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1 **Title:** The “isohydric trap”: a proposed feedback between water shortage, stomatal  
2 regulation and nutrient acquisition drives differential growth and survival of European  
3 pines under climatic dryness

4 **Running head:** Climate-induced nutrient imbalance in pines

5  
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24

25 **Keywords:** Climatic change, hotter drought, stable isotopes, nutrients, stoichiometry,  
26 stomatal behaviour, water use efficiency

27

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**29 Abstract**

30 Climatic dryness imposes limitations on vascular plant growth by reducing stomatal  
31 conductance, thereby decreasing CO<sub>2</sub> uptake and transpiration. Given that transpiration-  
32 driven water flow is required for nutrient uptake, climatic stress-induced nutrient deficit  
33 could be a key mechanism for decreased plant performance under prolonged drought.  
34 We propose the existence of an “isohydric trap”, a dryness-induced detrimental  
35 feedback leading to nutrient deficit and stoichiometry imbalance in strict isohydric  
36 species. We tested this framework in a common garden experiment with 840 individuals  
37 of four ecologically-contrasting European pines (*Pinus halepensis*, *P. nigra*, *P.*  
38 *sylvestris*, and *P. uncinata*) at a site with high temperature and low soil water  
39 availability. We measured growth, survival, photochemical efficiency, stem water  
40 potentials, leaf isotopic composition ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ), and nutrient concentrations (C, N, P,  
41 K, Zn, Cu). After two years, the Mediterranean species *Pinus halepensis* showed lower  
42  $\delta^{18}\text{O}$  and higher  $\delta^{13}\text{C}$  values than the other species, indicating higher time-integrated  
43 transpiration and water-use efficiency (WUE), along with lower predawn and midday  
44 water potentials, higher photochemical efficiency, higher leaf P and K concentrations,  
45 more balanced N:P and N:K ratios, and much greater dry-biomass (up to 63-fold) and  
46 survival (100%). Conversely, the more mesic mountain pine species showed higher leaf  
47  $\delta^{18}\text{O}$  and lower  $\delta^{13}\text{C}$ , indicating lower transpiration and WUE, higher water potentials,  
48 severe P and K deficiencies and N:P and N:K imbalances, and poorer photochemical  
49 efficiency, growth, and survival. These results support our hypothesis that vascular  
50 plant species with tight stomatal regulation of transpiration can become trapped in a  
51 feedback cycle of nutrient deficit and imbalance that exacerbates the detrimental  
52 impacts of climatic dryness on performance. This overlooked feedback mechanism may  
53 hamper the ability of isohydric species to respond to ongoing global change, by

54 aggravating the interactive impacts of stoichiometric imbalance and water stress caused  
55 by anthropogenic N deposition and hotter droughts, respectively.

56

## 57 **Introduction**

58 Plant survival and performance is determined largely by soil water availability, since  
59 water is a major limiting resource for primary production in many regions (Adams et  
60 al., 2017; Cramer & Hoffman, 2015; Lawlor & Cornic, 2002). The responses of plant  
61 species and individuals to water shortage span a variety of mechanisms that either tend  
62 to increase water uptake (e.g. enhanced root growth) or reduce water loss (e.g. stomatal  
63 closure). Stomatal regulation is the quickest mechanism to cope with drought, leading to  
64 two contrasting strategies termed anisohydry and isohydry, characterized by relaxed vs.  
65 tight stomatal control of transpiration in response to decreases in plant water potential  
66 (Moran, Lauder, Musser, Stathos, & Shu, 2017; Tardieu & Simonneau, 1998; but see  
67 Martínez-Vilalta & Garcia-Forner, 2017). These strategies are clearly the opposite  
68 extremes of a continuous ecophysiological gradient (Klein, 2014) but, overall, the  
69 degree of stomatal regulation of transpiration is currently considered a key functional  
70 trait that explains not only individual plant response to drought, but also forest  
71 persistence under current and future climatic conditions (McDowell et al., 2011).

72         Although the role of stomatal control as a key mechanism in the regulation of  
73 plant carbon balance and hydraulic functioning under drought and heat stress has been  
74 the focus of much research attention in recent years (Garcia-Forner, Biel, Savé, &  
75 Martínez-Vilalta, 2016; Martínez-Vilalta & Garcia-Forner, 2017; McDowell et al.,  
76 2013), less attention has been paid to plant nutrient dynamics as a potential key factor  
77 influencing plant survival and growth under conditions of prolonged climatic dryness  
78 (Gessler, Schaub, & McDowell, 2017; Hartmann et al., 2018). Nutrient availability and

79 plant nutrient status influence forest productivity through photosynthetic, allocation,  
80 and stoichiometric effects (Marschner, Kirkby, & Cakmak, 1996; Sardans & Peñuelas,  
81 2012). Drought decreases soil nutrient availability for plants due to reduced ion  
82 mobility and microbial activity, which can lead to impairment of the plant's nutrient  
83 status and growth (Kreuzwieser & Gessler, 2010). Plant nutrient acquisition from the  
84 soil is tightly linked to water uptake and movement in soils, as plants rapidly deplete  
85 nutrients from the rhizosphere, which must be replenished by dissolved nutrients carried  
86 in the transpiration-driven mass flow of water to plant roots (Cabrera-Bosquet, Sánchez,  
87 & Araus, 2009; Lambers, Chapin, & Pons, 2008; Voltas, Romagosa, Muñoz, & Araus,  
88 1998). Plant nutrient uptake is therefore heavily dependent on the existence of a  
89 negative water potential gradient from the soil to the roots that is driven by leaf  
90 transpiration (Lambers, Chapin, & Pons, 2008). Furthermore, plants need to maintain  
91 particular nutrient stoichiometric relations in their tissues for proper ecophysiological  
92 functioning (Güsewell, 2004; Koerselman & Meuleman, 1996; Marschner et al., 1996;  
93 Sardans & Peñuelas, 2012). Given that nutrient mobility in the soil matrix may differ by  
94 several orders of magnitude among various essential macro- and micronutrients  
95 (Lambers et al., 2008), environmental conditions of dryness forcing stomatal closure  
96 may severely impair the nutrient balance and stoichiometric ratios of plant tissues.  
97 Thus, the interplay and interdependence between plant water relations, nutrient status  
98 and stoichiometric relations should be considered a potential important mechanism  
99 contributing to plant mortality or reduced performance in models that seek to predict the  
100 impact of drier climatic conditions on plant communities.

101         Here, we propose the existence of an “isohydric trap” that occurs when vascular  
102 plant species with a strict isohydric behaviour fall under prolonged climatic dryness  
103 conditions, leading to a detrimental feedback loop between water stress, tight stomatal

104 control, and nutrient uptake and status that can be detrimental to plant physiological  
105 functioning including growth and survival (Figure 1). In this conceptual model, strict  
106 isohydric species exhibit early and prolonged stomatal closure under dry conditions  
107 (Klein, 2014; Moran et al., 2017), thereby drastically reducing cumulative transpiration,  
108 and hence mass flow of water and in-solution nutrients to roots. As a result, nutrient  
109 uptake decreases and plants become prone to macro- and micronutrient deficiency and  
110 stoichiometric imbalance, which in turn further decreases stomatal conductance and  
111 carbon assimilation through reductions in photochemical efficiency and water use  
112 efficiency (Figure 1; blue arrow). These feedbacks eventually decrease carbon  
113 availability for supporting root and ectomycorrhizal activity and growth, further  
114 reducing the capacity for plant water and nutrient uptake (León-Sánchez et al., 2017)  
115 and thereby further impairing the plant's ability to cope with prolonged climatic dryness  
116 (Figure 1; green arrow).

117 In this study, we seek to demonstrate that the detrimental impact of this  
118 “isohydric trap” on plant nutrient status and stoichiometry is a key mechanism behind  
119 the response of drought-sensitive plant species to prolonged climatic dryness. To test  
120 this theoretical framework, we conducted a two-year common garden experiment where  
121 juveniles of four pine species with contrasting ecological niche and ecophysiological  
122 behaviour were grown under the same xeric environmental conditions. Plants were  
123 monitored for survival and growth, along with photochemical efficiency, water  
124 potential, leaf nutrient concentrations, and leaf  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  signatures, which were  
125 used as surrogates of time-integrated stomatal conductance and water use efficiency,  
126 respectively. We predicted that exposure to prolonged climatic dryness will impose  
127 strong constraints on cumulative transpiration and nutrient uptake in pine species with  
128 strict isohydric behaviour. Due to the tight coupling between water and nutrient uptake

129 by roots, reduced transpiration will hamper the mass flow and diffusion of nutrients to  
130 roots and the uptake of dissolved nutrients, eventually leading to nutrient deficiency and  
131 severe N:P:K stoichiometric imbalance. This will hinder plant carbon balance,  
132 transpiration and water use efficiency, ultimately leading to decreased growth and  
133 survival. In contrast, drought-tolerant pine species with less strict stomatal control of  
134 transpiration will escape this “isohydric trap” by allowing water potential to drop while  
135 maintaining greater stomatal aperture and transpiration under prolonged drought stress,  
136 which will allow greater nutrient uptake and a more balanced plant nutrient status and  
137 stoichiometry. We seek to expand current knowledge on plant responses to increasing  
138 frequency of hotter droughts (Allen, Breshears, & McDowell, 2015) by examining the  
139 importance of drought-induced nutrient starvation and stoichiometric imbalance, which  
140 to-date has been largely overlooked or underestimated in both conceptual and empirical  
141 models of plant responses to climate change (Gessler et al., 2017).

142

## 143 **Material and methods**

### 144 *Species and plant material*

145 The studied species were *Pinus halepensis* Mill., *P. nigra* Arnold, *P. sylvestris* L., and  
146 *P. uncinata* Ram. These species are native in Europe, altogether cover a wide  
147 geographic range across the continent (*circa* 30% of its forest area; Köble & Seufert,  
148 2001) and segregate clearly along aridity gradients in the order *P. halepensis* > *P. nigra*  
149 > *P. sylvestris* > *P. uncinata*, whereas their resistance to cold stress follows the opposite  
150 trend (Fernández-Pérez, Villar-Salvador, Martínez-Vilalta, Toca, & Zavala, 2018; Ruiz  
151 de la Torre, 2006; Tapias, Climent, Pardos, & Gil, 2004). *Pinus halepensis* is widely  
152 distributed throughout the Mediterranean basin from sea level to 1200 m a.s.l., *P. nigra*  
153 from 800 to 2000 m a.s.l. (Mediterranean and Alpine distribution), *P. sylvestris* from

154 1000 to 2100 m a.s.l. (Boreo-Alpine/Eurosiberian distribution), and *P. uncinata* is  
155 distributed from 1400 to 2200 m a.s.l. in the Alps, Pyrenees, and other high Iberian  
156 mountains (Richardson 2000; altitudinal ranges for Southern Europe). In general, pines  
157 are considered isohydric species compared to other taxa such as oaks or junipers  
158 (Meinzer, Woodruff, Marias, Mcculloh, & Sevanto, 2014; Zweifel, Steppe, & Sterck,  
159 2007). However, several evidences support that there is a gradient in stomatal behaviour  
160 in response to water stress among the studied species. First, these species show  
161 differences in the regulation of plant water potential, which is associated with stomatal  
162 control (Klein, 2014; Sperry, Hacke, Oren, & Comstock, 2002; Tardieu & Simonneau,  
163 1998), with *P. halepensis* reaching the lowest water potentials, followed by *P. nigra*, *P.*  
164 *sylvestris* and *P. uncinata* (Choat et al., 2012; Matias, Castro, Villar-Salvador, Quero, &  
165 Jump, 2017; Oliet, Planelles, López Arias, & Artero, 2002). Second, leaf-level  
166 measurements suggest the existence of large interspecific differences in stomatal  
167 sensitivity to low plant water potentials, with *P. halepensis* showing the lowest water  
168 potentials at stomatal closure, followed by *P. nigra* and *P. sylvestris* (Martin-StPaul,  
169 Delzon, & Cochard, 2017). Finally, previous measurements at whole-plant level in the  
170 study site showed that *P. halepensis* exhibits less tight stomatal control and higher  
171 transpiration rates under dry conditions than *P. nigra* and *P. sylvestris* (Salazar-Tortosa  
172 et al., 2018). Therefore, we may assume that the studied species can be ordered along an  
173 iso-anisohydry gradient from *P. uncinata* (most isohydric), *P. sylvestris*, *P. nigra* to *P.*  
174 *halepensis* (most anisohydric).

