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1 **Functional diversity reduces the risk of hydraulic**  
2 **failure in tree mixtures through hydraulic disconnection**

3

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## 33 **Abstract**

34 Forest ecosystems are increasingly threatened by anthropogenic pressures, especially by  
35 the increase in drought frequency and intensity. Tree species mixtures could improve  
36 resilience to diverse global anthropogenic pressures. However, there is still little  
37 consensus on how tree diversity affects water stress. Although some studies suggest that  
38 mixing species with different drought response strategies could be beneficial, the  
39 underlying mechanisms have seldom been identified. By combining a greenhouse  
40 experiment and a soil-plant-atmosphere hydraulic model, we explored whether mixing a  
41 drought avoidant (*Pinus halepensis*) and a drought tolerant (*Quercus ilex*) tree species  
42 could reduce plant water stress (defined as the risk of hydraulic failure) during extreme  
43 drought, compared to their respective monocultures. Our experiment showed that mixing  
44 species with divergent drought response strategies had a neutral effect on the drought-  
45 avoidant species and a positive effect on the drought-tolerant species. The model  
46 simulations further suggested that the beneficial effect of mixture on plant water stress  
47 during extreme drought was related to changes in the hydraulic connection of the plant  
48 from both the soil and the atmosphere. The ability of the drought-avoidant species to  
49 disconnect from the soil and the atmosphere limits its exposure to water stress, whereas  
50 the ability of the drought-tolerant species to increase its hydraulic connection to the soil  
51 lowers its hydraulic risk. This study brings a new insight on the mechanisms and traits  
52 combinations improving drought resistance in diversified forests and plantations, with  
53 important implications for forest management under climate change.

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## 56 **Keywords**

57 Forest, functional diversity, drought resistance, tree hydraulic, safety margins.

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59

## 60 **Main Text**

### 61 **Introduction**

62

63 The rising frequency and intensity of extreme drought is impacting tree survival and  
64 forest functions worldwide (Allen et al., 2010; Breshears et al., 2013; Senf et al., 2020),  
65 jeopardizing crucial forest ecosystem services. Tree species diversity has been promoted  
66 as an important nature-based solution to improve the resilience of forests and tree  
67 plantations (Messier et al., 2022). The effects of species mixing on drought resistance  
68 could result from different mechanisms, such as competitive reduction for water through  
69 resource partitioning or facilitation – for instance hydraulic redistribution (Grossiord,  
70 2020). Yet, there is no consensus regarding the effects of tree diversity on forest  
71 resistance to drought (Grossiord et al., 2014; Grossiord, 2020). Indeed, recent review  
72 showed that diversity can have positive (de-Dios-García et al., 2015; Lebourgeois et al.,  
73 2013; Ruiz-Benito et al., 2017), neutral (Grossiord et al., 2014; Merlin et al., 2015) or  
74 even negative impacts (C. Grossiord et al., 2014; Vitali et al., 2018). These conflicting  
75 results suggest that it is not the species richness that matters, but rather the functional

76 composition (i.e., species with different drought response strategies) of the mixtures  
77 (Forrester and Bauhus, 2016; Grossiord, 2020). Such hypothesis was supported by recent  
78 research that found that the diversity of hydraulic traits determines the resilience to  
79 drought of forest water fluxes globally (Anderegg et al., 2018; Haberstroh and Werner,  
80 2022). Similarly, results from a large-scale tree diversity experiment showed that the  
81 diversity of drought resistance strategies is a good predictor of the stability of tree growth  
82 and forest productivity (Schnabel et al., 2021). However, we crucially miss a mechanistic  
83 understanding of the way the diversity of drought resistance strategies mediates tree  
84 mortality under extreme drought.

85 Tree drought resistance strategies result from a set of functional traits that determine how  
86 rapidly the different tree functions will be impaired by drought stress (often quantified as  
87 water potential thresholds inducing dysfunction). In particular, it determines the risk of  
88 xylem hydraulic failure, caused by a high rate of embolism in xylem conduits (Tyree and  
89 Sperry, 1989), which is a leading mechanism in drought-induced tree mortality (Adams et  
90 al., 2017). It is common in the literature to distinguish species strategies based on  
91 stomatal regulation - and associated water potential dynamics - and the xylem  
92 vulnerability to embolism (Chen et al., 2021). Drought-tolerant species tend to maintain  
93 gas exchanges during drought by delaying stomatal regulation, which implies important  
94 soil water depletion and large decrease in the soil and tree water potential during drought  
95 (Delzon, 2015; López et al., 2021). Their high resistance to xylem embolism limits the  
96 risk of hydraulic failure. By contrast, drought-avoidants are generally more vulnerable to  
97 xylem embolism, but they close their stomata earlier during drought, thereby reducing  
98 soil water depletion, which in turn limits the soil and tree water potential decrease and the  
99 risk of hydraulic failure (Delzon, 2015; López et al., 2021).

100 By assuming that trees are hydraulically connected to the soil (*i.e.*, soil and tree water  
101 potential are at equilibrium if transpiration is null such as under predawn conditions) and  
102 that the root system fully occupies a given soil volume, one can hypothesize how mixing  
103 species with distinct drought response strategies impacts soil and tree water potentials,  
104 and the risk of hydraulic failure under extreme drought:

105 **H1.** For a drought-tolerant species, it should be beneficial to compete for water with a  
106 drought-avoidant neighbour, because the soil water saved by earlier stomatal regulation  
107 of the avoidant is available to delay the decrease in water potential and the overall  
108 hydraulic failure risk (Fig. 1).

109 **H2.** When grown in mixture with a drought tolerant neighbour, a drought-avoidant  
110 species should be disadvantaged, as it would experience lower soil water potential due to  
111 sustained water-use by the companion tolerant species. This would lead to a decrease in  
112 its water potential, thereby increasing the risk of hydraulic failure (Fig. 1). The scenario  
113 presented in Fig. 1 - which suggests that a drought tolerant always “win the fight” during  
114 drought under mixture - holds only if the water potential of the mixed species is at  
115 equilibrium with the soil water potential.

116 **H3.** If the root systems of the two neighbour species are segregated in space, water  
117 consumption by the tolerant species does not affect the avoidant species and difference of  
118 water potentials between tree species in the mixture could occur given their isolation

119 (Fig. 1). In support to this hypothesis, root niche separation is often assumed in the  
120 literature to explain coexistence between co-occurring species (Grossiord, 2020; Jose et  
121 al., 2006).

122 To test these hypotheses, we conducted a greenhouse experiment where seedlings of  
123 *Pinus halepensis*, a drought avoidant (Baquedano and Castillo, 2007) and *Quercus ilex*, a  
124 drought tolerant (Baquedano and Castillo, 2007) were grown in pairs in small pots (12 L).  
125 Seedlings were planted in either monoculture or mixture with or without root separation.  
126 We applied an extreme drought by stopping the watering and we regularly monitored the  
127 overall pot soil ( $\Psi_{\text{soil}}$ ) and tree predawn ( $\Psi_{\text{pd}}$ ) water potentials, along with soil resistivity  
128 and tree gas exchanges. These data were combined with a state-of-the-art soil-tree-  
129 atmosphere hydraulic model (Cochard et al., 2021) to further identify the mechanisms  
130 and traits involved in the co-existence of drought avoidant/tolerant species during  
131 extreme drought.

132

## 133 **Results and Discussion**

134

### 135 **a) Empirical evidence for hydraulic commensality between *Quercus ilex* and** 136 ***Pinus halepensis* during extreme drought**

137 Tree predawn water potential ( $\Psi_{\text{pd}}$ ) decreased markedly during drought (SI appendix,  
138 Table S1, P-value < 0.001), but the dynamics differed between species across treatments  
139 (SI appendix, Table S1, P-value < 0.001). Due to their differential drought resistance  
140 strategies,  $\Psi_{\text{pd}}$  reached more negative values in the drought tolerant *Q. ilex* than in the  
141 drought avoidant *P. halepensis*, regardless of the pot composition. In agreement with  
142 hypothesis H1, *Q. ilex* had significantly more negative  $\Psi_{\text{pd}}$  in monoculture than in  
143 mixture at the drought peak (Fig. 2A; SI appendix, Table S2, P-value < 0.01), except for  
144 the mixture with root separation. By contrast, in *P. halepensis*,  $\Psi_{\text{pd}}$  at the drought peak  
145 remained above both soil water potential ( $\Psi_{\text{soil}}$ ) and *Q. ilex*'s  $\Psi_{\text{pd}}$  in mixture (Fig. 2A),  
146 contradicting hypothesis H2.

147 Therefore, the hydraulic risk, estimated as the hydraulic safety margins at the drought  
148 peak (HSM, computed as the difference between P50, the water potential causing 50%  
149 xylem embolism and the average  $\Psi_{\text{pd}}$  at the driest date) was significantly improved in  
150 mixture compared to monoculture only for *Q. ilex* (Fig. 2B). Hence, as expected from  
151 hypothesis H1, the mixture had a positive effect on the reduction of the risk of hydraulic  
152 failure for the drought tolerant species *Q. ilex* but contrary to hypothesis H2, the mixture  
153 had a neutral effect on the drought avoidant *P. halepensis*. This result reflects a  
154 commensalism relationship in terms of hydraulic risk between drought avoidant and  
155 tolerant species under drought conditions, that to our knowledge, has never been  
156 demonstrated until now. Hence, coexistence between a drought avoidant and a drought  
157 tolerant species is not the exclusive result of spatial segregation of their root niche, even  
158 if such phenomenon can occur (Bello et al., 2019), but depend rather on other  
159 mechanisms that we further discuss below.

