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Feedbacks between earlywood anatomy and non-structural carbohydrates affect spring phenology and wood production in ring-porous oaks

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Abstract. Non-structural carbohydrates (NSC) play a central role in the construction and maintenance of a tree's vascular system, but feedbacks between the NSC status of trees and wood formation are not fully understood. We aimed to evaluate multiple dependencies among wood anatomy, winter NSC, and phenology for coexisting temperate (*Quercus robur*) and sub-Mediterranean (*Q. pyrenaica*) oaks along a water-availability gradient in the NW Iberian Peninsula. Sapwood NSC concentrations were quantified at three sites in December 2012 ($N = 240$). Leaf phenology and wood anatomy were surveyed in 2013. Structural equation modelling was used to analyse the interplay among hydraulic diameter (D_h), winter NSC, budburst date, and earlywood vessel production (EVP), while the effect of D_h and EVP on latewood width was assessed by using a mixed-effects model. NSC and wood production increased under drier conditions for both species. *Q. robur* showed a narrower D_h and lower soluble sugar (SS) concentration (3.88–5.08 % dry matter) than *Q. pyrenaica* (4.06–5.57 % dry matter), but *Q. robur* exhibited larger EVP and wider latewood (1403 µm) than *Q. pyrenaica* (667 µm). Stem diameter and D_h had a positive effect on SS concentrations, which were related to an earlier leaf flushing in both species. Sapwood sugar content appeared to limit EVP exclusively in *Q. pyrenaica*. In turn, D_h and EVP were found to be key predictors of latewood growth. Our results confirm that sapwood SS concentrations are involved in modulating growth resumption and xylem production in spring. *Q. pyrenaica* exhibited a tighter control of carbohydrate allocation to wood formation than *Q. robur*, which would play a role in protecting against environmental stress in the sub-Mediterranean area.

1 Introduction

Non-structural carbohydrates (NSC) have multiple key functions in trees, such as maintenance respiration, osmoregulation, cryoprotection, or growth control (Morin et al., 2007; Sala et al., 2012; Wang and Ruan, 2013; Deslauriers et al., 2014). The asynchrony between carbon assimilation and consumption is solved by the active accumulation of NSC (Chapin et al., 1990; Dietze et al., 2014), which are mostly stored in stem, branches, and coarse roots as soluble sugars (SS) and starch (Salomon et al., 2016). A large part of the NSC budget of the tree is invested in construction and maintenance of the vascular system, as well as in turnover of fine roots and crown development (Wang and Ruan, 2013; DeSoto et al., 2016). The hydraulic network in ring-porous oaks is highly vulnerable to dysfunction due to cavitation of their large vessels, which operate at a narrow safety margin (Delzon and Cochard, 2014; Urli et al., 2015). The refilling of embolized vessels needs restoration of osmotic gradients by releasing SS into the conduits (Salleo et al., 2009; Brodersen and McElrone, 2013). Alternatively, the hydraulic function can be recovered through the formation of new conduits in the following spring (Brodribb et al., 2010). In ring-porous species, earlywood vessels are generally functional during only 1 year (Urli et al., 2015), and cambial resumption precedes leaf formation (Pérez-de-Lis et al., 2016). Stored NSC are therefore needed to provide energy and materials for leaf expansion and cambial activity at the onset of the growing season (El Zein et al., 2011).

Large vessels are presumed to boost carbon gain because stomatal conductance increases with the hydraulic capacity (Fichot et al., 2009). Concurrently, more carbohydrates may be allotted to hydraulic purposes in trees with wider but more vulnerable vessels (Salleo et al., 2009; Brodersen and McElrone, 2013). However, little is known about feedbacks between wood anatomy and short-distance NSC mobilization, which are probably influenced by tree vigour. Large, dominant trees commonly exhibit higher NSC levels and a faster NSC turnover (Sala and Hoch, 2009; Carbone et al., 2013; DeSoto et al., 2016), as well as wider vessels at the tree base to compensate for height-related hydraulic resistance in the stem (Petit et al., 2008). In turn, entangled relationships between tree vigour and growth are influenced by the timing of tree phenology, given that dominant trees show larger growing periods and more intense cambial activity (Rathgeber et al., 2011). Although the break in dormancy is mostly controlled by temperature and photoperiod (Basler and Körner, 2014), some studies suggest that high NSC concentrations in developing buds speed up leaf-out dates (Maurel et al., 2004). In winter, phloem of deciduous trees is probably non-functional, whereby sapwood might be involved in carbon translocation through the plant (Lacointe et al., 2004). The influx of sucrose from xylem conduits in branches into the buds was reported to be tightly correlated to bud swelling rates (Bonhomme et al., 2010), whilst high sucrose concentrations in the stem of mutant poplars have been associated with an advanced budburst (Park et al., 2009). Cascade effects of leaf phenology on secondary growth would consequently be expected, given that leaf and cambial phenology are coordinated at the whole-tree level (Pérez-de-Lis et al., 2016). In ring-porous oaks, winter temperature has been reported to affect earlywood formation, which has been attributed to thermally induced changes in respiratory demands and NSC levels (Gea-Izquierdo et al., 2012). However, the interplay between NSC, phenology, and tree growth is poorly understood.

Ecological requirements modulating phenology are linked to functional species-specific strategies (Basler and Körner, 2014). This is the case of the ring-porous oaks *Quercus robur* L. and *Q. pyrenaica* Willd., which coexist in the NW Iberian Peninsula. The former is widespread in Europe, being abundant in areas with mild-oceanic climate. By contrast, *Q. pyrenaica* is dominant in various mountain ranges of the sub-Mediterranean area, hence exhibiting multiple adaptations to cope with summer drought and winter frost, such as late flushing (Pérez-de-Lis et al., 2016). These differences could impact carbon metabolism and allocation to growth (Valladares et al., 2000; Piper, 2011; Guillemot et al., 2015), as well as the rate of developmental processes (Deslauriers et al., 2009), affecting the adaptive capacity to track rapid climate change (Jump and Peñuelas, 2005). Water shortage is deemed to influence carbon metabolism in a complex manner by constraining the activity of both source and sink organs (Sala et al., 2012), and by changing sugar fractions (Deslauriers et al., 2014). While some studies have suggested that declining growth demand under drought causes NSC to be accumulated (Sala and Hoch, 2009; Lempereur et al., 2015; DeSoto et al., 2016), other authors have found a reduction in starch concentration under long-term drought (Rosas et al., 2013). Therefore, we need to understand how phenology and growth are coordinated with NSC in order to better predict plant responses to climate in the context of global warming.

