

1 **Stem hydraulic capacitance decreases with drought stress: implications for modelling tree**  
2 **hydraulics in the Mediterranean oak *Quercus ilex***

3 Running title: Seasonality in stem hydraulic capacitance

4 Salomón, Roberto L<sup>1,2</sup>; Limousin, Jean Marc<sup>3</sup>; Ourcival, Jean Marc<sup>3</sup>; Rodríguez-Calcerrada, Jesús<sup>1</sup>;  
5 Steppe, Kathy<sup>2</sup>

6 <sup>1</sup> Forest Genetics and Ecophysiology Research Group, E.T.S. Forestry Engineering, Technical  
7 University of Madrid, Ciudad Universitaria s/n, 28040, Madrid, Spain

8 <sup>2</sup> Laboratory of Plant Ecology, Department of Applied Ecology and Environmental Biology, Faculty of  
9 Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium

10 <sup>3</sup> Centre d'Ecologie Fonctionnelle et Evolutive CEFE, CNRS, UMR 5175, 1919 route de Mende, F-34293  
11 Montpellier, Cedex 5, France

12 Corresponding author: Salomón, Roberto L.; email address:

13 RobertoLuis.SalomonMoreno@UGent.be

## Abstract

Hydraulic modelling is a primary tool to predict plant performance in future drier scenarios. However, as most tree models are validated under non-stress conditions they may fail when water becomes limiting. To simulate tree hydraulic functioning under moist and dry conditions, the current version of a water flow and storage mechanistic model was further developed by implementing equations that describe variation in xylem hydraulic resistance ( $R_x$ ) and stem hydraulic capacitance ( $C_s$ ) with predawn water potential ( $\Psi_{PD}$ ). The model was applied in a Mediterranean forest experiencing intense summer drought, where six *Quercus ilex* trees were instrumented to monitor stem diameter variations and sap flow, concurrently with measurements of predawn and midday leaf water potential. Best model performance was observed when  $C_s$  was allowed to decrease with decreasing  $\Psi_{PD}$ . Hydraulic capacitance decreased from 62 to 25 kg m<sup>-3</sup> MPa<sup>-1</sup> across the growing season. In parallel, tree transpiration decreased to a greater extent than the capacitive water release and the contribution of stored water to transpiration increased from 2.0% to 5.1%. Our results demonstrate the importance of stored water and seasonality in  $C_s$  for tree hydraulic functioning, and they suggest that  $C_s$  should be considered to predict the drought-response of trees with models.

**Keywords:** radial stem growth, drought, hydraulic conductance, process-based modelling, holm oak, stem water storage, hydraulic function

## 1 Introduction

As soil dries and atmospheric vapor pressure deficit intensifies, xylem conduits are subjected to lower water potential and may eventually cavitate, thus limiting tree water transport. Xylem vulnerability to drought-induced cavitation has been measured in hundreds of species as the main attribute to quantify tree resistance to drought (Choat *et al.*, 2012; Sperry & Love, 2015). A growing body of evidence points, however, to a complementary factor involved in drought resistance: the radial water flow in stems to maintain tree hydraulic integrity by buffering changes in xylem water potential and limiting cavitation of xylem conduits (Goldstein *et al.*, 1998; Meinzer *et al.*, 2003; Steppe & Lemeur, 2004; Scholz *et al.*, 2007; Steppe *et al.*, 2012; McCulloh *et al.*, 2014). In this way, plant tolerance to drought does not solely rely on inherent xylem resistance to cavitation, but also on the radial capacitive release of stored water from elastic-living tissues that transiently reduces xylem tension at a given rate of flow and hence cavitation. Accordingly, a trade-off between hydraulic capacitance and structural traits involved in xylem resistance to cavitation has been observed across a wide range of woody species (Meinzer *et al.*, 2008, 2009). Nevertheless, the role of hydraulic capacitance in plant hydraulics has been traditionally overshadowed by the study of drought-induced cavitation and the vulnerability of xylem to changes in water potential (Meinzer *et al.*, 2009; McCulloh *et al.*, 2014; Epila *et al.*, 2017).