### 160 **b) Species coexistence relies on species-specific modifications of the soil-tree** 161 **hydraulic conductance**

162 The relationship between  $\Psi_{pd}$  and  $\Psi_{soil}$  (Fig. 3) in *P. halepensis* was unaffected by  
163 mixture, with  $\Psi_{pd}$  equal to  $\Psi_{soil}$  until  $\Psi_{soil}$  decreased below -4 MPa. For  $\Psi_{soil}$  lower than -  
164 4MPa,  $\Psi_{pd}$  remained constant at ca. -4 MPa. For *Q. ilex* the slope between  $\Psi_{pd}$  and  $\Psi_{soil}$   
165 was greater than one ( $> 1.7$ , Fig. 3) for monocultures and for the mixture with root  
166 separation. By contrast, the slope of the  $\Psi_{pd}$ - $\Psi_{soil}$  relationship was equal to one for *Q. ilex*  
167 in mixture without root separation (i.e.,  $\Psi_{pd} \sim \Psi_{soil}$  throughout the experiment, Fig. 3).  
168 Overall, these empirical results indicate that (i) the  $\Psi_{pd}$  vs.  $\Psi_{soil}$  relationships varied  
169 between the two studied species with different drought resistance strategies and (ii) plant-  
170 soil water potentials were modified by mixture only in *Q. ilex*, the drought tolerant  
171 species.

172 It could be assumed that differences between  $\Psi_{pd}$  and  $\Psi_{soil}$  reflect shifts in the root profile  
173 in mixtures compared to monocultures. Indeed, if roots explore only a part of the  
174 available soil,  $\Psi_{pd}$  would equilibrate with this soil subspace, possibly differing from the  
175 overall  $\Psi_{soil}$  measured at plot level. However, we used small pots (12 L) to impose a  
176 complete occupation of the whole soil volume by the trees' root system, making this  
177 assumption unlikely. Furthermore, the fact that we found no significant differences  
178 between the average soil resistivity at the top and bottom profiles of the pots for each  
179 modality suggests that water is absorbed uniformly throughout the soil and definitely  
180 rules out this hypothesis (SI appendix, Fig. S1). Alternatively, we can postulate that  
181 differences between  $\Psi_{pd}$  and  $\Psi_{soil}$  result from changes in the hydraulic conductance  
182 between the soil and the trees.

183 Following the experimental component of our study, we carried out simulations with the  
184 hydraulic process-based model SurEau (Cochard et al., 2021) to test the possible  
185 mechanisms that could explain such empirical patterns. The model computes the water  
186 fluxes along the soil-tree-atmosphere continuum by accounting for the different  
187 resistances of the soil, the symplasm and apoplasm of the root, trunk, branch and leaf,  
188 and calculate the water potential and the water content of the corresponding  
189 compartments. By considering xylem vulnerability to cavitation, the model can estimate  
190 the loss of hydraulic conductance of the tree xylem in relation to water potentials and  
191 predicts the death of the tree by hydraulic failure when 100 % loss of hydraulic  
192 conductivity is reached. The model was improved to represent two different individuals  
193 competing for a same amount of soil water (see Materials and Methods).

194 We first conducted three benchmark simulations corresponding to Figure 1  
195 (monocultures of *Q. ilex* and *P. halepensis* and the mixture without root separation). In  
196 such simulations, we assumed that the hydraulic conductance of the rhizosphere ( $K_{rhizo}$ )  
197 and of the fine roots ( $K_{root}$ ) were the same for all species and pot compositions. More  
198 specifically, we applied the widespread “single root” approach that assumes that soil  
199 conductivity relates to the soil water content (van Genuchten, 1980) and is scaled up to  
200 the rhizosphere according to the length of fine roots per unit soil volume (Gardner, 1964;  
201 I. R. Cowan, 1965)

202 By doing so, the model predicted behaviours consistent with our initial hypotheses H1  
203 and H2 (Fig. 1): the drop of  $\Psi_{pd}$  in *P. halepensis* and *Q. ilex* growing in mixture are  
204 respectively faster and slower than for the corresponding monocultures (Fig. 4A),  
205 indicating that mixture should have a negative effect on *P. halepensis* and a positive

206 effect on *Q. ilex*, which contradict our results. We thus conducted sensitivity analyses on  
207 different traits to explore mechanisms explaining our empirical observations.

208 **c) The drought-avoider *P. halepensis* isolates from the soil through a decrease**  
209 **in both root hydraulic and cuticular conductance**

210 The fact that *P. halepensis* exhibits higher  $\Psi_{pd}$  than  $\Psi_{soil}$  during drought suggests that (i)  
211 this species can isolate from the soil (i.e., reducing the soil to tree hydraulic conductance)  
212 and (ii) is able to limit its dehydration. Several studies have suggested that plant isolation  
213 from the soil allows limiting the exposure to water stress (Aguadé et al., 2015; Brito et  
214 al., 2019; Cuneo et al., 2016). Different non-exclusive belowground mechanisms were  
215 proposed to explain tree isolation from the soil, such as the formation of cortical lacunae  
216 under fine roots (Cuneo et al., 2021; Duddek et al., 2022), which reduces water transfer to  
217 the root stele and hence affect roots hydraulic conductance. Roots shrinkage might also  
218 explain the plant-soil hydraulic disconnection by creating gaps between soil and fine  
219 roots interrupting the hydraulic conductance between both interfaces. Furthermore, the  
220 inhibition of the synthesis of proteins such as aquaporins facilitating water transport in  
221 the transcellular pathway (Domec et al., 2021), or even fine roots mortality (Leonova et  
222 al., 2022). could also explain hydraulic isolation. To evaluate whether tree isolation from  
223 the soil could explain the observed water potential patterns in *P. halepensis*, we  
224 hypothesized in the model a decrease in root hydraulic conductance ( $K_{root}$ ) as the tree  
225 water potential decreases (SI appendix, Fig. S2). Simulations were performed under a  
226 mixture condition with *Q. ilex* as a companion species (parametrized as in benchmark  
227 simulations) (Fig. 4B). This allows to force soil water potential to drop even after *P.*  
228 *halepensis* has closed its stomata and has isolated from the soil. Model simulations  
229 indicate that reducing only  $K_{root}$  does not allow to simulate higher  $\Psi_{pd}$  than  $\Psi_{soil}$  for *P.*  
230 *halepensis* (Fig. 4B). This means that the water losses that occur after stomatal closure –  
231 which result from the leaf cuticular conductance ( $g_{cuti}$ ), set in the model using the average  
232 value measured for *P. halepensis*, was high enough to cause tree water potential drops  
233 after a strong decrease in  $K_{root}$  (Fig. 4B). We thus implemented in the model a down-  
234 regulation of  $g_{cuti}$  with decreasing tree relative water content, which is in accordance with  
235 empirical data obtained in *P. halepensis* using the drought-box methods (Billon et al.,  
236 2020) (SI appendix, Fig. S3). Simulations showed that, although the reduction of  $g_{cuti}$   
237 alone attenuated the decrease in tree water potentials, the tree keeps dehydrating. Finally,  
238 when implementing a decrease of both  $K_{root}$  and  $g_{cuti}$  under drought, *P. halepensis* water  
239 potential departs from soil water potentials (Fig. 4B), in line with our observations. This  
240 suggests that these two mechanisms jointly could allow *P. halepensis* to prevent  
241 dehydration under drought. In the natural forest context, tree isolation from the soil  
242 during drought has already been proposed to explain the coexistence of drought-avoidant  
243 and drought-tolerant trees (Aguadé et al., 2015; Moreno et al., 2021; Pangle et al., 2012;  
244 Plaut et al., 2012). Yet, to our knowledge, the mechanisms leading to complete plant  
245 disconnection from the soil and the atmosphere had never been proposed until now.

246 **d) The drought-tolerant *Quercus ilex* increases root hydraulic conductance to**  
247 **the soil in mixture through increased root length**

248 For *Q. ilex*,  $\Psi_{pd}$  was respectively lower or comparable to  $\Psi_{soil}$  under monoculture and  
249 mixture conditions (Fig. 2A) which suggests that (i) contrary to *P. halepensis*, *Q. ilex* is

250 not able to limit its dehydration and (ii) the mixture likely impact the hydraulic  
251 conductance between the soil and the trees under drought.

252 According to the diffusion law, the lower departure between  $\Psi_{pd}$  and  $\Psi_{soil}$  that we  
253 observed for *Q. ilex* in mixture compared to monoculture, could result from an increase in  
254 the conductance of the rhizosphere, which could lower the water potential drops required  
255 for a given flux between the soil and the tree (39).

