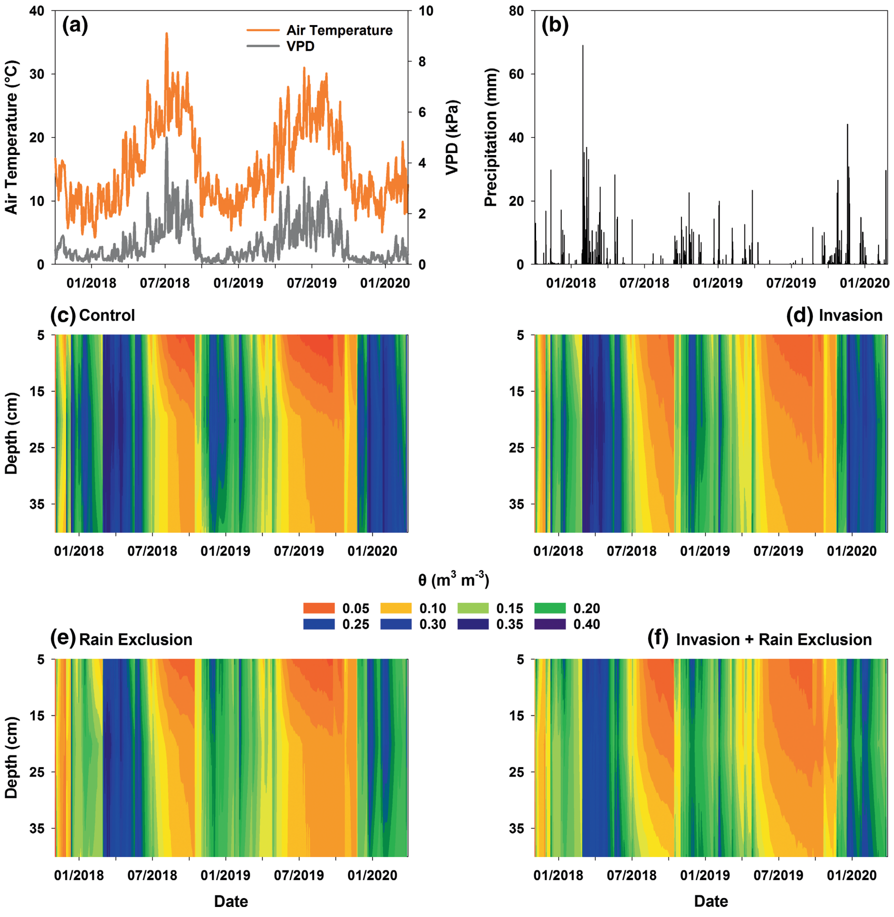

FIGURE 1 Air temperature and vapour pressure deficit (VPD) (a), precipitation (b) and volumetric water content (θ) for Control (c), Invasion (d), Rain Exclusion (e) and Invasion + Rain Exclusion (f) within the soil profile. θ was measured in 0.05, 0.20 and 0.40 m depth ($n = 6$ per treatment and depth), which is in the range of the rooting depths for both investigated species. In plots (c–e), θ (colour scale) is shown in a soil profile (y-axis) over time (x-axis). Red and orange colours indicate low water availability, green colours depict medium water availability and bluish colours show high water availability.

(Figure 1d,f), where soil water resources were stronger depleted in the dry summer periods (e.g. from July 2019 onwards) than in the non-invaded treatments (Figure 1c,e). For example, Invasion + Rain Exclusion ($0.11 \pm 0.04\,\mathrm{m^3/m^3}$) led to an overall reduction of 18% in θ in the dry summer periods compared to the Control ($0.13 \pm 0.06\,\mathrm{m^3/m^3}$) in 0.20 m depth.

3.2 | Leaf water potential

Significant species-specific differences were observed in predawn (Ψ_{PD}) and midday (Ψ_{MD}) leaf water potential ($p < 0.001$; Figure 2a–d), indicating different hydraulic behaviours. Leaf water potential (Ψ_L) strongly decreased in *C. ladanifer* (Figure 2b,d) with minimum values of −3.5 ± 0.1 MPa (Ψ_{PD}) and −5.5 ± 0.1 MPa (Ψ_{MD}) compared to *Q. suber* ($\Psi_{PD} = -1.7 \pm 0.1$ MPa/$\Psi_{MD} = -2.3 \pm 0.1$ MPa; Figure 2a,c). The seasonal patterns revealed that Ψ_{PD} and Ψ_{MD} declined strongly in dry phases and recovered quickly in wet phases. The magnitude of these seasonal changes was significantly larger for *C. ladanifer* compared to *Q. suber* (note different scales in Figure 2a–d).

For *Q. suber*, significant invasion effects were found for Ψ_{PD} ($p < 0.01$) and Ψ_{MD} ($p < 0.05$), while rain exclusion effects were negligible ($p > 0.05$) over the whole study period. Invasion effects were especially evident in the dry-down periods with lower Ψ_{PD} and higher Ψ_{MD} for invaded trees (Figure 2a,c, grey asterisk). The effect of the rain exclusion (black asterisks) for trees and shrubs was more prevalent and limited to phases with sufficient water supply (Figure 2a–d), which matched the patterns observed for θ (Figure 1c–f).

3.3 | Sap flow and leaf area index

Cistus ladanifer mostly had higher sap flow rates ($p < 0.001$) than *Q. suber* over the study period (Figure 3a,b). For both species, water fluxes decreased in the dry year 2019, although to a higher extent