Hydraulic modelling is a key tool to mechanistically understand how trees cope with severe and intense drought events (Mencuccini *et al.*, 2015; Steppe *et al.*, 2015a). Mechanistic models developed for well-watered conditions commonly use static parameters to describe constant xylem hydraulic conductance (Steppe *et al.*, 2006, 2008a; Verbeeck *et al.*, 2007; Zweifel *et al.*, 2007; De Schepper & Steppe, 2010), whereas variable hydraulic conductance as a function of water potential has been successfully implemented as the soil dries out (Sperry *et al.*, 1998; Baert *et al.*, 2015; Mencuccini *et al.*, 2015). Likewise, static parameters are used to describe hydraulic capacitance (e.g. Sperry *et al.*, 1998; Steppe *et al.*, 2006; Zweifel *et al.*, 2007; Baert *et al.*, 2015); however, none of these models consider the variability in stem hydraulic capacitance, although stem water reservoirs

are progressively depleted under dry conditions (Scholz *et al.*, 2007, 2008; Verbeeck *et al.*, 2007; Betsch *et al.*, 2011; Kocher *et al.*, 2013; Matheny *et al.*, 2015). Accounting for the dynamic drought response of both hydraulic conductance and capacitance could improve model performance under a wide range of environmental conditions and extended time frames (Steppe *et al.*, 2008b). Model refinement via integration of tree drought responses has therefore been encouraged to advance in our mechanistic understanding of tree hydraulic functioning in future drier scenarios (Verbeeck *et al.*, 2007; Baert *et al.*, 2015; Mencuccini *et al.*, 2015; Steppe *et al.*, 2015a, 2015b).

In this study, we aimed at modelling tree hydraulic functioning in field-grown *Quercus ilex* L. under wet to dry conditions by taking into account the dynamic nature of both xylem resistance to water transport ( $R_x$ ) and stem hydraulic capacitance ( $C_s$ ). For this purpose, process-level equations that describe the relationship of  $R_x$  and  $C_s$  with predawn water potential ( $\Psi_{PD}$ ) have been implemented in the current version of a sophisticated mechanistic model that integrates tree water transport dynamics and stem diameter variations (Steppe *et al.*, 2006, 2008a; De Swaef *et al.*, 2015). Water reservoirs are defined here as the elastic tissues subjected to reversible diel cycles of water release and refill to avoid xylem cavitation. Inelastic water release from cavitated xylem conduits under negative water potential (Tyree & Ewers, 1991; Hölttä *et al.*, 2009) is neglected because the rapid reversibility of this phenomenon remains a matter of debate (Brodersen & McElrone, 2013; Cochard & Delzon, 2013). An advantage of this approach is that measurements are not destructive, since the model is driven by sap flow and  $\Psi_{PD}$ , and calibrated against stem diameter variations and xylem water potential at midday. As these variables can be monitored with peripheral devices and by sampling a small number of leaves, the hydraulic functioning of surveyed trees can be continuously modelled and research is not restricted to discrete observations.

We hypothesize that including daily variations in  $R_x$  and  $C_s$  as a function of  $\Psi_{PD}$  improves model accuracy in predicting stem diameter variations and xylem water potential during the dry season. Contrarily, we predict that variability in  $R_x$  and  $C_s$  may not improve model performance during the wet season as  $R_x$  and  $C_s$  are expected to remain constant. The integrated root-to-leaf  $R_x$  was

modelled as function of  $\Psi_{PD}$  using a negative exponential curve (Baert *et al.*, 2015), an alternative approach to estimate the integrated root-to-leaf hydraulic conductance ( $K_x = 1/R_x$ ) and generate vulnerability curves. Furthermore, a new equation defining the relationship between  $C_s$  and  $\Psi_{PD}$  was developed using the shape of water desorption curves and cumulative water release curves reported for several species (Zweifel *et al.*, 2000; Meinzer *et al.*, 2003, 2009; Steppe *et al.*, 2006; Barnard *et al.*, 2011; McCulloh *et al.*, 2014). Both magnitude of the decrease in  $C_s$  during the dry season and contribution of stored water to the transpiration stream were evaluated. Simulated cumulative water release from internal storage pools was compared with estimates of stem water refilling at the end of the dry season after first autumn heavy rains. Likewise, the simulated vulnerability curve was compared to the vulnerability curves obtained by measurements at the organ scale in the same experimental site (Limousin *et al.*, 2010a; Martin-StPaul *et al.*, 2014).

