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► To cite this version:

Myriam Moreno, Guillaume Simioni, Maxime Cailleret, Julien Ruffault, Eric Badel, et al.. Consistently lower sap velocity and growth over nine years of rainfall exclusion in a Mediterranean mixed pine-oak forest. *Agricultural and Forest Meteorology*, 2021, 308-309, pp.108472. 10.1016/j.agrformet.2021.108472 . hal-03321323

HAL Id: hal-03321323

<https://hal.inrae.fr/hal-03321323v1>

Submitted on 22 Aug 2023

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1 Consistently lower sap velocity and growth over nine years of
2 rainfall exclusion in a Mediterranean mixed pine-oak forest

3

4 Myriam Moreno^{a,b,*}, Guillaume Simioni^a, Maxime Cailleret^c, Julien Ruffault^a, Eric Badel^d,
5 Simon Carrière^f, Hendrik Davi^a, Jordane Gavinet^e, Roland Huc^a, Jean-Marc Limousin^e,
6 Olivier Marloie^a, Ludovic Martin^d, Jesús Rodríguez-Calcerrada^g, Michel Vennetier^c, Nicolas
7 Martin-StPaul^a

8

9

10 ^a INRAE, URFM, Domaine Saint Paul, Centre de recherche PACA, 228 route de
11 l'Aérodrome, CS 40509, Domaine Saint-Paul, Site Agroparc, France.

12 ^b French Environment and Energy Management Agency 20, avenue du Grésillé- BP
13 90406 49004 Angers Cedex 01 France.

14 ^c INRAE, UMR RECOVER, University of Aix-Marseille, 13182 Aix-en-Provence, France.

15 ^d Université Clermont Auvergne, INRAE, PIAF, F-63000 Clermont-Ferrand, France.

16 ^e CEFÉ, Univ Montpellier, CNRS, EPHE, IRD, Univ. Paul Valéry Montpellier 3,
17 Montpellier, France.

18 ^f Sorbonne Univ., UPMC Univ Paris 06, CNRS, EPHE, UMR 7619 METIS, 4 place Jussieu,
19 75005 Paris, France.

20 ^g Departamento de Sistemas y Recursos Naturales, Escuela Técnica Superior de Ingeniería de
21 Montes, Forestal y del Medio Natural, Universidad Politécnica de Madrid. Ciudad
22 Universitaria s/n, 28040, Madrid, Spain.

23

24 * Corresponding author:

25 Email address: myriam.moreno@inrae.fr

26 **Abstract**

27 Mediterranean forests face an intensification of droughts caused by ongoing climate change.
28 To improve our understanding of tree and forest responses to increasing drought, we explored
29 over nine years, the effects of a 30% rainfall exclusion experiment on the water potential, sap
30 velocity and primary and secondary growth of two co-occurring species (*Quercus*
31 *ilex* and *Pinus halepensis*) in a French Mediterranean forest. In addition, native embolism was
32 measured after six and nine years of exclusion onset. Water potentials decreased earlier
33 during summer drought for both species in the rainfall exclusion plot, and to a higher extent
34 during the drought peak for *Q. ilex*, involving earlier stomatal closure and reduced sap
35 velocity. Sap velocities reduction persisted throughout the years in the exclusion plot. Outside
36 summer the water potential difference between predawn and midday was similar between
37 treatment which indicate that reduced water transport efficiency may be linked to decrease
38 hydraulic conductance. Such differences were neither related to differences in xylem
39 embolism, that remained similar between treatments, nor to change in secondary growth. In
40 contrast primary growth measurements indicate that *P. halepensis* trees, and *Q. ilex* to a lesser
41 extent, experienced reduction in total leaf areas in response to the rainfall exclusion. Globally,
42 our results suggest that increase drought lead, for both species, to a decrease in sap velocity
43 not mediated by increase embolism but rather by a reduction in primary growth. So far little
44 conclusion can be drawn regarding the competitive advantage of one species over the other in
45 the context of increasing drought related to climate change.

46

47 *Keywords:*

48 Throughfall exclusion, acclimation, embolism, plant hydraulics, diversity, electrical resistivity
49 tomography.

50 **1. Introduction**

51 Forests cover almost a third of the world's land surface and are involved in most
52 biogeochemical processes, the water cycle and the earth's energy balance. In particular, forest
53 trees constitute a net carbon sink and thus act as a buffer against the anthropogenic
54 accumulation of CO₂ in the atmosphere, storing about 25 % of annual global CO₂ emissions
55 (Friedlingstein et al., 2019). However, global warming has already altered and will continue
56 to affect how forests function, in particular because of the increased frequency and severity of
57 droughts in many regions of the world (Dai, 2012). This effect could be especially strong in
58 the Mediterranean basin, which has been identified as a potential "climate change hotspot"
59 (Diffenbaugh & Giorgi, 2012), due to larger increases in temperature (Cramer et al., 2018)
60 and larger decreases in rainfall compared with the global mean, especially during the summer
61 (Lionello & Scarascia, 2018). Mediterranean forests are thus at the forefront of climate
62 changes (FAO and Plan Bleu, 2018) and constitute a key natural laboratory to explore future
63 effects of drought on forest and tree functions.

64 During a drought, soil and tree water potentials drop because of soil water depletion, which
65 leads to a cascade of physiological reactions (Breda et al., 2006; Choat et al., 2018;
66 McDowell, 2011). Growth decreases rapidly as a result of reduced turgor and meristem
67 activity at relatively high water potential (Lempereur et al., 2015; Muller et al., 2011).
68 Stomata closure then limits the transpiration rate, and thereby the plant water potential
69 decline, but at the detriment of photosynthetic activity (Flexas et al., 2004). If the drought is
70 prolonged and increases in intensity, two phenomena can occur that alter plant function and
71 impair plant vitality (N. McDowell et al., 2008; Nathan G. McDowell, 2011). First, an
72 imbalance between the supply and demand of non-structural carbohydrates can lead to
73 "carbon starvation", leaving the plant unable to maintain its metabolism and/or to defend

74 against pathogens. Second, a catastrophically low water potential can trigger “hydraulic
75 failure”, leading to xylem embolism followed by tissue desiccation and ultimately plant death
76 (Adams et al., 2017; N. Martin-StPaul et al., 2017). However, in spite of the relevance of
77 these processes in drought induced tree death, this simplified view has emerged mainly from
78 short-term greenhouse experiments on seedlings and saplings that are difficult to transfer to
79 more complex, heterogeneous natural conditions and longer timescales (Kawaletz et al.,
80 2014). For instance, a tree’s ontogenetic development, size, and drought acclimation history
81 can modify its drought sensitivity over the long run (e.g., Martín-Benito et al., 2008). In
82 addition, the differential drought responses of co-occurring species to multi-year drought and
83 disturbances, may lead to progressive changes in forest succession and dynamic (Nate G.
84 McDowell et al., 2020).

85 An approach to explore long term changes is via gradient studies, in which time is substituted
86 by space (Klein et al., 2013; Martin-Stpaul et al., 2012). However confounding factors (e.g.,
87 differences in soil properties or in stand structure and density between sites) can make
88 comparisons difficult. Retrospective approaches based on tree-ring width or isotopes can also
89 be used to explore long-term drought responses (Gessler et al., 2018; Pellizzari et al., 2016;
90 Timofeeva et al., 2017), but the dependence of these metrics on several physiological
91 mechanisms precludes a comprehensive view of tree drought responses. In addition, some
92 external drivers of tree responses to drought, such as stand composition and density, are hard
93 to trace back and may bias the results of retrospective studies. Rainfall exclusion experiments
94 overcome some of the limitations of the gradient and retrospective studies and are a valuable
95 approach to study long-term drought responses (Beier et al., 2012; Benson et al., 2019; Song
96 et al., 2019)

97 Several plot-scale rainfall exclusion experiments have been conducted since the 2000s, with
98 most reporting a decrease in tree productivity (Wu et al., 2011). However, not all species

99 respond in the same way to drought intensification. For example, Ogaya and Penuelas (2007)
100 found that *Arbutus unedo* and *Quercus ilex* had lower stem diameter growth after a five-year
101 30% reduction in precipitation, but that *Phillyrea latifolia* did not. Moreover, the same species
102 can also respond differently to water stress depending on the site and the experiment duration.
103 Contrary to the previous study, Rodríguez-Calcerrada et al. (2011) did not find any reduction
104 in the radial increment of *Q. ilex* trees when these were subjected to a 27% reduction in
105 rainfall for six years, although they did observe a significant reduction in the crown leaf area.
106 Such species-, time- and site-specific responses highlight the need to monitor rainfall
107 exclusion effects on primary and secondary growth over long periods, and in co-occurring
108 species in different field experiments.

109 Rainfall exclusion systems used to experimentally increase drought have been found to
110 consistently alter plant water function. Trees exposed to rainfall reduction for more than a
111 year have shown reductions in sap flux ranging from 10 to 60% depending on the amount of
112 rainfall excluded (Fisher et al., 2007; Grossiord et al., 2018; Grossiord, Sevanto, Adams, et
113 al., 2017; Köhler et al., 2010; Limousin et al., 2009; Ward et al., 2015; Wullschleger &
114 Hanson, 2006; Zhang et al., 2018). Interestingly, transpiration reduction in rainfall exclusion
115 experiments tends to persist, even during well-watered periods. This persistent alteration of
116 tree transpiration may result from the combination of several mechanisms operating on
117 different timescales (Martin-StPaul et al., 2013). These include short-term stomatal regulation
118 of transpiration related to earlier summer water stress in drought treatments (Borghetti et al.,
119 1998), and modifications in the tree hydraulic system, which limit water transport on the
120 longer term, for example via a reduction in whole-tree hydraulic conductance (Limousin et
121 al., 2009; Pangle et al., 2015). These changes can be accompanied by changes in the leaf area
122 and the leaf to sapwood area ratio (Hudson et al., 2017).

123 In this study, we explored how long-term partial (30%) rainfall exclusion affects water
124 stress, growth and water transport in *Pinus halepensis* and *Quercus ilex*, two species that co-
125 occur in the mature mixed Mediterranean forest of Font-Blanche in France. These two forest
126 species are among the most drought-tolerant forest trees in Europe and dominate
127 Mediterranean forests (Delzon et al., 2010; Lobo et al., 2018). They often co-occur in post-
128 disturbed forests such as our study site, following a wildfire for instance. In such cases, seeder
129 *P. halepensis* trees form an upper canopy below which an understory of *Q. ilex* trees
130 develops. *P. halepensis* and *Q. ilex* react differently to drought, the former being more water-
131 saving (isohydric) than the latter, which is more anisohydric, with less tight transpiration
132 control (Baquedano & Castillo, 2006).

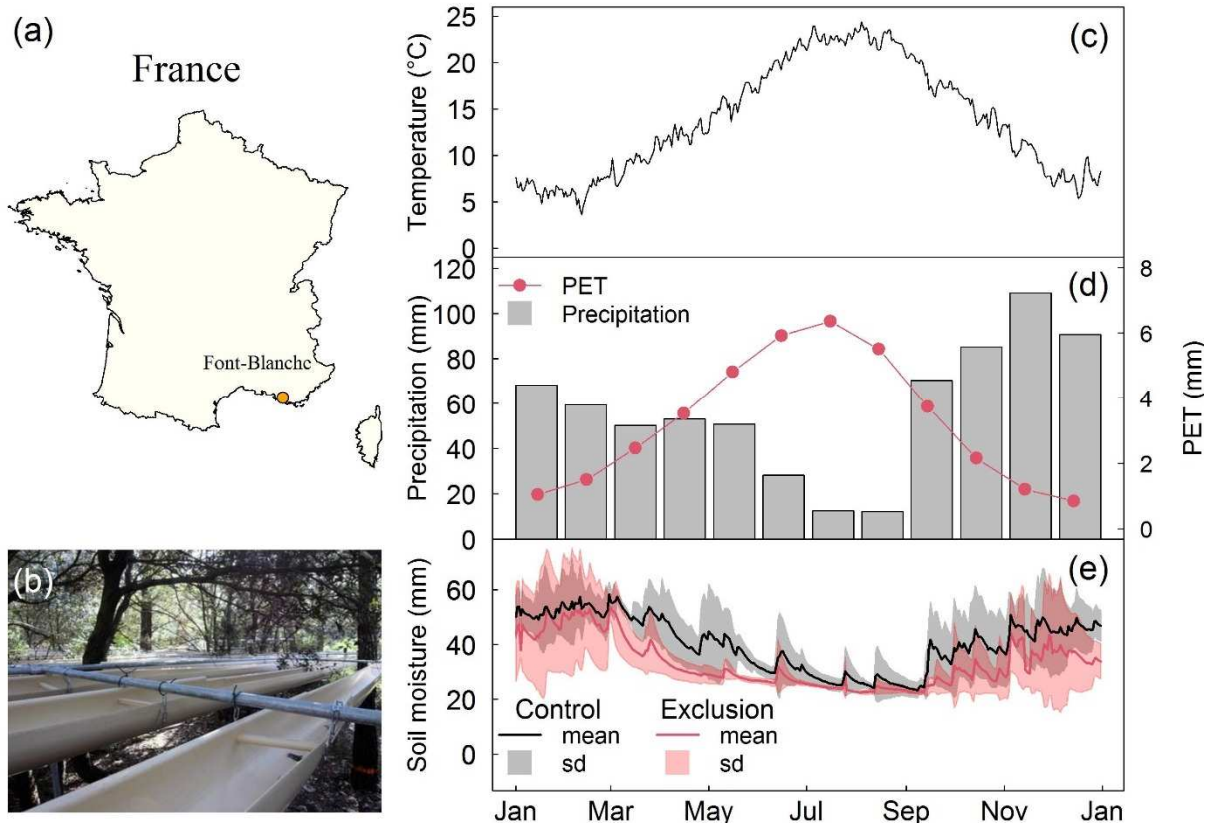
133 Based on measurements of sap velocity, leaf water potential, native xylem embolism,
134 plant area index, and primary and secondary growth (basal area increment and sapwood area
135 estimated through Electrical resistivity tomography measurements on trunks) carried out for
136 nine years, we tested how the two species would be affected by longer and more intense
137 summer droughts, which would be reflected by (i) a more negative water potential compared
138 with control conditions (with *Q. ilex* experiencing lower water potentials than *P. halepensis*,
139 because of the latter's tighter regulation of transpiration and water potential); (ii) reduced sap
140 velocities in response to earlier stomatal closure; (iii) reduced sap velocities in response to
141 reduced tree hydraulic conductance, because of increased summer cavitation and/or long-term
142 adjustments (i.e. a reduced leaf to sapwood area ratio); and (iv) reduced primary and
143 secondary growth and a lower overall stand leaf area index.

