Title: Climatic regulation of leaf and cambial phenology in *Quercus pubescens*: their interlinkage and impact on xylem and phloem conduits

Running Title: Cambial and leaf phenology of *Q. pubescens*

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Abstract

Increased frequency and severity of stressful events affects the growth patterns and functioning of trees which adjust their phenology to given conditions. Here, we analysed environmental effects (temperature, precipitation, VPD and SWC) on the timing of leaf phenology, seasonal stem radial growth patterns, and xylem and phloem anatomy of *Quercus pubescens* in the sub-Mediterranean in the period 2014–2019, when various adverse weather events occurred, i.e. spring drought in 2015, summer fire in 2016 and summer drought in 2017. Results showed that the timings of leaf and cambium phenology do not occur simultaneously in *Q. pubescens*, reflecting different environmental and internal constraints. Although year-to-year variability in the timings of leaf and cambial phenology exists, their chronological sequence is fairly fixed. Different effects of weather conditions on different stages of leaf development in spring were observed. Common climatic drivers (i.e., negative effect of hot and dry summers and a positive effect of increasing moisture availability in winter and summer) were found to affect the widths of xylem and phloem increments with more pronounced effect on late formed parts. A legacy effect of the timing of leaf and cambial phenology of the previous growing season on the timing of phenology of the following spring was confirmed. Rarely available phloem data permitted a comprehensive insight into the interlinkage of the timing of cambium and leaf phenology and adjustment strategies of vascular tissues in Mediterranean pubescent oak to various environmental constraints, including frequent extreme events (drought, fire). Our results suggest that predicted changes in autumn/winter and spring climatic conditions for this area could affect the timings of leaf and stem cambial phenology of *Q. pubescens* in the coming years, which would affect stem xylem and phloem structure and hydraulic properties, and ultimately its performance.

1 Introduction

Environmental changes will affect phenology and consequently growth patterns and the functioning of trees (Chuine, 2010). The leaf phenology of deciduous trees has proven to be a reliable bio-indicator for climate change (Menzel et al., 2006). An important advantage of leaf phenological monitoring is the possibility of remote sensing-based phenology data acquisition (Hufkens et al., 2012). Rapid progress in these technologies has greatly improved the understanding of vegetation phenology, providing data from local to global scales (Piao et al., 2019; Fang et al., 2020). This approach is not yet suitable for observing phenology on some other levels, e.g., non-leaf tissue such as cambium activity, which as a consequence is less frequently reported in the literature (Antonucci et al., 2017; Correa-Díaz et al., 2019). Although the activities of different tree organs need to be coordinated for whole-tree function, the phenology of each organ/tissue is regulated by a combination of different environmental and internal factors (Delpierre et al., 2016). It is unclear how the interplay of these covarying factors will affect the ability of different tree species to respond to environmental constraints. In this respect phenology and functioning of structures which contribute/preserve hydraulic functions and support plant carbon budget (leaves, secondary vascular tissues) are of particular importance.

Temperature and photoperiod are generally considered to be the main drivers for spring leaf and xylem phenology (Huang et al., 2020; Savage and Chuine, 2021; Way and Montgomery, 2015). Summer-autumn pheno-phases, such as leaf autumn colouring and cessation of cambium activity have been reported to be influenced either directly by environmental factors such as temperature, soil water availability and mean vapour pressure deficit (Pérez-de-Lis et al., 2017), but even more so, these factors seem to act indirectly on plant assimilation output (Zani et al., 2020). However, the research findings on the importance of these factors are far from consistent. For example, summer drought periods with higher temperatures and evaporative demand are reported to either accelerate autumn senescence (Estiarte and Peñuelas, 2015), have no effects (Mariën et al., 2021) or delay this process (Leuzinger et al., 2005; Zani et al., 2020). The

83 climate sensitivity of spring and autumn plant phenological events (leaf unfolding and falling, flowering,

84 fruit ripening and harvesting) also differ; relative to other seasons, spring events (leaf unfolding and

85 flowering) are more sensitive to climatic variables, particularly temperature and precipitation (which are

86 also reflected in more direct plant factors, such as soil water availability and vapour pressure deficit (VPD)),

87 and consequently more climate induced alterations can be expected (Gordo and Sanz, 2010). Nevertheless,

88 observations have revealed that the climate sensitivity of spring leaf phenology of temperate deciduous

89 species has declined significantly over the past three decades, which indicates a reduced impact of global

90 warming on the length of the growing season, and a reduced risk of late spring frost damage by avoiding

91 premature leaf unfolding (Fu et al., 2015). In addition to external drivers, the sequence of leaf and cambial

92 phenology depends on other factors, such as tree species, leaf habitat, wood porosity, tree part (e.g.,

93 Suzuki et al., 1996; Takahashi et al., 2015). The interactions between multiple drivers are quite complex

94 because they are often correlated, which complicates the modelling and predictions of future phenological

95 changes. Monitoring radial growth is crucial for understanding the impact of climate on phenology and

96 consequent changes of xylem and phloem structure, which are good indicators of tree success and

97 performance (Deslauriers et al., 2017).

98 For the function of the whole tree, the activities of the various tree organs, tissues must be coordinated.

99 In this respect, the phenology and the function of the structures that contribute/maintain hydraulic

100 functions and support the carbon balance of the plant are of particular importance. As plant develops, the

101 requirements of various parts of the plant for water and carbohydrates change considerably. While buds

102 and developing leaves are strong sinks for carbohydrates, they convert to sources when they are fully

103 developed and produce an excess of photoassimilates. At the same time, their water requirements

104 increase due to greater leaf area and transpiration losses. In seasonal environments, resumption of

105 deciduous tree growth after winter dormancy requires remobilization of resources (water, nutrients) and

106 transport of signalling molecules (hormones and proteins) that regulate carbon allocation between sources

107 (soil, roots, stems, branches) and sinks (growing buds, leaves, flowers, fruits) (Savage and Chuine, 2021).

108 All of these transport functions must be supported by vascular tissues. In spring, efficient transport is

109 achieved in part by the use of preformed conduits, their reactivation (e.g., callose breakdown in sieve cells)

110 but in many species it is mainly supported by de novo formation of xylem and phloem. Xylem and phloem

111 formation must therefore be synchronized with leaf phenology. It has been clearly shown that vascular

112 phenology can be a limiting factor for certain phenological stages, such as leaf emergence. These

113 relationships are quite well documented for xylem formation (e.g., Takahashi et al., 2015), whereas less

114 information exists for phloem phenology and transport capacity (Gričar et al., 2017). However, for both

115 tissues, research indicates that plants with xylem and phloem conduits that remain functional or rapidly

116 resume function prior to cambial productivity in the spring may be able to support earlier growth.

117 The transport capacity of vascular tissues depends not only on their extent (e.g., cross-sectional area in

118 the stem) but also on their functionality. This is of particular importance when a tree encounters

119 environmental conditions that disrupt or even threaten its function. In arid environments, traits such as

120 the size (diameter) of the vessels, the anatomy of the pits, the transition from earlywood to latewood are

121 important for the maintenance of hydraulic functions (Gričar et al., 2017). In relation to leaf phenology or

122 a whole tree ecophysiology, these traits are rarely reported (Lavrič et al. 2017; Vodnik et al. 2019).

123 In the study, environmental data (temperature, precipitation, VPD and SWC) were used to evaluate their

124 effect on the timing of leaf phenology, seasonal radial growth patterns, and xylem and phloem anatomy

125 in *Quercus pubescens* in the sub-Mediterranean region during six complete growing seasons, i.e., 2014–

126 2019 and initial leaf and cambium phases in 2020. The extended 6-year dataset is unique because it also

127 contains phloem phenology data. During the study period, various adverse weather events occurred, i.e.

128 spring drought in 2015, summer fire in 2016 and summer drought in 2017, which offer an opportunity to

129 study the responses of trees to such events. We hypothesised that: (H1) the timing of the studied leaf

130 phenological phases and radial growth patterns are influenced by different environmental conditions, but

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131 the chronological sequence of the timing of leaf and cambial phenology is fairly fixed; (H2) impacts of

132 adverse environmental conditions on the timing of leaf and cambial phenology are reflected in xylem and

133 phloem anatomies; (H3) due to the legacy effect, weather-induced variation in leaf and cambial phenology

134 in the previous growing season affect the timing of spring phenology in the following year.

135

136

137 **2 Material and methods**

138 **2.1 Study site description**

139 The study was conducted at Podgorski Kras (45°32'56.3"N, 13°54'36.1"E, 430 m a.s.l.), a karst region in

140 South-Western Slovenia. The site was used as pasture but abandoned about 30 years ago. Since then it

141 has been encroached by various woody plant species with pubescent oak (*Quercus pubescens* Willd.) being

142 one of the dominant tree species growing either in patches or as a solitary stand. The climate at the study

143 site is sub-Mediterranean, characterised by harsh winter conditions and frequent dry periods in summer.

144 In the 2014–2019 period, the average annual air temperature was 12.2 °C (T_{jan} = 3.4 °C, T_{jul} = 21.9 °C).

145 Precipitation is relatively abundant, about 1390 mm per year (2014–2019) usually with two peaks, in

146 autumn and late spring. Shallow soil and regular wind reduce the impact of the relatively large volume of

147 rainfall, which results in a large proportion of deep percolation loss of soil water and frequent droughts,

148 especially in summer (Ferlan et al., 2016).

149 In the 2016–2019 study period, the wettest year was 2014, with annual precipitation exceeding 1700 mm

150 (i.e. 24% above the long-term average), while the driest year was 2015 with only 875 mm of precipitation

151 (i.e. 35% below the long-term average) (Supplementary Figure 1). In 2016, a forest fire occurred at the

152 study site in early August. It was a low intensity fire with a high spread rate and non-uniform effects on

153   vegetation; certain areas of the site suffered little or no damage, whereas woody vegetation was

154   significantly damaged in other areas (Gričar et al., 2020a). Trees for this study were selected in the

155   undamaged areas of the site. In 2017, we observed severe drought between May and July with less than

156   200 mm of precipitation, which is 40% less than the long-term average. Despite the well-documented

157   extreme summer drought in central and northern Europe in 2018 (Buras et al., 2020), the summer of 2018

158   was rather wet at our site, but with above-average temperatures.

