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1 **Rooting big and deep rapidly: the ecological roots of pine species distribution**  
2 **in southern Europe**

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24 **Author Contribution Statement:** RS, FdH and PV designed the study, experiment setup and  
25 data collection was made by BG, FdH and PZ, data was analyzed by EA and PZ, first draft  
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27

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35

## 36 **Conflict of Interest**

37 The authors declare that they have no conflict of interest.

38

## 39 **Data availability**

40 The datasets generated during and/or analysed during the current study are available from the  
41 corresponding author on reasonable request.

42

43 **Abstract**

44 Root properties can influence plant drought resistance, and consequently plant species  
45 distribution. Root structure strongly varies across biomes partly as a result of phylogeny.  
46 However, whether the spatial distribution of phylogenetically close plant species is linked to  
47 differences in root properties remains unclear. We examined whether root properties mediate  
48 the strong correlation between summer drought intensity and the spatial segregation of pine  
49 species native to southern Europe. For this, we compared the seedling root growth and structure  
50 of five ecologically distinct pine species grown in 360L rhizotrons for 19 months under typical  
51 hot and dry Mediterranean conditions. We studied the mountain and boreo-alpine pines *Pinus*  
52 *sylvestris* and *Pinus nigra*, and the Mediterranean pines *Pinus pinaster*, *Pinus pinea* and *Pinus*  
53 *halepensis*. Mediterranean pines formed deep roots faster than mountain pines, their shoots and  
54 roots grew faster and had higher root growth, especially *P. halepensis*, at low air temperature.  
55 By the end of the study, Mediterranean pines had larger root systems than mountain pines.  
56 Neither distribution of root mass with depth nor root-to-shoot mass ratio varied significantly  
57 among species. Across species, minimal annual rainfall to which species are exposed in their  
58 range related negatively to root growth but positively to specific root length and the time needed  
59 for roots to reach a depth of 40 cm. This study highlights the importance of root growth as a  
60 driver of pine distribution in southern Europe and suggests that rapidly producing a large, deep  
61 root system may be a key attribute for pines to colonize dry Mediterranean locations.

62

63 **Keywords:** Drought resistance; *Pinus*; Rhizotron; Root growth; Root structure; Rooting  
64 depth; Specific root length

65

66 **Key message:** The rapid production of a large, deep root system during seedling  
67 establishment is critical for pines to colonize dry Mediterranean locations

## 68 **1. Introduction**

69 Water stress constrains plant life in many terrestrial ecosystems (Vicente-Serrano et al. 2013).  
70 Plants show a wide variety of adaptations to survive in dry ecosystems (Levitt 1980; Chaves et  
71 al. 2003; Brodribb et al. 2014). Roots vary widely in structure among plant species, and several  
72 root properties have been related to drought resistance of plants (Padilla and Pugnaire 2007;  
73 Alsina et al. 2011; Comas et al. 2013; Brunner et al. 2015). The size of the root system and  
74 rooting depth determine the plant's ability to access deep soil moisture reserves during dry  
75 periods (Schulze et al. 1996). Across species, the size of the root system, rooting depth and root  
76 hydraulic conductance increase with the size of growth forms (Canadell et al. 1996; Schenk and  
77 Jackson 2002; De Herralde et al. 2010). However, for a specific growth form, species inhabiting  
78 water-limited environments tend to have deeper roots than their mesic counterparts (Jackson et  
79 al. 1996).

80 The proportion of mass allocated to roots has also been related to plant drought  
81 resistance. The proportion of root mass relative to either shoot mass (R/S) or entire plant mass  
82 (root mass fraction, RMF) are indicators of the potential balance between water uptake and  
83 evaporative capacity of a plant (Grossnickle 2012). Globally, R/S (Mokany et al. 2006), RMF  
84 (Poorter et al. 2012b) and the relative distribution of root mass in depth (Schenk and Jackson  
85 2002) usually increase with aridity. In addition, plants species differ substantially in fine root  
86 production (Ostonen et al. 2007; Valverde-Barrantes et al. 2017). Fine root growth is often  
87 studied using the proxy variable of specific root length (SRL), defined as the length of roots per  
88 root mass unit (Ostonen et al. 2007). High-SRL root systems have a large root surface and  
89 consequently high capacity to take up soil resources (Comas et al. 2013). In a global review of  
90 studies of fine-root traits, SRL was greater in plants from cold and temperate climatic areas  
91 than in plants from arid and tropical areas (Freschet et al. 2017). Fast growing woody plants,

92 which have high nutrient demand, grow more high-SRL roots than slow growing plants (Reich  
93 et al. 1998; Comas and Eissenstat 2004; Hernández et al. 2010).

94 The seedling stage is a major bottleneck in the life history of plants and consequently in  
95 population dynamics (Pulido et al. 2010). Rooting depth determines the capacity of seedlings  
96 to access deep soil layers (Padilla and Pugnaire 2007), which usually hold stable water reserves  
97 during the dry season (Brum et al. 2017). This fact likely explains why the ability of seedlings  
98 in seasonal dry climates to survive their first dry season depends on root system size and rooting  
99 depth (Grossnickle 2005; Padilla and Pugnaire 2007; Villar-Salvador et al. 2012). Plants can  
100 achieve a deep and extensive root system either by starting to grow early in the wet season (De  
101 Luis et al. 2008) and/or by growing rapidly during the wet season (Holmgren et al. 2006; Stella  
102 and Battles 2010). Root growth in the wet season depends on soil and air temperature. Response  
103 of root growth to temperature varies among species (Lyr 1996; Pregitzer et al. 2000). Most plant  
104 species slow root growth greatly when soil temperature is  $< 10\text{ }^{\circ}\text{C}$  and root growth cessation  
105 occurs at  $2\text{-}6^{\circ}\text{C}$  (Alvarez- Uria and Körner 2007). Species differences in the response of root  
106 growth to temperature seems to be related to the temperature to which species are exposed in  
107 their range (Lyr 1996).

108 The spatial segregation of native pine species in southern Europe is correlated with  
109 summer drought and winter temperature (Barbero et al. 1998). *Pinus nigra* J.F. Arnold and *P.*  
110 *sylvestris* L. (hereafter referred to as mountain and boreo-alpine pines) inhabit cold winter sites  
111 in the high mountains of southern Europe, where rainfall is high and summer drought is mild  
112 and short. At these locations, cold is the main limitation for plant life (Barbero et al. 1998). In  
113 contrast, *Pinus halepensis* Mill., *Pinus pinaster* Ait., and *Pinus pinea* L. (hereafter referred to  
114 as Mediterranean pines) thrive in low- and mid-altitude locations in a typical Mediterranean  
115 climate, where winter is mild-to-cool and humid, while the summer is hot and dry (Barbero et  
116 al. 1998). At these sites, summer water stress is the main limiting factor for plant life (Mitrakos

117 1980). The spatial segregation of these pine species can be explained in part by their capacity  
118 to withstand frost events. *Pinus halepensis*, *P. pinea* and to a lesser extent *P. pinaster* are less  
119 frost-tolerant than the high-mountain pines *P. sylvestris* and *P. nigra* (Climent et al. 2009;  
120 Fernández et al. 2017; Toca et al. 2017). However, the fact that *P. sylvestris* and *P. nigra* do  
121 not occur at low-altitude locations where summers are dry and hot could be explained by a  
122 lower capacity to survive water stress than Mediterranean pines in the early life stages (Salazar-  
123 Tortosa et al. 2018).

124 The objective of this study was to compare the growth and structure of root systems of  
125 five ecologically distinct pine species native to the Iberian Peninsula (*P. sylvestris*, *P. nigra*, *P.*  
126 *pinaster*, *P. pinea* and *P. halepensis*) under lowland Mediterranean conditions. For this, we  
127 grew seedlings of these pine species in a common garden experiment simulating the low  
128 precipitation and high temperature regime typical of low- and mid-altitude Mediterranean  
129 location. Plants were grown in 360-L rhizotrons to avoid small rooting volume constraints  
130 (Poorter et al. 2012a), and we analyzed the root dynamics and structure for 19 months. We  
131 hypothesized that Mediterranean pines will grow faster a larger and deeper root system,  
132 showing higher SRL than mountain and boreo-alpine pines under these experimental  
133 conditions. This study will contribute to explain why mountain and boreo-alpine pines species  
134 fail to colonize low altitude Mediterranean environments.

