

1 **A new look at water transport regulation in plants**

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34 **Summary**

35

- 36 • Plant function requires effective mechanisms regulating water transport at a
37 variety of scales. Here we develop a new theoretical framework describing plant
38 responses to drying soil, based on the relationship between midday and predawn
39 leaf water potentials. The intercept of the relationship (Λ) characterizes
40 maximum transpiration rate per unit of hydraulic transport capacity; whereas the
41 slope (σ) measures the relative sensitivity of transpiration rate and plant
42 hydraulic conductance to declining water availability.
- 43 • This framework was applied to a newly compiled global database of leaf water
44 potentials to estimate the values of Λ and σ for 102 plant species.
- 45 • Our results show that our characterization of drought responses is largely
46 consistent within species, and that parameters Λ and σ show meaningful
47 associations with climate across species. Parameter σ was ≤ 1 in most species,
48 indicating a tight coordination between the gas and the liquid phases of water
49 transport, in which canopy transpiration tended to decline faster than hydraulic
50 conductance during drought, thus reducing the pressure drop through the plant.
- 51 • The quantitative framework presented here offers a new way of characterizing
52 water transport regulation in plants that can be used to assess their vulnerability
53 to drought under current and future climate conditions.

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56 **Keywords:** Drought stress, Isohydic/anisohydric behaviour, Leaf water potential, Plant
57 hydraulics, Stomatal responses, Water availability, Water transport, Xylem embolism

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67 **Introduction**

68 Plants are able to survive and function under extremely variable environmental
69 conditions, including dramatic changes in soil water availability and atmospheric
70 evaporative demand. This could not be achieved without powerful regulatory
71 mechanisms allowing plants to modulate water transport in response to those changes.
72 At relatively short time scales (*i.e.*, less than seasonal) this regulation is primarily
73 physiological rather than structural, and takes place mostly at two sites within the soil-
74 plant-atmosphere continuum (SPAC): stomata and the hydraulic transport system
75 connecting the soil with the leaves (Sperry *et al.*, 2002). Several components of the
76 hydraulic system of plants are known to vary as a function of water availability,
77 including the hydraulic conductivity of roots and the root-soil interface (*e.g.*, Maurel *et*
78 *al.*, 2010), as well as the xylem and extraxylary tissues (Hacke, 2014). The xylem is one
79 of the plant tissues where largest hydraulic conductivity losses occur during drought,
80 and it is certainly the tissue where these losses have been more thoroughly studied
81 (Tyree & Zimmermann, 2002).

82

83 The xylem of plants offers a low resistance pathway for water movement from roots to
84 the evaporation sites in leaves. Certain stress factors, such as drought and freezing,
85 induce the formation of emboli in xylem conduits, resulting in an overall loss of plant
86 hydraulic conductance and, therefore, increasing the pressure drop required to sustain a
87 certain transpiration rate (Tyree & Sperry, 1988; Tyree & Zimmermann, 2002). Under
88 severe water deficit hydraulic failure may result in the complete loss of plant hydraulic
89 conductance and the consequent desiccation of aboveground tissues (Choat *et al.*,
90 2012), eventually leading to drought-induced mortality (McDowell *et al.*, 2008).
91 Although there seems to be a certain level of reversibility in xylem embolism, even at
92 relatively short timescales (Zwieniecki & Holbrook, 2009; Brodersen *et al.*, 2010), most
93 of the evidence for novel refilling is controversial and should be treated with caution, as
94 it could be affected by measurement artifacts (Sperry, 2013; Rockwell *et al.*, 2014).

95

96 Stomata provide the most obvious mechanism allowing plants to control water transport
97 and loss under drought conditions. The aperture of stomata responds to both
98 atmospheric water demand and soil water content through a complex array of processes
99 that eventually result in changes in the turgor pressure of the guard cells that form the
100 pores or that of adjacent epidermal cells (Buckley, 2005). Plants have been classified

101 into two broad categories based on the ability of stomata to regulate leaf water potential
102 (Ψ_L) (Stocker, 1956; Jones, 1998; Tardieu & Simonneau, 1998). Isohydic species
103 adjust their stomatal opening in such a way as to maintain midday Ψ_L relatively stable
104 as environmental conditions change. On the contrary, anisohydric species have a less
105 strict stomatal control, with no discernible threshold of minimum Ψ_L . As a result, the Ψ_L
106 of anisohydric species tracks environmental fluctuations whereas in isohydric species
107 Ψ_L is highly buffered against those fluctuations. The fact that most plants are likely to
108 lay somewhere in between these two extreme theoretical behaviours is problematic if
109 the iso-anisohydric dichotomy is to be used to characterize drought response strategies,
110 and has lead to inconsistent classifications of the same species across or even within
111 studies (*e.g.*, Domec & Johnson, 2012). However, the iso-anisohydric categorization has
112 been used as a central tenet to describe different strategies by which plants cope with
113 drought stress and also to characterize the mechanisms underlying drought-induced
114 mortality in plants (McDowell *et al.*, 2008).

115

116 Overall, plant responses to limited water availability are complex and include
117 adjustments at a variety of organizational (stomata, leaf, whole plant...) and time scales
118 (Chaves *et al.*, 2003; Maseda & Fernández, 2006). This variety of behaviours and the
119 multiplicity of exceptions to any tentative general rule likely explain why a definitive
120 classification of plant water-use strategies and responses to drought has remained
121 somewhat elusive, despite the huge research effort that has been devoted to that topic.
122 Our first objective here is to develop a new theoretical framework to describe plant
123 responses to drying soil conditions based on the relationship between two commonly
124 measured ecophysiological parameters: midday and predawn leaf water potentials.
125 Secondly, we apply this scheme to a newly compiled global database of leaf water
126 potentials.

127

128 ***Theoretical framework***

129 Under steady-state conditions, water transport through the xylem (J) must balance
130 transpiration losses from leaves (E). This equality can be expressed as (Whitehead *et al.*,
131 1984):

132

$$133 \quad E = g_L \cdot A_L \cdot D = -k_s \cdot A_s \cdot (\psi_L - \psi_s) = J \quad (\text{Eqn 1})$$

134

135 where g_L is leaf conductance for water vapour, D is the vapour pressure deficit of the
136 atmosphere, k_S is whole plant hydraulic conductance per unit of basal sapwood cross-
137 sectional area, A_L and A_S are leaf area and basal sapwood area, and Ψ_L and Ψ_s are the
138 water potential in leaves and in the soil, respectively. The gravitational component of
139 the water potential gradient is omitted for simplicity and for consistency with the
140 nomenclature used below. We also assume here that steady-state conditions are a
141 reasonable approximation at seasonal or longer timescales, whereas capacitance needs
142 to be considered when studying shorter-term responses (Meinzer *et al.*, 2009).

143

144 Eqn 1 above can be rearranged to obtain:

145

$$146 \quad \psi_L - \psi_s = -\frac{E}{k_S \cdot A_S} = -\frac{g_L \cdot A_L \cdot D}{k_S \cdot A_S}. \quad (\text{Eqn 2})$$

147

148 As soil drought develops (*i.e.*, Ψ_s declines, becoming more negative), some of the plant
149 parameters in Eqn 2 remain constant or vary typically over relatively long time scales
150 (A_S , A_L), whereas others can vary in the short term. In particular, k_S is likely to decline
151 due to the occurrence of xylem embolism (among other processes) and g_L will be
152 reduced by stomatal closure. The changes in k_S and g_L with drought have been described
153 using many different equations (*e.g.*, Pammenter & Willigen, 1998, for k_S ; Oren *et al.*,
154 1999; and Granier *et al.*, 2000, for g_L). Here, we describe both relationships as a
155 function of Ψ_s . These functions are not intended to model a direct mechanistic link, but
156 to capture a strong empirical pattern that arises from potentially complex mechanisms.
157 Our argument only requires that an overall response of k_S and g_L to Ψ_s (f_k and f_g ,
158 respectively) can be defined. Eqn 2 can thus be rewritten as:

159

$$160 \quad \psi_L = \psi_s - \frac{A_L \cdot D \cdot g_{L,\max} \cdot f_g(\psi_s)}{A_S \cdot k_{S,\max} \cdot f_k(\psi_s)} = \psi_s + \Lambda \cdot \frac{f_g(\psi_s)}{f_k(\psi_s)} = \psi_s + \Lambda \cdot \Theta(\psi_s), \quad (\text{Eqn 3})$$

161

162 where Λ is a measure of (maximum) transpiration rate per unit of water transport
163 capacity or, equivalently, the leaf water potential at $\Psi_s \approx 0$ (the pressure drop or
164 ‘pulling’ capacity of the plant when there is plenty of water available in the soil), f_g and

165 f_k are the functions describing the reductions in g_L and k_S , respectively, with declining
166 Ψ_s , and Θ is the ratio of these functions. Several types of f_g and f_k functions have been
167 used in the literature, with two-parameter sigmoid or Weibull functions being among
168 the most frequent (e.g., Neufeld *et al.*, 1992; Pammenter & Willigen, 1998; Sperry *et*
169 *al.*, 1998; Hoffmann *et al.*, 2011).

170 Note that when $\Psi_s = 0$ by definition $f_g = f_k = 1$, so that Λ is the intercept of the
171 relationship in Eqn 3. Interestingly, if Λ is assumed to be relatively constant, at the
172 temporal scales of interest, compared to g_L and k_S , it follows that the relative sensitivity
173 of stomata and plant hydraulic conductance to declining soil water potentials (f_g / f_k)
174 determines whether the water potential gradient in the plant declines, increases or stays
175 approximately constant as drought progresses (Fig. 1). In more general terms (i.e.,
176 without making any assumptions on how or where water transport is regulated), Eqns 2
177 and 3 imply that the pressure drop in the plant will increase if hydraulic conductance
178 declines faster than transpiration rate as drought progresses, whereas it will be reduced
179 if transpiration rate declines faster than plant hydraulic conductance.