159

160   **2.2 Tree selection, sample collection and leaf phenology**

161   At the beginning of each growing season, we selected six dominant pubescent oaks without any visible

162   injuries on the tree surface. Due to the small diameters of the trees, new trees nearby were selected each

163   year for sampling to avoid wound effects on their radial growth patterns. Tree-level properties were

164   comparable: average diameter at breast height was 20.9 ± 1.6 cm, average height was 10.6 ± 1.3 m and

165   average age was 57.3 ± 7.3 years. Each growing season, leaf phenology (i.e. bud break, leaf emergence,

166   leaf unfolding and autumn colouring) was observed on all oaks at 7–10-day intervals from March until

167   September, and at 14-day intervals from September until December. A branch was selected on the north

168   side of each tree for phenological observations. We observed the stages of leaf phenology according to a

169   10-stage scale: 1 – dormant buds, 2 – swollen buds, 3 – bud break, 4 – leaf emergence, 5 – leaf

170   development, 6 – full leaf unfolding, 7 – flowering (beginning of opening of male flowers), 8 – initiation of

171   autumn colouring (first leaves begin to turn from green to yellow, red and orange), 9 – abundant autumn

172   colouring (>50% of the leaves of the observed crown turn from green to yellow, red, orange and brown)

173   and 10 – leaf fall (also divided as 10 – abundant leaf fall and 11 – full leaf fall) (Gričar et al., 2017). The

174   extent and pattern of autumn senescence-related chlorosis differed from sporadic summer chlorosis. To

175   document leaf development, images of a selected portion of crown were captured on each sampling date

176   with a digital camera.

177

178   **2.3 Radial growth analysis**

179   To assess the timings of xylem and phloem formation, 2.4 mm diameter microcores were collected using

180   the Trephor tool (Rossi et al., 2006) on the dates of leaf phenological observations. The samples were taken

181   from stems at 0.7–1.7 m above the ground in a helical pattern and separated by 3–5 cm to mitigate wound

182   effects. Each microcore contained inner phloem, cambium, and at least the three youngest xylem rings.

183   Immediately after removal, the microcores were put in 70% ethanol. In the Laboratory for Wood Anatomy

184   at the Slovenian Forestry Institute, sample cross-sections were prepared and stained with safranin and

185   astra blue for light microscope observations of developing xylem and phloem tissues. The procedure is

186   described by Gričar et al. (2017, 2020a) along with appropriate definitions of the phenological phases and

187   analysis protocol. The following developmental phases of xylem and phloem formation expressed in days

188   of the year (DOY), were assessed: (1) onset/end date of cambial cell production; (2) appearance of first

189   expanding xylem and phloem cells; (3) final size of initial early phloem sieve tube; (4) appearance of initial

190   earlywood vessels in expansion phase; (5) onset date of secondary wall formation and lignification of initial

191   earlywood vessels; (6) first mature initial earlywood vessels; (7) transition from earlywood to latewood;

192   (8) transition from early to late phloem; (10) cessation date of wood formation.

193   Growth ring boundaries between phloem increments are visible only in the uncollapsed part of the

194   phloem, i.e., the youngest and narrowest part of the secondary phloem. The uncollapsed phloem lies

195   between the cambium and the wide collapsed phloem. Based on the differences in the morphological

196   characteristics (i.e., dimensions) of the early and late phloem sieve tubes, it was possible to determine the

197   boundaries of the growth rings and the early and late phloem in oak. Namely, the early phloem sieve tubes

198 generally have wider radial dimensions than the late phloem sieve tubes. In addition, the tangential walls

199 of the initial sieve tubes of early phloem adjacent to the phloem increment formed the previous year are

200 slightly rounded, whereas the terminal late phloem sieve tubes formed the previous year are often

201 crushed. The transition from early to late phloem was characterised by the appearance of small,

202 tangentially oriented groups of phloem fibres, usually 2-5 cell layers wide, separating the two parts of the

203 phloem. The phloem fibres have thick lignified cell walls that stain red and exhibit birefringence in

204 polarised light (Gričar et al., 2015).

205 On the cross-sections taken at the end of each growing season, the final widths of xylem and phloem

206 increments were measured along three radial files (parallel to the rays) and then averaged. To assess the

207 year-to-year differences in conduit size, the tangential diameter and area of the initial earlywood vessels

208 (IEV) at the growth ring boundary were measured and mean values calculated. In phloem, the tangential

209 diameter and area of 10 randomly selected initial sieve tubes of early phloem were measured and mean

210 values calculated. All variables were calculated on a tree level. Observations and measurements of tissues

211 were performed with an image analysis system comprising an Olympus BX51 (Tokyo, Japan) light

212 microscope, a PIXElink, PL-A66Z digital camera, and the NIS-Elements Basic Research V.2.3 image analysis

213 program (Tokyo, Japan).

214

215 **2.4 Environmental data and weather-phenology relationships**

216 For greater comparability with other studies our main source of environmental data, i.e., daily

217 precipitation sums and mean daily temperatures, for our study site were extracted from the E-OBS gridded

218 climate dataset (Cornes et al., 2018). E-OBS version 21.0e on a 0.1-degree regular grid was used. All climate

219 variables were spatially interpolated by cokriging, with elevation as an additional explanatory variable

220 (Adhikary et al., 2017). To account for specific soil effects of the karst terrain on rainfall availability, soil

221 water content (SWC, in $m^3 m^{-3}$) data were acquired from the eddy covariance tower, located on the

222 investigated area. The ecophysiological effects of air humidity were assessed based on vapour pressure

223 deficit (VPD in kPa) also acquired from the eddy tower (see more details in Ferlan et al., 2011).

224 To examine the effect of accumulated heat on initial and terminal cambium and leaf phenology, we

225 calculated Growing Degree Days with T_{base} of 5 °C (GDD_5), Heat Degree Days (HDD) and Cooling Degree

226 Days (CDD) (Richardson et al., 2006a; Richardson et al., 2006b). To calculate HDD, we set the parameter

227 T_{base} to 5 °C (HDD_5) and considered the heat accumulated during the 30-day period preceding the mean

228 DOY of bud break and mean DOY of the onset of cambial cell production. We used the fixed-window

229 approach to avoid spurious conclusions resulting from direct dependence between temperature and time.

230 HDD_5 for onset of cambium and bud break was calculated as the accumulated heat between DOY 45 and

231 75, and DOY 75 and 105 respectively. CDD represents the thermal sum below the base temperature (Gill

232 et al., 2015; Richardson et al., 2006a) and was used to evaluate the effect of accumulated heat on late

233 phenological and cambium phases. To calculate CDD we considered all days from 1 August and 31 October

234 and set T_{base} to 20 °C (CDD_{20}), as suggested by (Gill et al., 2015). We are aware that the selected time-

235 windows may also be slightly variable, but based on our preliminary explanatory attempts, they optimally

236 reflect the accumulated heat required to initiate the studied phases.

237 **2.5 Data analyses**

238 Statistical relationships between all pairs of variables were assessed using the non-parametric Kendall's τ

239 coefficients, which account for non-normally distributed data. The complete correlation plot is given as

240 Supplementary Figure 2. For the selected key pairs of variables, we presented scatter plots. Principle

241 component analysis (PCA) (Supplementary Figure 3) was used to transform variables into a new feature

242 space to address their common variability, and to infer about the dependence of studied physiological

243 processes from loadings plot. Unfortunately, PCA does not allow for the inclusion of variables with missing

11

244   values, so for this part of analysis we included data from the 2014–2019 period, skipping 2018 due to

245   missing bud break information. Also, variable autumn colouring was not included due to missing

246   observations in 2017. We show alternative loadings plot as Supplementary Figure 4 and argue that the

247   effect of missing data is negligible.

248   In addition to HDD_5 and CDD_{20} (see Section 2.4), we correlated the short-term effects of mean

249   temperature, mean VPD and soil moisture availability (precipitation and SWC) on key phenological leaf

250   and cambial phases. We tested time windows of different lengths, and obtained optimal results in terms

251   of data flexibility with a 10-day window. We thus considered the 10-day window preceding the

252   phenological event for the calculation of aggregate weather parameters. Furthermore, the selected time

253   window is in accordance with Gričar et al. (2006), who reported the onset of cambial activation from winter

254   dormancy 10 days after heat treatment. One exception was implemented here: due to greater variability

255   of autumn colouring (see Table 1) and seasonal weather dependence, we could not use a 10-day window,

256   but instead considered weather conditions in the fixed season between DOY 210 and 240 to evaluate the

257   weather effect for autumn colouring.

258   Finally, to evaluate the weather effect on xylem and phloem traits, we calculated mean site chronologies

259   and compared those with weather using the variable response widow from the dendroTools R package

260   (Jevšenak, 2020; Jevšenak and Levanič, 2018). We did not apply a standardization method because we had

261   only six years of xylem and phloem measurements, none of which showing a problematic trend.

262

263   **3 Results**

264   **3.1 The inter-annual variability of cambial and leaf phenological phases**

265 Among all studied phases, the timing of bud break showed the lowest variability with min-max range of 18

266 days (Table 1). The onset date of cambial cell production always occurred prior to bud break and had a

267 min-max range of 25 days. The transition dates from earlywood to latewood and from early phloem to late

268 phloem had the same mean and standard deviation, indicating close occurrence, although they showed

269 only minor inter-dependence (Supplementary Figure 2). In general, variability in the timing of leaf

270 phenology, xylem phenology, and phloem formation increases with season. The greatest variability was

271 observed for the timing of autumn colouring, which could occur at the beginning of September or end of

272 November.

273

274 *[Table 1 preferred position]*

275

276 **3.2 Relationship between leaf phenology and radial growth**

277 The timings of spring leaf phenological phases interrelated, especially the timings of bud break and leaf

278 emergence (Figure 1). Thus, the earlier bud break occurred, the earlier also leaves emerged. A longer

279 duration of cambial cell production had positive effect on all analysed xylem and phloem traits

280 (Supplementary Figure 2). We found negative correlations between the timing of leaf unfolding and the

281 transition date from earlywood to latewood; thus, the earlier leaf unfolding occurred, the later the

282 transition from earlywood to latewood was detected. The transition date from early phloem to late phloem

283 showed no relation with the timing of leaf phenological phases in spring (Figure 1). Xylem and phloem

284 widths showed a stronger dependence on the end date of cambial cell production rather than on the date

285 of its onset, indicating that the difference in ring widths resulted mainly from cambial phenological events

286 in late summer. The timing of previous autumn colouring showed a weak positive effect on the timing of

287 bud break, but strong negative effect on the onset date of cambial cell production (Figure 2). Therefore,

288 compared to the timing of the early stages of leaf development in spring, favourable weather conditions

289 from the previous autumn, were more important for the timing of the onset of cambial cell production,

290 which also varied more than the timing of bud break (Table 1). Initial earlywood vessel and early phloem

291 sieve tube areas were more dependent on the timing of the early stages of leaf development (bud break,

292 leaf emergence) than on the timing of the onset of cambial cell production. The end date of cambial cell

293 production in the previous year showed a strong negative effect on the timing of autumn leaf colouring in

294 the following year. Thus, the earlier cambial activity stopped, the later autumn leaf colouring began the

295 next year.