144 2. Materials and methods

145 2.1. Study site

146 The Font-Blanche long-term experimental monitoring site is located in a mixed
147 Mediterranean forest in south-eastern France (43°14'27''N, 5°40'45''E; altitude 425 m above
148 sea level) ca. 20 km east of Marseille (Fig. 1a). The experimental area covers 7650 m² and is
149 dominated by *P. halepensis* in the upper tree stratum (average height, 13 m) and *Q. ilex* in the
150 intermediate tree stratum (average height, 5 m). The understorey is composed mainly of five
151 species that do not exceed 4 m in height: *Phillyrea angustifolia*, *Quercus pubescens*, *Quercus*
152 *coccifera*, *Arbutus unedo* and *Pistacia terebinthus*. The climate is Mediterranean with hot,
153 dry summers. The mean annual temperature and cumulative precipitation over the study
154 period were 14°C and 701 mm, respectively, between 2008 and 2017. The bedrock is
155 karstified Cretaceous limestone with urgonian facies including rudists. The soil is a typical
156 thin and rocky rendzina with a maximum depth of around 50 cm, and a volumetric rock
157 fraction of about 50% at the top, and up to 90% at the bottom. Font-Blanche is part of the
158 Integrated Carbon Observation System (ICOS) and the Analysis and Experiments on
159 Ecosystems (AnaEE-France) networks. The site is equipped with a 17 m eddy-covariance flux
160 tower on top of which meteorological variables (including radiation, rainfall, temperature,
161 vapour pressure deficit), and carbon, water and energy fluxes are continuously monitored at
162 30 min intervals. Since January 2009, temperature and air relative humidity have been
163 monitored with an HMP45C probe (Vaisala) and precipitation with an automatic rain gauge
164 (R 3029 0.5 mm tipping bucket, Précis Mécanique). In July 2013, more precise sensors were
165 installed for air humidity and temperature (Vaisala HMP155), and rainfall (R 3029 0.2 mm
166 tipping bucket, Précis Mécanique). The soil water content estimated with automatic soil

167 moisture probes (Decagon EC-5 Volumetric Water Content sensors) within the first 50 cm of
 168 soil averages 50 mm (Fig. 1e), while the total soil water available for plants estimated from
 169 eddy covariance fluxes average 160 mm. The stone-free fine fraction of the soil is a
 170 homogeneous silty clay loam that contains around a third of the available water capacity. The



171 remaining extractable water is located in the bedrock, within fractures and clay pockets.

172

173 **Fig. 1.** Description of the Font-blanche site. (a) geographical location. (b) gutters used for rainfall exclusion. (c)
 174 mean daily temperature between 2008 and 2017. (d) mean monthly rainfall and potential evapotranspiration
 175 (PET) between 2008 and 2017 and (e) Mean and standard deviation of daily soil moisture measured with
 176 automatic soil moisture probes at depths of 5–50 cm in the control and rainfall exclusion plots between 2013 and
 177 2017.

178 In December 2008, a single rainfall exclusion plot was set up, covering an area of 25 × 25
 179 m. It was effective in January 2009 (Fig. 1b). Parallel PVC gutters were hung with a slight

180 slope approximately 2 m above the ground to cover ~30% of the ground area and thus reduce
181 the precipitation reaching the ground by approximately 30%. Two control plots with the same
182 surface area were also established at the same time. One was equipped with the same gutters
183 as in the rainfall exclusion plot but hung upside down so as not to intercept rainfall. This was
184 done to account for the potential effects of the gutters on the ecosystem albedo and
185 understorey microclimate conditions. The other control plot was left without gutters (the
186 characteristics of the plots are listed in Table S1). Statistical analysis showed no significant
187 difference between the two control plots in terms of sap velocity, water potentials, or stem
188 diameter increments, so the corresponding data were grouped together as a single control
189 treatment. To minimize border and spatial effects and maximize the rainfall exclusion effects,
190 monitored trees were selected in small area within each plot and in the centre of the plot for
191 the exclusion treatment.

192 *2.2. Soil moisture*

193 From 2008 to 2013, soil moisture was monitored every two weeks using time domain
194 reflectometry probes (Moisture.Point model MP-917). Five sensors positioned at soil depths
195 ranging from 5 to 55 cm were installed in each plot. In 2013, four additional soil profiles
196 (three in the control treatment plots and one in the rainfall exclusion plot) were monitored at
197 half-hour intervals using automatic probes (Decagon's EC-5 Volumetric Water Content
198 sensor). The four new profiles consisted of measurements from five sensors positioned at 5 to
199 50 cm depth. In each sampled layers of the corresponding soil pits, the stones were separated
200 from the soil and immersed in water in a container. Stone volume was estimated using the
201 variation of the water level. The total volume for that layer was measured by measuring the
202 amount of water needed to fill a waterproof bag lining the pit. Stones and soil were put back in
203 the pit, respecting the layer order. The volumetric water content measured by each sensor was

204 converted to millimetres after accounting for the proportion of stones in the corresponding
205 soil layer.

206 Given the high soil heterogeneity, the limited number of probes may not be sufficient to
207 yield representative soil moisture. Additionally, these measurements do not cover the full
208 depth explored by the roots. Actually, two thirds of the water holding capacity being
209 contained deeper under the bedrock. We thus used additional simulations of soil moisture
210 dynamic under control and exclusion treatment by using the forest stand water balance model
211 Biljou© (Granier et al., 1999). This model computes the different component of the water
212 balance at a daily time scale using a set of meteorological data (wind speed, relative humidity,
213 precipitation, radiation and temperature) as well as soil and stand characteristics (number of
214 layer and their soil bulk density, maximum extractable water and wilting point by layer,
215 Maximum LAI). All these required parameters were measured on the site (Table S2).
216 Simulations were made for both treatments. For the rainfall exclusion treatment, the daily
217 precipitation was reduced by 30% and the rest of the parameters, except LAI, were kept
218 constant. The relative extractable water calculated by the model was used in the study to
219 represent soil water dynamic in each treatment. The online platform was used to perform the
220 simulations (<https://appgeodb.nancy.inra.fr/biljou/>).

221 Strong relationships were found between predicted relative extractable water (REW) and
222 predawn water potential (Ψ_{pd}) (Fig. S1, $R^2 = 0.8$ for both species) giving confidence that the
223 model is relevant for estimating and comparing the available water of both treatments.

224 *2.3. Plant area index*

225 Plant area index was regularly monitored using hemispherical photographs taken every
226 one to two months. For the present study, we used only data taken during summer, at the peak
227 of foliage growth. Photos were taken at eight locations in each plot, roughly following a

228 regular 5 × 5 meters grid (i.e. the distance between photos could vary between 7 and 10 m
229 depending on the presence of stumps that precluded the installation of the camera). From
230 2008 to 2015, we used a Canon 5D camera equipped with a sigma 8 mm EX DG fisheye lens,
231 and from 2015 to 2018 a Nikon D3200 camera with a sigma 4.5 mm EX DC HSM fisheye
232 lens. The camera was positioned skywards and oriented so that the north would be at the top
233 of the picture. The camera was mounted 2 m above the ground on a self-levelling platform
234 (manufactured at INRAE-URFM, Avignon, France), to avoid any influence of the gutters or
235 of understorey vegetation. Unusually tall understorey plants proved nonetheless to be a
236 problem in some locations, and these were excluded from the analysis. The analysed data
237 come from 6 locations in the rainfall exclusion plot and 12 locations in the control plots.
238 Photos were taken when the sun was low (mostly at sunset) or, very rarely, when the sky was
239 uniformly overcast. They were taken in RAW format, and later converted with the software
240 DCRAW with no gamma correction. The images were processed and analysed using a set of
241 macros written for the ImageJ software (Schneider et al., 2012): extraction of the blue
242 channel, automatic contrast adjustment, manual double thresholding, and calculation of the
243 gap fraction and of the plant area index (PAI). Thresholding was carried out as described by
244 Leblanc et al. (2005) and the PAI was calculated using Miller's formula, as approximated by
245 Welles and Norman (1991).

246 *2.4. Primary growth*

247 Primary branch growth was monitored monthly from 2008 to 2010 for *Q. ilex*, and from
248 2008 to 2017 for *P. halepensis*. For *Q. ilex*, measurements were made on 9 and 12 trees in the
249 exclusion and control plots, respectively (on 38 and 62 branches, respectively). Branches were
250 analysed at two heights in the canopy (bottom and top canopy branches). Measurements
251 included the number of ramifications and the shoot length.

252 For *P. halepensis*, measurements were performed on 4 trees in the exclusion plot and on 7
253 trees in the control plot. In total, 481 branches of different vigour (assessed from annual shoot
254 length) (Girard et al., 2012), different architectural order (second and third), and from
255 different locations in the canopy (bottom, middle and top of the crown) were chosen to span
256 the range of possible growth rates within a tree. For each branch, we measured (i) the annual
257 shoot length, which is a good proxy for the number of needles carried by the shoot once the
258 presence of male flowers was accounted for (Fig. A1); (ii) the annual branching rate (i.e. the
259 number of branches per shoot length); and (iii) the mean annual needle length. As *P.*
260 *halepensis* is highly polycyclic (Girard et al., 2012), the annual needle length was calculated
261 by averaging the needle length of the different growth units weighted by the length of the
262 respective unit. On these branches, we also retrospectively calculated annual shoot lengths
263 and branching rates as far back as 2000 based on morphological markers, such as the size and
264 density of scales at the base of the growth units (Pardos et al., 2003).

265 *2.5. Secondary growth*

266 *2.5.1. Basal area increment*

267 Tree stem circumferences at breast height were measured in the rainfall exclusion and control
268 plots in 2007, 2009, and annually from 2011 onwards. However, because of the low growth
269 rates, especially of *Q. ilex*, we aggregated annual circumference increments over the entire
270 period since the start of rainfall exclusion (i.e. from 2009 to 2017) and converted them into
271 basal area increment (BAIs). To limit edge effects resulting from tree roots extending outside
272 the rainfall exclusion area, trees located less than 5 m from the border of the rainfall exclusion
273 plot were excluded from the analysis.

274 *2.5.2 Sapwood area /basal area ratio based on Electrical Resistivity Tomography of tree*
275 *trunk.*

276 Fifteen (7 *P. halepensis*, 8 *Q. ilex*) and ten (5 *P. halepensis*, 5 *Q. ilex*) healthy trees were
277 selected from the control and the rainfall exclusion plots respectively. Electrical resistivity
278 tomography (ERT) was performed using a commercial multichannel, multielectrode
279 resistivity system (PICUS TreeTronic 3, Argus Electronic gmbh, Rostock, Germany) during
280 February 2020. For each tree, depending on their circumference, between 8 and 24 nail probes
281 were hammered at breast height (130 cm) equally distributed around the trunk until they
282 reached the sapwood of the xylem. Nails were inserted counterclockwise and starting with a
283 northward orientation. Exact positions of nail probes and trees geometry were measured using
284 an electronic calliper (PICUS Calliper Version 3, Argus Electronic GmbH) connected via
285 Bluetooth to the PICUS Treetric 3 tomograph. For each ERT measurement, nail probes
286 were connected via electrodes to the resistivity system. The system automatically determined
287 the appropriate electric voltages to apply between all MPs, measured the intensity and
288 deduced the mean resistivity between the nails. Then, all data (geometry and ERT) were sent
289 to the TreeTronic expert software Q74 (Argus Electronic GmbH) on a laptop for a 2D

290 reconstruction step. The R script provided by Benson et al. (2019) under the supplementary
291 material was then used to build electrical resistivity tomographs (Fig. S2-S3) with a colour
292 scale ranging from blue (low resistivity) to red (high resistivity) and calculate sapwood depth
293 and area, considering that the heartwood-sapwood boundary is characterized by a threshold
294 resistivity value. This value is identified by the algorithm as the steepest change in resistivity
295 with depth in radials profiles (Guyot et al., 2013). To cross-validate sapwood estimation from
296 the algorithm, we compared resistivity profile with water content profiles for one *P.*
297 *halepensis* and one *Q. ilex* sampled outside the plots area. One sample for each of the two
298 trees were taken at DBH height after ERT profiles were measured. Water content profile (per
299 unit dry mass) was measured at a 1cm resolution on the core. The comparison of water
300 content and resistivity profiles indicate that the decline in water content match closely the
301 increase in resistivity (Fig. S4).

302 *2.6. Tree water potential*

303 Predawn and midday water potentials were measured every summer season from 2008 to
304 2017. Measurement dates varied between years from three to six field campaigns depending
305 on drought duration. They were always performed on days with clear sky and little wind.
306 Measurements were made on twigs from 4 to 6 trees per species and per plot using a
307 Scholander pressure chamber (manufactured at INRAE, Avignon, France). Trees were chosen
308 so as to cover the range of sizes present in each plot. To limit edge effects derived from roots
309 of border trees extending outside the rainfall exclusion area, only trees located more than 5 m
310 from the border of the rainfall exclusion plot were selected. Midday water potentials were
311 measured on well-lit, sun-exposed shoots. Samples were collected between midday and 2 pm
312 for midday potentials, and during the two hours preceding sunrise for predawn potentials.
313 Measurements were made on-site. Generally, one sample was measured per tree. In the very

314 few cases when a difference of more than 1 MPa was observed between trees in the same plot,
315 additional measurements were performed on the tree(s) with suspicious value(s). Sap flow
316 driving force ($\Delta\Psi$) was calculated as the difference between predawn and midday water
317 potentials.

318 2.7. Sap velocity

319 Sap velocity was measured in 24 trees from 2009 to 2016 using the thermal dissipation
320 method (Granier, 1988). These trees were the same ones whose water potentials were
321 monitored. Four *Q. ilex* and four *P. halepensis* trees were monitored in each of the three plots
322 (Table S3). Continuous measurements of sap velocity were performed with sensors
323 constructed at our laboratory, consisting of two probes (2 mm in diameter and 2 cm long),
324 each containing a thermocouple to measure the stem temperature. The two probes were
325 inserted radially into the sapwood of each tree at 1.50 m above the ground and 2 cm deep. The
326 probes were placed 10 cm apart and covered by an aluminium reflective foil to protect them
327 from radiation and rain.

328 According to sapwood length estimated with trunk ERT measurements, Granier probes
329 measured at least 50 % of the active sapwood water flow for both species. Based on Cohen et
330 al. (2008), sap velocities decreases with sapwood depth and is highest inner the first 2 cm of
331 sapwood for both species, where probes are located. This gives confidence that granier
332 sensors can detect shifts in sap velocity due to environmental factors.

333 The upper probe was heated constantly using a 0.12 A current. Reference measurements
334 of the sapwood temperature were taken from the lower probe. The difference in temperature
335 between the probes (ΔT) was recorded at 30 min intervals. Disfunctionning sensors were
336 replaced and the affected data were excluded. Data were also lost because of power failure
337 (animal predation, battery failure). Anomalies in ΔT fluctuations were visually identified by

338 overlaying the measurements with potential evapotranspiration (PET) data (calculated using
339 the Penman-Monteith equation). When the PET increases (during daytime), ΔT decreases
340 because of the cooling effect of sap flow on the probes.

341 The sap velocity (u , in $\text{m}\cdot\text{s}^{-1}$) was then calculated using Granier's (1988) empirical equation:

$$u = 119 \times K^{1.231}, \text{ with } K = (\Delta T_{\text{max}} - \Delta T) / \Delta T \quad (1)$$

342 where ΔT_{max} is the daily maximum temperature difference between the probes, that
343 represents the zero-sap velocity. It is supposed to occur night-time. Days with daytime
344 occurrence of ΔT_{max} were suppress from the analysis so that only night-time values were
345 used. In addition, to limit errors in the sap velocities due to the thermal resistivity of the wood
346 and healing effects around the probes, two linear correction factors were applied. The first
347 (F1) was obtained by calibrating the Granier equation (1988) to data from *P. halepensis* trees
348 from the Font-blanche site. The correction factor for the healing effect (F2) was taken from
349 (Wiedemann et al., 2016):

$$u_{\text{corrected}} = u \times F1 \times F2 \quad (2)$$

350 with $F1 = 1.35$ and $F2 = 1.53$.