Table 1. Climatic information on the study sites in 2012 and 2013.

Site	P (mm)	Rainy days	T_m (°C)	T_{max} (°C)	T_{min} (°C)
2012					
Hyperhumid	1346.8	210	11.7	16.4	7.8
Humid	858.3	169	10.4	14.9	7.1
Subhumid	555.0	172	12.4	16.8	8.5
2013					
Hyperhumid	1979.0	225	11.6	15.8	8.0
Humid	1351.6	190	10.2	14.2	7.2
Subhumid	856.3	168	12.3	16.4	8.8

P, mean precipitation; T_m, mean temperature; T_{max}, mean maximum temperature; T_{min}, mean minimum temperature.

In this research, data from stem sapwood NSC concentration in winter 2012, xylem anatomical traits, and leaf phenology in spring 2013 (as a proxy for cambial phenology) are used to disentangle the influence of their mutual interactions on the radial growth of ring-porous oaks growing along a water-availability gradient in the NW Iberian Peninsula. We focus on the possible differences in xylem anatomy and NSC levels between *Q. robur* and *Q. pyrenaica* along the gradient. In this regard, wood production and stem sapwood NSC concentration are expected to vary along the gradient, with reduced growth but increasing NSC storage under drier conditions. We also hypothesize that the more drought-tolerant *Q. pyrenaica* will have xylem growth that is more reduced than *Q. robur* but larger stem sapwood NSC concentrations. At the species level, we aim to test the following hypotheses: (i) stem diameter influences earlywood vessel size, which in turn affects NSC content in the stem; (ii) higher sapwood SS content in winter predisposes trees to advance growth resumption in spring, as well as to produce more earlywood vessels; and (iii) earlywood vessel number and size are key predictors of latewood growth in oaks.

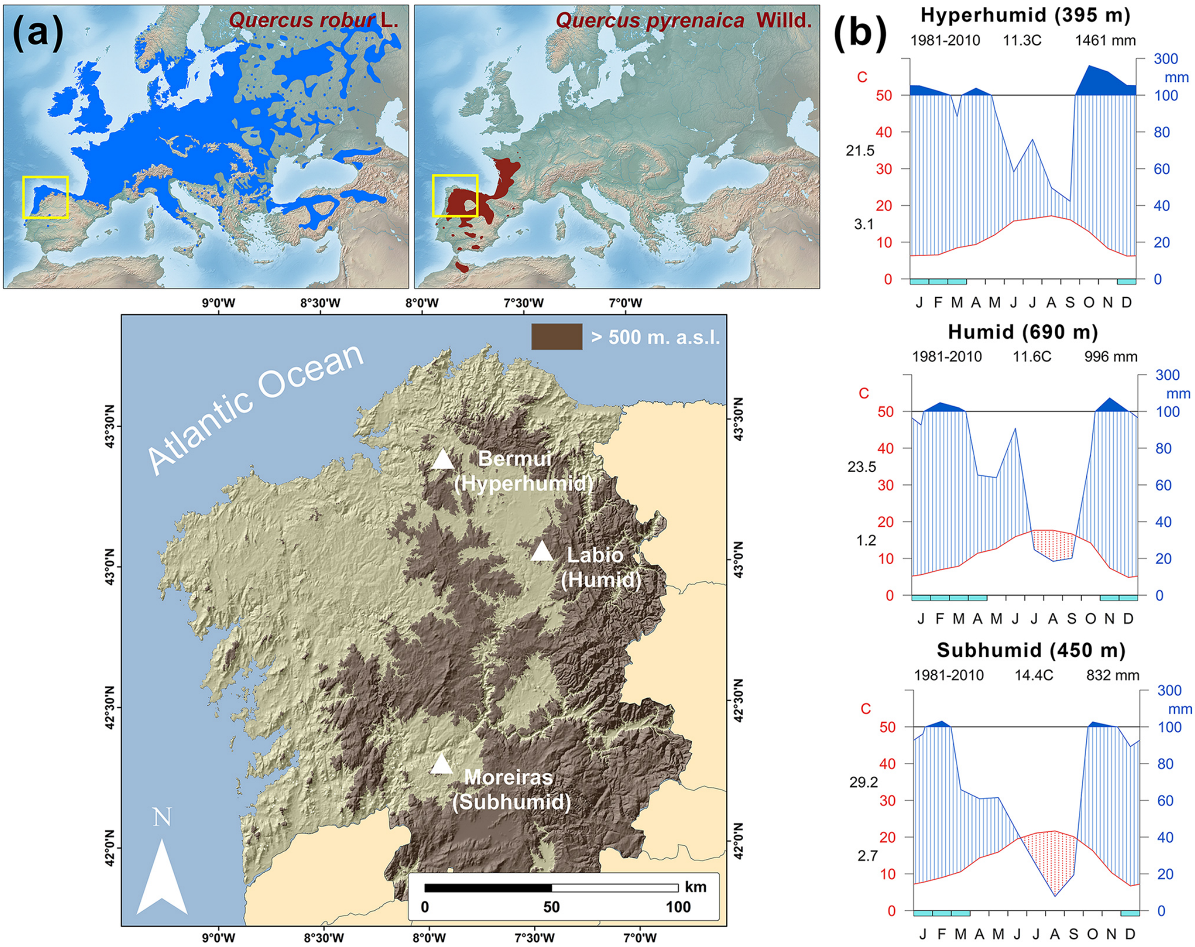

Figure 1. (a) Location of the study sites in the NW Iberian Peninsula and distribution range of *Quercus robur* and *Q. pyrenaica* (base map: http://www.euforgen.org). **(b)** Climatic diagrams of the sites including site altitude in m a.s.l, mean annual temperature, and total annual precipitation for the specified period.