296

297 **3.3 Effect of weather conditions on leaf and cambial phenology, and xylem and phloem anatomy**

298 The effect of accumulated heat on initial cambium and leaf phenology, was first assessed using the GDD_5.

299 The calculated GDD_5 for the onset date of cambial cell production it ranged from 105 to 247 °C and

300 averaged 164 °C, while for the timing of bud break ranged from 279 to 456 °C with an average of 364 °C

301 (Supplementary Table 1).

302 GDD_5 therefore significantly varied among the years, but it was not effective in explaining the timings of

303 spring and autumn cambial and leaf phenological events. We therefore introduced Heat Degree Days

304 (HDD_5) and Cooling Degree Days (CDD_{20}). The onset date of cambial cell production was negatively

305 correlated with 10-day precipitation sums and SWC means, whereas temperature and VPD did not show

306 significant effect on the timing of this phenological phase. Of all the variables, the onset date of cambial

307 cell production was most strongly associated with the timing of the previous autumn colouring and was

308 therefore more strongly related to the weather and phenological events at the end of the previous growing

309 season. Thus, the later the autumn leaf colouring occurred, the earlier was the onset date of cambial cell

310 production the following spring. When comparing the timing of bud break with HDD_5 or 10-day mean

temperature prior to each phenological observation, significant negative correlations were obtained (Figure 3), which indicates that short-term warm periods triggered the beginning of leaf development, rather than GDD_5, which measures accumulated heat in longer periods. The timing of leaf emergence and leaf unfolding phases were positively correlated with 10-day precipitation sums and SWC means (Figure 3 and Supplementary Figure 5), which indicates delay of leaf development in the case of wet spring. Transition date from earlywood to latewood showed negative and positive correlations with aggregated 10-day moisture availability and temperature and VPD means, respectively. Similarly, the transition date of early phloem to late phloem was positively correlated with 10-day temperature means. Both transition dates were therefore triggered by relatively warm and dry weather in the second half of May. The timing of the end of the cambial cell production period showed a positive correlation with 10-day moisture availability and a negative correlation with 10-day temperature and VPD means. This positive effect of a cool and wet August on radial growth was later confirmed with daily correlations between aggregated environmental data and xylem (total and latewood) and phloem (total and late phloem) widths (Figure 4). The timing of autumn colouring also positively correlated with accumulated heat during the three-month period of August-October (CDD_{20}), clearly showing a delayed timing of autumn colouring in the case of a warmer end of the growing season.

All pairwise comparisons of xylem and phloem traits, i.e. widths of earlywood, latewood, early phloem and late phloem, and areas of initial earlywood vessels and early phloem sieve tubes, were positively correlated (Supplementary Figure 2) and had large positive loadings on component 1 (Figure 1), which primarily measures increase in radial increment and growing season length. Variables related to the widths of the xylem and phloem tissues showed common climatic drivers, with xylem and phloem widths being positively correlated with higher winter moisture availability and mean temperature, while late spring drought significantly reduced both, xylem and phloem widths (Figure 4). The latter effect was more

335   obvious for latewood and late phloem widths. Areas of earlywood vessel and early phloem sieve tubes

336   showed only minor dependence on weather conditions. Earlywood vessel areas were sensitive to late

337   winter droughts, which resulted in their smaller areas.

338

339   **4 Discussion**

340   In this study, observations of the timing of leaf and cambial phenology, as well as xylem and phloem

341   formation in pubescent oak, collected in the 2014–2019 (2020) period are presented. The observations

342   were linked to xylem and phloem anatomy and environmental conditions to highlight their common

343   drivers and interdependence. Despite fairly large between-tree variability in the timing of leaf and cambial

344   phenological phases and wood and phloem anatomical traits we have succeeded to extract some

345   important inferences about phenological regulation of *Quercus pubescens* trees at our study site.

346   Generally, rarely available data on phloem phenology and anatomy, including early phloem conduits,

347   permitted a comprehensive insight into adjustment strategies of vascular tissues in Mediterranean oak to

348   various environmental constraints, including frequent extreme events (drought, fire). We confirmed our

349   first hypothesis (H1), that the timing of the studied leaf phenological phases and radial growth patterns

350   was influenced by different environmental conditions. However, although temporal variability existed

351   within each phenological phase, chronological sequence of the timing of leaf and cambial phenology was

352   fairly fixed. In the second part of the growing season, variability in cambial and leaf phenology was higher

353   compared to the first part of the growing season. The second hypothesis (H2) that the impacts of adverse

354   environmental conditions on the timing of leaf and cambial phenology were reflected in xylem and phloem

355   anatomies was confirmed. We found different effects of weather conditions on different stages of leaf

356   development in spring. Common climatic drivers (i.e., negative effect of hot and dry summers and a

357   positive effect of increasing moisture availability in winter and summer) were found to affect the widths

358 of xylem and phloem increments. Furthermore, weather effect was more pronounced on latewood and

359 late phloem widths (i.e., on the end date of cambial cell production), while earlywood and early phloem

360 were generally less sensitive to weather conditions. The third hypothesis (H3) on the legacy effect of the

361 previous growing season on the following spring phenology was also confirmed. This effect was the

362 strongest for the onset date of cambial cell production, which was strongly affected by the timing of

363 autumn colouring in the previous growing season. In addition, the end date of cambial cell production in

364 the previous year showed a strong negative effect on the timing of autumn leaf colouring in the current

365 year.

366

367 **4.1 Chronological sequence of initial leaf and cambial developmental phases**

368 Our study showed that the onset date of leaf phenology was not correlated with the onset date of cambial

369 cell production. It seems that the onset dates of these two phenological phases in the crown (bud break)

370 and in the stem (cambial cell production, and subsequent initial xylem and phloem formation phases) are

371 partly controlled by different environmental factors. Consequently, the temporal sequences of the studied

372 phenological phases of leaves and stem radial growth do not occur simultaneously in *Q. pubescens*. Our

373 results contrast with the close relationship between the timing of bud break, cambial resumption and

374 initial earlywood vessel formation in spring reported by Pérez-de-Lis et al. (2016) for temperate *Quercus*

375 *robur* and *Quercus pyrenaica* growing in mixed stands along a transitional gradient in the northwest Iberian

376 Peninsula. This discrepancy could be explained by: (i) differences in site conditions, in particular soil water

377 holding capacity or (ii) species-specifics, where (sub)-Mediterranean oaks adjust growth patterns to local,

378 often stress conditions, while the hydraulic properties of temperate *Q. robur* are more associated with

379 weather conditions.

380   Generally, lower year-to-year variability in the timing of leaf and cambial phenology was observed in spring

381   than in summer/ autumn (Table 1). Inter-annual differences were especially pronounced in leaf phenology;

382   a difference of 19 days was observed in the case of bud break, and 78 days in the case of the timing of

383   autumn colouring, which in our case proved to be primarily driven by accumulated heat in the second half

384   of summer. In the case of warm August and September, the timing of autumn colouring is delayed up to

385   the middle of November. For cambial cell production, a difference of 25 days was observed in the case of

386   onset date, and 32 days in the case of its end date. In addition to weather conditions, other drivers (e.g.

387   photoperiod, crown social status) might thus influence the timing of leaf and cambial phenological events.

388   As previously observed in *Q. pubescens* (Lavrič et al., 2017; Zweifel et al., 2006), cambial cell production

389   started a month before bud break. The formation of initial earlywood vessels starts before bud break and

390   their development was completed before full leaf unfolding to be ready for water transport by that time

391   (Gričar et al., 2020a). This order is important in ring-porous species because earlywood conduits from the

392   previous growing seasons are mostly blocked with tyloses therefore hydraulic functioning largely depends

393   on the conduits formed in the current growing season (Kitin and Funada, 2016; Savage and Chuine, 2021).

394   Similar to the initial earlywood vessels, the development of early phloem sieve tubes in ring-porous oak

395   also relies on storage reserves and reach their final size at the time of swollen buds (Gričar et al., 2020a).

396   Sieve tubes are functional for only 1–2 growing seasons (Prislan et al., 2018); hence, their rapid formation

397   in early spring is essential for ensuring the long-distance transport of photosynthates and signalling

398   molecules among different developing organs/tissues (Savage, 2020). Although well-known involvement

399   of sieve tubes in non-collapsed phloem in source (leaves)-to-sink (meristems) transport of carbohydrates,

400   phloem developmental patterns are generally very rarely compared with leaf phenology. In this sense, our

401   6-year phloem phenology data for pubescent oak from the sub-Mediterranean in this sense represent an

402   important contribution to the understanding of the links among phloem phenology and anatomy in

403   relation to environmental cues.

404

4.2 Transition from earlywood to latewood and from early phloem to late phloem

The transition dates from earlywood to latewood and from early phloem to late phloem were generally

close to each other, but they showed different dependence on leaf phenology. In xylem, the transition

date was negatively related to the timing of leaf unfolding. The transition date from earlywood to latewood

was positively correlated with the onset date of cambial cell production and the appearance of the first

xylem cells. Formation of initial xylem cells starts after the onset of cambial cell production in spring, which

is reflected in the correlation of these processes. In contrast, the timing of the transition in the phloem

was not dependent on the timing of spring leaf and cambial phenology. Initial sieve tube development

generally begins at the time of cambial cell production from overwintered cells formed in the previous

autumn, which starts to differentiate (e.g., cell expansion) in the spring of the next growing season (Prislan

et al., 2018). Our analyses revealed that the differentiation of initial sieve tubes appears to be independent

of the timing of leaf and cambial phenology.

Structural changes in xylem and phloem associated with these transitions are generally explained by

differences in hormonal signals induced by young and mature leaves. Auxin produced in young leaves

stimulates the development of xylem and phloem conduits, whereas gibberellins induced by mature leaves

provoke fibre formation (Aloni, 2015). In agreement with our findings that the analysed phenological

stages of leaves and radial growth were influenced by different weather conditions (see chapters 4.5 and

4.6.), it follows that the interplay of internal and external factors influences developmental processes in

trees. Since hormonal regulation affects cell characteristics (wall thickness and cell size) and the

proportions of different cell types (vessel, axial parenchyma, fibres), this aspect should be considered in

future studies of vascular differentiation and tree development. Such information would help to better

decipher the process-related response of trees to climatic-phenological changes, and their effects on

427 xylem and phloem structures, which are good indicators of tree success and performance (Deslauriers et

428 al., 2017).