351 In the end, there was 47.7 % of missing data for the control treatment and 45.4 % for the
352 rainfall exclusion one's. The proportion of data when sensors were working on less than three
353 trees amounted to 24/27 % for *P. halepensis* and *Q. ilex* respectively in the control treatment,
354 against 71/34 % in the exclusion one's. The higher proportion of missing data in the exclusion
355 treatment was due to the lower number of monitored trees (4 against 8 in the control
356 treatment) and to the use of CR10X Campbell to acquire the data, which are older and more
357 subjected to power outage. It was higher for *P. halepensis* because one tree had a
358 malfunctioning sensor for two years.

359 To smooth out the impact of gaps in sap velocities, we applied a moving average on sap
360 velocity data using a 2 weeks window for each tree monitored. The PAR quantile 0.9 was
361 calculated for each two-week period and sap velocity data was kept if they corresponded to a

362 PAR > q0.9 PAR. This allowed to increase the time period for which a maximum number of
363 trees data were available.

364 2.8. Measurement of native embolism by direct X-ray microtomography observation

365 Branch segments were collected in October 2015 for *P. halepensis* and in March 2018 for
366 both species. To avoid cutting artefacts, branches larger than 40 cm for *P. halepensis* and 90
367 cm for *Q. ilex* were cut just before dawn, immediately recut under water, and the cut surface
368 was kept under water. Short (length < 4 cm length and diameter < 0.7 cm), 2-year-old sub-
369 samples were then recut under water, immersed in liquid paraffin and stored at 4°C until
370 analysis. The measurements were performed using the standard protocol described by
371 Cochard et al. (2015). Each sample was positioned in the X-ray microtomograph (Nanotom
372 180 XS; GE, Wunstorf, Germany) and was analysed using the following settings: field of
373 view, $5 \times 5 \times 5 \text{ mm}^3$; X-ray voltage and current, 60 kV and 240 μA ; scan time, 21 min. After
374 3D-reconstruction, the final spatial resolution of the 3D images was 2.5 μm . One transversal
375 2D slice was extracted from the middle of the volume using VGStudio Max© software
376 (Volume Graphics, HeiΔberg, Germany). The slice was then analysed using the software
377 ImageJ (Schneider et al., 2012), to estimate the surface area of embolized conduits.

378 To estimate the embolism ratio for *P. halepensis*, we developed a process consisting in
379 separating areas with embolized tracheids from areas with water-filled tracheids, which are
380 easy to identify based on colour thresholds. The embolism ratio was then calculated as the
381 proportion of the total cross-sectional area containing embolized tracheids (Choat et al., 2015;
382 Torres-Ruiz et al., 2016). This procedure assumes that all tracheids have similar diameters
383 and thus similar hydraulic conductivities. For *Q. ilex*, which is semi ring-porous, and has a
384 lognormal distribution of conduit sizes, we first isolated large embolized vessels (which
385 contribute the most to hydraulic conductivity) and measured their surface area to estimate the

386 corresponding mean diameter and hydraulic conductivity. We then used a second scan,
387 performed on cut samples, after the free water was removed from the lumens, to estimate the
388 diameter of all large vessels and thus the maximal hydraulic conductivity. Finally, the level of
389 embolism was calculated as the ratio of the embolized and maximum hydraulic conductivity.

390 *2.9. Statistics*

391 All statistical tests were performed using the tree as the independent statistical unit, except for
392 PAI for which it was the treatment (2 merged plots for the control treatment and one for the
393 rainfall exclusion). Tests were only conducted if a minimum of three trees per species and
394 treatment were available.

395 We used a linear mixed effect model to test the rainfall exclusion effect on sap velocity, in
396 order to account for a potential bias causing by the low number of trees monitored, especially
397 in the exclusion plot. Treatments, years and the interactions between treatment and years were
398 chosen as fixed factors and trees as random factor. For the sap velocities, we also investigated
399 whether the difference between treatments changed over time by computing the standardized
400 difference between the maximal (spring/autumn times) values. To assess the period of the
401 year when differences between treatments were significant, we also performed Wilcoxon rank
402 sum tests for sap velocity considering daily moving average values of at least 3 trees per
403 treatment under a period of 15 days.

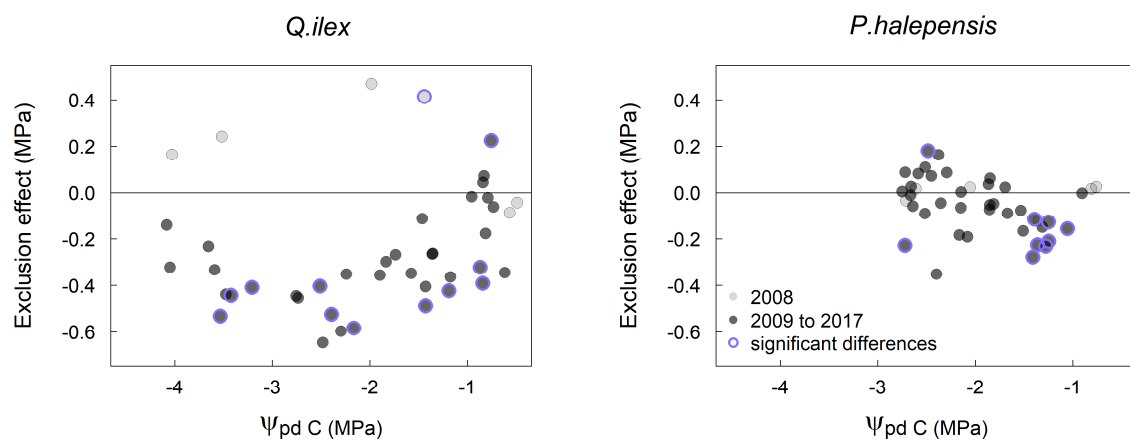
404 In addition, we used a linear mixed effect model to test for a rainfall exclusion and species
405 effect on $\Delta\Psi$. We used treatment, species, predawn water potential and included the
406 interaction between these variables as explanatory variables, and trees as random factor. We
407 additionally tested the rainfall exclusion effect at each measurement date for shoot water
408 potentials, sap flow driving force ($\Delta\Psi$), native embolism and primary growth using non-
409 parametric Wilcoxon rank sum test to overcome small sample size limitations.

410 Secondary growth was modelled for the two species by multiple linear regression, using the
411 BAI calculated from the annual forest inventories as the dependent variable. The explanatory
412 variables were the treatment, the diameter at breast height (DBH) at the beginning of the
413 rainfall exclusion period (i.e. 2009), and the interaction between these variables. The primary
414 growth data were analysed by comparing annual values for each branch type. The first two
415 years of data were excluded as they contain most of the age-related signal (Fig. A2). Finally,
416 we tested for a time effect on the evolution of PAI under both treatments, and on the
417 difference in PAI between treatments, using a simple linear model with time as the
418 explanatory factor. All statistical analyses were performed with the software R (3.5.2, R
419 Development Core Team 2018).

420 3. Results

421 3.1. Effect of rainfall exclusion on tree water status

422 In 2008, before rainfall exclusion began, predawn water potentials (Ψ_{pd}) were similar
423 between treatments either for *P. halepensis* or *Q. ilex*. Among the 5 sampling dates of 2008
424 (Fig. S5), only one was significant ($p < 0.05$), at the beginning of the dry season, when Ψ_{pd}
425 was higher in the exclusion plot. In subsequent years, rainfall exclusion was associated with a
426 general, although not permanent, decrease in water potentials for both species. For *Q. ilex*,
427 Ψ_{pd} differed significantly between treatments all along the gradient of Ψ_{pd} values (Fig. 2),
428 although the differences in Ψ_{pd} between treatments were largest (0.6 MPa) at intermediary



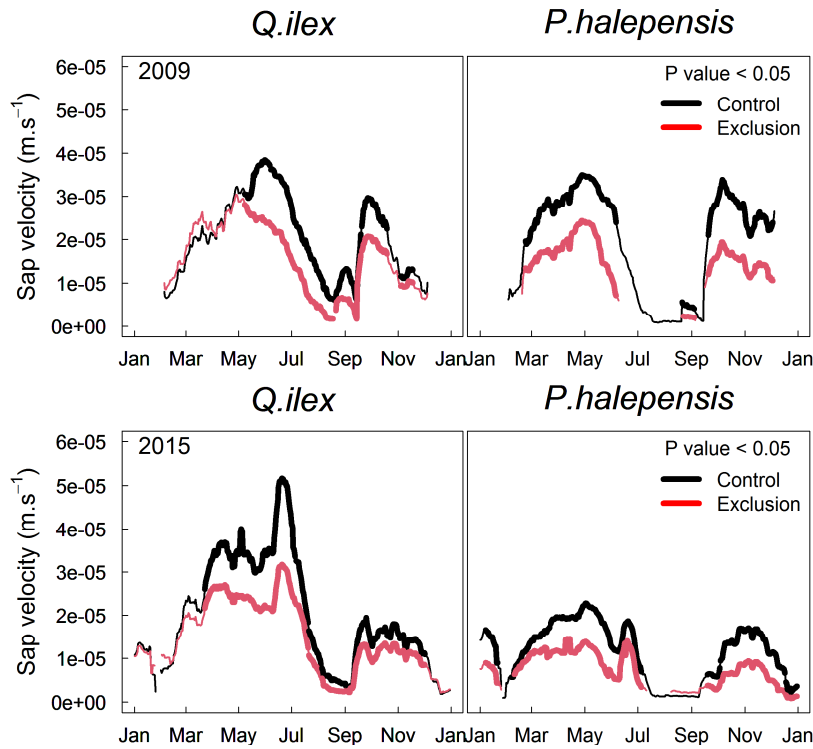
429 stress levels (Ψ_{pd} between -2 and -3 MPa). At more pronounced stress levels, the difference
430 between treatments decreased to 0.2 MPa. For *P. halepensis*, Ψ_{pd} differed significantly
431 between treatments mainly at low stress levels ($\Psi_{pd} > -2$ Mpa) and only occasionally at
432 lower values (Fig. 2). The maximum difference between treatments was 0.3 MPa and
433 occurred at higher Ψ_{pd} values than for *Q. ilex* (Ψ_{pd} of around -1.5 MPa).

434 **Fig. 2.** Effect of rainfall exclusion on the predawn leaf water potentials of *Q. ilex* and *P. halepensis*. The
435 exclusion effect is computed as the difference between the predawn water potentials ($\Psi_{pd}^e - \Psi_{pd}^c$) in exclusion
436 (Ψ_{pd}^e) and control trees (Ψ_{pd}^c). Each point represents the average value in a given plot on a given date. The light
437 grey circles represent data from 2008, before rainfall exclusion was initiated, and the dark grey circles represent
438 the values measured between 2009 and 2017. Significant differences between treatments ($p < 0.05$) are shown as
439 purple circles.

440 3.2. Effect of rainfall exclusion on sap velocities

441 Bimodal seasonal variations in sap velocity were observed every year for both species
442 and treatments, the highest values occurring during spring (from April to June), followed by a
443 sharp decrease in summer in parallel to the corresponding decrease in soil water levels (Fig.
444 S6-S7). A second peak occurred in autumn, concurrently with increased rainfall, and was
445 followed by another decrease in winter (Fig. 3 and Fig. S8). Sap velocities were significantly
446 lower in the rainfall exclusion plot for both species ($p < 0.001$ for both species considering
447 linear mixed effect model, Table 1). Sap velocity was lower in the rainfall exclusion plot from
448 the very first year of treatment (2009, Fig. 3-S8 reporting Wilcoxon rank sum test results),
449 although differences between treatments were not significant from January to March for
450 *P.halepensis* and from January to May for *Q.ilex* (3 to 5 months after the onset of rainfall
451 exclusion). The magnitude of the average reduction in sap velocity caused by rainfall
452 exclusion differed between years and species. The mean standardized difference in maximal
453 (spring and autumn times) sap velocities between the control and the dry treatments shows

454 that *P. halepensis* sap velocities were more affected by the rainfall exclusion, for which values
 455 are between -0.3 and -0.6 while for *Q. ilex* they are comprise between -0.2 and -0.4 (Fig. S9).
 456 In addition, the mean standardized difference in maximal sap velocities remains in the same
 457 range for both species without showing any trends across years. (Fig. S9).



458 **Fig. 3.** Variations in sap velocity for *Pinus halepensis* and *Quercus ilex* trees from the control (black lines) and
 459 rainfall exclusion (red lines) plots, during a moderately dry year (2009) and a severely dry year (2015). For each
 460 treatment and species, the moving mean sap velocity was calculated based on data from at least three trees, over
 461 with a 2-week window. The bold lines represent periods where significant ($p < 0.05$) differences were found
 462 between treatments using Wilcoxon rank sum test. Data for all other years are shown in Fig. S8.

463 **Table 1**

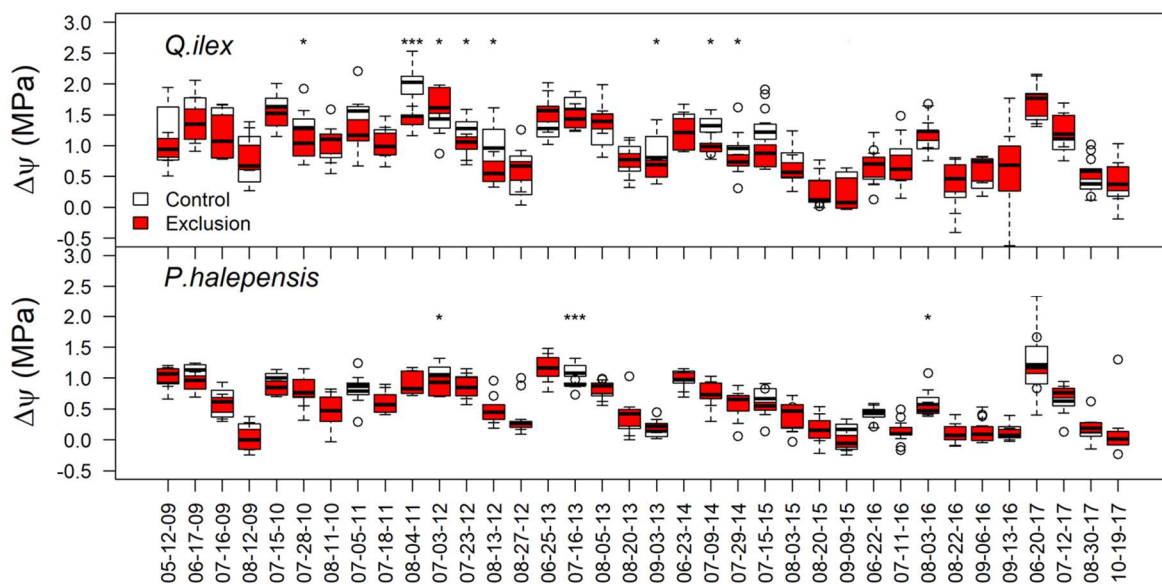
464 Analyse of deviance table using Type III Wald chisquare tests for linear mixed effect model
 465 of sap velocity with a tree random factor.