135

## 136 **2. Material and methods**

### 137 **2.1 Experimental setup and plant growth**

138 Seeds of the five pine species were collected from populations in the southern part of the Iberian  
139 Range, eastern Spain (see Table S1 for locations and their environmental details). This region  
140 covers around 8500 km<sup>2</sup> and natural populations of all studied species segregate along an  
141 altitudinal gradient from 650 to 2050 m a.s.l. (Blanco et al. 1998). Seeds were seeded in

142 February 2012 and seedlings were cultivated in trays (190/300-45, Plasnor, Spain) of 300 ml.  
143 Growing medium was fertilized peat (White 420 F6, Kekkilä, Finland) containing 0.8-1 kg/m<sup>3</sup>  
144 of slow-release fertilizer NPK 16-10-20. Seedlings were initially grown in a greenhouse of the  
145 Centro Nacional de Recursos Genéticos Forestales “El Serranillo” (Guadalajara, Spain) until  
146 late March 2012, then transported to the IRTA in Caldes de Montbui (Barcelona, Spain), where  
147 the rest of the study was performed. From late March to early June 2012, seedlings were kept  
148 inside a greenhouse and periodically watered until transplanted to rhizotrons.

149 In early June 2012, six four-month-old seedlings per species were transplanted into 30  
150 rhizotrons (1 seedling per rhizotron; rhizotron dimensions were height: 1.2 m, width: 0.5 m and  
151 depth: 0.6 m) filled with washed river sand. The side and rear walls of the rhizotrons were made  
152 of galvanized iron sheets, while the front wall was glass, which was covered by reflective plastic  
153 to avoid radiation (light and temperature) at root level. The rhizotrons were placed inside an  
154 open greenhouse tunnel, which reduced ambient photosynthetic photon flux density by 30%.  
155 At the time of transplanting seedling height and diameter ( $\pm$ SE) were: 11.4  $\pm$  0.7 cm and 2.3  $\pm$   
156 0.1 mm for *P. halepensis*; 15.9  $\pm$  0.2 cm and 3.1  $\pm$  0.2 mm for *P. pinea*; 14.7  $\pm$  1.3 cm and 2.8  
157  $\pm$  0.1 mm for *P. pinaster*; 8.7  $\pm$  0.2 cm and 2.1  $\pm$  0.1 mm for *P. nigra*; and 7.4  $\pm$  0.2 cm and 2.5  
158  $\pm$  0.1 mm for *P. sylvestris*, respectively. Seedlings were water-supplied with approximately the  
159 average rainfall rate of Caldes de Montbui (altitude: 203 m a.s.l.). This location has a typical  
160 Mediterranean climate with a mean temperature of 15.5 °C and a mean annual rainfall of 633  
161 mm. Water was supplied daily during the first month and then every 2-3 days thereafter. The  
162 cumulative amount of water supplied per plant during the study was 572 L. Soil volumetric  
163 water content (VWV, %) was measured every 2 weeks using a 70-cm frequency domain  
164 reflectometry probe (Diviner 2000, Sentek Sensor Technologies Stepney, Australia). The  
165 accession tube was inserted before planting of seedlings, and soil moisture was measured at 10-  
166 cm intervals. For simplicity, we performed data analysis using soil VWC for the depth intervals



167 of 0-20, 20-40 and 40-70 cm. Mean VWC increased with depth and was higher in mountain  
168 and boreal pines than in Mediterranean pines (Figure S1). In general, soil VWC decreased  
169 slightly over time in the depth layers of 20-40 and 40-70 cm, whereas it fluctuated without any  
170 clear pattern in the 0-20 cm layer.

171 Air temperature was measured daily using electronic sensors (PASSRHT, Decagon  
172 Devices, Washington, USA) placed next to the rhizotrons. Mean air temperature during the  
173 study was 16.6 °C, fluctuating between 30.3 °C in mid-August 2012 and 3.2 °C in early  
174 December 2012 (Figure S2). The lowest air temperature was -2.6 °C in late February 2013; the  
175 highest temperature was 40.9 °C in late July 2012.

## 176 **2.2 Root and shoot measurements**

177 Root elongation measurement was initiated after seedlings were established in early October  
178 2012 and were taken every two weeks until May 2014. The length of the visible roots growing  
179 against the rhizotron wall was measured in four soil layers, each of which was 20-40 cm thick:  
180 these layers covered depths of 0-20, 20-40, 40-80, and 80-110 cm. These layers were  
181 photographed with a digital camera, and root length was determined using WinRHIZO (Regent  
182 Instruments, Canada).

183 At the end of the experiment in early June 2014, plants were extracted from the  
184 rhizotrons by gently removing the growing medium with water. Plants were separated into  
185 shoots and roots and washed with tap water. The roots were cut at three depth layers (0-40, 40-  
186 80 and 80-120 cm), scanned and their length quantified using WinRHIZO. Then all plant  
187 fractions were dried at 60 °C until constant weight. SRL was calculated as the ratio between  
188 total root length and total root mass, while R/S was calculated by dividing root mass by shoot  
189 mass.

190 Shoot growth was monitored by periodically measuring stem height and diameter,  
191 which was measured at 1 cm from the ground. Both measurements were performed at planting

192 and on the following days after transplanting: 336 (18 June 2013), 456 (16 October 2013), 545  
193 (13 January 2014), 618 (26 March 2014) and 686 (02 June 2014). We also used the relative  
194 growth rate (RGR) as a surrogate of plant growth. RGR was calculated as:

$$195 \quad RGR = \frac{\ln(m_{t2}) - \ln(m_{t1})}{t2 - t1}$$

196 where  $m$  is the plant mass at the time of transplantation into the rhizotron ( $t1$ ) and at the end of  
197 the experiment ( $t2$ ). The mass at  $t1$  was measured on 10 seedlings per species randomly sampled  
198 after drying at 60 °C for 48 h.

### 199 **2.3 Data analysis**

200 Differences in height and diameter growth rates between species were assessed using a linear  
201 mixed model in which the random effect was individuals and the fixed effect was the interaction  
202 between species factor and the covariate time (days since planting). Significant differences  
203 ( $P < 0.05$ ) in the model slope meant that growth rates were different among species.

204 The effect of species on root and shoot measurements after harvesting were analyzed  
205 using one-way ANOVA. The response variables were shoot mass, total root mass, total root  
206 length, R/S, and SRL. We also evaluated the effect of species on the total root mass, and on the  
207 root mass proportion at the three different soil layers (0-40, 40-80 and 80-120 cm). Significant  
208 differences between species were evaluated using a Tukey HSD test ( $P < 0.05$ ).

209 Root elongation rate was analyzed using a Generalized Additive Mixed Model  
210 (GAMM) in which the random factor was individuals, the smoothed term was time (days since  
211 planting), and the fixed effect was species. We fitted four different GAMM models, one for  
212 each soil layer. To evaluate whether species had a significant effect on root elongation, we  
213 compared the model with a model in which species was omitted. If the difference between the  
214 two models was  $\leq 2$  in the corrected Akaike's information criterion (AICc), then the simpler  
215 model was selected (Burnham and Anderson 2002).

216 We also analyzed the effect of air temperature on species root growth. For this, root  
217 length of all soil layers was added up to yield the total root length per seedling and measurement  
218 date. Then, we calculated root length increment ( $\text{mm day}^{-1}$ ) as the difference in root length  
219 between two consecutive measurement dates divided by the number of days between both  
220 measurements. As the magnitude of root length increment greatly differs among species, it was  
221 species-scaled to 0-1 values (hereafter RLI). We used a linear mixed model to evaluate whether  
222 the relationship between RLI and temperature was species idiosyncratic. This model included  
223 the interaction between species and temperature as fixed effect, and each individual as random  
224 term. First, we compared -based on the AICc- this model with a model in which temperature  
225 was considered as a second order polynomial. The selected model in this step was then  
226 compared with a model in which species factor was dropped.

227 To assess whether species influenced root growth speed through depth, we quantified  
228 the time taken by each individual to reach a depth of 40 cm after transplanting into the  
229 rhizotrons. We considered that the roots of an individual reached a depth of 40 cm when the  
230 roots became visible in the layer at a depth of 40-50 cm. Data were analyzed using one-way  
231 ANOVA in which the response variable was the time taken by roots to reach a depth of 40 cm.