180 If it is assumed that the Θ function in Eqn 3 is approximately linear within biologically
181 reasonable ranges of water potentials, the relationship between Ψ_L and Ψ_s becomes also
182 a linear function and its slope (σ) determines the magnitude of the reduction in Ψ_L as Ψ_s
183 declines (see Supporting Information, Notes S1):

184

$$185 \quad \psi_L = \psi_s + \Lambda \cdot \Theta(\psi_s) \approx \Lambda + \sigma \cdot \psi_s. \quad (\text{Eqn 4})$$

186

187 In this equation the value of σ determines the behaviour of plants according to the
188 classical iso/anisohydry paradigm. A $\sigma = 0$ implies strict isohydry (constant Ψ_L as Ψ_s
189 declines), whereas $\sigma = 1$ would imply strict anisohydry (the difference between Ψ_L and
190 Ψ_s stays constant). Note that two other behaviours are possible (*cf.* Fig. 1): for $\sigma > 1$
191 there is extreme anisohydry, implying an increase in the pressure drop through the plant
192 as Ψ_s declines; whereas $0 < \sigma < 1$ implies a sort of partial isohydry, by which the
193 difference between Ψ_L and Ψ_s is reduced as Ψ_s declines (please note that our
194 nomenclature differs from that proposed by Franks *et al.* (2007) to describe similar
195 forms to control water status).

196 We aimed at using this theoretical framework and a newly developed global database of
197 leaf water potentials to test the following hypotheses: (1) the relationship described in
198 Eqn 3 can be approximated by a linear function, as expressed in Eqn 4, and its
199 parameters are mostly consistent within species (within limits of statistical
200 detectability); (2) the slope (σ) of the relationship between Ψ_L and Ψ_s is close to 1 in
201 most species, reflecting a close coordination between stomatal and hydraulic responses
202 to drought; (3) variation in σ across species would reflect differences in environmental
203 conditions and plant hydraulic traits, so that low values of σ (transpiration reduced
204 much faster than plant hydraulic conductance during drought) will occur in
205 environments characterized by high evaporative demands or in species showing large
206 pressure drops under well watered conditions (low Λ) and high vulnerability to xylem
207 embolism (overcompensation).

208

209

210 **Material and methods**

211

212 *Literature searches*

213 A literature search was conducted in July 2010 using Google Scholar and the terms:
214 (leaf OR needle) + midday + “predawn water potential”. All the > 400 references in the
215 initial list were checked for measured values of leaf water potential. The software
216 TechDig (Version 2.0, Ronald B Jones) was used to retrieve individual data points from
217 published figures. The following criteria were used to include individual papers in the
218 final database: (1) they had to be published in the primary scientific literature; (2) no
219 direct modifications of water potentials in the plant were conducted, whereas
220 experimental treatments such as irrigation or drought simulation were allowed; (3) leaf
221 water potentials had been monitored over a period of more than one month (*i.e.*, short
222 term studies focusing on diurnal changes were excluded); (4) predawn and midday
223 water potentials were measured concurrently over time and the number of data pairs
224 was > 5. One study (Hamerlynck *et al.*, 2000) was not considered because it was unique
225 in showing consistently lower (more negative) predawn than midday leaf water
226 potentials, suggesting that the measurements were conducted under very particular
227 conditions that may not be representative. In all the analyses midday (Ψ_{MD}) and
228 predawn leaf water potentials (Ψ_{PD}) were used as proxies of Ψ_L and Ψ_s , respectively. In

229 doing so we assumed that plant and soil water potential equilibrate overnight, which is
230 not always the case (Donovan *et al.*, 2003).

231

232 A total of 83 articles fulfilled the previous criteria and were included in our database
233 (see Table S1). In most cases the studies were conducted in natural conditions in the
234 field (83%), although some studies carried out on crop fields, potted plants or
235 experimental containers were included. An additional filtering was carried out at the
236 species level, so that only species for which the overall range of predawn leaf water
237 potential was > 0.6 MPa were retained. The final database contained data for 102
238 species sampled in five continents (see Fig. S1), including representatives from the
239 Temperate ($N = 44$, including one Boreal species), Mediterranean ($N = 33$), Tropical (N
240 $= 15$) and Desert ($N = 10$) biomes. All Tropical species except one corresponded to the
241 Dry Tropical biome (Table S1). The predominance of species from relatively dry
242 regions corresponds to the fact that leaf water potentials have been widely used to study
243 plant responses to drought, whereas they have been measured less frequently in wet
244 environments (*e.g.*, Tropical rainforests). Regarding growth habits and functional types
245 15 species were conifers, 46 were angiosperm trees (broadleaves), 28 angiosperm
246 shrubs and 13 were herbaceous (see Table S1).

247

248 ***Additional datasets***

249 Average climatic variables for the reference period 1961-1990 for each study location
250 were obtained from the CRU CL 2.0 gridded dataset, with a spatial resolution of 10'
251 (New *et al.* 2002). The following variables were considered: mean annual temperature
252 (MAT), mean summer temperature (June-August in the Northern hemisphere and
253 December-February in the Southern hemisphere, MST), mean diurnal temperature range
254 (MDTR), mean annual precipitation (MAP), coefficient of variation of monthly
255 precipitation (CVMP), mean summer precipitation (June-August, MSP), mean annual
256 vapour pressure difference (MAD), and mean summer vapour pressure difference (June-
257 August, MSD). These values were averaged across all the locations in which each
258 species had been sampled to obtain a mean value for each species. A preliminary
259 analysis including correlation and principal component analyses showed that some of
260 the previous climatic variables contained highly redundant information and we selected
261 MAT, CVMP, MSP and MSD as four relatively independent ($r < 0.5$ in all cases)
262 climate descriptors. These four variables were used in all further analyses.

263

264 Wood hydraulic traits were obtained from the Xylem Functional Traits dataset compiled
265 by Choat *et al.* (2012). The following variables were extracted from the database (the
266 number of common species with our dataset, which determined the sample size of the
267 corresponding analyses, is also given for each variable): the water potential at which
268 50% of hydraulic conductivity is lost due to xylem embolism (P_{50}) ($N = 49$), the water
269 potential at which 88% of hydraulic conductivity is lost due to xylem embolism (P_{88}) (N
270 $= 47$), the slope of the vulnerability curve ($N = 48$), maximum specific hydraulic
271 conductivity (K_S) ($N = 41$), maximum leaf-specific hydraulic conductivity (K_L) ($N =$
272 27), and leaf-to-sapwood area ratio ($A_L:A_S$) ($N = 25$). All variables correspond to
273 measurements taken on branches. When more than one value was available for a given
274 species the values were averaged to obtain a single, representative value per species.

275

276 ***Data analysis***

277 Mixed linear models were used to fit the (seasonal) relationship between predawn and
278 midday leaf water potential within and across species (Figs 1 and 2). Species and the
279 combination of study by treatment nested within species were included as random
280 effects and the species-specific slopes and intercepts of the model were allowed to co-
281 vary. By treatment here we refer to sets of plants of a given species that were measured
282 under different environmental conditions in a particular study, regardless of the nature
283 of the treatment (*e.g.*, different experimental drought treatments, but also different
284 measured populations in observational studies). In a preliminary analysis, different one-
285 parameter functions were used to fit the relationship between Ψ_{MD} and Ψ_{PD} , including
286 linear, logarithmic and exponential relationships. However, a linear function gave a
287 much better fit in terms of AIC and explained variance (see also Fig. 2) and was finally
288 selected. Higher order functions (*e.g.*, quadratic) were also tried but were abandoned
289 because the resulting model coefficients were highly correlated between each other ($r =$
290 0.99), indicating that they could not be resolved with the empirical data available. The
291 species-level random coefficients of the fitted model were used to estimate the value of
292 the intercept (Λ) and slope (σ) of the relationship between Ψ_{MD} and Ψ_{PD} for each
293 species in our dataset.

294

295 In order to test for the consistency of the estimated parameter values within species, we
296 selected all those species for which we had data for at least two study by treatment

297 combinations. For this subset of species ($N = 61$) we ran a variance components analysis
298 based on the same mixed model described above. Additionally, for each of these species
299 (separately) we ran a linear model (without random effects) of the relationship between
300 predawn and midday leaf water potentials in which study by treatment combinations
301 (ranging between 2 and 21, depending on the species) were introduced as a fixed factor
302 affecting the intercept and the slope of the relationship. This model was compared to the
303 base model assuming homogeneous intercept and slope across study by treatment
304 combinations.

305

306 We used linear models to study the relationships between the species-level parameters
307 Λ and σ and the climatic variables described above. For hydraulic traits we used
308 correlation analysis to study their association with parameters Λ and σ . Factors coding
309 for functional type (conifer, angiosperm tree, angiosperm shrub, herbaceous) and biome
310 (Temperate, Mediterranean, Tropical, Desert) were also included in models. In all cases,
311 we compared these base models with the equivalent model including phylogenetic
312 effects, by means of phylogenetic generalized least squares (PGLS; Paradis, 2006). In
313 addition, phylogenetic effects on response variables were explored using the coefficient
314 λ as a measure of phylogenetic correlation (Freckleton *et al.*, 2002), based on a
315 Brownian motion evolutionary model ($\lambda = 0$ indicates evolution of traits independent of
316 phylogeny, whereas $\lambda = 1$ indicates that traits have evolved according to a Brownian
317 motion model) and Moran's autocorrelation index I applied to different taxonomic
318 levels (Paradis, 2006).