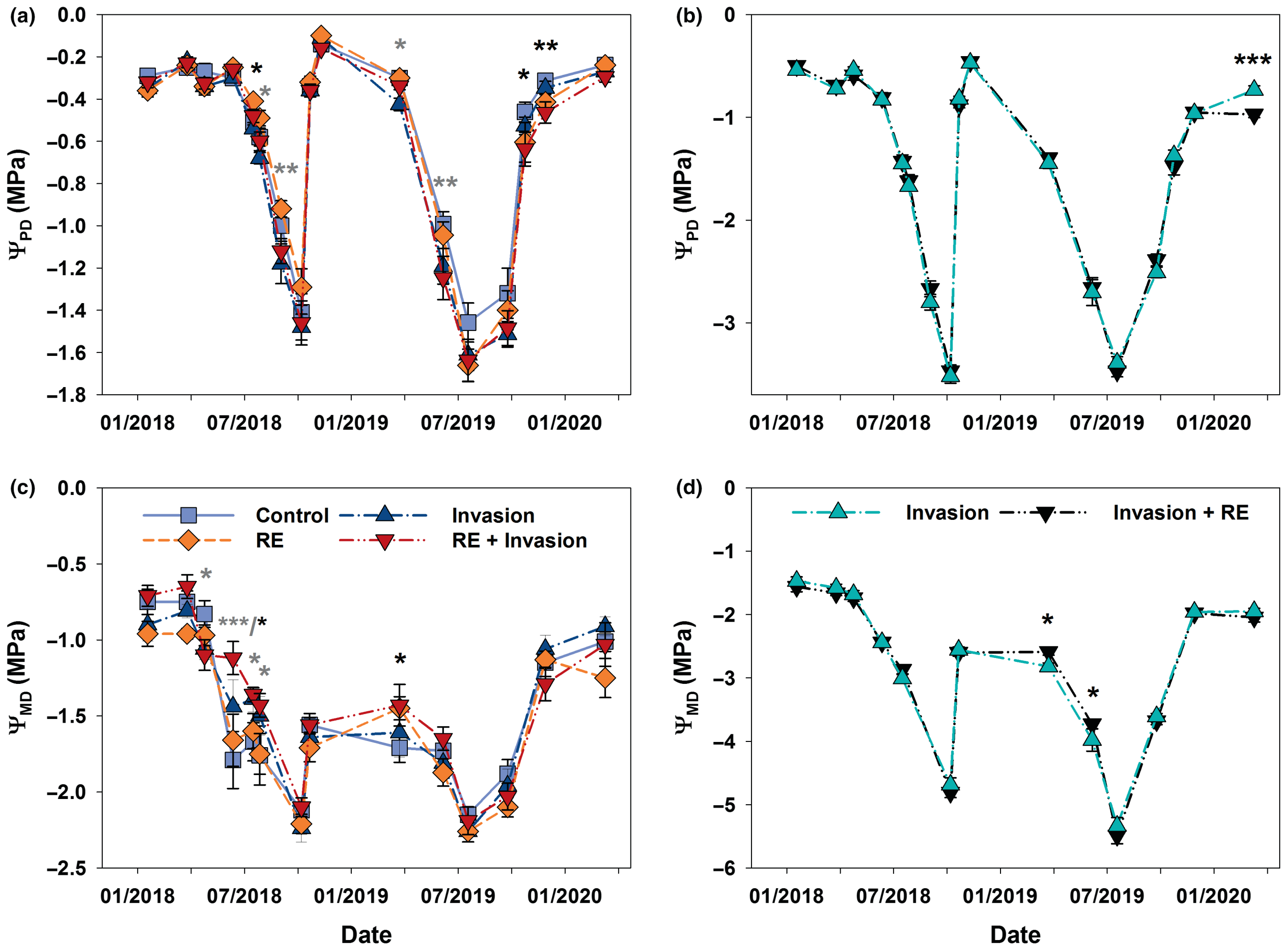

FIGURE 2 Pre-dawn leaf water potential (Ψ_{PD}) for *Q. suber* (a) and *C. ladanifer* (b), and midday leaf water potential (Ψ_{MD}) for *Q. suber* (c) and *C. ladanifer* (d) for all experimental treatments ($n = 9$ per treatment). Asterisks (*) indicate p-values (linear mixed effect model): <0.05*, <0.01**, <0.001***, where black asterisks indicate rain exclusion effects and grey asterisks indicate invasion effects. Note the different scales in (c–f) for the different species. RE, rain exclusion. See also Haberstroh et al. (2021).

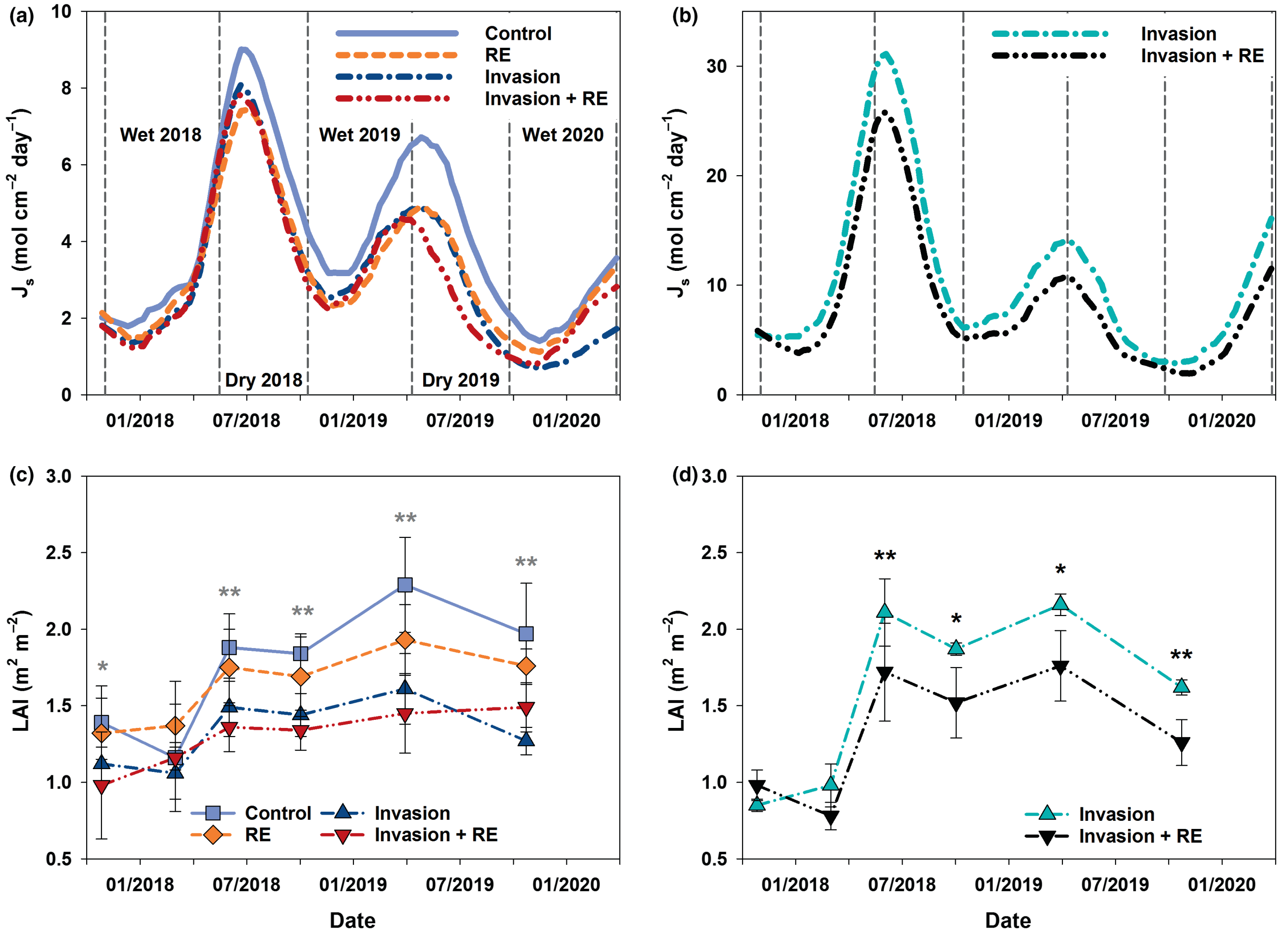

FIGURE 3 Sap flow density (J_s) for *Q. suber* (a) and *C. ladanifer* (b) and leaf area index (LAI) for *Q. suber* (c) and *C. ladanifer* (d) for all experimental treatments ($n = 9$ for all treatments). Asterisks (*) indicate *p*-values (linear mixed effect model): <0.05*, <0.01**, <0.001***, where black asterisks indicate rain exclusion effects and grey asterisks indicate invasion effects. The vertical dashed lines indicate the limits of the seasonal classification phases (Table 1). Sap flow density (a, b) was smoothed (negative exponential). Note the different scales for the species in (a) and (b). RE, Rain exclusion. The seasonal patterns of sap flow density are discussed in detail in Haberstroh et al. (2021).

for *C. ladanifer*. Seasonally, the highest sap flow rates were reached each year at the end of the wet season/beginning of the dry phase respectively. Notably, *C. ladanifer* approached these rates earlier and suffered faster declines in water fluxes compared to *Q. suber* (Figure 3).

Negative treatment effects in sap flow (Figure 3a) were mostly visible for *Q. suber* trees under invasion ($p = 0.08$), such as in the dry phase of 2019, where trees subjected to Invasion + Rain Exclusion reduced their sap flow earlier in the dry period. In the following wet phase 2020, trees under Invasion and ambient precipitation reached lower rates than the other treatments (Figure 3a). This negative invasion effect was also clear in the leaf area index (LAI) of invaded trees, independently of the rain exclusion treatment ($p < 0.01$; Figure 3c). On the other hand, *Q. suber* was able to maintain sap flow ($p = 0.20$) under Rain Exclusion alone (Figure 3a). For *C. ladanifer* strong Rain Exclusion effects were present in sap flow ($p < 0.001$) and LAI ($p < 0.05$; Figure 3b,d).