2 Materials and methods

2.1 Study sites

The study area is located in the NW Iberian Peninsula, at the transition between the Atlantic and Mediterranean biogeographical regions (Fig. 1a). The experiment was conducted at three mixed stands of *Q. robur* (hereafter *Qrob*) and *Q. pyrenaica* (hereafter *Qpyr*) located along a north-to-south transect of 120 km, following a gradient of decreasing water supply (Fig. 1b). Annual rainfall declines from 1461 mm at the northernmost site Bermui (hyperhumid), to 996 mm at Labio (humid), and to 832 mm at the southernmost site Moreiras (subhumid). Mean annual temperature is lower at the hyperhumid (11.3 °C) and humid (11.6 °C) sites than at the subhumid site (14.4 °C). Sampled stands are dominated by *Qrob* at the hyperhumid and humid sites, whereas *Qpyr* is more frequent at the subhumid location. Forests at the hyperhumid and humid sites include temperate trees and understorey shrubs, such as *Betula alba* L., *Castanea sativa* Mill., *Pyrus cordata* Desy., *Ilex aquifolium* L., *Daboecia cantabrica* (Huds.) K. Koch, and *Vaccinium myrtillus* L. By contrast, thermophilic Mediterranean flora, such as *Q. suber* L., *Laurus nobilis* L., *Arbutus unedo* L., *Osyris alba* L., and *Daphne gnidium* L., dominates the subhumid location. Stand tree densities are 1178, 1082, and 530 ha^{-1} at the hyperhumid, humid, and subhumid sites, respectively. Drought episodes can occur at the study region in summer, particularly at the subhumid site (Fig. 1b). Incident rainfall in 2012 was 8, 14, and 33 % lower than the 1981–2010 average, at the hyperhumid, humid, and subhumid locations respectively, whereas it was 35, 36, and 3 % higher than the average in 2013 (Table 1). However, in summer 2013, there were only 34 (45 mm), 11 (20 mm), and 8 (35 mm) days of precipitation at the hyperhumid, humid and subhumid locations, respectively. Furthermore, mean maximum temperature was 22.0 °C for the same period at the hyperhumid and humid sites but 25.3 °C at the subhumid location.

2.2 Phenology and NSC concentration

At each study site, 40 trees per species were randomly selected from those belonging to the dominant and intermediate crown classes (overall $N = 240$), while highly suppressed and juvenile individuals were disregarded. In October 2012, we measured stem diameter from all selected individuals with a diameter tape, while tree height was estimated with a Blume-Leiss hypsometer. Leaf phenology was weekly monitored during 2013 using binoculars (10 ×) at ca. 10 m distance from the stem. For each tree, budburst was identified as the day of year (DOY) in which the apical buds on the uppermost part of the crown were green and expanding, but no leaves were distinguishable yet. We considered budburst to be an indicator for cambial resumption in the stem, according to the high correlations observed between these two events in our study sites (Pérez-de-Lis et al., 2016). In late autumn, leaf shedding was identified as the date in which at least 50 % of the leaves were shed from the crown. In addition, foliage density was visually estimated in July 2013 by counting the proportion of gaps in the crown and was expressed as a percentage of the theoretical maximum foliage density.

In order to analyse feedbacks between NSC and xylem anatomy, we quantified the content of NSC in sapwood by sampling one 5 mm diameter wood core per tree with an increment borer at breast height. Cores were taken in mid-December 2012, soon after the completion of leaf abscission, which occurred between mid- and late November for both species. After extraction, cores were immediately placed into a cool box, and subsequently stored at −20 °C to prevent carbohydrate degradation. Before NSC extraction, we identified the boundary between sapwood (pale-coloured) and heartwood (brown-coloured). Bark and traces of heartwood were removed, and the cores were oven-dried at 60 °C for 72 h. Sapwood was then finely grounded with a mixer mill (Retsch MM 400, Düsseldorf, Germany). We quantified NSC concentration for the whole sapwood by using the anthrone method (Olano et al., 2006). SS were extracted from 20 mg of dry mass in 1 mL of ethanol (80 %) at 80 °C for 30 min. The extract was centrifuged for 10 min at 4000 rpm, and the supernatant was collected for the spectrophotometrical determination of SS concentrations, for which we used the anthrone reagent. Starch contained in the residue was hydrolysed with 1 mL of perchloric acid (35 %) for 1 h, and determination was conducted by using the anthrone reagent, as previously described for SS. Total NSC, SS, and starch concentrations were expressed as a percentage of dry matter.

2.3 Wood anatomical measurements

In October 2013, one additional core was collected from all the selected trees to perform wood anatomical measurements. Cores were air-dried and mounted on wooden supports to be cut using a microtome (WSL Core Microtome, Zurich, CH) and polished. Cross-sectional surfaces were photographed with a digital camera (Canon EOS 600D, Tokyo, Japan) attached to a transmitted light microscope (Olympus BX40, Tokyo, Japan). Image analysis was applied on the rings formed in 2012 and 2013 using ImageJ 1.48v (Schneider et al., 2012) in order to quantify the lumen area of earlywood vessels, latewood width, and the number of earlywood vessels, which is a proxy of earlywood vessel production (EVP). For each vessel, we estimated the diameter of the equivalent circle, obtaining the hydraulic diameter (D_h) at the tree level according to the following equation:

$$D_\mathrm{h} = \frac{\sum\limits_{n=1}^{N} d_n^5}{\sum\limits_{n=1}^{N} d_n^4}, \tag{1}$$

where d_n is the diameter of the n vessel (Sperry et al., 1994). According to the Hagen–Poiseuille equation, D_h is proportional to the hydraulic capacity.

