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Declining hydraulic performances and low carbon investments
predate Scots pine drought-induced mortality as inferred from
wood anatomy

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22 Abstract

23 Widespread episodes of drought-associated tree mortality are predicted to become more
24 frequent as climate becomes warmer and drier. Nevertheless, growth trends and their
25 links to changes in wood anatomical features before tree death occurs are still poorly
26 understood. Wood anatomical features may provide valuable information that can be
27 extracted and used retrospectively to infer the mechanisms leading to tree death. In this
28 study we use this approach to characterize drought-induced mortality events affecting two
29 Scots pine (*Pinus sylvestris*) sites (Prades and Arcalís) located in the North Eastern
30 Iberian Peninsula. To this end, co-occurring now-dead and living Scots pine trees were
31 sampled and their wood anatomical features were measured and compared. We aimed to
32 detect differences in anatomical features between living and dead trees, and to infer past
33 physiological performances that might have determined their subsequent death or
34 survival. Now-dead trees showed lower tracheid and resin duct production, and smaller
35 radial lumen diameters than co-occurring living trees. At the more xeric Prades site, these
36 anatomical differences were large and chronic, *i.e.* were observed over the three studied
37 decades, while they were less pronounced at the other, more mesic Arcalís site, where
38 **tree mortality episodes were more recent. This indicates that dead trees' hydraulic**
39 conductivity was severely affected and that carbon investment in xylem formation and
40 resin duct production was constrained prior to tree death. Our findings show that both
41 hydraulic deterioration and low carbon allocation to xylem formation were associated to
42 drought-induced mortality in Scots pine. Nevertheless, the temporal dynamics of these
43 processes differed between populations as a function of site climatic conditions.

44 Keywords: Scots pine; mortality; drought; tree ring; tracheid; wood anatomy.

45 Introduction

46 Episodes of drought-associated tree mortality have been reported in all major forest
47 biomes of the Earth (Allen et al., 2010), and are likely to become more frequent under a
48 progressively warmer and drier climate (IPCC, 2013). Consequently, understanding the
49 mechanisms that underlie tree mortality has become a research priority in drought-prone
50 areas. In Mediterranean regions like the Iberian Peninsula, water availability is the main
51 limiting factor for tree growth (Cherubini et al., 2003; Martínez-Vilalta et al., 2008 and
52 references therein). In these regions, temperature and evapotranspiration have increased
53 during the last decades, in concert with the frequency and intensity of severe droughts
54 (Piñol et al., 1998; IPCC, 2013). Thus, Mediterranean forests are considered to be
55 especially vulnerable to this predicted increase of severe drought events (Giorgi and
56 Lionello, 2008). This is particularly the case for forests dominated by species reaching
57 **their southern (and dry) distribution limit in this region (e.g., Martínez-Vilalta et al.,**
58 **2012; Matías and Jump, 2012).**

59 The mechanisms that underlie drought-induced tree mortality are still poorly
60 understood and highly debated (McDowell et al., 2008, 2011; Sala et al., 2010;
61 McDowell and Sevanto, 2010; McDowell, 2011; Sevanto et al., 2014). The efficiency and
62 safety of the water transport through the xylem is critical for tree performance, especially
63 under stressful climatic conditions such as those imposed by droughts (e.g., Choat et al.,
64 2012). Trees growing in dry areas must maintain a functional water transport system by
65 keeping the xylem water potentials above cavitation thresholds when facing drought
66 events (Bréda et al., 2006; Brodribb and Cochard, 2009). In the case of conifers, building
67 tracheids with narrow lumens and thick walls might be advantageous under these

68 circumstances, as the risk of cavitation and cell collapse generally decreases with smaller
69 lumen diameter and thicker cell walls (Hacke et al., 2001; Cochard et al., 2004). Conduit
70 lumen size is reduced when trees face water stress, because radial enlargement of
71 tracheids is particularly sensitive to water deficit (Hsiao and Acevedo, 1974; von Wilpert,
72 1991). However, tracheids that have a reduced lumen diameter are less efficient for water
73 transport, as the hydraulic conductivity increases with the fourth power of lumen
74 diameter according to the Hagen-Poiseuille law (Tyree and Zimmermann, 2002). Finally,
75 prolonged water deficit can affect tracheid division and xylogenesis to such a degree that
76 later on precipitations might not be able to compensate for past cumulative stress. As a
77 consequence, narrow rings formed by low numbers of narrow tracheids are built,
78 resulting into reduced water supply to the crown (Zweifel et al., 2006). The formation of
79 narrow rings involves a low carbon investment in radial growth. Regardless of whether
80 this low carbon investment under drought reflects low carbon availability, or simply the
81 direct effect of lower water availability (Hsiao and Acevedo, 1974; Sala et al., 2010), it
82 **has relevant implications for the tree's** carbon economy and for the likelihood of carbon
83 starvation (McDowell, 2011).