3.4 | Environmental controls on sap flow

To further identify the impact of invasion and rain exclusion on the seasonal water fluxes of *Q. suber* and *C. ladanifer*, we applied nonlinear mixed effect models with the dominant sap flow drivers, which were identified as θ in dry and $\mathrm{VPD}_{\max}$ in wet phases (Figure 4; see Table S2 for detailed analysis). In dry periods, $\mathrm{VPD}_{\max}$ was replaced as dominant sap flow driver by θ, as soon as soil water resources declined. The relationship of θ and sap flow was well described with a sigmoidal curve in dry phases (Figure 4a,c), where $J_{s,\max}$ represents the maximum sap flow density in a given period and θ_{50} the θ where plants reach 50% of modelled $J_{s,\max}$. Overall, modelled $J_{s,\max}$ was higher in 2018 than in 2019 for both species (Figure 4a,c, Table S2). $J_{s,\max}$ and θ_{50} were significantly higher for *C. ladanifer* in both years ($p < 0.001$), supporting the notion of a faster sap flow decline for shrubs in response to soil drying. In both dry phases, but especially in 2019, invaded trees ($p < 0.05$) had a lower modelled $J_{s,\max}$

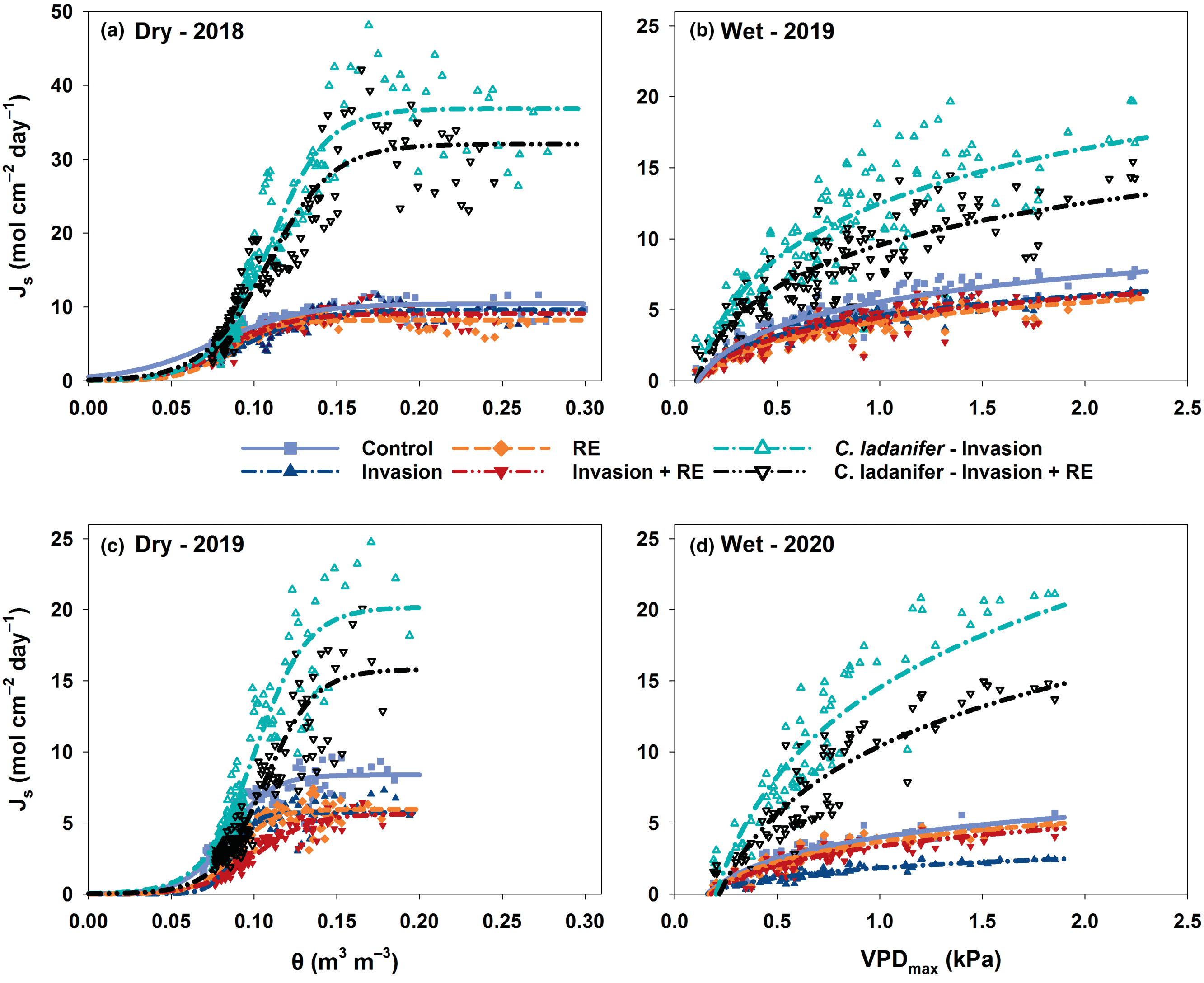

FIGURE 4 Nonlinear relationship of sap flow density (J_s) with volumetric soil water content (θ) for dry phases of 2018 (a) and 2019 (c) and nonlinear relationship of sap flow density with maximum vapour pressure deficit (VPD$_{max}$) for wet phases 2019 (b) and 2020 (d) for all experimental treatments ($n = 9$ per treatment) of *Q. suber* and *C. ladanifer*. Different fitting models were chosen for the dry and wet phases due to the inherently different response of sap flow density to rising VPD$_{max}$ and decreasing θ. Note the different scale in the dry phases 2018 (a) compared to the other seasons. The exact dates for the seasonal classification in wet and dry can be retrieved from Table 1. Average daily values are shown for each species and treatment. RE, rain exclusion.

(Figure 4a,c). For shrubs, the Rain Exclusion led to significantly lower modelled $J_{s,max}$ in 2019 ($p < 0.01$).

In wet periods, sap flow was rather driven by VPD$_{max}$ than θ for both species. This relationship was assessed with a logarithmic curve, where a is the sap flow density at VPD$_{max}$ = 1 kPa and b is the absolute sensitivity of sap flow to VPD$_{max}$. In both wet phases, *C. ladanifer* had a higher sensitivity to VPD$_{max}$ ($p < 0.001$) and higher sap flow ($p < 0.001$) at 1 kPa (VPD$_{max}$) compared to *Q. suber* (Figure 4b). Shrubs under the Rain Exclusion had a significantly lower sensitivity to VPD$_{max}$ and lower sap flow at 1 kPa in both years ($p < 0.05$; Figure 4a–d; Table S2).

Differences between invaded (Invasion and Invasion + RE) and Control *Q. suber* trees in their sap flow response to environmental drivers, such as in the dry periods (Figure 4a,c), persisted in both wet phases (Figure 4b,d), with a lower sensitivity of invaded trees to VPD$_{max}$ and lower sap flow density at 1 kPa (VPD$_{max}$, $p < 0.05$ in 2020; Table S2). *Quercus suber* trees under Rain Exclusion expressed a similar behaviour to invaded trees (Figure 4b,d). The relationship of VPD and sap flow density for the dry phases can be found in Appendix S2 and Figures S3–S5 for both species.

3.5 | Species-specific regulation of water relations in dry and wet periods

The combination of sap flow density, environmental conditions and Ψ_L elucidated seasonal shifts in the plants' hydraulic strategies for *Q. suber* and *C. ladanifer* (Figure S6). The relationship of these parameters revealed two different response patterns, which, after assessment of the measurement dates, were defined as 'wet' (blue) and 'dry' (red) responses. This classification was subsequently used to assess the seasonal species- and treatment-specific hydraulic strategies defined as the relationship of Ψ_{MD} and Ψ_{PD} (Figure 5; Table S3).

In Figure 5a,b it becomes evident that there was indeed a change in the slope (σ) and intercept (Λ) of the relation of Ψ_{MD} and Ψ_{PD}, indicating a shift in the hydraulic behaviour for both species between dry and wet seasons. During dry periods, the slope (σ) in *Q. suber* (Figure 5c) ranged between 0.32 and 0.57 MPa/MPa, which is defined as partial isohydric, but shifted to a more anisohydric behaviour in the wet period with a lesser control of soil moisture on Ψ_{MD} (Figure 5e; Table S3). In dry phases, σ of *C. ladanifer* was close to 1 MPa/MPa under ambient precipitation and rain exclusion (Figure 5d), which implies strict anisohydricity following the definition of Martínez-Vilalta et al. (2014). On the other hand, σ decreased in wet phases, indicating a shift to partial isohydricity (Figure 5f; Table S3).