319

320 A phylogenetic tree for the study species was constructed in Phylomatic 2 (Webb &
321 Donoghue, 2005), http://phylodiversity.net/phyloomatic/html/pm2_form.html), starting
322 from an updated megatree based on a recent classification by the Angiosperm
323 Phylogeny Group (APGIII; [https://github.com/camwebb/tree-of-](https://github.com/camwebb/tree-of-trees/blob/master/megatrees/R20100701.new)
324 [trees/blob/master/megatrees/R20100701.new](https://github.com/camwebb/tree-of-trees/blob/master/megatrees/R20100701.new)). Phylogenetic relationships within
325 families were resolved using published phylogenies for Anacardiaceae (Pell, 2004;
326 Wannan, 2006), Asteraceae (Bayer & Starr, 1998; Roberts & Urbatsch, 2004),
327 Betulaceae (Chen *et al.*, 1999), Ericaceae (Kron *et al.*, 2002), Oleaceae (Wallander &
328 Albert, 2000), Pinaceae (Wang *et al.*, 2000; Gernandt *et al.*, 2005), Poaceae (Hsiao *et*
329 *al.*, 1995; Hilu *et al.*, 1999; Catalán *et al.*, 2004) and Rosaceae (Potter *et al.*, 2007).

330 Additional phylogenetic trees were used to solve other politomies at genus level for
331 *Acacia* (Miller & Bayer, 2001; Bouchenak-Khelladi *et al.*, 2010), *Populus* (Hamzeh &
332 Dayanandan, 2004) and *Quercus* (Manos *et al.*, 1999; Bellarosa *et al.*, 2005). Family-
333 level politomies only remained unresolved for Poaceae; some politomies within genera
334 for *Acacia*, *Quercus*, *Ceanothus* and *Rhamnus* were also left unresolved.

335

336 We fitted each PGLS model assuming an Ornstein–Uhlenbeck (OU) model of character
337 evolution. All analyses were conducted with the software R (v. 2.12, the R Foundation
338 for Statistical Computing) using the packages *nlme*, *lme4*, *ape*, *ade4* and *pglm3.2*. The
339 residuals of all reported models showed no obvious pattern. Significance for all
340 statistical analyses was accepted at $\alpha = 0.05$. The R^2 (explained variance) of the mixed
341 models was estimated including both fixed and random factors using a likelihood ratio
342 statistic (Magee, 1990).

343

344

345 **Results**

346 The study species covered a wide range of minimum predawn leaf water potentials
347 ($\Psi_{PD,min}$), ranging from -0.7 MPa in the Tropical tree *Schima wallichii* to -11.4 MPa in
348 the Mediterranean shrub *Rhamnus crocea*. $\Psi_{PD,min}$ was affected by functional type, with
349 significantly lower values in shrubs (2.3–3.0 MPa more negative than the other
350 functional types, on average); and it also varied by biome, with Mediterranean species
351 showing the lowest $\Psi_{PD,min}$, followed by Desert species and by Temperate and Tropical
352 species, the latter two having similar values (Fig. S2).

353

354 Our linear model of the relationship between predawn and midday leaf water potentials
355 (Eqn 4) provided a good fit to the data. Overall, the model explained 90% of the
356 variability in Ψ_{MD} . The overall slope of the relationship between midday and predawn
357 water potential was $0.86 \text{ MPa} \cdot \text{MPa}^{-1}$ (*i.e.*, a value < 1 implies that the pressure drop
358 through the plant diminishes with drought), and varied across species between 0.19 for
359 *Acacia etbaica* (a Tropical tree) and $1.36 \text{ MPa} \cdot \text{MPa}^{-1}$ for *Prosopis glandulosa* (a Desert
360 tree). The value of the intercept (Λ) varied substantially across species, ranging between
361 -3.2 (*Balanites aegyptiaca*, a Tropical tree) and -0.2 MPa (*Eragrostis curvula*, a
362 Tropical grass).

363

364 Overall, the estimated σ values for most species was consistent with either strict
365 anisohdry ($\sigma \approx 1$, $N = 55$) or partial isohdry ($0 < \sigma < 1$, $N = 42$). Only five species
366 showed σ values significantly greater than 1 (extreme anisohdry) and none showed
367 strict isohdry as defined in this study ($\sigma \approx 0$) (Figs 3 and S3). In 80 of 102 species
368 (78%) estimated $\sigma < 1$, implying a faster decline of canopy transpiration than plant
369 hydraulic conductance (hence a decline in the plant pressure drop) in response to drying
370 soil, although the value of σ was not significantly different from 1 in all cases (Fig. S3).
371 The slope parameter σ was unaffected by functional type or biome ($P > 0.05$ in all
372 cases). The intercept Λ was also similar across functional types ($P > 0.05$), but its value
373 was ~ 0.4 MPa lower (*i.e.*, more negative) in Desert species compared to either
374 Temperate or Mediterranean ones ($P < 0.05$), while Tropical species showed
375 intermediate values (see Fig. S2).

376

377 There were 61 species for which measurements were available for different studies or
378 treatments. Parameter Λ differed significantly across study by treatment combinations
379 (*i.e.*, within species) in 20 of the corresponding linear models, whereas σ was different
380 across study by treatment combinations in only 8 of 61 cases. A variance components
381 analysis on the mixed model fitting the relationship between predawn and midday leaf
382 water potentials but using only the data for this 61 species showed that the species level
383 accounted for 56% of the total variance in midday leaf water potential, compared to
384 10% explained by different study by treatment combinations within species. Finally, an
385 error analysis to assess the error in the estimation of σ and Λ as a function of sample
386 size (number of Ψ_{PD} , Ψ_{MD} data pairs) revealed that these values could be estimated
387 relatively well using sample sizes of 8 data pairs or more (Notes S2). Taken as a whole,
388 and considering the large variability in measured water potentials, these results indicate
389 that the obtained parameter values were largely consistent within species and can be
390 used to characterize species responses to varying soil water availability.

391

392 With regard to phylogenetic effects, similar results were obtained for the two fitted
393 parameters, indicating no evidence of phylogenetic correlation (see also Fig. S4). For σ ,
394 $\lambda = 0.16$, which was significantly different from 1 ($\chi^2 = 46.7$, $P < 0.001$) but not from 0
395 ($\chi^2 = 1.1$, $P = 0.29$); and for Λ the estimated λ was 0.04, which was again significantly

396 different from 1 ($\chi^2 = 50.9$, $P < 0.001$) but not from 0 ($\chi^2 = 0.2$, $P = 0.67$). The same
397 test applied to $\Psi_{PD,\min}$ showed a higher value of $\lambda = 0.84$, which was significantly
398 different from 0 ($\chi^2 = 5.8$, $P = 0.02$) but not from 1 ($\chi^2 = 1.0$, $P = 0.33$). These results
399 were largely consistent with those of autocorrelative models showing significant
400 Moran's I indices at the genus and family levels for $\Psi_{L,\min}$ ($I \sim 0.6$ in both cases), non-
401 significant values for the slope parameter (σ) and significant values only at the family
402 level for Λ ($I = 0.18$).

403

404 The parameters Λ and σ were significantly related to each other (slope = 0.29, $r = 0.74$,
405 $P < 0.001$; Fig. 4), regardless of whether phylogenetic effects were taken into account.
406 The result was also very similar (slope = 0.28, $r = 0.68$, $P < 0.001$) if the relationship
407 between midday and predawn leaf water potentials (Eqn 4) was fitted using centered
408 instead of raw Ψ_{PD} values (results not shown). The parameters Λ and σ were both
409 unrelated to the minimum predawn leaf water potential measured on each species
410 ($\Psi_{PD,\min}$), regardless of whether phylogenetic effects were included or not ($P > 0.05$ in
411 all cases). However, Λ and σ were clearly associated to climate and these relationships
412 were robust to phylogenetic effects (Fig. 5, Table 1). Parameter Λ declined with annual
413 temperature and summer vapour pressure deficit and increased as a function of annual
414 precipitation variability and summer precipitation (Table 1b), whereas σ was negatively
415 related to mean summer vapour pressure deficit (Table 1a). Adding functional type into
416 the relationships between parameters Λ and σ and climate resulted in non-significant
417 coefficients for functional types and nearly identical coefficients for climate variables
418 (results not shown).

419

420 Parameter σ was negatively related to the vulnerability to xylem embolism at the
421 species level, with lower σ values occurring in more vulnerable species (i.e., more
422 vulnerable species showed a greater reduction in their pressure drop than more resistant
423 ones as Ψ_{PD} declined; Fig. 6). There was also a strong relationship with the slope of the
424 vulnerability curve, so that the relationship was tighter between σ and the water
425 potential causing 88% embolism (P_{88}) than with the water potential causing 50% loss of
426 hydraulic conductivity (P_{50}). These relationships remained significant and very similar
427 if phylogenetic effects were included in the models (results not shown). In all cases, the
428 fit of the models worsened if functional type or biome were included as additional

429 factors (with effects on the intercept and slope). There was no significant association
430 between σ and specific hydraulic conductivity (K_S), leaf-specific hydraulic conductivity
431 (K_L) or leaf-to-sapwood area ratio ($A_L:A_S$) ($P > 0.4$ in all cases). Finally, parameter Λ
432 was unrelated to any of the hydraulic traits considered in this study ($P > 0.1$ in all
433 cases).

