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i. Title

Plant invasion modifies isohydricity in Mediterranean tree species

ii. Authors

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23 **v. Abstract**

24 Knowledge of plant hydraulic strategies (isohydric vs anisohydric) is crucial to predict the
25 response of plants to changing environmental conditions, such as climate-change induced
26 extreme drought. Several abiotic factors, such as evaporative demand, have been shown to
27 seasonally modify the isohydricity of plants, however, the impact of biotic factors, such as
28 plant-plant interactions on hydraulic strategies has seldom been explored. Here, we
29 investigated adaptations in hydraulic strategies for two woody species in response to seasonal
30 abiotic conditions, experimental drought and plant invasion in a Mediterranean cork oak
31 (*Quercus suber*) ecosystem with a combined shrub invasion (*Cistus ladanifer*) and rain
32 exclusion experiment. From summer to winter, the degree of isohydricity shifted from partial
33 isohydric to anisohydric in *Q. suber* and inversely from strict anisohydric to partial isohydric
34 for *C. ladanifer*. During drought, plant invasion significantly modified the hydraulic strategy
35 of invaded *Q. suber* to a higher degree of anisohydricity with severe negative consequences
36 for tree functioning, implying progressive leaf and xylem damage. The rain exclusion alone
37 led to a non-significant increase in anisohydricity for both species. We demonstrate that the
38 degree of isohydricity of plants is dynamically determined by the interplay of species-specific
39 hydraulic traits and their abiotic and biotic environment.

40 **vi. Keywords**

41 Mediterranean ecosystem, transpiration, leaf water potential, isohydric, anisohydric, *Quercus*
42 *suber*, *Cistus ladanifer*, shrub invasion, plant competition

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Title

Plant invasion modifies isohydricity in Mediterranean woody species

Abstract

Knowledge of plant hydraulic strategies (isohydric vs anisohydric) is crucial to predict the response of plants to changing environmental conditions, such as climate-change induced extreme drought. Several abiotic factors, such as 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. Here, we investigated adaptations in hydraulic strategies for 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. From summer to winter, the degree of isohydricity shifted from partial isohydric to anisohydric in *Q. suber* and inversely from strict anisohydric to partial isohydric for *C. ladanifer*. During drought, plant invasion significantly modified the hydraulic strategy of invaded *Q. suber* to a higher degree of anisohydricity with severe negative consequences for tree functioning, implying progressive leaf and xylem damage. The rain exclusion alone led to a non-significant increase in anisohydricity for both species. We demonstrate that the degree of isohydricity of plants is dynamically determined by the interplay of species-specific hydraulic traits and their abiotic and biotic environment.

Keywords

Mediterranean ecosystem, transpiration, leaf water potential, isohydric, anisohydric, *Quercus suber*, *Cistus ladanifer*, shrub invasion, plant competition

71 Plant water regulations have long been a central focus of research (Landsberg & Waring,
72 2016). However, while stomatal regulation to environmental changes is well understood (e.g.
73 Jarvis & Morison, 1981; Medlyn *et al.*, 2011), there are still many open questions regarding
74 plasticity in hydraulic strategies in response to environmental stressors, such as extreme
75 drought (Sperry, & Love, 2015). Plants have developed a broad range of water regulation
76 strategies under drought with the aim to maximise their carbon assimilation at minimum
77 water cost (Cowan, 1982; Anderegg *et al.*, 2018), while simultaneously avoiding hydraulic
78 conductivity loss (Sperry, Hacke, Oren & Comstock, 2002) and runaway cavitation (Tyree &
79 Sperry, 1988; Cochard, Bréda, & Granier, 1996). Yet, there is a wide spectrum of responses
80 to soil drying, including conservative water use strategies with early stomatal closure at the
81 expense of reduced carbon gain during the onset of drought and less conservative strategies
82 with maintenance of high stomatal conductance, which implies the risk of increased hydraulic
83 damage or failure under drought conditions (Jones & Sutherland, 1991; McDowell *et al.*,
84 2008). Tardieu & Simmoneau (1998) reintroduced the concept of isohydricity, originally
85 defined by Berger-Landefeldt (1936), for these hydraulic strategies with isohydric plants
86 exerting a strong stomatal control and maintaining comparatively constant leaf water
87 potentials (Ψ_L) over the day. Contrarily, anisohydric plants exhibit less stomatal control and,
88 thus, tolerate larger variations in Ψ_L (Tardieu & Simmoneau, 1998).

89 Thereafter, many studies have followed to assess the species-specific drought adaptation
90 strategies of plants (cf. Hochberg *et al.*, 2018; Feng *et al.*, 2019). The simplified definition of
91 Martínez-Vilalta *et al.* (2014) explores the seasonal response of the minimum Ψ_L , measured
92 at midday (Ψ_{MD}), to declining soil water potentials (Ψ_s), which are reflected by pre-dawn Ψ_L

93 (Ψ_{PD}). This approach has the advantage that the required parameters are easy to measure, yet
94 other influential factors such as evaporative demand or leaf area, are set constant. This
95 implies that the degree of isohydricity can change and be modified by abiotic and biotic
96 factors other than soil water resources (Martínez-Vilalta & Garcia-Forner, 2017; Hochberg *et*
97 *al.*, 2018; Novick, Konings, & Gentine, 2019; Guo, Hultine, Koch, Kropp & Ogle, 2020; Wu
98 *et al.*, 2020). Relevant abiotic factors include deep water access (Feng *et al.*, 2019), soil
99 hydraulic properties (Javaux & Carminati, 2021), vapour pressure deficit (VPD) (Guo, &
100 Ogle, 2019; Novick *et al.* 2019), air temperature (Guo *et al.*, 2020) or light intensity (Guyot,
101 Scoffoni, & Sack, 2012; Meinzer *et al.*, 2016). For example, Guo *et al.* (2020) demonstrated
102 that the desert shrub *Larrea tridentata* shifted from partial isohydric under drought to an
103 extreme anisohydric behaviour under wet conditions. *Quercus douglasii* growing at a dry site
104 behaved more anisohydric than at a wet site during the summer periods (Feng *et al.*, 2019).
105 These examples illustrate that plant responses result from the interaction of plant
106 physiological strategies with their local, abiotic environment (Hochberg *et al.*, 2018; Feng *et*
107 *al.*, 2019). Knowledge of the impact of abiotic drivers is important to predict the response of
108 plants to recurrent extreme drought events (Sperry, & Love, 2015), which are projected to
109 increase in the Mediterranean basin with ongoing climate change in the near future (Spinoni
110 *et al.*, 2020).