175       Seeds of the four species were collected when ripe from certified provenance  
176 regions of the Iberian Peninsula (Appendix S1, Table S1). The seeds were stored under  
177 cold, dry conditions until sowing. Seeding was done in winter 2012 using 300-mL  
178 plastic containers filled with fertilized peat (White 420 F6 Kekkila, Finland; pH 4.7)



179 containing 0.8-1 kg/m<sup>3</sup> of a slow-release fertilizer NPK 16-10-20. They were initially  
180 grown in a greenhouse of the Centro Nacional de Recursos Genéticos Forestales “El  
181 Serranillo” (Guadalajara, Spain, 40° 39' 56.14" N, 3° 10' 15.20" W) to avoid frost  
182 damage. In mid-May 2012, the seedlings were moved outdoors and cultivated under  
183 optimal forest nursery conditions until 15 February 2013, when they were transferred to  
184 the common garden site. Nutrient content and isotopic composition at the time of  
185 transplanting indicates that seedlings had not been subjected to any water or nutrient  
186 stress during the nursery stage (Appendix S1, Table S1).

187

#### 188 *Study site and experimental design*

189 The common garden experiment was conducted at the “Huerta de La Paloma” farm (37°  
190 10' 03.43" N, 3° 36' 57.80" W; Granada, Southern Spain), a flat (slope ca. 2%),  
191 agricultural terrain at 649 m a.s.l. The climate is Mediterranean with hot, dry summers  
192 and precipitation concentrated in autumn and spring. The mean annual rainfall is  
193 394±38 L m<sup>2</sup> y<sup>-1</sup> and the mean annual temperature is 15.3±0.1°C, with a mean  
194 maximum of the hottest month of 35.7±0.2°C and a mean minimum of the coldest  
195 month of -0.1±0.2°C (period 2006-2015; climatic data from a meteorological station  
196 located 1.5 km away at IFAPA Research Field Station). These climatic conditions can  
197 be regarded as dry and hot for *P. nigra*, *P. sylvestris* and *P. uncinata* when compared to  
198 the prevailing climatic conditions in their native ranges (Christensen, 1987; Enescu, de  
199 Rigo, Caudullo, Mauri, & Houston-Durrant, 2016; Houston-Durrant, de Rigo, &  
200 Caudullo, 2016), whereas they fall within the optimal ecological range of *P. halepensis*  
201 (Mauri, Di Leo, de Rigo, & Caudullo, 2016). The soil is deep with a loamy texture, and  
202 average values of 44.8% sand, 41.8% silt, and 13.3% clay, and a soil water content of  
203 13% at wilting point and 33% at field capacity (-1.5 and -0.033 MPa, respectively;

204 means for the profile down to 1 m deep; no marked horizons in soil profile; analyses  
205 done in the *Laboratorio Agroalimentario de la Junta de Andalucía*, Atarfe, Granada,  
206 official laboratory for the Regional Agricultural Service). The soil-nutrient content at 0-  
207 15 cm depth (N, P and K) showed adequate values for plant growth (Appendix S1,  
208 Table S2).

209 On 15 February 2013, the one-year-old seedlings grown under nursery  
210 conditions were transplanted to the common garden site using a randomized-block  
211 design. Three blocks of 500 m<sup>2</sup> were located side by side, separated by 2.5 m. In each  
212 block, we planted a total of 70 individuals of each species (70 x 3 blocks x 4 species =  
213 840 seedlings in total). Within each block, seedlings were regularly planted at 1.25-m  
214 distance from each other, and individuals of each pine species were distributed  
215 randomly within the planting scheme. Weeds were removed manually and with a  
216 cultivator as needed to prevent competition. We did not find any competition effect  
217 from neighbours on either survival or growth ( $P > 0.53$  in both cases; Appendix S1,  
218 Table S3). The initial size of each seedling (length of the leader shoot and stem-root  
219 collar diameter) was measured just after planting as a baseline for aboveground growth  
220 estimations (Appendix S1, Table S1). Soil-water content was measured regularly  
221 throughout the summers (June-September) at 10, 20, 30, 40, 60, and 100 cm depth using  
222 a PR-2/6 Soil Moisture Profile Probe (Delta T, Cambridge, UK). Water content in the  
223 soil profile remained above the permanent wilting point throughout the hot, dry  
224 (summer) season (Appendix S1, Table S4).

225

#### 226 *Seedling survival and growth*

227 Survival was monitored eight times from 13 June 2013 to 9 September 2014. Seedling  
228 growth was measured non-destructively for all the plants in September 2013 and 2014,

229 considering leader shoot length and stem diameter (increment relative to initial values  
230 measured after planting). Stem volume was calculated for each year assuming a conical  
231 shape for the stem, with basal diameter given by the average of two perpendicular  
232 measurements at the root collar and height given by the maximum height of the leader  
233 shoot. Growth patterns for both years were similar and hence only the data from 2014  
234 are reported. In the third year (September 2015), height, stem-root collar diameter, and  
235 fresh weight were measured in a random subsample of five pines per species and block  
236 (*P. uncinata* not included due to small sample size; 45 pines in total). Survival was not  
237 monitored in the third growing season of the experiment, given that a destructive  
238 harvesting of seedlings was performed the previous year for leaf isotopic and nutrient  
239 analyses (see below), and thus the remaining plants might not represent a random  
240 sample for this variable.

241

#### 242 *Physiological variables*

243 The effective photochemical quantum yield of photosystem II (Y(II), termed quantum  
244 yield from now on), relative electron transport rate (rETR), photochemical quenching  
245 (qP), non-photochemical quenching (qN), maximum photochemical efficiency of  
246 photosystem II ( $F_v/F_m$ ), and leaf-water potential ( $\Psi$ ) were measured for a subsample of  
247 nine randomly selected seedlings per species and block in July of 2014. Quantum yield,  
248 qP, and qN were measured by means of a portable junior PAM fluorometer (Heinz  
249 Walz GmbH Germany), and rETR was calculated by means of the following equation  
250 (Schreiber, 2004):

251

$$252 \text{ rETR} = \text{PAR} \times \text{ETR-Factor} \times P_{\text{PSII}}/P_{\text{PPS}} \times Y(\text{II}),$$

253

254 where PAR is the photosynthetically active radiation during the measurements; ETR-  
255 Factor is the absorptance of photons by photosynthetic pigments which is considered to  
256 be 0.84 as a reasonable match to the average absorptance in the visible range (400-700  
257 nm);  $P_{PSII}/P_{PPS}$  is the ratio between the photons absorbed by PS II and photons absorbed  
258 by photosynthetic pigments, with a value of 0.5 assuming only linear electron transport,  
259 that is, equal transfer rates through PS I and PS II, and comparable photochemical  
260 quantum yields of PS I and PS II under strongly light-limiting conditions; and Y(II) is  
261 the effective photochemical quantum yield of PS II, as described above.

262 The  $F_v/F_m$  was measured at predawn and midday using a portable fluorometer  
263 (FMS2, Hansatech Instruments, UK). Plant water potential ( $\Psi$ ) was also measured at  
264 predawn and midday (except for *P. uncinata* which was only measured at predawn due  
265 to the low number of surviving individuals) with a pressure chamber (SKPM 1400,  
266 Skye Instruments, UK). Measurements were made in lateral branches in most cases.  
267 Photosynthetic fluorescence parameters were always measured between 12:00 and  
268 16:00 h (solar time), except  $F_v/F_m$  predawn measurements.

269

#### 270 *Leaf isotopic composition*

271 We used leaf  $\delta^{13}C$  and  $\delta^{18}O$  as time-integrated proxy measures of intrinsic water-use  
272 efficiency (iWUE, which is the ratio between net photosynthetic rate and stomatal  
273 conductance; Farquhar et al., 1989) and stomatal conductance, respectively (Barbour,  
274 2007; Farquhar et al., 2007). Given that all the target pine species had very similar  
275 needle size and morphology and were exposed to the same environmental conditions in  
276 the common garden (including air temperature, vapour-pressure deficit, and soil-  
277 moisture content), we assume that interspecific differences in leaf  $\delta^{18}O$  should primarily  
278 reflect differences in time-integrated cumulative transpiration derived from species-

279 specific patterns of stomatal regulation of leaf-gas exchange (Barbour, 2007; Farquhar  
280 et al., 2007). Likewise, we assume that interspecific differences in leaf  $\delta^{13}\text{C}$  should  
281 primarily reflect differences in iWUE, rather than differences in irradiance or soil-water  
282 availability (as these were the same across species; Dawson et al., 2002; Farquhar et al.,  
283 1989).

284 Both  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  were measured on fully expanded leaves harvested in late  
285 August 2014 (thus after two growing seasons in the field) from 15 individuals per  
286 species and replication block (thus totalling 45 individuals per species); in the case of *P.*  
287 *uncinata* we could only sample 13 individuals due to low survival rate. We harvested  
288 pine needles produced during the current year 2014, discarding those from previous  
289 year cohorts to minimize potential legacy effects from the nursery. The pine individuals  
290 were sampled randomly, although we disregarded those with clear symptoms of decay  
291 (close to death) and those previously used to monitor physiological performance in  
292 order to avoid any bias due to experimental manipulation (e.g. increased physiological  
293 stress after cutting of branches for water-potential measurements in the previous  
294 month). After harvesting the needles for isotopic and nutrient analysis, the whole-  
295 aboveground biomass of the plant was harvested for dry-biomass production  
296 measurement. Samples were oven dried at 60 °C until constant weight and afterwards  
297 the two weights (leaves used for isotopic analysis plus the rest of the plant) of each  
298 individual were added together for the statistical analyses of aboveground dry-biomass  
299 production.

300 The subsample of pine needles used for isotopic analyses was finely ground to a  
301 fine powder with a ball mill. For leaf  $\delta^{13}\text{C}$ , we used 4 mg weighed in tin capsules.  
302 Samples were analysed using an Isoprime isotope ratio mass spectrometer (IRMS;  
303 Isoprime Ltd, Cheadle Hulme, Stockport, UK) coupled to a CN elemental analyser (EA;

304 Eurovector, Pavia, Italy) with continuous flow, at the Department of Biology,  
305 University of Copenhagen. For foliar  $\delta^{18}\text{O}$  analyses, 0.7-0.8 mg were weighed in silver  
306 capsules and analysed at the Stable Isotope Facility of the University of California at  
307 Davis (USA). A Heckatech HT Oxygen Analyzer interfaced to a PDZ Europa 20-20  
308 isotope ratio mass spectrometer (Sercon, Cheshire, UK) was used following the method  
309 described in Kornexl, Gehre, Höfling, & Werner (1999). Leaf samples were  
310 decomposed in a glassy carbon reactor at 1400°C to CO and H<sub>2</sub>O, and oxygen was  
311 analysed as CO. We expressed the isotopic composition of the samples in delta notation  
312 as:

313

$$314 \quad \delta^{xx} = \left( \frac{R_{\text{samp}}}{R_{\text{stand}}} - 1 \right) * 1000$$

315

316 where <sup>xx</sup>E is the heavy isotope which is compared to the lighter one; R refers to the  
317 molar ratio of the heavy to the light isotope (i.e. <sup>13</sup>C:<sup>12</sup>C or <sup>18</sup>O:<sup>16</sup>O); “samp” refers to  
318 the sample; and “stand” refers to an international standard (V-PDB for C and V-SMOW  
319 for O). International and internal standards were used for validating the quality and  
320 precision of isotopic analyses.

321

### 322 *Nutrient analysis*

323 The concentrations of several essential macro- and micronutrients (C, N, P, K, Zn, and  
324 Cu) were measured on the same finely ground leaf samples used for isotopic analyses.  
325 Foliar C and N concentrations were measured with a CN elemental analyser as  
326 described above. Leaf K, P, Cu and Zn concentrations were measured by inductively  
327 coupled plasma emission spectrometry using a Perkin-Elmer 5500 ICP.

328

### 329 *Data analysis*

330 Analyses were performed using R, version 3.3.2 (R Core Team, 2016). We explored  
331 different approaches to control for spurious statistical effects induced by spatial  
332 heterogeneity. Note that the number of blocks is too low to consider this variable a  
333 random factor in a standard mixed-model approach (random factors need to have at  
334 least five levels; Crawley, 2002). Therefore, we controlled for spatial heterogeneity  
335 including the number of columns and rows as continuous variables in linear models  
336 (Appendix S2). Differences across species for all the variables measured, in any case,  
337 followed similar patterns whatever the model used.

338 Differences in seedling survival were estimated with a Cox regression model  
339 using the *survival* R package (Therneau, 2015; Therneau & Grambsch, 2000). The rest  
340 of response variables (growth, physiological variables, leaf isotopic composition and  
341 nutrient concentrations) were analysed with linear models. Significant differences  
342 between species were tested using Tukey's test. The stem-volume increment was used  
343 as a proxy of overall plant growth because it exhibited a close correlation with both  
344 plant height ( $P \leq 0.001$ ;  $\rho \geq 0.8$  in all species) and stem diameter ( $P \leq 0.001$ ;  $\rho \geq 0.6$  in all  
345 species).