434

435

436 **Discussion**

437 In this paper we propose a simple and novel approach to study water transport
438 regulation in plants, based on the relationship between midday and predawn leaf water
439 potentials. This relationship is characterized using two parameters: an intercept (Λ)
440 measuring the transpiration stream relative to the plant hydraulic capacity under well
441 watered conditions (or, equivalently, the leaf water potential at $\Psi_s \approx 0$), and a slope (σ)
442 characterizing the relative sensitivity of transpiration rate and plant hydraulic
443 conductance to declining soil water potential (Eqn 4). We postulate that this latter
444 parameter is more relevant than either stomatal sensitivity to drought or vulnerability to
445 xylem embolism in determining the temporal dynamics of leaf water potentials and that
446 our analysis provides synthetic, quantitative measures that can be used to characterize
447 water use strategies and drought responses in plants.

448

449 ***The link between stomatal function and plant hydraulics***

450 At the core of our approach is the realization that the response of the plant's water
451 potential gradient to declining soil water availability is not determined directly by
452 stomatal sensitivity to drought or, more generally, to the sensitivity of transpiration rate
453 to drought, but by the ratio between this sensitivity and the vulnerability of the plant
454 hydraulic system (due to, *e.g.*, xylem embolism). This fact has several important
455 implications, as it makes the link between stomatal function and the dynamics of leaf
456 water potential (in terms, for instance, of the isohydric vs. anisohydric strategies) less
457 straightforward than implied in previous reports (*e.g.*, Jones, 1998; Tardieu &
458 Simonneau, 1998). In our view, a plant with highly sensitive stomata closing at
459 relatively high water potentials could still show a strict anisohydric behaviour (*i.e.*, ever
460 declining leaf water potentials until the minimum tolerable value is reached at any given

461 point in the plant's hydraulic continuum) provided that its hydraulic transport system is
462 even more sensitive than stomata to declining water availability (Fig. 1).

463

464 The notion that leaf gas exchange and plant hydraulics are tightly linked is not new
465 (Meinzer, 2002; Sperry *et al.*, 2002; Mencuccini, 2003). Previous studies have shown
466 that xylem hydraulic conductivity is positively related to leaf photosynthetic capacity
467 (e.g. Brodribb & Feild, 2000) and stomatal conductance (e.g., Nardini & Salleo, 2000;
468 Zhang & Cao, 2009; Héroult *et al.*, 2013) across species, and also that tree species with
469 higher vulnerability to xylem embolism tend to have higher stomatal conductance
470 (Maherali *et al.*, 2006). What is less abundant in the literature is the direct comparison
471 between stomatal and plant hydraulic responses to declining water potential. In one of
472 the few articles taking this approach, Brodribb *et al.* (2003) found that the water
473 potential inducing 50% stomatal closure was linearly correlated with the water potential
474 inducing a 20% loss of xylem hydraulic conductivity in the stem, and that the slope of
475 the corresponding relationship was close to one, implying that stomatal conductance
476 was more sensitive to water potential than stem hydraulic conductivity. Similar results
477 were obtained by Arango-Velez *et al.* (2011) across poplar clones.

478

479 We believe that Eqns 3 and 4 above provide a quantitative framework to interpret the
480 close coordination between stomatal regulation and plant hydraulics. Our results show
481 that stomatal closure tends to occur somewhat faster than hydraulic conductivity loss in
482 response to declining soil water potential, as implied by the slope (σ) values, which
483 were slightly below 1 in most species (Figs 3 and S3). Very shallow slope values ($\sigma \approx$
484 0) would imply a safe strategy in terms of embolism avoidance, as stomata would close
485 completely before substantial loss of hydraulic conductivity is observed. However, such
486 a strategy would incur a high cost in terms of reduced carbon gain and it is thus likely to
487 be selected against. At the opposite extreme, $\sigma > 1$ implies that hydraulic transport
488 limitations (e.g., xylem embolism) occur faster than stomatal closure in response to
489 declining water potential. This result was observed in nearly one fourth of the species in
490 our database (although the standard error of the estimated slopes included 1 for 17 of
491 these 22 species; Figs 3 and S3). Such a strategy might seem disadvantageous because
492 stomatal conductance loss is more easily reversed than hydraulic conductivity loss in
493 the xylem, which may eventually lead to whole-plant mortality through hydraulic

494 failure (Tyree & Sperry, 1988; McDowell *et al.*, 2008). However, some level of xylem
495 embolism may be advantageous in terms of maximizing transpiration and assimilation
496 rates (Jones & Sutherland, 1991; Manzoni *et al.*, 2013a), and may occur in
497 phreatophytes with direct access to ground water (*e.g.*, *Prosopis glandulosa*; Fig. S3) or
498 in other cases where extremely low Ψ_s values are unlikely to occur. A value of $\sigma > 1$
499 could also occur during the process of disconnecting hydraulically from the soil in
500 drought-deciduous species.

501

502 ***Climatic and hydraulic correlates of the regulation of leaf water potential***

503 Our two descriptors of the relationship between predawn and midday leaf water
504 potentials (parameters Λ and σ) were associated to climate at the species level (Fig. 5).
505 Overall, species living in drier areas (high temperature and evaporative demand,
506 consistently low rainfall) tended to have higher pressure drops under well watered
507 conditions (more negative values of Λ). This result implies a greater gradient of water
508 potential within the plant at drier sites, even under well-watered conditions. This pattern
509 is likely related to the fact that plant species growing in drier environments are generally
510 able to sustain more negative water potentials (Maherali *et al.*, 2004; Choat *et al.*, 2012;
511 Manzoni *et al.*, 2013b) and tend to have lower hydraulic conductivity (Manzoni *et al.*,
512 2013b; but see Maherali *et al.*, 2004). The fact that σ responded only to D , with lower
513 values (more sensitive stomata relative to the hydraulic transport system) at sites with
514 higher evaporative demands, is consistent with the well known negative relationship
515 between stomatal conductance and D (*e.g.*, Oren *et al.*, 1999). Similarly, the tight and
516 positive relationship between Λ and σ (Fig. 4) may be interpreted analogously to the
517 association between reference stomatal conductance (at $D = 1$ kPa) and the (absolute)
518 sensitivity of stomatal conductance to D (Oren *et al.*, 1999); that is, species with higher
519 transpiration per unit of hydraulic transport capacity (more negative values of Λ) require
520 a stricter regulation of water loss to limit the decline in Ψ_L as soil water availability
521 declines (lower σ).

522

523 The negative relationship between σ and the vulnerability to xylem embolism (Fig. 6)
524 implies that species with more vulnerable xylem tend to compensate by having even
525 more sensitive stomata. Regardless of the mechanism behind this relationship (Nardini
526 & Salleo, 2000) its end result is the prevention of catastrophic levels of hydraulic

527 conductivity loss. The fact that the parameters σ and Λ did not show a significant
528 phylogenetic signal contrasts with previous results showing that there is some level of
529 phylogenetic conservatism in the vulnerability to xylem embolism (Maherali *et al.*,
530 2004). It should be noted, however, that this result might be confounded to some degree
531 by the tight association between σ and Λ (Fig. 4). When we tried to isolate the effect of
532 the relative sensitivity of stomata and the hydraulic system using Eqn S5 (*cf.* Notes S1),
533 the corresponding parameter (c_{gk} in Notes S1) showed phylogenetic conservatism ($\lambda =$
534 0.32, significantly different from 0 and from 1). A tight phylogenetic coordination
535 between σ and Λ would also tend to blur the phylogenetic signal in either parameter.

536

537 ***Limitations and potentialities***

538 The approach proposed here, as any similar attempt, is based on several assumptions.
539 Perhaps the most important one is that a response to soil (or predawn) water potential
540 can be defined for both plant hydraulic conductance and stomatal conductance or, more
541 generally, transpiration rate. This seems to be the case for the xylem, as embolism is
542 believed to be a direct response to water potential (Tyree & Zimmermann, 2002). Note
543 also that we do not make any particular assumption as to where hydraulic conductivity
544 losses start to develop and, as long as an overall relationship between whole-plant
545 hydraulic conductance and predawn water potential can be defined, our approach should
546 be robust regardless of where the main hydraulic bottleneck is (*cf.* Jackson *et al.*, 2000;
547 Johnson *et al.*, 2011; Nardini *et al.*, 2012). A similar reasoning can be applied to
548 stomatal conductance, although the situation is even more complex there as stomata
549 respond to soil water availability and leaf water status through a complex set of
550 chemical and hydromechanic signals (Buckley, 2005; Damour *et al.*, 2010).

551

552 In addition, the fact that leaf area may vary at the temporal scale of our analysis and that
553 vapour pressure deficit (D) frequently co-varies with soil water potential implies that we
554 are not necessarily characterizing a stomatal response to soil water potential, but an
555 overall response of transpiration rate to water availability (*cf.* Eqns 2 and 3). It is also
556 well known that soil and plant water potentials may not be in equilibrium, particularly
557 in dry soils (Donovan *et al.*, 2003). However, this decoupling is likely to affect in a
558 similar way the responses for both transpiration and plant hydraulic conductance. The
559 previous considerations do not invalidate our analysis but altogether they imply that the

560 $\sigma < 1$ values observed in most species should be interpreted to mean that water loss
561 regulation begins before hydraulic transport limitations start to occur, not necessarily
562 that stomata are more sensitive than the hydraulic system to water potential measured at
563 one particular point within the SPAC.