## 2 Materials and methods

### 2.1 Site description

The study site is located in the Puéchabon State Forest (Montpellier, France) in a stand dominated by *Q. ilex* (43°44'29" N, 3°35'45" E, 270 m.a.s.l.). The stand has been historically subjected to periodic coppicing, with the last cut being performed in 1942. Nowadays, top canopy height is 5.5 m, stand density is 4700 stems ha<sup>-1</sup>, and most stems (> 70%) range in diameter at breast height (DBH) from 4 to 10 cm. *Buxus sempervirens* L., *Phyllirea latifolia* L., *Pistacia terebinthus* L. and *Juniperus oxycedrus* L. are the main species of the understory layer. The area has a Mediterranean-type climate: annual mean temperature is 13.4 °C, annual precipitation is 907 mm, and 80% of this amount falls during winter and autumn, when temperatures are lower (Limousin *et al.*, 2009). A weather station located at the experimental site was used to monitor meteorological conditions. Air temperature, relative humidity (MP100, Rotronic, Bassersdorf, Switzerland), and rainfall (tipping bucket rain gauge ARG100, Environmental Measurements Ltd, Sunderland, UK) were measured every minute and

averaged every 30 minutes with a data logger (model 21X, Campbell Scientific Ltd, Shepshed, UK).  
More details on the experimental site are available in Rambal *et al.* (2014).

## 2.2 Tree and soil measurements

Six *Q. ilex* trees (see Table 1) were instrumented to continuously monitor sap flow and stem diameter variations. We selected data from the year 2009 because it was characterized by a strong and typical summer drought between mid-July and mid-September, and due to data availability on leaf water potential at predawn and midday in the instrumented trees during the summer.

Sap flux density ( $\text{g cm}^{-2} \text{ h}^{-1}$ ) was continuously monitored with thermal dissipation probes (Granier, 1985). Probe pairs were inserted at 1.2 m height with a vertical separation of 10 cm. Probes were oriented facing north to avoid direct solar heating, and were protected from rain and radiation by aluminum cover. Temperature difference between the probes was registered every 5 minutes, and averaged and recorded every 30 minutes with a data logger (model CR10X, Campbell Scientific). Sap flux density was calculated considering zero flow from the absolute maximum temperature difference over 2-day running periods. Sap flux density was upscaled to the tree level to obtain sap flow ( $F_{\text{STEM}}$ ,  $\text{g h}^{-1}$ ) by multiplying sap flux density by sapwood area. Sapwood area was estimated from an allometric relationship between tree DBH and sapwood area obtained from 18 additional trees (Limousin *et al.*, 2009). For further details about  $F_{\text{STEM}}$  measurements see Limousin *et al.* (2009).

Stem circumference variations were recorded using automatic band dendrometers (ELPA-98, University of Oulu, Oulu, Finland). Dendrometers were just below the thermal dissipation probes, and the outer layer of dead bark was removed prior to installation. Circumference variations were registered every 5 minutes and recorded every 30 minutes with a data logger (model CR1000; Campbell Scientific Ltd, Shepshed, UK) and transformed to stem diameter variations ( $\Delta D$ ,  $\mu\text{m}$ ). For further details about  $\Delta D$  measurements see Lempereur *et al.* (2015).

Leaf water potential ( $\Psi_{\text{LEAF}}$ , MPa) was measured with a pressure chamber (PMS1000, PMS Instruments, Corvallis, OR, USA) on DOYs 114, 176, 208, 234 and 310 during the 2009 growing season

(23 April, 24 June, 26 July, 21 August, 5 November, respectively) at predawn (before 06:00 h) and midday (14:00 h). Two leaves were sampled per tree and a third leaf was sampled if the observed difference between measurements was higher than 0.2 MPa. Samples were taken at similar tree heights and measurements were performed within 1 min after leaf excision. Leaf water potential was measured in four of the six trees instrumented for  $F_{STEM}$  and  $\Delta D$  measurements. Average  $\Psi_{LEAF}$  of these four trees was used for the two remaining trees.