346

## 347 **Results**

### 348 *Demography*

349 Across species, a total of 627 (74.7%) seedlings survived after two growing seasons.  
350 Survival rate differed among species ( $P < 2.2e-16$ ), with an overall value of 100<sup>a</sup> %  
351 for *P. halepensis*, 92.9<sup>b</sup> % for *P. nigra*, 80<sup>c</sup> % for *P. sylvestris*, and 25.8<sup>d</sup> % for *P.*  
352 *uncinata* (different superscript letters indicate significant differences among species,  $P$   
353  $< 0.05$  after Tukey's multiple comparison).

354 Growth (stem-volume increment) after two growing seasons also differed  
355 greatly among species ( $P < 2.2e-16$ ), with a much higher value for *P. halepensis*  
356 ( $223.14 \pm 11.47^a \text{ cm}^3$ ), followed by *P. nigra* ( $9.41 \pm 0.48^b \text{ cm}^3$ ), *P. sylvestris* ( $5.06 \pm 0.29^c$   
357  $\text{ cm}^3$ ), and *P. uncinata* ( $2.91 \pm 0.43^d \text{ cm}^3$ ). Interspecific differences in growth increased  
358 even further after the third growing season (September 2015), with mean stem volume  
359 of  $3000.56 \pm 330.25^a$ ,  $45.26 \pm 5.90^b$ , and  $16.50 \pm 2.20^c \text{ cm}^3$ , and mean fresh weight of  
360  $11723 \pm 700^a$ ,  $322 \pm 4^b$ , and  $110 \pm 14^c \text{ g}$  for *P. halepensis*, *P. nigra*, and *P. sylvestris*,  
361 respectively. *Pinus uncinata* was not sampled in 2015 due to the small number of  
362 surviving individuals, but their size was visually the lowest of all the species. See  
363 Appendix S1, Table S5 for species mean values of stem-root collar and leader shoot  
364 height in both years.

365

### 366 *Physiological variables*

367 Overall, there were large differences in physiological parameters between *P. halepensis*  
368 and the rest of the species, with *P. uncinata* in particular showing the poorest  
369 performance under the common garden conditions (Table 1). *Pinus halepensis* showed  
370 significantly lower predawn and mid-day stem water potentials than the other species  
371 during the summer dry season (Table 1), indicating a more anisohydric behaviour.  
372 *Pinus halepensis* also showed the highest values for most photochemical parameters  
373 including predawn and midday  $F_v/F_m$ , quantum yield, rETR, and photochemical  
374 quenching, as well as the lowest values of non-photochemical quenching (although  
375 without statistically significant differences from *P. nigra* and/or *P. sylvestris* for the  
376 latter two variables). On the contrary, *P. uncinata* showed the lowest values for most of  
377 these photochemical parameters, with significant differences from the rest of species for  
378 quantum yield and rETR but without significant differences from *P. nigra* and/or *P.*



379 *sylvestris* for the remaining fluorescence variables (Table 1).

380

### 381 *Leaf isotopic composition and nutrient concentrations*

382 Mean leaf  $\delta^{13}\text{C}$  values differed significantly among pine species (Table 2), with *P.*

383 *halepensis* showing the highest value (indicative of higher time-integrated water use

384 efficiency), followed by *P. sylvestris*, *P. nigra* and *P. uncinata*. There were also large

385 differences in mean leaf  $\delta^{18}\text{O}$  values among species, with *P. halepensis* showing the

386 lowest mean value by far (indicative of higher stomatal conductance and cumulative

387 transpiration), followed by *P. nigra*, *P. sylvestris*, and *P. uncinata* (Table 2).

388 Leaf N concentration differed only slightly among pine species, with values

389 ranging from 12.8 mg g<sup>-1</sup> for *P. nigra* to 18.3 mg g<sup>-1</sup> for *P. sylvestris* (Table 2). In

390 contrast, leaf P, K, Cu and Zn concentrations differed sharply among species, with *P.*

391 *halepensis* showing about 2-fold (P), 1.7-fold (K), 1.5-fold (Zn) and 1.8-fold (Cu)

392 higher mean concentrations than the other species (Table 2). As a result, mean foliar

393 N:P and N:K ratios were over 2-fold lower in *P. halepensis* than in the other pine

394 species (Table 2). Leaf C:N ratios were less variable across species and ranged from

395 26.5 in *P. sylvestris* to 35.6 in *P. nigra*.

396 The dry-biomass of pines harvested for isotopic analysis followed the same

397 trend described above for shoot volume and fresh weight after three years: 660.9±32.2

398 for *P. halepensis*, 32.0±2.0 for *P. nigra*, 19.2±1.1 for *P. sylvestris* and 10.4±1.6 g for *P.*

399 *uncinata*.

400

### 401 *Relationships among plant response variables across and within species*

402 Across pine species, leaf  $\delta^{18}\text{O}$  was strongly negatively correlated with aboveground

403 biomass and leaf P, K, Cu, and Zn concentrations (Figure 2), while it was positively

404 associated with leaf N and N:P ratio (Figure 3), overall indicating increased nutrient  
405 status, stoichiometric balance and growth with increasing time-integrated stomatal  
406 conductance and transpiration. Conversely, leaf  $\delta^{13}\text{C}$  exhibited strong positive  
407 correlations with biomass production and leaf P, K, Cu, and Zn concentrations across  
408 species, indicating increasing water use efficiency with increasing leaf nutrient status  
409 and growth. Leaf  $\delta^{13}\text{C}$  was negatively associated with N:P ratios, but was unrelated to  
410 leaf N concentration across species. Interestingly, leaf  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  were negatively  
411 associated with each other across all four pine species ( $\rho = -0.48$ ;  $P = 1.70\text{E-}09$ ), which  
412 suggests that interspecific variation in  $\delta^{13}\text{C}$  was primarily driven by variation in  
413 photosynthesis (rather than in stomatal conductance).

414         Similar correlation patterns were found also at the within-species level for *P.*  
415 *nigra* and *P. sylvestris*: aboveground biomass and stem volume increment were  
416 positively associated with leaf P concentration and negatively associated with leaf  $\delta^{18}\text{O}$ ,  
417 N, and N:P in at least one of the two species (Appendix S1, Figure S1). Similarly, leaf  
418  $\delta^{13}\text{C}$  was positively correlated with leaf P and Cu concentrations and negatively  
419 associated with N:P ratio. Leaf  $\delta^{18}\text{O}$  was negatively associated with leaf P, Cu, and Zn  
420 concentrations and positively correlated with N:P ratio in one or the two species  
421 (Appendix S1, Figure S2).

422         Plant water potentials and photochemical fluorescence variables were also  
423 significantly correlated with leaf nutrients and isotopes across species. Mean midday  
424 water potential was negatively associated with mean leaf K concentration  
425 ( $\rho = -0.991$ ;  $P = 0.0088$ ). Mean leaf Zn concentration was positively associated with  
426 mean quantum yield ( $\rho = 0.968$ ;  $P = 0.032$ ) and predawn  $F_v/F_m$  ( $\rho = 0.994$ ;  $P = 0.0063$ ),  
427 while it was negatively associated with  $q_N$  ( $\rho = -0.984$ ;  $P = 0.016$ ), which overall  
428 suggests increased photochemical performance with increased leaf Zn status across

429 species. In addition, mean leaf  $\delta^{13}\text{C}$  was positively correlated with mean quantum yield  
430 and predawn  $F_v/F_m$ , and was negatively associated with qN (Figure 5), which suggests  
431 increased water use efficiency with increased photochemical efficiency across species.  
432 Finally, mean leaf  $\delta^{18}\text{O}$  was negatively associated with mean quantum yield, predawn  
433  $F_v/F_m$  and rETR (and positively with mean qN), thus revealing an improved  
434 photochemical efficiency with increasing stomatal conductance and cumulative  
435 transpiration across species (Figure 6).

436

### 437 **Discussion**

438 We found that the thermophilous, drought-tolerant pine species *P. halepensis* exhibited  
439 100% survival and far greater growth than the other more drought-sensitive mountain  
440 pine species evaluated in the common garden experiment. Unsurprisingly, pine species  
441 originating from wetter and cooler habitats such as *P. uncinata* and, to a lesser extent, *P.*  
442 *sylvestris*, showed the poorest growth and survival under the xeric common garden  
443 conditions. These results are expected according to the contrasting ecological  
444 requirements of each species and the relatively warm and dry climatic conditions at the  
445 study site. However, our study provides insights into the physiological mechanisms  
446 underlying the contrasting responses among pine species, and supports the contention  
447 that the tight stomatal regulation typical of drought-sensitive mountain pine species  
448 makes them fall into an “isohydric trap”, in which high stomatal sensitivity to soil water  
449 shortage and high atmospheric evaporative demand trigger stomatal closure and lead to  
450 a detrimental feedback loop that eventually causes severe nutrient starvation and  
451 stoichiometric imbalance under prolonged dry conditions (Figure 1).

452         The large interspecific differences in leaf isotopic ratios and stem water potential  
453 indicate that pine species differ in stomatal control under prolonged climatic dryness.

454 According to the dual isotope conceptual model (Grams, Kozovits, Häberle, Matyssek,  
455 & Dawson, 2007; Scheidegger, Saurer, Bahn, & Siegwolf, 2000), the combination of  
456 high  $\delta^{18}\text{O}$  and low  $\delta^{13}\text{C}$  values in the drought-sensitive mountain pine species  
457 (compared to *P. halepensis*) indicates low time-integrated stomatal conductance and  
458 water use efficiency, as well as low photosynthesis rates (Querejeta, Allen, Caravaca, &  
459 Roldán, 2006), which is consistent with the poor growth of these species. This  
460 interpretation of isotope data is in strong agreement with gas exchange measurements  
461 conducted at whole plant level with transient-state closed chambers in the same  
462 common garden experiment, which showed higher stomatal conductance and  
463 transpiration, photosynthetic rates and water use efficiency in *P. halepensis* than in the  
464 other pine species during the dry season (Salazar-Tortosa et al., 2018). Furthermore, the  
465 potential influence of the use of different water sources among species can be discarded  
466 as the lower predawn water potential of *P. halepensis* compared to other species would  
467 be incompatible with the alternative explanation that it was using a more  $\delta^{18}\text{O}$  depleted  
468 source water stored in deeper, wetter soil layers (Nardini et al., 2016; Voltas,  
469 Lucabaugh, Chambel, & Ferrio, 2015; West et al., 2012).

470         The combination of high predawn and midday stem water potentials at the peak  
471 of the dry season with low stomatal conductance and cumulative transpiration (inferred  
472 from high leaf  $\delta^{18}\text{O}$  values) indicates a typical isohydric behaviour (Martínez-Vilalta &  
473 Garcia-Forner, 2017) in *P. nigra*, *P. sylvestris* and *P. uncinata*. Under low water  
474 availability and high temperature and evaporative demand conditions, plants with  
475 isohydric behaviour close their stomata early to maintain relatively high water potentials  
476 and prevent dehydration (Klein, 2014; McDowell et al., 2008; Tardieu & Simonneau,  
477 1998). Ultimately, this reduction of cumulative transpiration negatively affects the  
478 plant's nutrient status given the importance of active transpiration flux along the soil-

479 plant-air continuum for effective soil nutrient uptake in drying soil (Cramer et al., 2009;  
480 Lambers et al., 2008; Roupael et al., 2012; Sardans, Peñuelas, Prieto, & Estiarte, 2008;  
481 Sardans, Peñuelas, Coll, Vayreda, & Rivas-Ubach, 2012). The strong negative  
482 correlations between leaf  $\delta^{18}\text{O}$  (proxy of stomatal conductance) and foliar P, K, Cu, Zn  
483 concentrations and positive correlation of leaf  $\delta^{18}\text{O}$  with N:P and N:K ratios across  
484 species (as well as within species for P and N:P; Figure 3, Appendix S1 Figure S2)  
485 indicate that soil nutrient uptake was severely constrained by low cumulative  
486 transpiration in the drought-sensitive mountain pine species under prolonged dryness,  
487 relative to *P. halepensis*.