84 **Defence is another important component of the tree's** response to drought stress
85 particularly when biotic agents are involved (e.g., Gaylord et al., 2013). Resin ducts are a
86 key component of tree defence against biotic agents (Paine et al., 1997; Rigling et al.,
87 2003). Low resin-duct production under drought might reflect reduced carbon availability
88 to defence (McDowell et al., 2008; Sala et al., 2010) and increases the vulnerability of
89 trees to biotic attack (Kane and Kolb, 2010; Gaylord et al., 2013).

90 Wood anatomy studies on dead/dying vs. living trees are still scarce (but see
91 **Levanič** et al., 2011), yet they can bring valuable information about mortality processes,
92 because xylem represents a reliable and long-term proxy of hydraulic performance. A
93 retrospective analysis of the potential hydraulic performance of trees is feasible through
94 wood-anatomical analyses, which constitute a powerful tool for investigating tree
95 responses to past stress conditions (Vaganov et al., 2006) at a higher temporal resolution
96 than tree rings (Fritts, 2001). The environmental conditions during wood formation
97 **determine xylem cells' features, leaving permanent imprints at the conduit level (Denne**
98 **and Dodd, 1981; Wimmer, 2002; Fonti et al., 2010).** The long-term theoretical hydraulic
99 performance of a tree can be thus reconstructed through the analyses of anatomical
100 features of transversal wood sections.

101 Several episodes of drought-induced mortality of Scots pine (*Pinus sylvestris* L.)
102 have been reported in Europe over the last decades (Martínez-Vilalta and Piñol, 2002;
103 Bigler et al., 2006; Eilmann et al., 2006; Galiano et al., 2010; Camarero et al., 2012;
104 **Hereş** et al., 2012; Vilà-Cabrera et al., 2013; Rigling et al., 2013). Scots pine is a boreal
105 tree species, considered the most widely distributed conifer in the world (Nikolov and
106 Helmisaari, 1992). It reaches its south-western (and dry) distribution limit in the Iberian
107 Peninsula (Barbéro et al., 1998), where about half of its range is represented by natural
108 populations (Catalan Bachiller et al., 1991; Martín et al., 2010). **Scots pine is a “drought-**
109 **avoiding” species, with a relatively high vulnerability to** xylem embolism (Cochard,
110 1992) and a fast stomatal response to reduce evaporative water loss under drought
111 conditions (Irvine et al., 1998; Poyatos et al., 2013). Consistent with its wide distribution,

112 it shows high intraspecific variability in many traits, including wood anatomical ones
113 (Martín et al., 2010).

114 In this study, we compare the wood anatomy of co-occurring now-dead and living
115 Scots pine trees sampled at two climatically contrasted sites located in North Eastern
116 Iberian Peninsula. The analyses are conducted at an annual resolution for a period of 34
117 years (1975 to 2008). Previous studies on the same individuals showed that tree mortality
118 was associated with severe drought periods, and that now-dead individuals started to
119 grow less than their **surviving neighbours 15 to 40 years before death (Hereş et al., 2012)**.
120 Our main objective here was to compare the stem xylem structure of now-dead trees and
121 their surviving neighbours during the period previous to death. We retrospectively
122 describe wood anatomical variability in terms of hydraulic conductivity and carbon
123 investment in xylem structure and defence. More specifically, we aimed at establishing
124 whether now-dead trees were more vulnerable to xylem embolism than living ones, had
125 an impaired hydraulic system due to the production of very narrow tracheids or reduced
126 their carbon investment in the xylem, which could reflect low carbon availability.