232 We used linear regression to analyze whether inter-species differences in root properties  
233 were related to climatic conditions normally encountered by the species in their distribution  
234 range in the Iberian Peninsula. We used distribution data for continental Spain from the third  
235 Spanish Forest Inventory, which analyzed adults and saplings of all woody species in plots  
236 distributed over forest ecosystems according to a 1- $\text{km}^2$  grid. We selected those plots classified  
237 as natural pine forests based on the Spanish Regions of Provenance (Ruiz-Benito et al. 2012).  
238 Then we selected the plots showing saplings for each studied pine species. Climatic variables  
239 were calculated from a map with 1- $\text{km}^2$  spatial resolution (Gonzalo 2008) for each species, we  
240 chose the 2.5% percentile values for mean annual precipitation. This value represents the mean

241 annual rainfall in the 2.5% most arid locations in the range of each species. All statistical  
242 analyses were performed using *R* 3.2.5.

243

### 244 **3. Results**

#### 245 **3.1 Aboveground growth**

246 At the end of the experiment on day 728 after planting, *P. halepensis*, *P. pinea* and *P. pinaster*  
247 showed greater shoot mass than *P. nigra* and *P. sylvestris* (Figure S3). We found a significant  
248 interaction between species and time (days since planting) on seedling stem height and diameter  
249 (both  $P < 0.001$ ). *Pinus halepensis* showed a significantly larger diameter and faster height  
250 growth (higher interaction slope) than the other pine species, whereas *P. sylvestris* showed the  
251 slowest growth (Figure S4). *Pinus pinaster* showed faster height growth than *P. pinea*, *P. nigra*  
252 and *P. sylvestris*, while no significant differences were found between *P. nigra* and *P. pinea*.  
253 Similarly, no significant differences in diameter growth rate were observed among *P. pinea*, *P.*  
254 *pinaster* and *P. nigra*. *P. halepensis* also showed the highest RGR and *P. nigra* and *P. sylvestris*  
255 showed the lowest RGR, while *P. pinea* and *P. pinaster* had similar RGR (Figure S5).

#### 256 **3.2 Belowground growth**

257 At the end of the experiment, *P. halepensis*, *P. pinea* and *P. pinaster* showed greater root mass  
258 than *P. nigra* and *P. sylvestris* (Figure 1a). However, species did not significantly differ in R/S.  
259 Root mass varied with depth in the same way that total root mass did (Figure 1). *Pinus nigra*  
260 and *P. sylvestris* showed the smallest root mass at all depths. However, the distribution of root  
261 mass with depth did not differ between species. Differences among species affected the mass  
262 of roots produced in the soil profile but not the relative distribution of root mass with depth.  
263 Total root length showed a similar pattern as root mass. *Pinus halepensis* and *P. pinaster* had  
264 longer roots than *P. nigra* and *P. sylvestris*; the latter two species had roots of similar length

265 (Figure 2a). Species SRL showed the opposite trend as root length and mass, with *P. sylvestris*  
266 showing the largest SRL and *P. halepensis* the smallest (Figure 2b).

267 Root length increased over time to different extents among the various Iberian pine species  
268 (Figure 3, Table S2). *Pinus halepensis* showed the greatest capacity to quickly colonize soil  
269 layers (Figure 3), especially the layer at 0-20 cm, followed by *P. pinaster* and *P. pinea*. In  
270 general, these three species showed the greatest root length increase in each soil layer, whereas  
271 *P. sylvestris* showed the smallest and slowest increase in root length (Figure 3). Root length in  
272 *P. nigra* increased more than in *P. sylvestris* but less than in Mediterranean pines except in the  
273 0-20-cm layer, where *P. nigra* showed the lowest increase in root length of all five pine species.  
274 From day 385 onwards, *P. pinea* showed the longest roots in the layers at depths of 20-40 and  
275 40-80 cm. Roots of *P. nigra* and *P. sylvestris* lengthened more slowly towards the end of the  
276 experiment than initially, except for soil layer at a depth of 80-110 cm, where growth remained  
277 active (Figure 3). The Mediterranean pines, in contrast, showed active growth across all the soil  
278 depth layers. Roots lengthened at similar rates across all species in the soil layer at 80-110 cm  
279 (Table S2).

280 Scaled root length increment (RLI) showed a quadratic and idiosyncratic species  
281 response to temperature (Table S3). RLI monotonically increased in *P. nigra* and *P. sylvestris*  
282 (Figure 4). *P. pinaster* and *P. pinea* showed a similar response to mountain pines but reaching  
283 constant values around 20°C. On the contrary, *P. halepensis* showed maximum RLI values at  
284 low temperatures and then decreased with temperature (Figure 4). RLI values at 7°C was highest  
285 in *P. halepensis* ( $0.18 \pm 0.03$ ) followed by *P. pinea* and *P. pinaster* ( $0.04 \pm 0.03$ , for both  
286 species). *P. sylvestris* and *P. nigra* showed the lowest scaled RLI values ( $0.02 \pm 0.03$  and  $0.01$   
287  $\pm 0.03$ , respectively).

288 Species differed in how long roots needed to reach a depth of 40 cm ( $F=3.21$ ,  $P=0.034$ )  
289 according to the trend: *P. halepensis* < *P. pinea* and *P. pinaster* < *P. nigra* (150 days after *P.*  
290 *halepensis*) < *P. sylvestris* (180 days after *P. halepensis*) (Figure 5).

### 291 **3.3 Relationship between root characteristics and rainfall in the distribution range**

292 Root mass at the end of the experiment and the stem diameter growth rate of each species  
293 correlated negatively with the 2.5% smallest annual precipitation in the natural range of that  
294 species (Figure 6). In contrast, SRL and the time for roots to reach a depth of 40 cm correlated  
295 positively with the 2.5% smallest annual rainfall in the natural range (Figure 6).

296

## 297 **4. Discussion**

298 Juveniles of pine species differ in how rapidly they grow during establishment and consequently  
299 in the final size of their root systems and shoots. Interestingly, root differences among species  
300 correlate strongly with the precipitation in the driest areas of the species range. In accordance  
301 with our hypothesis, seedlings of the Mediterranean pine species, which are exposed to high  
302 drought stress in their range (especially *P. halepensis*), show greater ability to rapidly colonize  
303 the soil profile and produce larger root systems than the seedlings of the mountain and the  
304 boreo-alpine pines under typical lowland Mediterranean conditions. These root differences may  
305 drive drought survival (Grossnickle 2005; Padilla and Pugnaire 2007; Villar-Salvador et al.  
306 2012). Consistent with this suggestion, survival and growth of *P. nigra* and *P. sylvestris* was  
307 significantly lower than *P. halepensis* survival in a common garden experiment in a dry hot  
308 Mediterranean location (Salazar-Tortosa et al. 2017; Salazar-Tortosa et al. 2018). Therefore,  
309 this study provides new insights into the functional basis of the latitudinal and altitudinal spatial  
310 segregation of pines in southern Europe. However, low field survival and growth of mountain  
311 and boreo-alpine pines might be also explained by poor physiological performance under water  
312 stress conditions (Salazar-Tortosa et al. 2017; Salazar-Tortosa et al. 2018). In addition, the use

313 of one provenance per species requires some caution when generalizing our results. Because  
314 we used provenances from the southern dry edge of the range of the mountain and boreo-alpine  
315 species, which are more drought resistant than northern and mesic provenances (Richter et al.  
316 2012; Matías et al. 2014), it is unlikely that the lower growth of these pine species compared to  
317 the Mediterranean pines is biased by provenances selection. Future studies, however, should  
318 evaluate provenances differences in root growth.