488         Stomatal conductance and transpiration will determine the rate of water  
489 extraction from soil, so that species with higher transpiration rates will deplete  
490 rhizosphere soil water faster and will hence experience greater reductions in predawn  
491 water potentials during dry periods (Martínez-Vilalta & Garcia-Forner, 2017), as found  
492 in *P. halepensis*. This ability to allow water potentials to drop while sustaining high  
493 stomatal aperture and conductance (i.e. a more anisohydric strategy) may have enabled  
494 *P. halepensis* to continue extracting water and dissolved nutrients from soil during  
495 prolonged dry periods. A recent study has also reported strong negative correlations  
496 between leaf  $\delta^{18}\text{O}$  and foliar nutrient concentrations across contrasting plant species  
497 exposed to the same environmental conditions, suggesting a heavy dependence of plant  
498 nutrient uptake and status on stomatal conductance and cumulative transpiration (Prieto,  
499 Querejeta, Segrestin, Volaire, & Roumet, 2017). Nitrogen, by contrast, did not seem to  
500 be involved in the observed pattern, as this nutrient was negatively associated with  
501 biomass and positively associated with  $\delta^{18}\text{O}$  (Figure 2, 3, Appendix S1 Figure S1),  
502 which suggests that the interspecific differences in leaf N concentration were mainly a  
503 consequence of differential growth dilution effects (Sabaté & Gracia, 1994) and not of

504 stomatal behaviour. In addition, the species with lower growth (*P. sylvestris* and *P.*  
505 *uncinata*) could have constitutively high leaf N as supported by higher N concentration  
506 for *P. uncinata* at nursery, and by previous studies for *P. sylvestris* (Sardans et al.,  
507 2011).

508         The unusually high leaf N:P and N:K ratios found in the more drought sensitive,  
509 mountain pine species are indicative of strong P and K limitation of growth (Güsewell,  
510 2004; Lawniczak, Güsewell, & Verhoeven, 2009; See Table 2 for a comparison with  
511 reference values of each species). The severe nutrient deficit and imbalance observed in  
512 these drought-sensitive pine species appeared to be a direct consequence of climatic  
513 drought stress, as seedling nutrient status was optimal at the beginning of the  
514 experiment across species (Appendix S1, Table S1), and given that the soil at the  
515 common garden site was not deficient in any nutrient (Appendix S1, Table S2). Indeed,  
516 soil nutrient availability in this fertile agricultural field was sufficient to support a  
517 balanced leaf stoichiometry with optimal N:P ratios and vigorous biomass growth in *P.*  
518 *halepensis*. Despite much larger growth-dilution effects, leaf P, K, Zn and Cu  
519 concentrations were higher and N:P and N:K ratios were much lower in *P. halepensis*  
520 than in the other species, indicating that severe nutrient starvation and stoichiometric  
521 imbalance in the other species was largely the result of climatic stress, rather than the  
522 result of low soil nutrient availability “per se”. Deficiency of P, K, Zn, and Cu and  
523 severe N:P:K stoichiometric imbalance can impair photosynthesis, transpiration (Figure  
524 1; blue arrow), water-use efficiency and growth, as reported in this study (see also  
525 Güsewell, 2004; Sardans & Peñuelas, 2015; Talbott & Zeiger, 1996). This idea is  
526 supported by the strong correlations of leaf nutrient concentrations and stoichiometric  
527 ratios with both leaf  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  ratios and with aboveground biomass across and  
528 within species (Figures 2, 3, 4, Appendix S1, Figures S1, S2). We also found a negative

529 association between leaf K concentration and water potential across species, which may  
530 be explained by the major role that K plays in drought tolerance (Rivas-Ubach, Sardans,  
531 Perez-Trujillo, Estiarte, & Penuelas, 2012; Sardans & Peñuelas, 2015).

532         The rather extreme N:P:K stoichiometric imbalance observed in the drought-  
533 sensitive mountain pine species may be explained by differences in mobility and  
534 availability in the soil solution among nutrients, given that  $\text{PO}_4^{--}$  and  $\text{K}^+$  have diffusion  
535 coefficients that are lower by orders of magnitude than that of  $\text{NO}_3^-$  (Lambers et al.,  
536 2008; Marschner & Rengel, 2012). Our study suggests that uptake of nutrients with  
537 limited mobility and diffusion rates in soil (e.g. phosphate, potassium, zinc, copper)  
538 may be particularly vulnerable to severe decreases in transpiration and mass flow to  
539 roots, compared to highly mobile nutrients like nitrate (Rengel & Marschner, 2005).  
540 Besides, atmospheric deposition is several orders of magnitude higher for N than for  
541 other nutrients (Peñuelas, Sardans, Rivas-Ubach, & Janssens, 2012), which may also  
542 lead to higher foliar N uptake. Therefore, in a global scenario of climate warming  
543 combined with increasing rates of anthropogenic N deposition (Güsewell, 2004; Jonard  
544 et al., 2015) we should expect plant P status (along with K and micronutrients like Cu or  
545 Zn) to be particularly vulnerable to decreases in transpiration fluxes during prolonged  
546 periods of climatic dryness, whereas plant N status may be less responsive. In addition,  
547 the reduced carbon assimilation exhibited by drought-sensitive mountain pine species at  
548 the common garden site (Salazar-Tortosa et al., 2018) could lead to low carbon  
549 availability to support the growth and activity of fine roots and ectomycorrhizal (EMF)  
550 fungi (Gessler et al., 2017; Matías et al., 2017; Moran et al., 2017). This could hamper  
551 even more the assimilation of low mobility nutrients, whose absorption has high energy  
552 and carbon costs such as the production of extramatrical EMF mycelium, the secretion  
553 of phosphatases and organic acids by roots and mycorrhizae for solubilisation and

554 mineralization of inorganic and organic P, or rhizosphere priming effects (Achat,  
555 Augusto, Gallet-Budynek, & Loustau, 2016; Kreuzwieser & Gessler, 2010). Moreover,  
556 limited carbohydrate availability and transfer to roots could also constrain the supply of  
557 energy and carbon skeletons for nutrient assimilation (Kreuzwieser & Gessler, 2010).

558       Soil moisture content remained above the permanent wilting point throughout  
559 the summer dry period in both years of the experiment (Appendix S1, Table S4), which  
560 suggests that high temperature and evaporative demand may have also been key drivers  
561 of the contrasting responses observed among pine species (McDowell et al., 2015;  
562 McDowell & Allen, 2015; Salazar-Tortosa et al., 2018; Williams et al., 2013). In fact,  
563 mean summer temperature at the common garden site was considerably higher than that  
564 experienced by the mountain pine species in their original habitat (*P. nigra*, *P.*  
565 *sylvestris*, and *P. uncinata*). Heat stress can limit stomatal conductance, as plants close  
566 their stomata to prevent excessive transpiration and water loss when atmospheric  
567 demand for water increases with rising temperatures (Novick et al., 2016; Urban,  
568 Ingwers, McGuire, & Teskey, 2017; Zhang, Wollenweber, Jiang, Liu, & Zhao, 2008).

569 Stomatal closure in response to heat and drought stress reduces evaporative leaf cooling  
570 (Cook, Dixon, & Leopold, 1964), which may favour heat-induced damage of the  
571 photosynthetic machinery that decreases photochemical efficiency (Sharkey, 2005).

572 Lower predawn  $F_v/F_m$  values as well as larger predawn  $F_v/F_m$  vs. midday  $F_v/F_m$   
573 differences in mountain pine species (compared to *P. halepensis*) support the idea that  
574 heat stress in combination with high light levels led to both chronic photoinhibition as  
575 well as reversible reduced photochemical efficiency during the hottest time of the day in  
576 summer (Table 1). Moreover, the detrimental impacts of the combination of high  
577 temperatures and drought stress on leaf photochemical efficiency and carboxylation  
578 capacity were likely compounded by the effects of macro- and micronutrient deficiency



579 and stoichiometric imbalance in mountain pine species (Cakmak, 2005; Casimiro,  
580 Barroso, & Pais, 1990; Eller, Jensen, & Reisdorff, 2016), which may explain the  
581 association of low photochemical efficiency (as indicated by low rETR, quantum yield  
582 and predawn  $F_v/F_m$  and high qN) with low cumulative transpiration (high  $\delta^{18}\text{O}$ ) and  
583 water use efficiency (low  $\delta^{13}\text{C}$ ) across species (Figures 5, 6). Overall, the results of this  
584 study suggest that intensified drought stress from higher air temperatures—the “hotter  
585 drought” effect (Allen et al., 2015)—might lead to severe nutrient deficit,  
586 stoichiometric imbalance, and photosynthetic impairment mediated by reduced  
587 cumulative transpiration in drought-sensitive species.

588         In summary, we found that the degree of stomatal regulation of transpiration is  
589 tightly linked to plant nutrient status and stoichiometry under dry conditions, and that  
590 reduced nutrient uptake is a key mechanism to consider when assessing poor plant  
591 growth and survival under prolonged climatic dryness. We propose the existence of a  
592 detrimental feedback loop leading to severe P and K starvation and N:P:K imbalance in  
593 strictly isohydric vascular plants undergoing prolonged drought stress, such as some of  
594 our study pine species. These drought-sensitive species close their stomata at relatively  
595 high soil and plant water potentials, and thus are not able to maintain adequate  
596 transpiration and nutrient uptake during prolonged drought periods, which leads to  
597 severe nutrient imbalance that might exacerbate and accelerate the onset of carbon  
598 starvation, hydraulic failure, phloem dysfunction, and their multiple interplays  
599 (McDowell et al., 2011; Sala, Piper, & Hoch, 2010; Sevanto, McDowell, Dickman,  
600 Pangle, & Pockman, 2014; Zwieniecki & Holbrook, 2009). In contrast, relatively  
601 drought-tolerant plant species with a more anisohydric behaviour such as *P. halepensis*  
602 can maintain open stomata and transpiration at comparatively lower soil and plant water  
603 potentials, thereby escaping this detrimental feedback and avoiding nutrient deficit and

604 imbalance. This study highlights the intimate interplay and interdependence between  
605 stomatal regulation, transpiration, carbon assimilation and nutrient status in the response  
606 of vascular plants to long periods of climatic dryness (Gessler et al., 2017). We  
607 advocate consideration of this proposed conceptual framework (Fig. 1) in order to better  
608 understand and predict the impacts of ongoing global change on the performance and  
609 survival of pines and other plant species with tight stomatal regulation and strict  
610 isohydric behaviour, with special attention to the role of macronutrients with low  
611 mobility in soil, such as P and K.

612

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622 **Bibliography**

- 623 Achat, D. L., Augusto, L., Gallet-Budynek, A., & Loustau, D. (2016). Future challenges  
624 in coupled C–N–P cycle models for terrestrial ecosystems under global change: a  
625 review. *Biogeochemistry*, *131*, 173–202. [https://doi.org/10.1007/s10533-016-0274-](https://doi.org/10.1007/s10533-016-0274-9)  
626 9
- 627 Adams, H. D., Zeppel, M. J. B., Anderegg, W. R. L., Hartmann, H., Landh usser, S. M.,  
628 Tissue, D. T., ... McDowell, N. G. (2017). A multi-species synthesis of  
629 physiological mechanisms in drought-induced tree mortality. *Nature Ecology &*  
630 *Evolution*, *1*, 1285–1291. <https://doi.org/10.1038/s41559-017-0248-x>
- 631 Allen, C. D., Breshears, D. D., & McDowell, N. G. (2015). On underestimation of  
632 global vulnerability to tree mortality and forest die-off from hotter drought in the  
633 Anthropocene. *Ecosphere*, *6*, 129. <https://doi.org/10.1890/ES15-00203.1>
- 634 Barbour, M. M. (2007). Stable oxygen isotope composition of plant tissue: a review.  
635 *Functional Plant Biology*, *34*, 83–94. <https://doi.org/10.1071/FP06228>
- 636 Cabrera-Bosquet, L., S anchez, C., & Araus, J. L. (2009). Oxygen isotope enrichment  
637 ( $\delta^{18}\text{O}$ ) reflects yield potential and drought resistance in maize. *Plant, Cell and*  
638 *Environment*, *32*, 1487–1499. <https://doi.org/10.1111/j.1365-3040.2009.02013.x>
- 639 Cakmak, I. (2005). The role of potassium in alleviating detrimental effects of abiotic  
640 stresses in plants. *Journal of Plant Nutrition and Soil Science*, *168*, 521–530.  
641 <https://doi.org/10.1002/jpln.200420485>
- 642 Casimiro, A., Barroso, J., & Pais, M. S. (1990). Effect of copper deficiency on  
643 photosynthetic electron transport in wheat plants. *Physiologia Plantarum*, 459–  
644 464.
- 645 Choat, B., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R., ... Zanne,  
646 A. E. (2012). Global convergence in the vulnerability of forests to drought. *Nature*,  
647 *491*, 752–5. <https://doi.org/10.1038/nature11688>
- 648 Christensen, K. Ib. (1987). Taxonomic revision of the *Pinus mugo* complex and *P.*  
649 *rhaetica* (*P. mugo sylvestris*) (Pinaceae). *Nordic Journal of Botany*, *7*, 383–408.  
650 <https://doi.org/10.1111/j.1756-1051.1987.tb00958.x>
- 651 Cook, G. D., Dixon, J. R., & Leopold, A. C. (1964). Transpiration: Its Effects on Plant  
652 Leaf Temperature. *Science*, *144*, 546–547.
- 653 Cramer, M. D., Hawkins, H.-J., & Verboom, G. A. (2009). The importance of  
654 nutritional regulation of plant water flux. *Oecologia*, *161*, 15–24.  
655 <https://doi.org/10.1007/s00442-009-1364-3>
- 656 Cramer, M. D., & Hoffman, M. T. (2015). The consequences of precipitation  
657 seasonality for mediterranean-ecosystem vegetation of South Africa. *PLoS ONE*,  
658 *10*, 1–21. <https://doi.org/10.1371/journal.pone.0144512>
- 659 Crawley, M. J. (2002). *Statistical Computing: An Introduction to Data Analysis using S-*  
660 *PLUS*. John Wiley & Sons, Ltd.
- 661 Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., & Tu, K. P. (2002).