Beside the species dissimilarities, there were some striking treatment differences observed for *Q. suber* with clear invasion effects on σ ($p < 0.01$) and intercepts (Λ; $p < 0.001$) in dry phases (Figure 5c). All trees under Invasion and Invasion + Rain Exclusion, decreased their Ψ_{MD} significantly stronger in response to a change of −1 MPa in Ψ_{PD} (Figure 5c) and thus, deployed, according to Martínez-Vilalta et al. (2014), a more anisohydric strategy in comparison to non-invaded trees. Additionally, Λ was significantly higher for trees under Invasion and Invasion + Rain Exclusion (Table S3). Rain Exclusion alone did not alter the relationship of Ψ_{MD} and Ψ_{PD} for trees (Figure 5c) or shrubs (Figure 5d,f) significantly ($p > 0.05$). Nevertheless, trees under the Rain Exclusion alone showed a non-significant tendency of a slightly higher σ and thus, more anisohydric strategy compared to Control trees in dry phases (Figure 5c).

4 | DISCUSSION

With this study, we demonstrate that *Q. suber* and *C. ladanifer* dynamically shift their degree of isohydricity between dry and wet phases in response to seasonal abiotic conditions. Remarkably, plant competition by *C. ladanifer* invasion significantly modified the hydraulic strategy of *Q. suber*, independently of the drought treatment, providing evidence that biotic factors indeed influence the degree of isohydricity in plants. The causes and implications of these modified hydraulic strategies provoked by abiotic and biotic factors will be discussed in the following.

4.1 | Degree of isohydricity in *Q. suber* and *C. ladanifer* shifts dynamically between seasons

The opposed shifts in isohydricity of *Q. suber* and *C. ladanifer* from dry to wet phases (Figure 6a) seem contradictory at first, but might be explained by a three-phase model (Kannenberg et al., 2022; Meinzer et al., 2016). In phase I, the decrease in Ψ_{MD} is almost independent from Ψ_{PD} (Figure 6a). In this phase, the water supply is high and Ψ_{MD}, as well as water fluxes, are controlled by other environmental factors than soil moisture, such as PPFD (Meinzer et al., 2016) or VPD (Novick et al., 2019). Plants behave anisohydric in this period with high σ and Λ and water fluxes not regulated by soil moisture (Hochberg et al., 2018). In phase II, water resources decline and plants develop a higher degree of isohydricity to limit water losses. This phase lasts until $\Psi_{PD} = \Psi_{MD}$ (phase III), where plants lose control of stomatal closure (Figure 6a; Meinzer et al., 2016). However, this point (phase III) was not reached in our study.

Although the range of Ψ_{PD} was low for *Q. suber* in wet phases (cf. Martínez-Vilalta et al., 2014), this three-phase model fits our observations particularly well. In the wet periods, the loose relationship of Ψ_{MD} and Ψ_{PD} under well-watered conditions (Figure 5e) matched phase I (Figure 6a), as also observed for *Pinus ponderosa* during times of sufficient water supply (Kannenberg et al., 2022). In addition, sap flow of trees was strongly controlled by VPD in wet phases. Under dry conditions, trees switched to a partial isohydric strategy (phase II, Figure 6a), as their sap flow was strongly coupled to soil moisture. This is a well-known behaviour of *Q. suber* with its dimorphic rooting system (David et al., 2013), common groundwater access (Dubbert et al., 2019; Kurz-Besson et al., 2006) and tight stomatal control (David et al., 2007; Kurz-Besson et al., 2014). In addition, high VPD has been suggested as a trigger of stomatal closure in *Q. suber* when soil water is still available (Lobo-do-Vale et al., 2019). It must be noted that the water fluxes of *Q. suber* decreased with declining water resources in the topsoil, even though trees had access to groundwater. This feature has also been observed in other deep rooted tree species (Werner et al., 2021).

Cistus ladanifer operated in a different Ψ_L (<−5 MPa) range and has been described as an anisohydric species (Quero et al., 2011), which strongly reacts to changing environmental conditions (Caldeira et al., 2015; Haberstroh et al., 2018). Ψ_{PD} of *C. ladanifer* was rarely higher than −0.5 MPa, even under well-watered conditions. However, this has been described as approximate value for the shift from phase I to phase II (Figure 6a), where plants start to regulate differently (Hochberg et al., 2018; Meinzer et al., 2016). It is likely that *C. ladanifer* shrubs are in phase II most of the year, due to their shallow rooting system (Rivest et al., 2011). The increase in anisohydricity in dry periods might implicate that shrubs are slowly progressing towards the point where stomatal control becomes less effective in controlling Ψ_{MD} (Meinzer et al., 2016), potentially also caused by higher VPD in the summer periods (Kannenberg et al., 2022). Indeed, sap flow for shrubs under extremely dry conditions did not decrease as strongly as for *Q. suber* trees in response to VPD (Figures S3–S5), which supports the notion of a lesser stomatal control. Such behaviour can be accompanied by leaf senescence (Wu et al., 2021), which is commonly observed for *C. ladanifer* under stressful conditions (Núñez-Olivera et al., 1996; Simões et al., 2008).