319 In water-limited ecosystems, seedling mortality due to water stress during the first dry  
320 season is a bottleneck for forest regeneration (Castro et al. 2004; Pulido et al. 2010). Resistance  
321 of seedlings to drought relies on adaptations that increase water uptake and /or reduce water  
322 loss during dry periods (De Micco and Aronne 2012; Brunner et al. 2015). In the present study,  
323 Mediterranean pines colonized through soil depth more rapidly, as evidenced by shorter time  
324 to reach a depth of 40 cm (Figure 5) and showed the fastest aboveground growth, growing larger  
325 than mountain pines at the end of the experiment. This indicates that seedling establishment  
326 under Mediterranean climate conditions depends on how rapidly the root system colonizes the  
327 soil profile. Similar to our findings, Climent et al. (2011) observed that *P. pinaster*, *P. pinea*  
328 and *P. halepensis* grew faster than *P. nigra* and *P. sylvestris* after 32 weeks of growth in 7-L  
329 containers. Higher shoot growth rate leads to greater foliage biomass and higher photosynthesis  
330 (Cuesta et al. 2010). This greater C assimilation will support the growth of new organs during  
331 the wet season, triggering a positive feedback loop in which shoot and root growth support each  
332 other (Burdett 1990; Villar-Salvador et al. 2012). In addition, Mediterranean pines, especially  
333 *P. halepensis* achieved maximum root growth potential at lower air temperature than mountain  
334 and boreo-alpine pines (Figure 4). Maximizing root elongation during the cool and wet season  
335 can facilitate seedling establishment before the summer drought, the most limiting season for  
336 seedling life in Mediterranean climates (Castro et al. 2004; Pulido et al. 2010). Mediterranean  
337 pines were also able to grow faster despite they reduced soil moisture more than the mountain

338 and boreo-alpine pines (Figure S1). Taken all together, our results indicate that Mediterranean  
339 pines avoid summer drought maximizing their growth during the wet and cool season, accessing  
340 rapidly deep water reserves and exploring a large soil volume (Jackson et al. 1996; Schulze et  
341 al. 1996; Padilla and Pugnaire 2007). We suspect that these pines also avoid drought because  
342 of early emergence: they disperse seeds in the summer and most seedlings emerge in autumn  
343 (Calama et al. 2017). This early emergence in the wet season, together with fast growth and  
344 higher root growth under cool air conditions, likely facilitates seedling establishment long  
345 before the onset of summer drought. In contrast, the two mountain pine species in our study  
346 disperse seeds in winter, and seedlings emerge in spring (Castro 2006; Tíscar and Linares 2011).  
347 This later emergence in the wet season, together with lower growth capacity, results in less root  
348 growth prior to summer drought and therefore greater seedling vulnerability to water stress  
349 (Castro 2006; De Luis et al. 2008).

350 Mediterranean pines showed shorter SRL than mountain pines. In other words, the  
351 mountain and boreo-alpine pines grew longer roots per root mass unit invested, which does not  
352 support our initial hypothesis. Körner and Renhardt (1987) reported that that perennial herb  
353 species that grow at low altitude had thicker fine roots and lower SRL than perennial herbs  
354 species inhabiting at high altitude location. Differences in SRL allows for coping with changes  
355 in soil resources. SRL variability reflects a trade-off between stress tolerance and resource  
356 exploitation (Comas and Eissenstat 2004; de la Riva et al. 2017): fast growing species, which  
357 usually thrive in rich resource environments, are expected to have higher SRL, whereas slow  
358 growing, stress-tolerant species are expected to have lower SRL. However, whether root traits  
359 are primarily aligned along the acquisition-conservation axis is under debate (de la Riva et al.  
360 2016; Kramer-Walter et al. 2016; de la Riva et al. 2017). Our data show that although  
361 Mediterranean pines inhabit drier locations they have lower SRL but higher growth rates than  
362 mountain and boreo-alpine pines. The high SRL in mountain and boreo-alpine pines is



363 consistent with the idea from recent global reviews that plants inhabiting low-temperature  
364 ecosystems tend to have higher SRL (Freschet et al. 2017; Valverde-Barrantes et al. 2017).  
365 High SRL in cold-climate plants may be an adaptation to enhance soil nutrient exploitation,  
366 counteracting the negative effects of low temperature on organic matter mineralization (Comas  
367 et al. 2012).

368         Plants can also maintain their water status through increased biomass allocation to roots  
369 (Grossnickle 2005; Brunner et al. 2015). However, we found no significant R/S differences  
370 among species, suggesting a conservative pattern in the allocation of biomass among pine  
371 species. These results contrast with previous reports of higher R/S in *P. nigra* and *P. sylvestris*  
372 than in our Mediterranean pines (Climent et al. 2011; Matías et al. 2017). Differences between  
373 our study and previous ones may reflect that our study used a much larger rooting volume  
374 (Poorter et al. 2012a): in our study it was 360L, compared to 7L in Climent et al. (2011) or 2.5L  
375 in Matías et al. (2017). Similarly, our data showed no inter-species differences in the  
376 distribution of root mass through depth, in contrast to quantitative reviews and experimental  
377 studies showing that tree species adapted to dry conditions generally invest more root mass in  
378 depth than species inhabiting mesic environments (Schenk and Jackson 2002; Mokany et al.  
379 2006; Markesteijn and Poorter 2009; Poorter et al. 2012b). It is possible that root depth and root  
380 system size are more important than R/S for survival under dry conditions (Padilla and Pugnaire  
381 2007). Plants can also respond to water stress by constructing root systems with a larger taproot  
382 and increasing the allocation of biomass to coarse roots at the expenses of fine roots. It is also  
383 possible that our results are phylogenetically biased: the species in our study belong to the same  
384 genus, and morphological root traits are phylogenetically structured (Valverde-Barrantes et al.  
385 2017). Indeed, *Pinus* species show a strong phylogenetic signal in some functional traits (He et  
386 al. 2012) and mass allocation may also have evolved conservatively.

387 In conclusion, seedlings of southern Europe pines differ in how rapidly they grow and  
388 colonize the soil profile, and the sensitivity of root growth to low temperature. Mediterranean  
389 pines showed faster growth rates, larger root systems, faster rooting through the soil depth and  
390 at lower temperature and soil moisture than mountain and boreo-alpine pines under lowland  
391 Mediterranean conditions. These differences related to the aridity to which the species are  
392 normally exposed in their range. SRL was higher in mountain and boreo-alpine pines than in  
393 Mediterranean pines. The distribution of root mass through soil depth and the ratio of mass  
394 allocation to roots and shoots did not differ among pines. Our results suggest that differences  
395 in growth rate play an important role in determining the capacity of pine species to colonize dry  
396 Mediterranean locations. However, other functional attributes related to plant's water economy  
397 and tolerance to high temperatures might also contribute to explain pine species distribution  
398 and should be addressed in future studies. Climate change projections for southern Europe  
399 predict an increase in aridity over the next century (Christensen and Christensen 2007), which  
400 can potentially trigger shifts in the tree species distribution. Based on our results, the higher  
401 growth capacity of Mediterranean pines may allow the colonization of zones at higher altitude  
402 and thereby displace mountain pines, which are also more vulnerable to warming and drought  
403 (Matías et al. 2017; Salazar-Tortosa et al. 2018).

404

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598 **Figure legends**

599 **Figure 1:** Root mass of pine species at the end of the experiment over the entire soil depth (a)  
600 and in layers at depths of 0-40 cm (b), 40-80 cm (c), and 80-110 cm (d). Boxes show the 95%  
601 and 5% percentile values, while the solid line indicates the median. Different letters show  
602 significant differences ( $p < 0.05$ ) between pine species.

603 **Figure 2:** Total root length (a) and specific root length (b) of pine species at the end of the  
604 experiment. Boxes are the 95% and 5% percentile values, while the solid line indicates the  
605 median. Different letters show significant differences ( $p < 0.05$ ) between pine species.

606 **Figure 3:** Root length at different soil depths in pine species. Lines depict the mean values  
607 ( $n=6$ ) for each species at each measurement date, while strips represent the SE. The scale of the  
608 root length axis differs among panels.

609 **Figure 4:** Relationship of pine species' scaled root length increment with temperature. Points  
610 show observed scaled root length increment. Lines depict the predictions of the fitted linear  
611 mixed model for each species, while strips represent the SE of these predictions.

612 **Figure 5:** Time needed for roots to reach a depth of 40 cm across pine species. Boxes are the  
613 95% and 5% percentile values, while the solid lines indicate the median. Different letters show  
614 significant differences ( $p < 0.05$ ) between pine species.