- 662 Stable Isotopes in Plant Ecology. *Annual Review of Ecology and Systematics*, 33,  
663 507–559. <https://doi.org/10.1146/annurev.ecolsys.33.020602.095451>
- 664 Eller, F., Jensen, K., & Reisdorff, C. (2016). Nighttime stomatal conductance differs  
665 with nutrient availability in two temperate floodplain tree species. *Tree Physiology*,  
666 1–13. <https://doi.org/10.1093/treephys/tpw113>
- 667 Enescu, C. M., de Rigo, D., Caudullo, G., Mauri, A., & Houston-Durrant, T. (2016).  
668 *Pinus nigra* in Europe: distribution, habitat, usage and threats. In J. San-Miguel-  
669 Ayanz, D. de Rigo, G. Caudullo, T. Houston-Durrant, & A. Mauri (Eds.),  
670 *European Atlas of Forest Tree Species*. Off. EU, Luxembourg.
- 671 Farquhar, G. D., Cernusak, L. a, & Barnes, B. (2007). Heavy water fractionation during  
672 transpiration. *Plant Physiology*, 143, 11–18. <https://doi.org/10.1104/pp.106.093278>
- 673 Farquhar, G. D., Ehleringer, J. R., & Hubick, K. T. (1989). Carbon Isotope  
674 Discrimination and Photosynthesis. *Annual Review of Plant Physiology and Plant*  
675 *Molecular Biology*, 40, 503–537.  
676 <https://doi.org/10.1146/annurev.pp.40.060189.002443>
- 677 Fernández-Pérez, L., Villar-Salvador, P., Martínez-Vilalta, J., Toca, A., & Zavala, M.  
678 A. (2018). Distribution of pines in the Iberian Peninsula agrees with species  
679 differences in foliage frost tolerance, not with vulnerability to freezing-induced  
680 xylem embolism. *Tree Physiology*, in press.  
681 <https://doi.org/10.1093/treephys/tpx171>
- 682 Garcia-Forner, N., Biel, C., Savé, R., & Martínez-Vilalta, J. (2016). Isohydric species  
683 are not necessarily more carbon limited than anisohydric species during drought.  
684 *Tree Physiology*, 1–15.
- 685 Gessler, A., Schaub, M., & McDowell, N. G. (2017). The role of nutrients in drought-  
686 induced tree mortality and recovery. *New Phytologist*, 214, 513–520.  
687 <https://doi.org/10.1111/nph.14340>
- 688 Grams, T. E. E., Kozovits, A. R., Häberle, K. H., Matyssek, R., & Dawson, T. E.  
689 (2007). Combining  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  analyses to unravel competition, CO<sub>2</sub> and O<sub>3</sub>  
690 effects on the physiological performance of different-aged trees. *Plant, Cell and*  
691 *Environment*, 30, 1023–1034. <https://doi.org/10.1111/j.1365-3040.2007.01696.x>
- 692 Güsewell, S. (2004). N : P Ratios in Terrestrial Plants: Variation and Functional  
693 Significance. *New Phytologist*, 164, 243–266.
- 694 Hartmann, H., Moura, C. F., Anderegg, W. R. L., Ruehr, N. K., Salmon, Y., Allen, C.  
695 D., ... O'Brien, M. (2018). Research frontiers for improving our understanding of  
696 drought-induced tree and forest mortality. *New Phytologist*, 218, 15–28.  
697 <https://doi.org/10.1111/nph.15048>
- 698 Houston-Durrant, T., de Rigo, D., & Caudullo, G. (2016). *Pinus sylvestris* in Europe:  
699 distribution, habitat, usage and threats. In J. San-Miguel-Ayanz, D. de Rigo, G.  
700 Caudullo, T. Houston-Durrant, & A. Mauri (Eds.), *European Atlas of Forest Tree*  
701 *Species*. Off. EU, Luxembourg.
- 702 Jonard, M., Fürst, A., Verstraeten, A., Thimonier, A., Timmermann, V., Potočić, N., ...  
703 Rautio, P. (2015). Tree mineral nutrition is deteriorating in Europe. *Global Change*

- 704 *Biology*, 21, 418–430. <https://doi.org/10.1111/gcb.12657>
- 705 Klein, T. (2014). The variability of stomatal sensitivity to leaf water potential across  
706 tree species indicates a continuum between isohydric and anisohydric behaviours.  
707 *Functional Ecology*, 28, 1313–1320. <https://doi.org/10.1111/1365-2435.12289>
- 708 Köble, R., & Seufert, G. (2001). Novel maps for forest tree species in Europe. In  
709 *Proceedings of the 8th European Symposium on the Physico-Chemical Behaviour*  
710 *of Air Pollutants: “A Changing Atmosphere!”* Torino (IT).
- 711 Koerselman, W., & Meuleman, A. F. M. (1996). The vegetation N:P ratio: a new tool to  
712 detect the nature of nutrient limitation. *Journal of Applied Ecology*, 33, 1441–  
713 1450. <https://doi.org/10.2307/2404783>
- 714 Kornexl, B. E., Gehre, M., Höfling, R., & Werner, R. A. (1999). On-line  $\delta^{18}\text{O}$   
715 measurement of organic and inorganic substances. *Rapid Communications in Mass*  
716 *Spectrometry*, 13, 1685–1693. [https://doi.org/10.1002/\(SICI\)1097-0231\(19990830\)13:16<1685::AID-RCM699>3.0.CO;2-9](https://doi.org/10.1002/(SICI)1097-0231(19990830)13:16<1685::AID-RCM699>3.0.CO;2-9)
- 718 Kreuzwieser, J., & Gessler, A. (2010). Global climate change and tree nutrition:  
719 Influence of water availability. *Tree Physiology*, 30, 1221–1234.  
720 <https://doi.org/10.1093/treephys/tpq055>
- 721 Lambers, H., Chapin, F. S., & Pons, T. L. (2008). Mineral Nutrition. In T. L. Pons  
722 (Ed.), *Plant Physiological Ecology* (2nd ed, pp. 255–320). New York, NY:  
723 Springer New York. [https://doi.org/10.1007/978-0-387-78341-3\\_9](https://doi.org/10.1007/978-0-387-78341-3_9)
- 724 Lawlor, D. W., & Cornic, G. (2002). Photosynthetic carbon assimilation and associated  
725 metabolism in relation to water deficits in higher plants. *Plant, Cell &*  
726 *Environment*, 25, 275–294. <https://doi.org/10.1046/j.0016-8025.2001.00814.x>
- 727 Lawniczak, A. E., Güsewell, S., & Verhoeven, J. T. A. (2009). Effect of N:K supply  
728 ratios on the performance of three grass species from herbaceous wetlands. *Basic*  
729 *and Applied Ecology*, 10, 715–725. <https://doi.org/10.1016/j.baae.2009.05.004>
- 730 León-Sánchez, L., Nicolás, E., Goberna, M., Prieto, I., Maestre, F. T., & Querejeta, J. I.  
731 (2017). Poor plant performance under simulated climate change is linked to  
732 mycorrhizal responses in a semiarid shrubland. *Journal of Ecology*,  
733 <https://doi.org/10.1111/1365-2745.12888>. <https://doi.org/10.1111/1365-2745.12888>
- 735 Marschner, H., Kirkby, E. a, & Cakmak, I. (1996). Effect of mineral nutritional status  
736 on shoot-root partitioning of photoassimilates and cycling of mineral nutrients.  
737 *Journal of Experimental Botany*, 47, 1255–1263.  
738 [https://doi.org/10.1093/jxb/47.Special\\_Issue.1255](https://doi.org/10.1093/jxb/47.Special_Issue.1255)
- 739 Marschner, P., & Rengel, Z. (2012). Nutrient Availability in Soils. In P. Marschner  
740 (Ed.), *Marschner’s Mineral Nutrition of Higher Plants: Third Edition* (pp. 315–  
741 330). Elsevier Ltd. <https://doi.org/10.1016/B978-0-12-384905-2.00012-1>
- 742 Martin-StPaul, N., Delzon, S., & Cochard, H. (2017). Plant resistance to drought  
743 depends on early stomatal closure. *Ecology Letters*, 1–23.  
744 <https://doi.org/10.1111/ele.12851>

- 745 Martínez-Vilalta, J., & Garcia-Forner, N. (2017). Water potential regulation, stomatal  
746 behaviour and hydraulic transport under drought: Deconstructing the  
747 iso/anisohydric concept. *Plant Cell and Environment*, *40*, 962–976.  
748 <https://doi.org/10.1111/pce.12846>
- 749 Matías, L., Castro, J., Villar-Salvador, P., Quero, J. L., & Jump, A. S. (2017).  
750 Differential impact of hotter drought on seedling performance of five ecologically  
751 distinct pine species. *Plant Ecology*, 1–12. [https://doi.org/10.1007/s11258-016-](https://doi.org/10.1007/s11258-016-0677-7)  
752 [0677-7](https://doi.org/10.1007/s11258-016-0677-7)
- 753 Mauri, A., Di Leo, M., de Rigo, D., & Caudullo, G. (2016). *Pinus halepensis* and *Pinus*  
754 *brutia* in Europe: distribution, habitat, usage and threats. In J. San-Miguel-Ayanz,  
755 D. de Rigo, G. Caudullo, T. Houston-Durrant, & A. Mauri (Eds.), *European Atlas*  
756 *of Forest Tree Species*. Off. EU, Luxembourg.
- 757 McDowell, N., Beerling, D. J., Breshears, D. D., Fisher, R. A., Raffa, K. F., & Stitt, M.  
758 (2011). The interdependence of mechanisms underlying climate-driven vegetation  
759 mortality. *Trends in Ecology and Evolution*, *26*, 523–532.  
760 <https://doi.org/10.1016/j.tree.2011.06.003>
- 761 McDowell, N., Fisher, R. A., Xu, C., Domec, J. C., Hölttä, T., Mackay, D. S., ...  
762 Pockman, W. T. (2013). Evaluating theories of drought-induced vegetation  
763 mortality using a multimodel-experiment framework. *New Phytologist*, *200*, 304–  
764 321. <https://doi.org/10.1111/nph.12465>
- 765 McDowell, N. G., & Allen, C. D. (2015). Darcy's law predicts widespread forest  
766 mortality under climate warming. *Nature Climate Change*, *5*, 669–672.  
767 <https://doi.org/10.1038/nclimate2641>
- 768 McDowell, N. G., Williams, A. P., Xu, C., Pockman, W. T., Dickman, L. T., Sevanto,  
769 S., ... Koven, C. (2015). Multi-scale predictions of massive conifer mortality due  
770 to chronic temperature rise. *Nature Climate Change*, *6*, 295–300.  
771 <https://doi.org/10.1038/nclimate2873>
- 772 McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., ...  
773 Yepez, E. A. (2008). Mechanisms of plant survival and mortality during drought:  
774 why do some plants survive while others succumb to drought? *New Phytologist*,  
775 *178*, 719–739. <https://doi.org/10.1111/j.1469-8137.2008.02436.x>
- 776 Meinzer, F. C., Woodruff, D. R., Marias, D. E., Mcculloh, K. A., & Sevanto, S. (2014).  
777 Dynamics of leaf water relations components in co-occurring iso- and anisohydric  
778 conifer species. *Plant, Cell and Environment*, 2577–2586.  
779 <https://doi.org/10.1111/pce.12327>
- 780 Moran, E., Lauder, J., Musser, C., Stathos, A., & Shu, M. (2017). The genetics of  
781 drought tolerance in conifers. *New Phytologist*, *216*.  
782 <https://doi.org/10.1111/nph.14774>
- 783 Nardini, A., Casolo, V., Dal Borgo, A., Savi, T., Stenni, B., Bertoncin, P., ...  
784 McDowell, N. G. (2016). Rooting depth, water relations and non-structural  
785 carbohydrate dynamics in three woody angiosperms differentially affected by an  
786 extreme summer drought. *Plant Cell and Environment*, *39*, 618–627.  
787 <https://doi.org/10.1111/pce.12646>