256 As we found a greater root system length in mixture than in monoculture (SI appendix,  
257 Fig. S4), we assumed that the increase in rhizosphere conductance might be achieved  
258 through an increase in exchange surface between soil and root (“single root” approach).  
259 We tested this hypothesis by varying the modelling parameters of fine roots length per  
260 unit soil volume. This sensitivity test shows that changing  $K_{rhizo}$  can change the  $\Psi_{pd}$  vs  
261  $\Psi_{soil}$  relationships between monoculture and mixture (Fig. 4C). Indeed, reducing the value  
262 of this parameter (graph “root length x 1/2”, Fig. 4C), results in a departure between  $\Psi_{pd}$   
263 and  $\Psi_{soil}$  as observed in monoculture, whereas increasing it results in  $\Psi_{pd}$  and  $\Psi_{soil}$  being  
264 comparable, as observed in mixture. Interestingly, some studies have already reported  
265 modifications of the root system under mixture toward higher fine roots density (Sun et  
266 al., 2017; Wambsganss et al., 2021), identifying this phenomenon to a complementarity  
267 effect between species associated.

#### 268 **e) Ecological implications**

269 Our results provide evidence that mixing drought-avoidant and drought-tolerant species  
270 reduces the risk of hydraulic failure under extreme drought conditions at the community  
271 level. According to model simulations, such mixing effect can be explained by changes in  
272 hydraulic connection between the plant, the soil and the atmosphere during drought. The  
273 avoidant species can sustain extreme drought through an isolation from the soil (decrease  
274 of  $K_{root}$ ) and the atmosphere (decrease of  $g_{cuti}$ ) whereas the tolerant species can increase  
275 hydraulic conductance of the rhizosphere through an increase in root length. Such results  
276 remained to be tested at larger scale but could change our view about the mechanisms of  
277 species co-existence. Whereas it is sometimes assumed that mixture has positive effect  
278 due to root system segregation in space (Bello et al., 2019; Grossiord et al., 2018), we  
279 provide evidence that the hydraulic connection of the plant to the soil and the atmosphere  
280 can also be involved, without the need to call for a spatial segregation of the root systems.

281 Our results also challenge the way vegetation models represent drought stress. To date,  
282 the majority of process-based models assume that soil water deficit in the rooting zone  
283 drives the water status of the plant. However, we provide evidence that changes in  
284 hydraulic connection from the soil can make the plant behave independently from soil  
285 water status. Implementing such processes in larger scale vegetation models could help  
286 explain and predict co-existence between species and drought induced effect on forest  
287 community. Such modelling approach could be a step toward the development of tools  
288 allowing to design drought resilient mixture.

289

290

291

## 292 **Materials and Methods**

### 293 **Seedlings and experimental design**

294 The experiment was set up during the summer 2021. It consisted in applying a drought  
295 treatment (watering stop) to potted *P. halepensis* and *Q. ilex* trees grown in monoculture  
296 or in mixture while monitoring ecophysiological variables at 5 different dates. Seedlings  
297 of *P. halepensis* and *Q. ilex* (one- and two-years old respectively) of equivalent  
298 dimensions were repoted in January 2020. 90 trees of each species were planted in 12 L  
299 containers, including two individuals per pot, either in monoculture or in mixtures. The  
300 soil was composed of sand (~20%) and organic matters. Half of the pots were equipped  
301 with a physical barrier made of acrylic fabric (with 30 $\mu$ m mesh) that precludes root  
302 colonization from one side to the other of the pot but allow water transfer between the  
303 two separated compartments. From 2019 to June 2021, saplings were grown at the  
304 National Forestry Office of France (ONF) nursery in Cadarache (Southeast of France)  
305 and were watered twice a week to field capacity and fertilized once a week. One month  
306 before the start of the experiment (June 2021), pots were brought on the campus of  
307 INRAe (Avignon, France) to acclimate in the experimental greenhouse. The greenhouse  
308 was equipped with air temperature, a humidity (HD 9817T1) and radiation loggers. It  
309 included an independent regulation of climate through aeration (opening of the glasses or  
310 forced ventilation in the compartment) and cooling (humidification of the air entering  
311 through a “cool□ box”). These systems allowed regulating the environment of the  
312 greenhouse according to the defined settings. In addition, the sidewalls of the greenhouse  
313 have been whitewashed to homogenize the radiation and the temperature. The  
314 temperature was kept between 25 and 35 °C, relative humidity (RH) between 40 and  
315 75%, and maximum diurnal photosynthetically active radiation (PAR) below 1000  
316  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  (SI appendix, Fig. S5).

317 During acclimation period, watering was applied as in the nursery. Among the initial  
318 batch of 90 pots, we selected 54 pots for which the two trees were alive and had reached  
319 a height between 40 and 60 cm with less than 10cm heights differences between the two  
320 trees. Pots were divided into two batches: a batch of 6 pots per composition (36 pots in  
321 total) that was assigned to the drought experiment, and a batch of 3 pots per treatment (18  
322 pots in total) that was assigned to a control treatment in which trees were maintained  
323 watered all along the season (two times a week). All pots were monitored once a week,  
324 from July 26 to August 18, for soil water and water potential and ecophysiological  
325 variables (leaf water potentials, leaf gas exchange, pots water loss- described below). The  
326 day before the beginning of the experiment, at the end of the afternoon, all pots were  
327 watered at saturation and weighted.

328

### 329 **Tree water potentials**

330 Water potential was estimated through leaf water potentials of all trees measured at  
331 predawn once a week across the experimental period..The evening before measurements,  
332 for each tree, one leaf (*Q. ilex*) or small twig (*P. halepensis*) was covered with an  
333 aluminium foil and placed in a ziplock plastic bag. In addition, to limit tree nocturnal  
334 transpiration and allow water potential equilibration between the tree and the soil

335 (Rodriguez-Dominguez et al., 2022), trees were covered with a plastic bag and a piece of  
336 wet paper was included under the plastic bag. Samples were collected before sunrise,  
337 between 4 to 5 am, kept into the ziplock and immediately placed in a cooler for water  
338 potential measurement. The 108 measurements were done randomly in less than 4 hours  
339 following sampling, with a scholander pressure chamber (PMS model 1505 D). At the  
340 beginning of the experimentation, midday water potentials of tree were measured  
341 between 1 and 2 PM, following the same procedures as described above for predawn  
342 water potential (leaf or twig covered with an aluminium foil and placed in a ziplock  
343 plastic bag). There were used to parametrize the model.

344

### 345 **Tree leaf gas exchanges**

346 Leaf level gas exchange was measured using two portable photosynthesis system (LI-  
347 6400XT) for all trees at all dates except the second one due to breakdown of the  
348 greenhouse system. Measurements were done between 11 am to 3 pm, period during  
349 which PAR in the green house is highest and stable (between 600 and 1000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ).  
350 Licor chamber conditions were set to keep close to the greenhouse while providing  
351 non-limiting conditions: PAR was set at 1000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , the block temperature was  
352 set at 25°C, flow rate and scrubbing were adjusted to maintain RH between 60 and 80%.  
353 The leaves were allowed to acclimate for at least 3 minutes in the chamber before  
354 measurement, to ensure gas exchange stability. For each leaf (*Q. ilex*) or needle bunch (*P.*  
355 *halepensis*), ten values were recorded during one minute and the average was used in the  
356 data analysis. After the measurement, the area of leaves or needles included in the  
357 chamber were cut and stored in a plastic bag inside a cooler. The day after, leaf area was  
358 measured to correct gas exchange computation with actual leaf area in the chamber.  
359 Samples were then dried during 48 hours at 70°C to estimate specific leaf area.

360

### 361 **Tree biomass and leaf area estimates**

362 We estimated leaf area of each tree at the beginning and the end of the experiment using  
363 a method relying on profile photographs, proposed by (Michael and Parker, 2000). It is  
364 based on a calibrated relationship between the projected area of the tree profile and the  
365 foliage biomass estimated destructively. For each species, we first built a calibration  
366 relationship between numbers of tree pixels in profile photographs and the foliage  
367 biomass. For the calibration relationship, trees were selected to span the range of sizes  
368 encountered in the experiment. We sampled trees before the beginning of the drought  
369 experiment (June 2021), and after the experiment (September 2021) to consider potential  
370 changes in size or leaf area or angulation that could have occurred during the summer and  
371 influenced the relationship. For each tree, the profile surface projected area was estimated  
372 by photography. All the settings were made to ensure a constant reproduction ratio (i.e.,  
373 constant dimensions of real object dimensions per pixel) among photographs. To obtain  
374 foliage dry mass, all trees used for this calibration were cut at the base of the stem after  
375 taking photographs. Tree parts were sorted to separate green foliage, dead foliage, and the  
376 rest which is almost entirely made of stems. Tree parts were then dried at 70°C for 3 days  
377 (leaves/ needles) or until there was no variation in dry mass (almost one week). The leaf

378 area of each tree with the estimation of total foliage dry mass at a specific date and  
379 specific leaf area estimated on leaf gas exchange measurement samples.

380 At the end of the experiment and for droughted pots, the belowground part of each tree  
381 were uprooted. The rooting system was washed to separate the soil particles for the roots.  
382 The rooting system extension (maximal length and width) were measured using a ruler,  
383 with a millimeter resolution. The root system was then dried out at 70°C in an oven for at  
384 least 10 days, until there are no more weight variations, and the total dry mass was  
385 estimated.