Species	Fixed factors	Chisq	df	Pr (>Chisq)
<i>Quercus ilex</i>	Intercept	0.5114	1	<0.001
	Treatment	10.2381	1	<0.001
	Years	0.5736	1	<0.001
	Treatment:years	10.1591	1	<0.001

	Intercept	54.0281	1	< 0.001
<i>Pinus halepensis</i>	Treatment	4.8824	1	< 0.001
	Years	53.5799	1	<0.001
	Treatment:years	4.7796	1	< 0.001

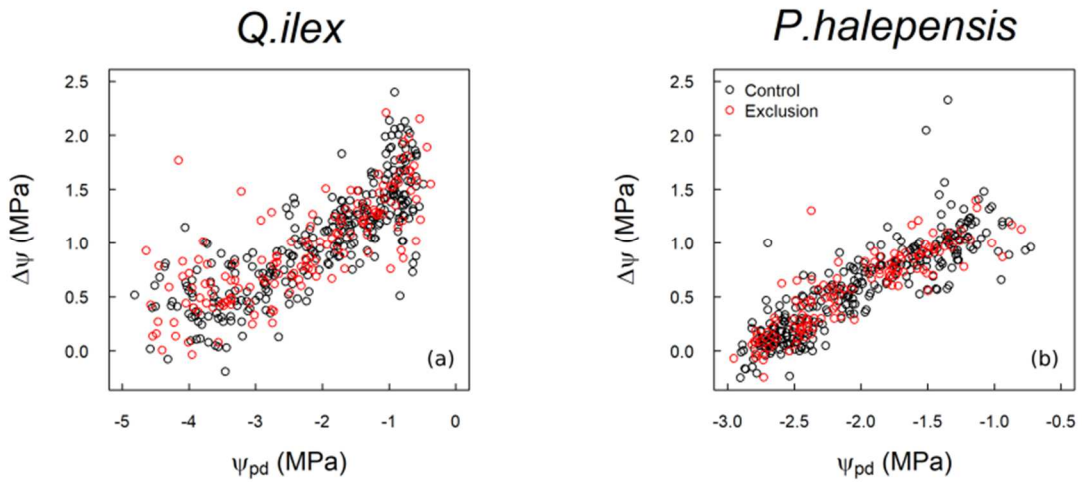
466 **3.3. Effect of rainfall exclusion on sap flow driving force and xylem native embolism**

467 To further investigate the decrease in sap velocities associated with rainfall exclusion,
468 we assessed the effect of Ψ_{pd} on the driving force for transpiration ($\Delta\Psi$). Summer $\Delta\Psi$ levels
469 tended to be lower in the exclusion plot for both species, with more significant differences for
470 *Q. ilex* than for *P. halepensis* (Fig. 4). $\Delta\Psi$ decreased strongly with decreasing values of Ψ_{pd} (p
471 < 0.001, Table S4), with a significant difference in this relationship between species (p <
472 0.001, Table S4). For *P. halepensis*, $\Delta\Psi$ decreased linearly down to zero at -3 MPa, whereas
473 for *Q. ilex* it decreased exponentially and then remained constant, at approximately 0.5 MPa,
474 for Ψ_{pd} lower than -3 MPa (Fig 5a, b). There was no significant association between rainfall
475 exclusion
476 and the decline in $\Delta\Psi$ with decreasing Ψ_{pd} (Fig. 5a, b and Table S4).



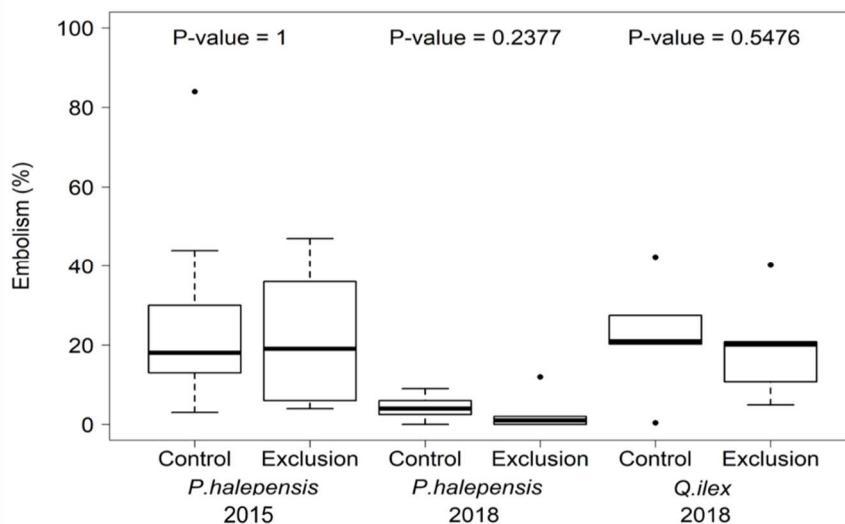
477 **Fig. 4.** Sap flow driving force (i.e. the difference between predawn and midday water potentials; $\Delta\Psi$) in
478 *Quercus ilex* and *Pinus halepensis* trees. Sap velocity was also monitored in these trees. Each point represents

479 one tree at one date. Significant differences (Wilcoxon rank sum tests) are indicated by asterisks (*, $0.01 \leq p <$
 480 0.05 ; ***, $p < 0.001$).



481
 482 **Fig 5.** Variations in the sap flow driving force (difference between predawn and midday water potentials, $\Delta\Psi$) as
 483 a function of Ψ_{pd} for *Quercus ilex* (a) and *Pinus halepensis* (b).

484 Stem native embolism did not differ significantly between treatments for any species ($p >$
 485 0.05 , Wilcoxon rank sum test, Fig. 6). The embolism ratio, calculated from the percentage
 486 loss of theoretical conductivity, averaged 21% in March 2018 for *Q. ilex*. For *P. halepensis*,
 487 the



488 embolism ratio averaged 25% after the summer drought of 2015 but was only 4% in March
 489 2018; this is likely due to autumn or winter growth, which is common for this species.

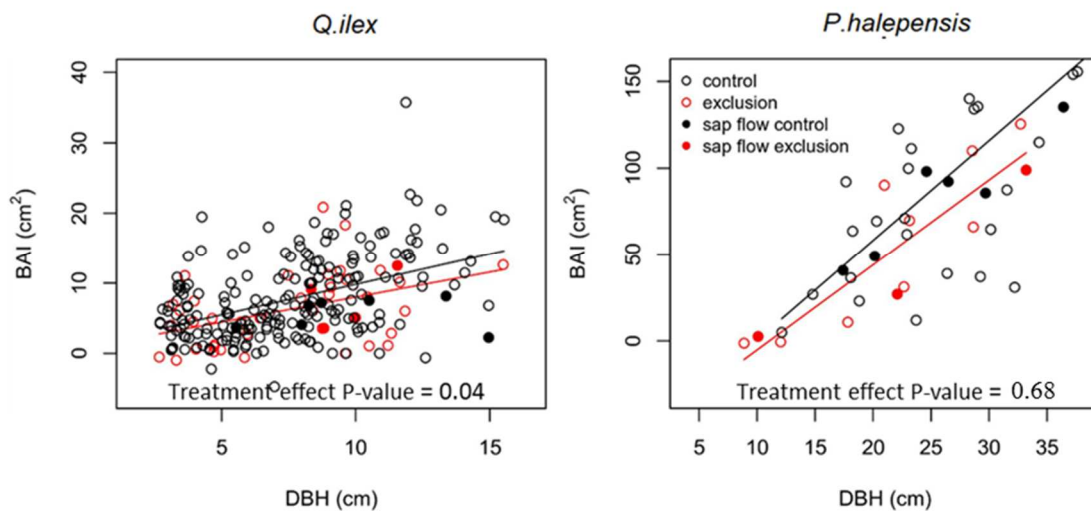
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500 **Fig. 6.** Comparison of native stem embolism in *Pinus halepensis* and *Quercus ilex* trees in the rainfall exclusion
501 and control plots, estimated from X-ray microtomography measurements performed in September 2015 (for both
502 species) and in March 2018 (for *P. halepensis*).

503 **3.4. Effect of rainfall exclusion on tree growth and plant area index**

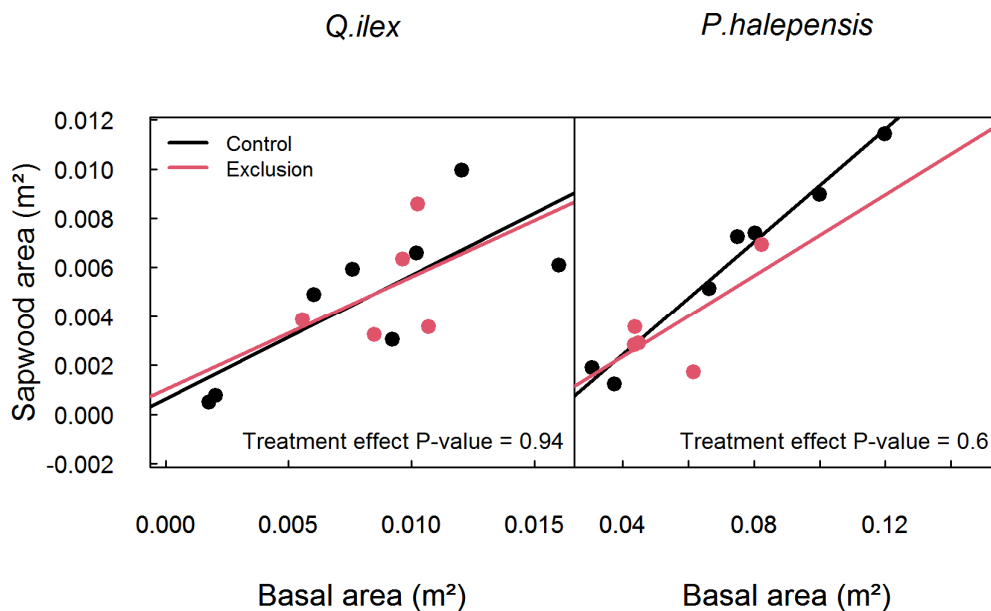
504 From 2009 to 2017, the annual basal area increment (BAI) of individual stems was
505 significantly lower in the rainfall exclusion plot for *Q. ilex* trees ($p = 0.04$, Fig. 7 and Table
506 S5) but not for *P. halepensis* ($p = 0.68$, Fig. 7 and Table S5).



507 **Fig. 7.** Relationships between basal area increment (BAI) from 2009 to 2017 and diameter at breast height
508 (DBH) in 2009 for *Quercus ilex* and *Pinus halepensis* trees in the control and rainfall exclusion plots. The data

509 from trees equipped with sap flow sensors are shown as filled circles. For *Q. ilex*, the data from coppiced stems
510 are shown as separate points. Note that the two panels have different scales.

511 According to the accuracy of the sapwood delineation using ERT measurements in
512 comparison to water profiles under cores (Fig. S4), sapwood ERT estimations were used to
513 see if sapwood area proportion under the stem was affected by the rainfall exclusion. For both
514 species, no significant difference between treatment was found for sapwood area/basal area
515 ratio ($p = 0.94$ for *Q. ilex*, $p = 0.6$ for *P. halepensis*, Fig. 8 and Table S6). The significant
516 radial growth reduction found for *Q. ilex* of the rainfall exclusion treatment imply reduced
517 sapwood area compared to control trees.



518 **Fig. 8.** Relationships between total sapwood area and basal area for *Quercus ilex* and *Pinus halepensis* trees in
519 the control (black lines and circles) and rainfall exclusion plots (red lines and circles).

520 For *P. halepensis*, needle lengths were shorter in the rainfall exclusion plot between 2009
521 and 2017, especially for third-order branches for whom differences between treatment and
522 control trees were significant for all years studied (Fig. 9a and Fig. A3 a). In the
523 retrospectively estimated primary growth data for the 2000–2008 period (before the start of
524 the experiment), annual shoot lengths and number of ramifications for third and second order
525 branches were higher in the exclusion plot than in the control plots (Fig. 9c; Fig A3 b, c, d).

526 Shoot lengths became similar in the two groups after the start of the experiment (Fig 9c and
527 Fig A3 b). The number of ramifications on second-order branches remained higher in the
528 exclusion plot, as was the case well before the start of the experiment, with no clear temporal
529 trend (Fig A3 c). The number of ramifications on third-order branches decreased over time in
530 the rainfall exclusion plot, and reached values similar to those in the control plots from 2010
531 (Fig. A3 d, $p < 0.05$ before 2010, $p > 0.05$ after 2010 for all but one year; Wilcoxon rank sum
532 tests). The decrease in the shoot length of third order branches followed a similar trend as
533 observed for the number of ramifications (Fig. 9c). For *Q. ilex*, when the data for all branches
534 were analysed together, there were no significant differences between treatments for either of
535 the primary growth metrics (number of ramifications and shoot length, not shown). However,
536 considering upper and lower canopy branches separately some differences appeared (Fig. 9
537 and Fig. A4). The number of ramifications on top canopy branches was significantly lower in
538 the exclusion plot after (but not before) the start of the experiment (Fig. A4). Shoot of upper
539 canopy branches were more numerous in the exclusion before the start of the experiment but
540 the difference tended to decrease after the setup of the exclusion (Fig. 9d).

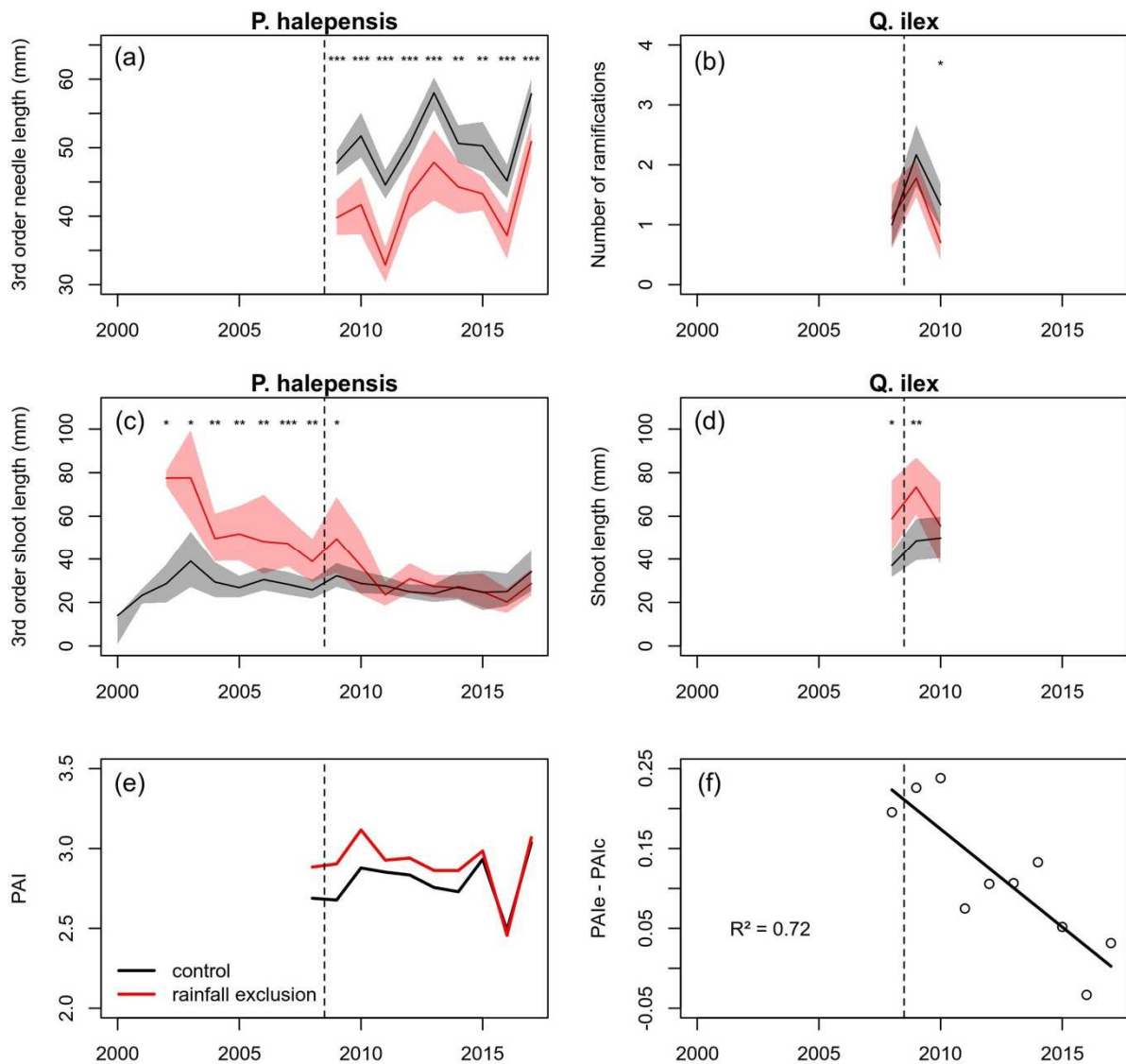
541 At the stand level, the plant area index (PAI), a surrogate of canopy cover, was higher in
542 the rainfall exclusion plot before the start of the experiment and remained higher during the
543 first 3 years of rainfall exclusion, after which it tended to converge with the value in the
544 control plot (Fig. 9e). None of the year-by-year differences in PAI between treatment groups
545 are statistically significant; however, the decrease in the PAI difference between groups
546 during the exclusion experiment, down to zero in 2017, was significant ($p < 0.05$; Fig. 9f).