111 Yet, the effect of biotic factors, such as plant-plant interactions, plant invasion and water
112 competition (Lu, Duursma, Farrior, Medlyn, & Feng, 2020), has rarely been considered
113 (Craine & Dybzinski, 2013). However, there is strong evidence that competition for water is
114 able to modify the hydraulic strategy of plants (e.g. Rascher, Große-Stoltenberg, Máguas, &
115 Werner, 2011; Caldeira *et al.*, 2015; Magh *et al.*, 2020). For example, *Fagus sylvatica* trees
116 were not able to recover to maximum transpiration after a drying-wetting cycle when co-
117 occurring with *Abies alba* (Magh *et al.*, 2020). *Quercus suber* trees invaded by *Cistus*

118 *ladanifer* shrubs had lower Ψ_{PD} and impaired sap flow rates during and after a severe drought
119 (Caldeira *et al.*, 2015). Grossiord *et al.* (2015) reported negative impacts of plant-plant
120 interactions for *Pinus sylvestris*, but positive effects for *Quercus faginea* and *Pinus nigra* in a
121 Mediterranean ecosystem. Thus, plant-plant interactions can alleviate or amplify drought
122 stress for co-existing plants (Grossiord, 2019). Therefore, it is likely that plant competition or
123 facilitation trigger modifications of the plants' hydraulic strategy in comparable ways to
124 abiotic factors.

125 The main objectives of this study are to (1) investigate seasonal shifts in isohydricity caused
126 by varying abiotic conditions, such as VPD and soil water resources and (2) explore
127 modifications in plant hydraulic strategies (e.g. isohydric vs anisohydric) in response to plant
128 invasion and experimental drought. To this end, we established a shrub invasion and rain
129 exclusion experiment in a savannah-type Mediterranean ecosystem including two co-
130 occurring species with different drought adaptation strategies and life forms. The first
131 investigated species, *Quercus suber* (cork oak), is characterised as an isohydric, water-saving
132 tree, which maintains relatively high Ψ_L (David *et al.*, 2007; Haberstroh *et al.*, 2018) and
133 often relies on groundwater resources during summer drought (Kurz-Besson *et al.*, 2014;
134 Mendes, Ribeiro, David, & Costa, 2016). A tight coupling between soil water content and
135 stomatal conductance was reported before (David *et al.*, 2007; Otieno *et al.*, 2007). Ψ_{50} ,
136 where plants lose 50% of their hydraulic conductivity was determined at -5.5 MPa (Lobo *et*
137 *al.*, 2018). *Cistus ladanifer* (gum rockrose) is a shallow-rooted, native Mediterranean shrub
138 (Talavera, Gibbs, & Herrera, 1993; Rivest, Rolo, López-Díaz, & Moreno, 2011) with
139 invasive characteristics (Acácio, Holmgren, Rego, Moreira, & Mohren, 2009; Costa, Pereira,
140 & Madeira, 2010), which can endure low Ψ_L and has a high water consumption (Caldeira *et*
141 *al.*, 2015; Haberstroh *et al.*, 2018). Low Ψ_L , low Ψ_{50} (-8.9 MPa; Torres-Ruiz *et al.*, 2017) and
142 high stomatal conductance under moderate drought (Quero, Sterck, Martínez-Vilalta, &

143 Villar, 2011; Haberstroh *et al.*, 2018) indicate an anisohydric behaviour of this species, that
144 can have a strong competitive impact on *Q. suber*.

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

149 **Material and Methods**

150 **Experimental Site**

151 The study was conducted in a savannah-type cork oak ecosystem in Vila Viçosa, south-
152 eastern Portugal. The Mediterranean climate (Cs; Kottek, Grieser, Beck, Rudolf, & Rubel,
153 2006) is dominated by frequent winter rainfalls and a typical summer drought period with
154 low precipitation. The long-term average air temperature is 16.8 °C with a long-term
155 precipitation of 515 mm (1981-2010). On site, we investigated different combinations of
156 shrub invasion and rain exclusion treatments including two woody Mediterranean species: the
157 tree *Quercus suber* (cork oak) and the shrub *Cistus ladanifer* (gum rockrose). Experimental
158 plots (180 m² each) were classified into four treatments: 1) *Q. suber* trees receiving ambient
159 precipitation (Control), 2) *Q. suber* trees with *C. ladanifer* in the understorey (Invasion), 3)
160 *Q. suber* trees with a rain exclusion (RE) and 4) *Q. suber* trees with *C. ladanifer* in the
161 understorey with a rain exclusion (Invasion + RE). Rainfall was excluded with PVC-tubes
162 (12.5 cm in diameter) mounted 10-40 cm above ground to secure water run-off, covering
163 30% of the plot area starting in November 2017, which was increased to 45% in April 2019.
164 Non-invaded plots were populated by a sparse grass layer in the understorey. The
165 experimental plots were replicated in three blocks. Trees were adult with a height of 6.5 ± 0.6
166 m, a diameter at breast height of 28.0 ± 4.3 cm and a crown area of 29.6 ± 1.5 m² in October

2017. In total, 36 trees and 18 shrubs (when present) were randomly selected for monitoring between November 2017 and March 2020. Shrubs reached a density of 11,000 individuals ha^{-1} in March 2018. The poorly developed, acidic soils were on average 0.40 m deep (Haberstroh *et al.*, 2018), classified as haplic Leptosols and contained a high gravel content of the bedrock material schist (Caldeira *et al.*, 2014). Information about pH, organic matter and nitrogen contents can be found in the Supporting Information (Table S1). More information about the field site is available from previous studies (Caldeira *et al.*, 2015; Haberstroh *et al.*, 2018; 2019; Dubbert, Caldeira, Dubbert, & Werner, 2019; Haberstroh, Caldeira, Lobo-do-Vale, Dubbert, & Werner, 2020).

Meteorological conditions

Meteorological conditions were monitored with three permanent meteorological stations located two meters above ground including air temperature and relative humidity sensors (RHT2, AT2, Delta-T Devices Ltd., Cambridge, UK; S-THB-M002, Onset, MA, USA), photosynthetic photon flux density (PPFD) sensors (S-LIA-M003, Onset, MA, USA) and rain gauges (ARG100, Campbell Scientific, Logan, UT, USA; RG1, Delta-T Devices Ltd., Cambridge, UK). Data were stored on independent data loggers (HOBO USB Micro Station Data Logger, Onset, MA, USA; ECH2O Em50, METER Group, Inc. USA, Pullman, WA, USA). Vapour pressure deficit (VPD) was calculated with half-hourly values of air temperature and relative humidity. For further analysis, daily maximum values for VPD (VPD_{max}) were computed.

