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Anatomical tree-ring chronologies and seasonal patterns of cambial dynamics are valuable indicators of tree performance of two oak species at the Atlantic-Mediterranean boundary

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ABSTRACT

Northwestern Iberia is characterized for being an Atlantic/Mediterranean transitional area, where the most natural forests contain certain species, typical from either biogeographic region, growing under limiting conditions due to their marginal location. In order to identify the main climatic factors controlling growth, and thus better understand how they impact wood formation processes of the key tree species in these ecosystems, we analyzed tree rings of two somehow contrasting oaks (*Quercus robur*, Atlantic; and *Q. pyrenaica*, sub-Mediterranean) at their distribution boundary towards the Mediterranean region. For this, two nearby sites with slightly different regime of water availability were selected for each species. We developed chronologies of radial increment (expressed as latewood width) and functional anatomical traits (size and number of earlywood vessels) for the last decades, and also monitored wood formation along two growing seasons. Our results suggest that the combination of anatomical traits and radial growth constitute a useful tool to understand the behavior of these species in boundary distribution areas. We found some differences between sites, especially for *Q. pyrenaica*, but the main factors controlling growth were clearly identified at all sites. Earlywood characteristics were mainly related to temperature, whereas latewood width responded to precipitation, regardless of the species. However, vessels of low-elevation *Q. robur* seemed to be controlled by factors affecting carbohydrate balance, while those of high-elevation *Q. pyrenaica* were associated to spring conditions for growth resumption. Summer water availability was linked to a wider latewood for both species. In addition, the analysis of xylogenesis carried out at all sites was essential to interpret climate responses by providing evidences for the existence of a cause-effect relationship.

1. Introduction

Oaks (*Quercus* spp.) are dominant species in European forests, extending from the Urals to the Mediterranean Basin. Among them, nemoral oaks (*Q. petraea* (Mattuschka) Liebl., and mainly *Q. robur* L.), adapted to mild conditions, are keystone components of temperate forests (Ellenberg, 2009), but reach their southern distribution boundary towards the Mediterranean Region. Afterwards, they are progressively replaced by more drought-tolerant species, such as evergreen oaks (*Q. ilex* subsp. *ilex* L., *Q. ilex* subsp. *ballota* (Desf.) Samp.) or other xerophile species (Amaral Franco, 1990). However, transition zones are often occupied by the so-called 'sub-Mediterranean' oaks

(Sánchez-de-Dios et al., 2009), which are usually more xerophytic, but deciduous with marcescent habit (e.g., *Q. pyrenaica* Willd., *Q. pubescens* Willd., *Q. faginea* Lam.…).

Summer drought is one of the main factors driving the distribution of these species, as well as an attribute of Mediterranean climate and its associated vegetation. According to the IPCC (2013), summer drought is expected to increase, which can lead to local extinctions, as some species populations override their ecological limitations. This is the case of the Iberian Peninsula, where prediction models anticipate the replacement of *Q. robur* and *Q. petraea* by sub-Mediterranean oaks, among which *Q. pyrenaica* is the most relevant species (Benito Garzón et al., 2008; Sánchez-de-Dios et al., 2009). At present, *Q. pyrenaica* dominates

boundary areas and subhumid mountains of the Mediterranean region in Iberia. Its characteristics to deal with these harsh environments, such as pubescent leaves, a thick bark, and a very late flushing season confers a higher drought tolerance than nemoral oaks, as well as the avoidance of late frost.

Among these boundary areas, northwestern Iberia is one of the most abrupt transitions between the Atlantic and the Mediterranean regions. Natural forests of both *Q. robur* and *Q. pyrenaica* coexist at their distribution edge, being therefore a very appropriate territory to understand their behavior under changing conditions. *Q. robur* woodlands are limited to Atlantic climate, almost spreading along the whole altitudinal range, but tend to be replaced by *Q. pyrenaica* as conditions become more Mediterranean and continental towards the inland. But Mediterranean influence is also present at low-elevation coastal oak forests, the so-called thermocoline belt (Rivas-Martínez, 1987). Thus, very mild conditions throughout the year, mainly driven by temperature rather drought, favor the presence of Mediterranean or even sub-tropical (Macaronesian) floristic elements (Izco et al., 1990), but with some differences between Atlantic and Cantabrian areas (Amigo et al., 2017). In contrast, *Q. pyrenaica* occurs as conditions become more xeric. Being well adapted to continentality, it tends to dominate mountain areas (Díaz González and Penas, 2017), but also coexists in many inland areas (Díaz-Maroto et al., 2007). Understanding the performance of these forests by inferring how they are expected to cope with climate change, and consequently how vegetation changes could occur, is of great relevance from a biogeographic point of view. Moreover, it is essential for conservation management, because this region suffers from a strong human pressure, and most natural forests have been destroyed by constructions, agricultural fields, or fast-growing tree plantations such as *Eucalyptus globulus* Labill. or *Pinus radiata* D. Don.

One of the most critical tree adaptations to climate change is the shifting of phenological events (Menzel et al., 2006), so that a lengthening of the growing season, and particularly an advancement of reactivation after winter dormancy have been observed (Vitasse et al., 2017). But an increment of spring temperature anticipating leaf unfolding can involve some risks, as trees become more vulnerable to late frost (Gu et al., 2008). On the other hand, there is a close link between foliar and vascular phenology, i.e., leaf development and cambial activity (Guada et al., 2018; Takahashi et al., 2013), and increasing spring temperatures are also associated to an anticipation in the onset of cambial activity (Begum et al., 2018; Yang et al., 2017). This association is especially relevant in the case of ring-porous trees as oaks, because the formation of the first earlywood vessels is linked to leaf phenology, whereby the expansion of the first elements is concomitant or just preceding bud break, and their maturation is required for full leaf expansion (Kitin and Funada, 2016). However, Guada et al. (2019) recently argued that spring temperature might alter the synchronicity between primary and secondary growth, whereby the yearly variation of maximum and minimum temperature modifies respectively the onset of cell enlargement and budburst in a different way.

The study of xylogenesis allows identifying the immediate responses of trees to changes along the growing season, and helps understanding how trees cope with climate change (Camarero et al., 2016; Cuny et al., 2015; Guada et al., 2016). In contrast, dendrochronological techniques add a retrospective point of view, and both approaches can be combined to better understand tree reactions. Furthermore, they become a very powerful tool when combined with anatomical studies (Pacheco et al., 2015), as quantitative wood anatomy has proven to be highly valuable for the study of climate change (Fonti et al., 2010). This is the case of the earlywood vessel size of oak, whose chronologies were able to reveal the main factors controlling wood formation (Gea-Izquierdo et al., 2012; Rita et al., 2016; Souto-Herrero et al., 2018b) even in areas where climate conditions are relatively mild (Souto-Herrero et al., 2018a), or under the lack of a clear single limiting factor (Fonti and Garcia-Gonzalez, 2008).

Recent investigations carried out in northwestern Iberia pinpoint that both the monitoring of xylogenesis (Guada et al., 2019; Pérez-de-Lis et al., 2017, 2016b) and dendrochronological wood anatomy (Souto-Herrero et al., 2018a, 2018b) are very useful tools to evaluate the performance of *Q. robur* and *Q. pyrenaica*, especially if combined to achieve a better understanding of the underlying mechanisms (González-González et al., 2015). The separate dendrochronological analysis of earlywood and latewood was much more successful than the single analysis of ring width (Souto-Herrero et al., 2017, 2018b), and only when both tree-ring compartments were analyzed together, did climate-growth relationships provide a feasible interpretation of the ongoing processes. In addition, monitoring wood dynamics allowed elucidating the role of temperature on spring reactivation (Guada et al., 2019), the close relationships between leaf phenology and vessel formation (Guada et al., 2018), the environmental control of vessel size and latewood growth (Pérez-de-Lis et al., 2017, 2016b), or the influence carbohydrate dynamics on early growth (Pérez-de-Lis et al., 2016a). All these studies evidence that both species do not follow the same strategy of wood formation, probably due to their different ecological requirements.