429

430 **4.3 Effect of cambial phenology on xylem and phloem increments**

431 The duration and the end of the period of cambial cell production showed a generally positive effect on all

432 analysed xylem and phloem variables. The effect of the onset date of cambial cell production was most

433 pronounced in subsequent stages of xylem formation, i.e., the first expanding xylem cells and the initial

434 earlywood conduits, while it did not affect the xylem and phloem widths nor the width of the early or late

435 formed parts. Thus, the earlier the cambial cell production started, the earlier the first expanding xylem

436 cells and the initial earlywood conduits appeared. We found that the end date of cambial cell production

437 had a strong impact on both, xylem and phloem increment widths. According to our data from 2014–2019,

438 cambial cell production in pubescent oak ended between mid-July and mid-August depending on weather

439 conditions (see chapter 4.6). In the Mediterranean region, bimodal xylem growth pattern was often

440 detected in various tree species (e.g., Campelo et al., 2018; de Luis et al., 2007; Pérez-de-Lis et al., 2017;

441 Vieira et al., 2019). It results from summer interruption of cambial cell production, typically triggered by

442 stress conditions due to high temperatures, low soil water availability and low air humidity, and an

443 autumnal resumption of cambial cell production in the case of favourable growing conditions. Bimodal

444 growth is reflected in intra-annual density fluctuations in xylem rings (e.g., de Luis et al., 2007). No such

445 pattern was observed in *Q. pubescens* at our sub-Mediterranean site, which could be explained by

446 relatively short favourable periods in the autumn (sufficient temperature combined with favourable SWC

447 and VPD regimes) and harsh winter conditions with frequent short snow events.

448 Thus, year-to-year differences in ring widths were strongly dependent on the end date of cambial cell

449 production, with the widths of latewood and late phloem closely related to the annual ring widths of the

450　xylem and phloem, respectively. Interestingly, the widths of earlywood and early phloem were more

451　strongly related to the duration of cambial activity suggesting that the rate of cell production and transition

452　dates also influence the widths and structure of xylem and phloem increments. The majority of xylem and

453　phloem increments in *Q. pubescens* was formed between April and mid-June, indicating its adaptation to

454　local conditions to avoid possible summer drought during the period of most intense radial growth (Gričar

455　et al., 2017; Zweifel et al., 2006). Thus, part of the stem growth (i.e., initial earlywood vessels and early

456　phloem sieve tubes) was finished before leaves started to unfold to be available for transport. Large

457　earlywood and early phloem conduits, which ensure an efficient conducting system in the tree

458　(Kiorapostolou and Petit, 2018; Tyree and Zimmermann, 2010) were thus formed during a more stable

459　period with less frequent stress events. Small latewood and late phloem conduits are less efficient but

460　important for transport as both, earlywood and early phloem conduits are dysfunctional within a year of

461　their formation (Kitin and Funada, 2016; Prislan et al., 2018). Latewood vessels can remain functional for

462　many years and prevent complete transport failure in the case of stress events (Taneda and Sperry, 2008),

463　while late phloem conduits formed in the previous growing season are still functional in the first weeks of

464　the following growing season to ensure an adequate phloem transport system for developing tree tissues

465　and organs (Prislan et al., 2018).

466

467　**4.4 Legacy effects in phenological processes**

468　The onset date of cambial cell production and the subsequent stages of xylem formation showed a strong

469　negative link with the timing of the previous autumn colouring; later leaf colouring resulted in earlier onset

470　date of cambial cell production. This carry-over effect is a well-documented phenomenon (Delpierre et al.,

471　2017; Marchand et al., 2020a,b), in which trees accumulate large amounts of carbohydrates during the

472　autumn in the case of favourable weather conditions, and these stored reserves are then available at the

473   beginning of the next growing season (Barbaroux and Bréda, 2002). Since radial growth in *Q. pubescens*

474   starts before leaf development, i.e. before the onset of assimilate production in leaves, stored

475   carbohydrates are crucial for the development of earlywood and early phloem (Gričar et al., 2018).

476   Moreover, the carry-over effect of the timing of previous autumn colouring was more important for the

477   beginning of cambial cell production than for the onset of leaf development. However, this relationship

478   may differ among tree species depending on porosity and site specifics (Delpierre et al., 2017; Marchand

479   et al., 2020b).

480   We found that the onset date of cambial cell production varied more than the timing of bud break between

481   study years. Although highly variable, the timing of autumn leaf colouring showed a strong negative link

482   to the end date of cambial cell production in the previous year. This argues for a strong inter- and intra-

483   annual linkage between the timings of leaf and cambial phenology in terms of carbon supply (leaves),

484   demand (meristems) and storage. It follows that large inter-annual variabilities at the onset date of autumn

485   colouring result in significant year-to-year differences of the annual carbon balance and carbon allocation

486   patterns in individuals, which depend on the timing of cessation of cambial cell production in the previous

487   growing season and affect the onset date of radial growth in the following growing season. Trees need to

488   coordinate the use of the limited supply of carbohydrates for metabolic and structural demands (Dietze et

489   al., 2014). In deciduous trees that shed their leaves seasonally, storage reserves are important for

490   maintenance during the leafless season, like respiration and dormancy-related physiological processes

491   (Fadón et al., 2020). Furthermore, our sub-Mediterranean site is characterised by frequent summer

492   droughts and fire events; storage reserves are important for coping with such disturbances (Martínez-

493   Vilalta et al., 2016), as demonstrated by altered radial growth dynamics and increment widths in fire-

494   damaged trees in 2017, one year after the occurrence of summer fire (Gričar et al., 2020a).

495

4.5 Effect of weather conditions on leaf phenology

The onset dates of bud break and cambial cell production were associated with different GDD_5 values, which were highly variable among the study years for both phenological phases, indicating that GDD_5, which measures accumulated heat from 1 January, is not the optimal variable for predicting the timings of initial cambium and leaf phenological phases. A few random warm days early in the year increased the GDD_5 variable, but had little effect on ecophysiological processes in trees. We therefore used HDD_5 and CDD_{20} variables, which are more flexible and measure accumulated heat in a pre-defined time window. To explain the effect of accumulated heat on phenological events in *Q. pubescens*, we tested different time windows, but the highest explanatory power was obtained with 30-day windows (see Section 2.4 for details).

We found different effects of weather conditions on different stages of leaf development in spring. Bud break was triggered by a short-term increase in temperature and VPD, while the timings of leaf emergence and leaf unfolding were negatively correlated with precipitation and SWC, which delayed leaf development. The positive effect of temperature on the initial stages of leaf development was previously confirmed, but with great variability in the strength of response among species (Fu et al., 2014; Lukasová et al., 2020; Morin et al., 2010; Savage and Chuine, 2021). Similarly, the effect of water availability on leaf phenology is inconsistently discussed in the literature (Lukasová et al., 2020; Morin et al., 2010; Sherry et al., 2007). Anticipated warmer spring temperature would potentially increase the risk of frost damage by triggering bud break (Augspurger, 2009), but in ring-porous pubescent oak bud break occurs one month later than in diffuse porous species *Ostrya carpinifolia* (Gričar et al., 2020b), which would be more exposed to such unfavourable events. However, Fu et al. (2015) reported that the sensitivity of leaf development to climate warming has generally decreased in recent decades indicating a slowdown in the advance of tree spring phenology to winter warming. The authors attribute this reduction in sensitivity to reduced

519   chilling and to mechanisms related to photoperiod, which may ultimately become limiting if leaf unfolding

520   occurs too early in the season.

521   The timing of autumn leaf colouring was positively influenced by higher temperature and accumulated

522   heat, while moisture availability and VPD showed no significant effect on this phenological stage. Summer

523   drought is characterised by lack of precipitation and high evapotranspirational demands, which can

524   advance leaf senescence (Lukasová et al., 2020). Although water deficit frequently occurs at our site during

525   the summer months (July-August), this did not seem to affect the timing of autumn leaf colouring. In this

526   respect our study does not support the results by Zani et al. (2020) who recently showed, using a massive

527   trans-European phenological database for five deciduous tree species, that more productive conditions

528   during growth period and less stress inflict earlier autumn senescence possibly due to tree sink limitation

529   (full carbohydrate reserves, seized radial growth and cell wall thickening etc.). In general, data in the

530   literature on the influence of environmental conditions on leaf phenology are contradictory. Morin et al.

531   (2010) found no effect of temperature and soil water content on the date of leaf colouring. In contrast,

532   Gill et al. (2015) found that in boreal and temperate deciduous forests, October temperature was the

533   strongest predictor of senescence date (i.e., positive relationship), especially at low latitudes. Warming

534   would delay leaf senescence, resulting in enhanced net carbon uptake (Keenan, 2015). The positive effect

535   of autumn temperature on leaf senescence was also confirmed in Mediterranean tree species. Del Río

536   García et al. (2015) reported delayed leaf senescence due to warmer autumn conditions in four coexisting

537   Mediterranean *Quercus* species. This may lead to smaller differences in the length of productive leaf life

538   of deciduous species compared to evergreen species, thereby improving the competitive ability of the

539   former (del Río García et al., 2015).

540

541   **4.6 Effect of weather conditions and extreme events on the growth and structure of vascular tissues**

542   We used daily response functions to analyse weather conditions and extreme events on the radial growth

543   and xylem and phloem anatomy. Although the traditional dendroclimatological analyses usually rely on

544   longer time series, the variable response window showed expected and significant results, indicating the

545   potential of applying these functions to shorter intra-annual growth series. The influence of weather

546   conditions was reflected in xylem and phloem widths, and to lesser extent in the conduit areas (Table 1

547   and Figure 4). The widths of xylem and phloem increments showed common climatic signals, i.e. negative

548   effect of hot and dry summers and a positive effect of increasing moisture availability in winter and

549   summer. In addition, latewood and late phloem widths were more sensitive to weather conditions than

550   earlywood and early phloem, respectively. Xylem and phloem widths were generally promoted by higher

551   winter precipitation and temperature, while late spring drought significantly inhibited their widths,

552   especially those of latewood and late phloem. Wet and cool summers resulted in a longer growing season

553   and, consequently in wider xylem and phloem increments. The difference between the timing of end of

554   cambial cell production in the study years was one month, between mid-July and mid-August. In

555   comparison to the least favourable year (2017), xylem and phloem were on average 2.5-fold and 1.5-fold

556   wider, respectively, in the most favourable year (2014). Our analyses confirmed our previous conjectures

557   (Gričar et al., 2018, 2020a) that weather conditions had more influence on the second part of the growing

558   season, i.e. the period of latewood and late phloem formation, than on the first part of the growing season,

559   which was less weather-dependent. Thus, the period of most intense growth, when the earlywood and

560   early phloem conduits were formed, was concentrated in months with favourable weather conditions,

561   which is a good prerequisite for successful growth in drought-prone Mediterranean environments,

562   avoiding potentially stressful summer conditions and not affecting the trees' hydraulic and carbohydrate

563   systems.