564

565 Another important assumption is that a linear function is a reasonable approximation of
566 the relationship between predawn and midday leaf water potential within species (Eqn
567 4). Although this is largely consistent with the empirical data used in this study (Fig. 2)
568 and statistically robust towards alternative assumptions, it seems clear that highly non-
569 linear relationships are possible, particularly when the hydraulic system is much more
570 sensitive than stomata or when stomatal conductance is close to zero (Notes S1). Our
571 results show that a linear function is a good descriptor of the relationship in Eqn 3, and
572 the best one using only two parameters. More complex functions (*e.g.*, with three
573 parameters) can be used in further studies focusing on drought responses on one or a
574 few intensively studied species. Importantly, the interpretation of the resulting
575 functions, even if more complex than those assumed here, would still be consistent with
576 our general framework, as their shape would always reflect the relative sensitivity of
577 transpiration rate and hydraulic conductance to declining water potentials. Note,
578 however, that the use of more complex functions is at the expense of simplicity and
579 generality, and could not be applied to our global water potentials database because
580 most individual datasets lacked the required level of detail.

581

582 Finally, our framework is based on the assumption that steady-state conditions are a
583 reasonable approximation of water flow through plants at seasonal time scales. The
584 importance of hydraulic capacitance is undeniable (*e.g.*, Meinzer *et al.*, 2009),
585 particularly at relatively short time scales, and could influence the shape of the
586 relationship between Ψ_{MD} and Ψ_{PD} even at the longer time scales considered here.
587 However, our approach seems a reasonable first approximation and it is consistent with
588 previous synthesis efforts at comparable temporal scales (*e.g.*, Oren *et al.*, 1999;
589 Manzoni *et al.*, 2013).

590

591 An important advantage of our approach is that it is based on predawn and midday leaf
592 water potentials, perhaps the most commonly measured variables in ecophysiological
593 studies (*e.g.*, Bhaskar & Ackerly, 2006). In addition, our analyses demonstrate that the

594 estimated parameters (Λ and σ) show meaningful associations with climate variables
595 and are largely consistent within species, implying that they can be used to characterize
596 species behaviour (see also Notes S2). In that regard, the slope parameter σ provides a
597 quantitative index to locate species along the continuum between isohydric ($\sigma \approx 0$) and
598 anisohydric ($\sigma \approx 1$) behaviour and a more precise characterization of drought responses
599 than the qualitative approaches used thus far. The fact that σ explicitly relates stomatal
600 sensitivity to plant hydraulic vulnerability to drought provides a useful descriptor in the
601 context of studying the mechanism of drought-induced mortality in plants, as it directly
602 relates to the carbon starvation and hydraulic failure hypotheses and their interaction
603 (McDowell *et al.*, 2008; McDowell, 2011). It helps to explain, for instance, why species
604 with a stronger stomatal control are not necessarily less prone to hydraulic failure (cf.
605 Martínez-Vilalta *et al.*, 2003; Meinzer *et al.*, 2009; Nardini *et al.*, 2013), as they may
606 reach very high levels of xylem embolism (measured for example as the P_{88}) before
607 their stomata are completely closed (measured as the point at which $\Psi_{PD} = \Psi_{MD}$, Fig. 1).
608 In that regard, our analysis could be expanded to include a third parameter to
609 characterize species: Ψ_{PD} at cessation of gas exchange. This value could be estimated as
610 $\Lambda/(1-\sigma)$ for relatively isohydric species ($\sigma < 1$) and as the water potential causing 100%
611 loss of hydraulic conductivity for anisohydric species ($\sigma \geq 1$).

612

613 We are still far from completely understanding plant strategies to cope with drought and
614 clearly there is no single metric that is able to synthesize the plethora of responses
615 observed across species. At the same time, however, climate change is increasing
616 drought stress over many regions of the Earth and reports of drought (and heat) related
617 forest die-off are becoming widespread (Allen *et al.*, 2010). In this context, we need to
618 identify the species and populations that are more likely to be vulnerable to increased
619 drought and there is an urgent need to develop simple but reliable metrics that could be
620 used in the context of dynamic global vegetation models (Bartlett *et al.*, 2012; Choat *et al.*,
621 2012; Klein *et al.*, 2014). We are convinced that the analyses presented here will
622 facilitate characterizing drought responses of plants and will therefore provide a
623 valuable and fruitful addition to these efforts.

624

625

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636

637

638 **References**

639

640 **Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M,**
641 **Kitzberger T, Rigling A, Breshears DD, Hogg EH et al. 2010.** A global overview of
642 drought and heat-induced tree mortality reveals emerging climate change risks for
643 forests. *Forest Ecology and Management* **259**: 660–684.

644

645 **Arango-Velez A, Zwiazek JJ, Thomas BR, Tyree MT. 2011.** Stomatal factors and
646 vulnerability of stem xylem to cavitation in poplars. *Physiologia Plantarum* **143**: 154–
647 165.

648

649 **Bartlett M, Scoffoni C, Sack L. 2012.** The determinants of leaf turgor loss point and
650 prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecology*
651 *Letters* **15**: 393–405.

652

653 **Bayer RJ, Starr JR. 1998.** Tribal Phylogeny of the Asteraceae Based on Two Non-
654 Coding Chloroplast Sequences, the trnL Intron and trnL/trnF Intergenic Spacer. *Annals*
655 *of the Missouri Botanical Garden* **85**: 242.

656

657 **Bellarosa R, Simeone MC, Papini A, Schirone B. 2005.** Utility of ITS sequence data
658 for phylogenetic reconstruction of Italian *Quercus* spp. *Molecular Phylogenetics and*
659 *Evolution* **34**: 355–370.

660

661 **Bhaskar R, Ackerly DD. 2006.** Ecological relevance of minimum seasonal water
662 potentials. *Physiologia Plantarum* **127**: 353–359.

663

664 **Bouchenak-Khelladi Y, Maurin O, Hurter J, van der Bank M. 2010.** The
665 evolutionary history and biogeography of Mimosoideae (Leguminosae): An emphasis
666 on African acacias. *Molecular Phylogenetics and Evolution* **57**: 495–508.

667

668 **Brodersen CR, McElrone AJ, Choat B, Matthews MA, Shackel KA. 2010.** The
669 dynamics of embolism repair in xylem: in vivo visualizations using high-resolution
670 computed tomography. *Plant Physiology* **154**: 1088–1095.

671
672 **Brodribb TJ, Feild TS. 2000.** Stem hydraulic supply is linked to leaf photosynthetic
673 capacity: evidence from New Caledonian and Tasmanian rainforests. *Plant, Cell &*
674 *Environment* **23**: 1381–1388.
675
676 **Brodribb TJ, Holbrook NM, Edwards EJ, Gutiérrez MV. 2003.** Relations between
677 stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees.
678 *Plant, Cell & Environment* **26**: 443–450.
679
680 **Buckley TN. 2005.** The control of stomata by water balance. *New Phytologist* **168**:
681 275–292.
682
683 **Catalán P, Torrecilla P, Rodríguez JÁL, Olmstead RG. 2004.** Phylogeny of the
684 festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS
685 and trnL–F sequences. *Molecular Phylogenetics and Evolution* **31**: 517–541.
686
687 **Chaves MM, Maroco JP, Pereira JS. 2003.** Understanding plant responses to drought
688 — from genes to the whole plant. *Functional Plant Biology* **30**: 239–264.
689
690 **Chen Z-D, Manchester SR, Sun H-Y. 1999.** Phylogeny and evolution of the
691 Betulaceae as inferred from DNA sequences, morphology, and paleobotany. *American*
692 *Journal of Botany* **86**: 1168–1181.
693
694 **Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Feild**
695 **TS, Gleason SM, Hacke UG, et al. 2012.** Global convergence in the vulnerability of
696 forests to drought. *Nature* **491**: 752–755.
697
698 **Damour G, Simonneau T, Cochard H, Urban L. 2010.** An overview of models of
699 stomatal conductance at the leaf level. *Plant, Cell & Environment* **33**: 1419–1438.
700
701 **Domec J-C, Johnson DM. 2012.** Does homeostasis or disturbance of homeostasis in
702 minimum leaf water potential explain the isohydric versus anisohydric behavior of *Vitis*
703 *vinifera* L. cultivars? *Tree Physiology* **32**: 245–248.
704
705 **Donovan LA, Richards JH, Linton MJ. 2003.** Magnitude and Mechanisms of
706 Disequilibrium between Predawn Plant and Soil Water Potentials. *Ecology* **84**: 463–
707 470.
708
709 **Franks PJ, Drake PL, Froend RH. 2007.** Anisohydric but isohydrodynamic:
710 seasonally constant plant water potential gradient explained by a stomatal control
711 mechanism incorporating variable plant hydraulic conductance. *Plant Cell &*
712 *Environment* **30**: 19–30.
713
714 **Freckleton RP, Harvey PH, Pagel M. 2002.** Phylogenetic analysis and comparative
715 data: a test and review of evidence. *The American Naturalist* **160**: 712–726.
716
717 **Gernandt DS, López GG, García SO, Liston A. 2005.** Phylogeny and classification of
718 *Pinus*. *Taxon* **54**: 29–42.
719

720 **Granier A, Loustau D, Bréda N. 2000.** A generic model of forest canopy conductance
721 dependent on climate, soil water availability and leaf area index. *Annals of Forest*
722 *Science* **57**: 755–765.
723

724 **Hacke UG. 2014.** Variable plant hydraulic conductance. *Tree Physiology* **34**: 105–108.
725

726 **Hamerlynck EP, Mcauliffe JR, Smith SD. 2000.** Effects of surface and sub-surface
727 soil horizons on the seasonal performance of *Larrea tridentata* (creosotebush).
728 *Functional Ecology* **14**: 596–606.
729