In this paper, we aim at elucidating the responses of radial growth of oaks at the transition between the Atlantic and the Mediterranean regions. Through two independent studies, we compared i) *Q. robur* at two thermophile sites of its lower distribution limit, but under more humid (Cantabrian coast) or drier (Atlantic coast) summer conditions; and ii) *Q. pyrenaica* at its upper boundary, also differing in water availability due to their opposite facing within a mountain range, where the southern slope had more Mediterranean conditions. For this, we developed tree-ring width and anatomical chronologies, and monitored wood formation at the four sites. Our main objectives are i) to assess how powerful these methodologies are in order to identify the main factors driving growth performance at the boundary to the Mediterranean region, and ii) to evaluate to what extent these factors are sensitive to small differences in the regime of water availability.

2. Materials and methods

2.1. Study area and sites

We selected two areas in the northwestern Iberian Peninsula, respectively corresponding to the lower limit of *Q. robur* (**Qrob**), and the upper limit or *Q. pyrenaica* (**Qpyr**); within each area, we chose two stands slightly differing in their summer water regime. **Qrob** was located close to the coastline, whereas **Qpyr** occupied mountain areas inland, above 1000 a.s.l. (Fig. 1). Although regional climate is mainly Atlantic with more than 1200 mm of annual precipitation (maximum during fall/ winter), its seasonal distribution is very variable spatially. Mediterranean influence is associated to a remarkable summer drought towards the inland, but also at some lowland areas along the Atlantic, where maximum temperature can be high (Table 1). As both areas had different characteristics, the analyses for **Qrob** and **Qpyr** sites were taken as two independent experiments, and all pairwise comparisons were performed just within species.

Qrob was studied in coastal areas (43.42–43.68 ºN, 7.71–8.06 ºW), where natural vegetation is dominated by oak (*Q. robur*) forests, and *Q. pyrenaica* usually absent. Both sites belong to the association *Blechno spicantis-Quercetum roboris*, and specifically to its most thermophile facies (Izco et al., 1990). The mild (13 ºC) and moist (ca. 1200 mm) conditions throughout the year are indicated by the presence of some subtropical elements, such as Macaronesian ferns (*Culcita macrocarpa* C. Presl., *Woodwardia radicans* (L.) Sm., *Hymenophyllum tunbrigense* (L.) Sm., *Asplenium onopteris* L., or *Dryopteris guanchica* Gibby & Jermy). These two woodlands correspond to the mouths of two rivers. River Eume is facing the Atlantic coast (*Qrob*-D), which results in a drier summer due to a higher temperature and consequently evapotranspiration, whereas the river Sor flows into the Cantabrian Sea, yielding more humid summers (*Qrob*-H), with mean maximum temperature ca. 3
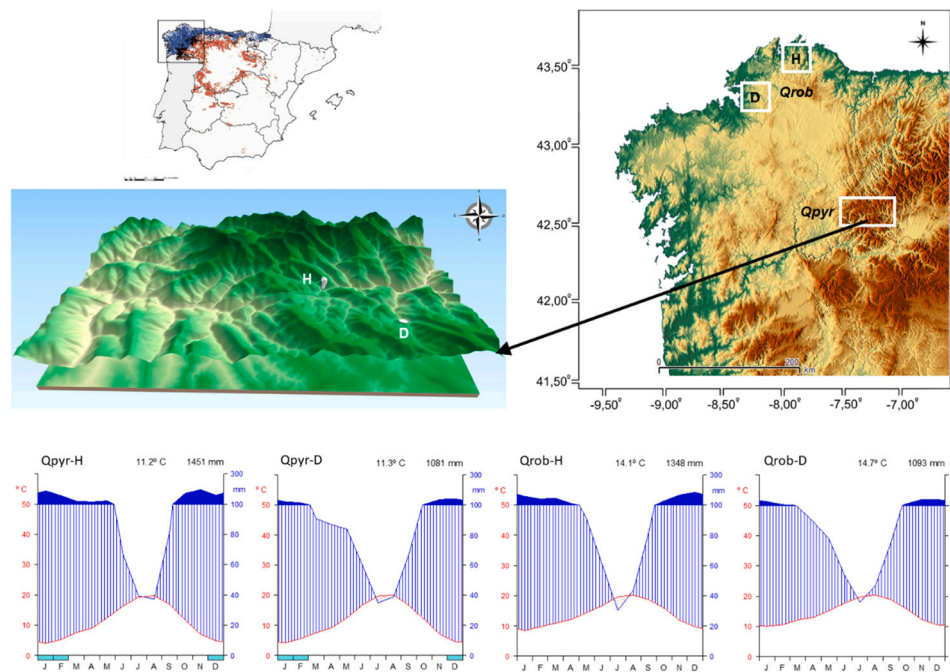
Fig. 1. Plot locations of *Quercus robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) for the dry (D) and humid (H) sites, with climate diagrams for each one. Blue painted area corresponds to distribution of *Quercus robur*, while red one corresponds to distribution of *Quercus pyrenaica*; the dark intermediate area indicates where both species coexist.
Source: Adapted from GIS-FOREST (INIA) 2016, https://sites.google.com/site/sigforestspecies/).

Table 1
Description of the sites included in the analysis, with their identification codes (ID), geographical location, and elevation, of *Q. robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) at the dry (D) and humid (H) sites. Normal annual temperature (Temp), precipitation (Prec), annual water balance (WB) and summer WB; values were obtained from Rodríguez-Lado et al. (2016) (source: digital database at http://rgis.cesga.es), Two-year temperature records correspond to sensors at the study sites, including mean (Tmean), minimum (Tmin), and maximum (Tmax) temperatures.

				Normal Values				2012/13 years values		
ID	Latitude	Longitude	Altitude	Temp	Prec	Annual WB	Summer WB	Tmean	Tmin	Tmax
	(N)	(E)	(m.a.s.l.)	(°C)	(mm)	(mm)	(mm)	(°C)	(°C)	(°C)
Qrob-D	43.416991	-8.064395	125	13.2	1158	562.2	-174.3	13.8	9.9	19.6
Qrob-H	43.676000	-7.708347	125	13.0	1194	475.3	-168.0	12.3	9.0	16.1
Qpyr-D	42.684528	-7.094356	950	8.5	1428	724.3	-161.1	10.3	6.5	16.1
Qpyr-H	42.664597	-7.054362	1050	8.82	1433	830.0	-146.2	9.5	5.3	14.8

°C lower during summer (Table 1).