564   Contrasting year-to-year weather conditions and frequent extreme events, such as drought periods (2017),

565   heat waves (2015), and fires (2016), which are typical of the region, enables an insight into the impact of

25

different extreme events on the timing of these processes and consequently on xylem and phloem anatomy. We found that initial earlywood vessels and early phloem conduits showed little dependence on weather conditions. Earlywood vessel areas were sensitive to late winter dry periods, which was reflected in their smaller areas. This can be explained by the positive effect of water availability on turgor pressure in the expanding vessels. In contrast, water deficit causes a reduction in turgor pressure, resulting in smaller xylem conduits (Zweifel et al., 2006; Hölttä et al., 2010). However, a relative independence of earlywood and early phloem conduits from weather conditions is consistent with the results of Pérez-de-Lis et al. (2016) in *Quercus pyrenaica* confirming the strategy of Mediterranean oaks to adjust their growth patterns to local environmental conditions with frequent stress events and maintain a more conservative hydraulic architecture. This contrasts with temperate oaks, where the hydraulic properties of earlywood vessels are influenced by the timing of their enlargement, which is determined by winter and spring weather conditions (Tardif and Conciatori 2006; Fonti et al., 2010; Pérez-de-Lis et al., 2016).

Our results revealed that the timings of the studied phenological phases of leaves and stem radial growth are partly controlled by different environmental factors, which is reflected in the anatomy of xylem and phloem and hydraulic properties. It was demonstrated that, in addition to inter-individual and year-to-year variation, there are strong inter- and intra-annual interlinkages between some phenological events. Here, cambial phenology was monitored at the stem base level. Whether similar connections can be found in other tree parts remains to be clarified in future analyses taking into account the whole-tree.

5. Conclusions

To the best of our knowledge, the extended 6-year dataset of cambium and leaf phenology used in this study is one of the longest of its kind. In addition, it includes phloem data, which provides a unique insight into the ecophysiological processes of the studied tree species and beyond. It provides an opportunity to

589 study the interlinkage of cambium and leaf phenology in combination with favourable and unfavourable

590 weather events and their effects on vascular tissues in mature trees. We found that the timings of leaf and

591 cambium phenology do not occur simultaneously in *Q. pubescens*, reflecting different environmental and

592 internal constraints. Although year-to-year variability of the timings of leaf and cambial phenology exists,

593 their chronological sequence is fairly fixed. Our results suggest that predicted changes in autumn/winter

594 and spring climatic conditions for this area (IPCC, 2014) could affect the timings of leaf and cambial

595 phenology of *Q. pubescens* in the coming years, which would affect xylem and phloem structure. Shifts in

596 plant phenology due to climate change have been reported in the Mediterranean region (Gordo and Sanz,

597 2010). This species is predicted to expand its range in the warmer future, thus a better understanding of

598 climate-growth interactions in *Q. pubescens* is important to more accurately assess its future economic

599 and ecological role (Buras and Menzel, 2019; Hanewinkel et al., 2013).

600

606

607 **Conflict of Interest:** The authors declare that they have no conflict of interest.

608

609 **Author contributions statements**

610 JG and JJ contributed equally to this work and are to be considered co-first authors. All authors conceived

611 and designed the work; all authors collected the samples; JG, ML and PH carried out analyses of radial

612   growth; ML and PH collected leaf phenological data; JJ and KE performed the statistical analyses, JJ

613   prepared the figures and tables; JG and JJ wrote the manuscript; all authors critically revised the

614   manuscript; all authors approved the final version of the manuscript to be published.

615

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TABLES

795 **Table 1:** Basic descriptive statistics with mean and standard deviation (Std), and minimum and maximum

796 range. Abbreviations: CA (cambial cell production), EW (earlywood), LW (latewood), EP (early phloem), LP

797 (late phloem), IEV (initial earlywood vessel).

	Observation	Unit	Mean	Std	Min	Max	Data availability
Leaf phenology	Bud break	DOY	108	5	97	116	2014 – 2020*
	Leaf emergence	DOY	114	7	104	127	2014 – 2019*
	Leaf unfolding	DOY	134	4	124	142	2014 – 2019
	Autumn colouring	DOY	287	28	246	324	2014 – 2019**
Phenology of radial growth	Onset CA	DOY	75	7	62	87	2014 – 2020
	First xylem cells	DOY	89	7	71	98	2014 – 2019
	First expanding EW vessels	DOY	98	6	87	109	2014 – 2019
	Transition EW-LW	DOY	144	9	129	156	2014 – 2019
	Transition EP-LP	DOY	144	9	127	160	2014 – 2019
	End CA	DOY	209	9	187	219	2014 – 2019
	Duration CA	DOY	133	11	105	149	2014 – 2019
Xylem	EW width	µm	422	106	242	634	2014 – 2019
	IEV area	$µm^2$ 10^2	598	133	315	865	2014 – 2019
	LW width	µm	412	335	94	1447	2014 – 2019
	Xylem width	µm	834	408	387	2039	2014 – 2019
Phloem	EP width	µm	145	27	89	191	2014 – 2019
	EP mean sieve tube area	$µm^2$ 10^2	115	15	73	142	2014 – 2019
	LP width	µm	122	43	48	202	2014 – 2019
	Phloem width	µm	267	58	150	360	2014 – 2019

798 * Observations missing for 2018

799 ** Observations missing for 2017 and 2018

800

FIGURE CAPTIONS
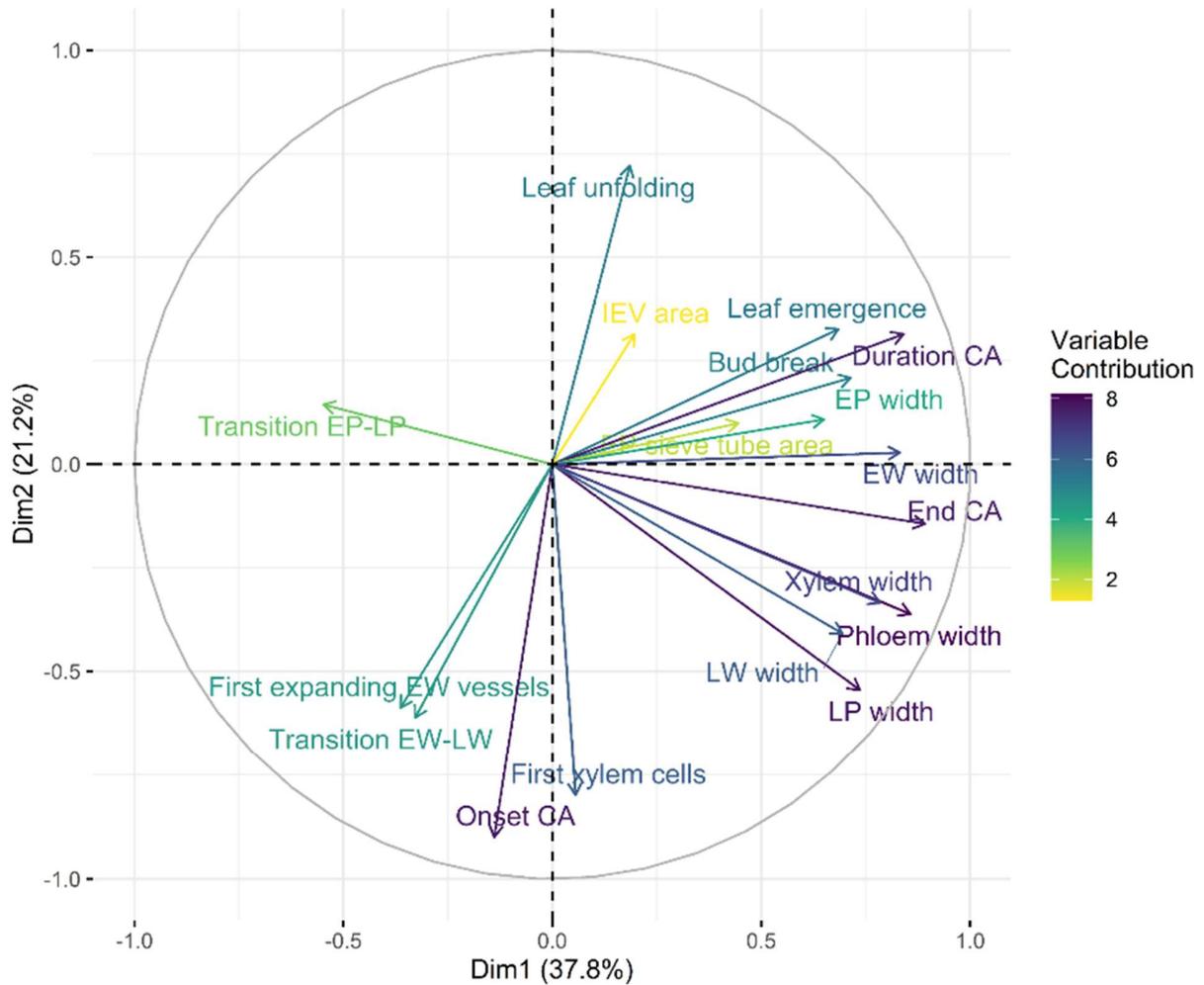
**Figure 1:** Loading plot of first two principal components (Dim1 and Dim2) of PCA showing the interdependence of studied ecophysiological variables. Positively correlated variables point to the same direction, negatively correlated variables diverge and form an angle close to 180°, while unrelated variables meet each other at approximately 90°. Abbreviations: CA (cambial cell production), EW (earlywood), LW (latewood), EP (early phloem), LP (late phloem), IEV (initial earlywood vessels).