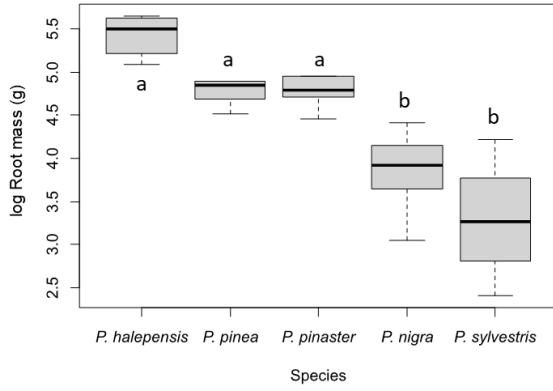
615 **Figure 6:** Relationships of a pine species' root mass at the end of the experiment, shoot diameter  
616 growth rate, specific root length (SRL) and time for roots to reach a depth of 40 cm with 2.5%  
617 percentile values of annual rainfall in the distribution range of that species. Equations of the  
618 adjusted linear models are shown together with  $r^2$  and P values. Each point represents mean  
619 values  $\pm 1$  SE. Ph=*P.halepensis*, Pa=*P.pinea*, Ppt=*P.pinaster*, Pn=*P.nigra* and Ps=*P.sylvestris*.

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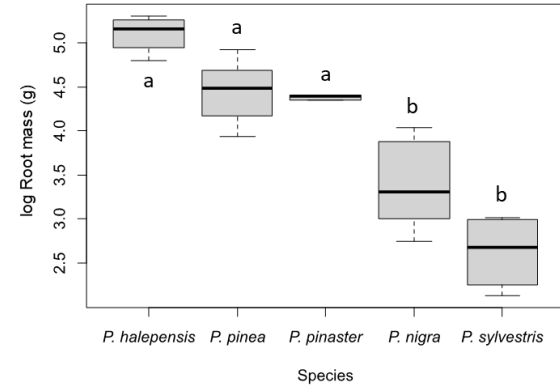
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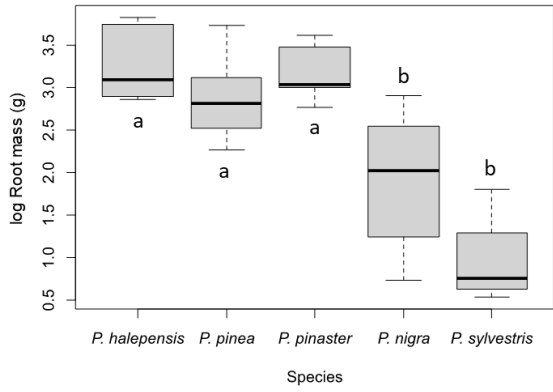
**a) Whole profile (0-110 cm depth)**



**b) 0-40 cm depth**



**c) 40-80 cm depth**



**d) 80-110 cm depth**

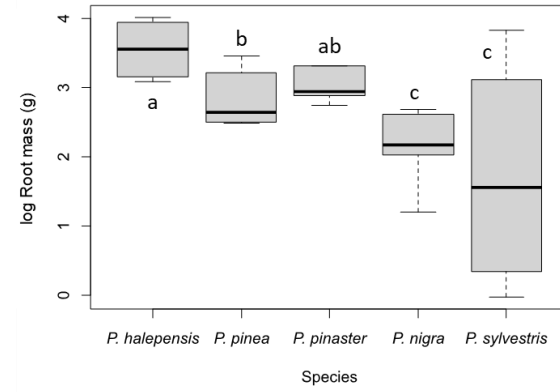
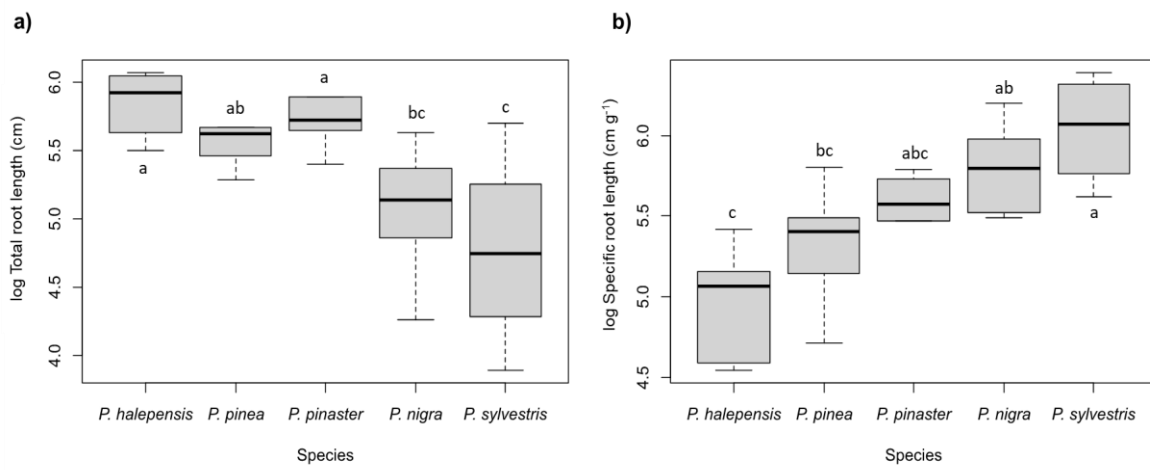


Figure 1

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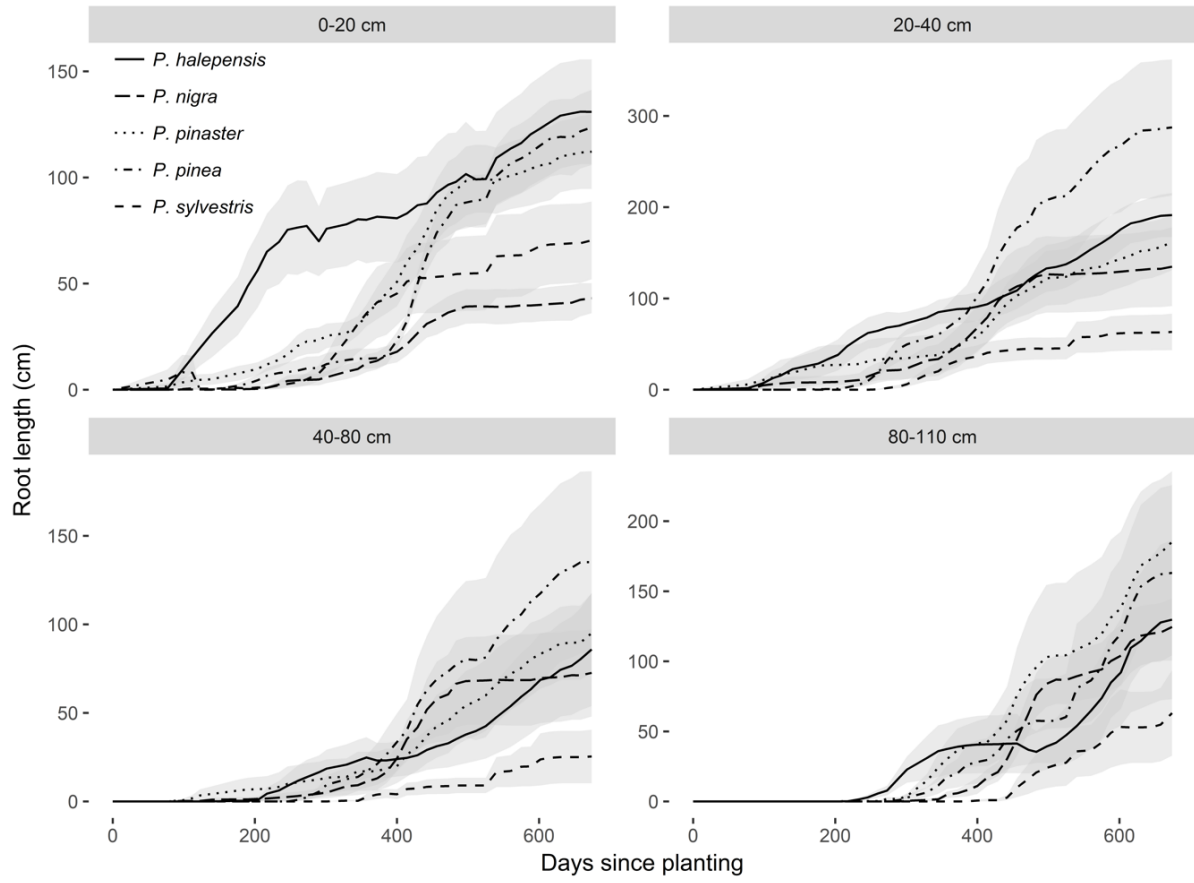