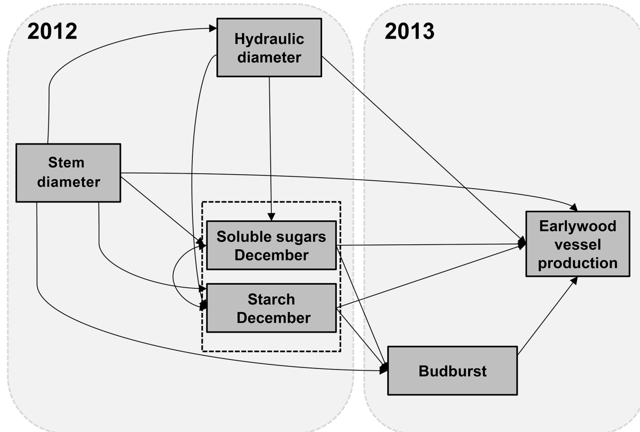

Figure 2. Structure of the hypothetical conceptual model showing interactions among stem diameter, hydraulic diameter in 2012, soluble sugars and starch concentrations in December 2012, and budburst date and earlywood vessel production in 2013.

2.4 Comparisons along the gradient

Variation among sites and between species for NSC, dates of budburst and leaf shedding, wood anatomical traits, and foliage density were evaluated by applying generalized linear models (GLMs) for gamma-distributed data. Multiple pairwise comparisons were also assessed to test differences among site factor levels. This analysis was performed by using the packages “lme4” and “multcomp” for R 3.1.1 (R Core Team, 2014). Moreover, we calculated Pearson’s correlation between stem diameter and tree height (one-tailed test of significance and 95 % confidence interval).

2.5 Connections among earlywood anatomy, sapwood NSC content, and spring phenology

We performed structural equation models (SEMs) to disentangle the role of winter NSC content as a possible regulator of budburst and EVP in 2013 at the species level. Thereby, data from all sites were pooled, and a unique model was fitted for each species. An SEM approach provides an adequate representation for interacting systems, in which simultaneous influences and responses, including direct and indirect effects, are explored (Grace, 2006). The structure of a hypothetical SEM, and its calculation, requires incorporating available a priori knowledge. According to the lines of evidence showed in the introduction, we hypothesized that larger trees show higher SS and starch concentrations due to their larger D_h (Fig. 2). In turn, high SS and starch concentrations in winter are expected to speed up tree phenology (budburst date) and boost EVP during the following year.

Standardized coefficients were estimated by the maximum likelihood method, and model evaluation was performed using a χ^2 test. A P value below 0.05 indicates that discrepancy between observed and expected covariance matrices is acceptable. The adjusted goodness of model fit index (AGFI) and the root mean square error of approximation (RMSEA) were complementarily performed in order to consider the effect of sample size on the model fit evaluation. Values of AGFI above 0.90 and RMSEA below 0.05 indicate an acceptable fit of the model in relation to the degrees of freedom. A χ^2 test for multi-group invariance was applied to evaluate differences between the models fitted for each species. SEM analyses were carried out with AMOS 18.0 software (AMOS Development Corp., Mount Pleasant, South Carolina, USA).

2.6 Predictors of latewood formation

We performed generalized linear mixed-effects models (GLMMs) to identify how earlywood anatomy, foliage density, phenology, and winter NSC levels affected latewood production in 2013. The effect of site was included as a random component, while winter NSC, earlywood anatomy (D_h and EVP in 2013), growing season length, and foliage density were the explanatory variables of the model. Collinearity was surveyed by calculating the generalized variance-inflation factors for each species. GLMMs were fitted by a log-link function with a gamma distribution, being ranked according to the corrected Akaike's information criterion (AICc; Bolker et al., 2009). We averaged the 95 % confidence set of models according to the Akaike's weights, and the relative importance of a given variable was calculated as the sum of the weights across all models in which it was contained (Burnham and Anderson, 2002). Marginal (fixed effects only) and conditional R^2 (both fixed and random effects) were calculated by using the variance components of fixed and random factors and the residuals. The proportion change in variance (PCV) was quantified to provide the variability in latewood width explained by the full model (containing fixed and random effects), as compared to the null model (only containing the random component). Complementarily, we estimated the percentage of the variance explained by each fixed factor, and both full and null models were ranked according to the minimum AICc and Bayesian information criterion (BIC) scores. Variance partitioning, R^2 and PCV calculations were performed by following the methods reported by Nakagawa and Schielzeth (2013). We used the packages "lme4" and "MuMIn" for R 3.1.1 (R Core Team, 2014) to assess GLMM estimates, variance partitioning, and statistics.

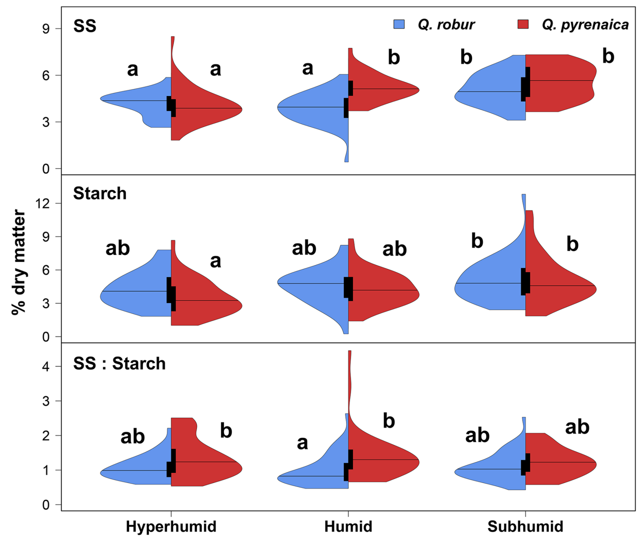

Figure 3. Distribution of soluble sugars (SS), starch concentrations, and SS-to-starch ratio, for *Quercus robur* ($N = 120$) and *Q. pyrenaica* ($N = 120$) at the three study sites. Horizontal lines represent the median, and black box plots show the extent of 25th and 75th percentiles. Lower-case letters indicate statistically significant differences along the gradient according to multiple pairwise comparisons. Note that different scales are used on the y axis for each set of distributions.