Volumetric soil water content

Volumetric soil water content (θ) was measured with ECH2O 5TM probes (METER Group, Inc. Pullman, WA, USA) in three depths (0.05, 0.20 and 0.40 m) and averaged over the profile. Per plot, we installed two profiles of volumetric soil water content sensors. The raw

191 data was stored half hourly and converted to θ in $\text{m}^3 \text{m}^{-3}$ with sensor-specific calibrations.
192 Calibrations were conducted in the laboratory before installation in the field and
193 supplemented by frequent soil coring under field conditions. Data gaps caused by logger or
194 sensor failure were gap filled with linear regressions, using close-by sensors at the same
195 depth.

196 **Leaf area measurements**

197 Leaf area index (LAI) was measured with the LAI-2000 plant canopy analyser (LICOR Inc.,
198 Lincoln, NE, USA) for trees and shrubs after the growing period in early summer and after
199 the natural drought period in autumn to capture the maximum and minimum values.
200 Measurements were taken at dusk and dawn with the 45° view cap to minimise the impact of
201 the operator on the measuring sensor (LI-COR, 1992). For the trees, we measured one point
202 in an open site, followed by ten measurements under the canopy. For the final computation,
203 we removed the outer 68° ring with the software FV2200 (LICOR Inc., Lincoln, NE, USA),
204 as recommended by LI-COR (1992). Shrub LAI was measured in three transects per plot ($n =$
205 15 per transect). Leaf area for *Q. suber* was determined by multiplying the crown area with
206 LAI measurements. Leaf area for *C. ladanifer* was calculated at the plot level by multiplying
207 LAI with the plot size (180 m^2). Subsequently, we modelled the dynamics of the leaf area
208 with a general additive model (GAM) for both species to obtain daily leaf area values. This
209 was required to convert daily sap flow measurements (next section) into daily transpiration
210 data on a leaf area basis.

211 **Sap flow measurements**

212 Tree sap flow was measured with the thermal dissipation method developed by Granier
213 (1985; Granier & Gross, 1987) with the sensor SFS2 (Type M-M12, UP GmbH, Ibbenbüren,
214 Germany). Sap flow was calculated according to Granier (1985). For the determination of the

215 maximum nocturnal temperature difference of the two measurement needles, we applied a
 216 three-day average moving window (see Haberstroh *et al.*, 2020). For shrub sap flow, we used
 217 custom-made sap flow gauges similar to the SGA13 (Dynamax Inc., TX, USA). Sap flow
 218 analysis followed the stem heat balance method (Sakuratani, 1984). Data were stored half-
 219 hourly on independent data loggers (CR300/CR800/CR1000 and AM16/32 multiplexer,
 220 Campbell Scientific, Logan, UT, USA). Data gaps were filled with linear regressions using
 221 data from shrubs and trees growing in close proximity according to the best fit ($R^2 = 0.60$ -
 222 0.97), taking into account measurement periods before and after sensor failure. Daily sap
 223 flow sums were computed in $\text{mol cm}^{-2} \text{ sapwood day}^{-1}$ and converted to transpiration in mol
 224 $\text{m}^{-2} \text{ leaf day}^{-1}$ with a dynamic ratio of leaf area to sapwood area. Additionally, to assess which
 225 species dominated ecosystem transpiration, we computed stand transpiration for each species
 226 in mm day^{-1} by multiplying sap flow in $\text{m}^3 \text{ m}^{-2} \text{ sapwood day}^{-1}$ with the sapwood area per
 227 ground area in $\text{m}^2 \text{ m}^{-2}$. For the exact determination of tree and shrub sapwood area, see
 228 Caldeira *et al.* (2015) and the Supporting Information. The transpiration was separated into
 229 five seasonal phases for the analysis of the abiotic controls on transpiration. Winter phases
 230 were characterized by increasing transpiration rates, while summer dry down phases were
 231 defined by decreasing transpiration. The exact dates for these periods were as following: 1)
 232 winter 2018: 1st of October 2017-14th of May 2018, 2) summer 2018: 15th of May-13th of
 233 October 2018, 3) winter 2019: 14th of October 2018-8th of April 2019, 4) summer 2019: 9th of
 234 April-20th of September 2019 and 5) winter 2020: 21st of September 2020-22nd of March
 235 2021. These periods are referred to as “winter” and “summer” periods for simplification.
 236 Defining the phases with thresholds on VPD_{max} and θ rather than using the trend in
 237 transpiration (cf. Sánchez-Costa, Poyatos, & Sabaté, 2015) led to similar relationships
 238 between transpiration and the abiotic controls.

239 **Abiotic controls on transpiration**

240 To elucidate the influence of the abiotic factors VPD_{max} and θ on transpiration, we conducted
 241 non-linear mixed effect models (Sánchez-Costa *et al.*, 2015) with the R package ‘saemix’
 242 (version 2.3) (Comets, Lavenu, & Lavielle, 2017) in R (R core team, 2021). In doing so, tree/
 243 shrub number was defined as random effect, with abiotic predictor (θ , VPD_{max}) as fixed effect
 244 to explain the variations in transpiration. Each abiotic predictor and defined period was
 245 analysed separately. To compare species and treatments, we defined respective covariates in
 246 the model and repeated the analysis. For all analysed periods, we excluded days where rain
 247 occurred. For VPD_{max} as predictor, we defined a logarithmic relationship as:

$$248 \quad (1)$$

$$249 \quad E = b \times \ln(VPD_{max}) + a$$

250 where E is the transpiration on the leaf level, a equals E at 1 kPa (VPD_{max}) and b is the
 251 sensitivity of E to VPD_{max} . For this analysis, we only considered days with $\theta > 0.15 \text{ m}^3 \text{ m}^{-3}$ to
 252 avoid co-limitation by θ (Sánchez-Costa *et al.*, 2015). The relationship of E and θ was fitted
 253 with a sigmoidal curve as:

$$254 \quad (2)$$

$$255 \quad E = \frac{E_{Asym}}{1 + \exp\left(\frac{\theta - \theta_{mid}}{\theta_{scal}}\right)}$$

256 In this equation, E_{Asym} is the horizontal asymptote of transpiration at the leaf level and θ_{mid}
 257 represents θ where E equals 50% of E_{Asym} . θ_{scal} is a measure of the steepness of the curve and
 258 describes the differences of θ and θ_{mid} , where about 75% of E_{Asym} is reached. Both equations
 259 were adopted from Sánchez-Costa *et al.* (2015), and the data were separated into five periods
 260 according to the transpiration patterns.