730 **Hamzeh M, Dayanandan S. 2004.** Phylogeny of *Populus* (Salicaceae) based on
731 nucleotide sequences of chloroplast trnT-trnF region and nuclear rDNA. *American*
732 *Journal of Botany* **91**: 1398–1408.
733

734 **Hérault A, Lin Y-S, Bourne A, Medlyn BE, Ellsworth DS. 2013.** Optimal stomatal
735 conductance in relation to photosynthesis in climatically contrasting *Eucalyptus* species
736 under drought. *Plant Cell & Environment* **36**: 262–274.
737

738 **Hilu KW, Alice LA, Liang H. 1999.** Phylogeny of Poaceae Inferred from matK
739 Sequences. *Annals of the Missouri Botanical Garden* **86**: 835.
740

741 **Hoffmann WA, Marchin RM, Abit P, Lau OL. 2011.** Hydraulic failure and tree
742 dieback are associated with high wood density in a temperate forest under extreme
743 drought. *Global Change Biology* **17**: 2731–2742.
744

745 **Hsiao C, Chatterton NJ, Asay KH, Jensen KB. 1995.** Molecular phylogeny of the
746 Pooideae (Poaceae) based on nuclear rDNA (ITS) sequences. *Theoretical and Applied*
747 *Genetics* **90**: 389–398.
748

749 **Jackson RB, Sperry JS, Dawson TE. 2000.** Root water uptake and transport: using
750 physiological processes in global predictions. *Trends in Plant Science* **5**: 482–488.
751

752 **Johnson DM, McCulloh KA, Meinzer FC, Woodruff DR, Eissenstat DM. 2011.**
753 Hydraulic patterns and safety margins, from stem to stomata, in three eastern US tree
754 species. *Tree Physiology* **31**: 659–668.
755

756 **Johnson DM, McCulloh KA, Woodruff DR, Meinzer FC. 2012.** Hydraulic safety
757 margins and embolism reversal in stems and leaves: Why are conifers and angiosperms
758 so different? *Plant Science* **195**: 48–53.
759

760 **Jones HG. 1998.** Stomatal control of photosynthesis and transpiration. *Journal of*
761 *Experimental Botany* **49**: 387–398.
762

763 **Jones HG, Sutherland RA. 1991.** Stomatal control of xylem embolism. *Plant, Cell &*
764 *Environment* **14**: 607–612.
765

766 **Klein T, Yakir D, Buchmann N, Grünzweig JM. 2014.** Towards an advanced
767 assessment of the hydrological vulnerability of forests to climate change-induced
768 drought. *New Phytologist* **201**: 712–716.
769

770 **Kron KA, Judd WS, Stevens PF, Crayn DM, Anderberg AA, Gadek PA, Quinn**
771 **CJ, Luteyn JL. 2002.** Phylogenetic classification of Ericaceae: Molecular and
772 morphological evidence. *The Botanical Review* **68**: 335–423.
773
774 **Magee L. 1990.** R^2 Measures Based on Wald and Likelihood Ratio Joint Significance
775 Tests. *The American Statistician* **44**: 250–253.
776
777 **Maherali H, Moura CF, Caldeira MC, Willson CJ, Jackson RB. 2006.** Functional
778 coordination between leaf gas exchange and vulnerability to xylem cavitation in
779 temperate forest trees. *Plant, Cell & Environment* **29**: 571–583.
780
781 **Maherali H, Pockman WT, Jackson RB. 2004.** Adaptive variation in the vulnerability
782 of woody plants to xylem cavitation. *Ecology* **85**: 2184–2199.
783
784 **Manos PS, Doyle JJ, Nixon KC. 1999.** Phylogeny, Biogeography, and Processes of
785 Molecular Differentiation in *Quercus* Subgenus *Quercus* (Fagaceae). *Molecular*
786 *Phylogenetics and Evolution* **12**: 333–349.
787
788 **Manzoni S, Vico G, Katul G, Palmroth S, Jackson RB, Porporato A. 2013a.**
789 Hydraulic limits on maximum plant transpiration and the emergence of the safety–
790 efficiency trade-off. *New Phytologist* **198**: 169–178.
791
792 **Manzoni S, Vico G, Porporato A, Katul G. 2013b.** Biological constraints on water
793 transport in the soil–plant–atmosphere system. *Advances in Water Resources* **51**: 292–
794 304.
795
796 **Martínez-Vilalta J, Mangirón M, Ogaya R, Sauret M, Serrano L, Peñuelas J, Piñol**
797 **J. 2003.** Sap flow of three co-occurring Mediterranean woody species under varying
798 atmospheric and soil water conditions. *Tree Physiology* **23**: 747–758.
799
800 **Maseda PH, Fernández RJ. 2006.** Stay wet or else: three ways in which plants can
801 adjust hydraulically to their environment. *Journal of Experimental Botany* **57**: 3963–
802 3977.
803
804 **Maurel C, Simonneau T, Sutka M. 2010.** The significance of roots as hydraulic
805 rheostats. *Journal of experimental botany* **61**: 3191–3198.
806
807 **McDowell NG. 2011.** Mechanisms linking drought, hydraulics, carbon metabolism, and
808 vegetation mortality. *Plant physiology* **155**: 1051–1059.
809
810 **McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J,**
811 **Sperry J, West A, Williams DG. 2008.** Mechanisms of plant survival and mortality
812 during drought: why do some plants survive while others succumb to drought? *New*
813 *Phytologist* **178**: 719–739.
814
815 **Meinzer FC. 2002.** Co-ordination of vapour and liquid phase water transport properties
816 in plants. *Plant, Cell & Environment* **25**: 265–274.
817

818 **Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR. 2009.**
819 Xylem hydraulic safety margins in woody plants: coordination of stomatal control of
820 xylem tension with hydraulic capacitance. *Functional Ecology* **23**: 922–930.
821

822 **Mencuccini M. 2003.** The ecological significance of long-distance water transport:
823 short-term regulation, long-term acclimation and the hydraulic costs of stature across
824 plant life forms. *Plant, Cell & Environment* **26**: 163–182.
825

826 **Miller JT, Bayer RJ. 2001.** Molecular phylogenetics of Acacia (Fabaceae:
827 Mimosoideae) based on the chloroplast matK coding sequence and flanking trnK intron
828 spacer regions. *American Journal of Botany* **88**: 697–705.
829

830 **Nardini A, Battistuzzo M, Savi T. 2013.** Shoot desiccation and hydraulic failure in
831 temperate woody angiosperms during an extreme summer drought. *New Phytologist*
832 **200**: 322–329.
833

834 **Nardini A, Pedà G, Rocca NL. 2012.** Trade-offs between leaf hydraulic capacity and
835 drought vulnerability: morpho-anatomical bases, carbon costs and ecological
836 consequences. *New Phytologist* **196**: 788–798.
837

838 **Nardini A, Salleo S. 2000.** Limitation of stomatal conductance by hydraulic traits:
839 sensing or preventing xylem cavitation? *Trees* **15**: 14–24.
840

841 **Neufeld HS, Grantz DA, Meinzer FC, Goldstein G, Crisosto GM, Crisosto C. 1992.**
842 Genotypic Variability in Vulnerability of Leaf Xylem to Cavitation in Water-Stressed
843 and Well-Irrigated Sugarcane. *Plant Physiology* **100**: 1020–1028.
844

845 **New M, Lister D, Hulme M, Makin I. 2002.** A high-resolution data set of surface
846 climate over global land areas. *Climate Research* **21**: 1–25.
847

848 **Oren R, Sperry JS, Katul GG, Pataki DE, Ewers BE, Phillips N, Schäfer KVR.**
849 **1999.** Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to
850 vapour pressure deficit. *Plant, Cell & Environment* **22**: 1515–1526.
851

852 **Pammenter NW, Willigen CV der. 1998.** A mathematical and statistical analysis of
853 the curves illustrating vulnerability of xylem to cavitation. *Tree Physiology* **18**: 589–
854 593.
855

856 **Paradis E. 2006.** *Analysis of Phylogenetics and Evolution with R*. New York, USA:
857 Springer.
858

859 **Pell SK. 2004.** Molecular systematics of the cashew family (Anacardiaceae). PhD
860 Thesis, Louisiana State University, 193 pages.
861

862 **Potter D, Eriksson T, Evans RC, Oh S, Smedmark JEE, Morgan DR, Kerr M,**
863 **Robertson KR, Arsenault M, Dickinson TA, et al. 2007.** Phylogeny and classification
864 of Rosaceae. *Plant Systematics and Evolution* **266**: 5–43.
865

866 **Roberts RP, Urbatsch LE. 2004.** Molecular phylogeny of Chrysothamnus and related
867 genera (Asteraceae, Astereae) based on nuclear ribosomal 3'ETS and ITS sequence
868 data. *Systematic botany* **29**: 199–215.
869

870 **Rockwell FE, Wheeler JK, Holbrook NM. 2014.** Cavitation and its discontents:
871 opportunities for resolving current controversies. *Plant Physiology*, in press.
872

873 **Sperry JS. 2013.** Cutting-edge research or cutting-edge artefact? An overdue control
874 experiment complicates the xylem refilling story. *Plant Cell & Environment* **36**: 116-
875 118.
876

877 **Sperry JS, Adler FR, Campbell GS, Comstock JP. 1998.** Limitation of plant water
878 use by rhizosphere and xylem conductance: results from a model. *Plant, Cell &*
879 *Environment* **21**: 347–359.
880

881 **Sperry JS, Hacke UG, Oren R, Comstock JP. 2002.** Water deficits and hydraulic
882 limits to leaf water supply. *Plant, Cell & Environment* **25**: 251–263.
883