Qpyr stands were located at the southwestern edge of the Cantabrian Mountain Range (42.64–42.70 ºN, 7.02–7.13 ºW), an area of a complex topography with peaks around 1300 m. With a mean annual temperature below 10 ºC, summers are mild (17 ºC), and winters cold (4.7 ºC), with frost periods, frequent fogs, and thermal inversions in the valleys; precipitation is high (>1400 mm/year), also concentrated in fall and winter (often as snow), with a negative water balance in summer. The selected sites constitute the upper distribution boundary of the species, still lie within the Atlantic biogeographical region (Rodríguez Guitián and Ramil Rego, 2008), and belong to the association *Linario triornithophorae-Quercetum pyrenaicae* (Rivas-Martínez, 1987), usually poor communities on acid soils. The humid site (*Qpyr*-H) was facing north on deep schistic soils with abundant organic matter, accompanied by *Fagus sylvatica* L. on the wettest depressions. This moister environment was indicated by species such as *Corylus avellana* L., *Ilex aquifolium*, *Vaccinium myrtillus* L., *Omphalodes nitida* (Willd.) Hoffmanns & Link or *Physospermum cornubiense* (L.) DC, and abundant bryophytes. In contrast, the dry site (*Qpyr*-D) was facing south on a moderate slope, and was a monospecific formation of *Q. pyrenaica* with a poor understory of *Rubus* sp. or *Pteridium aquilinum* (L) Kuhn in Kerst., with *Pyrus cordata* Desv. and *Erica arborea* L. towards the forest edge; no elements

evidenced a high humidity; furthermore, the Mediterranean *Q. ilex* subsp. *ballota* occurred not far from this site.

2.2. Xylem sampling and wood phenophases

We monitored wood formation during two consecutive years, from March to November in 2012, and from February to September in 2013, with a sampling interval of 12–15 days in spring, and 20 in summer. During each visit, we randomly selected ten trees per site within an area of ca. 1 ha, in order to have a representative sample of the whole forest. Sampled trees had a diameter at breast height (DBH) ranging 20–40 cm (average was 28.2 ± 4.9 cm, with no significant differences between sites), and trees with polycormic stems, partially dead crowns, or evident damage, were avoided.

We used a Trephor tool (Rossi et al., 2006) to extract at least two wood microcores at breast height (1.3 m), perpendicularly to the slope, which contained mature and currently developing xylem, the cambial zone and adjacent phloem, and one to a few complete tree rings. At the lab, we handled the samples following the protocol by (Guada et al., 2018; Pérez-de-Lis et al., 2016b) to obtain permanent slides, which includes fixation in alcohol, paraffin embedding, cutting by a rotatory microtome, and differential staining.

We took images of the microscopic slides (0.926 μm/pixel) using a digital camera (Canon EOS 600D, Tokyo, Japan), coupled to a transmitted light microscope (Olympus BX40, Tokyo, Japan; 40 × objective); a white light polarizing filter help detecting secondary cell wall deposition. On these images, we identified cambial phenological phases, counted the number of cambial cells, and measured the width of expansion and maturation zones along three radial files. Vessel enlargement was recorded when the most recent ring contained at least one enlarging vessel element; the onset of earlywood vessel maturation was defined by the deposition of secondary cell wall, detected by birefringence under polarizing light.

2.3. Leaf phenological observations

Leaf phenology of each tree sampled for wood formation was recorded by observing the top of the main branches using binoculars (10 × magnification), and expressed as a particular day of year (DOY).

We identified four phenophases, namely, a) budburst, b) leaf unfolding, c) appearance of small leaves, and d) full leaf extension. Budburst was characterized by expanded buds with no unfolded leaves; leaf unfolding ended as soon as the leaf blade was clearly visible, but not the petiole; appearance of small leaves was considered when at least one leaf was completely out of the bud, and the petiole could be visually appreciated; and full extension was recorded when leaves attained at least the 50% of their apparent final size.

2.4. Dendrochronological sampling, ring width, and vessel measurements

At each site, we selected 11–26 dominant or isolated oaks for developing tree-ring chronologies during the late spring of 2016, and extracted at least two 5-mm increment cores at breast height. Cores were air-dried, mounted on wooden supports, and prepared for an optimal visualization of tree rings and vessel elements. We obtained a regular cross-sectional surface with a WSL sliding microtome (Gärtner and Nievergelt, 2010), manual polishing (sandpaper P220 to P1200, FEPA Abrasives), removal of dust and tyloses by high-pressure water blast, staining with black printer ink, and finally rubbing chalk on the surface (Souto-Herrero et al., 2018b).

We measured earlywood (EW) and latewood widths (LW) to the nearest 0.001 mm, with a tree-ring measuring linear stage (Velmex TA UniSlide, Velmex Inc., Bloomfield NY, USA) and a binocular microscope (Olympus SZ60) at 20–40 × magnification. Crossdating was obtained by first comparing individual curves, and then statistically verified using COFECHA (Grissino-Mayer, 2001). Further measurements of the earlywood vessels were performed on sequences of high-resolution digital images (5,184×3456, 17.9 Mpx) taken with a Canon EOS 600D camera coupled to the binocular microscope and a mechanically-driven platform, stitched (PTGui ver. 9.1.8 Pro, New House Internet Services B.V., Rotterdam, The Netherlands) into a single file per core, and saved into TIFF files. These images were processed in ImageJ (Schneider et al., 2012), using the VesselJ plugin and Autovasos (for further details on the process of image analysis, see Souto-Herrero et al. (2017) and (2018b)). We only retained vessels larger than 10,000 μm^2, and separated those in the first row from the others. All raw data (ring widths and vessel measurements) from cores of the same tree were respectively averaged or pooled for each ring.

2.5. Variable survey and tree-ring analyses

We obtained chronologies for latewood width (LW), and earlywood vessel number (NV) and size, which was expressed as the hydraulically-weighted diameter (D_H), following Sperry et al. (1994); D_H estimates the average diameter for the theoretical conductivity of a given stem (Fichot et al., 2009), and is probably the most appropriate variable to relate earlywood vessels and environment (Souto-Herrero et al., 2017, 2018b), for being robust to the presence of small vessels (García-González et al.,

2016). Vessel size was considered separately for the first row (D_H-r1), and for vessels outside this row (D_H-nr1).

Individual series were detrended by first adjusting a 32-year cubic smoothing spline with 50% cutoff (Cook et al., 1992), which was flexible enough to minimize both age trend and forest dynamics. Afterwards, we computed growth indices by division (Fritts, 1976), applied autoregressive modeling to remove persistence, and averaged the indices into a chronology for each variable and site using a biweight robust mean (Mosteller and Tukey, 1977). The obtained chronologies were subsequently assessed for their quality, using the most common parameters in dendrochronology, namely the mean sensitivity (*MS*), an indicator of interannual variability; the first order autocorrelation coefficient (*AR1*), which measures the influence of previous year on growth; the mean correlation between trees (*Rbt*), i.e., the mean value of all possible Pearson's cross-correlation coefficients; and the expressed population signal (*EPS*), indicating the extent to which the sample size is representative of a theoretical population with an infinite number of individuals (Wigley et al., 1984).

2.6. Comparison of series and climate-growth relationships

For each species and variable, we ordinated individual tree-ring series by varimax-rotated principal component analysis (PCA), calculated from the correlation matrix of all selected trees. Series were pairwise compared along their entire length, and the correlation coefficient corrected for a theoretical common period. All **Qpyr** trees overlapped for a longer period, but the length of **Qrob** series was shorter (especially at **Qrob-H**), and consequently only series overlapping at least 25 years with all trees considered; thus, five trees had to be excluded (two at **Qrob-D**, three at **Qrob-H**; out of a total of 40 **Qrob** trees).