127

128 Materials and methods

129 *Study sites*

130 Two Scots pine sites located in the North Eastern Iberian Peninsula were selected: Prades
131 (**Prades Mountains, 41° 19'N, 1° 0'E**) and Arcalís (**Soriguera, Central Pyrenees, 42°**
132 **22'N, 1° 11'E**). **At these two sites, high mortality rates following particularly dry years**
133 have been observed starting in the 1990s (Martínez-Vilalta and Piñol, 2002; Galiano et
134 al., 2010; Poyatos et al., 2013). In addition, a direct association between tree mortality

135 and severe drought periods characterized by low summer water availability has been
136 reported (Hereş et al., 2012). The climate in Prades is typically Mediterranean while in
137 Arcalís it is characterized by cool-summer Mediterranean conditions (Köppen, 1936).
138 Mean annual temperature in Prades is around 11.2°C, and the mean annual rainfall is 611
139 mm. In Arcalís, the mean annual temperature is lower (9.7°C), and the mean annual
140 rainfall is slightly higher (653 mm) than in Prades (Climatic Digital Atlas of Catalonia,
141 period [1951](#)–2006 in both cases) (Pons, 1996; Ninyerola et al., 2000). Since the 1970s
142 there has been a significant warming trend in both study sites, while drier conditions have
143 been more frequent recently in Arcalís (Supplementary Figure 1). The vegetation type at
144 the two sites follows an altitudinal gradient, with Mediterranean species at low altitudes
145 and Scots pine appearing above 800 m in Prades and between 600 and 1500 m in Arcalís.
146 The soils in Prades are xerochrepts with a clay-loam texture (Hereter and Sánchez, 1999),
147 and in Arcalís they are calcareous with a clay-loam texture (Galiano et al., 2010). [-At](#)
148 both sites, soils have a low water retention capacity.

150 *Field sampling*

151 Scots pine trees used in this study were sampled in late autumn 2008 (Prades) and early
152 spring 2009 (Arcalís) along constant altitude transects (1000 m a.s.l for both sites) located
153 on north facing slopes. Sampling consisted in coring co-occurring living and dead
154 individuals at breast height (1.3 m), using increment borers, orthogonally to the slope.
155 Here, we use 20 trees (10 per site, i.e. five living and five dead trees) that were visually
156 cross-dated using pointer years in a previous study, from where basal area increment
157 (BAI, cm²) and **tree-ring width (mm) data were available** (Hereş et al., 2012). [Dead](#)

158 | [individuals used in this study](#) died (last ring formed) in the 2000s ([between 2001 and](#)
159 | [2008](#)). Living and [dead trees did not differ significantly in terms of diameter at breast](#)
160 | [height \(DBH\) neither in Prades \(P = 0.793, mean ± SD = 32.0 ± 5.1 cm\) nor in Arcali's](#)
161 | [\(P = 0.533, mean ± SD = 33.0 ± 5.0 cm cm\). The age of the living and dead trees was](#)
162 | [also similar both in Prades \(P = 0.144, mean ± SD = 98 ± 30 years\) and Arcali's \(P =](#)
163 | [0.411, mean ± SD = 69 ± 9 years\). Sampled trees were separated from each other or from](#)
164 | [other adult Scots pines, by a distance of at least 5 m. See Heres, et al. \(2012\) for](#)
165 | [additional sampling details.](#)

166

167 *Wood anatomy measurements*

168 The segments of the cores that included the 1975–2008 period were separated into small
169 blocks (about 1 cm long) that were further cut transversally with a sliding microtome
170 (Leica SM 2010R; Leica Microsystems, Germany) to obtain thin wood sections (12–18
171 μm thick). These sections were stained with a mixture of safranin (0.5%) and astrablue
172 (1%) to get a better contrast between tracheid lumen and walls, dehydrated repeatedly in
173 an alcohol concentration gradient (50% to 96%), and mounted using a synthetic resin
174 (Eukitt; Merck, Darmstadt, Germany) onto permanent glass microscope slides. Images of
175 | the [thin wood](#) sections were taken at magnifications of $\times 40$ using a camera (Leica
176 | DFC290; Leica Microsystems, Germany) attached to a transmitted light microscope
177 | (Olympus BH2; Olympus, Hamburg, Germany). When tree rings were too wide to fit in
178 | one image, several adjacent pictures were taken and then merged using Adobe Photoshop
179 | CS4 (Adobe Systems; San Jose, USA). The images were later on used to analyze a total
180 | of 646 annual rings using DACiA (Dendrochronological Analysis of Conifer Wood

181 Anatomy), a new Matlab[®]-based (version 7.10 R2010a, MathWorks, Natick, MA)
182 software developed specifically for this study (see next section on [Software used to](#)
183 [quantify wood anatomy, DACiA](#)).