386

### 387 **Soil water content and soil water potentials**

388 Pots were weighted at each measurement dates in the morning (8am) and at the end of the  
389 measurement day (5pm). Soil water content was estimated at the pot level, by subtracting  
390 the total pot weight, performed at each measurement dates in the morning (ca. 8 am), the  
391 soil dry mass and the total fresh tree biomass. Soil water potential ( $\Psi_{soil}$ ) was then  
392 estimated at the pot level from the normalized soil water content of the pots ( $W_{norm}$ ) and  
393 water retention curves determined in the laboratory on soil samples ( $V= 6 \text{ cm}^3$ ). The  
394 determination of the retention curve was made with the combination of suction table  
395 ( $\Psi_{soil} > -0.01 \text{ MPa}$ ), pressure plate ( $\Psi_{soil} > -1.5 \text{ MPa}$ ) and dew point hygrometer (WP4C,  
396 Decagon-  $\Psi_{soil} < -1.5 \text{ MPa}$ ) methods (Dane and Hopmans, 2002). Five soil sample  
397 replicates were used for each point of the retention curve and the gravimetric water  
398 content was determined from fresh and dry weight obtained after drying in an oven at  
399 70°C (limit temperature to avoid organic matter degradation) for about one week. To  
400 perfectly match the data, two different retention curves were fitted. A first retention curve  
401 was fitted with two set of van-Genuchten relationships (van Genuchten, 1980)  
402 intersecting at a gravimetric water content of  $0.116 \text{ g.g}^{-1}$  (corresponding to  $\Psi_{soil} = -1.4$   
403 MPa). A second set of retention curve was fitted with only one van-Genuchten  
404 relationships (van Genuchten, 1980) . The retention curves take the following form:

$$\psi_{soil} = \frac{\left( \left( \frac{1}{W_{norm}} \right)^{\frac{1}{m}} - 1 \right)^{\frac{1}{n}}}{\alpha}$$

405 where  $m$ ,  $n$  and  $\alpha$  are empirical parameters describing the typical sigmoidal shape of the  
406 function and  $W_{norm}$  is the normalized water content. Water potential was calculated from  
407 this fit using the gravimetric water content of pots estimated at each measurement dates.  
408 The parameters of the curves are provided in the SI appendix, Table S3.

409 The normalized water content was computed for each pot as:

$$W_{norm} = \frac{W - W_r}{W_{sat} - W_r}$$

410 With  $W$  the soil mass of the pot at a given time,  $W_r$  the soil mass at residual water  
411 content. It was measured at the end of the experiment after drying the soil at 70°C.  $W_{sat}$   
412 is the saturated mass of the soil which was estimated from the first weight measurement

413 of the experiment, after the pots were irrigated at saturation.  $W$  and  $W_{sat}$  were computed  
414 by removing the mass of the tree and the pot to the total weight measured during the  
415 experiment (either from the balance or continuous load cell measurements). The total tree  
416 fresh was measured at the end of the experiment, by assuming that tree growth that could  
417 have occur during the experiment can be neglected due to the extreme drought  
418 experienced by the tree.  $W_{norm}$  was not measured on the control (irrigated) pots.

419

### 420 **Soil resistivity measurement**

421 Electrical resistivity of soil in pots was measured using electrical resistivity tomography  
422 (ERT). 4 pots (including one control) per modality (monoculture or mixture, with or  
423 without root separation system) were selected. On these pots, electrical resistivity was  
424 monitored with time over 2 radial planes, located at 1/3 and 2/3 of the pots' height, by  
425 inserting 20 stainless steel screws (2cm long) equally spaced (3.9cm) along the column's  
426 circumference. ERT measurement were done using an ABEM SAS 4000 resistivity meter  
427 connected to all these electrodes. All quadrupole combinations were used, including  
428 reciprocal measurements for assessing error and measurement quality. The resistivity  
429 measurements were taken before the start of the experiment (when the pot substrates  
430 were at field capacity), in the middle and at the end of the experiment. In the late dry  
431 situations, it was necessary to add a small amount of water at electrodes to enable soil-  
432 electrode electrical contact and resistivity measurements. Soil resistivity distribution at  
433 the two heights was obtained from the inversion of apparent resistivity using ResIPy  
434 software (Blanchy et al., 2020).

435

### 436 **Statistics**

437 We evaluated globally the effect of species and measurement date and their interaction on  
438 the water potential of trees by using a linear mixed model. Then, for each species  
439 independently and root separation modalities (root separation or not), we assessed the  
440 effect of the pot composition (mixture or monoculture association) on tree water  
441 potentials by considering date, composition and their interaction as explanatory factors.  
442 As we did not find any significant differences between water potentials of monoculture  
443 with and without root separation for each species (SI appendix, Fig. S6), we decided to  
444 pool them for the analysis. We also test the differences between soil and water potentials  
445 of tree at each measurement dates using Student T test. Finally, we applied post-hoc  
446 Tuckey HSD tests to evaluate differences between pots modalities (composition and root  
447 separation modalities) for leaf area and gas exchanges variables (leaf conductance and  
448 transpiration, Fig. S7). All statistical analyses were performed with the R software (3,5,2,  
449 R Development Core Team 2018) with the package LME4 and *agricolae*.

450

### 451 **Model analysis using SurEau**

452 We used the SurEau model to explore how the species composition in pots influenced  
453 soil and water potential dynamics during extreme drought (Cochard et al., 2021). SurEau  
454 is a soil-tree-atmosphere model that simulates water fluxes and stocks with the soil-tree  
455 atmosphere continuum by accounting for conductance, water potential gradient and

456 capacitances. It is dedicated to model extreme drought and accounts for the processes  
457 occurring after the point of stomatal closure (*i.e.*, cuticular water losses and hydraulic  
458 conductance and water stocks losses due to xylem embolism). It is discretized in the soil  
459 layers and four tree compartments (roots, trunk, branches, and leaves) which are each  
460 described by an apoplasmic and a symplasmic water volume. At each time step, the  
461 model computes leaf stomatal and cuticular transpiration as the product between leaf-to-  
462 air vapor pressure deficit and stomatal and cuticular conductance. Knowing the soil water  
463 content, soil water potential and hydraulic conductance are computed. This along with  
464 leaf stomatal and cuticular fluxes, can be used to compute tree water potential in the  
465 different tree compartments while accounting for the symplasmic capacitance and the  
466 hydraulic conductance losses due to xylem embolism. The resulting water potential is  
467 used to compute stomatal closure, water content, and xylem embolism. The model is  
468 driven by hourly climate data, tree traits (water pools dimensions, stomatal response to  
469 water potential, cuticular transpiration, capacitance, and vulnerability curve to cavitation  
470 (SI appendix, Table S4) and soil properties (volume and water retention curves). In the  
471 present study, the model was improved to include the possibility for two trees to absorb  
472 water in the same soil volume. In principle, two codes corresponding to two trees,  
473 parameterized for monoculture of *P. halepensis*, monoculture of *Q. ilex* or for mixture,  
474 were run in parallel.

475 To test the hypotheses presented in the introduction (illustrated in Fig. 1) and evaluate  
476 whether the model can also explain the patterns of soil and water potential dynamics  
477 found experimentally. First, we performed *benchmark* simulations, for monoculture and  
478 mixture, with default parameters, to reproduce the hypotheses presented in Figure 1. As  
479 explained above, the patterns of Figure 1 hold only under the assumptions that (i) the two  
480 individuals in the pots exploit the same soil water stock (physical coexistence of the  
481 roots) and are perfectly connected to the soil (large hydraulic conductance between the  
482 soil and the fine roots). In SurEau the flow of water between the soil and the roots is  
483 modelled using two different types of conductance in series, (i) the hydraulic  
484 conductance between the soil and the root surface ( $K_{\text{soil}}$ ) which depends on fine roots  
485 density and the soil water content (Martin-StPaul et al., 2017), and (ii) the hydraulic  
486 conductance between the root surface and the inner root ( $K_{\text{root}}$ ) which depends on the fine  
487 roots area ( $R_a$ ) and fine roots conductivity ( $K_{\text{root}}$ ), both set constant by default (Cochard  
488 et al., 2021). The first hypothesis (root occupy the same volume) was fulfilled by setting  
489 the same quantity of fine roots in all three soil layers for the two individuals in the pot.  
490 The second hypothesis (equilibration between tree and soil water potential) was fulfilled  
491 by setting a fine roots length so that the soil hydraulic conductance is high enough for the  
492 night-time water potential equalled the soil water potential all along the drought range  
493 and before hydraulic failure (where water potential drops to minus infinity).

494 However, two different empirical results conflicted the expected patterns and we used the  
495 model to explain the divergences observed.