For both species, we selected the period 1960–2013 (53 years) to establish climate-growth relationships, covered by at least 10 trees per site, using monthly series of climatic variables of temperature (mean, minimum, and maximum), and water regime (total precipitation, standardized precipitation-evapotranspiration index – SPEI – at one and three months). All data were obtained from CRU TS 4.01, a gridded source at 0.5° × 0.5° of geographical resolution available from the KNMI Climate Explorer (http://climexp.knmi.nl/). The SPEI is a multiscalar drought index that combines precipitation and potential evapotranspiration (Vicente-Serrano et al., 2010), successfully applied to understand the response of trees using both dendrochronology (Peña-Gallardo et al., 2018) and xylogenesis (Ren et al., 2015).

We computed Pearson's correlation coefficients between the site chronologies and the monthly meteorological records. Confidence intervals for the correlation functions were achieved by the bootstrap technique (Guiot, 1991), using the correction proposed by Mason and Mimmack (1992) for the percentile method. According to own observations of cambial activity and leaf phenology (Guada et al., 2018, 2019; Pérez-de-Lis et al., 2017), and the analyses of xylogenesis in this paper, we considered for Pearson's correlation from previous to current May for earlywood, and from previous May to September for LW. In addition, we used seasonal means for the periods of dormancy (December-February), late dormancy-quiescence (February-March), or early spring (March-April, April-May, March-May) for earlywood variables; and late spring-early summer (May-July), or current summer (June-July, July-September) for latewood width.

3. Results

3.1. Xylogenesis and leaf phenology

For **Qrob**, the dormant cambium consisted of 5–7 cells for both sites and years (Fig. 2), but increased during the growing season, reaching a maximum (9.52 ± 0.28 in 2012; 8.33 ± 0.29 in 2013) around the summer solstice (178 DOY). The pattern of cell enlargement was bimodal, with maxima at earlywood vessel formation, and at the
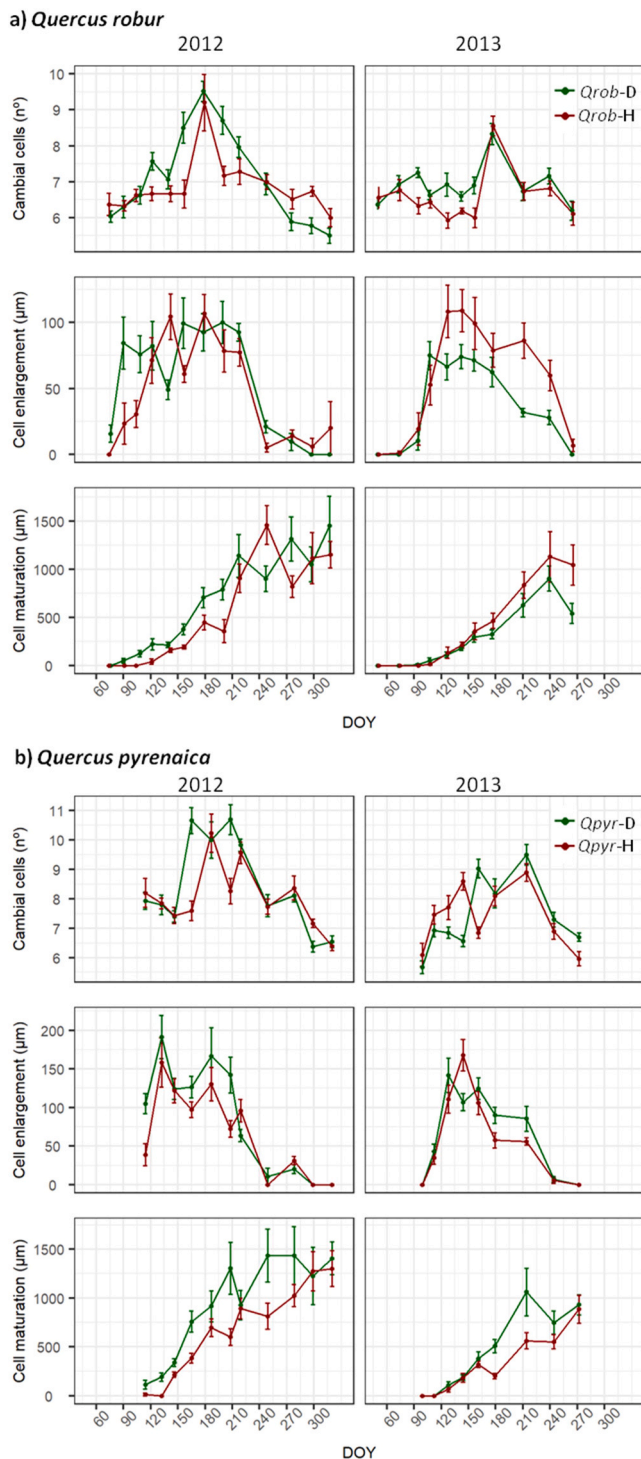
Fig. 2. Seasonal dynamics of radial growth in 2012 and 2013 of a) *Quercus robur* (*n*=10) and b) *Q. pyrenaica* (*n*=10) at the 'dry' (D) and 'humid' (H) sites. Vertical bars represent the standard error, DOY day of the year.

development of summer latewood. In 2012, the first and second peak occurred earlier at **Qrob-D** (90–120 DOY and 150–210 DOY) than at **Qrob-H** (120–150 DOY and 180–210 DOY), whereas this pattern was less evident in 2013, when the highest values occurred in May (120–150 DOY) at both sites; but showing an overall larger width increment for the Cantabrian **Qrob-H** (108.83 ± 16.01 μm vs. 74.08 ± 9.13 μm). In addition, growth cessation occurred later at **Qrob-H**, with two trees still undergoing cell enlargement at the end of September 2012 (265 DOY), and even one in November 2012 (318 DOY).

The onset of cell maturation in 2012 was anticipated for **Qrob-D** (90–120 DOY vs. 120–150 DOY) and delayed in 2013 at this site (May, 120–150 DOY). The total maturation zone was wider in 2012 (1452 ± 307.89 μm) than in 2013 (540.98 ± 105.63 μm) at **Qrob-D**, and similar for both years at **Qrob-H** (1150.55 ± 139.35 μm in 2012; 1043.70 ± 207.63 μm in 2013).

As regards leaf phenology (Fig. 3), timings from budburst to full leaf development were earlier and shorter at **Qrob-D** (90–122 DOY vs. 91–157 in 2012; 73–126 DOY vs. 95–143 in 2013). But for trees already undergoing enlargement of the first vessels, we found all phenophases at **Qrob-D**, while trees at **Qrob-H** were mostly at budburst, or even less developed.

Dormant cambium of **Qpyr** was similar to **Qrob** (5–7 cells) in 2013, but it was already active on the first sampling date in 2012 (Fig. 2b). The number of cambial cells increased until reaching a maximum in June and July (DOY 150–210), with a higher average number of cells in 2012 (10.13 ± 0.44; 187 DOY) than in 2013 (9.19 ± 0.23; 214 DOY), and no differences between sites. The pattern of cell enlargement was also bimodal, coupled to earlywood vessel formation and summer latewood increment, and more remarkable in 2012 (early May, 132 DOY; early July, 188 DOY), than in 2013, when just a single maximum occurred in May (128–144 DOY). As a result, the average width of the cell maturation zone was wider for 2012 than for 2013 (1406.80 ± 169.06 μm vs. 887.20 ± 143.27 μm), but with no differences between sites. Cambial activity ceased at the end of summer (249 DOY, early September in 2012; 244 DOY, late August in 2013), but reactivated in autumn (278 DOY; early October) in 2012, for seven out of ten trees at each site.