184 Tracheids were measured along three to five complete radial rows per ring.
185 Measured variables, analyzed at the whole ring level (RW) and separately for the
186 earlywood (EW) and latewood (LW), included number of tracheids, radial lumen
187 diameter (LD) and cell-wall thickness (CWT). The visual identification of LW, based on
188 the abrupt shifts in colour and tracheid size, was preferred over the delineation based on
189 the Mork index (Denne, 1988), which proved to largely overestimate LW in our samples.
190 Radial dimensions were chosen because they vary along time, while the tangential
191 dimensions are effectively constant (Vysotskaya and Vaganov, 1989). Using the
192 measured anatomical features listed above, we calculated the theoretical hydraulic
193 conductivity (K_h) according to the Hagen-Poiseuille law (Tyree and Zimmermann, 2002),
194 the $(CWT/LD)^2$ ratio (Hacke et al., 2001), here used as a surrogate of the xylem
195 vulnerability to embolism, and the tracheid carbon cost investments (C_{cost}). We are fully
196 aware that the $(CWT/LD)^2$ ratio may be a biased predictor of vulnerability to xylem
197 cavitation in dying trees, but we assumed it is a reasonable anatomical proxy of xylem
198 resistance against embolism (Hacke et al., 2001). C_{cost} was estimated by multiplying the
199 number of tracheids by CWT for each tree ring. In order to estimate the carbon allocation
200 for defence ([Kane and Kolb, 2010](#)), we counted the number of resin ducts produced per
201 annual ring (RD).

202 A selection of the measured [wood](#) anatomical features was used in further
203 analyses, their subscript indicating if they refer to RW, EW or LW (Table 1). This

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204 selection was mainly based on the concept that EW has predominantly a water conductive
205 function, while LW has mainly a mechanical one (Eilmann et al., 2006; Vaganov et al.,
206 2006).

207

208 *Software used to quantify wood anatomy (DACiA)*

209 To obtain the [wood](#) anatomical features across transversal wood sections, we developed a
210 new semi-automatic Matlab®-based software (DACiA), which is available upon request.

211 Based on state-of-the-art thresholding techniques, the software automatically identifies
212 the tracheid features of the radial rows initially marked along the tree rings, using
213 segmented flexible lines. Further on, a manual procedure corrects pixel by pixel possible
214 measurement errors through an interactive graphic interface that helps to precisely
215 delimitate tracheid lumens and walls. Finally, the software exports the measured
216 anatomical features directly into their corresponding units of measurements to an Excel©
217 or plain text file.

218

219 *Climatic and environmental data*

220 Monthly mean temperature and total precipitation values (period 1975–2006) were
221 modelled at a spatial resolution of 180 m from discrete climatic data provided by the
222 Spanish Meteorological Agency (www.aemet.es, accessed 18 December 2013) (Ninyerola
223 et al., 2007a, 2007b). Missing data for the 2007 and 2008 years were estimated by means
224 of regression models using a second climatic dataset that was available from the Catalan
225 Weather Service (www.meteo.cat, accessed 23 July 2013). Based on the available
226 climatic data, the ratio between precipitation (P) and potential evapotranspiration (PET)

227 was calculated (P/PET) and used as a drought index. The PET was estimated using the
228 Hargreaves method (Hargreaves and Samani, 1982).

229 Preliminary correlation analyses had shown that corresponding RW, EW and LW
230 wood anatomical features responded to climatic variables averaged over different time
231 periods. On the basis of these results and xylogenesis studies on Scots pine (Camarero et
232 al., 1998, 2010), three different sets of P/PET measures, obtained from the meteorological
233 data, were used in further analyses, covering the following time intervals: 1) from August
234 (previous to growth year) to October of the year of tree-ring formation (named current
235 year) for RW wood anatomical features (P/PET_{RW}); 2) from August (previous to growth
236 year) to current June for EW wood anatomical features (P/PET_{EW}), and 3) from current
237 May to current October for LW wood anatomical features (P/PET_{LW}).

238 Additionally, the standardized precipitation evapotranspiration index (SPEI)
239 (Vicente-Serrano et al., 2010a, 2010b) was used to explore the correlations between
240 drought and the measured wood anatomical features at different time scales (1 to 12
241 months). The SPEI is a multi-scalar drought index that accounts for both the effects of
242 temperature and precipitation on drought severity. The lower the SPEI value is, the drier
243 the conditions are (Vicente-Serrano et al., 2010a, 2010b). Based on previous analyses
244 ([Supplementary Figure 2](#); Pasho et al. 2011), different SPEI time intervals were
245 considered and used in further analyses to quantify time-dependent responses of wood
246 anatomical features to drought stress (Supplementary Table 1).