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553 **Fig. 9.** Time evolution of (a) needle length, (b) number of ramifications and (c, d) shoot length for (a, c) *Pinus*
 554 *halepensis* and (b, d) *Quercus ilex* trees in the control (black) and rainfall exclusion (red) plots; (e) plant area
 555 index (PAI, stand-level canopy coverage) in the two plots, and (f) the difference between the PAI in the
 556 exclusion (PAI_e) and control (PAI_c) plots. The dashed lines indicate the start of rainfall exclusion (2009). For
 557 *Quercus ilex* only data for upper canopy branches are shown; data are presented for lower canopy branches in
 558 Fig. A4. In graphs (a) to (d), significant differences between treatments are displayed for $P < 0.001$ (***), $P < 0.01$
 559 (**), and $P < 0.05$ (*). The slope of the difference in PAI between treatments in (f) is significant ($P < 0.05$).

560 **4. Discussion**

561 Assessing how trees in mixed Mediterranean forests respond to an experimental increase
562 in drought over long timescales can provide a better understanding of how increasingly dry
563 conditions associated to climate change in some areas is affecting forest function and
564 succession. This 9-year rainfall exclusion experiment in a mixed pine–oak Mediterranean
565 forest explored long-term changes in water potential, sap velocity, sap flow driving force and
566 growth at the tree and forest level. In line with previous long-term rainfall exclusion studies
567 (Hudson et al., 2017; Limousin et al., 2009; Maggard et al., 2016; Saunier et al., 2018) our
568 exclusion design lead to a decrease in summer tree water potential from the very first year of
569 the experiment in both species. We first discuss the direct effects of the rainfall exclusion
570 treatment on summer water potentials and their consequences for tree growth and sap
571 velocities. We then discuss the indirect effects of rainfall exclusion on the long-term decrease
572 in sap velocities in relation to hydraulic adjustments that potentially reflect acclimation to
573 higher drought stress.

574 *4.1. Exclusion effects on growth*

575 Our data support that the rainfall exclusion negatively affected the different estimates of
576 growth we considered, in accordance with the high sensitivity of cell growth to drought
577 (Guillemot et al., 2015; Lempereur et al., 2015). For *P. halepensis*, lower needle lengths were
578 recorded from the very first year of the experiment onwards. On average, needles were shorter
579 in the exclusion plot by 15.0% in 2009, and by 15.7% over the entire period. This direct effect
580 on needle length is consistent with the results of several studies (e.g. Adams et al., 2015;
581 Myers, 1988) that have shown that needle growth depends strongly on the water potential,
582 notably for *P. halepensis* (Borghetti et al., 1998). The impact of rainfall exclusion on shoot
583 length, and to a lesser extent, on the number of lateral ramifications, was delayed by at least

584 one year (Fig. 9, Fig. A3, Fig. A4). This delayed effect fits with the fact that shoot length and
585 ramification are partly predetermined in buds (Girard et al., 2012). From 2009 to 2017, trees
586 in the exclusion plot did not have shorter shoots or fewer ramifications than those in the
587 control plot, but this is mainly because they were more vigorous before the treatment started.
588 Nevertheless, there was a strong reduction in both variables once rainfall exclusion was
589 initiated for third order branches. These reductions in shoot length and needle length clearly
590 support a reduction in the leaf area for this category of branches, which are the most abundant
591 at the crown level, and account for most of the transpiration and the photosynthesis, while
592 second order branches are more involved in space exploration (Barthélémy & Caraglio,
593 2007). For *Q. ilex*, the dataset was more limited and a drought effect was only observed in the
594 number of ramifications of top branches, which slightly decreased in trees subjected to the dry
595 treatment. This decrease is in agreement with previous studies on this species (Limousin et al.,
596 2012; Martin-StPaul et al., 2013; Ogaya et al., 2006). Measurements of the PAI were in line
597 with the primary growth data, indicating a progressive reduction in the overall stand foliage
598 cover in the exclusion plot. Indeed, while the PAI in the exclusion plot was higher before the
599 start of the experiment, this difference decreased as the experiment went on, indicating a
600 progressive reduction in leaf area under rainfall exclusion.

601 The results obtained for basal area increment were not fully consistent with those on
602 primary growth, as basal area growth was only significantly reduced under rainfall exclusion
603 in *Q. ilex* and not in *P. halepensis*. *P. halepensis* and *Q. ilex* probably allocate their biomass
604 differently in response to increased drought. It is difficult to be more specific in the absence of
605 more adequate data. However, both species are known to display some plasticity in their
606 growth patterns in relation to drought. For instance, Gavinet et al. (2019) found that for *Q.*
607 *ilex*, a long-term rainfall exclusion impacted net primary productivity through a reduction in
608 leaf growth and acorn production, but not wood production. Alfaro-Sánchez et al. (2015)

609 studied differences in allometric relationships for Aleppo pines at three sites of varying levels
610 of aridity. They found that some variables were more affected by long term site conditions,
611 such as biomass and the height-stem diameter relationship, while others were more influenced
612 by recent weather conditions, such as leaf and branch biomass, and the leaf area-stem
613 diameter relationship. As expected, root growth remains a major unknown.

614 *4.2. Effects of rainfall exclusion on sap velocity*

615 Regarding water transport, we found that sap velocities were lower in trees subjected to
616 rainfall exclusion. This is also a widely reported response (Besson et al., 2014; Grossiord,
617 Sevanto, Borrego, et al., 2017; Limousin et al., 2009; Pangle et al., 2015; Zhang et al., 2018).
618 Here, sap velocities were lower under exclusion treatment both during the summer dry period
619 and during well-watered periods (Fig. 3 and Fig. S8). This behaviour is probably the
620 consequence of two separate phenomena. First, short-term reduction in sap velocity can be
621 due to stomatal closure, one of the primary mechanisms of plants to limit transpiration and
622 thus water potential decreases (Bréda et al., 2006; Maseda & Fernandez, 2006). The lower
623 leaf water potentials experienced by trees in the exclusion plot during summer is thus likely
624 triggered greater stomatal closure. Second, the reduced sap velocity in the exclusion plot
625 during well-watered conditions (spring and autumn) periods may be related to changes in
626 plant hydraulic conductance, that can decrease water transport capacity even when the
627 stomata are fully open (see following section).

628 **4.3. The effects of rainfall exclusion on the tree water relation can be explained by**
629 *hydraulics and growth*

630 To better understand the parameters involved in the adjustment of the sap velocity (u , in
631 $\text{m}\cdot\text{s}^{-1}$) to rainfall exclusion, u can be expressed in terms of Ohm's law analogy (Tyree &
632 Ewers, 1991):

$$633 \qquad \qquad \qquad u = \Delta\Psi \cdot ks = E \cdot LA/SA \qquad (3)$$

634 with $\Delta\Psi$ being the sap flow driving force of a tree (in MPa), ks the sapwood based specific
635 hydraulic conductance (in $\text{m}\cdot\text{s}^{-1}\cdot\text{MPa}^{-1}$), E the transpiration per unit leaf area (in $\text{m}^3\cdot\text{m}^{-2}\cdot\text{s}^{-1}$),
636 LA the tree leaf area (in m^2) and SA the tree sapwood area (in m^2). In the following
637 paragraphs, assuming that (i) water potentials are representative of the whole tree canopy and
638 (ii) a steady state sap flow, we discuss how changes in $\Delta\Psi$, ks and LA/SA ratio can explain
639 the observed differences in sap velocities between treatments, and identify mechanisms imply
640 in these changes.

641 During summer drought, for both species, $\Delta\Psi$ decrease earlier in the exclusion plot, which is
642 consistent with the reduction in sap velocity due to an earlier drought and thus an earlier
643 stomatal closure. However, this mechanism cannot explain the reduction in sap velocities in
644 the exclusion plot that occurs during wet periods, at high Ψ_{pd} , when $\Delta\Psi$ was similar in all
645 treatments.

646 The lower sap velocities measured during wet periods for trees in the rainfall exclusion may
647 therefore be related to a decrease in ks . This can occur through several mechanisms. Firstly, it
648 is well known that xylem embolism can alter hydraulic conductance (Tyree & Sperry, 1989).
649 However, the levels of native stem embolism were similar in the experimental and control
650 trees. Similar levels of embolism were expected for *P. halepensis* trees, because the water
651 stress in the two plots at the end of the summer were also similar. For *Q. ilex*, these similar
652 embolism levels suggest either that water potential levels reached were not enough to induce

653 significant embolism differences between treatment, or that the trees in the dry plot may have
654 become more resistant to embolism. Further measurements of tree vulnerability to drought-
655 induced embolism would help to explore these questions. According to the vulnerability
656 segmentation hypothesis, leaves and roots may be more vulnerable to cavitation than branches
657 or the main stem (Johnson et al., 2016; Skelton et al., 2019; Melvin T. Tyree & Ewers, 1991;
658 Zhu et al., 2016), so cavitation events in distal parts of the plant cannot be excluded.
659 Nevertheless recent studies comparing the xylem vulnerabilities of different organs
660 (Lamarque et al., 2018; Li et al., 2020; Peters et al., 2020), report no segmentation over a
661 range of deciduous and evergreen tree species. Additionally, recent results from ongoing study
662 (Moreno et al, in prep.) show no difference between leaf and stem vulnerabilities to cavitation
663 for *Quercus ilex*. All this suggests that segmentation is unlikely to occur for both studied
664 species.

665 A second possible explanation for the decrease in k_s is that the higher drought intensity may
666 have affected the xylem anatomy of the trees in the exclusion plot (Maseda & Fernandez,
667 2006), narrowing the xylem conduits and/or increasing cell-wall thickness and wood density
668 (Dalla-Salda et al., 2011; de Luis et al., 2011; Ladjal et al., 2005; Pasho et al., 2012). These
669 changes, which have all been observed in rainfall exclusion studies (Belien et al., 2012;
670 D'Orangeville et al., 2013), reduce the risk of cavitation, but because of the trade-off between
671 xylem efficiency and safety (Venturas et al., 2017), they may also reduce xylem-specific
672 hydraulic conductance. These modifications are not systematic however, as Limousin et al.
673 (2010a) found no such adjustments in the xylem properties of *Q. ilex* trees subjected to
674 rainfall exclusion for six years. For *P. halepensis*, Pasho et al. (2012) found that severe
675 drought reduced the production of early wood and thus the global conductivity of the xylem.
676 Further anatomical analyses will help to test these hypotheses.

677 Differences in sap velocity may also be related to changes in the leaf area to sapwood area

678 ratio (LA/SA). Many studies have reported a decrease in the LA/SA ratio in trees subjected to
679 increased water stress (Carter & White, 2009; Hudson et al., 2017; Limousin et al., 2010;
680 Mencuccini & Grace, 1995). This adjustment, which is often interpreted as a mean to improve
681 water availability and thus the gas exchange per unit leaf area, can also reduce persistently the
682 overall tree transpiration. While we did not directly measure the LA/SA ratio here, indirect
683 measurements at the branch level (number and size of needles) suggest that leaf areas
684 decreased in response to exclusion treatment. At the stand level, the initial excess in PAI in the
685 exclusion plot disappeared progressively during the experiment, which is also indicative of a
686 decrease in LA compared with the control plot. These trends, together with the observation
687 that *P. halepensis* basal area growth was barely affected by the treatment, suggest that rainfall
688 exclusion led to a decrease in the LA/SA ratio. The results for *Q. ilex* are harder to interpret
689 because of the shorter time series of data available. Nevertheless, the conservation of the tree
690 SA/BA ratio between treatment with decreasing primary growth under the exclusion plot
691 attest that sapwood growth was reduced by the aggravated water stress. Hence, reduction of
692 sap velocity could probably result of an imbalance between LA/SA, with a highest reduction
693 of LA than SA. The greater impact of rainfall exclusion on the sap velocity of *Pinus*
694 *halepensis* could also be explained by LA/SA ratio spectrum. *P. halepensis* should have
695 lowest LA/SA ratio because of a greater decrease of LA. To prospect this hypothesis, further
696 measurements are required. It is noteworthy to add that other factors, such as canopy
697 microclimate, not explored under this study could also influence sap velocity of both studied
698 species.

699 *4.4. Conclusions and perspectives*

700 Our study shows that a nine-year 30% reduction in rainfall in a mixed forest impacted broadly
701 similarly adult *Q. ilex* and *P. halepensis* trees, by causing an early decrease in water potential

702 during the summer and reducing persistently sap velocities. These effects have been
703 frequently reported, but here we prove that such changes are certainly not caused by
704 cavitation but rather by changes in leaf area.

705 However, there are also some differences in the way species have been affected. For example,
706 it seems that the decrease in water potential during extreme drought was much more
707 pronounced for *Q. ilex* than for *P. halepensis* and it deserves to be explored how this translates
708 in terms of hydraulic safety margins. This is all the more intriguing since the intensity of the
709 reduction in sap velocity in the exclusion treatment was greater for *P. halepensis* than for
710 *Q.ilex*, which may indicate greater resilience of the later that could be related to interactions
711 between the two species. This should be explored in the future.

712 Additionally, it has to be noted that studies conducted in natural conditions are inherently
713 affected by differences in soil (Carrière et al., 2020; Nourtier et al., 2011) and stand structure.
714 The fine-scale spatial structure of soils can be measured by electrical tomography resistivity
715 (Carrière et al., 2020) and canopy structures can be characterized by terrestrial LiDAR (Soma
716 et al., 2018), and these data could be combined with 3D eco-physiological models (Simioni et
717 al., 2016). Future studies over longer timescales with data on physiological traits involved in
718 drought response (e.g. turgor loss points and vulnerability to cavitation) will also help to
719 elucidate how forest function and successional trajectories are likely to be affected by climate
720 change.