261 Leaf water potential

Leaf water potential was measured at pre-dawn (Ψ_{PD}) between 2 and 6 am and midday (Ψ_{MD}) between 12 am and 3 pm on a regular basis with a Scholander-type pressure chamber (PMS 1000, PMS Instruments, Corvallis, OR, USA). For each plant measurement, we randomly selected two leaves (*Q. suber*) or terminal shoots (*C. ladanifer*). In case of strong discrepancies, we conducted a third 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 strongly varying environmental conditions. Leaves were taken from the south-facing side of the sunlit crown. For shrubs, we chose high sunlit shoots. 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). For defining the species-specific hydraulic strategy as isohydric or anisohydric, 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:

$$(3)$$

$$\Psi_{MD} = \sigma \Psi_{PD} + \Lambda$$

where σ is the slope and Λ 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, which includes the majority of plants investigated by Martínez-Vilalta *et al.* (2014). Values above 1 can be interpreted as extreme anisohydric (Martínez-Vilalta *et al.*, 2014). It must be emphasised that this definition only describes the reaction of Ψ_L to soil water resources (Martínez-Vilalta *et al.*, 2014). Therefore, changes in other parameters, such as VPD, PPFD or leaf area will be reflected in alterations of σ and Λ in equation 3.

Data analysis

For the evaluation of species, seasonal and treatment effects, we applied linear mixed effect models (R package ‘nlme’). For θ , sap flow and transpiration data, invasion and rain exclusion were set as fixed factors. For LAI, Ψ_{PD} , Ψ_{MD} , and seasonal variations in transpiration, we additionally added the measurement day/season as fixed effect. To retrieve the parameters (σ and Λ) of the relationship of Ψ_{MD} and Ψ_{PD} (Eq. 3), we conducted a linear mixed effect model for each species and season separately with Ψ_{PD} and treatment as fixed factor. Tree/shrub identity nested in block number were set as random effects in all of these linear mixed effect models. To capture species differences and reduce the model’s complexity, we repeated all models with species as additional fixed and treatment (invasion and rain exclusion) as random effects. Leaf area index (LAI) values were not compared between species due to differing measurement procedures. In the case of autocorrelation, we introduced a continuous covariate (corAR1). Model assumptions were validated visually and by the Shapiro Wilks- (normal distribution of residuals) and Levenes tests (variance homogeneity). If assumptions were not met, we applied a square root or log-transformation to the data. Significant differences between species, treatments and/or measurement periods were assessed with post hoc tests available in the R package ‘emmeans’ with a Tukey adjustment. All data were analysed with the statistical software R (R core team, 2021).

Results

Environmental conditions

Meteorological conditions between hydrological years (October-September) were only comparable in regard to air temperature, maximum vapour pressure deficit (VPD_{max}) (Fig. 1a) and maximum photosynthetic photon flux density (PPFD), but different strongly in terms of precipitation (Fig. 1b). Average air temperatures were similar to the long-term average (16.8 °C, 1981-2010) with 15.9 °C (2018) and 16.4 °C (2019). In 2018, precipitation on the field

310 site was above the long-term average (515 mm, 1981-2010) for ambient treatments (713 mm)
311 but reduced to 520 mm for the rain exclusion treatments. Contrastingly, 2019 was a dry year
312 with 387 mm (ambient) and 257 mm (rain exclusion) of precipitation. The effect of the rain
313 exclusion was visible in the volumetric soil water content (θ) ($p < 0.001$), which was
314 particularly reduced in the winter periods compared to the control treatment, such as in winter
315 2019/2020 after the severe drought (Fig. 1b). Invasion effects on θ on the other hand were
316 less prominent ($p = 0.54$).

317 **Leaf water potential**

318 Significant species-specific differences were present in pre-dawn (Ψ_{PD}) and midday (Ψ_{MD})
319 leaf water potentials ($p < 0.001$) (Fig. 1c-f), indicating different hydraulic behaviour. Leaf
320 water potentials (Ψ_L) strongly decreased in *C. ladanifer* (Fig. 1d, f) with minimum values of
321 -3.5 ± 0.07 MPa (Ψ_{PD}) and -5.5 ± 0.05 MPa (Ψ_{MD}) compared to *Q. suber* ($\Psi_{PD} = -1.7 \pm 0.08$
322 MPa / $\Psi_{MD} = -2.3 \pm 0.08$ MPa) (Fig. 1c, e). The seasonal patterns followed the soil moisture
323 (Fig. 1b) with a fast decline of Ψ_{PD} and Ψ_{MD} in summer and a quick recovery in autumn (Fig.
324 1c, d). However, the magnitude of these seasonal changes was significantly larger for *C.*
325 *ladanifer* compared to *Q. suber* (see different scales in Fig. 1c-f).

326 For *Q. suber*, significant invasion effects were found for Ψ_{PD} and Ψ_{MD} (grey asterisks), which
327 were particularly prominent in the dry-down periods (Fig. 1c, e), while the effect of the rain
328 exclusion for trees and shrubs was more relevant in phases with sufficient water supply, such
329 as autumn recovery or spring periods (Fig. 1c-f).

330 **Sap flow, transpiration and the influence of leaf area**

331 *C. ladanifer* had clearly higher sap flow rates ($p < 0.001$) than *Q. suber* over the study period
332 (Fig. 2a, b). Assessing the water flux at the canopy leaf level (Fig. 2c, d) revealed that
333 differences between species vanished ($p > 0.05$). Scaling the data to stand transpiration (data

not shown) elucidated that shrubs and trees contributed equally to total ecosystem transpiration, when shrubs were present. For both species, water fluxes were reduced in the dry year 2019, although to a higher extent for *C. ladanifer*. Seasonally, the highest sap flow and transpiration rates were reached at the beginning of summer each year. Notably, *C. ladanifer* approached these rates earlier in the year and suffered a faster decline in water fluxes compared to *Q. suber* (Fig. 2a-d).