247 Values of the CO₂ atmospheric concentration (C_a) were also used to account for
248 their potential effects on the measured wood anatomical features. They were taken from
249 the literature for the 1975 to 2003 period (Robertson et al., 2001a; McCarroll and Loader,

250 2004), and were estimated by means of linear regressions, based on the above mentioned
251 datasets, for the 2004–2008 period.

252

253 *Statistical analyses*

254 All variables were first checked for normality (Kolmogorov–Smirnov test) and logarithm
255 transformed when necessary (No. tracheids_{RW}, K_{hRW} , K_{hEW} , $(CWT/LD)_{EW}^2$, C_{costRW} ,
256 BAI). In the case of RD_{RW} (a count response variable) no transformation was applied, but
257 a Poisson generalized mixed model was used (see below). The No. tracheids_{RW} variable
258 was not normalized to a standard number for all trees (Vaganov, 1990), as raw data
259 clearly showed large differences for this variable between living and dead trees.

260 Independent samples *t*-tests were used to analyse differences in DBH and age
261 between living and dead trees from Prades and Arcalís. Pearson and Spearman correlation
262 coefficients (the Spearman coefficient was used only in the case of RD_{RW}) were used to
263 quantify the associations between wood anatomical features and climatic variables
264 (temperature, precipitation and SPEI), while linear regressions were conducted to assess
265 temporal trends of annual climatic variables ([Supplementary Figure 1](#)). To evaluate the
266 time-related variability of each of the selected wood anatomical features, the coefficient
267 of variation (CV) was calculated by dividing the standard deviation of each variable by
268 its mean.

269 We used linear mixed-effects models to analyse the time trends of the wood
270 anatomical features (No. tracheids_{RW}, LD_{EW} , CWT_{LW} , K_{hEW} , $(CWT/LD)_{EW}^2$ and C_{costRW}),
271 the influence of the environmental variables (C_a and P/PET or SPEI) on them and the
272 relationship between BAI and wood anatomical features (No. tracheids_{RW}, LD_{RW} ,

273 CWT_{RW}, K_{hRW} and C_{costRW}). A first set of models was fitted for each of the studied
274 anatomical variables (No. tracheids_{RW}, LD_{EW}, CWT_{LW}, K_{hEW}, (CWT/LD)²_{EW} and C_{costRW})
275 to study their time trends. In each case, the fixed part of the model included the effects of
276 tree condition (living vs. dead tree), [site](#) (Prades, Arcalís), the interaction condition × site,
277 the covariate year (from 1975 to 2008), and the interactions condition × year, site × year,
278 and condition × site × year.

279 A second set of models was fitted to analyze wood anatomy features (No.
280 tracheids_{RW}, LD_{EW}, CWT_{LW}, K_{hEW}, (CWT/LD)²_{EW} and C_{costRW}) as a function of
281 condition, site, the interaction condition × site, the covariate C₀ and its interactions
282 condition × C₀, site × C₀, and condition × site × C₀, and the covariate P/PET (or SPEI)
283 and its interactions condition × P/PET (or SPEI), site × P/PET (or SPEI), and condition ×
284 site × P/PET (or SPEI).

285 Finally, a third set of models was fitted to study the response of BAI to different
286 wood anatomical features (No. tracheids_{RW}, LD_{RW}, CWT_{RW}, K_{hRW} or C_{costRW}). In each
287 case, the fixed part of the models included the effect of condition, site, the interaction
288 condition × site, the corresponding wood anatomical feature and its interactions with
289 condition, site, and condition × site.

290 In the case of the RD_{RW}, three Poisson generalized mixed models were fitted. The
291 first model accounted for RD_{RW} time trends, and the second model evaluated the
292 influence of the environmental variables (C₀ and P/PET or SPEI) on RD_{RW}. These two
293 models had the same structures as described above for the other selected wood
294 anatomical features. The third model accounted for the relationship between RD_{RW} and
295 ring width, including the fixed effects of condition, site, the interaction condition × site,

296 the covariate ring width, and the interactions condition × ring width, site × ring width,
297 and condition × site × ring width.