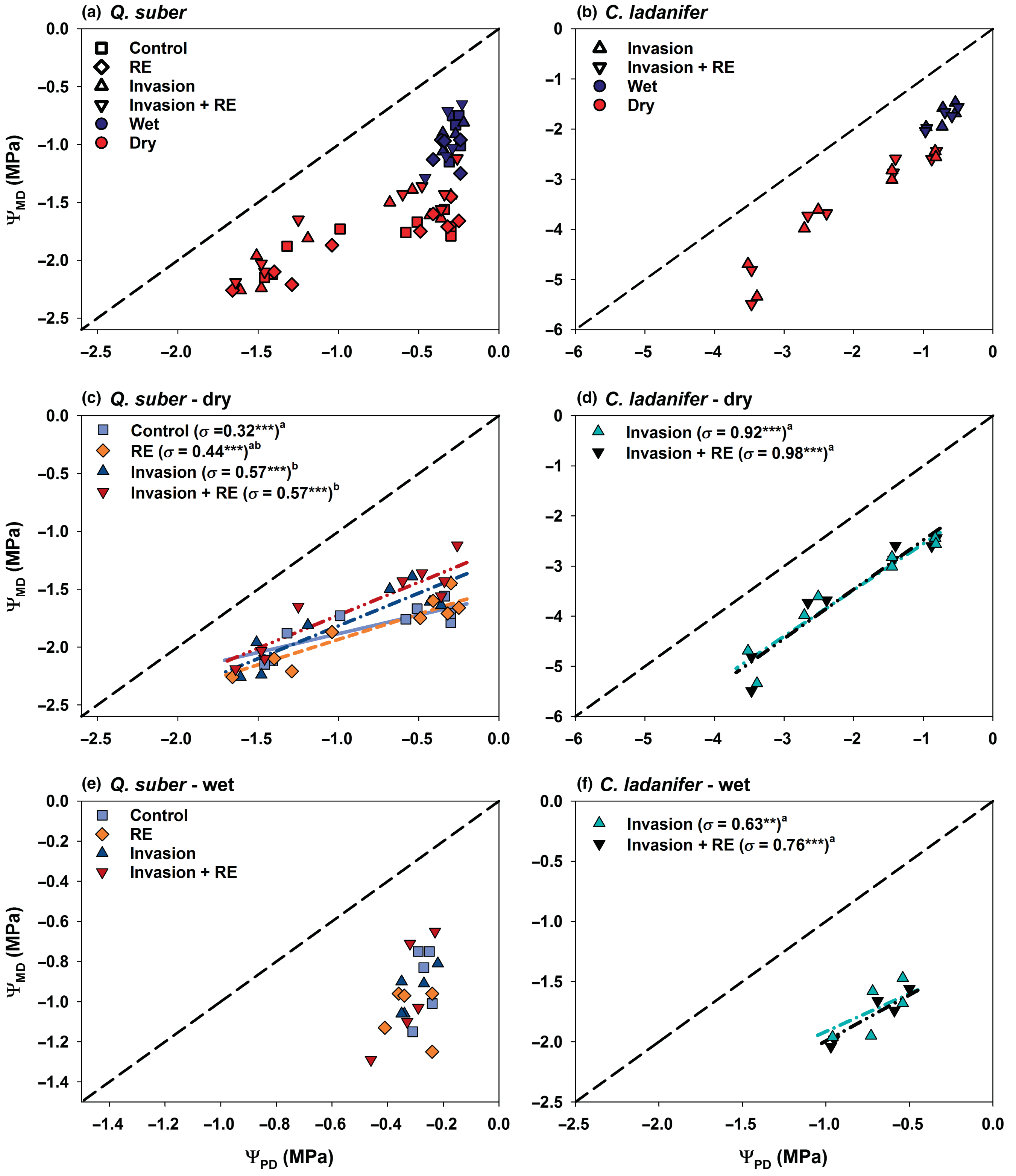

FIGURE 5 Relationship of pre-dawn leaf water potential (Ψ_{PD}) and midday leaf water potential (Ψ_{MD}) including a 1:1 line (dashed) adopted from Martínez-Vilalta et al. (2014) for *Q. suber* (a) separated into dry (c) and wet (e) and *C. ladanifer* (b) separated into dry (d) and wet phases (f). Asterisks (*) indicate *p*-values for the regression lines: <0.05*, <0.01**, <0.001***. Letters indicate differences between the slope (σ) of the experimental treatments ($n = 9$ per treatment) for each season ($n = 5$, wet; $n = 9$, dry) and species separately at a level of $p < 0.05$. Note the different *x*- and *y*-scales in all panels. In panel (e), the regression lines and slopes are not shown, due to a weak statistical relationship of Ψ_{PD} and Ψ_{MD} (Table S3). RE, rain exclusion.

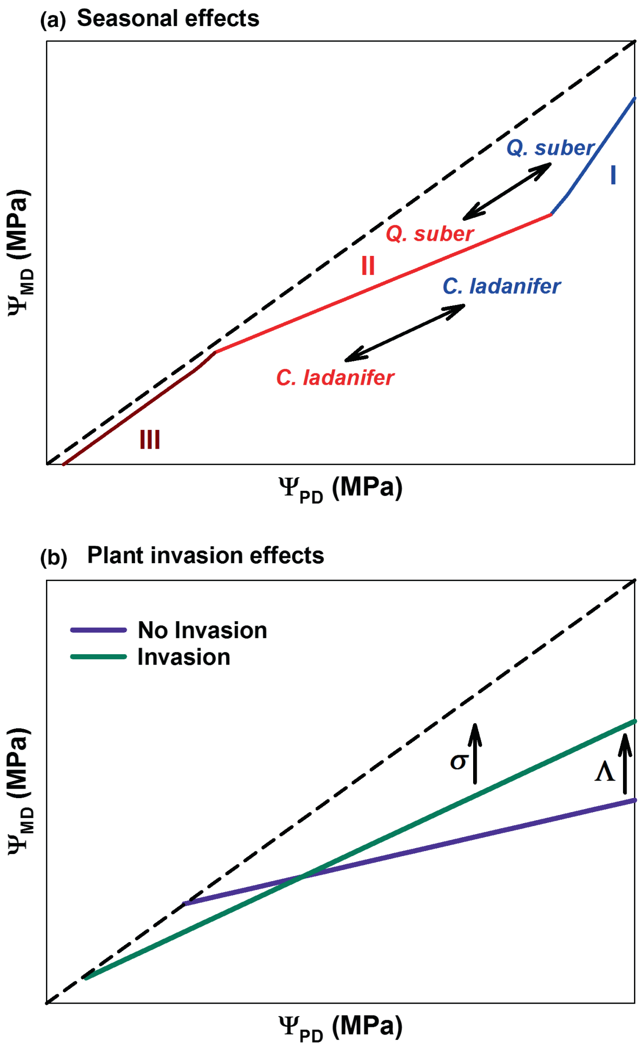

FIGURE 6 Conceptual illustration of seasonal (a) and plant invasion effects (b) on the slope (σ, isohydricity) and intercept (Λ) of the relationship between pre-dawn (Ψ_{PD}) and midday leaf water potential (Ψ_{MD}). Colours in (a) depict the wet (blue) and dry phases (red, dark red). Adapted and modified after Wu et al. (2021).

Thus, our first hypothesis that seasonal changing abiotic conditions play an important role in determining the degree of isohydricity of *Q. suber* and *C. ladanifer* can be confirmed.

4.2 | Hydraulic strategy of *Q. suber* is modified by plant invasion, but not rain exclusion

Invaded trees, independent of the additional drought treatment, expressed similar responses in Ψ_L, sap flow density, LAI and shifts in isohydricity over the study period. Thus, we will discuss the invasion effects on *Q. suber* trees independently from the additional drought treatment. *C. ladanifer* is a strong competitor for *Q. suber* trees, especially for shallow soil water resources during summer drought (Caldeira et al., 2015), as evident from soil water content profiles (Figure 1). Here, it could be argued that *Q. suber* and *C. ladanifer* are not competing for water resources in the same depth during summer drought due to differences in their rooting systems. However, both species do have the highest proportion of fine roots in the upper soil and results from stable water isotope analysis indicate that *Q. suber* takes up water from shallow soil layers in variable quantities in all seasons throughout the year (Dubbert et al., 2019). This would also explain why *Q. suber* sap flow and stomatal conductance is tightly coupled to declining soil water resources during summer drought (David et al., 2007; Kurz-Besson et al., 2014), despite groundwater access. Thus, it is likely that *Q. suber* and *C. ladanifer* do compete for the same water resources all year round, especially when water is scarce.

As a result, invaded trees adjusted, according to the definition of Martínez-Vilalta et al. (2014), to a more anisohydric hydraulic strategy in comparison to non-invaded trees. This shift (Figure 6b) most likely occurred as a result of the more stressful conditions under shrub invasion. This is supported by lower Ψ_{PD}, yet higher Ψ_{MD}, during drought and lower sap flux densities in response to soil moisture and increasing evaporative demand (Figure 4). Thus, our second hypothesis that biotic plant–plant interactions, as caused by plant invasion, can modify the hydraulic strategy of *Q. suber* is supported by our data (Figure 6b).

However, it should be denoted that although the definition (σ) of Martínez-Vilalta et al. (2014) points towards a more anisohydric strategy, other parameters indicate a more conservative behaviour of invaded trees. These include lower sap flow densities, higher Ψ_L at fully water-saturated soil conditions (Λ, Figure 6b) and overall lower stomatal conductance (data not shown). Indeed, other authors have deemed different definitions of isohydricity problematic and not straightforward (e.g. Martínez-Vilalta & Garcia-Forner, 2017; Meinzer et al., 2016). Thus, we conclude that several metrics have to be taken into account and care has to be taken, when discussing the isohydricity of plants. Nevertheless, invasion modified the hydraulic strategy of *Q. suber* and the potential reasons and consequences, besides the direct below-ground competition by *C. ladanifer*, are discussed in the following.

A possible explanation for the modified isohydricity of *Q. suber* might involve the fine root biomass present in the topsoil. Profiles of fine root weight and length per soil volume taken in May 2019 (Figure S2) did not reveal significant differences between invaded and non-invaded plots, contrary to the expectation that additional invasion might increase the below-ground biomass of ecosystems. Potentially, exudates of *C. ladanifer*, which are known to inhibit seed germination (Herranz et al., 2006), might be able to obstruct or reduce fine root growth of *Q. suber*. A lower fine root biomass in the topsoil can also translate into increased water stress and a more water conservative behaviour. However, such processes are speculative and have to be confirmed by future research.