Negative treatment effects in sap flow (Fig. 2a) were mostly visible for *Q. suber* trees under invasion ($p = 0.07$), such as in summer 2019, where trees subjected to invasion and rain exclusion (dark red line) reduced their sap flow earlier in the summer period. In the following winter, trees under invasion and ambient precipitation (blue line) reached lower rates than the other treatments (Fig. 2a). This negative invasion effect was also prominent in the leaf area index (LAI) of invaded trees ($p < 0.01$) (Fig. 2e). On the other hand, *Q. suber* was able to maintain sap flow ($p = 0.20$) and transpiration rates ($p = 0.97$) under the rain exclusion treatment (Fig. 2a, c). In contrast, for *C. ladanifer* rain exclusion effects were present in sap flow ($p < 0.001$) transpiration ($p < 0.05$) and LAI ($p < 0.05$) (Fig. 2b, d, f).

Environmental controls on leaf transpiration

To further identify the impact of the invasion and rain exclusion treatments on the seasonal water fluxes of *Q. suber* and *C. ladanifer*, we applied non-linear mixed effect models with the dominant transpiration drivers, which were identified as θ in summer and VPD_{max} in winter (Fig. 3) (please see Table 1 for detailed analysis). In summer, VPD_{max} was replaced as dominant transpiration driver by θ , as soon as soil water resources were declining. The relationship of θ and transpiration was well described with a sigmoidal curve in summer (Fig. 3a, c), where E_{Asym} represents the maximum transpiration in a given period and θ_{mid} , the θ , where plants reach 50% of modelled E_{Asym} . Overall, modelled E_{Asym} was higher in 2018 than

in 2019, but did not differ significantly between *Q. suber* and *C. ladanifer* (Fig. 3a, c, Table 1). However, θ_{mid} was significantly higher for *C. ladanifer* in both years ($p < 0.05$), supporting the notion of a faster transpiration decline for shrubs in response to soil drying. As for Ψ_L and sap flow rates, there were clear invasion effects for the *Q. suber* trees, which were expressed by higher modelled E_{Asym} in both investigated years (Fig. 3a, c) (significant in 2018, $p < 0.05$).

In the winter periods, the transpiration driver for both species shifted from θ to VPD_{max} . This relationship was assessed with a logarithmic curve, where a is the transpiration rate at $\text{VPD}_{\text{max}} = 1$ kPa and b the absolute sensitivity of transpiration to VPD_{max} (Table 1). In winter 2018, *C. ladanifer* had a lower sensitivity to VPD_{max} ($p < 0.05$) and lower transpiration rates ($p = 0.07$) at 1 kPa (VPD_{max}) (Fig. 3b), which was inversed one year later, where shrubs expressed a higher sensitivity ($p < 0.01$) and higher transpiration rates (Fig. 3d) compared to *Q. suber*. Differences between invaded and control *Q. suber* trees, such as in the summer periods (Fig. 3a, c), persisted in both winter periods (Fig. 3b, d), with a higher sensitivity of invaded trees to VPD_{max} and higher transpiration rates at 1 kPa (VPD_{max}) (Table 1), with the exception of invaded trees under ambient precipitation (blue line) in winter 2020 (Fig. 3d). *Q. suber* trees under rain exclusion expressed a similar behaviour to invaded trees in the winter periods (Fig. 3b, 3d). For *C. ladanifer*, no clear effects of the rain exclusion were observed for the modelled transpiration response parameters (Fig. 3a-d, Table 1).

Species-specific regulation of water relations in summer and winter

In Figure 4, the relationship of transpiration, θ and Ψ_{MD} is illustrated for all days, where Ψ_{MD} was measured to elucidate seasonal alterations and shifts in the plants' hydraulic strategies. For *Q. suber* and *C. ladanifer*, the combination of these three parameters revealed two different response patterns (Fig. 4), which, after assessment of the measurement dates, were

382 defined as ‘winter’ (blue) and ‘summer’ (red) response. Both species elucidated different
 383 responses from winter to summer: For *Q. suber* low transpiration rates at a high water supply
 384 and low Ψ_{MD} (−0.7–−1.3 MPa) in winter shifted to high transpiration rates under decreasing
 385 soil water supply and moderate Ψ_{MD} (−1.1–−2.3 MPa) in early summer (Fig. 4a). *C. ladanifer*
 386 revealed already moderate Ψ_{MD} (−1.5–−2.0 MPa) and high transpiration rates in the wet winter
 387 response period, but shifted to lower transpiration values in conjunction with decreasing Ψ_{MD}
 388 (−2.4–−5.5 MPa) and soil water resources in summer (Fig. 4b). This classification in winter
 389 and summer responses was subsequently used to assess the seasonal species- and treatment-
 390 specific hydraulic strategies defined as the relationship of Ψ_{MD} and Ψ_{PD} (Martínez-Vilalta *et*
 391 *al.*, 2014) (Fig. 5a-f, Table 2).

392 In Fig. 5a and b it becomes evident that there was indeed a change in the slope (σ) and
 393 intercept (Λ) of the relation of Ψ_{MD} and Ψ_{PD} , indicating a shift in the hydraulic behaviour for
 394 both species between seasons. During summer periods, the slope (σ) in *Q. suber* (Fig. 5c)
 395 ranged between 0.32–0.57 MPa MPa^{−1}, which is defined as partial isohydric, but shifted to a
 396 more anisohydric strategy in the winter period with a lesser control of soil moisture on Ψ_{MD}
 397 (Fig. 5e, Table 2). In summer, σ of *C. ladanifer* was close to 1 MPa MPa^{−1} under ambient
 398 precipitation and rain exclusion (Fig. 5d), which implies strict anisohydricity (Table 2).
 399 Contrarily, σ decreased in winter, indicating a shift to partial isohydricity (Fig. 5f, Table 2).

400 Beside the species dissimilarities, there were some striking treatment differences observed for
 401 *Q. suber* with clear invasion effects on σ ($p < 0.01$) and intercepts (Λ) ($p < 0.001$) in summer
 402 (Fig. 5c, Table 2). Invaded trees, independent of the rain exclusion, decreased their Ψ_{MD}
 403 significantly stronger in response to a change of −1 MPa in Ψ_{PD} (Fig. 5c) and thus, deployed a
 404 more anisohydric strategy in comparison to non-invaded trees. Additionally, Λ was
 405 significantly higher for invaded trees under ambient precipitation and rain exclusion (Table
 406 2). The rain exclusion treatment alone did not alter the relationship of Ψ_{MD} and Ψ_{PD} for trees

407 (Fig. 5c) or shrubs (Fig. 5d, f) significantly ($p > 0.05$) in winter or summer (Table 2).
408 Nevertheless, trees under the rain exclusion showed a non-significant tendency of a slightly
409 higher σ and thus, more anisohydric strategy compared to control trees in summer (Fig. 5c).