The discrete measurements of  $\Psi_{PD}$  were interpolated on a daily basis using modelled soil water storage (SWS, mm). Daily soil water storage was modelled using the soil water balance module of the SIERRA vegetation model driven by daily values of solar radiation, minimum and maximum temperature and precipitation (Mouillot *et al.*, 2001; Ruffault *et al.*, 2013). Soil water storage and  $\Psi_{PD}$  were related by a Campbell-type retention curve (Campbell, 1974). The soil water storage model was validated against measurements of SWS integrated over a rooting depth of 4.5 m and performed at approximately monthly intervals from 1998 to 2009 using a neutron moisture gauge (see Rambal *et al.* 2003 for further details). The relationship between modelled and measured SWS displayed a good agreement ( $R^2 = 0.92$ ;  $p < 0.0001$ ; RMSE = 16 mm;  $n = 90$ ), as well as the relationship between modelled and measured  $\Psi_{PD}$  ( $R^2 = 0.73$ ;  $p < 0.0001$ ; RMSE = 0.71 MPa;  $n = 95$ ). Despite the good fit obtained between SWS and  $\Psi_{PD}$  on an annual basis,  $\Psi_{PD}$  was underestimated by the soil water balance model during summer drought. To correct daily simulated values of  $\Psi_{PD}$  in summer, optimized parameters for each tree were selected to account for spatial heterogeneity in leaf area index, soil texture and stone fraction.

## **2.3 Mechanistic tree modelling**

### **2.3.1 Model description**

The current version of a mechanistic water flow and storage model (Steppe *et al.*, 2008a) was used to study the hydraulic functioning of *Q. ilex* trees. Briefly, the model is composed of two interconnected sub-models describing dynamics in tree water transport and stem diameter variations, in which  $F_{STEM}$

dynamics are intimately linked to stem  $\Delta D$  by radial water flow between the xylem and the outer tissues. Note that “stem” refers to the tree trunk, so that branches are excluded for model simplicity. The model assumes the xylem as a rigid cylinder (xylem compartment) surrounded by an elastic outer ring composed of cells of cambium, phloem and bark (storage compartment) responsible for diel shrinkage and swelling (Steppe *et al.*, 2006). Therefore, the water stored in outer elastic cells constitutes the only capacitive water source; water release from the xylem parenchyma and cavitared conduits is neglected by this model. Sap flow ( $F_{STEM}$ ) integrates the axial water flow through the xylem compartment via root water uptake ( $f_x$ ) and the radial water exchange between xylem and storage compartment ( $f_s$ ). Axial water flow ( $f_x$ ) is calculated as the water potential gradient between the roots and the stem divided by  $R_x$ , and radial water flow ( $f_s$ ) is calculated as the first derivative defining the change in water content of the storage compartment, which is influenced by the resistance to radial flow between xylem and storage compartment ( $R_s$ , MPa h g<sup>-1</sup>). The model equations are shown in the supplementary material (Note S1). When transpiration starts, a water potential gradient between xylem and storage compartment ( $\Psi_x - \Psi_s$ ) leads to radial flow from outer cells to the xylem to fulfill the transpiration need;  $\Psi_x$  is inferred from  $\Psi_{LEAF}$  measurements (see below), whereas  $\Psi_s$  is estimated as a function of the water content and capacitance of the storage compartment (see Note S1). Water depletion in the morning results in reduction in cell turgor and reversible stem shrinkage. Conversely, water refilling in the afternoon, when atmospheric vapor pressure deficit and transpiration start to decline, results in reversible stem swelling. Water potential in the storage compartment is the algebraic sum of the osmotic potential ( $\Psi_s^O$ ) and the turgor pressure ( $\Psi_s^P$ ). Irreversible stem growth occurs when carbon requirements are met (Daudet *et al.*, 2005) and turgor pressure exceeds a threshold value for cell wall yielding ( $\Gamma$ , Lockhart 1965). For a detailed explanation of the principles of the model see Steppe *et al.* (2006, 2008a) and De Pauw *et al.* (2008).

To allow variation in xylem hydraulic resistance of the root-to-leaf continuum ( $R_x$ , MPa h g<sup>-1</sup>) and stem hydraulic capacitance ( $C_s$ , g MPa<sup>-1</sup>), the model was further developed by implementing two equations and their corresponding parameters:



184 The hydraulic resistance was described to vary exponentially with  $\Psi_{PD}$  (Baert *et al.*, 2015), which  
 185 accounted for day to day variations in  $R_x$  with soil drying while assuming no daily refilling of cavitaded  
 186 xylem:

$$187 \quad R_x = r_1 e^{(\Psi_{PD}^2) r_2} \quad \text{Eqn. 1}$$

188 where  $r_1$  (MPa h g<sup>-1</sup>) and  $r_2$  (MPa<sup>-2</sup>) are the proportionality parameters influenced by plant  
 189 characteristics.