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728 **References**

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- 730 Adams, H. D., Collins, A. D., Briggs, S. P., Vennetier M., Dickman, L. T., Sevanto, S. A.,
731 Garcia-Forner, N., Powers, H. H., & McDowell, N. G. (2015). Experimental drought and
732 heat can delay phenological development and reduce foliar and shoot growth in semiarid
733 trees. *Global Change Biology*, 21(11), 4210–4220. <https://doi.org/10.1111/gcb.13030>
- 734 Adams, H. D., Zeppel, M. J. B., Anderegg, W. R. L., Hartmann, H., Landhäusser, S. M.,
735 Tissue, D. T., Huxman, T. E., Hudson, P. J., Franz, T. E., Allen, C. D., Anderegg, L. D.
736 L., Barron-Gafford, G. A., Beerling, D. J., Breshears, D. D., Brodribb, T. J., Bugmann,
737 H., Cobb, R. C., Collins, A. D., Dickman, L. T., ... McDowell, N. G. (2017). A multi-
738 species synthesis of physiological mechanisms in drought-induced tree mortality. *Nature*
739 *Ecology and Evolution*, 1(9), 1285–1291. <https://doi.org/10.1038/s41559-017-0248-x>
- 740 Alfaro-Sánchez, R., López-Serrano, F. R., Rubio, E., Sánchez-Salguero, R., Moya, D.,
741 Hernández-Tecles, E., & De Las Heras, J. (2015). Response of biomass allocation
742 patterns to thinning in *Pinus halepensis* differs under dry and semiarid Mediterranean
743 climates. *Annals of Forest Science*, 72(5), 595–607. [https://doi.org/10.1007/s13595-015-](https://doi.org/10.1007/s13595-015-0480-y)
744 0480-y
- 745 Baquedano, F. J., & Castillo, F. J. (2006). Comparative ecophysiological effects of drought on
746 seedlings of the Mediterranean water-saver *Pinus halepensis* and water-spenders *Quercus*
747 *coccifera* and *Quercus ilex*. *Trees - Structure and Function*, 20(6).
748 <https://doi.org/10.1007/s00468-006-0084-0>
- 749 Barthélémy, D., & Caraglio, Y. (2007). Plant architecture: A dynamic, multilevel and
750 comprehensive approach to plant form, structure and ontogeny. In *Annals of Botany*.
751 <https://doi.org/10.1093/aob/mcl260>
- 752 Beier, C., Beierkuhnlein, C., Wohlgemuth, T., Penuelas, J., Emmett, B., Körner, C., de Boeck,
753 H., Christensen, J. H., Leuzinger, S., Janssens, I. a, & Hansen, K. (2012). Precipitation
754 manipulation experiments--challenges and recommendations for the future. *Ecology*
755 *Letters*, 15(8), 899–911. <https://doi.org/10.1111/j.1461-0248.2012.01793.x>
- 756 Belien, E., Rossi, S., Morin, H., & Deslauriers, A. (2012). Xylogenesis in black spruce
757 subjected to rain exclusion in the field ¹ This article is one of a selection of papers from
758 the 7th International Conference on Disturbance Dynamics in Boreal Forests. *Canadian*
759 *Journal of Forest Research*, 42(7), 1306–1315. <https://doi.org/10.1139/x2012-095>

760 Benson, A. R., Koeser, A. K., & Morgenroth, J. (2019). Estimating conductive sapwood area
761 in diffuse and ring porous trees with electronic resistance tomography. *Tree Physiology*,
762 39(3), 484–494. <https://doi.org/10.1093/TREEPHYS/TPY092>

763 Besson, C. K., Lobo-Do-Vale, R., Rodrigues, M. L., Almeida, P., Herd, A., Grant, O. M.,
764 Soares David, T., Schmidt, M., Otieno, D., Keenan, T. F., Gouveia, C., Mériaux, C.,
765 Chaves, M. M., & Pereira, J. S. (2014). Cork oak physiological responses to manipulated
766 water availability in a Mediterranean woodland. *Agricultural and Forest Meteorology*,
767 184, 230–242. <https://doi.org/10.1016/j.agrformet.2013.10.004>

768 Borghetti, M., Cinnirella, S., Magnani, F., & Saracino, A. (1998). Impact of long-term
769 drought on xylem embolism and growth in *Pinus halepensis* Mill. *Trees*, 12(4), 187–195.
770 <https://doi.org/10.1007/pl00009709>

771 Bréda, N., Huc, R., Granier, A., & Dreyer, E. (2006). Temperate forest trees and stands under
772 severe drought: a review of ecophysiological responses, adaptation processes and long-
773 term consequences. *Annals of Forest Science*, 63(6), 625–644.
774 <https://doi.org/10.1051/forest:2006042>

775 Carrière, S. D., Ruffault, J., Pimont, F., Doussan, C., Simioni, G., Chalikakis, K., Limousin,
776 J.-M., Scotti, I., Courdier, F., Cakpo, C.-B., Davi, H., & Martin-StPaul, N. K. (2020).
777 Impact of local soil and subsoil conditions on inter-individual variations in tree responses
778 to drought: insights from Electrical Resistivity Tomography. *Science of The Total*
779 *Environment*, 698, 134247. <https://doi.org/10.1016/J.SCITOTENV.2019.134247>

780 Carter, J. L., & White, D. A. (2009). Plasticity in the Huber value contributes to homeostasis
781 in leaf water relations of a mallee Eucalypt with variation to groundwater depth. *Tree*
782 *Physiology*, 29(11), 1407–1418.

783 Choat, B., Brodersen, C. R., & Mcelrone, A. J. (2015). Synchrotron X-ray microtomography
784 of xylem embolism in *Sequoia sempervirens* saplings during cycles of drought and
785 recovery. *New Phytologist*, 205(3), 1095–1105. <https://doi.org/10.1111/nph.13110>

786 Choat, B., Brodribb, T. J., Brodersen, C. R., Duursma, R. A., López, R., & Medlyn, B. E.
787 (2018). Triggers of tree mortality under drought Drought and forest mortality. *Nature*,
788 558, 531–539. <https://doi.org/10.1038/s41586-018-0240-x>

789 Cochard, H., Delzon, S., & Badel, E. (2015). X-ray microtomography (micro-CT): A
790 reference technology for high-resolution quantification of xylem embolism in trees.
791 *Plant, Cell and Environment*, 38(1), 201–206. <https://doi.org/10.1111/pce.12391>

792 Cohen, Y., Cohen, S., Cantuarias-Aviles, T., & Schiller, G. (2008). Variations in the radial
793 gradient of sap velocity in trunks of forest and fruit trees. *Plant and Soil*, 305(1–2), 49–

794 59. <https://doi.org/10.1007/s11104-007-9351-0>

795 Cramer, W., Guiot, J., Fader, M., Garrabou, J., Gattuso, J. P., Iglesias, A., Lange, M. A.,
796 Lionello, P., Llasat, M. C., Paz, S., Peñuelas, J., Snoussi, M., Toreti, A., Tsimplis, M. N.,
797 & Xoplaki, E. (2018). Climate change and interconnected risks to sustainable
798 development in the Mediterranean. In *Nature Climate Change* (Vol. 8, Issue 11, pp. 972–
799 980). Nature Publishing Group. <https://doi.org/10.1038/s41558-018-0299-2>

800 D'Orangeville, L., Cote, B., Houle, D., & Morin, H. (2013). The effects of throughfall
801 exclusion on xylogenesis of balsam fir. *Tree Physiology*, 33(5), 516–526.
802 <https://doi.org/10.1093/treephys/tpt027>

803 Dai, A. (2012). Increasing drought under global warming in observations and models. *Nature*
804 *Climate Change*, 3(1), 52–58. <https://doi.org/10.1038/nclimate1633>

805 Dalla-Salda, G., Martinez-Meier, A., Cochard, H., & Rozenberg, P. (2011). Genetic variation
806 of xylem hydraulic properties shows that wood density is involved in adaptation to
807 drought in Douglas-fir (*Pseudotsuga menziesii* (Mirb.)). *Annals of Forest Science*, 68(4),
808 747–757. <https://doi.org/10.1007/s13595-011-0091-1>

809 de Luis, M., Novak, K., Raventós, J., Gričar, J., Prislán, P., & Čufar, K. (2011). Cambial
810 activity, wood formation and sapling survival of *Pinus halepensis* exposed to different
811 irrigation regimes. *Forest Ecology and Management*, 262(8), 1630–1638.
812 <https://doi.org/10.1016/J.FORECO.2011.07.013>

813 Delzon, S., Douthe, C., Sala, A., & Cochard, H. (2010). Mechanism of water-stress induced
814 cavitation in conifers: Bordered pit structure and function support the hypothesis of seal
815 capillary-seeding. *Plant, Cell and Environment*, 33(12). <https://doi.org/10.1111/j.1365-3040.2010.02208.x>

817 Diffenbaugh, N. S., & Giorgi, F. (2012). Climate change hotspots in the CMIP5 global
818 climate model ensemble. *Climatic Change*, 114(3–4), 813–822.
819 <https://doi.org/10.1007/s10584-012-0570-x>

820 FAO and Plan Bleu. (2018). *State of Mediterranean Forests 2018* (FAO and Plan Bleu (ed.)).
821 <http://www.fao.org/docrep/017/i32226e/i32226e.pdf>

822 Fisher, R. A., Williams, M., da Costa, A. L., Malhi, Y., da Costa, R. F., Almeida, S., & Meir,
823 P. (2007). The response of an Eastern Amazonian rain forest to drought stress: Results
824 and modelling analyses from a throughfall exclusion experiment. *Global Change*
825 *Biology*, 13(11), 2361–2378. <https://doi.org/10.1111/j.1365-2486.2007.01417.x>

826 Flexas, J., Bota, J., Loreto, F., Cornic, G., & Sharkey, T. D. (2004). Diffusive and Metabolic
827 Limitations to Photosynthesis under Drought and Salinity in C3 Plants. *Plant Biology*, 6,

828 269–279.

829 Friedlingstein, P., Jones, M. W., O’Sullivan, M., Andrew, R. M., Hauck, J., Peters, G. P.,
830 Peters, W., Pongratz, J., Sitch, S., Le Quéré, C., DBakker, O. C. E., Canadell, J. G.,
831 Ciais, P., Jackson, R. B., Anthoni, P., Barbero, L., Bastos, A., Bastrikov, V., Becker,
832 M., ... Zaehle, S. (2019). Global carbon budget 2019. *Earth System Science Data*, 11(4),
833 1783–1838. <https://doi.org/10.5194/essd-11-1783-2019>

834 Gavinet, J., Ourcival, J. M., & Limousin, J. M. (2019). Rainfall exclusion and thinning can
835 alter the relationships between forest functioning and drought. *New Phytologist*, 223(3),
836 1267–1279. <https://doi.org/10.1111/nph.15860>

837 Gessler, A., Cailleret, M., Joseph, J., Schönbeck, L., Schaub, M., Lehmann, M., Treydte, K.,
838 Rigling, A., Timofeeva, G., & Saurer, M. (2018). Drought induced tree mortality - a tree-
839 ring isotope based conceptual model to assess mechanisms and predispositions. *New*
840 *Phytologist*, 219(2), 485–490. <https://doi.org/10.1111/nph.15154>

841 Girard, F., Vennetier, M., Guibal, F., Corona, C., Ouarmim, S., & Herrero, A. (2012). *Pinus*
842 *halepensis* Mill. crown development and fruiting declined with repeated drought in
843 Mediterranean France. *European Journal of Forest Research*.
844 <https://doi.org/10.1007/s10342-011-0565-6>

845 Granier, A. (1988). Evaluation of transpiration in a Douglas-fir stand by means of sap flow
846 measurements. *Tree Physiology*, 3(4), 309–320. <https://doi.org/10.1093/treephys/3.4.309>

847 Granier, A., Bréda, N., Biron, P., & Villette, S. (1999). A lumped water balance model to
848 evaluate duration and intensity of drought constraints in forest stands. *Ecological*
849 *Modelling*, 116(2–3), 269–283. [https://doi.org/10.1016/S0304-3800\(98\)00205-1](https://doi.org/10.1016/S0304-3800(98)00205-1)

850 Grossiord, C., Sevanto, S., Adams, H. D., Collins, A. D., Dickman, L. T., McBranch, N.,
851 Michaletz, S. T., Stockton, E. A., Vigil, M., & McDowell, N. G. (2017). Precipitation,
852 not air temperature, drives functional responses of trees in semi-arid ecosystems. *Journal*
853 *of Ecology*, 105(1), 163–175. <https://doi.org/10.1111/1365-2745.12662>

854 Grossiord, C., Sevanto, S., Borrego, I., Chan, A. M., Collins, A. D., Dickman, L. T., Hudson,
855 P. J., McBranch, N., Michaletz, S. T., Pockman, W. T., Ryan, M., Vilagrosa, A., &
856 McDowell, N. G. (2017). Tree water dynamics in a drying and warming world. *Plant*,
857 *Cell & Environment*, 40(9), 1861–1873. <https://doi.org/10.1111/pce.12991>

858 Grossiord, C., Sevanto, S., Limousin, J. M., Meir, P., Mencuccini, M., Pangle, R. E.,
859 Pockman, W. T., Salmon, Y., Zweifel, R., & McDowell, N. G. (2018). Manipulative
860 experiments demonstrate how long-term soil moisture changes alter controls of plant
861 water use. *Environmental and Experimental Botany*, 152, 19–27.

862 <https://doi.org/10.1016/j.envexpbot.2017.12.010>

863 Guillemot, J., Martin-StPaul, N. K., Dufrière, E., François, C., Soudani, K., Ourcival, J. M., &
864 Delpierre, N. (2015). The dynamic of the annual carbon allocation to wood in European
865 tree species is consistent with a combined source–sink limitation of growth: implications
866 for modelling. *Biogeosciences*, *12*(9), 2773–2790. [https://doi.org/10.5194/bg-12-2773-](https://doi.org/10.5194/bg-12-2773-2015)
867 2015

868 Guyot, A., Ostergaard, K. T., Lenkopane, M., Fan, J., & Lockington, D. A. (2013). Using
869 electrical resistivity tomography to differentiate sapwood from heartwood: Application
870 to conifers. *Tree Physiology*, *33*(2), 187–194. <https://doi.org/10.1093/treephys/tps128>

871 Hudson, P. J., Limousin, | J M, Krofcheck, | D J, Boutz, | A L, Pangle, | R E, Gehres, | N,
872 Mcdowell, | N G, & Pockman, | W T. (2017). *Impacts of long-term precipitation*
873 *manipulation on hydraulic architecture and xylem anatomy of piñon and juniper in*
874 *Southwest USA*. <https://doi.org/10.1111/pce.13109>

875 Johnson, D. M., Wortemann, R., McCulloh, K. A., Jordan-Meille, L., Ward, E., Warren, J.
876 M., Palmroth, S., & Domec, J.-C. (2016). A test of the hydraulic vulnerability
877 segmentation hypothesis in angiosperm and conifer tree species. *Tree Physiology*, *36*(8),
878 983–993. <https://doi.org/10.1093/treephys/tpw031>

879 Kawaletz, H., Mölder, I., Annighöfer, P., Terwei, A., Zerbe, S., & Ammer, C. (2014). Pot
880 experiments with woody species - A review. In *Forestry*.
881 <https://doi.org/10.1093/forestry/cpu017>

882 Klein, T., Di Matteo, G., Rotenberg, E., Cohen, S., & Yakir, D. (2013). Differential
883 ecophysiological response of a major Mediterranean pine species across a climatic
884 gradient. *Tree Physiology*, *33*(1), 26–36. <https://doi.org/10.1093/treephys/tps116>

885 Klepsch, M., Zhang, Y., Kotowska, M. M., Lamarque, L. J., Nolf, M., Schuldt, B., Torres-
886 Ruiz, J. M., Qin, D. W., Choat, B., Delzon, S., Scoffoni, C., Cao, K. F., & Jansen, S.
887 (2018). Is xylem of angiosperm leaves less resistant to embolism than branches? Insights
888 from microCT, hydraulics, and anatomy. *Journal of Experimental Botany*, *69*(22).
889 <https://doi.org/10.1093/jxb/ery321>

890 Köhler, M., Schwendenmann, L., & Hölscher, D. (2010). Throughfall reduction in a cacao
891 agroforest: tree water use and soil water budgeting. *Agricultural and Forest*
892 *Meteorology*, *150*, 1079–1089. <https://doi.org/10.1016/j.agrformet.2010.04.005>

893 Ladjal, M., Huc, R., & Ducrey, M. (2005). Drought effects on hydraulic conductivity and
894 xylem vulnerability to embolism in diverse species and provenances of Mediterranean
895 cedars. *Tree Physiology*, *25*(9), 1109–1117.