3 Results

3.1 Variation in NSC, wood anatomy, and leaf phenology along the gradient

Mean SS concentrations along the gradient ranged from 3.88 to 5.08 % dry matter in *Qrob* and from 4.06 to 5.57 % dry matter in *Qpyr*, being similar to those of starch, which ranged from 4.28 to 5.11 % dry matter in *Qrob* and from 3.47 to 5.11 % in *Qpyr* (Fig. 3). As a result, *Qpyr* exhibited greater SS concentrations than *Qrob* ($F_{[1,238]} = 18.27$, $P < 0.001$), while both starch and NSC levels did not differ between species (starch $F_{[1,238]} = 2.14$, NSC $F_{[1,238]} = 0.62$, $P > 0.050$). Such a pattern resulted in a higher SS-to-starch ratio for *Qpyr* than for *Qrob* ($F = 18.07$, $P < 0.001$), espe-

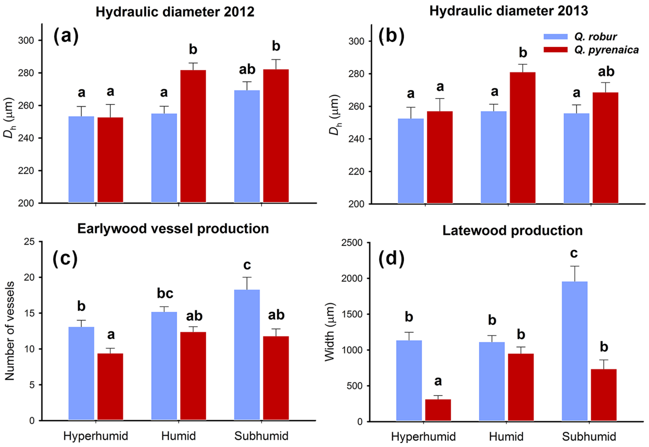

Figure 4. Mean values and SE of **(a)** hydraulic diameter in 2012, **(b)** hydraulic diameter in 2013, **(c)** earlywood vessel production, and **(d)** latewood production in 2013 for *Quercus robur* ($N = 120$) and *Q. pyrenaica* ($N = 120$). Lower-case letters indicate statistically significant differences along the gradient according to multiple pairwise comparisons.

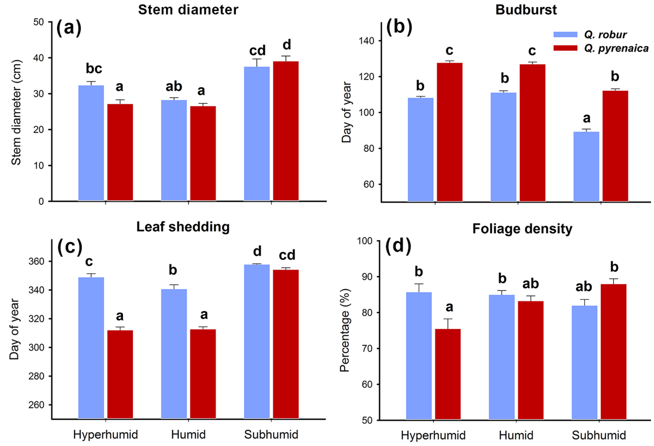

Figure 5. Mean values and SE of **(a)** stem diameter, **(b)** budburst date, **(c)** date of leaf shedding, and **(d)** foliage density in 2013 for *Quercus robur* ($N = 120$) and *Q. pyrenaica* ($N = 120$). Lower-case letters indicate statistically significant differences along the gradient according to multiple pairwise comparisons.

cially at the humid location (Fig. 3), although there was no variation along the gradient ($F = 0.21$, $P = 0.814$). In contrast, SS content decreased in both species from the subhumid to the hyperhumid site (*Qrob* $F_{[2,117]} = 17.72$, *Qpyr* $F_{[2,\,117]} = 21.89$, $P < 0.001$). The subhumid site exhibited a higher starch content than the hyperhumid location for *Qpyr* ($F_{[2,\,117]} = 8.59$, $P < 0.001$), whereas no clear pattern was found for *Qrob* ($F_{[2,\,117]} = 2.52$, $P = 0.085$).

Overall, *Qpyr* exhibited a higher D_h than *Qrob* (2012 $F_{[1,\,236]} = 7.76$, 2013 $F_{[1,\,236]} = 8.31$, $P < 0.010$; Fig. 4a). The highest D_h values were found for *Qpyr* at the humid (both years) and subhumid sites (2012), while *Qrob* had a more reduced variation along the gradient (2012 $F_{[2,\,117]} = 2.89$, 2013 $F_{[2,\,117]} = 0.18$, $P > 0.050$; Fig. 4b). EVP and latewood width were higher in *Qrob* than in *Qpyr* at the hyperhumid and subhumid sites, whereas differences were non-significant at the humid location (Fig. 4c, d). Trees at the subhumid site exhibited wider latewood (both species) and higher EVP (*Qrob*) than those at the hyperhumid location (Fig. 4c, d).

Qrob had a larger stem diameter than *Qpyr* at the hyperhumid site, whereas similar values were found at the humid and subhumid locations (Fig. 5a). Stem diameter was positively correlated with tree height in *Qrob* ($r_{[118]} = 0.60$, $P < 0.001$) and *Qpyr* ($r_{[118]} = 0.58$, $P < 0.001$). Trees of both species were taller at the hyperhumid and subhumid sites than at the humid location (*Qrob* $F_{[2,\,117]} = 22.85$, *Qpyr* $F_{[2,\,117]} = 29.46$, $P < 0.001$). *Qrob* exhibited an earlier budburst than *Qpyr* ($F_{[1,\,236]} = 527.83$, $P < 0.001$), occurring from early March to late April for the former and from mid-April to late May for the latter. In both species, budburst occurred earlier at the subhumid site than at humid and hyperhumid locations (Fig. 5b). By contrast, leaf shedding was first recorded at the hyperhumid and humid sites for *Qpyr* (DOY 312 on average; Fig. 5c), whereas some green leaves could be perceived until late December at the subhumid site (*Qrob* DOY 357, *Qpyr* DOY 354). The leaf period was on average 42 days longer for *Qrob* than for *Qpyr* ($F_{[1,\,236]} = 450.90$, $P < 0.001$) in 2013. Foliage density was similar along the gradient for *Qrob*, but significantly lower at the hyperhumid site for *Qpyr* (Fig. 5d). It is also relevant that numerous *Qpyr* trees at the hyperhumid site had their leaves infected with powdery mildew in spring 2013.