298 In all mixed-effects models, tree identity was introduced as random effect and a
299 first-order autoregressive covariance structure was used to account for temporal
300 autocorrelation. To characterize differences between living and dead trees, the estimated
301 marginal means were analysed, applying a Bonferroni correction to compare the main
302 effects. If second order interactions were significant, separate relationships for every site
303 were considered. In all cases, coefficients were estimated using restricted maximum
304 likelihood methods (REML), and relationships were considered significant at $P < 0.05$.
305 Statistical analyses were carried out with SPSS (version 15.0, SPSS Inc., Chicago, IL) or
306 R (version 3.0 packages, The R Foundation for Statistical Computing 2013).

307

308 Results

309 *Patterns and trends of wood anatomical features in living and dead trees*

310 The majority of wood anatomical features presented a significant negative time trend, the
311 values of the dead trees being usually lower than those of the living trees, particularly at
312 the more xeric Prades site (Figure 1, Tables 1 and 2). Overall, statistically significant
313 differences ($P < 0.01$) were found between the living and dead trees for most anatomical
314 features (No. tracheids_{RW}, LD_{EW}, K_{hEW}, C_{costRW} and RD_{RW}) (Figure 1, Table 2). For all
315 the aforementioned features, the predicted values (estimated marginal means) were
316 always lower for the dead than for the living trees (results not shown). No statistically
317 significant differences were found between living and dead trees in the case of the
318 CWT_{LW} ($P = 0.086$) and (CWT/LD)²_{EW} features ($P = 0.454$). In Prades, the temporal

319 variability (CV) of the wood anatomical features was always higher for the dead trees as
320 compared with their living counterparts, whereas this was also observed in Arcalís except
321 for LD_{RW} , CWT_{RW} and CWT_{LW} (Table 1).

322 The intercept and slope of the significant positive relationship between the
323 number of resin ducts RD_{RW} and tree-ring width were similar for living and dead trees in
324 Arcalís, whereas in Prades the intercept tended to be slightly higher for living trees and
325 the slope was steeper for dead trees (Figure 2, Supplementary Table 2).

326

327 *Environmental influences on wood anatomical features*

328 Both C_0 and P/PET significantly influenced most wood anatomical features. C_0 had a
329 predominantly negative effect, while P/PET had a predominantly positive one (Table 3).

330 The significant effects of P/PET did not depend on condition, site or the condition by site
331 interaction for the majority of analyzed wood anatomical features (No. tracheids $_{RW}$,
332 CWT_{LW} , K_{hEW} , $(CWT/LD)_{EW}^2$ and C_{costRW}). In the case of the LD_{EW} , the significant effect
333 of P/PET depended only on site (Table 3). The significant effects of C_0 did not depend on
334 condition, site or the condition by site interaction for most wood anatomical features (No.
335 tracheids $_{RW}$, C_{costRW} , RD_{RW}) (Table 3). In the case of CWT_{LW} , the significant relationship
336 with C_0 depended on the condition and its interaction with site (Table 3). For this
337 anatomical feature, the two sites differed significantly between them ($P < 0.05$), with
338 Prades presenting a significant positive difference between living and dead trees ($P < 0.01$)
339 (results not shown). The association between C_0 and K_{hEW} depended on site, but not on
340 tree condition (Table 3).

341 The results of linear mixed-effects models were very similar if SPEI was used
342 instead of P/PET to characterize climatic stress (Supplementary Table 3). Again, as in the
343 case of P/PET most wood anatomical features were positively related to the SPEI drought
344 index_r (Supplementary Table 3). In general, the strongest relationships between wood
345 anatomical features and SPEI were observed from May to August, and dead trees tended
346 to respond over longer time scales (by ca. 3 months) than living trees for most anatomical
347 features (Supplementary Table 1, Supplementary Figure 2). This means that dead trees
348 showed a higher responsiveness in anatomical terms to long-duration droughts than living
349 trees.

350

351 *BAI association with wood anatomical features*

352 BAI was significantly and positively related to several wood anatomical features
353 (Supplementary Table 4), and this association was particularly strong with [No.](#)
354 [tracheids_{RW}](#) (Figure 3). For all the relationships of BAI with wood anatomical features,
355 the estimated marginal means were always lower for the dead than for the living trees
356 (results not shown), but this difference was significant only for LD_{RW} and CWT_{RW}
357 ($P < 0.01$ in both cases).