496 Firstly, *P. halepensis* showed that water potential can be higher than soil water potential  
497 during extreme drought under all composition monoculture and mixture, suggesting that  
498 this species can behave independently from the soil and maintain its water potential  
499 constant even if soil water potential decreases. Recent work suggests that disconductance  
500 between the soil and root can occur for some species (Cuneo et al., 2016; Duddek et al.,

501 2022; North and Nobel, 1997). This can be represented in the model by decreasing the  
502 root conductance relative to water potential in the root. We thus implemented an equation  
503 relating the root hydraulic conductance to the root water potential (SI appendix, Fig. S2)  
504 for *P. halepensis* and realized simulation in mixture conditions with *Q. ilex* parametrized  
505 as in benchmark conditions. It appeared that this implementation led to an acceleration of  
506 hydraulic failure for *P. halepensis*. This is explained by the excessive water losses, that  
507 occur through the cuticle at this stage of water stress, and that cannot anymore be  
508 compensated by the supply from the root. Under the same mixture conditions, we  
509 therefore also tested whether a decrease in leaf cuticular conductance with relative water  
510 content (RWC, SI Appendix, Fig. S3), a phenomenon already observed on cut branches  
511 of *P. halepensis*, could explain -- alone or in combination with the reduction in root  
512 conductance -- the observed pattern.

513 Secondly, for *Q. ilex*, we noticed lower water stress under mixture linked to a change of  
514 the soil water potential ( $\Psi_{\text{soil}}$ ) vs plant predawn water potential ( $\Psi_{\text{pd}}$ ) relationship. Higher  
515 plant water potential for a given soil water potential was found under mixture compared  
516 to monoculture. Such pattern could be explained by an increase of the soil hydraulic  
517 conductance that, as explained above, can be related to the density of fine roots ( $L_a$ , the  
518 length of fine roots per m<sup>2</sup> of soil). We thereby performed a sensitivity analysis on this  
519 trait under monoculture conditions to support that its variation could explain the  
520 empirical observation.

521

## 522 **References**

523 Adams, H.D., Zeppel, M.J.B., Anderegg, W.R.L., Hartmann, H., Landhäusser, S.M.,  
524 Tissue, D.T., Huxman, T.E., Hudson, P.J., Franz, T.E., Allen, C.D., Anderegg,  
525 L.D.L., Barron-Gafford, G.A., Beerling, D.J., Breshears, D.D., Brodrigg, T.J.,  
526 Bugmann, H., Cobb, R.C., Collins, A.D., Dickman, L.T., Duan, H., Ewers, B.E.,  
527 Galiano, L., Galvez, D.A., Garcia-Forner, N., Gaylord, M.L., Germino, M.J.,  
528 Gessler, A., Hacke, U.G., Hakamada, R., Hector, A., Jenkins, M.W., Kane, J.M.,  
529 Kolb, T.E., Law, D.J., Lewis, J.D., Limousin, J.M., Love, D.M., Macalady, A.K.,  
530 Martínez-Vilalta, J., Mencuccini, M., Mitchell, P.J., Muss, J.D., O'Brien, M.J.,  
531 O'Grady, A.P., Pangle, R.E., Pinkard, E.A., Piper, F.I., Plaut, J.A., Pockman, W.T.,  
532 Quirk, J., Reinhardt, K., Ripullone, F., Ryan, M.G., Sala, A., Sevanto, S., Sperry,  
533 J.S., Vargas, R., Vennetier, M., Way, D.A., Xu, C., Yezzer, E.A., McDowell, N.G.,  
534 2017. A multi-species synthesis of physiological mechanisms in drought-induced  
535 tree mortality. *Nat Ecol Evol* 1, 1285–1291. <https://doi.org/10.1038/s41559-017-0248-x>

537 Aguadé, D., Poyatos, R., Rosas, T., Martínez-Vilalta, J., Aguadé, D., Poyatos, R., Rosas,  
538 T., Martínez-Vilalta, J., 2015. Comparative Drought Responses of *Quercus ilex* L.  
539 and *Pinus sylvestris* L. in a Montane Forest Undergoing a Vegetation Shift. *Forests*  
540 6, 2505–2529. <https://doi.org/10.3390/f6082505>

541 Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier,  
542 M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H. (Ted), Gonzalez, P.,

- 543 Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.H., Allard, G., Running,  
544 S.W., Semerci, A., Cobb, N., 2010. A global overview of drought and heat-induced  
545 tree mortality reveals emerging climate change risks for forests. *For Ecol Manage*  
546 259, 660–684. <https://doi.org/10.1016/j.foreco.2009.09.001>
- 547 Anderegg, W.R.L., Konings, A.G., Trugman, A.T., Yu, K., Bowling, D.R., Gabbitas, R.,  
548 Karp, D.S., Pacala, S., Sperry, J.S., Sulman, B.N., Zenes, N., 2018. Hydraulic  
549 diversity of forests regulates ecosystem resilience during drought. *Nature* 561, 538–  
550 541. <https://doi.org/10.1038/s41586-018-0539-7>
- 551 Baquedano, F.J., Castillo, F.J., 2007. Drought tolerance in the Mediterranean species  
552 *Quercus coccifera*, *Quercus ilex*, *Pinus halepensis*, and *Juniperus phoenicea*.  
553 *Photosynthetica* 45. <https://doi.org/10.1007/s11099-007-0037-x>
- 554 Bello, J., Hasselquist, N.J., Vallet, P., Kahmen, A., Perot, T., Korboulewsky, N., 2019.  
555 Complementary water uptake depth of *Quercus petraea* and *Pinus sylvestris* in  
556 mixed stands during an extreme drought. *Plant Soil* 437.  
557 <https://doi.org/10.1007/s11104-019-03951-z>
- 558 Billon, L.M., Blackman, C.J., Cochard, H., Badel, E., Hitmi, A., Cartailier, J., Souchal,  
559 R., Torres-Ruiz, J.M., 2020. The DroughtBox: A new tool for phenotyping residual  
560 branch conductance and its temperature dependence during drought. *Plant Cell*  
561 *Environ* 43, 1584–1594. <https://doi.org/10.1111/PCE.13750>
- 562 Blanchy, G., Saneiyan, S., Boyd, J., McLachlan, P., Binley, A., 2020. ResIPy, an intuitive  
563 open source software for complex geoelectrical inversion/modeling. *Comput Geosci*  
564 137. <https://doi.org/10.1016/j.cageo.2020.104423>
- 565 Breshears, D.D., Adams, H.D., Eamus, D., Mcdowell, N.G., Law, D.J., Will, R.E.,  
566 Williams, A.P., Zou, C.B., 2013. The critical amplifying role of increasing  
567 atmospheric moisture demand on tree mortality and associated regional die-off.  
568 *Front Plant Sci*. <https://doi.org/10.3389/fpls.2013.00266>
- 569 Brito, C., Dinis, L.-T., Moutinho-Pereira, J., Correia, C.M., 2019. Drought Stress Effects  
570 and Olive Tree Acclimation under a Changing Climate. *Plants (Basel)* 8.  
571 <https://doi.org/10.3390/plants8070232>
- 572 Chen, Z., Zhang, Y., Yuan, W., Zhu, S., Pan, R., Wan, X., Liu, S., 2021. Coordinated  
573 variation in stem and leaf functional traits of temperate broadleaf tree species in the  
574 isohydric-anisohydric spectrum. *Tree Physiol* 41.  
575 <https://doi.org/10.1093/treephys/tpab028>
- 576 Cochard, H., Pimont, F., Ruffault, J., Martin-StPaul, N., 2021. SurEau: a mechanistic  
577 model of plant water relations under extreme drought. *Ann For Sci* 78, 1–23.  
578 <https://doi.org/10.1007/S13595-021-01067-Y/TABLES/7>
- 579 Cuneo, I.F., Barrios-Masias, F., Knipfer, T., Uretsky, J., Reyes, C., Lenain, P., Brodersen,  
580 C.R., Walker, M.A., McElrone, A.J., 2021. Differences in grapevine rootstock