- 788 Novick, K. A., Ficklin, D. L., Stoy, P. C., Williams, C. A., Bohrer, G., Oishi, A. C., ...  
789 Phillips, R. P. (2016). The increasing importance of atmospheric demand for  
790 ecosystem water and carbon fluxes. *Nature Climate Change*, *6*, 1023–1027.  
791 <https://doi.org/10.1038/nclimate3114>
- 792 Oliet, J., Planelles, R., López Arias, M., & Artero, F. (2002). Soil water content and  
793 water relations in planted and naturally regenerated *Pinus halepensis* Mill.  
794 seedlings during the first year in semiarid conditions. *New Forests*, *23*, 31–44.  
795 <https://doi.org/10.1023/A:1015668815037>
- 796 Peñuelas, J., Sardans, J., Rivas-Ubach, A., & Janssens, I. A. (2012). The human-  
797 induced imbalance between C, N and P in Earth's life system. *Global Change*  
798 *Biology*, *18*, 3–6. <https://doi.org/10.1111/j.1365-2486.2011.02568.x>
- 799 Prieto, I., Querejeta, J. I., Segrestin, J., Volaire, F., & Roumet, C. (2017). Leaf carbon  
800 and oxygen isotopes are coordinated with the leaf economics spectrum in  
801 Mediterranean rangeland species. *Functional Ecology*.  
802 <https://doi.org/doi:10.1111/1365-2435.13025>
- 803 Querejeta, J. I., Allen, M. F., Caravaca, F., & Roldán, A. (2006). Differential  
804 modulation of host plant  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  by native and nonnative arbuscular  
805 mycorrhizal fungi in a semiarid environment. *The New Phytologist*, *169*, 379–387.  
806 <https://doi.org/10.1111/j.1469-8137.2005.01599.x>
- 807 R Core Team. (2016). R: A Language and Environment for Statistical Computing.  
808 Retrieved from <https://www.r-project.org/>
- 809 Rengel, Z., & Marschner, P. (2005). Nutrient availability and management in the  
810 rhizosphere: Exploiting genotypic differences. *New Phytologist*, *168*, 305–312.  
811 <https://doi.org/10.1111/j.1469-8137.2005.01558.x>
- 812 Richardson, D. M. (2000). *Ecology and Biogeography of Pinus*. Cambridge University  
813 Press.
- 814 Rivas-Ubach, A., Sardans, J., Perez-Trujillo, M., Estiarte, M., & Penuelas, J. (2012).  
815 Strong relationship between elemental stoichiometry and metabolome in plants.  
816 *Proceedings of the National Academy of Sciences*, *109*, 4181–4186.  
817 <https://doi.org/10.1073/pnas.1116092109>
- 818 Roupael, Y., Cardarelli, M., Schwarz, D., Franken, P., & Colla, G. (2012). Effects of  
819 Drought on Nutrient Uptake and Assimilation in Vegetable Crops. In *Plant*  
820 *Responses to Drought Stress* (pp. 171–195). Berlin, Heidelberg: Springer Berlin  
821 Heidelberg. [https://doi.org/10.1007/978-3-642-32653-0\\_7](https://doi.org/10.1007/978-3-642-32653-0_7)
- 822 Rühl, E. H. (1992). Effect of K supply and relative humidity on ion uptake and  
823 distribution on two grapevine rootstock varieties. *Vitis*, *31*, 23–33.
- 824 Ruiz de la Torre, J. (2006). *Flora mayor*. Madrid, Spain: Organismo Autónomo Parques  
825 Nacionales, Dirección General para la Biodiversidad.
- 826 Sabaté, S., & Gracia, C. A. (1994). Canopy nutrient content of a *Quercus ilex* L. forest:  
827 fertilization and irrigation effects. *Forest Ecology and Management*, *68*, 31–37.  
828 [https://doi.org/10.1016/0378-1127\(94\)90135-X](https://doi.org/10.1016/0378-1127(94)90135-X)

- 829 Sala, A., Piper, F., & Hoch, G. (2010). Physiological mechanisms of drought-induced  
830 tree mortality are far from being resolved. *New Phytologist*, *186*, 274–281.
- 831 Salazar-Tortosa, D., Castro, J., Rubio de Casas, R., Viñepla, B., Sánchez-Cañete, E., &  
832 Villar-Salvador, P. (2018). Gas exchange at whole plant level shows that a less  
833 conservative water use is linked to a higher performance in three ecologically  
834 distinct pine species. *Environmental Research Letters*, in press.  
835 <https://doi.org/10.1088/1748-9326/aab18f>
- 836 Sardans, J., & Peñuelas, J. (2012). The Role of Plants in the Effects of Global Change  
837 on Nutrient Availability and Stoichiometry in the Plant-Soil System. *Plant*  
838 *Physiology*, *160*, 1741–1761. <https://doi.org/10.1104/pp.112.208785>
- 839 Sardans, J., & Peñuelas, J. (2015). Potassium : a neglected nutrient in global change.  
840 *Global Ecology and Biogeography*, *24*, 261–275.  
841 <https://doi.org/10.1111/geb.12259>
- 842 Sardans, J., Peñuelas, J., Coll, M., Vayreda, J., & Rivas-Ubach, A. (2012).  
843 Stoichiometry of potassium is largely determined by water availability and growth  
844 in Catalanian forests. *Functional Ecology*, *26*, 1077–1089.  
845 <https://doi.org/10.1111/j.1365-2435.2012.02023.x>
- 846 Sardans, J., Peñuelas, J., Prieto, P., & Estiarte, M. (2008). Changes in Ca, Fe, Mg, Mo,  
847 Na, and S content in a Mediterranean shrubland under warming and drought.  
848 *Journal of Geophysical Research: Biogeosciences*, *113*, 1–11.  
849 <https://doi.org/10.1029/2008JG000795>
- 850 Sardans, J., Rivas-Ubach, A., & Peñuelas, J. (2011). Factors affecting nutrient  
851 concentration and stoichiometry of forest trees in Catalonia ( NE Spain ). *Forest*  
852 *Ecology and Management*, *262*, 2024–2034.  
853 <https://doi.org/10.1016/j.foreco.2011.08.019>
- 854 Scheidegger, Y., Saurer, M., Bahn, M., & Siegwolf, R. (2000). Linking Stable Oxygen  
855 and Carbon Isotopes with Stomatal Conductance and Photosynthetic Capacity : A  
856 Conceptual Model. *Oecologia*, *125*, 350–357.  
857 <https://doi.org/10.1007/S004420000466>
- 858 Schreiber, U. (2004). Pulse-Amplitude-Modulation (PAM) Fluorometry and Saturation  
859 Pulse Method: An Overview. In G. C. Papageorgiou & Govindjee (Eds.), *Advances*  
860 *in Photosynthesis and Respiration* (pp. 279–319). Dordrecht: Springer  
861 Netherlands. [https://doi.org/10.1007/978-1-4020-3218-9\\_11](https://doi.org/10.1007/978-1-4020-3218-9_11)
- 862 Sevanto, S., McDowell, N. G., Dickman, L. T., Pangle, R., & Pockman, W. T. (2014).  
863 How do trees die? A test of the hydraulic failure and carbon starvation hypotheses.  
864 *Plant, Cell and Environment*, *37*, 153–161. <https://doi.org/10.1111/pce.12141>
- 865 Sharkey, T. D. (2005). Effects of moderate heat stress on photosynthesis: Importance of  
866 thylakoid reactions, rubisco deactivation, reactive oxygen species, and  
867 thermotolerance provided by isoprene. *Plant, Cell and Environment*, *28*, 269–277.  
868 <https://doi.org/10.1111/j.1365-3040.2005.01324.x>
- 869 Sperry, J. S., Hacke, U. G., Oren, R., & Comstock, J. P. (2002). Water deficits and  
870 hydraulic limits to leaf water supply. *Plant, Cell and Environment*, *25*, 251–263.



- 871 <https://doi.org/10.1046/j.0016-8025.2001.00799.x>
- 872 Talbott, L. D., & Zeiger, E. (1996). Central Roles for Potassium and Sucrose in Guard-  
873 Cell Osmoregulation. *Plant Physiology*, *111*, 1051–1057.  
874 <https://doi.org/10.1111/4/1051> [pii]
- 875 Tapias, R., Climent, J., Pardos, J. A., & Gil, L. (2004). Life histories of Mediterranean  
876 pines. *Plant Ecology*, *171*, 53–68.  
877 <https://doi.org/10.1023/B:VEGE.0000029383.72609.f0>
- 878 Tardieu, F., & Simonneau, T. (1998). Variability among species of stomatal control  
879 under fluctuating soil water status and evaporative demand: modelling isohydric  
880 and anisohydric behaviours. *Journal of Experimental Botany*, *49*, 419–432.  
881 [https://doi.org/10.1093/jxb/49.Special\\_Issue.419](https://doi.org/10.1093/jxb/49.Special_Issue.419)
- 882 Therneau, T. M. (2015). A Package for Survival Analysis in S. Retrieved from url:  
883 <http://CRAN.R-project.org/package=survival>
- 884 Therneau, T. M., & Grambsch, P. M. (2000). *Modeling Survival Data: Extending the*  
885 *Cox Model*. New York: Springer.
- 886 Urban, J., Ingwers, M. W., McGuire, M. A., & Teskey, R. O. (2017). Increase in leaf  
887 temperature opens stomata and decouples net photosynthesis from stomatal  
888 conductance in *Pinus taeda* and *Populus deltoides x nigra*. *Journal of*  
889 *Experimental Botany*, *68*, 1757–1767. <https://doi.org/10.1093/jxb/erx052>
- 890 Voltas, J., Lucabaugh, D., Chambel, M. R., & Ferrio, J. P. (2015). Intraspecific  
891 variation in the use of water sources by the circum-Mediterranean conifer *Pinus*  
892 *halepensis*. *The New Phytologist*, *208*, 1031–41. <https://doi.org/10.1111/nph.13569>
- 893 Voltas, J., Romagosa, I., Muñoz, P., & Araus, J. L. (1998). Mineral accumulation,  
894 carbon isotope discrimination and indirect selection for grain yield in two-rowed  
895 barley grown under semiarid conditions. *European Journal of Agronomy*, *9*, 147–  
896 155. [https://doi.org/10.1016/S1161-0301\(98\)00032-X](https://doi.org/10.1016/S1161-0301(98)00032-X)
- 897 West, A. G., Dawson, T. E., February, E. C., Midgley, G. F., Bond, W. J., & Aston, T.  
898 L. (2012). Diverse functional responses to drought in a Mediterranean-type  
899 shrubland in South Africa. *New Phytologist*, *195*, 396–407.  
900 <https://doi.org/10.1111/j.1469-8137.2012.04170.x>
- 901 Williams, A. P., Allen, C. D., Macalady, A. K., Griffin, D., Woodhouse, C. A., Meko,  
902 D. M., ... McDowell, N. G. (2013). Temperature as a potent driver of regional  
903 forest drought stress and tree mortality. *Nature Climate Change*, *3*, 292–297.  
904 <https://doi.org/10.1038/nclimate1693>
- 905 Zhang, X., Wollenweber, B., Jiang, D., Liu, F., & Zhao, J. (2008). Water deficits and  
906 heat shock effects on photosynthesis of a transgenic *Arabidopsis thaliana*  
907 constitutively expressing ABP9, a bZIP transcription factor. *Journal of*  
908 *Experimental Botany*, *59*, 839–848. <https://doi.org/10.1093/jxb/erm364>
- 909 Zweifel, R., Steppe, K., & Sterck, F. J. (2007). Stomatal regulation by microclimate and  
910 tree water relations : interpreting ecophysiological field data with a hydraulic plant  
911 model, *58*, 2113–2131. <https://doi.org/10.1093/jxb/erm050>

912 Zwieniecki, M. A., & Holbrook, N. M. (2009). Confronting Maxwell's demon:  
913 biophysics of xylem embolism repair. *Trends in Plant Science*, 14, 530–534.  
914 <https://doi.org/10.1016/j.tplants.2009.07.002>

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942 *Tables*

943 Table 1 Mean values  $\pm$  SE of physiological variables measured in the four target pine  
 944 species during mid-summer (dry season) of the second year after planting. F and P  
 945 values of the “species” factor are shown for each variable. Significant p values are  
 946 indicated in bold, while differences between species ( $P < 0.05$  after Tukey’s multiple  
 947 comparison) are shown with different superscripts.