358

359 Discussion

360 Surviving and now-dead Scots pine trees from Prades and Arcalís showed significant
361 differences in their wood anatomical features in response to drought stress. Now-dead
362 individuals usually presented smaller tracheids and a lower tracheid and resin ducts
363 production per growth ring than living trees, indicating that lower hydraulic capacity and

364 reduced investment of carbon into growth and defence characterized these mortality
365 processes. Our results bring support to the idea that tree mortality is a complex process
366 associated to changes in the carbon and water economy (McDowell, 2011; McDowell et
367 al., 2011; Sevanto et al., 2014). It should be noted, however, that the two study sites
368 showed important differences between them. In Prades, living and now-dead trees
369 showed a long-term divergent hydraulic performance (chronic decline), whereas this
370 divergence was less accentuated and more recent in Arcalís (acute decline).

371 High growth variability has been associated to increased mortality risks (e.g.,
372 Ogle et al., 2000). Our results show that lower and more variable growth in now-dead
373 compared to surviving individuals at the studied sites (Hereş et al., 2012) is associated to
374 a higher variability in wood anatomical traits in now dead trees. This is consistent with
375 Levanič et al. (2011), who also found a greater variability for the anatomical features of
376 dying pedunculate oak (*Quercus robur* L.) trees in comparison with surviving individuals
377 of the same species. Nevertheless, and in contrast to our findings, dying pedunculate oak
378 trees studied by Levanič et al. (2011) presented wider conduits and higher specific
379 hydraulic conductivity than surviving individuals until five years before death.

380 Our results show lower hydraulic conductivity in the stem xylem of now-dead
381 trees, reflecting a lower water transport capacity over the whole studied period,
382 particularly at the more xeric Prades site. This lower hydraulic capacity at the growth ring
383 level does not necessarily translate into lower capacity to supply leaves with water, as
384 concurrent changes in sapwood and leaf area need to be taken into account. However, the
385 lower theoretical hydraulic conductivity in the earlywood of dead trees from Prades was
386 observed throughout the study period and was greater in magnitude than the average

387 defoliation levels currently observed in trees that are suffering drought-induced mortality
388 at this site (Poyatos et al., 2013). At the same time, the leaf-to-sapwood area ratio of
389 defoliated and healthy trees at this site is similar (Poyatos, unpublished results), strongly
390 suggesting that the measured reduction in hydraulic conductivity at the ring level was
391 associated with lower capacity to support canopy water demands. Those findings
392 observed in the “desiccation avoiding” Scots pine contrast with those discussed before for
393 a “desiccation tolerant” **oak species**. Such apparently contradictory anatomical patterns
394 between both functional groups of tree species may be linked to contrasting xylem
395 responses and stomatal control of photosynthesis, carbon use and growth dynamics
396 during drought. In pedunculate oak, tree death seems to be a response to drought-induced
397 xylem cavitation, hydraulic failure and a fast growth decline due to loose stomatal control
398 of photosynthesis. In Scots pine, dead trees had lower hydraulic conductance (less
399 tracheids with smaller lumen diameters) and growth rates than living trees, suggesting a
400 more strict stomatal control of photosynthesis and a lower carbon investment in dead than
401 in living trees. The [more xeric Prades](#) site illustrates well this last comment.

402 The analysis of the response of wood anatomical features to SPEI showed that
403 now-dead trees tended to respond to drought over longer time scales than surviving
404 individuals. This result is consistent with a general physiological slowdown previous to
405 death and suggests a carryover effect on growth and hydraulic performance that
406 constrains drought responses of trees prone to die. The mechanisms underlying this
407 pattern are not clear, but it is likely that the long-distance transport systems of the plant
408 are involved (Anderegg et al., 2012) and could **imply drought ‘legacy’ effects on the**
409 plant hydraulic system as recently reported for sudden aspen decline (Anderegg et al.,

410 2013). We are aware that our correlational approach precludes investigating the ultimate
411 mechanisms of tree death but still we consider our research as a valuable tool to
412 understand how changes in wood production and anatomy predate tree death. For
413 instance, the negative correlations observed between atmospheric CO₂ concentrations and
414 xylem growth apparently contradict the evidence that increasing carbon availability
415 increases tree growth as long as water is not limiting. However, we suggest that such
416 negative association is due to growth reduction in response to warmer and drier
417 conditions in the study area (Martínez-Vilalta et al., 2008).