410 **Discussion**

411 With this study, we demonstrate that *Q. suber* and *C. ladanifer* dynamically shift their degree
412 of isohydricity between summer and winter in response to seasonal abiotic conditions.
413 Remarkably, plant competition by *C. ladanifer* shrub invasion significantly modified the
414 hydraulic strategy of *Q. suber*, providing evidence that biotic factors indeed influence the
415 degree of isohydricity in plants. The causes and implications of this modified hydraulic
416 strategies caused by abiotic and biotic factors will be discussed in detail in the following.

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

418 The opposed shifts in isohydricity of *Q. suber* and *C. ladanifer* from summer to winter (Fig.
419 6a) seem contradictory at first, but might be explained by a three-phase model described by
420 Meinzer *et al.* (2016). In phase I, Ψ_{MD} declines almost independently from Ψ_{PD} (Fig. 6a), as
421 the water supply is high and Ψ_{MD} , as well as transpiration, are controlled by other
422 environmental factors, such as PPFD (Meinzer *et al.*, 2016) or VPD (Novick *et al.*, 2019).
423 Plants behave anisohydric in this period with high σ and transpiration not regulated by soil
424 moisture (Hochberg *et al.*, 2018). In phase II, water resources decline and plants develop a
425 higher degree of isohydricity to limit water losses. This phase lasts until $\Psi_{PD} = \Psi_{MD}$ (phase
426 III), where plants lose control of stomatal closure (Fig. 6a) (Meinzer *et al.*, 2016).

427 Although the range of Ψ_{PD} was low for *Q. suber* in winter (cf. Martínez-Vilalta *et al.*, 2014),
428 this three-phase model fits our observations explicitly well. In winter, the loose relationship
429 of Ψ_{MD} and Ψ_{PD} under well-watered conditions (Fig. 5e) matched phase I (Fig. 6a), as also
430 observed for Mediterranean *Q. douglasii* at a wet site in spring periods (Feng *et al.*, 2019). In

431 addition, transpiration of trees was strongly controlled by VPD_{max} . In summer, trees switched
432 to a partial isohydric strategy (phase II, Fig. 6a), as their transpiration was strongly coupled to
433 soil moisture. This is a well-known behaviour of *Q. suber* with its deep rooting system
434 (David *et al.*, 2013), common groundwater access (Kurz-Besson *et al.*, 2014; Mendes *et al.*,
435 2016; Dubbert *et al.*, 2019) and tight stomatal control (David *et al.*, 2007; Otieno *et al.*,
436 2007).

437 *C. ladanifer* operated in a different Ψ_L (< -5 MPa) range and has been described as an
438 anisohydric species (Quero *et al.*, 2011), which reacts strongly to changing environmental
439 conditions (Correia, Catarino, Tenhunen, & Lange, 1987; Correia & Ascensao 2016;
440 Haberstroh *et al.*, 2018). Ψ_{PD} of *C. ladanifer* was rarely higher than -0.5 MPa, even under
441 well-watered conditions. However, this has been described as approximate value for the shift
442 from phase I to phase II (Fig. 6a), where plants start to regulate differently (Meinzer *et al.*,
443 2016; Hochberg *et al.*, 2018). It is likely that *C. ladanifer* shrubs are permanently in phase II
444 all year round, due to their shallow rooting system (Talavera *et al.*, 1993; Rivest *et al.*, 2011).
445 The increase in anisohydricity in the summer periods might implicate that shrubs are slowly
446 progressing towards the point where $\Psi_{PD} = \Psi_{MD}$ (Fig. 6a) and, hence stomatal control
447 becomes less effective in controlling Ψ_{MD} (Meinzer *et al.*, 2016). This can be accompanied by
448 above-ground leaf senescence (Wu *et al.*, 2020), which is commonly observed for *C.*
449 *ladanifer* (Núñez-Olivera, Martínez-Abaigar, & Escudero, 1996; Simões, Madeira, &
450 Gazarini, 2008). Thus, our second hypothesis that seasonal changing abiotic conditions play
451 an important role in determining the degree of isohydricity of *Q. suber* and *C. ladanifer* can
452 be confirmed.

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

454 *C. ladanifer* is a strong competitor for *Q. suber* trees, especially for soil water resources
455 during summer drought (Caldeira *et al.*, 2015). Invaded trees, independent of the rain
456 exclusion, adjusted to a riskier, more anisohydric hydraulic strategy to counteract the water
457 competition effects caused by shrub invasion (Fig. 6b), which is supported by the higher
458 transpiration in response to soil water moisture and increasing evaporative demand (Fig. 3).
459 Thus, our second hypothesis that biotic plant-plant interactions, as caused by plant invasion,
460 can modify the hydraulic strategy of plants can be confirmed (Fig. 6b).

461 Modified belowground water uptake and competition has also important implications for
462 nutrient uptake patterns (Dubbart *et al.*, 2014; Dubbart & Werner, 2019) and could thus,
463 provide an additional explanation for the shift of invaded *Q. suber* trees to a more anisohydric
464 behaviour. Given that Mediterranean oak ecosystems are often found on nutrient deficient
465 soils (Gómez-Gutiérrez, & Pérez-Fernández, 1996; Marañón, Pugnaire, & Callaway, 2009)
466 and soils on site are in fact nutrient poor (Table S1), invaded *Q. suber* trees need to be more
467 competitive to not fall prey to the “isohydric trap”, where isohydric species experience a
468 severe nutrient deficit provoked by prolonged drought conditions (Salazar-Tortosa *et al.*,
469 2018). Indeed, the negative effect of *C. ladanifer* invasion on the nutrient status of *Q. ilex* has
470 been demonstrated (Rolo, López-Díaz, & Moreno, 2012).