884 **Stocker O. 1956.** Die Abhängigkeit der Transpiration von den Umweltfaktoren. In:
885 Adriani DMJ, Aslyng DDHC, Burström PDH, Geiger DR, Gessner DF, Härtel U-PDO,
886 Huber DB, Hülsbruch DM, Kalle PD rer nat K, Kern DH, *et al.*, eds. Handbuch der
887 Pflanzenphysiologie / Encyclopedia of Plant Physiology. Pflanze und Wasser / Water
888 Relations of Plants. Berlin Heidelberg, Germany: Springer, 436–488.
889

890 **Tardieu F, Simonneau T. 1998.** Variability among species of stomatal control under
891 fluctuating soil water status and evaporative demand: modelling isohydric and
892 anisohydric behaviours. *Journal of Experimental Botany* **49**: 419–432.
893

894 **Tyree MT, Sperry JS. 1988.** Do Woody Plants Operate Near the Point of Catastrophic
895 Xylem Dysfunction Caused by Dynamic Water Stress? Answers from a Model. *Plant*
896 *Physiology* **88**: 574–580.
897

898 **Tyree MT, Zimmermann MH. 2002.** *Xylem Structure and the Ascent of Sap*. Springer.
899

900 **Wallander E, Albert VA. 2000.** Phylogeny and classification of Oleaceae based on
901 rps16 and trnL-F sequence data. *American Journal of Botany* **87**: 1827–1841.
902

903 **Wang X-Q, Tank DC, Sang T. 2000.** Phylogeny and Divergence Times in Pinaceae:
904 Evidence from Three Genomes. *Molecular Biology and Evolution* **17**: 773–781.
905

906 **Wannan BS. 2006.** Analysis of generic relationships in Anacardiaceae. *Blumea-*
907 *Biodiversity, Evolution and Biogeography of Plants* **51**: 165–195.
908

909 **Webb CO, Donoghue MJ. 2005.** Phylomatic: tree assembly for applied phylogenetics.
910 *Molecular Ecology Notes* **5**: 181–183.
911

912 **Whitehead D, Edwards WRN, Jarvis PG. 1984.** Conducting sapwood area, foliage
913 area, and permeability in mature trees of *Picea sitchensis* and *Pinus contorta*. *Canadian*
914 *Journal of Forest Research* **14**: 940–947.
915

916 **Zhang JL, Cao KF. 2009.** Stem hydraulics mediates leaf water status, carbon gain,
917 nutrient use efficiencies and plant growth rates across dipterocarp species. *Functional*
918 *ecology* **23**: 658–667.

919
920 **Zwieniecki MA, Holbrook NM. 2009.** Confronting Maxwell’s demon: biophysics of
921 xylem embolism repair. *Trends in plant science* **14**: 530–534.

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952 **Supporting Information**

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954 **Notes S1.** The theoretical framework expanded, including an error analysis.

955

956 **Notes S2.** Error analysis of the estimation of the slope and intercept of the relationship
957 between predawn and midday leaf water potentials.

958

959 **Figure S1.** Mean annual temperature map of the World showing the sampling locations
960 of all the studies included in our final water potentials database.

961

962 **Figure S2.** Species-level values of minimum measured predawn leaf water potential,
963 the slope parameter σ , and the intercept Λ as a function of Biome.

964

965 **Figure S3.** Species-level values of the intercept (parameter Λ), and the slope (parameter
966 σ).

967

968 **Figure S4.** Phylogenetic tree of the studied species.

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970 **Table S1.** Characteristics of the species considered in this study and list of references
971 used to build the database of leaf water potentials.

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985 **Tables**

986

987 **Table 1.** Models of parameters σ (Table 1a) and Λ (Table 1b) of the relationship
 988 between predawn and midday leaf water potentials as a function of climatic variables.
 989 MAT = mean annual temperature; CVMP = coefficient of variation of monthly
 990 precipitation; MSP = mean summer precipitation; MSD = mean summer vapour
 991 pressure difference. Significant relationships are marked with asterisks (*: $0.05 > P >$
 992 0.01 , **: $0.01 > P > 0.001$, ***: $P < 0.001$; ns: not significant).

993

994 **(a) Response variable: σ**

Explanatory variables	Without phylogenetic effects		Including phylogenetic effects	
	Coefficient \pm standard error	t-value	Coefficient \pm standard error	t-value
Intercept	0.96 \pm 0.08	12.12***	0.96 \pm 0.08	12.12***
MAT	-0.0036 \pm 0.0046	-0.78 ^{ns}	-0.0036 \pm 0.0046	-0.78 ^{ns}
CVMP	0.0010 \pm 0.0008	1.30 ^{ns}	0.0010 \pm 0.0008	1.30 ^{ns}
MSP	0.0001 \pm 0.0002	0.44 ^{ns}	0.0001 \pm 0.0002	0.44 ^{ns}
MSD	-0.14 \pm 0.04	-3.60***	-0.14 \pm 0.04	-3.60***
	Model AIC = 15.3; $R^2 = 0.15$		Model AIC = 17.3	

995

996 **(b) Response variable: Λ**

Explanatory variables	Without phylogenetic effects		Including phylogenetic effects	
	Coefficient \pm standard error	t-value	Coefficient \pm standard error	t-value
Intercept	-1.01 \pm 0.19	-5.24***	-1.01 \pm 0.19	-5.24***
MAT	-0.032 \pm 0.011	-2.86**	-0.032 \pm 0.011	-2.88**
CVMP	0.0053 \pm 0.0019	2.76**	0.0053 \pm 0.0019	2.77**
MSP	0.0013 \pm 0.0005	2.54*	0.0013 \pm 0.0005	2.55*
MSD	-0.28 \pm 0.09	-3.06**	-0.28 \pm 0.09	-3.05**
	Model AIC = 188.3; $R^2 = 0.22$		Model AIC = 190.2	

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1000 **Figure captions**

1001

1002 **Figure 1.** Relationship between predawn and midday leaf water potentials according to
1003 our theoretical model, which assumes a linear relationship. Four different behaviours
1004 are depicted, all sharing the same intercept (Λ): strict isohydric ($\sigma = 0$), partial isohydric
1005 ($0 < \sigma < 1$), strict anisohydric ($\sigma = 1$), and extreme anisohydric ($\sigma > 1$). The point of
1006 cessation of gas exchange is also represented: for isohydric behaviours it occurs when
1007 $\Psi_{PD} = \Psi_{MD}$; for anisohydric relationships it occurs when Ψ_{MD} reaches the water
1008 potential inducing complete loss of plant hydraulic conductance. The 1:1 line is also
1009 depicted.

1010

1011 **Figure 2.** Relationship between predawn and midday leaf water potentials for all the
1012 species included in this study. Three species representative of contrasting responses are
1013 highlighted in colour: *Salix gooddingii*, with rapidly shrinking water potential difference
1014 as predawn water potential declines (~isohydric); *Ceanothus crassifolius*, with nearly
1015 constant difference between predawn and midday water potentials (~anisohydric); and
1016 *Prosopis glandulosa*, with increasing water potential difference as predawn water
1017 potential declines (~extreme anisohydric).

1018

1019 **Figure 3.** Frequency histogram of the type of water potential regulation according to the
1020 observed σ values (cf. Fig. 1). Species are classified as strict isohydric if the confidence
1021 interval of σ (CI, defined as the estimated σ value ± 1 standard error) included 0, as
1022 strict anisohydric if the CI included 1, as partial isohydric if $0 < \sigma < 1$ and the CI did not
1023 include 0 or 1, and as extreme anisohydric if $\sigma > 1$ and its CI did not include 1.

1024

1025 **Figure 4.** Relationship between the estimated values of parameters σ and Λ . Each data
1026 point corresponds to a different species ($N = 102$).

1027

1028 **Figure 5.** Relationship between σ and Λ and climate variables, including mean annual
1029 temperature (MAT), the coefficient of variation of monthly precipitation (CVMP), mean
1030 summer precipitation (June-August, MSP), and mean summer vapour pressure
1031 difference (June-August, MSD). Each data point corresponds to a different species ($N =$
1032 102). Fitted functions correspond to significant coefficients as estimated in the

1033 corresponding linear models of σ or Λ as a function of all four climate variables (see
1034 text).

1035

1036 **Figure 6.** Relationship between σ and two different measures of vulnerability to xylem
1037 embolism: P_{50} , the water potential causing a 50% loss in xylem hydraulic conductivity;
1038 and P_{88} , the water potential causing a 88% loss in xylem hydraulic conductivity. Each
1039 data point corresponds to a different species ($N = 102$). Simple regression curves are
1040 fitted to the data in each panel.