- 581 sensitivity and recovery from drought are linked to fine root cortical lacunae and  
582 root tip function. *New Phytologist* 229. <https://doi.org/10.1111/nph.16542>
- 583 Cuneo, I.F., Knipfer, T., Brodersen, C.R., McElrone, A.J., 2016. Mechanical failure of  
584 fine root cortical cells initiates plant hydraulic decline during drought. *Plant Physiol*  
585 172. <https://doi.org/10.1104/pp.16.00923>
- 586 Dane, J.H., Hopmans, J.W., 2002. *Methods of Soil Analysis, Part 4, Physical Methods*,  
587 in: *Soil Science Society of America Book Series*. Madison.
- 588 de-Dios-García, J., Pardos, M., Calama, R., 2015. Interannual variability in competitive  
589 effects in mixed and monospecific forests of Mediterranean stone pine. *For Ecol*  
590 *Manage* 358, 230–239. <https://doi.org/10.1016/J.FORECO.2015.09.014>
- 591 Delzon, S., 2015. New insight into leaf drought tolerance. *Funct Ecol* 29, 1247–1249.  
592 <https://doi.org/10.1111/1365-2435.12500>
- 593 Domec, J.C., King, J.S., Carmichael, M.J., Overby, A.T., Wortemann, R., Smith, W.K.,  
594 Miao, G., Noormets, A., Johnson, D.M., 2021. Aquaporins, and not changes in root  
595 structure, provide new insights into physiological responses to drought, flooding,  
596 and salinity. *J Exp Bot* 72. <https://doi.org/10.1093/jxb/erab100>
- 597 Duddek, P., Carminati, A., Koebnick, N., Ohmann, L., Lovric, G., Delzon, S.,  
598 Rodriguez-Dominguez, C.M., King, A., Ahmed, M.A., 2022. The impact of  
599 drought-induced root and root hair shrinkage on root–soil contact. *Plant Physiol*.  
600 <https://doi.org/10.1093/plphys/kiac144>
- 601 Forrester, D.I., Bausch, J., 2016. A Review of Processes Behind Diversity—Productivity  
602 Relationships in Forests. *Current Forestry Reports* 2, 45–61.  
603 <https://doi.org/10.1007/S40725-016-0031-2/FIGURES/3>
- 604 Gardner, W.R., 1964. Relation of Root Distribution to Water Uptake and Availability 1 .  
605 *Agron J* 56. <https://doi.org/10.2134/agronj1964.00021962005600010013x>
- 606 Grossiord, C., 2020. Having the right neighbors: how tree species diversity modulates  
607 drought impacts on forests. *New Phytologist* 228, 42–49.  
608 <https://doi.org/10.1111/NPH.15667>
- 609 Grossiord, C., Gessler, A., Granier, A., Pollastrini, M., Bussotti, F., Bonal, D., 2014.  
610 Interspecific competition influences the response of oak transpiration to increasing  
611 drought stress in a mixed Mediterranean forest. *For Ecol Manage* 318, 54–61.  
612 <https://doi.org/10.1016/j.foreco.2014.01.004>
- 613 Grossiord, C., Sevanto, S., Bonal, D., Borrego, I., Dawson, T.E., Ryan, M., Wang, W.,  
614 McDowell, N.G., 2018. Prolonged warming and drought modify belowground  
615 interactions for water among coexisting plants. *Tree Physiol* 39.  
616 <https://doi.org/10.1093/treephys/tpy080>
- 617 Grossiord, Granier, A., Ratcliffe, S., Bouriaud, O., Bruelheide, H., Chečko, E., Forrester,  
618 D.I., Dawud, S.M., Finér, L., Pollastrini, M., Scherer-Lorenzen, M., Valladares, F.,

- 619 Bonal, D., Gessler, A., 2014. Tree diversity does not always improve resistance of  
620 forest ecosystems to drought. *Proceedings of the National Academy of Sciences*  
621 111, 14812–14815. <https://doi.org/10.1073/pnas.1411970111>
- 622 Haberstroh, S., Werner, C., 2022. The role of species interactions for forest resilience to  
623 drought. *Plant Biol.* <https://doi.org/10.1111/PLB.13415>
- 624 I. R. Cowan, 1965. Transport of Water in the Soil-Plant-Atmosphere System. *Journal of*  
625 *Applied Ecology* 2, 221–239.
- 626 Jose, S., Williams, R., Zamora, D., 2006. Belowground ecological interactions in mixed-  
627 species forest plantations. *For Ecol Manage* 233.  
628 <https://doi.org/10.1016/j.foreco.2006.05.014>
- 629 Lebourgeois, F., Gomez, N., Pinto, P., Mérian, P., 2013. Mixed stands reduce *Abies alba*  
630 tree-ring sensitivity to summer drought in the Vosges mountains, western Europe.  
631 *For Ecol Manage* 303, 61–71. <https://doi.org/10.1016/J.FORECO.2013.04.003>
- 632 Leonova, A., Heger, A., Vásconez Navas, L.K., Jensen, K., Reisdorff, C., 2022. Fine root  
633 mortality under severe drought reflects different root distribution of *Quercus robur*  
634 and *Ulmus laevis* trees in hardwood floodplain forests. *Trees - Structure and*  
635 *Function* 36. <https://doi.org/10.1007/s00468-022-02275-3>
- 636 López, R., Cano, F.J., Martin-StPaul, N.K., Cochard, H., Choat, B., 2021. Coordination  
637 of stem and leaf traits define different strategies to regulate water loss and tolerance  
638 ranges to aridity. *New Phytologist* 230. <https://doi.org/10.1111/nph.17185>
- 639 Martin-StPaul, N., Delzon, S., Cochard, H., 2017. Plant resistance to drought depends on  
640 timely stomatal closure. *Ecol Lett* 20, 1437–1447.
- 641 Merlin, M., Perot, T., Perret, S., Korboulewsky, N., Vallet, P., 2015. Effects of stand  
642 composition and tree size on resistance and resilience to drought in sessile oak and  
643 Scots pine. *For Ecol Manage* 339, 22–33.  
644 <https://doi.org/10.1016/J.FORECO.2014.11.032>
- 645 Messier, C., Bauhus, J., Sousa-Silva, R., Auge, H., Baeten, L., Barsoum, N., Bruelheide,  
646 H., Caldwell, B., Cavender-Bares, J., Dhiedt, E., Eisenhauer, N., Ganade, G.,  
647 Gravel, D., Guillemot, J., Hall, J.S., Hector, A., Hérault, B., Jactel, H., Koricheva, J.,  
648 Kreft, H., Mereu, S., Muys, B., Nock, C.A., Paquette, A., Parker, J.D., Perring,  
649 M.P., Ponette, Q., Potvin, C., Reich, P.B., Scherer-Lorenzen, M., Schnabel, F.,  
650 Verheyen, K., Weih, M., Wollni, M., Zemp, D.C., 2022. For the sake of resilience  
651 and multifunctionality, let's diversify planted forests! *Conserv Lett* 15.  
652 <https://doi.org/10.1111/conl.12829>
- 653 Michael, T.-M., Parker, W., 2000. Estimating biomass of white spruce seedlings with  
654 vertical photo imagery. *New For (Dordr)* 145–162.  
655 <https://doi.org/10.1023/A:1006716406751>

- 656 Moreno, M., Simioni, G., Cailleret, M., Ruffault, J., Badel, E., Carrière, S., Davi, H.,  
657 Gavinet, J., Huc, R., Limousin, J.-M.M., Marloie, O., Martin, L., Rodríguez-  
658 Calcerrada, J., Vennetier, M., Martin-StPaul, N., 2021. Consistently lower sap  
659 velocity and growth over nine years of rainfall exclusion in a Mediterranean mixed  
660 pine-oak forest. *Agric For Meteorol* 308–309, 108472.
- 661 North, G.B., Nobel, P.S., 1997. Drought-induced changes in soil contact and hydraulic  
662 conductivity for roots of *Opuntia ficus-indica* with and without rhizosheaths. *Plant*  
663 *Soil* 191. <https://doi.org/10.1023/A:1004213728734>
- 664 Pangle, R.E., Hill, J.P., Plaut, J.A., Yepez, E.A., Elliot, J.R., Gehres, N., McDowell,  
665 N.G., Pockman, W.T., 2012. Methodology and performance of a rainfall  
666 manipulation experiment in a piñon–juniper woodland. *Ecosphere* 3, art28.  
667 <https://doi.org/10.1890/ES11-00369.1>
- 668 Plaut, J.A., YEPEZ, E.A., Hill, J., Pangle, R., SPERRY, J.S., Pockman, W.T., Mcdowell,  
669 N.G., Ssperry, J.S., Pockman, W.T., Mcdowell, N.G., 2012. Hydraulic limits  
670 preceding mortality in a piñon-juniper woodland under experimental drought. *Plant*  
671 *Cell Environ* 35, 1601–1617. <https://doi.org/10.1111/j.1365-3040.2012.02512.x>
- 672 Rodriguez-Dominguez, C.M., Forner, A., Martorell, S., Choat, B., Lopez, R., Peters,  
673 J.M.R., Pfautsch, S., Mayr, S., Carins-Murphy, M.R., McAdam, S.A.M.,  
674 Richardson, F., Diaz-Espejo, A., Hernandez-Santana, V., Menezes-Silva, P.E.,  
675 Torres-Ruiz, J.M., Batz, T.A., Sack, L., 2022. Leaf water potential measurements  
676 using the pressure chamber: Synthetic testing of assumptions towards best practices  
677 for precision and accuracy. *Plant Cell Environ* 45, 2037–2061.  
678 <https://doi.org/10.1111/PCE.14330>
- 679 Ruiz-Benito, P., Ratcliffe, S., Jump, A.S., Gómez-Aparicio, L., Madrigal-González, J.,  
680 Wirth, C., Kändler, G., Lehtonen, A., Dahlgren, J., Kattge, J., Zavala, M.A., 2017.  
681 Functional diversity underlies demographic responses to environmental variation in  
682 European forests. *Global Ecology and Biogeography* 26, 128–141.  
683 <https://doi.org/10.1111/GEB.12515>
- 684 Schnabel, F., Liu, X., Kunz, M., Barry, K.E., Bongers, F.J., Bruelheide, H., Fichtner, A.,  
685 Härdtle, W., Li, S., Pfaff, C.-T., Schmid, B., Schwarz, J.A., Tang, Z., Yang, B.,  
686 Bauhus, J., von Oheimb, G., Ma, K., Wirth, C., 2021. Species richness stabilizes  
687 productivity via asynchrony and drought-tolerance diversity in a large-scale tree  
688 biodiversity experiment. *Sci Adv* 7, 11–13. <https://doi.org/10.1126/sciadv.abk1643>
- 689 Senf, C., Buras, A., Zang, C.S., Rammig, A., Seidl, R., 2020. Excess forest mortality is  
690 consistently linked to drought across Europe. *Nat Commun* 11, 6200.  
691 <https://doi.org/10.1038/s41467-020-19924-1>
- 692 Sun, Z., Liu, X., Schmid, B., Bruelheide, H., Bu, W., Ma, K., 2017. Positive effects of  
693 tree species richness on fine-root production in a subtropical forest in SE-China.  
694 *Journal of Plant Ecology* 10. <https://doi.org/10.1093/jpe/rtw094>