There were slight differences in leaf development, with an earlier budburst at **Qpyr-D** in 2012 (114–165 DOY vs. 132–165 DOY), and apparently no differences in 2013 (128–179 DOY). But most trees at **Qpyr-D** had not undergone budburst when we observed the enlargement of the first earlywood vessels, and neither did half of them at **Qpyr-H**. The others were found to be at budbreak; but in no case were leaves expanded, confirming that vessels start forming before this stage. Furthermore, xylem maturation was concomitant to leaf unfolding in 2012, and to budburst in 2013 (Figs. 2 and 3).

3.2. Tree-ring analyses and climate-growth relationships

The comparison of individual series by PCA (Fig. 4) showed that the similarity among trees from both sites was considerably higher for **Qpyr**, as expressed by the highest variance in the first eigenvector. Among variables, LW and D_H-r1 present the clearest results. In contrast, the variables depending on vessels outside the first row (NV and D_H-nr1) have a much lower signal, with a negligible explained variance (12.1% and 15.0%, respectively) for **Qrob**, and slightly higher for **Qpyr** (23.6% and 27.8%), but always lower than that of D_H-r1, and especially, LW. As regards the ordination of individuals, at least for D_H-r1 and LW, there is in general a trend to split each contrasting site along the first eigenvector, or even along the second one for **Qpyr**. However, separation is not clear, as always a few trees from every other site are scattered, though more evident for D_H-r1.

Chronology quality confirms D_H-r1 and LW as the most reliable variables for further dendrochronological analysis (Table 2). Common signal can be considered strong for LW, and high for D_H-r1, for both **Qrob** and **Qpyr**. For the former, signal is higher for **Qrob-H**, where the values of EPS are even higher with a considerably lower number of trees; in contrast, D_H-nr1 and especially NV have a small common signal. As regards **Qpyr**, differences between sites are negligible (except for series length), and signal is very high for LW, and high for D_H-r1; for the other variables, the values are still moderate, especially for D_H-nr1. MS is high for LW, and low for vessel size, whereas AR1 evidences a notable persistence, and justifies the application of the autoregressive modeling.

The results of climate-growth relationships demonstrate the similarities among sites for both species, but also evidence the existence of some differences. As a rule, vessel size (D_H-r1) is mainly related to
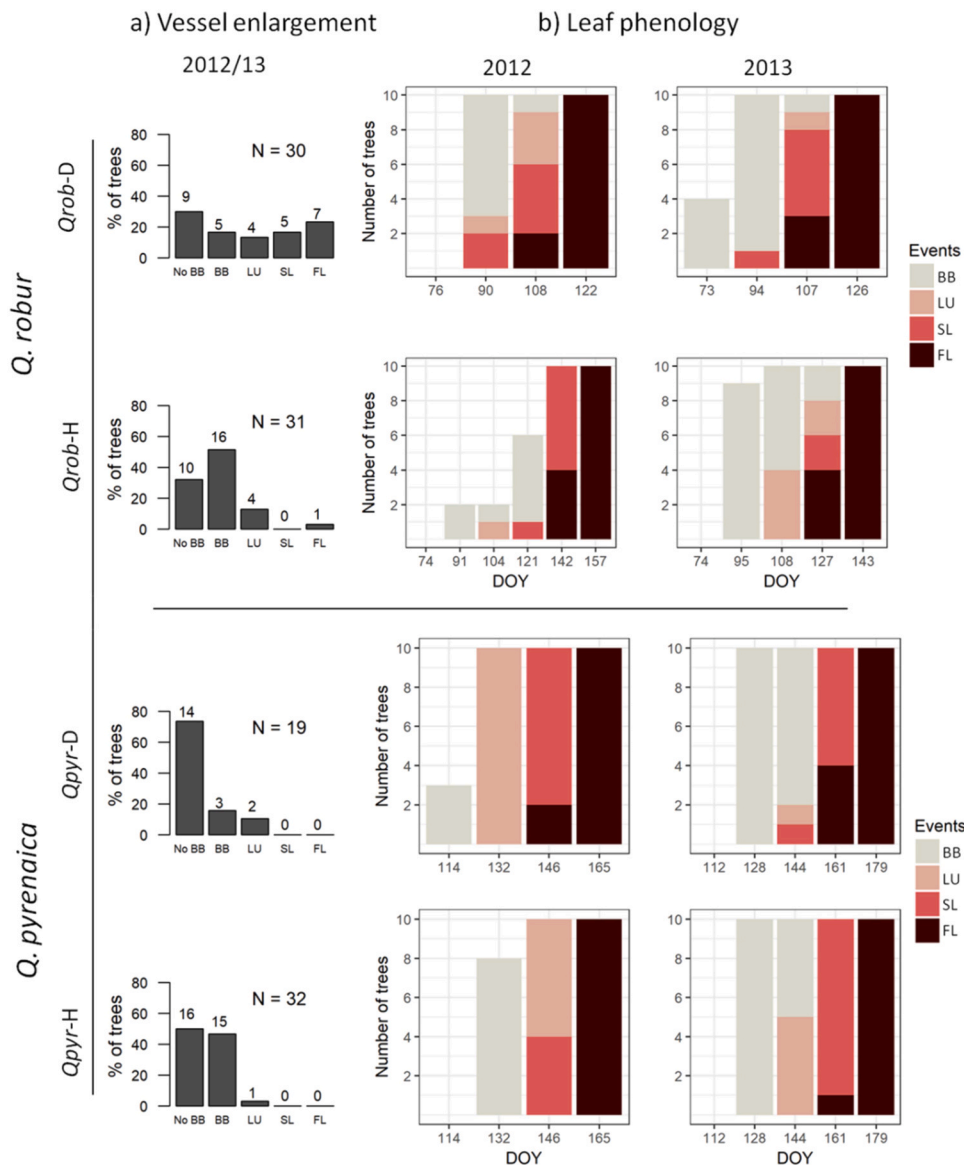
Fig. 3. Vessel enlargement and leaf phenology of *Quercus robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) at the 'dry' (D) and 'humid' (H) sites. (a) Percentage of different crown phenophases at the onset of the enlargement of the first row of vessels in 2012 and 2013; number of trees per each crown phenophase are indicated above the column. No budburst (No BB), budburst (BB), leaf unfolding (LU), small leaves with < 50% of their apparent final size (SL), full leaf expansion with > 50% of their apparent final size (FL). (b) Temporal variation of the number of trees for each leaf phenophase and year. Leaf phenology events are indicated in the legend, with lighter colors progressively referring to earlier stages (budburst, leaf unfolding, leaves with < 50% of their apparent final size, and leaves with > 50% of their apparent final size).

temperature, whereas LW to precipitation (Fig. 5); the climatic association of the variables D_H-nr1 and NV is weaker (Sup.1–2).

For **Qrob**, there is a strong negative correlation between D_H-r1 and the temperature during dormancy and quiescence (December-February), i.e, high temperatures during this period are coupled to small vessels and vice versa. There are however some differences between sites, because the signal is maximized in early winter (December) at **Qrob-H**, but at the end dormancy (February) at **Qrob-D**. Furthermore, **Qrob-D** even records some association during March, and a stronger response to maximum temperature, whereas the results at **Qrob-H** are more driven by mean and minimum temperature. Responses of earlywood are not observed later in the season, except for a weak positive correlation to minimum temperature for D_H-nr1 at **Qrob-H**, where NV is also highly related to temperature in previous spring; however, the statistical quality of the chronology is very low for this variable. With regard to LW, only a slight response to temperature is observed at early summer, probably coupled to precipitation.