190 The hydraulic capacitance of the stem was calculated as the derivative of the water release curve  
 191 (Steppe *et al.*, 2006):

$$192 \quad C_s = \frac{dW}{d\Psi} \quad \text{Eqn. 2}$$

193 where  $dW$  is the variation in water content in the storage compartment (g h<sup>-1</sup>) and  $d\Psi$  the  
 194 corresponding variation in  $\Psi$  (MPa h<sup>-1</sup>). In this study, the cumulative water ( $W$ ) release curve was  
 195 defined by a logarithmic equation of similar shape to those previously reported (e.g. Meinzer *et al.*,  
 196 2003, 2009; McCulloh *et al.*, 2014):

$$197 \quad W = -\log_{w_1}(w_2\Psi + 1) \quad \text{Eqn. 3}$$

198 where  $w_1$  and  $w_2$  are the parameters defining the shape of the water release curve. The derived form  
 199 of Eqn. 3 results in an inverse equation between  $C_s$  and  $\Psi_{PD}$ :

$$200 \quad C_s = \frac{1}{(c_1 \Psi_{PD} + c_2)} \quad \text{Eqn. 4}$$

201 where  $c_1$  (g<sup>-1</sup>) and  $c_2$  (MPa g<sup>-1</sup>) are the proportionality parameters dependent on plant properties,  
 202 which are related to the parameters defining the water release curve (Eqn. 3) as follows:  $w_1 = e^{c_1}$ ,

203 and  $w_2 = \frac{\ln(w_1)}{c_2}$ . Note that the purpose of this study is to evaluate seasonality in  $R_x$  and  $C_s$ .

204 Therefore,  $R_x$  and  $C_s$  in this model fluctuates on a daily basis with  $\Psi_{PD}$ , so that both are assumed  
 205 constant within 24 h periods as diel variability in  $R_x$  and  $C_s$  is expected to be comparatively negligible  
 206 (see Baert *et al.*, 2015).

Four models were calibrated for each tree: [1]  $R_x$  and  $C_s$  were assumed to be constant (the simplest framework, used for model calibration of  $R_x$  and  $C_s$ ), [2]  $R_x$  was allowed to vary and  $C_s$  was assumed to be constant (model calibration of  $r_1$ ,  $r_2$  and  $C_s$ ), [3]  $C_s$  was allowed to vary and  $R_x$  was assumed to be constant (model calibration of  $R_x$ ,  $c_1$  and  $c_2$ ), and [4] both  $R_x$  and  $C_s$  were allowed to vary (model calibration of  $r_1$ ,  $r_2$ ,  $c_1$  and  $c_2$ ). The resistance to radial flow between xylem and storage compartment ( $R_s$ ) was also calibrated in each model.

### 2.3.2 Model simulation, calibration, and identifiability analyses

Model simulations, calibrations and identifiability analyses were performed using the plant modelling software PhytoSim (version 2.1, Phyto-IT, Mariakerke, Belgium). Simulations were conducted with a fourth order variable step size solver of an accuracy of  $10^{-6}$  and a maximum step size of 1 h. Calibrations were done using the simplex method to minimize the weighted sum of squared errors (SSE) for  $\Delta D$  and  $\Psi_x$ . Identifiability analyses were performed to check whether the subset of model parameters to calibrate was not correlated and sensitive enough, and thus was identifiable (De Pauw *et al.*, 2008). A collinearity index (CI) above 15 indicated an unidentifiable subset of parameters. Values of non-calibrated model parameters were directly measured or assigned from literature (Table S1 in supplementary material).

Predawn water potential and  $F_{STEM}$  were used as model inputs, and  $\Delta D$  and  $\Psi_x$  were used for calibration purposes. The best model was selected using the final predicted error (FPE) criteria (Steppe *et al.*, 2006):

$$FPE = \frac{SSE}{N} + \frac{2pSSE}{(N-p)N} \quad \text{Eqn. 5}$$

where SSE is the weighted sum of squared errors for  $\Delta D$  and  $\Psi_x$ ,  $N$  is the number of observations, and  $p$  the number of calibrated parameters. The first term of the FPE evaluates the goodness of fit between measured and simulated data, whereas the second term penalizes over-parameterized models. Thus, the smaller the FPE value, the better the model.

Because rain events and tree trembling resulted in irregular signals recorded by the automatic band dendrometers, data was manually inspected to select a set of days that displayed reliable diel patterns, i.e., smooth morning shrinkage and afternoon swelling. As the number of  $\Delta D$  observations after manual inspection differed among trees, FPE values also differed. To remove any effect associated to the number of observations, values of FPE were normalized relative to the simplest framework (constant  $R_x$  and  $C_s$ ) for each tree.