3.2 Species-specific models on functional relationships affecting wood production

SEMs showed a good fit for both species (*Qrob*, $\chi^2_{[1,N=120]} = 0.202$, $P = 0.653$; *Qpyr*, $\chi^2_{[1,N=120]} = 0.118$, $P = 0.732$), with AGFI > 0.90 and RMSEA < 0.1 (Fig. 6a, b). Stem diameter had a positive indirect effect on SS levels that was mediated by D_h in 2012. Large trees exhibited an earlier budburst date, partially related to their higher SS concentrations in winter. In *Qrob*, SS content affected EVP by mediating budburst date, although our model explained a low amount of the observed variability in EVP (Fig. 6a). In *Qpyr*, SS concentrations had a positive direct effect on EVP, with an acceptable R^2 value for EVP ($R^2 = 0.20$; Fig. 6b). Neither EVP nor budburst date responded to winter starch concentration, although our model failed to account for the observed variability in the latter variable.

Our GLMM had a sufficiently good performance ($R^2_{[\text{conditional}]} > 36\,\%$). The fixed factors explained most of the variability in latewood width ($R^2_{[\text{marginal}]} > 34\,\%$), while considerably high PCV values confirmed the better fit of the full

Table 2. Statistics of the null and full generalized linear models for *Quercus robur* (left) and *Q.pyrenaica* (right).

	Q. robur ($N = 120$)			*Q. pyrenaica* ($N = 120$)		
	Null model	Full model	PVE (%)	Null model	Full model	PVE (%)
Fixed effects						
Intercept	7.209***	4.348***		6.404***	2.696**	
GS_{13}		0.003	4.84		0.003	5.22
NSC_{12}		−0.022	1.06		0.003	5.81
D_{h13}		0.004*	8.26		0.005**	16.64
EVP_{13}		0.050***	83.37		0.092***	69.03
FD_{13}		0.006	2.47		0.008	3.31
Variance components						
Site	0.033	0.013		0.183	0.060	
Residuals	0.380	0.332		0.829	0.605	
Fixed effects		0.181			0.363	
$PCV_{[site]}$		83.79 %			89.18 %	
$PCV_{[residuals]}$		26.81 %			28.30 %	
$R^2_{GLMM_{(m)}}$		34.42 %			35.28 %	
$R^2_{GLMM_{(c)}}$		36.95 %			41.14 %	
− log likelihood	968.20	946.44		889.84	865.13	
AIC	1942.41	1908.89		1785.67	1746.25	
AICc	1942.62	1910.19		1785.88	1747.55	
BIC	1950.77	1931.19		1794.03	1768.55	

N, sample size for each species. PVE, relative proportion of variance explained by each fixed factor. GS_{13}, length of the growing season in 2013. NSC_{12}, total non-structural carbohydrates in December 2012. D_{h13}, hydraulic diameter in 2013. EVP_{13}, earlywood vessel production in 2013. FD_{13}, foliage density in 2013. PCV, proportion change in variance. $R^2_{GLMM_{(m)}}$, marginal coefficient of determination. $R^2_{GLMM_{(c)}}$, conditional coefficient of determination. AIC, Akaike's information criterion. AICc, corrected Akaike's information criterion. BIC, Sawa's Bayesian information criterion. *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$.

model with respect to the null one. A similar result was obtained after ranking the models according to their AICc, log likelihood, and BIC scores (Table 2). Larger and more numerous earlywood vessels strongly favoured latewood production (Table 2). In fact, EVP and D_h were the most relevant predictors, attaining a relative influence above 85 % (Fig. 7). However, EVP accounted for 69 (83) % of the variability in latewood width predicted by the fixed part of the model for *Qpyr* (*Qrob*), whereas D_h only explained 8–16 % (Table 2). Variables related to tree vigour were less relevant, such as foliage density (40–60 %) and length of the growing season (31–40 %), with no significant effects on latewood width (Table 2). Winter NSC only accounted for a marginal weight in both species, having a negative slope for *Qrob* but positive for *Qpyr* (Table 2, Fig. 7). Problems of collinearity were not detected among the predictors included in the model (Supplement, Table S1).

4 Discussion

4.1 NSC allocation to xylem growth reflects contrasting stress-tolerance strategies in oaks

According to our expectations, sapwood SS content decreased from the subhumid to the hyperhumid site for both species, and starch content followed a similar pattern for *Qpyr*. More immediate effects of water shortage on stem growth than on photosynthesis likely favoured carbohydrate accumulation in the previous summer (Sala et al., 2012; Lempereur et al., 2015; DeSoto et al., 2016). Such a response should be adaptive in dry environments because sugars contribute to prevent desiccation through osmotic regulation and cavitation repair (Salleo et al., 2009; Brodersen and McElrone, 2013; Pantin et al., 2013; Deslauriers et al., 2014). The fact that *Qpyr* showed larger vessels at the humid and subhumid locations cannot be explained by corresponding differences in tree height, whereby long-distance water transport was probably prompted under drier conditions, which concurrently increased the risk of vessel dysfunction (Urli et al., 2015). Although the prevalence of vessel refilling is still under discussion (Delzon and Cochard, 2014), we hypothesize