896 Lamarque, L. J., Corso, D., Torres-Ruiz, J. M., Badel, E., Brodribb, T. J., Burlett, R.,
897 Charrier, G., Choat, B., Cochard, H., Gambetta, G. A., Jansen, S., King, A., Lenoir, N.,
898 Martin-StPaul, N., Steppe, K., Van den Bulcke, J., Zhang, Y., & Delzon, S. (2018). An
899 inconvenient truth about xylem resistance to embolism in the model species for refilling
900 *Laurus nobilis* L. *Annals of Forest Science*, *75*(3). [https://doi.org/10.1007/s13595-018-](https://doi.org/10.1007/s13595-018-0768-9)
901 [0768-9](https://doi.org/10.1007/s13595-018-0768-9)

902 Leblanc, S. G., Chen, J. M., Fernandes, R., Deering, D. W., & Conley, A. (2005).
903 Methodology comparison for canopy structure parameters extraction from digital
904 hemispherical photography in boreal forests. *Agricultural and Forest Meteorology*,
905 *129*(3–4), 187–207. <https://doi.org/10.1016/j.agrformet.2004.09.006>

906 Lempereur, M., Martin-stpaul, N. K., Damesin, C., Joffre, R., Ourcival, J.-M., Rocheteau, A.,
907 & Rambal, S. (2015). Growth duration rather than carbon supply explains the stem
908 increment of *Quercus ilex*: Implication for vulnerability assessment under climate
909 change. *New Phytologist*, *33*(0), 1–42. <https://doi.org/10.1111/nph.13400>

910 Li, X., Delzon, S., Torres-Ruiz, J., Badel, E., Burlett, R., Cochard, H., Jansen, S., King, A.,
911 Lamarque, L. J., Lenoir, N., St-Paul, N. M., & Choat, B. (2020). Lack of vulnerability
912 segmentation in four angiosperm tree species: evidence from direct X-ray
913 microtomography observation. *Annals of Forest Science*, *77*(2).
914 <https://doi.org/10.1007/s13595-020-00944-2>

915 Limousin, J.-M., Longepierre, D., Huc, R., & Rambal, S. (2010). Change in hydraulic traits of
916 Mediterranean *Quercus ilex* subjected to long-term throughfall exclusion. *Tree*
917 *Physiology*, *30*(8), 1026–1036. <https://doi.org/10.1093/treephys/tpq062>

918 Limousin, J.-M., Misson, L., Lavoit, A.-V., Martin, N. K., & Rambal, S. (2010). Do
919 photosynthetic limitations of evergreen *Quercus ilex* leaves change with long-term
920 increased drought severity? *Plant, Cell & Environment*, *33*(5), 863–875.
921 <https://doi.org/10.1111/j.1365-3040.2009.02112.x>

922 Limousin, J.-M., Rambal, S., Ourcival, J.-M., Rodríguez-Calcerrada, J., Pérez-Ramos, I. M.,
923 Rodríguez-Cortina, R., Misson, L., & Joffre, R. (2012). Morphological and phenological
924 shoot plasticity in a Mediterranean evergreen oak facing long-term increased drought.
925 *Oecologia*, *169*(2), 565–577. <https://doi.org/10.1007/s00442-011-2221-8>

926 Limousin, J. M., Rambal, S., Ourcival, J. M., Rocheteau, A., Joffre, R., & Rodriguez-Cortina,
927 R. (2009). Long-term transpiration change with rainfall decline in a Mediterranean
928 *Quercus ilex* forest. *Global Change Biology*, *15*(9), 2163–2175.
929 <https://doi.org/10.1111/j.1365-2486.2009.01852.x>

- 930 Lionello, P., & Scarascia, L. (2018). The relation between climate change in the
931 Mediterranean region and global warming. *Regional Environmental Change*, 18(5),
932 1481–1493. <https://doi.org/10.1007/s10113-018-1290-1>
- 933 Lobo, A., Torres-Ruiz, J. M., Burlett, R., Lemaire, C., Parise, C., Francioni, C., Truffaut, L.,
934 Tomášková, I., Hansen, J. K., Kjær, E. D., Kremer, A., & Delzon, S. (2018). Assessing
935 inter- and intraspecific variability of xylem vulnerability to embolism in oaks. *Forest*
936 *Ecology and Management*, 424. <https://doi.org/10.1016/j.foreco.2018.04.031>
- 937 Maggard, A., Will, R., Wilson, D., & Meek, C. (2016). Response of Mid-Rotation Loblolly
938 Pine (*Pinus taeda* L.) Physiology and Productivity to Sustained, Moderate Drought on
939 the Western Edge of the Range. *Forests*, 7(12), 203. <https://doi.org/10.3390/f7090203>
- 940 Martín-Benito, D., Cherubini, P., Del Río, M., & Cañellas, I. (2008). Growth response to
941 climate and drought in *Pinus nigra* Arn. trees of different crown classes. *Trees -*
942 *Structure and Function*, 22(3), 363–373. <https://doi.org/10.1007/s00468-007-0191-6>
- 943 Martin-StPaul, N., Delzon, S., & Cochard, H. (2017). Plant resistance to drought depends on
944 timely stomatal closure. *Ecology Letters*, 20(11), 1437–1447.
945 <https://doi.org/10.1111/ele.12851>
- 946 Martin-StPaul, N. K., Limousin, J.-M., Vogt-Schilb, H., Rodríguez-Calcerrada, J., Rambal,
947 S., Longepierre, D., & Misson, L. (2013). The temporal response to drought in a
948 Mediterranean evergreen tree: comparing a regional precipitation gradient and a
949 throughfall exclusion experiment. *Global Change Biology*, 19(8), 2413–2426.
950 <https://doi.org/10.1111/gcb.12215>
- 951 Martin-Stpaul, N. K., Limousin, J. M., Rodríguez-Calcerrada, J., Ruffault, J., Rambal, S.,
952 Letts, M. G., & Misson, L. (2012). Photosynthetic sensitivity to drought varies among
953 populations of *Quercus ilex* along a rainfall gradient. *Functional Plant Biology*, 39(1),
954 25–37. <https://doi.org/10.1071/FP11090>
- 955 Maseda, P. H., & Fernandez, R. J. (2006). Stay wet or else: three ways in which plants can
956 adjust hydraulically to their environment. *Journal of Experimental Botany*, 57(15),
957 3963–3977. <https://doi.org/10.1093/jxb/erl127>
- 958 McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., Plaut, J.,
959 Sperry, J., West, A., Williams, D. G., & Yezpez, E. A. (2008). Mechanisms of plant
960 survival and mortality during drought: why do some plants survive while others succumb
961 to drought? *New Phytologist*, 178(4), 719–739. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.2008.02436.x)
962 [8137.2008.02436.x](https://doi.org/10.1111/j.1469-8137.2008.02436.x)
- 963 McDowell, Nate G., Allen, C. D., Anderson-Teixeira, K., Aukema, B. H., Bond-Lamberty,

964 B., Chini, L., Clark, J. S., Dietze, M., Grossiord, C., Hanbury-Brown, A., Hurtt, G. C.,
965 Jackson, R. B., Johnson, D. J., Kueppers, L., Lichstein, J. W., Ogle, K., Poulter, B.,
966 Pugh, T. A. M., Seidl, R., ... Xu, C. (2020). Pervasive shifts in forest dynamics in a
967 changing world. *Science (New York, N.Y.)*, 368(6494).
968 <https://doi.org/10.1126/science.aaz9463>

969 McDowell, Nathan G. (2011). Mechanisms linking drought, hydraulics, carbon metabolism,
970 and vegetation mortality. *Plant Physiology*, 155(3), 1051–1059.
971 <https://doi.org/10.1104/pp.110.170704>

972 Mencuccini, M., & Grace, J. (1995). Climate influences the leaf area/sapwood area ratio in
973 Scots pine. *Tree Physiology*, 15(1), 1–10.

974 Muller, B., Pantin, F., Génard, M., Turc, O., Freixes, S., Piques, M., & Gibon, Y. (2011).
975 Water deficits uncouple growth from photosynthesis, increase C content, and modify the
976 relationships between C and growth in sink organs. *Journal of Experimental Botany*,
977 62(6), 1715–1729. <https://doi.org/10.1093/jxb/erq438>

978 Myers, B. J. (1988). Water-stress Integral - A Link Between Short-term Stress and Long-term
979 Growth. *Tree Physiology*, 4(4), 315–323.

980 Nourtier, M., Chanzy, A., Granier, A., & Huc, R. (2011). Sap flow measurements by thermal
981 dissipation method using cyclic heating: a processing method accounting for the non-
982 stationary regime. *Annals of Forest Science*, 68(7), 1255–1264.
983 <https://doi.org/10.1007/s13595-011-0065-3>

984 Ogaya, R., & Penuelas, J. (2007). Tree growth, mortality, and above-ground biomass
985 accumulation in a holm oak forest under a five-year experimental field drought. *Plant*
986 *Ecology*, 189(2), 291–299. <https://doi.org/10.1007/s11258-006-9184-6>

987 Ogaya, R., Penuelas, J., Peñuelas, J., Csic-ceab-creaf, U. E., & Barcelona, U. A. De. (2006).
988 Contrasting foliar responses to drought in *Quercus ilex* and *Phillyrea latifolia*. *Biologia*
989 *Plantarum*, 50(3), 373–382.

990 Pangle, R. E., Limousin, J.-M., Plaut, J. A., Yopez, E. A., Hudson, P. J., Boutz, A. L., Gehres,
991 N., Pockman, W. T., & McDowell, N. G. (2015). Prolonged experimental drought
992 reduces plant hydraulic conductance and transpiration and increases mortality in a piñon-
993 juniper woodland. *Ecology and Evolution*, 5(8), 1618–1638.
994 <https://doi.org/10.1002/ece3.1422>

995 Pardos, M., Climent, J., Gil, L., & Pardos, J. A. (2003). Shoot growth components and
996 flowering phenology in grafted *Pinus halepensis* Mill. *Trees - Structure and Function*.
997 <https://doi.org/10.1007/s00468-003-0259-x>

998 Pasho, E., Julio Camarero, J., & Vicente-Serrano, S. M. (2012). Climatic impacts and drought
999 control of radial growth and seasonal wood formation in *Pinus halepensis*. *Trees*, 26(6),
1000 1875–1886. <https://doi.org/10.1007/s00468-012-0756-x>

1001 Pellizzari, E., Camarero, J. J., Gazol, A., Sangüesa-Barreda, G., & Carrer, M. (2016). Wood
1002 anatomy and carbon-isotope discrimination support long-term hydraulic deterioration as
1003 a major cause of drought-induced dieback. *Global Change Biology*, 22(6), 2125–2137.
1004 <https://doi.org/10.1111/gcb.13227>

1005 Peters, J. M. R., Gauthey, A., Lopez, R., Carins-Murphy, M. R., Brodribb, T. J., & Choat, B.
1006 (2020). Non-invasive imaging reveals convergence in root and stem vulnerability to
1007 cavitation across five tree species. *Journal of Experimental Botany*, 71(20), 6623–6637.
1008 <https://doi.org/10.1093/jxb/eraa381>

1009 Rodríguez-Calcerrada, J., Pérez-Ramos, I. M., Ourcival, J.-M., Limousin, J.-M., Joffre, R., &
1010 Rambal, S. (2011). Is selective thinning an adequate practice for adapting *Quercus ilex*
1011 coppices to climate change? *Annals of Forest Science*, 68(3), 575–585.
1012 <https://doi.org/10.1007/s13595-011-0050-x>

1013 Saunier, A., Ormeño, E., Havaux, M., Wortham, H., Ksas, B., Temime-Roussel, B., Blande, J.
1014 D., Lecareux, C., Mévy, J. P., Bousquet-Mélou, A., Gauquelin, T., & Fernandez, C.
1015 (2018). Resistance of native oak to recurrent drought conditions simulating predicted
1016 climatic changes in the Mediterranean region. *Plant Cell and Environment*.
1017 <https://doi.org/10.1111/pce.13331>

1018 Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of
1019 image analysis. In *Nature Methods* (Vol. 9, Issue 7, pp. 671–675). Nature Publishing
1020 Group. <https://doi.org/10.1038/nmeth.2089>

1021 Simioni, G., Marie, G., & Huc, R. (2016). Influence of vegetation spatial structure on growth
1022 and water fluxes of a mixed forest: Results from the NOTG 3D model. *Ecological*
1023 *Modelling*, 328, 119–135. <https://doi.org/10.1016/j.ecolmodel.2016.02.004>

1024 Skelton, R. P., Anderegg, L. D. L., Papper, P., Reich, E., Dawson, T. E., Kling, M.,
1025 Thompson, S. E., Diaz, J., & Ackerly, D. D. (2019). No local adaptation in leaf or stem
1026 xylem vulnerability to embolism, but consistent vulnerability segmentation in a North
1027 American oak. *New Phytologist*, 223(3), 1296–1306. <https://doi.org/10.1111/nph.15886>

1028 Soma, M., Pimont, F., Durrieu, S., & Dupuy, J.-L. (2018). Enhanced Measurements of Leaf
1029 Area Density with T-LiDAR: Evaluating and Calibrating the Effects of Vegetation
1030 Heterogeneity and Scanner Properties. *Remote Sensing*, 10(10), 1580.
1031 <https://doi.org/10.3390/rs10101580>

- 1032 Song, J., Wan, S., Piao, S., Knapp, A. K., Classen, A. T., Vicca, S., Ciais, P., Hovenden, M.
1033 J., Leuzinger, S., Beier, C., Kardol, P., Xia, J., Liu, Q., Ru, J., Zhou, Z., Luo, Y., Guo,
1034 D., Adam Langley, J., Zscheischler, J., ... Zheng, M. (2019). A meta-analysis of 1,119
1035 manipulative experiments on terrestrial carbon-cycling responses to global change.
1036 *Nature Ecology and Evolution*, 3(9), 1309–1320. [https://doi.org/10.1038/s41559-019-](https://doi.org/10.1038/s41559-019-0958-3)
1037 0958-3
- 1038 Timofeeva, G., Treydte, K., Bugmann, H., Rigling, A., Schaub, M., Siegwolf, R., & Saurer,
1039 M. (2017). Long-term effects of drought on tree-ring growth and carbon isotope
1040 variability in Scots pine in a dry environment. *Tree Physiology*, 37(8), 1028–1041.
1041 <https://doi.org/10.1093/TREEPHYS/TPX041>
- 1042 Torres-Ruiz, J. M., Cochard, H., Mencuccini, M., Delzon, S., & Badel, E. (2016). Direct
1043 observation and modelling of embolism spread between xylem conduits: a case study in
1044 Scots pine. *Plant Cell and Environment*, 39(12), 2774–2785.
1045 <https://doi.org/10.1111/pce.12840>
- 1046 Tyree, M T, & Sperry, J. S. (1989). Vulnerability of Xylem to Cavitation and Embolism.
1047 *Annual Review of Plant Physiology and Plant Molecular Biology*, 40(1), 19–36.
1048 <https://doi.org/10.1146/annurev.pp.40.060189.000315>
- 1049 Tyree, Melvin T., & Ewers, F. W. (1991). The hydraulic architecture of trees and other woody
1050 plants. *New Phytologist*, 119(3), 345–360. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.1991.tb00035.x)
1051 8137.1991.tb00035.x
- 1052 Ward, E. J., Domec, J. C., Laviner, M. A., Fox, T. R., Sun, G., McNulty, S., King, J., &
1053 Noormets, A. (2015). Fertilization intensifies drought stress: Water use and stomatal
1054 conductance of *Pinus taeda* in a midrotation fertilization and throughfall reduction
1055 experiment. *Forest Ecology and Management*, 355, 72–82.
1056 <https://doi.org/10.1016/j.foreco.2015.04.009>
- 1057 Welles, J. M., & Norman, J. M. (1991). (1991) *Instrument for Indirect Measurement of*
1058 *Canopy Architecture (AJ)*.
- 1059 Wiedemann, A., Marañón-Jiménez, S., Rebmann, C., Herbst, M., & Cuntz, M. (2016). An
1060 empirical study of the wound effect on sap flux density measured with thermal
1061 dissipation probes. *Tree Physiology*, 36(12), 1471–1484.
1062 <https://doi.org/10.1093/treephys/tpw071>
- 1063 Wu, Z., Dijkstra, P., Koch, G. W., Peñuelas, J., & Hungate, B. A. (2011). Responses of
1064 terrestrial ecosystems to temperature and precipitation change: A meta-analysis of
1065 experimental manipulation. In *Global Change Biology* (Vol. 17, Issue 2, pp. 927–942).