Modified below-ground water uptake and competition has also important implications for nutrient uptake patterns (Dubbert et al., 2014; Dubbert & Werner, 2019). Mediterranean oak ecosystems are often found on nutrient deficient soils (e.g. Marañón et al., 2009) and soils on site are in fact nutrient poor (Table S1).

Given the high competitiveness of *C. ladanifer*, invaded *Q. suber* trees might suffer from enhanced nutrient deficiencies. Indeed, the negative effect of *C. ladanifer* invasion on the nutrient status of the closely related tree *Q. ilex* has been shown (Rolo et al., 2012). In addition, it has been demonstrated that trees, which behave too conservative under drought might suffer from the so-called 'isohydric trap'. In isohydric species, the transpirational flow, which is required for nutrient transport, is reduced under prolonged drought conditions. As a consequence, these species can experience severe nutrient deficits (Salazar-Tortosa et al., 2018).

Independently of the root biomass and potential nutrient deficiencies, the modified strategy of invaded *Q. suber* trees could potentially transfer into sustained negative impacts during dry and wet phases: Decreased whole tree water fluxes and reduced stomatal conductance point towards diminished carbon assimilation rates, which is supported by lower leaf areas (Figure 3e) and trunk increment rates (Haberstroh et al., 2021) for invaded *Q. suber*. These features in combination with lower Ψ_{PD} during drought point towards chronic stress for invaded trees, which may result in a potential out-competition by *C. ladanifer* shrubs and detrimental effects for *Q. suber* trees and ecosystems. Hence, these results support the notion that shrub invasion could contribute to increased mortality rates of *Q. suber* (Costa et al., 2010) in severe drought years, by the means of carbon depletion (McDowell et al., 2008).

Contrastingly, our Rain Exclusion experiment alone did not modify the hydraulic strategies of *Q. suber* nor *C. ladanifer* significantly, potentially because the rain exclusion of 30–45% was still in the range where both species were able to adjust their water relations in the short term. *C. ladanifer* shrubs drought response was mainly regulated by a reduced leaf area in dry phases (Núñez-Olivera et al., 1996; Simões et al., 2008). For *Q. suber* trees under Rain Exclusion, higher σ suggests potential modifications of the hydraulic strategy under long-term extreme drought, which have to be explored in detail in the future. Such modifications of the hydraulic behaviour under recurrent drought are of high importance, as the Mediterranean basin, but especially the Iberian Peninsula, is expected to face longer and more frequent drought events with ongoing climate change (Moemken et al., 2022; Spinoni et al., 2020).

5 | CONCLUSIONS

With this study, we demonstrate that biotic and abiotic factors can influence the degree of isohydricity in two woody Mediterranean plant species. Plant invasion plays a critical role when two species with different hydraulic strategies compete for the same resources. This modification of the hydraulic strategy of *Q. suber* can potentially increase the risk for carbon depletion and thus, might contribute to an increase in tree mortality in cork oak, which in turn could favour the conversion of *Q. suber* woodlands into persistent shrubland (Acácio et al., 2009). In the future, plant–plant interactions will likely be intensified by ongoing plant invasion and climate-change driven alterations, such as increase in severe droughts. Thus, this calls for a better understanding of the potentially detrimental effects of invasion and plant competition on hydraulic strategies in ecosystems endangered by droughts.

AUTHORS' CONTRIBUTIONS

C.W., M.C.C. and S.H. conceived the ideas and designed methodology; S.H., M.C.C. and R.L.-d.-V. collected the data; S.H. and M.C. analysed the data; S.H. led the writing of the manuscript with input from C.W., M.D., M.C.C., R.L.-d.-V. and M.C. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

We acknowledge funding from the DFG (WE 2681/10-1, CU 173/4-1, MD 1688/1-1), DAAD (57396941) and FCT (Lisboa-01-0145-FEDER-030406–PTDC/ASP-SIL/3406/2017). R.L.d.V. was funded by FCT researcher contracts (PTDC/ASP-SIL/3406/2017 and CEECIND/02735/2018). M.C. was supported by a grant overseen by the French National Research Agency (ANR) as part of the 'Investissements d'Avenir' program (ANR-11- LABX-0002-01, Lab of Excellence ARBRE). Centro de Estudos Florestais (CEF) is a research unit funded by FCT, Portugal (UID/AGR/00239/2019). To Fundação da Casa de Bragança for permission to undertake research in Tapada Real de Vila Viçosa. S.H. was partly funded by the scholarship of the German Academic Foundation. We thank Joaquim Mendes and Joana I. Martins for maintaining the field site and support during the intense field campaigns. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.v15dv41zf (Haberstroh et al., 2022).

ORCID

Simon Haberstroh https://orcid.org/0000-0002-6097-6633
Raquel Lobo-do-Vale https://orcid.org/0000-0003-4566-5420
Maria C. Caldeira https://orcid.org/0000-0002-3586-8526
Maren Dubbert https://orcid.org/0000-0002-2352-8516
Matthias Cuntz https://orcid.org/0000-0002-5966-1829
Christiane Werner https://orcid.org/0000-0002-7676-9057

REFERENCES

Acácio, V., Holmgren, M., Rego, F., Moreira, F., & Mohren, G. M. J. (2009). Are drought and wildfires turning Mediterranean cork oak forests into persistent shrublands? *Agroforestry Systems*, *76*, 389–400. https://doi.org/10.1007/s10457-008-9165-y

Anderegg, W. R. L., Wolf, A., Arango-Velez, A., Choat, B., Chmura, D. J., Jansen, S., Kolb, B., Li, S., Meinzer, F. C., Pita, P., Resco de Dios, V., Sperry, J. S., Wolfe, B. T., & Pacala, S. (2018). Woody plants optimise stomatal behaviour relative to hydraulic risk. *Ecology Letters*, *21*, 968–977. https://doi.org/10.1111/ele.12962

Berger-Landefeldt, U. (1936). *Der Wasserhaushalt der Alpenpflanzen (Bibliotheca Botanica Vol. 115)*. E. Schweizerbart'sche Verlagsbuchhandlung.

Caldeira, M. C., Lecomte, X., David, T. S., Pinto, J. G., Bugalho, M. N., & Werner, C. (2015). Synergy of extreme drought and shrub invasion reduce ecosystem functioning and resilience in water-limited climates. *Scientific Reports*, *5*, 15110. https://doi.org/10.1038/srep15110

Cochard, H., Bréda, N., & Granier, A. (1996). Whole tree hydraulic conductance and water loss regulation in *Quercus* during drought: Evidence for stomatal control of embolism? *Annales des Sciences Forestieres*, *53*, 197–206. https://doi.org/10.1051/forest:19960203

Comets, E., Lavenu, A., & Lavielle, M. (2017). Parameter estimation in nonlinear mixed effect models using saemix, an R implementation of the SAEM algorithm. *Journal of Statistical Software*, *80*, 1–41. https://doi.org/10.18637/jss.v080.i03

Correia, O., & Ascensao, L. (2016). Summer semi-deciduous species of the Mediterranean landscape: A winning strategy of Cistus species to face the predicted changes of the Mediterranean climate. In A. A. Ansari, S. S. Gill, Z. K. Abbas, & M. Naeem (Eds.), *Plant biodiversity. Monitoring, assessment and conservation* (pp. 195–217). CAB International.

Correia, O., Catarino, F. M., Tenhunen, J. D., & Lange, O. L. (1987). Regulation of water use by four species of Cistus in the scrub vegetation of the Serra da Arrábida, Portugal. In J. D. Tenhunen, F. M. Catarino, O. L. Lange, & W. C. Oechel (Eds.), *Plant responses to stress. Functional analysis in mediterranean ecosystems* (pp. 247–258). Springer.

Costa, A., Pereira, H., & Madeira, M. (2010). Analysis of spatial patterns of oak decline in cork oak woodlands in Mediterranean conditions. *Annals of Forest Science*, *67*, 204. https://doi.org/10.1051/forest/2009097

Cowan, I. R. (1982). Regulation of water use in relation to carbon gain in higher plants. In O. L. Lange, P. S. Nobel, C. B. Nobel, & H. Ziegler (Eds.), *Physiological plant ecology II* (pp. 589–613). Springer.