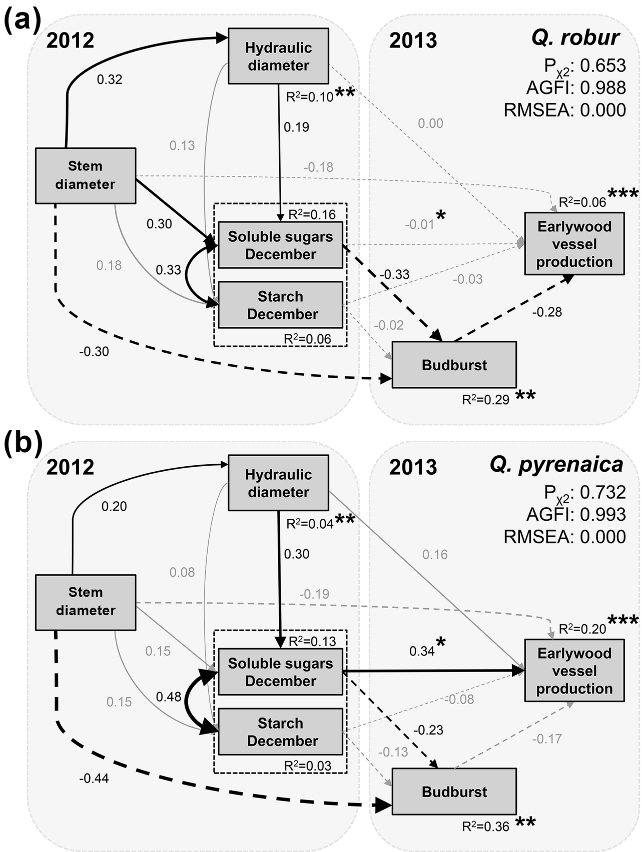

Figure 6. Structural equation models fitted for **(a)** *Quercus robur* ($N = 120$) and **(b)** *Q. pyrenaica* ($N = 120$). Variables of the conceptual model are stem diameter, hydraulic diameter in 2012, soluble sugars and starch concentrations in December 2012, budburst date, and earlywood vessel production (number of vessels) in 2013. Explained deviances of endogenous variables are shown near the boxes. Solid black (positive effects) and dashed (negative effects) arrows denote significant relations, while non-significant relations are shown as grey coefficients and arrows. The χ^2 test, the adjusted goodness of model fit index (AGFI), and the root mean square error of approximation (RMSEA) are shown for each model. Asterisks indicate paths or error values significantly different between the models of both species. *** $P \leq 0.001$, ** $P \leq 0.01$, and * $P \leq 0.05$.

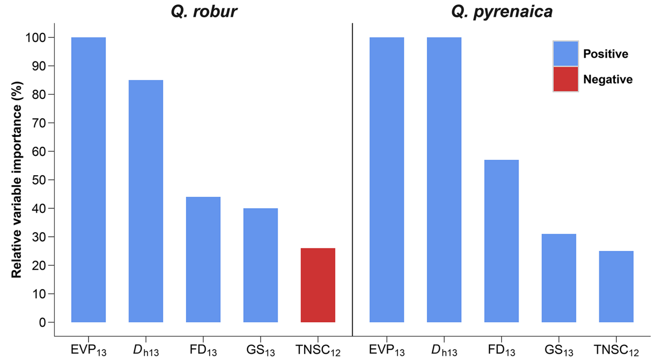

Figure 7. Relative importance of the variables driving latewood production in 2013, expressed as a percentage, for *Quercus robur* ($N = 120$) and *Q. pyrenaica* ($N = 120$). Site effect was included as a random factor in the model. NSC_{12} are total non-structural carbohydrates in December 2012, D_{h13} is hydraulic diameter in 2013, EVP_{13} is earlywood vessel production in 2013, GS_{13} is length of the growing season in 2013, and FD_{13} is foliage density in 2013. Different colours of bars denote variables with either a positive or negative effect.

that enhanced SS concentration might be involved in compensating hydraulic vulnerability in this species.

Wood formation declined along with NSC content from the subhumid toward the hyperhumid site, which contradicts our expectations, and also a possible trade-off between NSC accumulation and growth. A shorter growing season at the hyperhumid and humid locations could restrict photosynthesis (Morecroft et al., 2003), as well as xylem formation (Rathgeber et al., 2011). A lower tree density at the subhumid site might be associated with a reduced intertree competition, which is assumed to favour both carbon uptake and xylem growth (Fernández-de-Uña et al., 2016). Strong differences between the two Atlantic sites for *Qpyr*, however, contrasted with their similar tree density. Soil water excess in winter at the hyperhumid site could exacerbate carbon consumption associated with fermentation processes and root anaerobic stress (Ferner et al., 2012). Moreover, *Qpyr* trees exhibited sparser foliage and more severe powdery mildew infestation at the hyperhumid site, which may reduce NSC levels and growth (Améglio et al., 2001; Martínez-Vilalta, 2014; Camarero et al., 2016). Growth decline and tree dieback were indeed recently reported for oaks suffering from both high competition levels and water excess after extremely rainy periods (Rozas and García-González, 2012).

The sub-Mediterranean *Qpyr* exhibited a higher SS-to-starch ratio at the onset of dormancy than the temperate *Qrob*, although NSC content was similar for both species. Sugars play a key role in the osmotic protection against freezing damage (Améglio et al., 2004), whereby higher symplastic SS concentrations would reflect stronger cold tolerance (Morin et al., 2007), as is the case for *Qpyr*. On the other hand, EVP and latewood width were generally lower for *Qpyr* than for *Qrob*, particularly under more Mediterranean climatic conditions. This contradictory outcome suggests that *Qpyr* is more conservative than *Qrob* in allocating NSC to wood production, hence reflecting a stronger drought tolerance (Valladares et al., 2000; Piper, 2011). Saving NSC may allow long-lived trees to mitigate detrimental effects of eventual wildfire and drought episodes (Sala et al., 2012), which are frequent at the Mediterranean area (Rosas et al., 2013; Camarero et al., 2016). However, such a strategy could entail a high opportunity cost under favourable conditions (Chapin et al., 1990). Therefore, *Qrob* probably outcompeted *Qpyr* in our study sites, demonstrating that temperate oaks are more competitive than sub-Mediterranean ones (Rodríguez-Calcerrada et al., 2008; Grossiord et al., 2014). However, validation of these hypotheses requires further research quantifying the whole-tree NSC pool size and the activity of complementary carbon sinks. In this regard, a recent study suggested that neighbouring *Qpyr* trees were able to share NSC through the root system, which is an important carbon reservoir within the tree (Salomon et al., 2016).