471 Maybe however, this modified strategy could potentially transfer into sustained negative
472 impacts for invaded *Q. suber* trees during summer drought and recovery periods: A more
473 anisohydric strategy of invaded trees can lead to progressive hydraulic damage (Kannenberg,
474 Novick, & Phillips, 2019), which can severely delay transpiration recovery (Skelton,
475 Brodribb, McAdam, & Mitchell, 2017; Kannenberg *et al.*, 2019), as detected for invaded *Q.*
476 *suber* trees under ambient precipitation in the winter recovery 2020. Furthermore, progressive
477 leaf and/or canopy damage, as observed for more anisohydric *Q. douglasii* (Feng *et al.*,
478 2019), results in an overall reduction of carbon assimilation, which is supported by lower leaf

area (Fig. 2e) and lower trunk increment (Supporting Information Fig. S1) for invaded *Q. suber* trees. All these features point towards chronic stress for trees which may results 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 be a driver for increased mortality rates of *Q. suber* (Costa *et al.*, 2010) in severe drought years, by the means of hydraulic failure and/or carbon starvation (McDowell *et al.*, 2008).

Contrastingly, our rain exclusion experiment alone did not modify the 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 summer (Núñez-Olivera *et al.*, 1996; Simões *et al.*, 2008). For *Q. suber* trees under the rain exclusion the higher σ and transpiration rates, especially in response to VPD_{max} in the winter periods point towards potential modifications of the hydraulic strategy under long-term recurrent extreme drought, which have to be explored in detail in the future. Such modifications of the hydraulic behaviour under drought are of high importance, as the Mediterranean basin is expected to develop into a meteorological drought hotspot (Spinoni *et al.*, 2020).

Conclusion

With this study, we demonstrate that biotic and abiotic factors influence the degree of isohydricity in plants. 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 hydraulic failure and thus, might contribute to an increase in tree mortality in cork oak, as has also been observed in many forest trees globally (Allen, Breshears, & McDowell, 2015), 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 of severe droughts. Thus, this calls for a better understanding of the potentially detrimental effects of invasion and plant competition on the hydraulic strategies in ecosystems endangered by droughts.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors have no conflict of interest to declare.

Author contribution

CW and MCC conceived the experimental design. SH, RLV and MCC conducted the fieldwork and field site maintenance. SH analysed the data and wrote the manuscript with input from CW, MCC, MD, RLV, and MC. All co-authors contributed significantly to data interpretation, discussion and revision of the manuscript.

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733 **Supporting information**

734 **Table S1.** Soil parameters for all experimental treatments (n = 27) with standard error for pH,
735 organic matter, nitrogen (N) and potassium (K) contents. Values are averaged over three
736 depths (0-10, 15-25, 30-40 cm).

737 **Figure S1.** Change in diameter at breast height (DBH) relative to DBH at 27/04/2018 for all
738 experimental treatments (n = 7-9) with standard error. Change in DBH was measured with
739 manual band dendrometers (DB20 dendrometer, EMS, Brno, Czech Republic). RE = Rain
740 exclusion.

741 **Table 1.** Parameters for the non-linear mixed effect models described in equation 1 and 2 with standard error (n = 9 per treatment and season)
742 for the relationship of transpiration (E) with volumetric soil water content (θ) in the summer periods and daily maximum vapour pressure deficit
743 (VPD_{max}) in the winter periods. The parameters were taken from the following equations: 1) $E = b \times \ln(VPD_{max}) + a$, where a is the transpiration (E)
744 at $VPD_{max} = 1$ kPa and b the sensitivity of the transpiration to VPD_{max} ; and 2) $E = \frac{E_{Asym}}{1 + \exp(\frac{\theta - \theta_{mid}}{\theta_{scal}})}$, where E_{Asym} is the horizontal asymptote of the
745 transpiration, θ_{mid} the θ , where E equals 50% of E_{Asym} and θ_{scal} describes the differences of θ and θ_{mid} , where about 75% of E_{Asym} is reached.

	Season	<i>Q. suber</i>				<i>C. ladanifer</i>	
Parameter	Treatment	Control	Rain Exclusion	Invasion	Invasion + Rain Exclusion	Invasion	Invasion + Rain Exclusion
E_{Asym} (mol m ⁻² day ⁻¹)	Summer 2018	58.3 ± 12.5 ^a	53.5 ± 11.3 ^a	75.6 ± 11.9 ^a	71.4 ± 10.3 ^a	73.7 ± 7.0 ^a	78.5 ± 9.4 ^a
	Summer 2019	32.6 ± 5.4 ^a	40.5 ± 15.8 ^a	36.6 ± 5.0 ^a	39.4 ± 7.3 ^a	33.5 ± 2.4 ^a	32.7 ± 5.4 ^a
θ_{mid} (%)	Summer 2018	8.9 ± 1.3 ^a	8.7 ± 0.5 ^a	9.9 ± 1.0 ^{ab}	9.0 ± 1.1 ^a	11.5 ± 0.7 ^b	11.4 ± 1.0 ^b
	Summer 2019	8.4 ± 0.5 ^a	8.9 ± 0.4 ^{ab}	8.6 ± 0.8 ^a	10.2 ± 0.9 ^{ab}	10.0 ± 0.8 ^{ab}	10.7 ± 1.1 ^b
θ_{scal} (%)	Summer 2018	3.4 ± 1.0 ^a	1.6 ± 0.2 ^b	2.1 ± 0.3 ^{ab}	2.2 ± 0.5 ^{ab}	1.9 ± 0.1 ^b	2.2 ± 0.3 ^{ab}
	Summer 2019	1.5 ± 0.5 ^{ab}	1.3 ± 0.4 ^{ab}	0.7 ± 0.04 ^a	1.7 ± 0.4 ^b	1.7 ± 0.2 ^b	1.7 ± 0.4 ^b
a (mol m ⁻² day ⁻¹)	Winter 2019	21.9 ± 3.3 ^a	28.4 ± 11.1 ^a	29.6 ± 6.6 ^a	31.0 ± 5.7 ^a	20.6 ± 1.0 ^a	19.4 ± 2.0 ^a
	Winter 2020	19.4 ± 2.9 ^{ab}	26.8 ± 7.4 ^{bc}	16.1 ± 4.5 ^a	23.1 ± 3.1 ^{abc}	30.8 ± 2.2 ^c	29.0 ± 4.7 ^{bc}
b	Winter 2019	9.7 ± 1.5 ^a	13.1 ± 4.6 ^a	11.9 ± 1.1 ^a	14.3 ± 2.8 ^a	9.0 ± 0.6 ^a	8.3 ± 1.2 ^a

	Winter 2020	10.8 ± 1.9^{ab}	16.2 ± 5.1^{bc}	9.0 ± 2.5^a	13.4 ± 1.7^{abc}	19.1 ± 1.2^c	19.0 ± 2.3^c
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Table 2. Slope (σ) and intercept (Λ) with standard error for the relationship of midday and pre-dawn leaf water potential according to Martínez-Vilalta *et al.* (2014) with p-value, marginal (m) and conditional (c) R^2 of the linear mixed effect model for summer (a) and winter (b).