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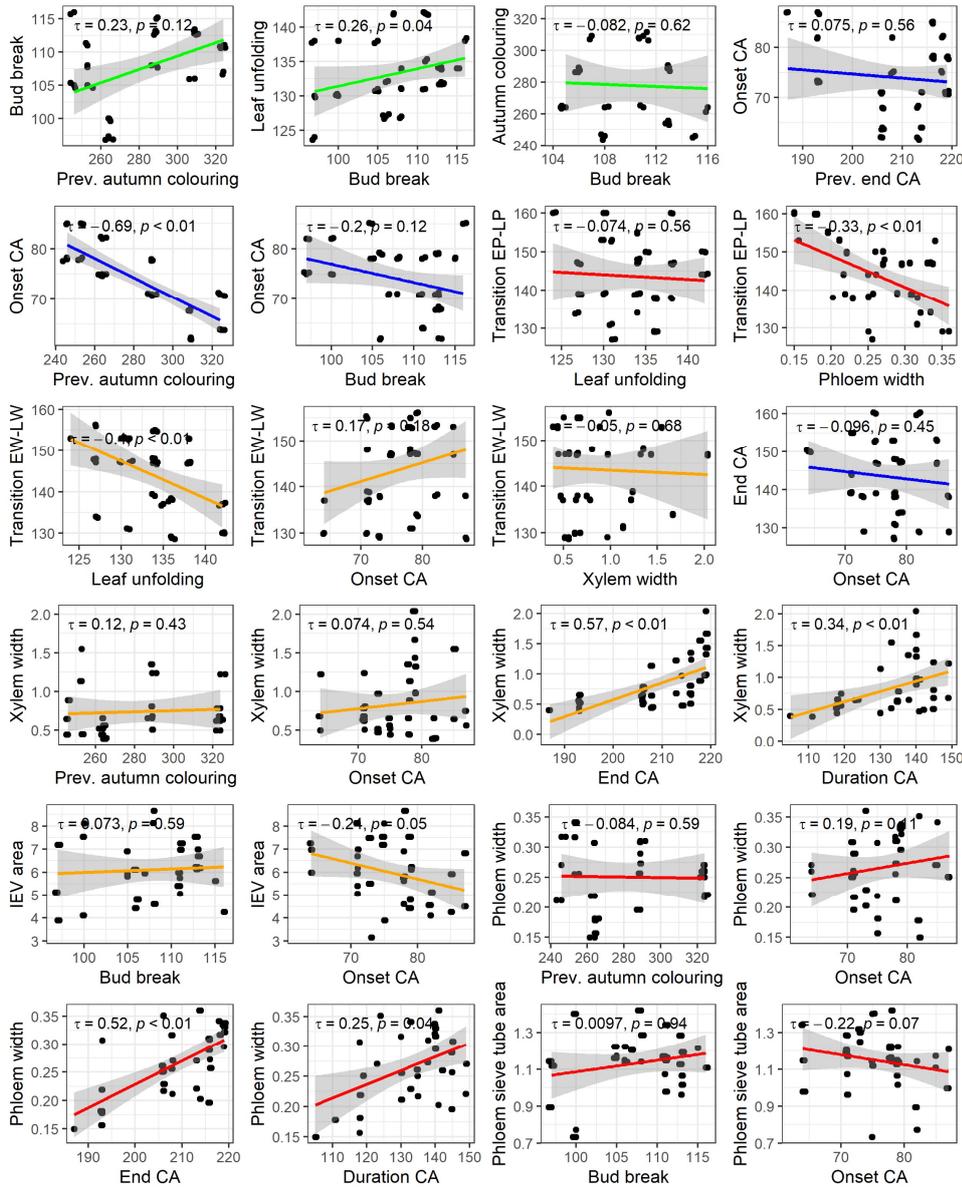
Figure 2: Kendall's tau correlations and their significance, and linear regression models with 95% confidence interval among selected key pairs of leaf and cambium phenological phases, and xylem and phloem traits. Individual observations are of sampled *Quercus pubescens* trees in each year during the 2014–2019 (2020) period. The colour of the regression lines indicates the group of variables on the y-axis: Leaf phenology (green), cambial phenology (blue), xylem phenology (orange) and phloem phenology (red). Abbreviations: Prev. (previous), CA (cambial cell production), EW (earlywood), LW (latewood), EP (early phloem), LP (late phloem), IEV (initial earlywood vessel).

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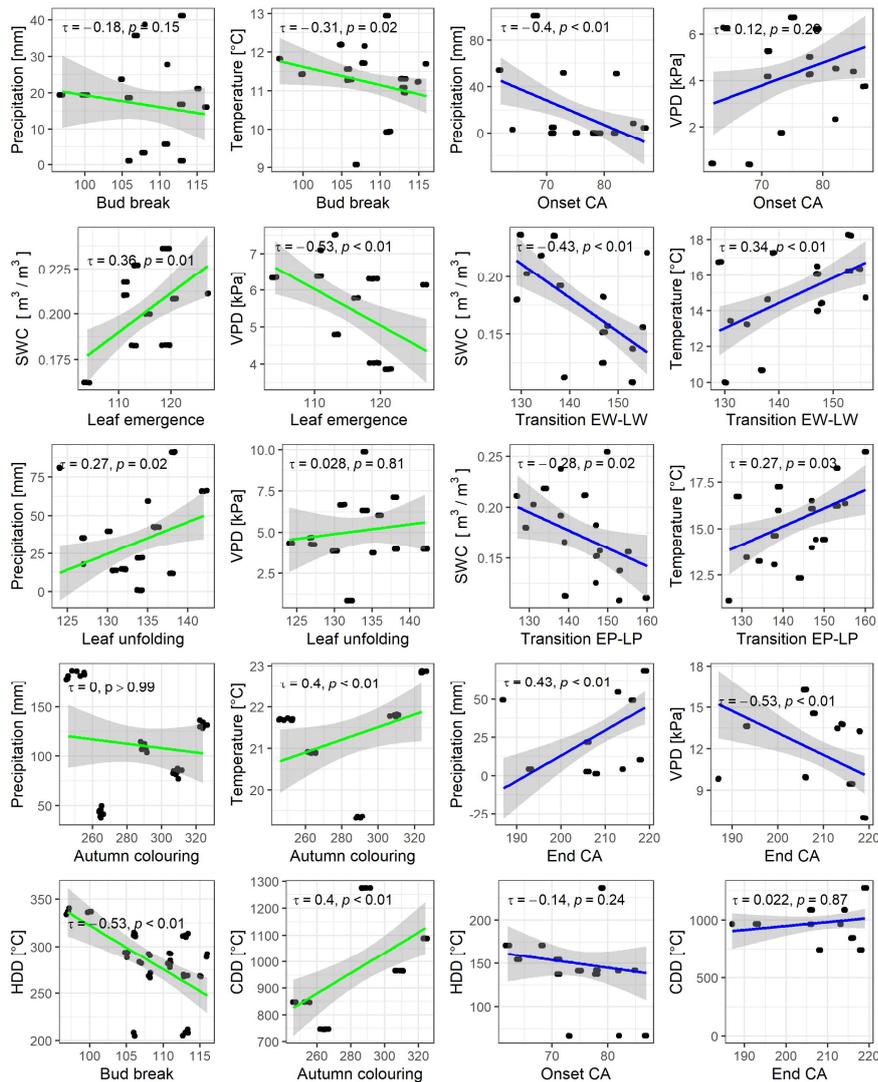
Figure 3: Scatterplots of leaf phenology (green colour) and radial growth (blue colour) plotted against different weather parameters (Heat Degree Days (HDD_5), Cooling Degree Days (CDD_{20}), 10-day mean temperature, vapour pressure deficit (VPD), soil water content (SWC) and precipitation sum before the phenological phase). Here we show only more significant variables of SWC/precipitation and temperature/VPD pairs, while remaining panels are shown in Supplementary Figure 5. For autumn colouring we considered a fixed season between DOY 210 and 240. Each observation is shown per tree. Abbreviations: CA (cambial cell production), EW (earlywood), LW (latewood), EP (early phloem), LP (late phloem).

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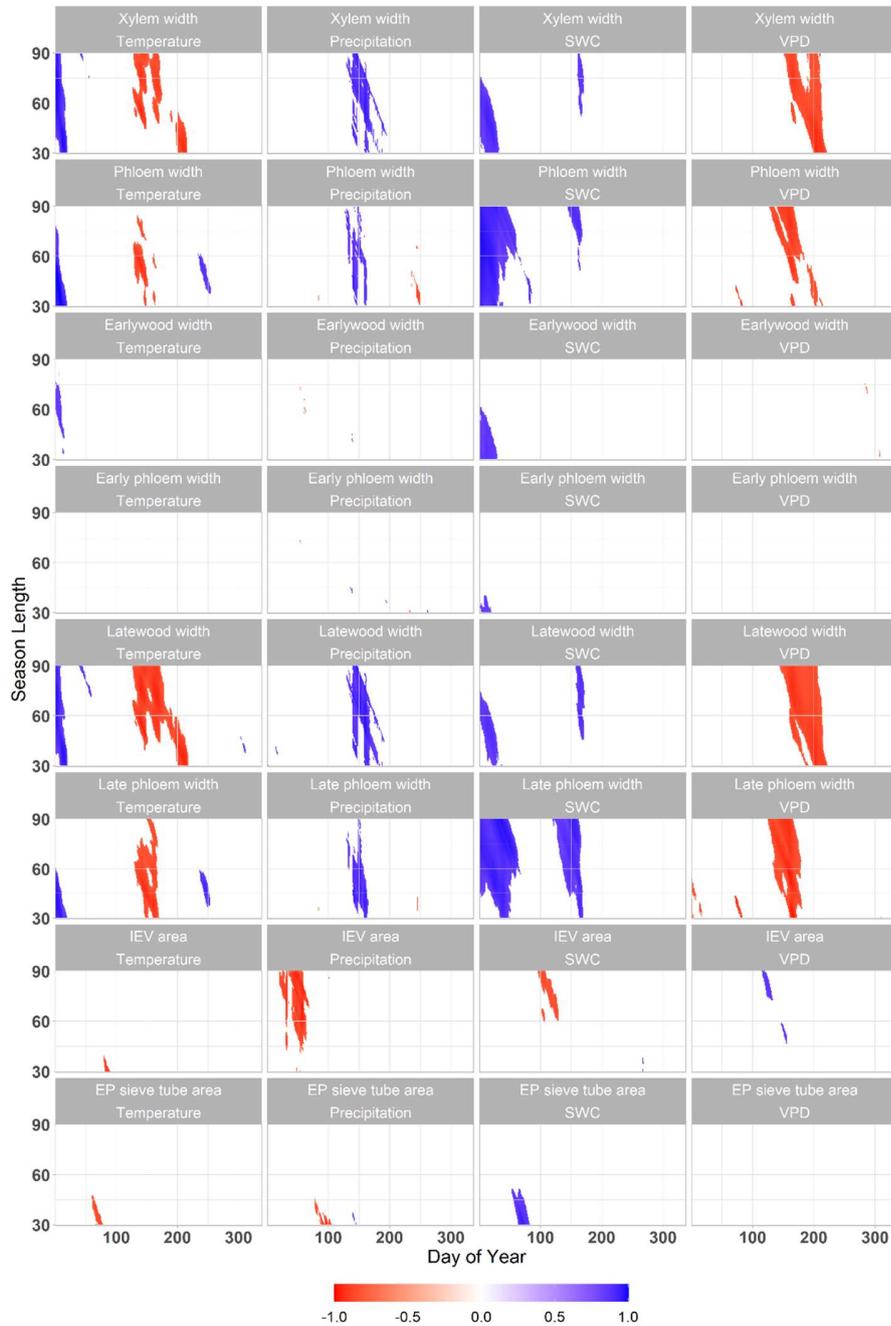
Figure 4: The effect of mean temperature, precipitation sums, soil water content (SWC) and vapour pressure deficit (VPD) on xylem and phloem traits in the 2014–2019 period. We show only significant correlations (p < 0.05) between the pairs of xylem/phloem variable and aggregated daily environmental data. Abbreviations: IEV (initial earlywood vessel), EP (early phloem).