4.2 Dependencies among NSC content, phenology, and earlywood vessels

Our SEMs confirmed the hypothesized functional relationships among earlywood anatomy, spring phenology, and NSC content. The growing season started earlier in large trees, which is in line with previous studies analysing cambial activity (Rathgeber et al., 2011). Stem diameter also had a positive effect on SS content at dormancy, which was mediated by the hydraulic capacity (i.e. vessel size). Enhanced water transport capacity in trees with large vessels may boost carbohydrate uptake under a high evaporative demand (Meinzer et al., 2005; Fichot et al., 2009). Alternatively, since large vessels are thought to be more vulnerable to cavitation (Sperry et al., 1994), higher SS concentrations may be required in the sapwood of trees bearing wider vessels to maintain long-distance water transport (Brodersen and McElrone, 2013). Trees with a higher SS concentration in the stem showed earlier budburst in the following spring, as reported in poplar (Park et al., 2009). Bud swelling depends on sugar influx from sapwood vessels (Maurel et al., 2004; Bonhomme et al., 2010), which have been suggested to be responsible for carbohydrate transport during the dormant period (Lacointe et al., 2004). In addition, xylem sap osmolarity plays a role in the generation of the stem pressure needed to reverse winter embolisms in early spring (Améglio et al., 2001). A reduced ability to repair embolism in trees with low xylem sap SS concentration could thus negatively affect the supply of water to swelling buds (Améglio et al., 2001).

EVP was in tune with SS concentration for *Qpyr*, whereas this effect was irrelevant for *Qrob*. Such a discrepancy in the effect of stored carbohydrates on growth is consistent with the aforesaid carbon use strategies, and could reflect the contrasting stress tolerance of the study species (Guillemot et al., 2015). Our results consequently suggest that *Qpyr* was able to limit construction costs in spring according to SS levels. Presumably, high overwintering SS levels in sapwood somehow increased energy and materials as well as water supplied to growing tissues in spring, even though starch mobilization may be initiated at that time (Améglio et al., 2001). Furthermore, sugars are elicitors of auxin biosynthesis and distribution (Lilley et al., 2012; Sairanen et al., 2012), as well as growth promoters (Stewart et al., 2011). Although relations between carbon accumulation and growth are complex, and mainly related to the activity of carbon sinks (Lempereur et al., 2015), a growing body of literature suggests that NSC availability is involved in growth regulation (Pantin et al., 2013; Dietze et al., 2014; Guillemot et al., 2015). This can be particularly true for earlywood given its reliance on stored carbohydrates (Skomarkova et al., 2006). This idea agrees with the direct association between tree vigour, NSC pool, and growth found in multiple species (Deslauriers et al., 2009; Carbone et al., 2013), as well as with the observed positive effect of CO_2 fertilization on tree growth (Nissinen et al., 2016). EVP did not respond to sapwood starch content, which suggests that thermal-induced changes in starch breakdown during cold hardening could be more decisive than the total amount of reserves. This is consistent with the connection between earlywood anatomy and autumn–winter temperature reported by dendrochronological studies (Gea-Izquierdo et al., 2012). However, the actual starch availability could be overestimated if starch contained in sapwood is partially inaccessible (Sala et al., 2012).

4.3 Earlywood anatomy is a predictor of latewood growth

The most influential predictors driving latewood growth did not differ between *Qrob* and *Qpyr*, suggesting common underlying mechanisms for both species. Latewood width was considerably influenced by earlywood properties within the same tree ring, whereas the effects of foliage density, length of the growing season, and winter NSC content were secondary. This result confirms the positive impact of enhanced water transport capacity on xylem formation (Fichot et al., 2009), which is largely related to both conduit size (Sperry et al., 1994) and total conductive area (Meinzer et al., 2005). A more efficient water supply to growing tissues probably allows trees to protect cambial activity against water shortage in summer (Wang and Ruan, 2013). Despite the higher construction costs, abundant earlywood vessels of distinct size could be useful to avoid hydraulic failure because functioning small conduits would serve as local water reservoirs to recover neighbouring collapsed ones (Brodersen and McElrone, 2013). Moreover, an efficient hydraulic network may enhance carbon gain under favourable conditions (Fichot et al., 2009), hence ensuring key processes in which sugars are involved, such as osmotic regulation (Sala et al., 2012; Deslauriers et al., 2014) and embolism repair (Salleo et al., 2009). Our results suggested that changes in cell division and differentiation rates are more relevant for wood production than the duration of the growing period, as has been previously reported for conifers (Rathgeber et al., 2011). Although defoliation is assumed to impair carbon gain and radial growth in evergreen (Rosas et al., 2013; Camarero et al., 2016) and deciduous species (Améglio et al., 2001), we found that foliage density had a minor effect on latewood growth, which may reflect the high overall foliage density levels at the study sites.

5 Conclusions

In this study, non-structural carbohydrates in sapwood, wood anatomy, and leaf phenology were comprehensively addressed for two ring-porous species during a 1 year period, along a broad geographical range in the NW Iberian Peninsula. Our results reveal that feedbacks between earlywood vessels and soluble sugars involve changes in wood production. Earlywood vessel formation showed a tighter control by soluble sugar content in *Q. pyrenaica* than in *Q. robur*, suggesting a more conservative carbon use strategy for the former species. These lines of evidence support the idea that non-structural carbohydrates play a role in the acquisition of resistance to cope with harsh environmental conditions in the sub-Mediterranean area. We acknowledge the need for further research comprising a longer time span, soluble sugar fractioning, additional tree compartments such as branches and roots, and a comprehensive data set on cambial phenology instead of isolated leaf phenophases. However, this study suggests the existence of stable functional interactions between sapwood carbohydrate levels, xylem anatomy, and phenology in ring-porous oaks. In the light of our results, we suggest that *Q. pyrenaica*, and to a lesser extent *Q. robur*, could mitigate increasing hydraulic vulnerability under climate warming by prioritizing carbon accumulation over growth. Nevertheless, such a mechanism would impose additional limitations for secondary growth if adverse climate episodes become more frequent in future decades.

6 Data availability

Data used in this article are available in the Supplement.

The Supplement related to this article is available online at doi:10.5194/bg-13-5499-2016-supplement.

Author contributions. Ignacio García-González, José Miguel Olano, and Vicente Rozas conceived and designed the experiment. Ignacio García-González, Vicente Rozas, and Gonzalo Pérez-de-Lis conducted fieldwork. Gonzalo Pérez-de-Lis performed sample processing and data collection. José Miguel Olano and Gonzalo Pérez-de-Lis executed model calculation. Gonzalo Pérez-de-Lis prepared the manuscript. Ignacio García-González, José Miguel Olano, and Vicente Rozas provided editorial advice.

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