Models were calibrated under wet and dry conditions. As stem growth during the summer season is limited by soil water availability in the surveyed site (Lempereur *et al.*, 2015), wet and dry periods were defined according to growing or non-growing conditions, respectively. Periods that cover two consecutive midday  $\Psi_{LEAF}$  measurements were initially considered for model calibration: DOYs 113-177 were selected for the wet period (spring and early summer) and DOYs 207-235 for the dry period (late summer). Inaccurate simulations were initially obtained during the wet period and further attempts to improve simulations were tested. First, the stem growth curve was adjusted to a Gompertz-shaped curve (Winsor, 1932) to smooth large fluctuations registered by the band dendrometers (Fig. 1D); and second, a shorter time period around a single  $\Psi_{LEAF}$  measurement campaign (DOY 176, early summer) was additionally examined.

To calibrate the models, midday  $\Psi_x$  was inferred from midday  $\Psi_{LEAF}$  measurements. Under dry conditions, measurements of midday  $\Psi_{LEAF}$  could be used as a surrogate of midday  $\Psi_x$ , because of the strong stomatal limitation observed in the monitored trees (see Fig. 3 in Limousin *et al.*, 2010b) which minimized the disequilibrium in water potential between xylem and leaves (Meinzer *et al.*, 2009). In contrast, substantial disequilibrium between midday  $\Psi_{LEAF}$  and midday  $\Psi_x$  might occur when water is not limiting, as has been observed in *Q. ilex* seedlings (Rodríguez-Calcerrada *et al.*, 2017) and in Neotropical savanna trees (see Fig. 2 in Scholz *et al.*, 2007). Under wet conditions, the difference between midday and predawn  $\Psi_x$  ( $\Delta\Psi_x = \text{midday } \Psi_x - \text{predawn } \Psi_x$ ) was assumed as a constant fraction of  $\Delta\Psi_{LEAF}$  ( $\Delta\Psi_{LEAF} = \text{midday } \Psi_{LEAF} - \text{predawn } \Psi_{LEAF}$ ). The ratio  $\Delta\Psi_x / \Delta\Psi_{LEAF}$  was obtained from measurements performed in *Q. ilex* seedlings ( $\Delta\Psi_x / \Delta\Psi_{LEAF} = 0.342$ ; Fig. S1 in

supplementary material), in which leaves were covered with aluminium foil for one hour to measure  $\Psi_x$ . Midday  $\Psi_x$  was therefore estimated as a function of  $\Psi_{PD}$  and  $\Delta\Psi_{LEAF}$  (midday  $\Psi_x = \Psi_{PD} + 0.342 * \Delta\Psi_{LEAF}$ ). By using this approximation of  $\Psi_x$ , we made the assumptions that leaf hydraulic resistance was a constant fraction of the tree hydraulic resistance independently of tree size (Sack *et al.*, 2003), that the water potential difference between leaves and stem depended mainly on leaf transpiration, and that the  $\Psi_x$  difference between the trunk and the branches was negligible.

## 2.4 Stem water refilling measurements

Stem water refilling was estimated by integrating sap flow over intense rain events at the end of summer drought. We only considered short (< 4 hours) and heavy (> 20 mm) rainfall events occurring at night or in the late evening to ensure that recorded sap flow could be attributed to stem water refilling and not to leaf transpiration (Betsch *et al.*, 2011), and to avoid rainfall events that resulted in slow refilling extending beyond night-time. Sap flow was integrated along the rain event and following night-time hours to estimate the volume of water refilled. Stem water refilling was then divided by the corresponding shift in  $\Psi_{PD}$  measured before and after the rain event to estimate  $C_s$  (Eqn. 2) and compare it with values obtained from model simulations. Available measurements of  $\Psi_{PD}$  obtained less than one week before and after the rainfall event were necessary to calculate  $C_s$ . As the first heavy rain in 2009 did not meet these requirements, different years were inspected and suitable rain events were found in 2006 and 2007 to estimate  $C_s$ . TREE4 was excluded from this analysis, as it was not monitored in 2006 and 2007. However, eighteen *Q. ilex* trees located in the study site and equipped with thermal dissipation probes were additionally included.