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Figure 2

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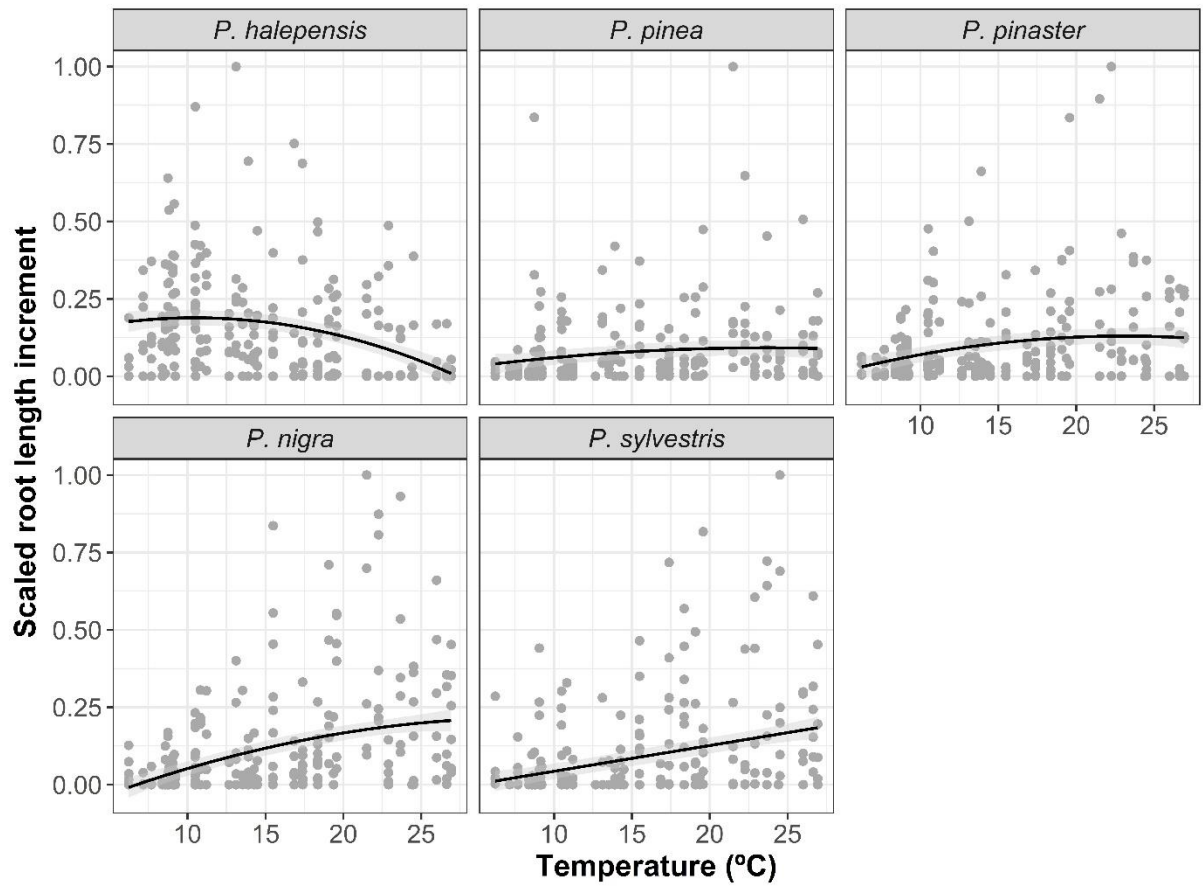


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Figure 3



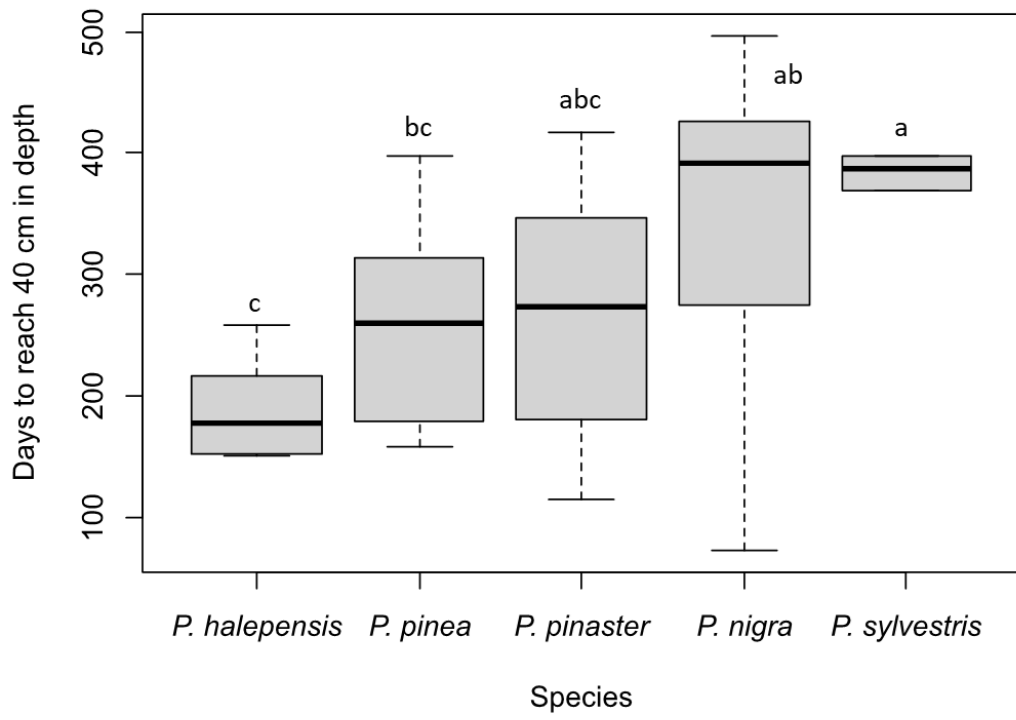
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Figure 4

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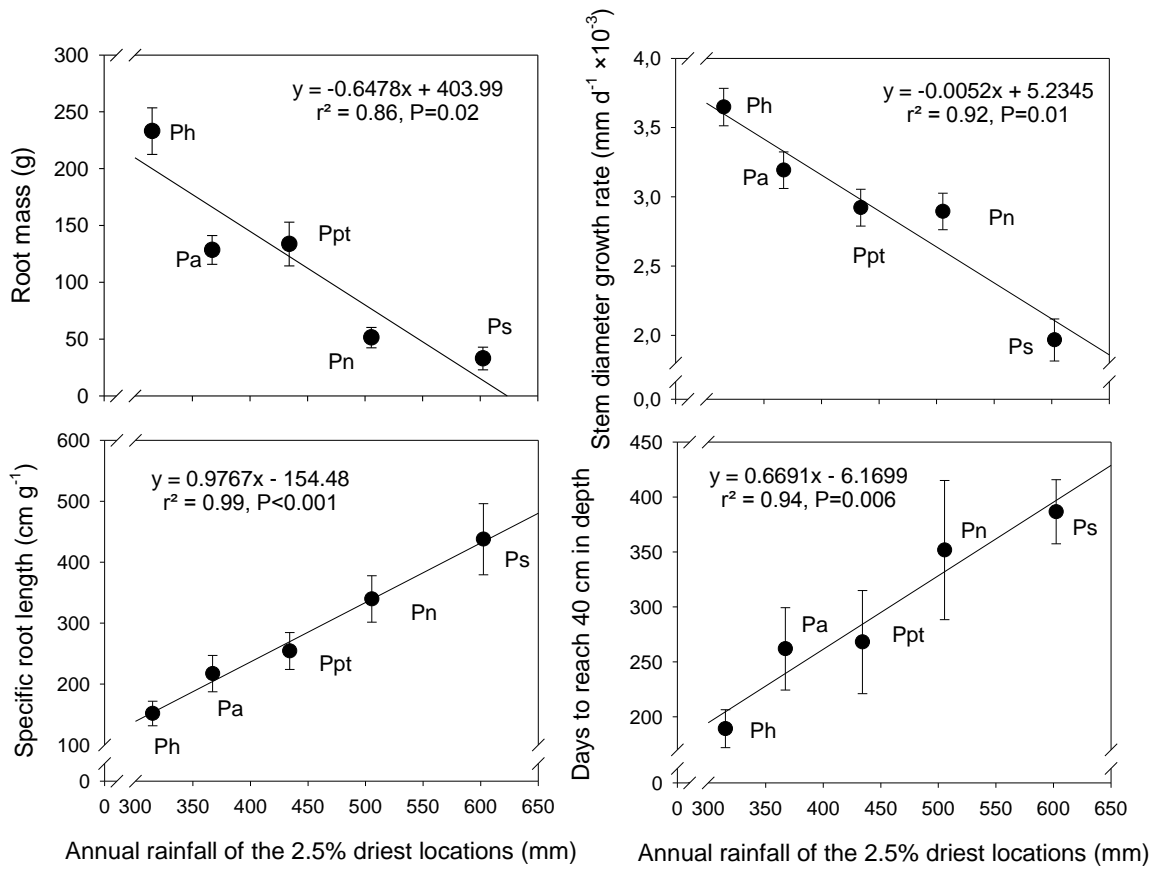
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Figure 5