695 Tyree, M.T., Sperry, J.S., 1989. Vulnerability of Xylem to Cavitation and Embolism.  
696 *Annu Rev Plant Physiol Plant Mol Biol* 40, 19–36.  
697 <https://doi.org/10.1146/annurev.pp.40.060189.000315>

698 van Genuchten, M.Th., 1980. A Closed-form Equation for Predicting the Hydraulic  
699 Conductivity of Unsaturated Soils. *Soil Science Society of America Journal* 44,  
700 892–898. <https://doi.org/10.2136/SSSAJ1980.03615995004400050002X>

701 Vitali, V., Forrester, D.I., Bahuus, J., 2018. Know Your Neighbours: Drought Response  
702 of Norway Spruce, Silver Fir and Douglas Fir in Mixed Forests Depends on Species  
703 Identity and Diversity of Tree Neighbourhoods. *Ecosystems* 21:6 21, 1215–  
704 1229. <https://doi.org/10.1007/S10021-017-0214-0>

705 Wambsganss, J., Beyer, F., Freschet, G.T., Scherer-Lorenzen, M., Bahuus, J., 2021.  
706 Tree species mixing reduces biomass but increases length of absorptive fine  
707 roots in European forests. *Journal of Ecology* 109.  
708 <https://doi.org/10.1111/1365-2745.13675>

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## 716 **Author Contributions**

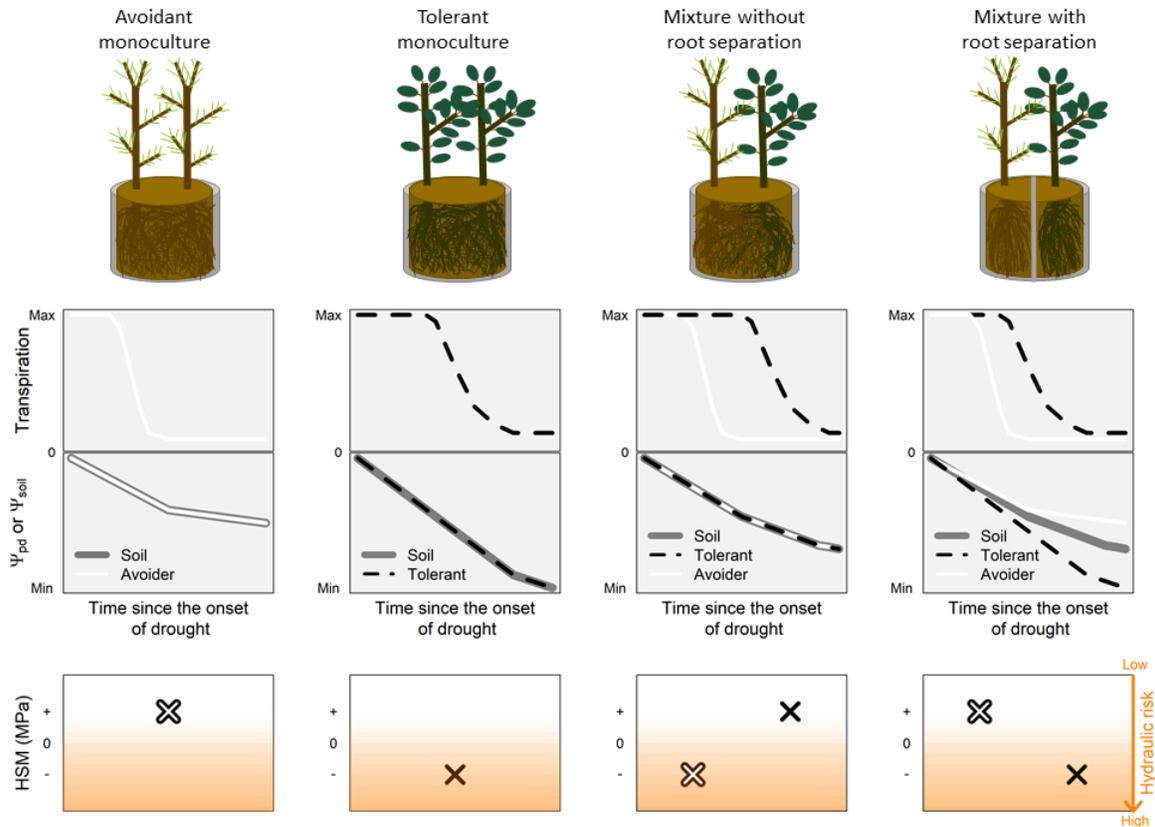
717 M.M., N.M. and H.C designed the research; M.M., G.S., N.M. C.D, P.F and R.D.  
718 performed research; H.C. and N.M. performed the model simulations; M.M analyzed the  
719 data, with the help of N.M, G.S, and CD; M.M and N.M wrote the paper, and all authors  
720 contributed to its review.

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## 722 **Competing Interest Statement**

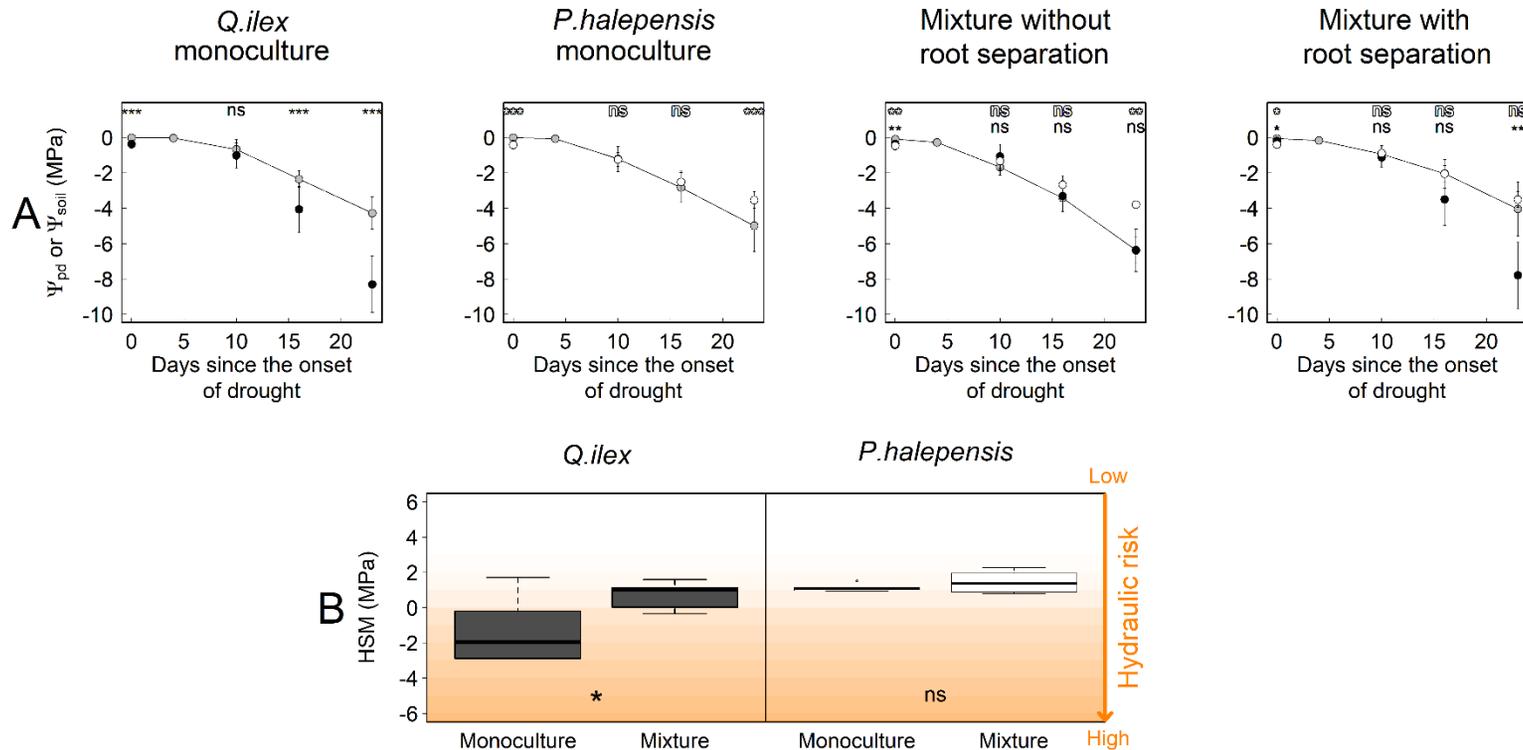
723 Authors declare no competing interest.