Craine, J. M., & Dybzinski, R. (2013). Mechanisms of plant competition for nutrients, water and light. *Functional Ecology*, *27*, 833–840. https://doi.org/10.1111/1365-2435.12081

David, T. S., Henriques, M. O., Kurz-Besson, C., Nunes, J., Valente, F., Vaz, M., Pereira, J. S., Siegwolf, R., Chaves, M. M., Gazarini, L. C., & David, J. S. (2007). Water-use strategies in two co-occurring Mediterranean evergreen oaks: Surviving the summer drought. *Tree Physiology*, *27*, 793–803. https://doi.org/10.1093/treephys/27.6.793

David, T. S., Pinto, C. A., Nadezhdina, N., Kurz-Besson, C., Henriques, M. O., Quilhó, T., Cermak, J., Chaves, M. M., Pereira, J. S., & David, J. S. (2013). Root functioning, tree water use and hydraulic redistribution in *Quercus suber* trees: A modeling approach based on root sap flow. *Forest Ecology and Management*, *307*, 136–146. https://doi.org/10.1016/j.foreco.2013.07.012

Dubbert, M., Caldeira, M. C., Dubbert, D., & Werner, C. (2019). A pool-weighted perspective on the two-water-worlds hypothesis. *New Phytologist*, *222*, 1271–1283. https://doi.org/10.1111/nph.15670

Dubbert, M., Piayda, A., Cuntz, M., Correia, A. C., Costa e Silva, F., Pereira, J. S., & Werner, C. (2014). Stable oxygen isotope and flux partitioning demonstrates understory of an oak savanna contributes up to half of ecosystem carbon and water exchange. *Frontiers in Plant Science*, *5*, 530. https://doi.org/10.3389/fpls.2014.00530

Dubbert, M., & Werner, C. (2019). Water fluxes mediated by vegetation: Emerging isotopic insights at the soil and atmosphere interfaces. *New Phytologist*, *221*, 1754–1763. https://doi.org/10.1111/nph.15547

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 and Environment*, *42*, 1104–1111. https://doi.org/10.1111/pce.13486

Granier, A. (1985). Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres. *Annales des Sciences Forestières*, *42*, 193–200.

Grossiord, C. (2020). Having the right neighbours: How tree species diversity modulates drought impacts on forests. *New Phytologist*, *228*, 42–49. https://doi.org/10.1111/nph.15667

Grossiord, C., Forner, A., Gessler, A., Granier, A., Pollastrini, M., Valladares, F., & Bonal, D. (2015). Influence of species interactions on transpiration of Mediterranean tree species during a summer drought. *European Journal of Forest Research*, *134*, 365–376. https://doi.org/10.1007/s10342-014-0857-8

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 Phytologist*, *225*, 713–726. https://doi.org/10.1111/nph.16196

Guo, J. S., & Ogle, K. (2019). Antecedent soil water content and vapor pressure deficit interactively control water potential in *Larrea tridentate*. *New Phytologist*, *221*, 218–232. https://doi.org/10.1111/nph.15374

Guyot, G., Scoffoni, C., & Sack, L. (2012). Combined impacts of irradiance and dehydration on leaf hydraulic conductance: Insights into vulnerability and stomatal control. *Plant, Cell and Environment*, *35*, 857–871. https://doi.org/10.1111/j.1365-3040.2011.02458.x

Haberstroh, S., Caldeira, M. C., Lobo-do-Vale, R., Dubbert, M., & Werner, C. (2020). Determination of zero-flow for the thermal dissipation method of sap flow measurements in Mediterranean climates. *Acta Horticulturae*, *1300*, 29–36. https://doi.org/10.17660/ActaHortic.2020.1300.5

Haberstroh, S., Caldeira, M. C., Lobo-do-Vale, R., Martins, J. I., Moemken, J., Pinto, J. G., & Werner, C. (2021). Non-linear plant-plant interactions modulate impact of extreme drought and recovery on Mediterranean ecosystem. *New Phytologist*, *231*, 1784–1797. https://doi.org/10.1111/nph.17522

Haberstroh, S., Kreuzwieser, J., Lobo-do-Vale, R., Caldeira, M. C., Dubbert, M., & Werner, C. (2018). Terpenoid emissions of two Mediterranean woody species in response to drought stress. *Frontiers in Plant Science*, *9*, 1071. https://doi.org/10.3389/fpls.2018.01071

Haberstroh, S., Lobo-do-Vale, R., Caldeira, M. C., Dubbert, M., Cuntz, M. C., & Werner, C. (2022). Data from: Plant invasion modifies isohydricity in Mediterranean tree species. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.v15dv41zf

Haberstroh, S., & Werner, C. (2022). The role of species interactions for forest resilience to drought. *Plant Biology*. https://doi.org/10.1111/plb.13415

Herranz, J. M., Ferrandis, P., Copete, M. A., Duro, E. M., & Zalacaín, A. (2006). Effect of allelopathic compounds produced by *Cistus ladanifer* on germination of 20 Mediterranean taxa. *Plant Ecology*, *184*, 259–272. https://doi.org/10.1007/s11258-005-9071-6

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

Javaux, M., & Carminati, A. (2021). Soil hydraulics affect the degree of isohydricity. *Plant Physiology*, *186*, 1378–1381. https://doi.org/10.1093/plphys/kiab154

Jones, H. G., & Sutherland, R. A. (1991). Stomatal control of xylem embolism. *Plant, Cell and Environment*, *14*, 607–612. https://doi.org/10.1111/j.1365-3040.1991.tb01532.x

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. *Functional Ecology*, *36*, 24–37. https://doi.org/10.1111/1365-2435.13945

Kannenberg, S. A., & Phillips, R. P. (2017). Soil microbial communities buffer physiological responses to drought stress in three hardwood species. *Oecologia*, *183*, 631–641. https://doi.org/10.1007/s00442-016-3783-2

Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. (2006). World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, *15*, 259–263.

Kurz-Besson, C. K., Lobo-do-Vale, R., Rodrigues, M. L., Almeida, P., Herd, A., Grant, O. M., David, T. S., Schmidt, M., Otieno, D., Keenan, T. F., Gouveia, C., Mériaux, C., Chaves, M. M., & Pereira, J. S. (2014). Cork oak physiological responses to manipulated water availability in a Mediterranean woodland. *Agricultural and Forest Meteorology*, *184*, 230–242. https://doi.org/10.1016/j.agrformet.2013.10.004

Kurz-Besson, C. K., Otieno, D., Lobo-do-Vale, R., Siegwolf, R., Schmidt, M., Herd, A., Nogueira, C., David, T. S., David, J. S., Tenhunen, J., Pereira, J. S., & Chaves, M. (2006). Hydraulic lift in cork oak trees in a savannah-type Mediterranean ecosystem and its contribution to the local water balance. *Plant and Soil*, *282*, 361–378. https://doi.org/10.1007/s11104-006-0005-4

Landsberg, J., & Waring, R. (2016). Water relations in tree physiology: Where to from here? *Tree Physiology*, *37*, 18–32. https://doi.org/10.1093/treephys/tpw102

LI-COR. (1992). *LAI-2000 plant canopy analyzer: Instruction manual*. LI-COR, Inc.