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Figure 6

1 **SUPPLEMENTARY MATERIAL**

2

3 **Rooting big and deep rapidly: the ecological roots of pine species distribution**  
4 **in southern Europe**

5

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23

**Table S1.** Geographic location and climatic characteristics of the provenances of the seeds used in the study.

Species	Provenance name*	Latitude (N)	Longitude (W)	Altitude (m a.s.l.)	Mean temperature (°C)	Annual rainfall (mm)
<i>P. halepensis</i>	Alcarria	40°24'52''	2°24'33''	860	12.6	580
<i>P. pinea</i>	La Mancha	39°12'02''	1°57'59''	675	14.2	397
<i>P. pinaster</i>	Cuenca	39°38'44''	1°13'52''	1135	12	540
<i>P. nigra</i> subsp. <i>salzmanii</i>	Sistema Ibérico Meridional	40°15'16''	1°58'22''	1515	10.4	617
<i>P. sylvestris</i>	Montes Universales	40°28'09"	1°38'42"	1725	9.2	894

24 \* According to Alía et al. (2009).

25

26 **Table S2:** Comparison of Generalized Additive Mixed Models to assess the effects of the factor  
 27 *species* on the seedling elongation rate of pine species at different soil depths (0-20, 20-40, 40-  
 28 80 and 80-110 cm). The model with the factor *species* is compared with a model without *species*  
 29 effect (- sp). All models included *time* (days since planting) as a smoothed term. Model  
 30 selection was performed based on the corrected Akaike's Information Criteria (AICc). The final  
 31 model for each soil layer is shown in boldface.

Soil depth		AICc	R <sup>2</sup>
<b>0-20 cm</b>			
	<i>full</i>	<b>11177.9</b>	<b>0.57</b>
	<i>-sp</i>	11186.61	0.42
<b>20-40 cm</b>			
	<i>full</i>	<b>13279.3</b>	<b>0.46</b>
	<i>-sp</i>	13282.8	0.37
<b>40-80 cm</b>			
	<i>full</i>	<b>12131.0</b>	<b>0.34</b>
	<i>-sp</i>	12134.7	0.29
<b>80-110 cm</b>			
	<i>full</i>	12816.5	0.40
	<i>-sp</i>	<b>12812.5</b>	<b>0.37</b>

32

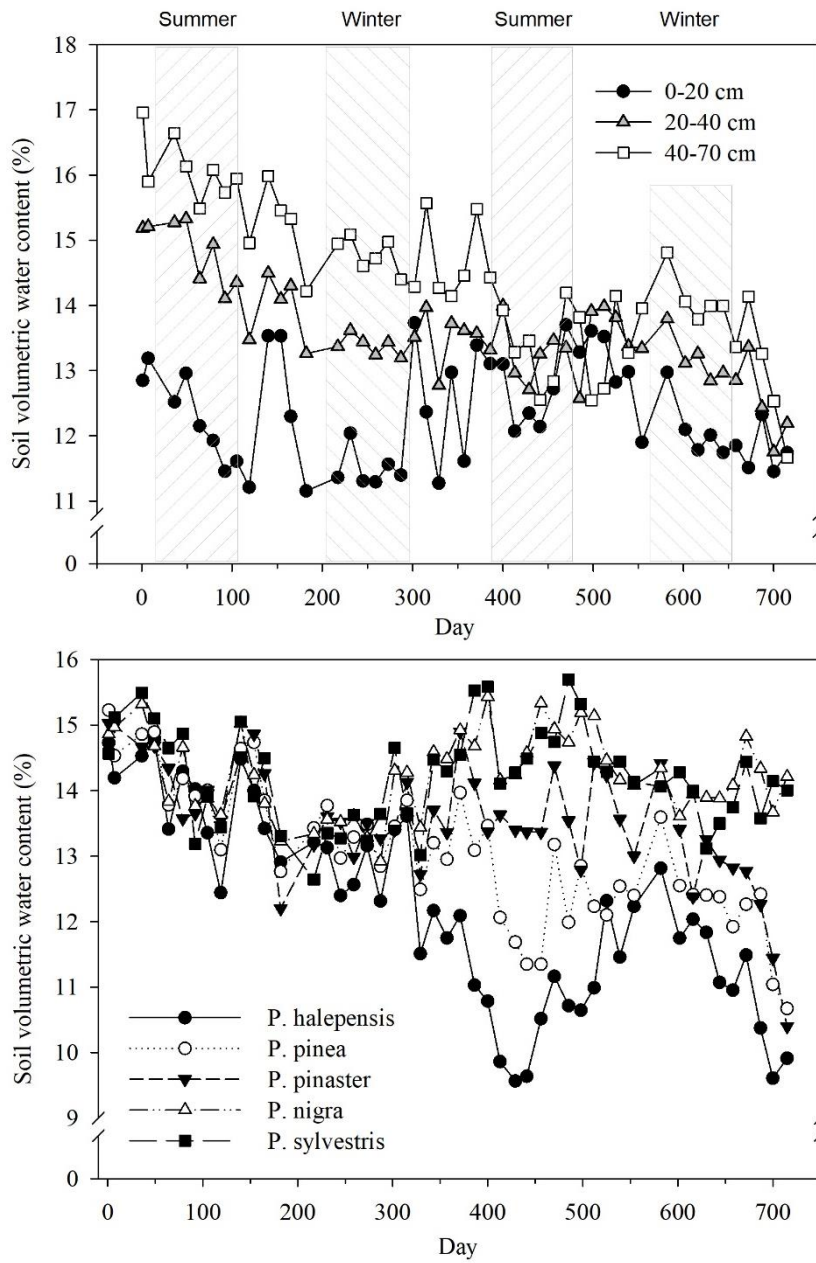
33

34 **Table S3:** Comparison of Linear Mixed Models to assess the relationship between scaled root  
 35 length increment and temperature for the studied pine species. First, we compared a model  
 36 including the interaction between temperature (T) and the factor *species* (Sp) with a model  
 37 including the interaction between temperature as a second polynomial order (polyT) and the  
 38 factor *species*. The model selected in this step is then compared with a model without the effect  
 39 of the interaction. Model selection was performed based on the corrected Akaike's Information  
 40 Criteria (AICc). The final model is shown in boldface.

<b>Model</b>	<b>AICc</b>	<b>R<sup>2</sup></b>
<i>T x Sp</i>	-1117.3	0.20
<b><i>polyT x Sp</i></b>	<b>-1167.4</b>	<b>0.24</b>
<i>polyT</i>	-1115.2	0.12