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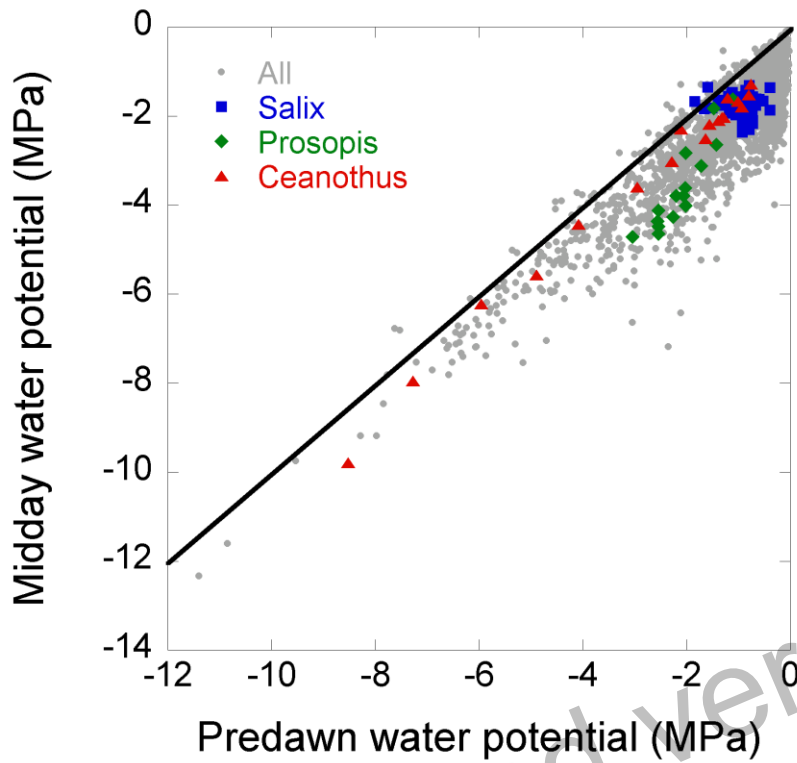
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1085 **Figure 2.**

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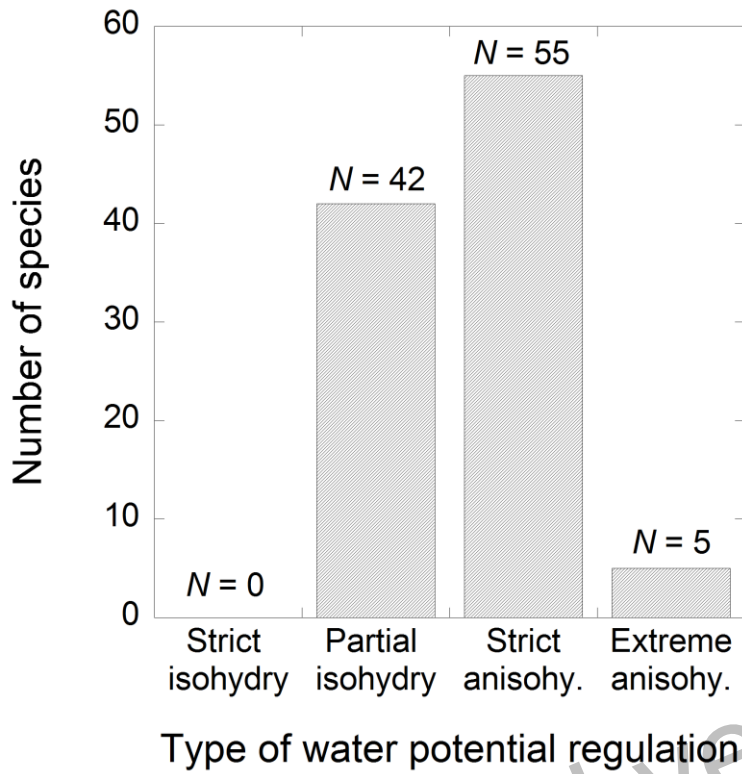
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1105 **Figure 3**

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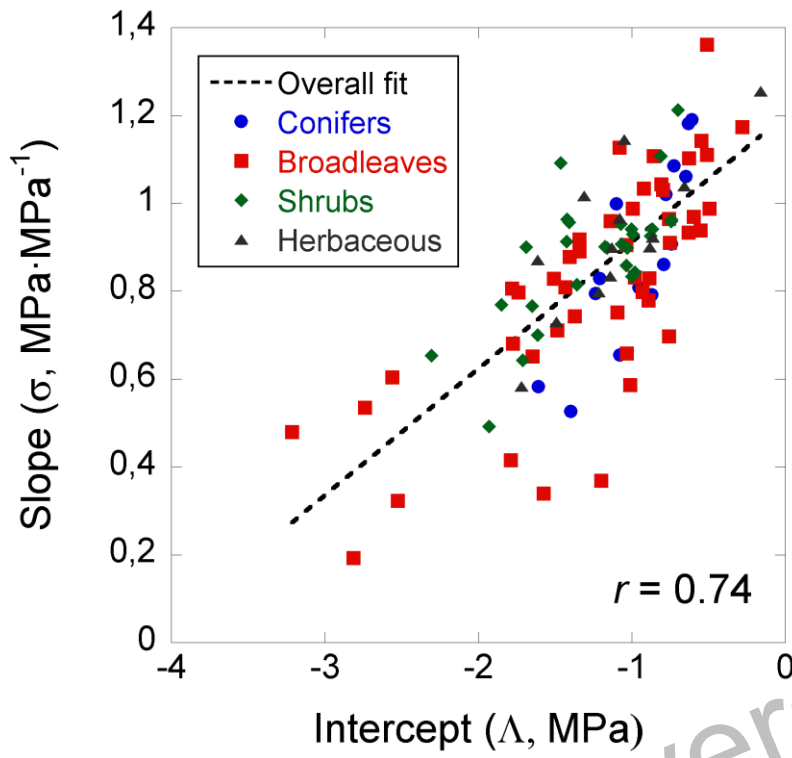
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1125 **Figure 4.**

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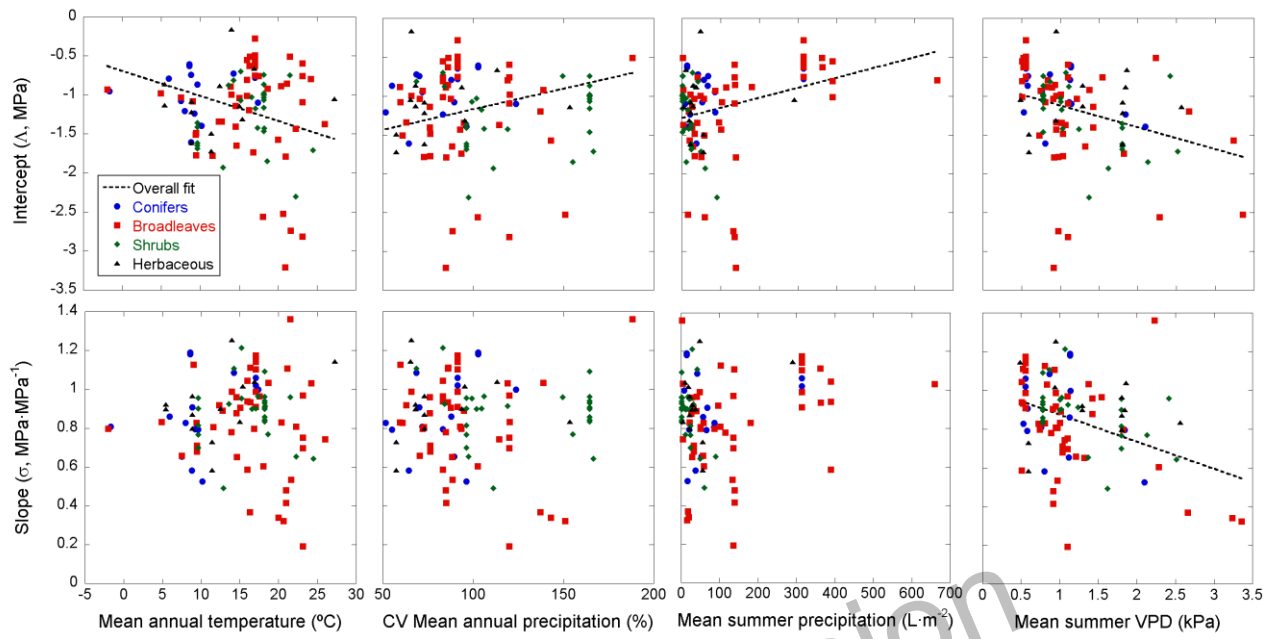
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1145 **Figure 5.**

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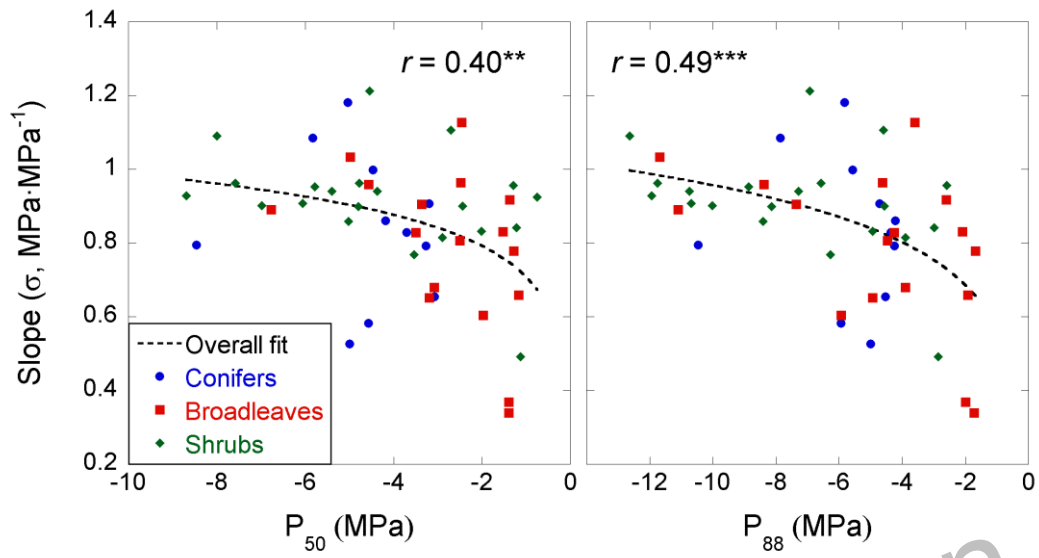
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1166 **Figure 6.**

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