418 We did not observe any difference between now-dead and surviving pines in the
419 ratio between cell-wall thickness and radial lumen diameter, here used as a proxy of
420 vulnerability to xylem embolism (Hacke et al., 2001). This was due to the fact that in
421 Prades tracheid lumens and cell-wall thickness co-varied, and both variables presented
422 lower values in now-dead individuals than in living trees (Figure 1). This result is
423 consistent with: (i) previous reports showing limited plasticity of the vulnerability to
424 embolism in Scots pine (Martínez-Vilalta and Piñol, 2002; Martínez-Vilalta et al., 2009),
425 and (ii) our own measurements at the Prades site showing no difference in vulnerability
426 to xylem embolism (in branches) between healthy and heavily defoliated pines (Poyatos
427 et al., 2013).

428 Reduced hydraulic conductivity in now-dead trees resulted from the formation of
429 narrow growth rings with narrow tracheids. At the same time, declining growth could be
430 an indicator of low carbon availability. Under drought, whole-tree carbon assimilation
431 tends to be impaired due to defoliation and stomatal closure (McDowell et al., 2008;
432 Galiano et al., 2011). Although trees subjected to drought may allocate assimilates

433 preferentially to other organs (e.g., buds, needles, roots) than to wood formation (Waring,
434 1987; Eilmann et al., 2009), our own measurements at the same two study sites indicate
435 also lower reproductive investment in defoliated than healthy pines (Vilà-Cabrera et al.,
436 2014). In our case, the low carbon investment (in terms of C_{costRW} and RD_{RW}) was
437 observed in the now-dead individuals from the more xeric Prades site. The lower
438 production of resin ducts in now-dead trees is consistent with previous results (Kane and
439 Kolb, 2010; Gaylord et al., 2013). Even though in our case bark beetles or other pests do
440 **not seem to be directly involved in the mortality process (authors' personal observation)**,
441 the reduced defence found in these chronically declining trees must be included as an
442 additional vulnerability factor. Depleted carbohydrate reserves have been reported in
443 dying trees at both study sites (Galiano et al., 2011; Poyatos et al., 2013), suggesting that
444 lower growth and resin duct production in dying trees might be associated to nearly
445 exhausted (or unavailable) carbon reserves (Galiano et al., 2011; Sala et al., 2012;
446 Poyatos et al., 2013).

447 The growth reductions previously observed for the now-dead trees (Hereş et al.,
448 2012) were more related to lower tracheid production than to a reduction in tracheid size
449 (see also Camarero et al., 1998; Martin-Benito et al., 2013), thus minimizing the impact
450 of reduced growth on hydraulic conductivity without increasing the carbon investment.
451 However, the positive relationship between BAI and lumen diameter found for both now-
452 dead and surviving individuals indicates that Scots pines from Prades and Arcalís reduce
453 radial growth also at the expense of decreasing conduit size and experience
454 proportionally higher declines in K_{hRW} . Interestingly, cell-wall thickness, a trait that is
455 usually less variable than lumen size (Vaganov et al., 2006), showed a tighter positive

456 relationship with BAI than lumen size. This result explains why $(CWT/LD)_{EW}^2$ did not
457 differ between living and now-dead trees and it is again consistent with the notion that
458 overall carbon availability may be constraining radial growth in the studied trees.

459 To conclude, we observed long-term changes in wood-anatomical features
460 predating tree death in Scots pine. We found different wood-anatomical patterns between
461 surviving and now-dead trees, [with dead trees showing lower tracheid and resin duct](#)
462 [production, and smaller lumen diameters than living trees](#). Those differences in wood
463 anatomy were more pronounced in the xeric ([Prades](#)) than in the mesic ([Arcalís](#)) site, and
464 they are consistent with the different forest decline dynamics observed at the two sites. At
465 the xeric site, long-term growth reduction started on average forty years before tree
466 death, whereas at the mesic site, instead, growth started decreasing on average fifteen
467 years before tree death ([Hereş et al., 2012](#)). Carbohydrate depletion in the [more](#) xeric
468 [Prades](#) site seems to be associated with long-term lowered hydraulic capacity in
469 anatomical terms, whereas this pattern is not so clear in the mesic [Arcalís](#) site. In any
470 case, the fact that the pace and pattern of the decline process differed substantially
471 between the two study sites indicates that the wood-anatomical responses and related
472 mechanisms underlying drought-induced tree mortality vary among populations of the
473 same species. This finding has implications for the monitoring and management of forests
474 in drought-prone areas since early symptoms of decline, including changes in wood
475 anatomy previous to tree death, differ markedly across sites.

476

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485

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