As opposed to temperature, precipitation is not related to vessel size in the same growing season, but highly to LW, especially during June and July, stronger for **Qrob-H**. Moreover, this appears to be a short-term effect, as it is clearer for 1-month SPEI than for a 3-month period; a few feeble correlations were also present for late winter and early spring. For

earlywood, vessels formed later in the season had a weak negative correlation to temperature in the previous season (D_H-nr1 to August at **Qrob-D**; NV to June at **Qrob-H**).

Qpyr also exhibited an association between earlywood and temperature, as well as LW and precipitation. D_H-r1 shows remarkable differences between sites, with a strong link at **Qpyr-H**, but low at **Qpyr-D**; in fact, warm spring (April, cambial reactivation) temperatures seem to enhance vessel size at **Qpyr-H**, with a stronger effect of minimum temperature, whereas there is a weak negative link to temperature in late winter (February-March, quiescence) at **Qpyr-D**. Besides, D_H-nr1 and NV still have a significant positive correlation to temperature at the end of the previous season (September). The same response to April temperature, but weaker, is observed for LW, but in this case also for **Qpyr-D**; in addition, warmer conditions in September appear to stimulate growth as well.

Relationships to water availability are clear, with a strong correlation during summer (June through August-September, but maximized at the beginning of summer) at both sites, though more remarkable at **Qpyr-D**. A weaker negative association to conditions in previous June were also detected for both LW at **Qpyr-H**, and for D_H-nr1 at **Qpyr-D**.
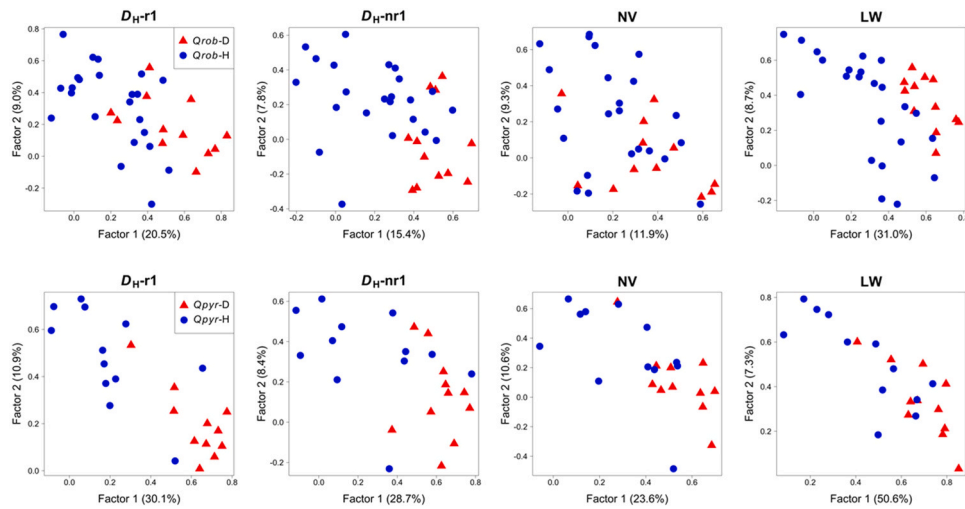
Fig. 4. Principal component analysis (PCA) of individual tree-ring series per variable, for *Q. robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) at the dry (D) and humid (H) sites. Growth variables are: D_H-r1: hydraulic diameter of the first row; D_H-nr1: hydraulic diameter for vessels not in the first row; NV: number of earlywood vessels; LW: latewood width.

Table 2
Statistics of the chronologies obtained for *Q. robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) at the dry (D) and humid (H) sites, and indicators of chronology quality for the common interval 1960–2015. SD: standard deviation; MS: mean sensitivity; AR1: first-order autocorrelation; Rbt: mean correlation between trees; EPS: expressed population signal; SNR: signal-to-noise ratio; %Var: variance in the first eigenvector. Growth variables are: D_H-r1: hydraulic diameter of the first row; D_H-nr1: hydraulic diameter for vessels not in the first row; NV: number of earlywood vessels; LW: latewood width.

	Variable	Start	End	Mean	SD	MS	AR1	Trees	Rbt	EPS	SNR	%Var
Qrob-D	D_H-r1	1906	2013	261.0	37.8	0.09	0.62	14	0.29	0.84	5.35	35.2
	D_H-nr1	1906	2013	206.0	35.4	0.12	0.51	14	0.21	0.77	3.30	29.1
	NV	1906	2013	42.3	16.6	0.20	0.55	14	0.09	0.56	1.25	20.7
	LW	1906	2013	2.08	1.28	0.42	0.64	14	0.46	0.92	10.80	50.80
Qrob-H	D_H-r1	1903	2013	281.8	40.4	0.07	0.65	26	0.14	0.80	3.92	22.3
	D_H-nr1	1903	2012	223.9	37.1	0.10	0.57	26	0.08	0.67	1.99	14.5
	NV	1903	2013	46.4	14.0	0.18	0.50	26	0.09	0.69	2.26	16.2
	LW	1903	2013	2.43	1.10	0.26	0.68	26	0.21	0.86	6.14	26.34
Qpyr-D	D_H-r1	1942	2015	282.9	40.9	0.07	0.69	11	0.38	0.87	6.59	44.6
	D_H-nr1	1942	2015	224.3	32.1	0.09	0.61	11	0.35	0.85	5.78	41.7
	NV	1942	2015	48.3	19.4	0.18	0.63	11	0.26	0.79	3.81	33.7
	LW	1942	2015	1.41	0.61	0.35	0.43	11	0.55	0.93	13.23	59.35
Qpyr-H	D_H-r1	1893	2015	283.5	38.4	0.07	0.74	12	0.26	0.81	4.21	33.1
	D_H-nr1	1893	2015	221.0	34.5	0.11	0.55	12	0.18	0.72	2.52	26.5
	NV	1893	2015	43.2	21.1	0.18	0.61	12	0.22	0.77	3.43	30.3
	LW	1893	2015	0.92	0.74	0.38	0.71	12	0.50	0.92	11.78	54.41

4. Discussion

In our study, we compared two different nearby sites for each one nemoral (*Q. robur*) and one sub-Mediterranean (*Q. pyrenaica*) oak species, growing at their distribution boundary. For this, we obtained tree-ring chronologies of radial increment (namely LW), and anatomical features (earlywood vessel size and number, expressed as D_H-r1, D_H-nr1, and NV), and monitored leaf phenology and cambial dynamics along two growing seasons. However, we did not intend to directly compare between species since they were growing under different climatic conditions (*Q. robur* in coastal lowland, *Q. pyrenaica* on inland mountains) but to understand i) how these species behave at their Atlantic/Mediterranean distribution boundaries, and ii) how the combined analysis of earlywood anatomy and radial increments contributes to understand their performance.

Chronologies of latewood had a much better statistical quality than those of earlywood. For the latter, only the first-row vessels (D_H-r1) reached high EPS values around 0.85, the most standard reference in dendrochronology (Wigley et al., 1984), and not far from those in ring width; in contrast to variables influenced by vessels formed later (D_H-nr1 and NV). Previous studies reported much lower climatic signals for

anatomical variables of ring-porous trees, especially for vessel size (Fonti and García-González, 2004; García-González et al., 2016; Kniesel et al., 2015). In fact, García-González et al. (2016) argued that the 'classical' dendrochronological assessment of the signal is probably not appropriate for vessel features, and several recent papers do not even report these values among their results (Castagneri et al., 2017; Matisons et al., 2012; Souto-Herrero et al., 2018a).