(a) Summer	<i>Q. suber</i>				<i>C. ladanifer</i>	
Treatment	Control	Rain Exclusion	Invasion	Invasion + Rain Exclusion	Invasion	Invasion + Rain Exclusion
Λ	-1.56 ± 0.08^a	-1.50 ± 0.11^{ab}	-1.25 ± 0.07^{bc}	-1.15 ± 0.11^c	-1.63 ± 0.10^a	-1.50 ± 0.14^a
σ	0.32 ± 0.07^a	0.44 ± 0.09^{ab}	0.57 ± 0.09^b	0.57 ± 0.09^b	0.92 ± 0.04^a	0.98 ± 0.06^a
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
R^2 (m/c)	0.17/0.34	0.35/0.44	0.42/0.56	0.59/0.59	0.87/0.88	0.87/0.88
Strategy	Partial isohydric	Partial isohydric	Partial isohydric	Partial isohydric	Strict anisohydric	Strict anisohydric
(b) Winter	Control	Rain Exclusion	Invasion	Invasion + Rain Exclusion	Invasion	Invasion + Rain Exclusion
Λ	-0.66 ± 0.17^{ab}	-1.06 ± 0.21^a	-0.66 ± 0.23^{ab}	-0.47 ± 0.21^b	-1.29 ± 0.13^a	-1.23 ± 0.17^a
σ	0.86 ± 0.59^{ab}	-0.02 ± 0.70^a	0.93 ± 0.75^{ab}	1.49 ± 0.69^b	0.63 ± 0.17^a	0.76 ± 0.23^a
p-value	0.27	0.87	<0.05	<0.01	< 0.01	< 0.001
R^2 (m/c)	0.02/0.29	0.00/0.00	0.11/0.28	0.21/0.28	0.20/0.23	0.44/0.44
Strategy	(Partial isohydric)	(Strict isohydric)	(Strict anisohydric)	(Extreme anisohydric)	Partial isohydric	Partial isohydric

750

751 Figure Legends

752 **Figure 1.** Air temperature and maximum vapour pressure deficit (VPD_{max}) (a), precipitation
753 and volumetric soil water content (θ) for all experimental treatments ($n = 6$ per treatment) (b),
754 pre-dawn leaf water potential (Ψ_{PD}) for *Q. suber* (c) and *C. ladanifer* (d), and midday leaf
755 water potential (Ψ_{MD}) for *Q. suber* (e) and *C. ladanifer* (f) for all measurement dates and
756 experimental treatments ($n = 9$ per treatment). Asterisks (*) indicate p-values (linear mixed
757 effect model): $< 0.05^*$, $< 0.01^{**}$, $< 0.001^{***}$, where black asterisks indicate rain exclusion
758 effects and grey asterisks indicate invasion effects. Note the different scales in (c-f) for the
759 different species. RE = Rain exclusion.

760 **Figure 2.** Sap flow for *Q. suber* (a) and *C. ladanifer* (b), transpiration for *Q. suber* (c) and *C.*
761 *ladanifer* (d), and leaf area index (LAI) for *Q. suber* (e) and *C. ladanifer* (f) for the study
762 period and all experimental treatments ($n = 9$ for all treatments). Asterisks (*) indicate p-
763 values (linear mixed effect model): $< 0.05^*$, $< 0.01^{**}$, $< 0.001^{***}$, where black asterisks
764 indicate rain exclusion effects and grey asterisks indicate invasion effects. Note the different
765 scales for the species in (a) and (b). RE = Rain exclusion.

766 **Figure 3.** Non-linear relationship of leaf transpiration with volumetric soil water content (θ)
767 for summer 2018 (a) and summer 2019 (c) and non-linear relationship of leaf transpiration
768 with maximum vapour pressure deficit VPD_{max} for winter 2019 (b) and winter 2020 (d) for all
769 experimental treatments ($n = 9$ per treatment) of *Q. suber* and *C. ladanifer*. The non-linear
770 mixed effect models are described in detail in the material and methods section. Note the
771 different scale in summer 2018 (a) compared to the other seasons. RE = Rain exclusion.

772 **Figure 4.** 3D plot with 2D projections of midday leaf water potential (Ψ_{MD}), volumetric soil
773 water content (θ) and transpiration ($n = 9$ per treatment) for *Q. suber* (a) and *C. ladanifer* (b)
774 in winter (blue, $n = 5$) and summer periods (red, $n = 9$). Note the different scales of the axis

775 for midday leaf water potential (Ψ_{MD}) in (a) and (b). Large symbols represent the 3D
776 projections; small symbols represent the 2D projections. RE = Rain exclusion.

777 **Figure 5.** Relationship of pre-dawn leaf water potential (Ψ_{PD}) and midday leaf water potential
778 (Ψ_{MD}) including a 1:1 line (dashed) adopted from Martínez-Vilalta et al. (2014) for *Q. suber*
779 (a) separated into summer (c) and winter (e) and *C. ladanifer* (b) separated into summer (d)
780 and winter (f). Asterisks (*) indicate p-values for the regression lines: < 0.05* (short-dash), <
781 0.01** (long-dash), < 0.001*** (solid). Letters indicate differences between the slope (σ) of
782 the experimental treatments (n = 9 per treatment) for each season (n = 5 in winter; n = 9 in
783 summer) and species separately at a level of $p < 0.05$. Note the different x- and y-scales in all
784 panels. In panel (e), the regression lines and slopes are not shown, due to a weak statistical
785 relationship of Ψ_{PD} and Ψ_{MD} and high variability in the data. RE = Rain exclusion.

786 **Figure 6.** Conceptual illustration of seasonal (a) and plant invasion effects (b) on the slope
787 (σ , isohydricity) and intercept (Λ) of the relationship between pre-dawn (Ψ_{PD}) and midday
788 leaf water potential (Ψ_{MD}). Adapted and modified after Wu *et al.* (2020).