Variables	Sample size	Species				F	Pr(>F)
		<i>P. halepensis</i>	<i>P. nigra</i>	<i>P. sylvestris</i>	<i>P. uncinata</i>		
Predawn Water Potential (MPa)	71	-1.26 $\pm$ 0.05 <sup>b</sup>	-0.86 $\pm$ 0.03 <sup>a</sup>	-0.87 $\pm$ 0.04 <sup>a</sup>	-	43.9	<b>2.63E-11</b>
Midday Water Potential (MPa)	131	-2.37 $\pm$ 0.04 <sup>b</sup>	-1.88 $\pm$ 0.05 <sup>a</sup>	-1.84 $\pm$ 0.04 <sup>a</sup>	-1.92 $\pm$ 0.04 <sup>a</sup>	30.6	<b>6.46E-14</b>
Predawn fluorescence (F <sub>v</sub> /F <sub>m</sub> )	132	0.8709 $\pm$ 0.0024 <sup>a</sup>	0.839 $\pm$ 0.005 <sup>b</sup>	0.836 $\pm$ 0.006 <sup>b</sup>	0.825 $\pm$ 0.006 <sup>b</sup>	17.2	<b>5.17E-09</b>
Midday fluorescence (F <sub>v</sub> /F <sub>m</sub> )	132	0.796 $\pm$ 0.007 <sup>a</sup>	0.685 $\pm$ 0.017 <sup>bc</sup>	0.721 $\pm$ 0.017 <sup>b</sup>	0.657 $\pm$ 0.022 <sup>c</sup>	16.3	<b>1.34E-08</b>
Yield	127	0.688 $\pm$ 0.005 <sup>a</sup>	0.599 $\pm$ 0.017 <sup>b</sup>	0.598 $\pm$ 0.017 <sup>b</sup>	0.545 $\pm$ 0.024 <sup>b</sup>	22.1	<b>7.35E-11</b>
rETR ( $\mu$ M e/m <sup>2</sup> s)	127	455.0 $\pm$ 10.0 <sup>a</sup>	396.1 $\pm$ 13.3 <sup>b</sup>	384.9 $\pm$ 13.7 <sup>b</sup>	324.4 $\pm$ 15.9 <sup>c</sup>	17.7	<b>3.85E-09</b>
Photochemical quenching	125	0.972 $\pm$ 0.003 <sup>a</sup>	0.968 $\pm$ 0.004 <sup>ab</sup>	0.958 $\pm$ 0.005 <sup>ab</sup>	0.953 $\pm$ 0.005 <sup>b</sup>	3.6	<b>1.66E-02</b>
Non-Photochemical quenching	126	0.044 $\pm$ 0.006 <sup>b</sup>	0.067 $\pm$ 0.010 <sup>ab</sup>	0.071 $\pm$ 0.009 <sup>ab</sup>	0.083 $\pm$ 0.013 <sup>a</sup>	3.2	<b>2.53E-02</b>

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953 Table 2 Mean values  $\pm$  SE of leaf isotopic composition, nutrient concentrations and  
 954 stoichiometric ratios measured in the four target pine species in mid-summer (dry  
 955 season) of the second year after planting. F and P values of the “species” factor are  
 956 shown for each variable. Significant p-values are indicated in bold, while differences  
 957 between species ( $P < 0.05$  after Tukey’s multiple comparison) are shown with different  
 958 superscripts. As a reference, macronutrient concentration and stoichiometric ratios  
 959 observed in natural populations of the studied species in Northeast of the Iberian  
 960 Peninsula are shown in brackets (data obtained from Sardans, Rivas-Ubach, &  
 961 Peñuelas, 2011).

Variables	Sample size	Species				F	Pr(>F)
		<i>P. halepensis</i>	<i>P. nigra</i>	<i>P. sylvestris</i>	<i>P. uncinata</i>		
Foliar $\delta^{13}\text{C}$ (‰)	186	-25.50 $\pm$ 0.09 <sup>a</sup>	-27.68 $\pm$ 0.17 <sup>b</sup>	-27.50 $\pm$ 0.16 <sup>b</sup>	-28.38 $\pm$ 0.32 <sup>b</sup>	60.8	<b>&lt; 2.2E-16</b>
Foliar $\delta^{18}\text{O}$ (‰)	186	26.72 $\pm$ 0.11 <sup>d</sup>	28.59 $\pm$ 0.17 <sup>c</sup>	29.55 $\pm$ 0.18 <sup>b</sup>	30.12 $\pm$ 0.3 <sup>a</sup>	97.3	<b>&lt; 2.2E-16</b>
Nitrogen (mg g <sup>-1</sup> )	181	14.3 $\pm$ 0.3 <sup>b</sup> (10.6 $\pm$ 0.1)	12.8 $\pm$ 0.3 <sup>c</sup> (9.4 $\pm$ 0.09)	18.3 $\pm$ 0.7 <sup>a</sup> (11.9 $\pm$ 0.1)	16.5 $\pm$ 0.8 <sup>ab</sup> (9.7 $\pm$ 0.15)	26.8	<b>1.06E-13</b>
Phosphorus (mg g <sup>-1</sup> )	185	0.85 $\pm$ 0.03 <sup>a</sup> (0.80 $\pm$ 0.01)	0.37 $\pm$ 0.02 <sup>c</sup> (0.83 $\pm$ 0.01)	0.43 $\pm$ 0.02 <sup>b</sup> (1.10 $\pm$ 0.01)	0.49 $\pm$ 0.03 <sup>bc</sup> (0.92 $\pm$ 0.01)	122	<b>&lt; 2.2E-16</b>
Potassium (mg g <sup>-1</sup> )	186	4.74 $\pm$ 0.14 <sup>a</sup> (4.00 $\pm$ 0.05)	2.95 $\pm$ 0.10 <sup>b</sup> (4.44 $\pm$ 0.08)	2.57 $\pm$ 0.12 <sup>c</sup> (5.55 $\pm$ 0.09)	2.83 $\pm$ 0.21 <sup>bc</sup> (4.16 $\pm$ 0.10)	58.4	<b>&lt; 2E-16</b>
Zinc (ppm)	185	18.8 $\pm$ 0.5 <sup>a</sup>	13.6 $\pm$ 0.8 <sup>b</sup>	12.3 $\pm$ 0.7 <sup>b</sup>	11.3 $\pm$ 1.3 <sup>b</sup>	19.1	<b>2.17E-10</b>
Copper (ppm)	184	3.09 $\pm$ 0.08 <sup>a</sup>	1.53 $\pm$ 0.07 <sup>b</sup>	1.69 $\pm$ 0.08 <sup>b</sup>	1.95 $\pm$ 0.20 <sup>b</sup>	74.2	<b>&lt; 2.2E-16</b>
C:N ratio	186	31.4 $\pm$ 0.7 <sup>b</sup> (52.2 $\pm$ 0.5)	35.62 $\pm$ 0.81 <sup>a</sup> (57.3 $\pm$ 0.6)	26.5 $\pm$ 0.7 <sup>c</sup> (45.0 $\pm$ 0.3)	27.96 $\pm$ 1.16 <sup>bc</sup> (55.6 $\pm$ 0.8)	27	<b>8.56E-14</b>
N:P ratio	180	17.5 $\pm$ 0.6 <sup>c</sup> (14.9 $\pm$ 1.1)	36.6 $\pm$ 1.5 <sup>b</sup> (11.8 $\pm$ 0.2)	46.4 $\pm$ 2.7 <sup>a</sup> (11.4 $\pm$ 0.1)	35.4 $\pm$ 2.7 <sup>ab</sup> (10.5 $\pm$ 0.2)	99	<b>&lt; 2.2E-16</b>
N:K ratio	181	3.13 $\pm$ 0.12 <sup>c</sup> (3.00 $\pm$ 0.07)	4.59 $\pm$ 0.20 <sup>b</sup> (2.55 $\pm$ 0.07)	7.84 $\pm$ 0.50 <sup>a</sup> (2.63 $\pm$ 0.07)	6.20 $\pm$ 0.50 <sup>a</sup> (2.70 $\pm$ 0.12)	62.1	<b>&lt; 2.2E-16</b>

962

963

964

965 *Figure captions*

966 Figure 1 Proposed “isohydric trap” conceptual model of reduced plant physiological  
967 performance and growth under hotter drought, in which vascular plants with a strong  
968 stomatal regulation of transpiration undergo early and prolonged closure of the stomata  
969 under heat and drought stress. This strict isohydric behaviour triggers a series of  
970 physiological changes that culminate in severe nutrient imbalance and a reduction of the  
971 capacity to cope with further drought stress, thereby becoming a feedback to in earlier  
972 stomatal closure under a subsequent event of water stress (the “isohydric trap”). Sub-  
973 loops are shown with arrows of different colours: i) Transpiration-nutrient loop in blue;  
974 ii) Transpiration-root growth loop in green.

975

976 Figure 2 Spearman’s rank coefficients ( $\rho$ ) for the correlations of pine sapling growth  
977 after two years with leaf  $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$  and nutrient concentration across species.  
978 Significant associations are indicated with asterisks (\* for  $P < 0.05$  and \*\* for  $P < 0.01$ ).

979

980 Figure 3 Correlations between leaf nutrient concentrations and foliar  $\delta^{18}\text{O}$  (proxy of  
981 stomatal conductance) across species. Spearman’s rank correlation coefficients along  
982 with p and S value are shown for each correlation. Abbreviations: H = *P. halepensis*, N  
983 = *P. nigra*, S = *P. sylvestris*, U = *P. uncinata*.

984

985 Figure 4 Correlations between foliar  $\delta^{13}\text{C}$  (proxy of water-use efficiency) and leaf  
986 nutrient concentrations across pine species. Spearman’s rank correlation

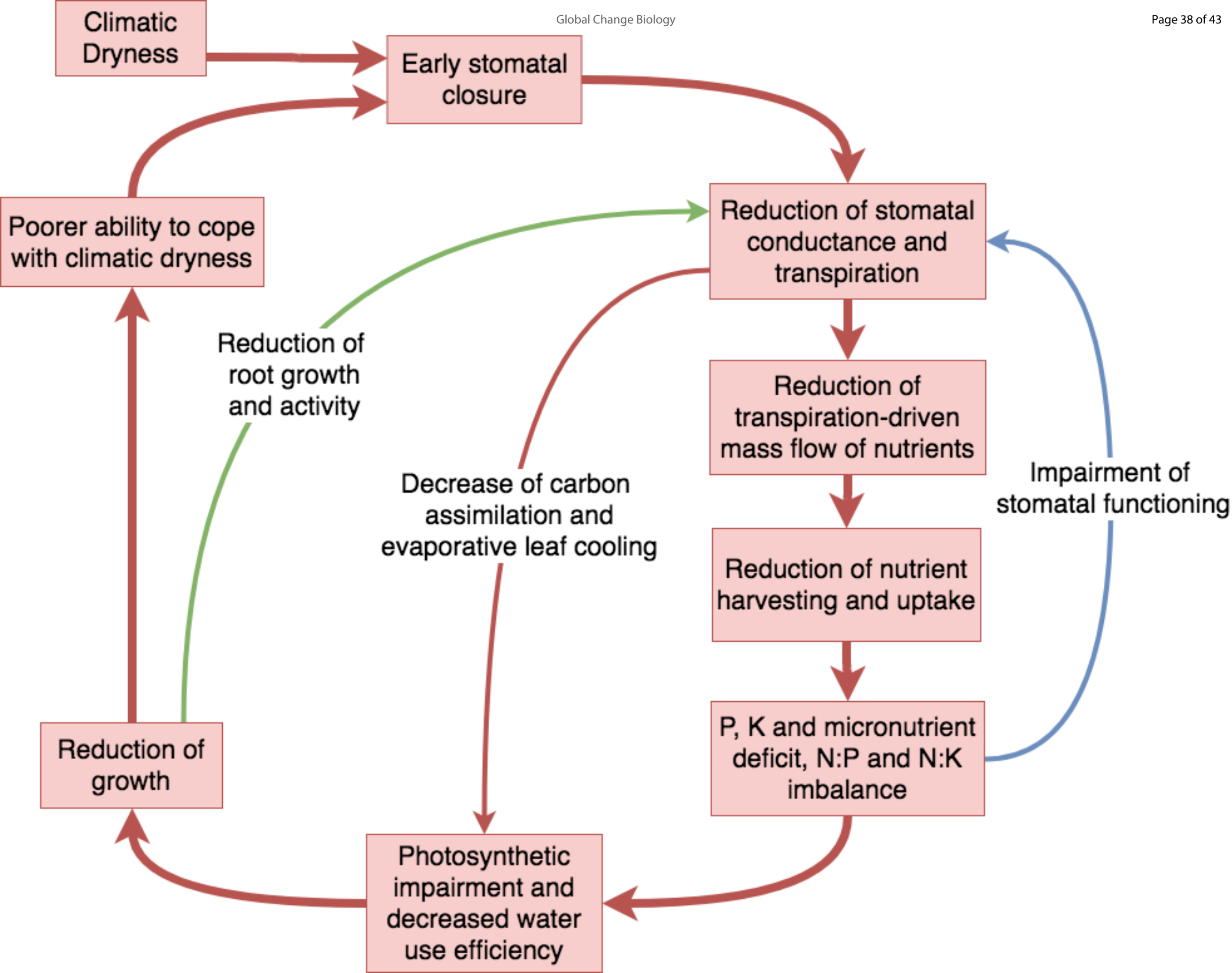
987 coefficients together with p and S value are shown for each correlation. Species  
988 abbreviations as in Figure 3.