Our tree-ring series recorded a clear climatic signal for both ***Qrob*** and ***Qpyr***, and the monitoring of xylogenesis during 2012 and 2013, complementing other regional surveys (Guada et al., 2018, 2019; Pérez-de-Lis et al., 2017, 2016b) pointed to a cause-effect relationship of meteorological conditions upon earlywood anatomical structure (D_H-r1) and radial increment (LW). Thus, PCA of individual trees indicated that these two variables recorded not only the main macroclimatic signal, expressed by their common variance explained; but also site-related conditions, as trees tended to split between sites for both species. This pattern of variation was also observed in the results of xylogenesis and the correlations to climate.

For ***Qrob***, D_H-r1 was strongly linked to temperature during the dormant season, and probably also during quiescence, whereby warm temperature was coupled to small vessels. An association between
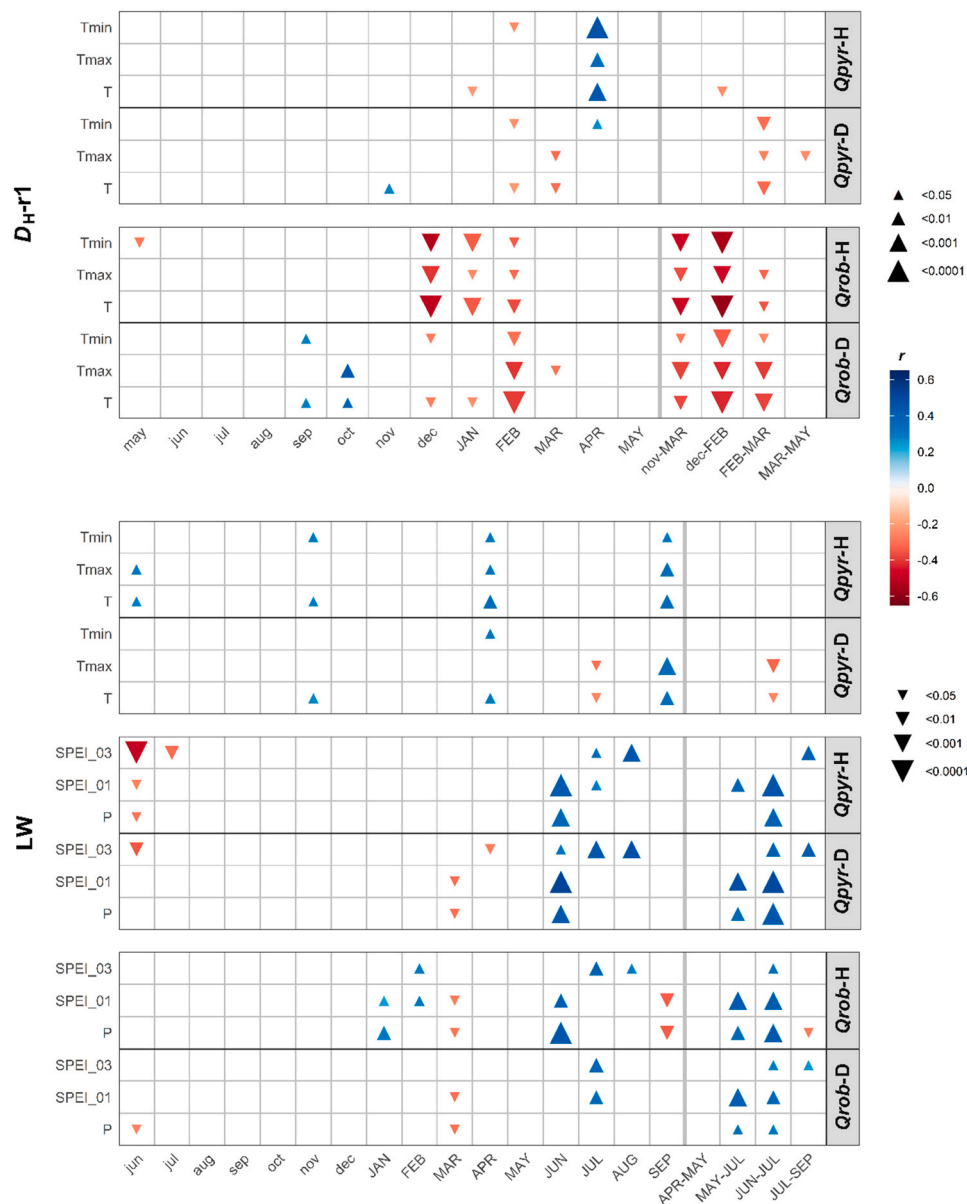
Fig. 5. Main climate-growth relationships for *Quercus pyrenaica* (Qpyr), and *Quercus robur* (Qrob) at the dry (D) and humid (H) sites, respectively; for hydraulically-weighted diameter of the first row of earlywood vessels (D_H-r1), and latewood width (LW). Climatic variables are: maximum (Tmax), minimum (Tmin), mean (T) temperature, standardized precipitation-evapotranspiration index (SPEI) at one (SPEI_1) and three months (SPEI_3), and total precipitation (P).

earlywood vessel size and temperature during dormancy has been observed for several regions (Fonti et al., 2007; Pritzkow et al., 2016), and is especially relevant for low-elevation woodlands in the study region (González-González et al., 2014; Souto-Herrero et al., 2018a) and other areas in northern Spain (Akhmetzyanov et al., 2019). Such a relationships was commonly associated to carbohydrate balance, hypothesizing that high winter temperatures would fuel sugar reserves by increasing respiration rates, which would lead to a shortage at growth resumption, resulting in smaller vessels (González-González et al., 2015; Souto-Herrero et al., 2018a). Ring-porous trees restart cambial activity before bud break (Copini et al., 2016; Kudo et al., 2014; Pérez-de-Lis et al., 2016b; Suzuki et al., 1996), and vessels need to become functional to contribute to full leaf expansion (Guada et al., 2018, 2019), following the lignification of the secondary wall and appearance of perforations (Kitin and Funada, 2016; Kudo et al., 2015). Consequently, initial vessel formation occurs before assimilation, and must entirely rely on previous year's storage (Barbaroux and Bréda, 2002; El Zein et al., 2011).

In view of our results, the explained mechanism is plausible at these

coastal ***Qrob*** sites, with the timings also in accordance with our observations of xylogenesis. Thus, the December-February climatic signal should not be directly related to vessel growth, because the registered the enlargement of the first elements during March-April at these sites. Reserve balance is probably the most reasonable process, as sugar concentration was found to decline from February to April in oaks (Pérez-de-Lis et al., 2017), presumably linked to earlywood vessel formation, notwithstanding other explanations proposed for a similar relation between vessel size and temperature, as e.g. auxin sensitivity (Fonti et al., 2007). Thus, we hypothesize that warm conditions in late winter would diminish the availability of carbohydrates for vessel onset as a result of their consumption by respiration. In this sense, (Klesse et al., 2020) found that reduced amounts of carbohydrates led to smaller earlywood vessels in ring-porous ash, stating that turgor for vessel enlargement depends not only on water availability but also on sugar concentrations. However, mechanisms could slightly differ between both sites, since the signal was delayed at ***Qrob*-D** (shifted from December to February, and more dependent on maximum temperature),

despite its earlier vessel enlargement and maturation, suggesting that other metabolic processes related to the onset of cambial activity and leaf flushing. Other signals in the vessels were less robust, but confirm no direct effect of climate on vessel growth, except for a weak link of favorable spring conditions for D_H-nr1, which could be somehow influenced by the prior growing season as well, again through regulation of storage processes.