Supplementary Material

Supplementary Table 1: Spring and Autumn cambium and leaf phenological observations with day of observation and respective Growing Degree

Days (GDD_5), Heat Degree Days (HDD_5) and Cooling Degree Days (CDD_{20}). Abbreviation: CA (cambial cell production).

| Year	Spring phenological observations	DOY	GDD_5 [°C]	HDD_5 [°C]	Autumn phenological observations	DOY	CDD_{20} [°C]
2014	Onset CA	79	247	236	End CA	219	1274
2015	Onset CA	73	143	137	End CA	216	847
2016	Onset CA	80	177	142	End CA	211	746
2017	Onset CA	77	143	141	End CA	192	967
2018	Onset CA	79	116	67	End CA	209	1088
2019	Onset CA	68	148	154	End CA	208	965
2020	Onset CA	66	175	171	End CA	-	-
2014	Bud break	110	434	312	Autumn colouring	289	1274
2015	Bud break	112	339	269	Autumn colouring	250	847
2016	Bud break	108	356	292	Autumn colouring	264	746
2017	Bud break	99	289	337	Autumn colouring	-	-
2018	Bud break	-	-	-	Autumn colouring	324	1088
2019	Bud break	110	366	282	Autumn colouring	309	965
2020	Bud break	112	399	208	Autumn colouring	-	-

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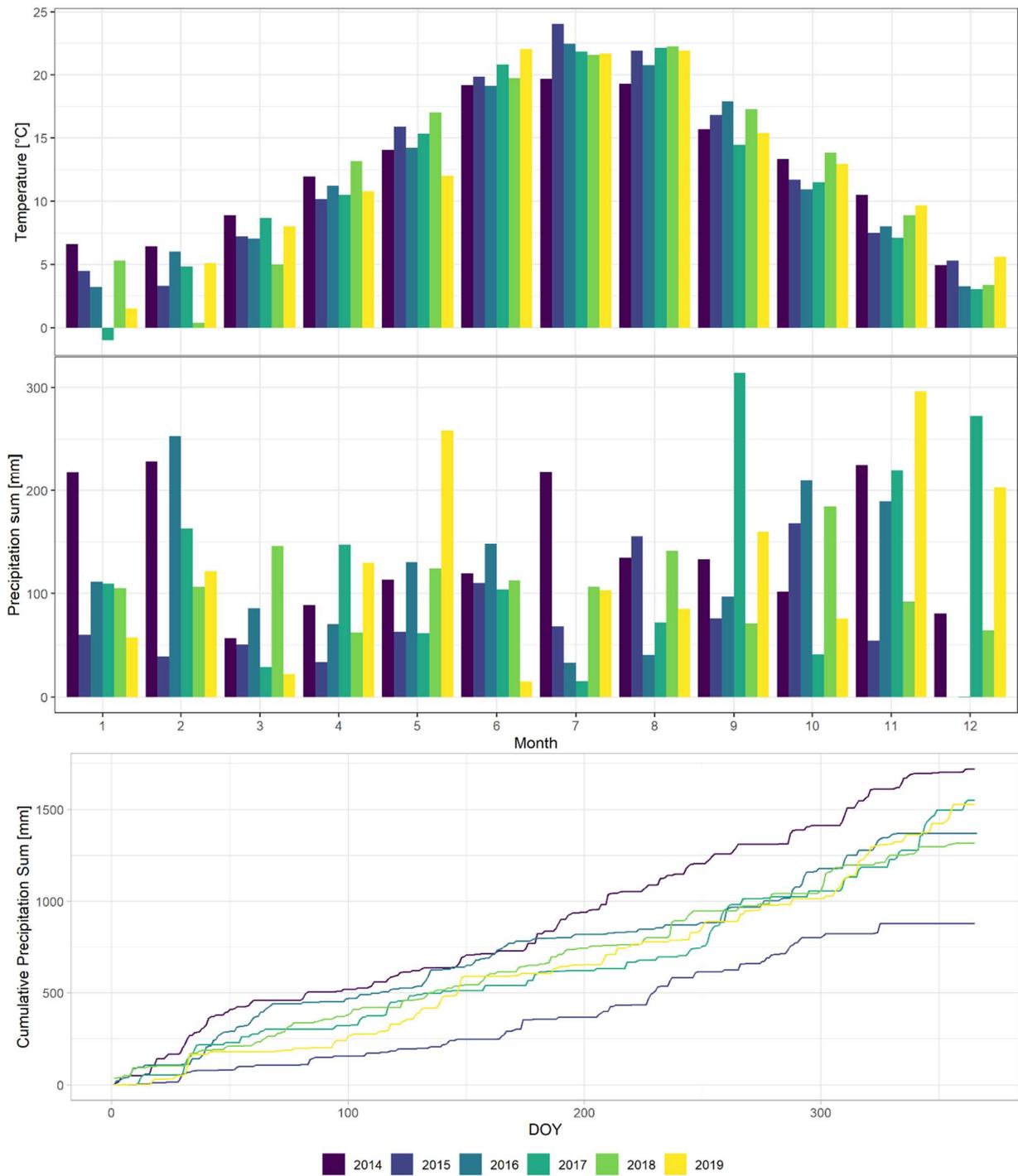
Supplementary Figure 1: Monthly mean temperature and precipitation sums, including cumulative sums based on daily precipitation data for the analysed years 2014–2019.
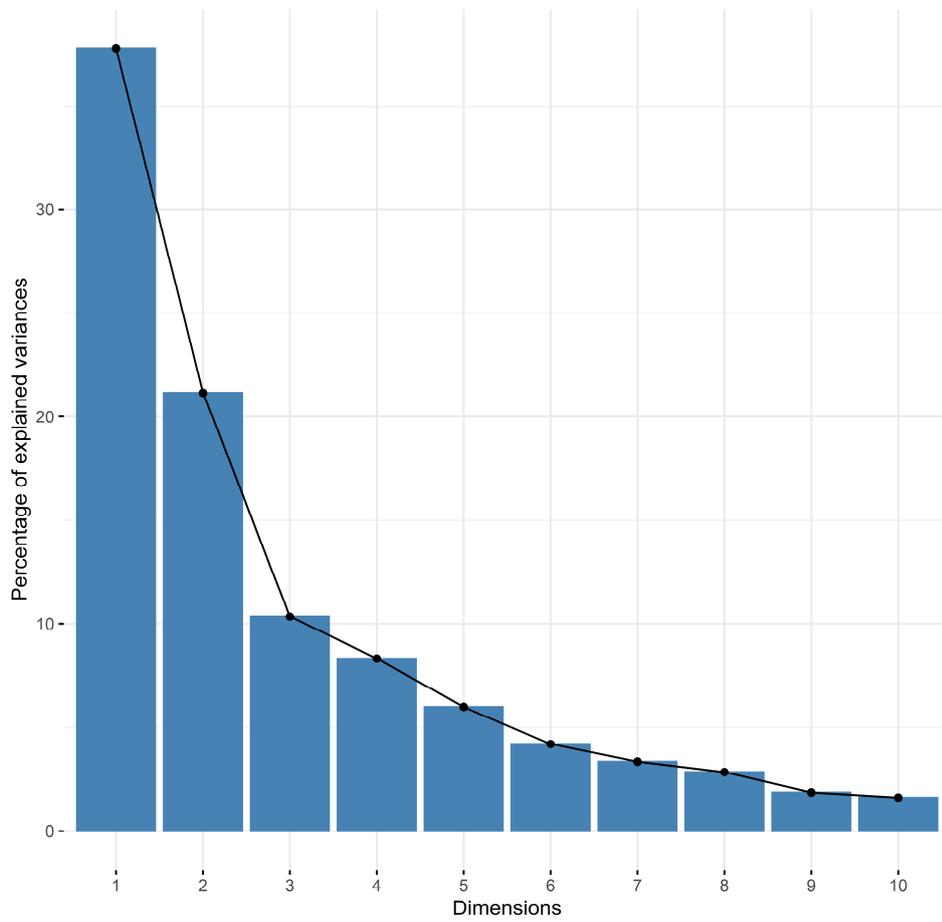
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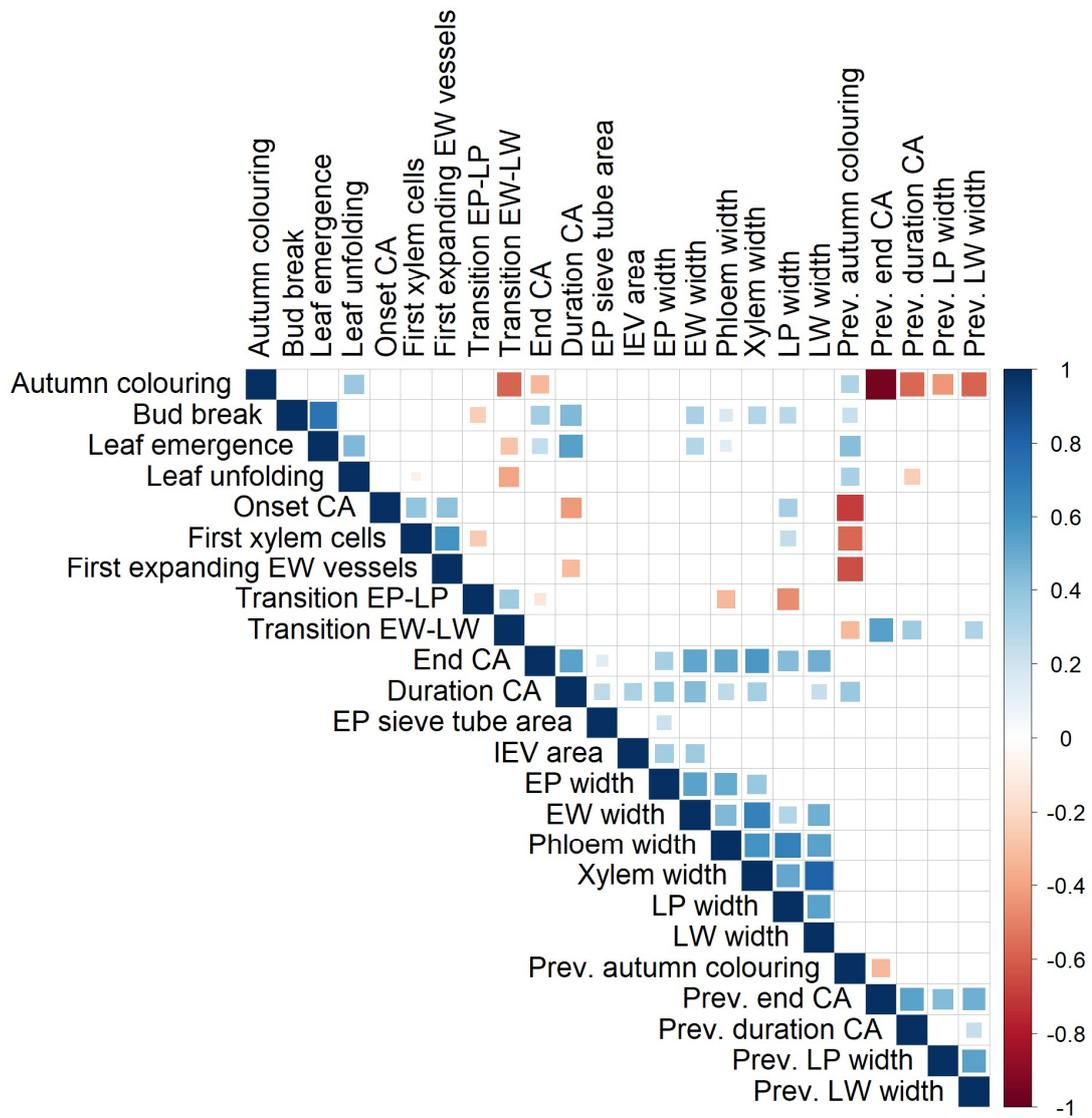
839