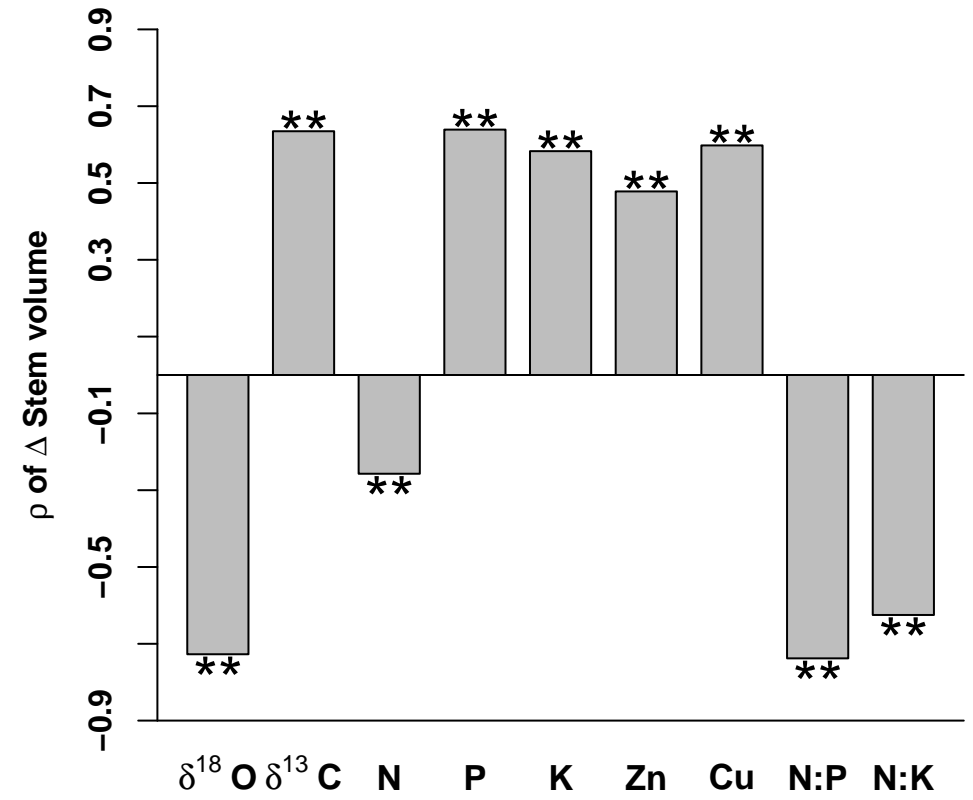
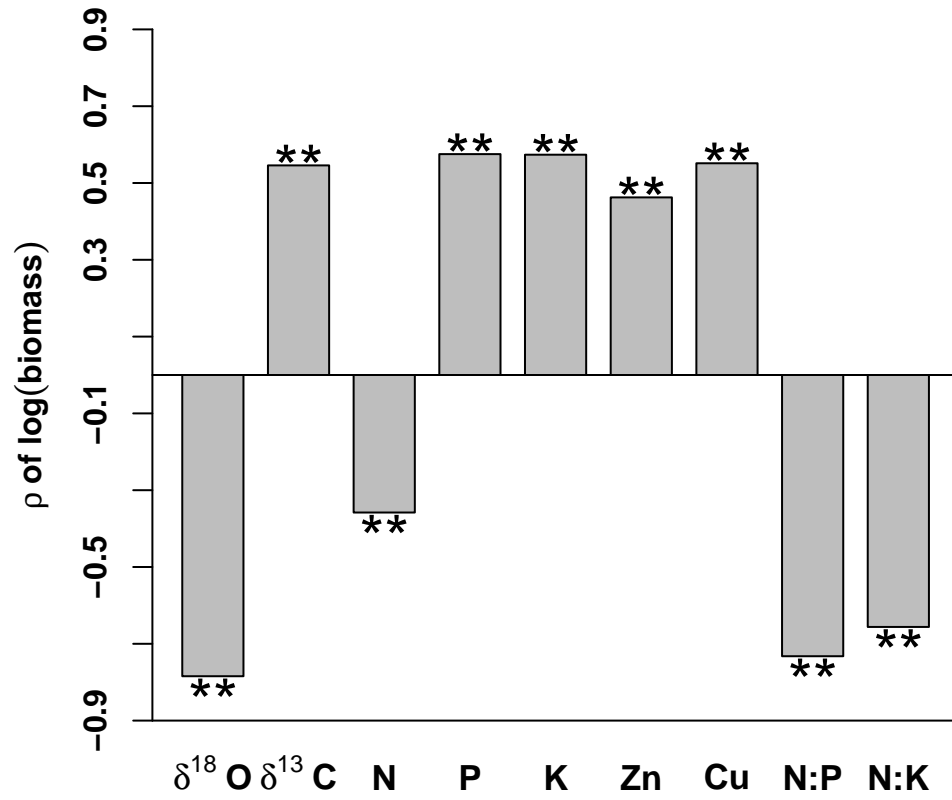
989

990 Figure 5 Correlation between mean values per species of leaf  $\delta^{13}\text{C}$  (proxy of water-use  
991 efficiency) with photosynthetic fluorescence parameters across pine species. Pearson's  
992 correlation coefficients along with p and t value are shown for each correlation. Error  
993 bars for both axes represent  $\pm\text{SE}$ . Species abbreviations as in Figure 3. Variables  
994 abbreviations: Yield = Quantum yield, qN = Non-photochemical quenching.

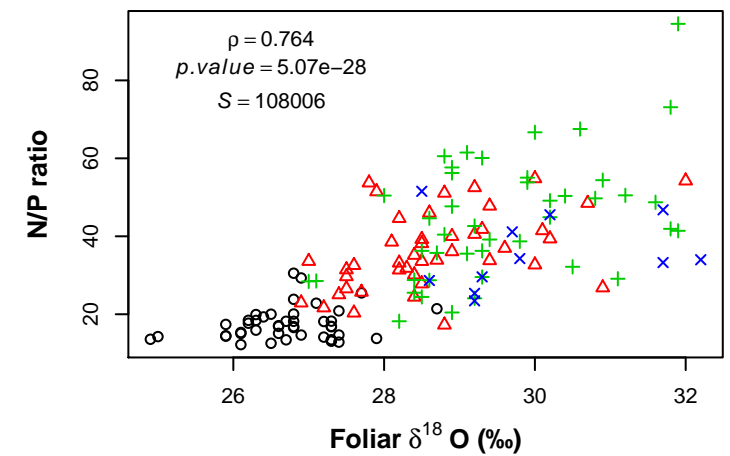
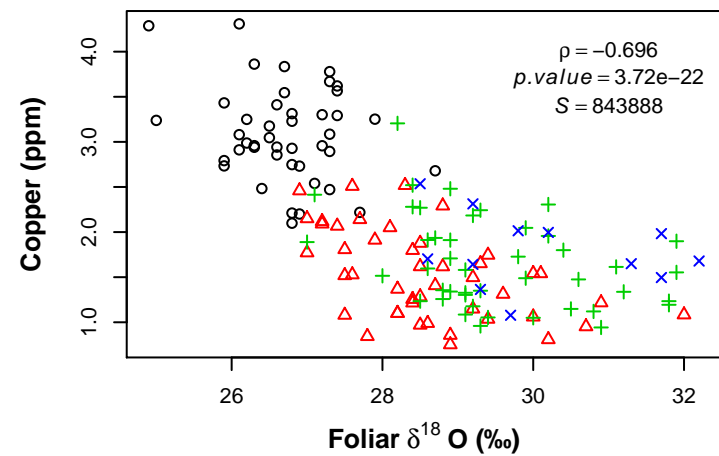
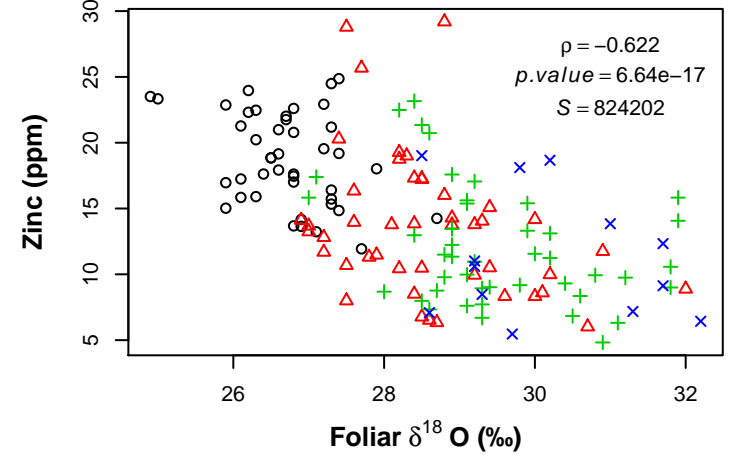
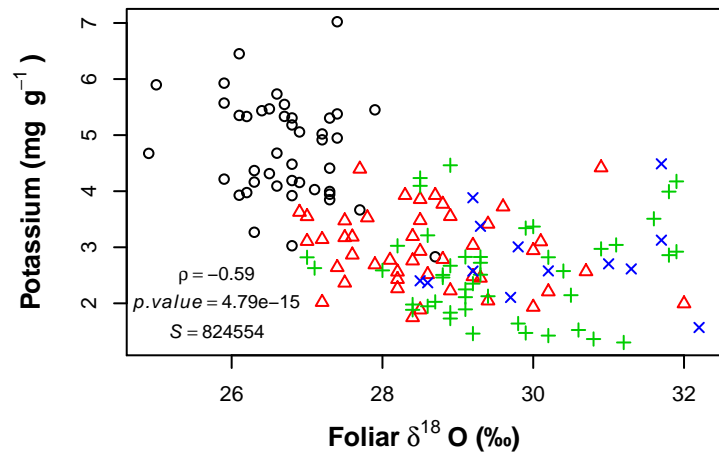
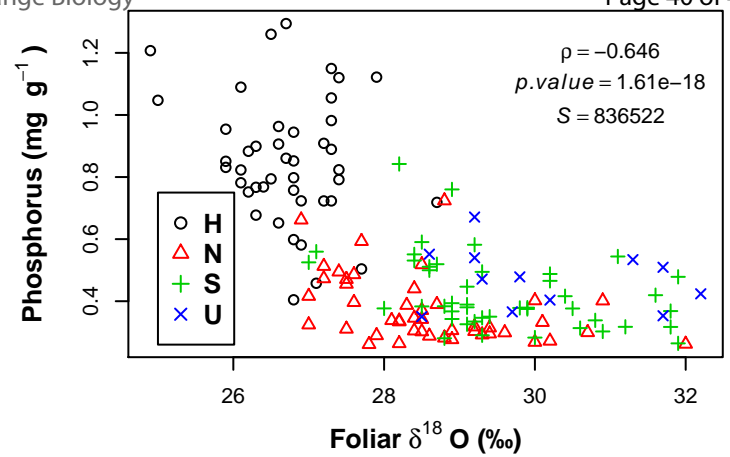
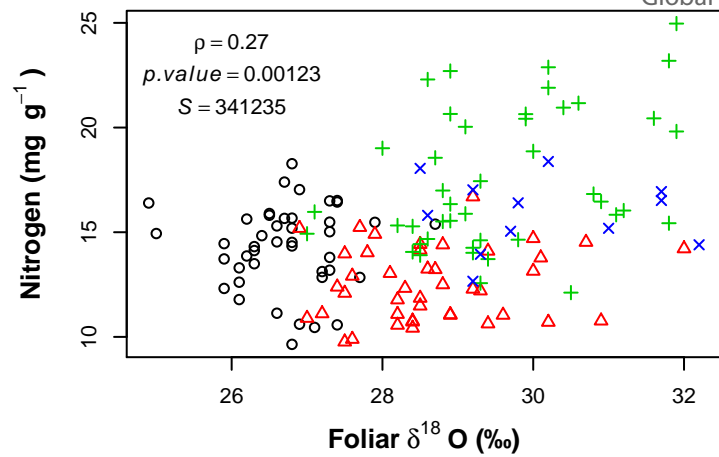
995

996 Figure 6 Correlation between mean values per species of leaf  $\delta^{18}\text{O}$  (proxy of stomatal  
997 conductance) with photosynthetic fluorescence parameters across pine species.  
998 Pearson's correlation coefficients together with p and t value are shown for each  
999 correlation. Error bars for both axes represent  $\pm\text{SE}$ . Species abbreviations as in Figure 3.  
1000 Variables abbreviations: Yield = Quantum yield, rETR = relative electron transport rate,  
1001 qN = Non-photochemical quenching.









← + Stomatal conductance - →

← + Stomatal conductance - →