724 **Figures and Tables**  
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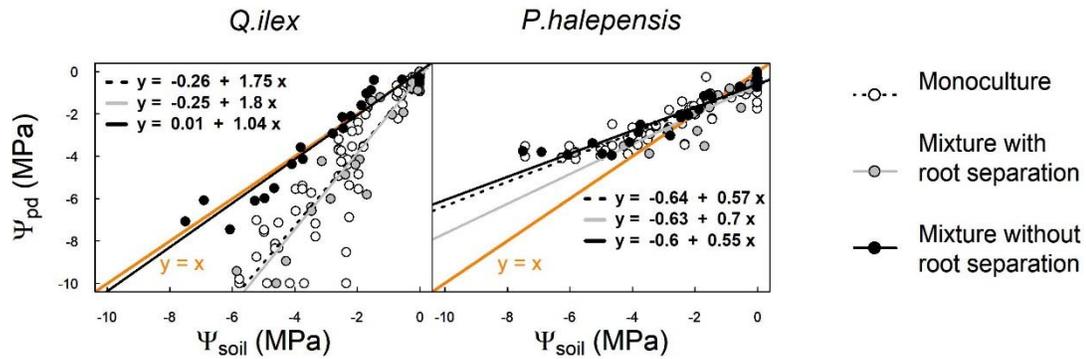


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728 **Figure 1.** Experimental design and hypothesized drought responses for monoculture and  
729 mixture of a drought avoidant and a drought tolerant species. The transpiration, water  
730 potentials ( $\Psi_{\text{soil}}$ : overall pot soil water potential;  $\Psi_{\text{pd}}$ : tree water potential) and hydraulic  
731 safety margins (ie. HSM: the difference between  $\Psi_{\text{pd}}$  and P50; the water potential causing  
732 50 % of embolism) expected according to pot modalities are presented. In the drought  
733 avoidant monoculture, trees transpiration are expected to reduce rapidly after the onset of  
734 drought, limiting  $\Psi_{\text{soil}}$  and  $\Psi_{\text{pd}}$  drop and hence hydraulic failure risk (positive HSM). In  
735 the drought tolerant monoculture, transpiration of the two trees is expected to reduce later  
736 than the one of the drought avoidant species, inducing a sharp decrease of  $\Psi_{\text{soil}}$  and  $\Psi_{\text{pd}}$ ,  
737 increasing the risk of hydraulic failure (negative HSM). In the mixture without root  
738 separation, transpiration of the drought avoidant species decreases earlier than for the  
739 drought tolerant, which improve the water potential and HSM of the drought tolerant  
740 compared to monoculture. However, because the two trees share the same volume of soil,  
741 the water consumption of the drought tolerant should decrease water potential and HSM  
742 of the drought avoidant thereby increasing its hydraulic failure risk compared to the  
743 monoculture. A mixture with root separation illustrates that when each species root  
744 system occupies its proper soil volume, the regulation of the transpiration, the water  
745 potentials dynamics and the HSM are expected to be the same as in monoculture. As  $\Psi_{\text{soil}}$   
746 represents the global pot soil water potential, it is here equal to the mean of both  
747 compartment soil water potential.



748 **Figure 2.** Positive and neutral effect of mixture on hydraulic failure risk of drought tolerant *Q. ilex* and drought avoidant *P.*  
 749 *halepensis*. (A) Soil and tree water potential for the different pot composition at each measurement dates. Soil water potentials  
 750 represent average values computed at the pot level from manual weightings (grey points). The average tree water potentials of *Q. ilex*  
 751 and *P. halepensis* correspond respectively to black and white dots. Standard deviations are represented and significant differences  
 752 between soil and water potentials are indicated (ns, non-significant differences; \*,  $0.01 \leq p < 0.05$ ; \*\*,  $0.001 \leq p < 0.01$ ; \*\*\*,  $p <$   
 753  $0.001$ ). Per measurement date, for  $\Psi_{pd}$ ,  $N = 24$  for monoculture (pooling monoculture with and without root separation/ two trees per  
 754 pots) and 6 for mixture. For  $\Psi_{soil}$ ,  $N = 12$  for monoculture (pooling monoculture with and without root separation) and 6 for mixtures  
 755 concerning soil water potentials. (B) Hydraulic safety margins (HSM) measured at the driest date of the experiment in monoculture  
 756 (with and without root separation) and mixture (only for pots designed without root separation). HSM were computed as the difference  
 757 between water potential at the driest date and the P50 (i.e., the water potential causing 50% embolism).



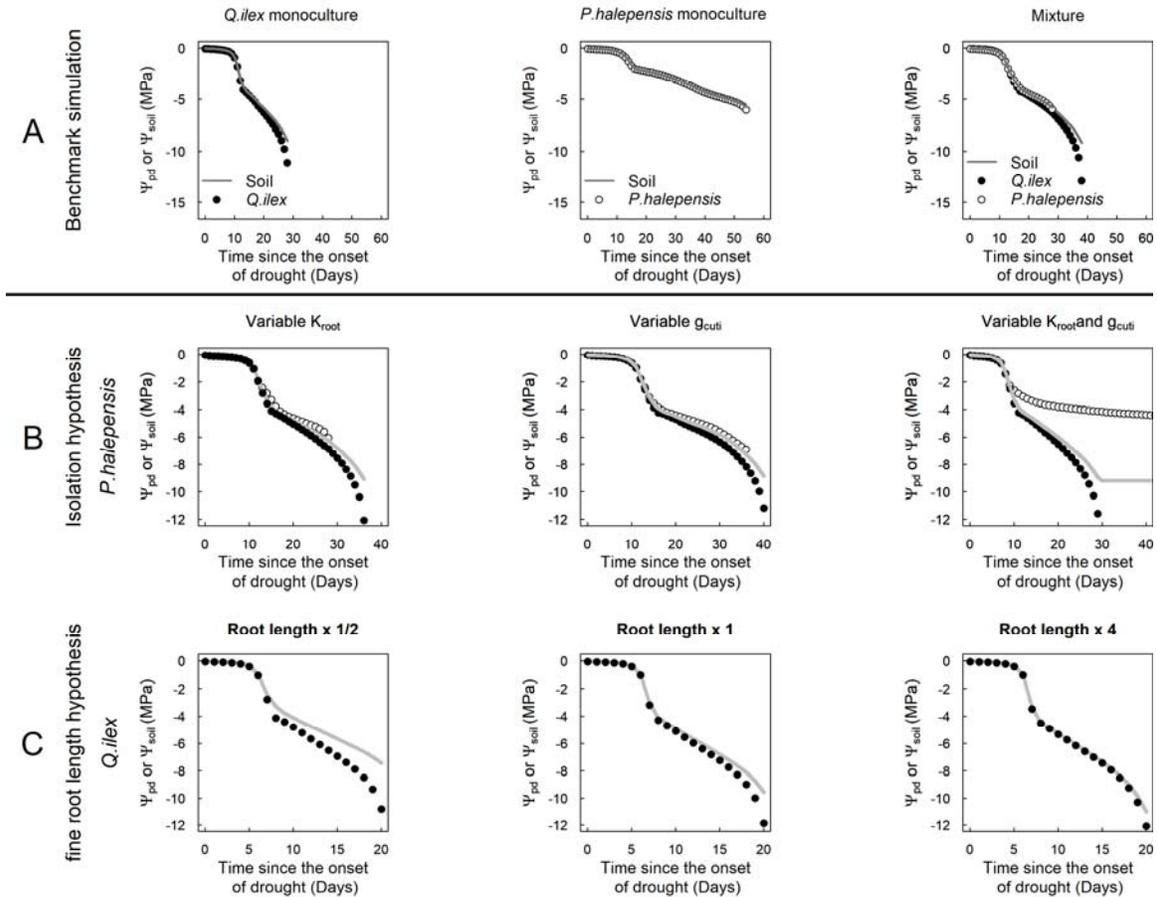
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761 **Figure 3.** Uncoupling between soil and tree water potentials suggest an improvement of  
762 the soil-tree hydraulic conductance for *Q. ilex* in mixture and soil isolation for *P.*  
763 *halepensis*. Different colours were used for monoculture (black dots), mixture with root  
764 separation (light grey dots) and mixture without root separation (dark grey dots). The  
765 isoline ( $y=x$ ) is reported in orange line. For each modality, linear fit between soil and  
766 water potentials is depicted and the equation is indicated on the plot.  $N = 96$  for  
767 monoculture (with and without root separation) and 24 for mixture for each root  
768 separation categories.

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**Figure 4.** Implication of the soil-tree hydraulic conductance in the coexistence of drought avoidant and drought tolerant species during extreme drought. (A) Benchmark SurEau simulations of the dynamics of leaf and soil water potentials for *P. halepensis* and *Q. ilex* grown in monoculture and mixture until tree death. In these simulations root hydraulic conductance and cuticular conductance were kept constant. The results are in adequation with H1 and H2 hypotheses postulated in the introduction (illustrated in Fig.1). (B) Test of sensibility of root conductance ( $K_{root}$ ) and leaf cuticular conductance ( $g_{cuti}$ ) parameters for *P. halepensis*. By reducing both parameters, trees can keep higher water potentials than the soil. (C) Test of sensibility of fine roots length parameter for *Q. ilex* (multiplying fine roots length by  $\frac{1}{2}$ , 1 and 4). The more the fine roots length, the closer are tree predawn and soil water potential. Note that the graduation of the x and y axis change according to plot. Model parameters are provided in the SI appendix, Table S4.