840 **Supplementary Figure 2**: Kendall's τ coefficients among xylem and phloem traits and phenology

841 of leaf, xylem and phloem. Only significant coefficients with *p < 0.05* are shown. Abbreviations:

842 Prev. (previous), CA (cambial cell production), EW (earlywood), LW (latewood), EP (early phloem), LP (late
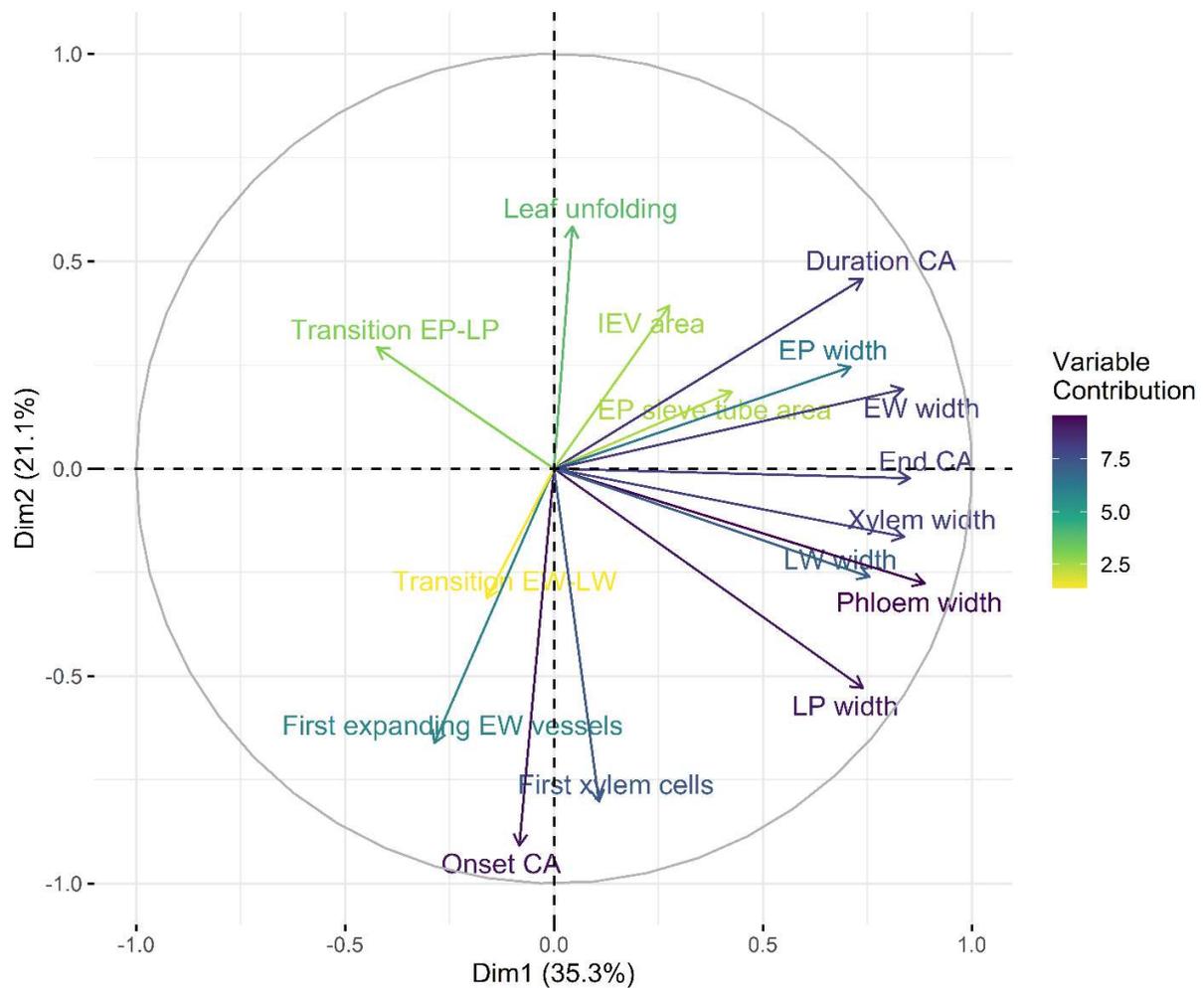
843 phloem), IEV (initial earlywood vessel).

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Supplementary Figure 3: Scree plot showing the percentage of explained total variance for the first ten

847   principal components.

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Supplementary Figure 4: The interdependence of the studied ecophysiological variables was analysed with the alternative loadings plot, where variables bud break and leaf emergence are excluded and the year 2018 is included. We can observe the effects of the studied variables on PC component one and two. Positively correlated variables point in the same direction, negatively correlated variables diverge and form an angle close to 180°, while unrelated variables meet each other at approximately 90°. Abbreviations: CA (cambial cell production), EW (earlywood), LW (latewood), EP (early phloem), LP (late phloem), IEV (initial earlywood vessels).

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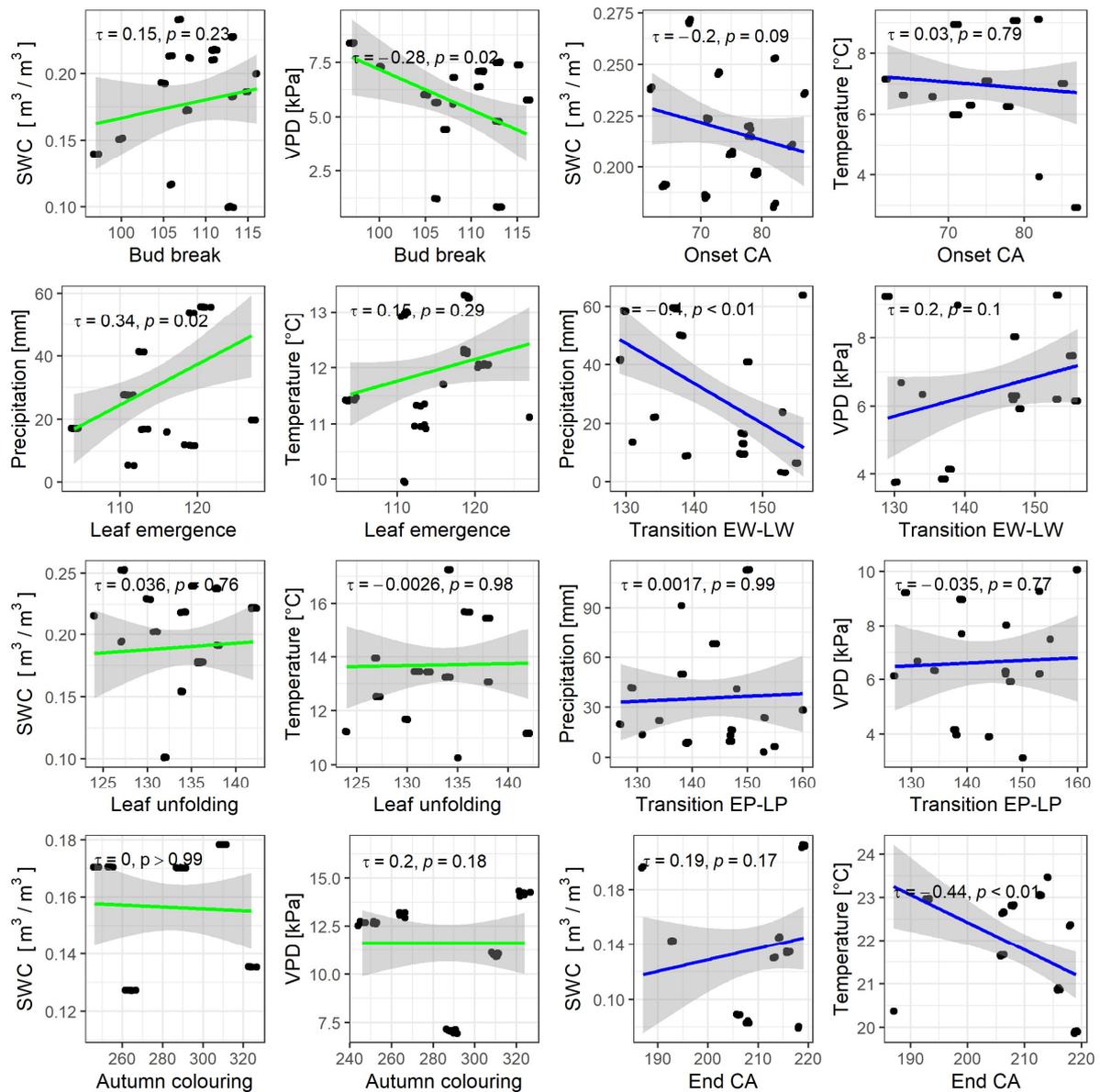
Supplementary Figure 5: Scatterplots of leaf phenology (green colour) and radial growth (blue colour) plotted against different weather parameters (Heat Degree Days (HDD$_5$), Cooling Degree Days (CDD$_{20}$), 10-day mean temperature, vapour pressure deficit (VPD), soil water content (SWC) and precipitation sum before the phenological phase). Here we show only less significant variables of SWC/precipitation and temperature/VPD pairs, while remaining panels are shown in Figure 3. For autumn colouring we considered a fixed season between DOY 210 and 240. Each observation is shown per tree. Abbreviations: CA (cambial cell production), EW (earlywood), LW (latewood), EP (early phloem), LP (late phloem).