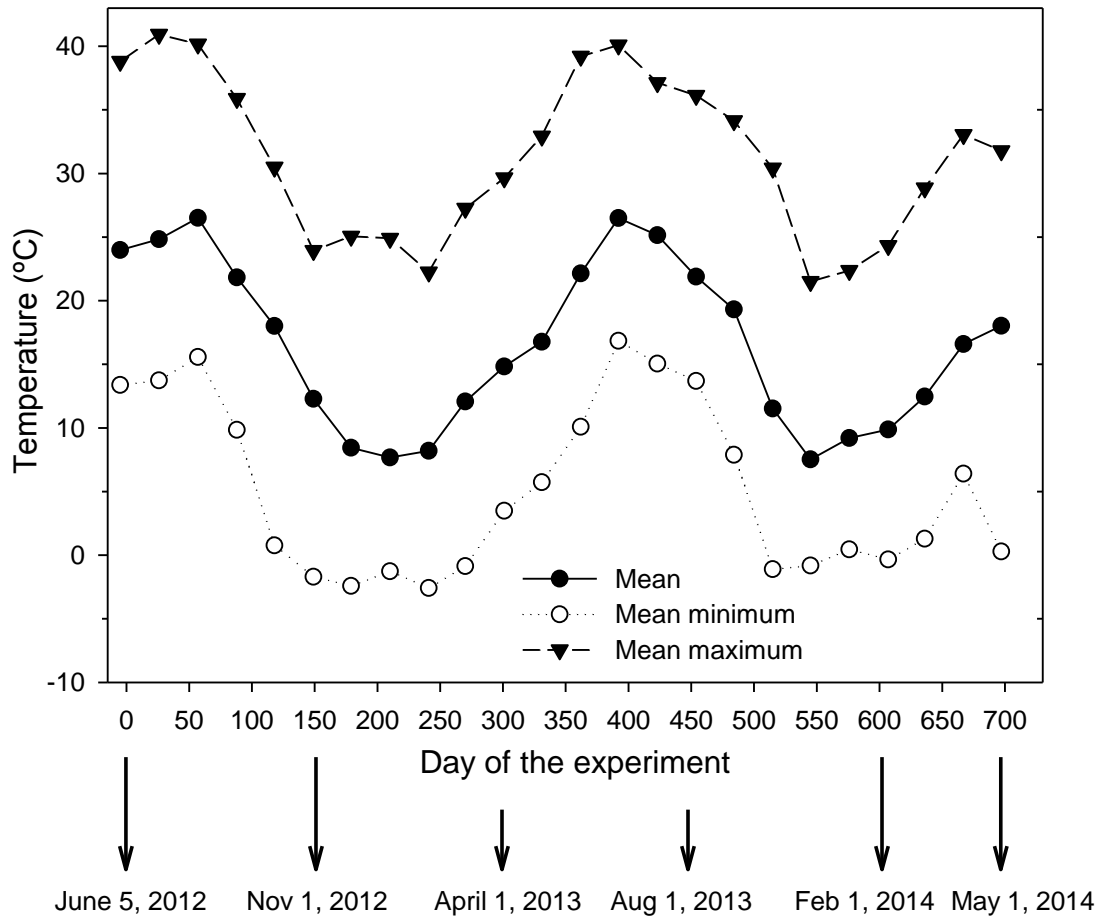
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42 **Figure S1:** Soil volumetric water content variation during the experiment at three soil depth  
 43 layers (upper figure) and per species (lower figure) as aggregate mean.



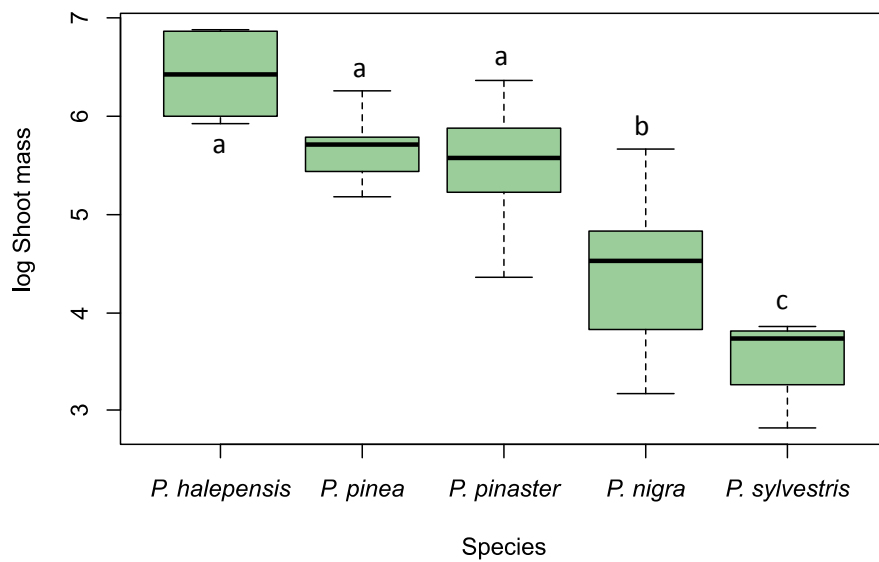
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45 **Figure S2:** Air temperature values during the experiment.



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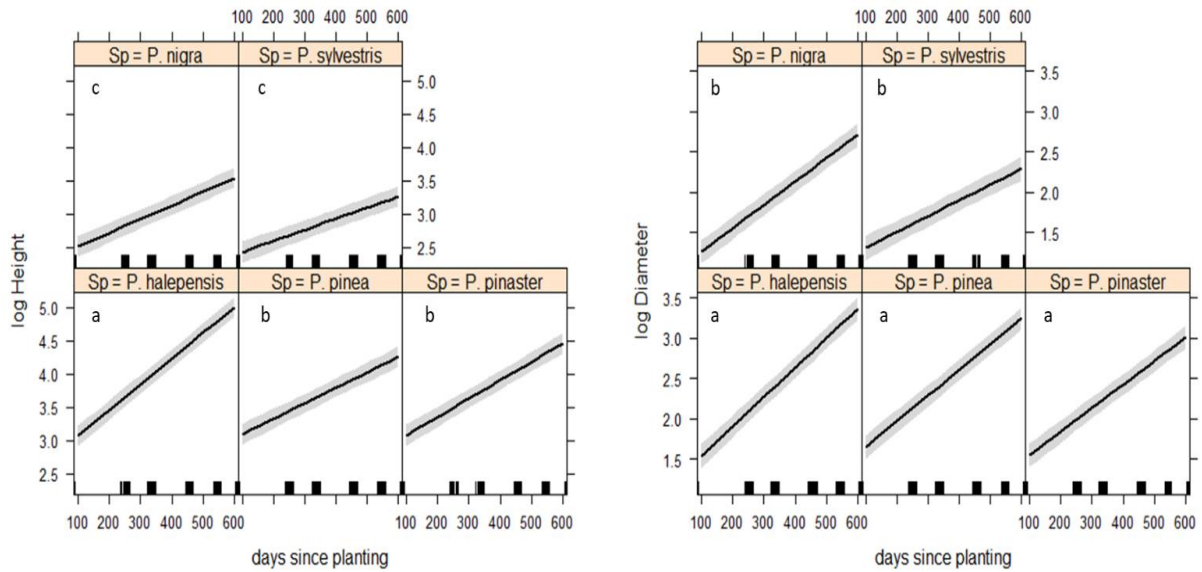
48 **Figure S3:** Shoot mass (g) of five pine species at the end of the experiment. Boxes are the  
49 95% and 5% percentile values, while the solid line indicates the median. Different letters  
50 show significant differences ( $p < 0.05$ ) in the mean values between pine species.



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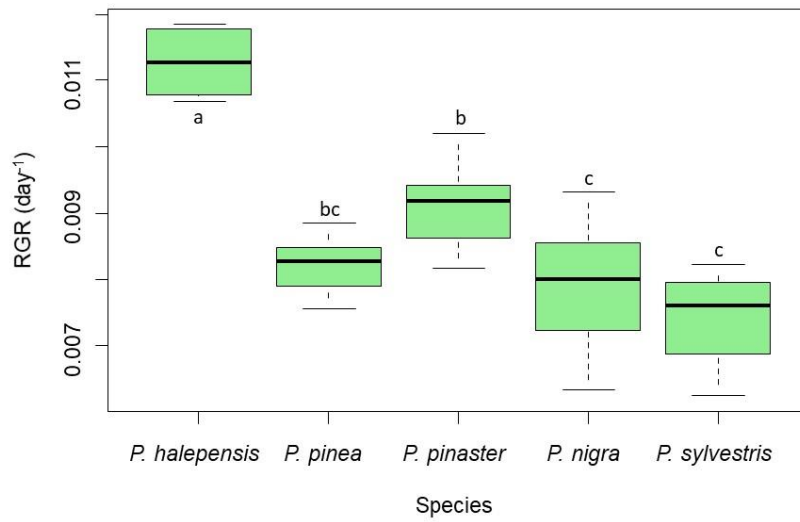


52 **Figure S4:** Model predictions for the height (cm) and diameter (mm) growth of seedlings of  
 53 five pine species. Grey strips depict the 95% confidence interval for model predictions.  
 54 Different letters show significant differences ( $p < 0.05$ ) in the slope of the model between pine  
 55 species.  
 56



57

58 **Figure S5:** Relative Growth Rate (RGR) ( $\text{day}^{-1}$ ) of five pine species during the experiment.  
59 Boxes are the 95% and 5% percentile values, while the solid line indicates the median.  
60 Different letters show significant differences ( $p < 0.05$ ) in the mean values between pine  
61 species.



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