As opposed to earlywood vessels, radial increment expressed as LW was enhanced by water availability during the growing season, especially in June and July. This result must be associated to assimilation, because water shortage limits photosynthesis, resulting in narrow rings (Bréda et al., 2006). Furthermore, the response period is concomitant to our own observations of wood formation, whose maximum growth rate peaks around summer solstice. Interestingly, we found no remarkable negative correlations to temperature, whereas the strongest responses were directly observed to precipitation, rather than to SPEI. As this index integrates the balance between rainfall and evapotranspiration, the results suggest that it is only a shortage of precipitation and not high temperatures that are detrimental for latewood growth for these Atlantic oaks. We hypothesize that summer drought occurs later in the season, when growth has already slowed down, an idea also supported by the lack of a late summer/early autumn cambial reactivation. In addition, **Qrob-D** could be more affected by drought in some years due to its warmer summer, but would also compensate with higher photosynthetic rates under favorable conditions.

Climate-growth relationships differed for **Qpyr**, especially in the earlywood, but there was a similar overall pattern, as earlywood vessels were controlled by temperature, and radial increment by precipitation. D_H-r1 clearly varied between sites, because high spring temperatures (April) were associated to larger vessels only at **Qpyr-H**. In contrast to **Qrob**, there seems to be no regulation during dormancy, probably because low winter temperatures reduce respiration rates. The positive response in April should be linked to the threshold temperature to initiate cambial activity, and to the first stages of vessel enlargement, as observed for other mountain areas (González-González et al., 2014; Souto-Herrero et al., 2017). Maximization of the signal by minimum temperature pinpoints the frost-avoidance strategy of *Q. pyrenaica*, through its late flushing (Pérez-de-Lis et al., 2016b), a result supported by the dates of xylogenesis along with the corresponding phenological events. However, this signal was absent for **Qpyr-D**, with a weak negative association to late winter temperature instead. These differences may be due to slightly warmer conditions for being on a sunny slope at a lower elevation, or to the lack of interspecies competition as opposed to **Qpyr-H**. In addition, previous autumn could affect vessel formation at **Qpyr-H**.

Unlike earlywood vessel size, LW was closely linked to summer precipitation, but stronger at the driest site **Qpyr-D**. As for **Qrob**, assimilation should be the most relevant process involved, and it was also around summer solstice that the response was maximized, concomitantly to the fastest growth rate. These results are sustained by xylogenesis, whose identification of growth cessation during 2013 suggests that summer drought can be enough to inhibit radial increment. In fact, summer water availability was reported as the main factor controlling radial growth of *Q. pyrenaica* in the Iberian Peninsula (Gea-Iz-quierdo and Cañellas, 2014), but not earlywood vessel size (Fernández-de-Uña et al., 2017). Another relevant feature revealed by both xylogenesis and dendrochronology is the existence of a certain growth pulse at the end of summer, observed in 2012, and evidenced by the positive correlation between LW and temperature in September. This somehow bimodal pattern is a typical plastic behavior in the Mediter-ranean Region (Sanchez-Salguero et al., 2010), and was previously reported for northwestern Iberian oaks (Guada et al., 2020; Pérez-de-Lis et al., 2017).

Our tree-ring analyses combining radial growth (LW) and earlywood anatomy (D_H-r1) revealed a detailed view of climate-growth relationships. This is especially important for areas where the lack of a single

prevailing factor often prevents tree-ring width from identifying the most important climatic constraints. Although earlywood vessel chronologies already proved to be promising under the absence of a single limiting factor (Fonti and Garcia-Gonzalez, 2008), especially if coupled to latewood analysis (Souto-Herrero et al., 2017, 2018a, 2018b), our paper highlights that it was their integration to xylogenesis that let us validate the climatic relations derived from the dendrochronological results. By monitoring wood formation, we ensure that our observations support the existence of cause-effect relationships, which in turn is one of the most important features that an ecological variable requires to be correctly used as an environmental proxy (Bradley, 1986).

The use of ecological variables that reflect functional traits is necessary to understand vegetation changes in boundary areas. The biogeographical limit between the Atlantic and Mediterranean regions is a complex area. Although the Mediterranean region is characterized by the existence of a prevailing limiting factor, because the long drought period can often span more than three months, transitions are often gradual, and include some characteristics of the Atlantic Region. This is probably the reason why the combination of LW (controlled by the single prevailing factor water availability) and D_H-r1 (an apparently powerful proxy of mesic regions) has been successful at our study boundary areas, as well as at other sites along this transitional zone (Souto-Herrero et al., 2018b). This idea is reinforced by the fact that LW demonstrated to be closely linked to water availability, whereas D_H-r1 was dependent on temperature, regardless of the species (*Q. robur* vs. *Q. pyrenaica*) or the geographical location (coast vs. mountain). Furthermore, the existence of very mild temperatures or a precipitation did not prevent these variables from recording the environmental signal.

Finally, we consider that our results are also of great biogeographical relevance. In the Iberian Peninsula, the limit between the Atlantic and the Mediterranean regions spans from the Pyrenees along the southern slope of the Cantabrian Range until the northern Atlantic coast (Rivas-Martínez, 1987), and changes from coastal areas towards the mountains often occur in a short distance. These areas are therefore very sensitive to environmental changes that can modify species balance. In fact, some models predict future changes in the distribution of rear-edge oak forests in Iberia (Benito Garzón et al., 2008), whereby the increased growth of some species could lead to mortality of others (Benito-Garzón et al., 2013), and with marcescent sub-Mediterranean oaks as *Q. pyrenaica* having a prevailing role in all predictions (Sánchez-de-Dios et al., 2009). In future studies involving the Mediterranean boundary, the tools we discussed here should be further considered. Radial growth has proved to be very powerful to study forest resilience in Iberia (Gazol et al., 2018), whereas xylem adjustment is one of the mechanisms that Mediterranean oaks use to cope with a dryer climate (Castagneri et al., 2017) and climatic extremes (Rita et al., 2016), resulting in responses in their vessel features at both local (Souto-Herrero et al., 2018b) and continental (Martínez-Sancho et al., 2017) scales. When complemented with studies on wood formation dynamics, a much better understanding of the influence of climate on tree growth will be inferred.

5. Conclusion

We carried out two different studies on oaks at the edge of their distribution boundary between the Atlantic and the Mediterranean regions, using chronologies of earlywood vessel size and latewood width. Despite being different species, with different ecological requirements, and also at diverging locations (coast vs. inland), there was a common pattern in both cases, as the size of the first earlywood vessels was controlled by temperature, whereas precipitation was coupled to radial increment. We found reliable cause-effect explanations for the identified climate-growth relationships, and the analysis of cambial dynamics was in agreement with the timings of the responses observed. In view of these results, we propose that a combined 'multiproxy' approach using earlywood anatomy and radial increment, supported by the physiological evidence of studies on wood formation, is a powerful indicator of the

growth performance of trees at their distribution boundaries.

Author contribution

I.G-G and G.G. planned and designed the research. G.G. and M.S-H performed experiments and conducted fieldwork. I.G-G analysed data. G.G, I.G-G., M. S-H, and U.S-K interpreted the results. G.G. and I.G-G. wrote the manuscript, with inputs from M.S-H and U.S-K.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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