1066 <https://doi.org/10.1111/j.1365-2486.2010.02302.x>

1067 Wullschleger, S. D., & Hanson, P. J. (2006). Sensitivity of canopy transpiration to altered
1068 precipitation in an upland oak forest: evidence from a long-term field manipulation
1069 study. *Global Change Biology*, *12*(1), 97–109. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2486.2005.001082.x)
1070 [2486.2005.001082.x](https://doi.org/10.1111/j.1365-2486.2005.001082.x)

1071 Zhang, Q., Jia, X., Shao, M., Zhang, C., Li, X., & Ma, C. (2018). Sap flow of black locust in
1072 response to short-Term drought in southern Loess Plateau of China. *Scientific Reports*,
1073 *8*(1), 1–10. <https://doi.org/10.1038/s41598-018-24669-5>

1074 Zhu, S.-D., Liu, H., Xu, Q.-Y., Cao, K.-F., & Ye, Q. (2016). Are leaves more vulnerable to
1075 cavitation than branches? *Functional Ecology*, *30*(11), 1740–1744.
1076 <https://doi.org/10.1111/1365-2435.12656>

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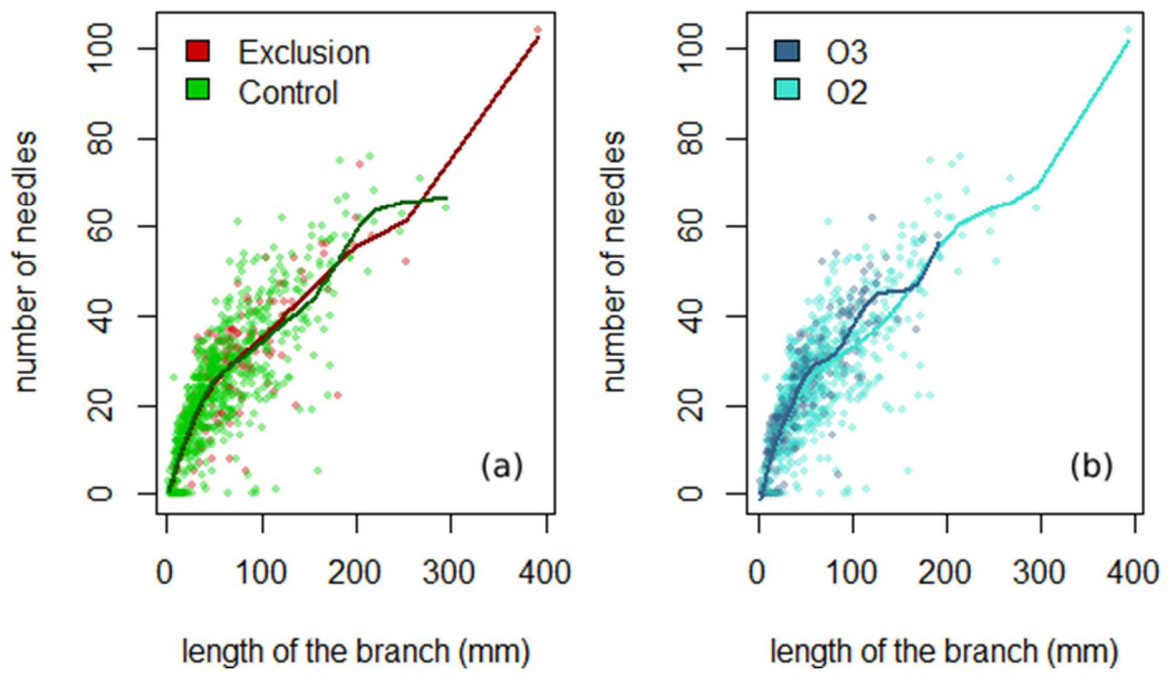
Acknowledgements:

The data analysis and writing for this study were supported by the French Environment and Energy Management Agency (ADEME) in the form of a PhD scholarship. During the long study period, experiments were funded by the French National Research Institute for Agriculture, Food and Environment (INRAE), the Agence Nationale pour la Recherche (ANR project Drought+), the long term French observatory networks SOERE F-ORE-T and Analysis and Experimentation on Ecosystems (AnaEE). The authors thank Damien Gounelle, Arnaud Jouineau, Jean-Michel Lopez, Mathieu Audouard, and Roland Esteve, from INRAE, for their contributions to the field work. The authors also thank the INRAE PHENOBOIS centre (Clermont-Ferrand, France) and Pierre Conchon for help with the X-ray microtomography analysis.

1134 **Appendices**

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1138 **Fig. A1.** Number of needles vs branch length compared between (A) rainfall exclusion and control trees and (B)
1139 branch architectural order (O2 vs O3). The lines are general additive models fitted to the data and show that the
1140 relationship between needle number and branch length does not depend on rainfall or architectural order. The data
1141 were collected in autumn 2009 on a sample of 953 branches that grew in 2008 and 2009.

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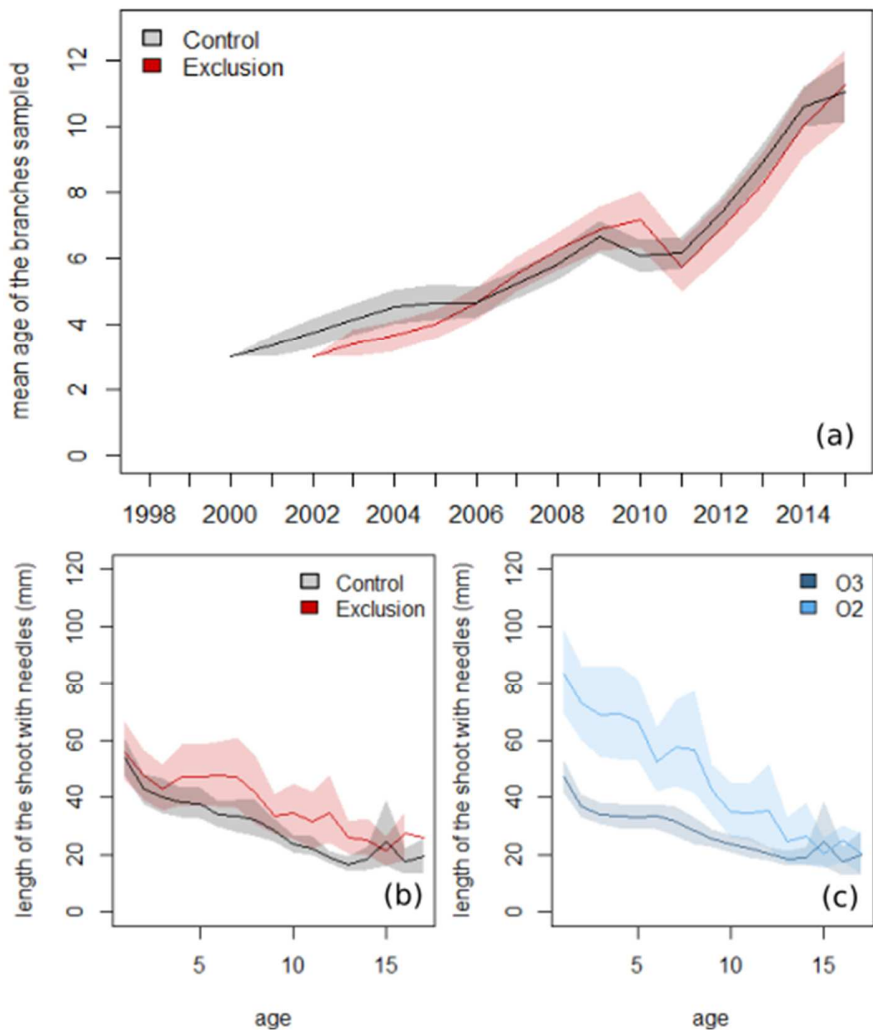
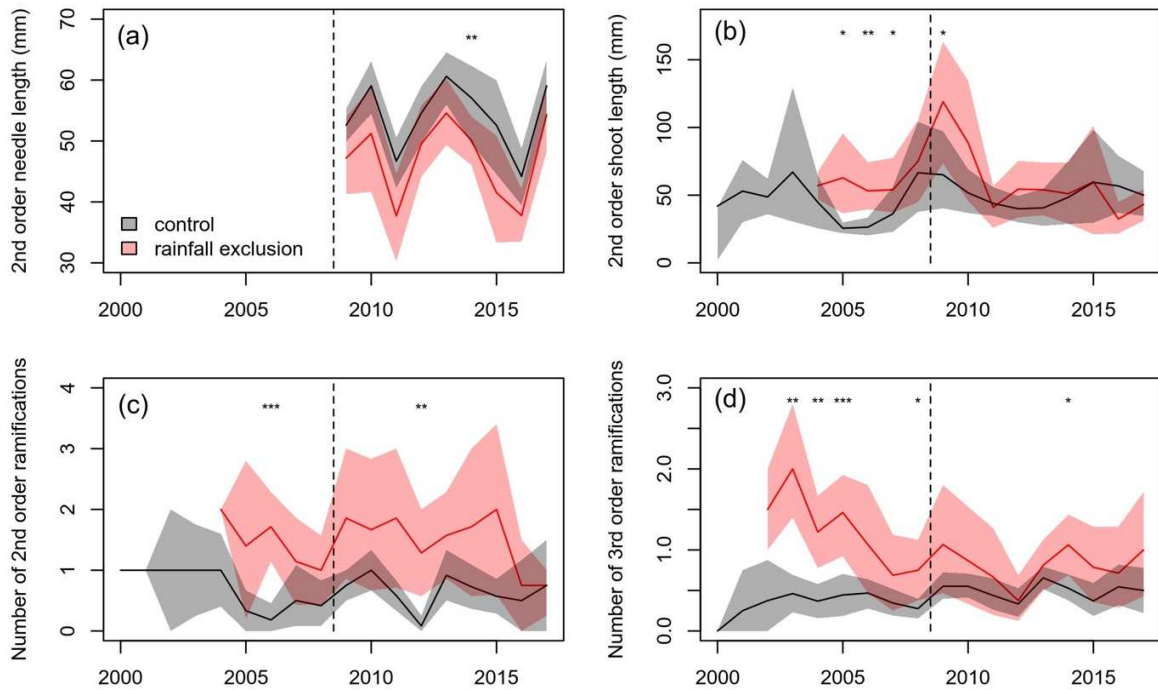


Fig. A2. (a) Mean age of the branches sampled in the control and exclusion plots. (b, c) Effect of branch age on the needle-carrying shoot length of *Pinus halepensis* trees (b) in the control and exclusion plots and (c) in terms of to the architectural order of the branch.

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1177 **Fig. A3.** Time evolutions of (a) the needle length and (b) the shoot length of second order branches and the (c)
1178 number of ramifications of second and (d) third order branches of *Pinus halepensis* in the control (black) and rainfall
1179 exclusion (red) plots. Significant differences between treatments are displayed for $P < 0.001$ (***), $P < 0.01$ (**) and
1180 $P < 0.05$ (*).

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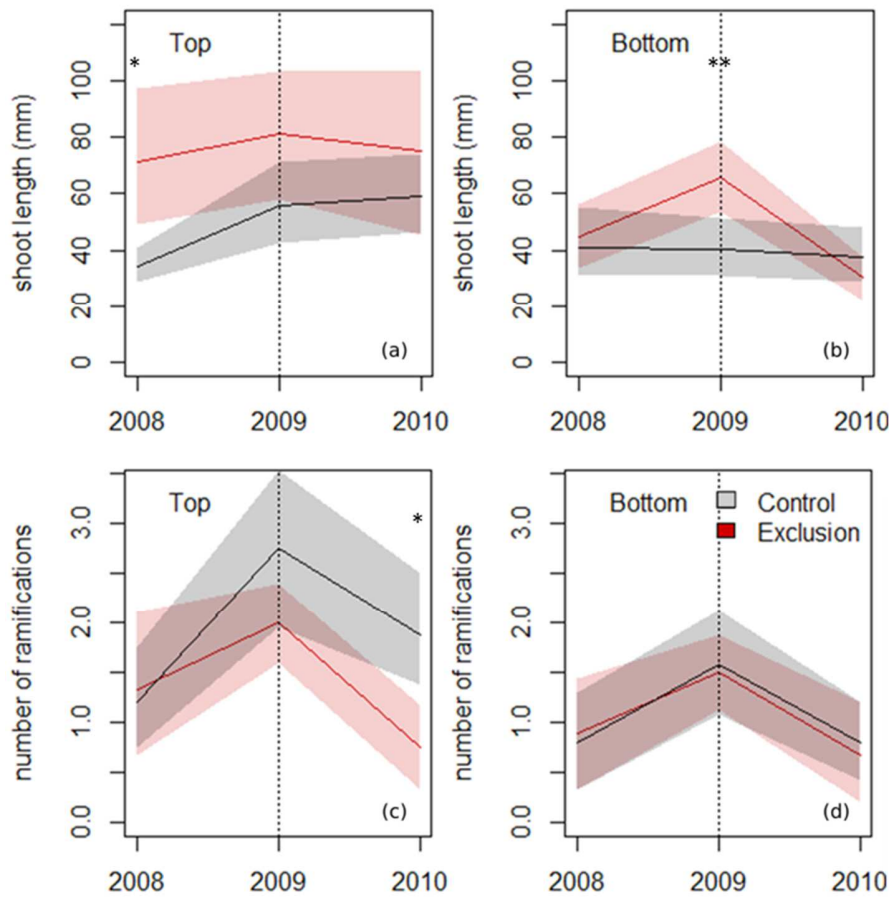
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1189 **Fig. A4.** Time evolutions of (a, b) the shoot length and (c, d) the number of ramifications of *Quercus ilex* branches

1190 (a, c) at the top and (b, d) at the bottom of the canopy in the control (black) and rainfall exclusion (red) plots.

1191 Significant differences between treatments are displayed for $P < 0.001$ (***), $P < 0.01$ (**) and $P < 0.05$ (*).

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