Lobo-do-Vale, R., Kurz Besson, C., Caldeira, M. C., Chaves, M. M., & Pereira, J. S. (2019). Drought reduces tree growing season length but increases nitrogen resorption efficiency in a Mediterranean ecosystem. *Biogeosciences*, *16*, 1265–1279. https://doi.org/10.5194/bg-16-1265-2019

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

Magh, R.-K., Eiferle, C., Burzlaff, T., Dannenmann, M., Rennenberg, H., & Dubbert, M. (2020). Competition for water rather than facilitation in mixed beech-fir forests after drying-wetting cycle. *Journal of Hydrology*, *587*, 124944. https://doi.org/10.1016/j.jhydrol.2020.124944

Marañón, T., Pugnaire, F. I., & Callaway, R. M. (2009). Mediterranean-climate oak savannas: The interplay between abiotic environment and species interactions. *Web Ecology*, *9*, 30–43. https://doi.org/10.5194/we-9-30-2009

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

Martínez-Vilalta, J., Poyatas, R., Aguadé, D., Retana, J., & Mencuccini, M. (2014). A new look at water transport regulation in plants. *New Phytologist*, *204*, 105–115. https://doi.org/10.1111/nph.12912

Matheny, A. M., Mirfenderesgi, G., & Bohrer, G. (2017). Trait-based representation of hydrological functional properties of plants in weather and ecosystem models. *Plant Diversity*, *39*, 1–12. https://doi.org/10.1016/j.pld.2016.10.001

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

Meinzer, F. C., Woodruff, D. R., Marias, D. E., Smith, D. D., Mcculloh, K. A., Howard, A. R., & Magedman, A. L. (2016). Mapping 'hydroscapes' along the iso- to anisohydric continuum of stomatal regulation of plant water status. *Ecology Letters*, *19*, 1343–1352. https://doi.org/10.1111/ele.12670

Mirfenderesgi, G., Matheny, A. M., & Bohrer, G. (2018). Hydrodynamic trait coordination and cost–benefit trade-offs throughout the isohydric–anisohydric continuum in trees. *Ecohydrology*, *12*, e2041. https://doi.org/10.1002/eco.2041

Moemken, J., Koerner, B., Ehmele, F., Feldmann, H., & Pinto, J. G. (2022). Recurrence of drought events over Iberia. Part II: Future changes using regional climate projections. *Tellus A: Dynamic Meteorology and Oceanography*, *74*, 262–279. https://doi.org/10.16993/tellusa.52

Moemken, J., & Pinto, J. G. (2022). Recurrence of drought events over Iberia. Part I: Methodology and application for present climate conditions. *Tellus A: Dynamic Meteorology and Oceanography*, *74*, 222–235. https://doi.org/10.16993/tellusa.50

Novick, K. A., Konings, A. G., & Gentine, P. (2019). Beyond soil water potential: An expanded view on isohydricity including land–atmosphere interactions and phenology. *Plant, Cell and Environment*, *42*, 1802–1815. https://doi.org/10.1111/pce.13517

Núñez-Olivera, E., Martínez-Abaigar, J., & Escudero, J. C. (1996). Adaptability of leaves of *Cistus ladanifer* to widely varying environmental conditions. *Functional Ecology*, *10*, 636–646.

Quero, J. D., Sterck, J., Martínez-Vilalta, J., & Villar, R. (2011). Water-use strategies of six co-existing Mediterranean woody species during a summer drought. *Oecologia*, *166*, 45–57. https://doi.org/10.1007/s00442-011-1922-3

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rascher, K. G., Große-Stoltenberg, A., Máguas, C., & Werner, C. (2011). Understory invasion by *Acacia longifolia* alters the water balance and carbon gain of a Mediterranean pine forest. *Ecosystems*, *14*, 904–919. https://doi.org/10.1007/s10021-011-9453-7

Richter, H. (1997). Water relations of plants in the field: Some comments on the measurement of selected parameters. *Journal of Experimental Biology*, *48*, 1–7. https://doi.org/10.1093/jxb/48.1.1

Rivest, D., Rolo, V., López-Díaz, L., & Moreno, G. (2011). Shrub encroachment in Mediterranean silvopastoral systems: *Retama sphaerocarpa* and *Cistus ladanifer* induce contrasting effects on pasture and *Quercus ilex* production. *Agriculture, Ecosystems and Environment*, *141*, 447–454. https://doi.org/10.1016/j.agee.2011.04.018

Rolo, V., López-Díaz, M. L., & Moreno, G. (2012). Shrubs affect soil nutrients availability with contrasting consequences for pasture understory and tree overstory production and nutrient status in Mediterranean grazed open woodlands. *Nutrient Cycling in Agroecosystems*, *93*, 89–102. https://doi.org/10.1007/s10705-012-9502-4

Sakuratani, T. (1984). Improvement of the probe for measuring water flow rate in intact plants with the stem heat balance method. *Journal of Agricultural Meteorology*, *40*, 273–277. https://doi.org/10.2480/agrmet.40.273

Salazar-Tortosa, D., Castro, J., Villar-Salvador, P., Viñegla, B., Matías, L., Michelsen, A., Rubio de Casas, R., & Querejeta, J. I. (2018). The 'isohydric trap': A proposed feedback between water shortage, stomatal regulation, and nutrient acquisition drives differential growth and survival of European pines under climatic dryness. *Global Change Biology*, *24*, 4069–4083. https://doi.org/10.1111/gcb.14311

Sánchez-Costa, E., Poyatos, R., & Sabaté, S. (2015). Contrasting growth and water use strategies in four co-occurring Mediterranean tree species revealed by concurrent measurements of sap flow and stem diameter variations. *Agricultural and Forest Meteorology*, *207*, 24–37. https://doi.org/10.1016/j.agrformet.2015.03.012

Simões, M. P., Madeira, M., & Gazarini, L. (2008). The role of phenology, growth and nutrient retention during leaf fall in the competitive potential of two species of mediterranean shrubs in the context of global climate changes. *Flora*, *203*, 578–589. https://doi.org/10.1016/j.flora.2007.09.008

Sperry, J. S., Hacke, U. G., Oren, R., & Comstock, J. P. (2002). Water deficits and hydraulic limits to leaf water supply. *Plant, Cell and Environment*, *25*, 251–263. https://doi.org/10.1046/j.0016-8025.2001.00799.x

Sperry, J. S., & Love, D. M. (2015). What plant hydraulics can tell us about responses to climate-change droughts. *New Phytologist*, *207*, 14–27. https://doi.org/10.1111/nph.13354

Spinoni, J., Barbosa, P., Bucchignani, E., Cassano, J., Cavazos, T., Christensen, J. H., Christensen, O. B., Coppola, E., Evans, J., Geyer, B., Giorig, F., Hadjinicolaou, P., Jacob, D., Katzfey, J., Koenigk, T., Laprise, R., Lennard, C. J., Kurnaz, M. L., Delei, L., et al. (2020). Future global meteorological drought hot spots: A study based on CORDEX data. *Journal of Climate*, *33*, 3635–3661. https://doi.org/10.1175/JCLI-D-19-0084.1

Tardieu, F., & Simonneau, T. (1998). Variability among species of stomatal control under fluctuating soil water status and evaporative demand: Modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany*, *49*, 419–432. https://doi.org/10.1093/jxb/49.Special_Issue.419

Tyree, M. T., & Sperry, J. S. (1988). Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? *Plant Physiology*, *88*, 574–580. https://doi.org/10.1104/pp.88.3.574

Werner, C., Meredith, L. K., Ladd, S. N., Ingrisch, J., Kübert, A., von Haaren, J., Bahn, M., Bailey, K., Bamberger, I., Beyer, M., Blomdahl, D., Byron, J., Daber, E., Deleeuw, J., Dippold, M. A., Fudyma, J., Gil-Loaiza, J., Honeker, L. K., Hu, J., et al. (2021). Ecosystem fluxes during drought and recovery in an experimental forest. *Science*, *374*, 1514–1518. https://doi.org/10.1126/science.abj6789

Wu, G., Guan, K., Li, Y., Novick, K. A., Feng, X., McDowell, N. G., Konings, A. G., Thompson, S. E., Kimball, J. S., De Kauwe, M. G., Aisnworth, E. A., & Jiang, C. (2021). Interannual variability of ecosystem iso/anisohydry is regulated by environmental dryness. *New Phytologist*, *229*, 2562–2575. https://doi.org/10.1111/nph.17040

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Haberstroh, S., Lobo-do-Vale, R., Caldeira, M. C., Dubbert, M., Cuntz, M., & Werner, C. (2022). Plant invasion modifies isohydricity in Mediterranean tree species. *Functional Ecology*, *36*, 2384–2398. https://doi.org/10.1111/1365-2435.14126