## 2.5 Data analyses

To compare model performance (i.e. normalized FPE) among the four tested models and the two surveyed periods, Tukey's multiple comparison tests were performed using the *TukeyHSD* function in the R software (version 3.2.3). The best model was then selected to obtain  $R_x$  and  $C_s$  along the gradient of  $\Psi_{PD}$  on a per-tree basis. To test whether  $F_{STEM}$  and radial water flow ( $f_s$ ) varied with  $\Psi_{PD}$ ,

mixed models were adjusted using the *lme* function in the nlme library, in which stem was considered as a random factor ( $n = 6$ ). As the model output for  $C_s$  refers to the whole tree,  $C_s$  was standardized per unit of storage volume. The storage volume was estimated as the product of stem height by stem basal area subtracting the xylem compartment. The integrated root-to-leaf hydraulic conductance was calculated as the inverse of the integrated root-to-leaf hydraulic resistance ( $K_x = 1/R_x$ ,  $\text{g MPa}^{-1} \text{h}^{-1}$ ). Predawn water potential causing 50% loss of  $K_x$  ( $\Psi_{50}$ ) was obtained following Baert *et al.* (2015). To obtain the mean curve and confidence intervals of  $C_s$ , cumulative water release, and integrated root-to-leaf  $R_x$  and  $K_x$  along the gradient of  $\Psi_{PD}$ , uncertainty analyses were performed in PhytoSim using the averaged parameters among the six studied trees yielded by the best model. All values presented in the text are means  $\pm$  SE.

### 3 Results

Averaged among the six surveyed stems and across the whole year, daily sap flow was  $4.11 \pm 0.81$  kg  $\text{day}^{-1}$  (Fig. 1C, Table 1), and mean annual diameter increment was  $0.53 \pm 0.20$  mm  $\text{year}^{-1}$  (Fig. 1D, Table 1). During spring and early summer (DOYs 113-177), average temperature was  $18.2$  °C and accumulated rainfall 107.2 mm. Late summer was hot and dry, with an average temperature of  $25.2$  °C and an accumulated rainfall of 11.8 mm during the dry modelled period (DOYs 207-235). The lowest  $\Psi_{LEAF}$  was measured at the end of this period, when it reached mean values of  $-3.3$  MPa at predawn and  $-3.9$  MPa at midday (Fig. 1B).

Four models with constant or variable  $R_x$  and  $C_s$  were adjusted per tree and period to simulate  $\Delta D$  and  $\Psi_x$  over time. A consistent pattern was observed under wet (Fig. 2A) and dry (Fig. 2B) conditions. Relative to the simplest model (constant  $R_x$  and  $C_s$ ), the normalized FPE was not reduced when  $R_x$  alone was allowed to vary ( $P > 0.1$ ). In contrast, the normalized FPE was reduced ( $P < 0.001$ ) when  $C_s$  was allowed to vary with  $\Psi_{PD}$ . The model with variable  $R_x$  and  $C_s$  showed the lowest normalized FPE, although its value did not differ significantly from that obtained with constant  $R_x$  and variable  $C_s$  ( $P > 0.1$ ). Therefore, calibration of the model with a variable  $C_s$  was the main improvement required to

307 accurately simulate  $\Delta D$  and  $\Psi_x$ . Only data yielded by the best model (variable  $R_x$  and  $C_s$ ) is shown  
308 hereafter.

309 Models were calibrated under wet and dry conditions. Only TREE5 was not modelled during the wet  
310 season due to the inconsistent dendrometer signal at the time of  $\Psi_{LEAF}$  measurement. Because of  
311 concurrent stem growth, inaccurate simulations of  $\Delta D$  were initially obtained during the wet period  
312 when considering long temporal spans (DOYs 113-177; data not shown). Shorter temporal spans of 3-  
313 4 days resulted in realistic simulations of  $\Psi_x$  and  $\Delta D$  (Fig. 3A-C, FPE =  $6.04 \pm 1.90$ ). The length of the  
314 simulation period was not an issue under dry conditions when stem growth was impeded, and  
315 accurate simulations were obtained for every tree throughout the one-month simulated period (Fig.  
316 3D-F, FPE =  $7.71 \pm 2.11$ ). During the wet season, cell turgor pressure at night-time was higher than  
317 the turgor threshold for cell wall yielding ( $\Psi_s^P > \Gamma$ , Fig. 3A) leading to irreversible stem growth (Fig.  
318 3B). During the dry season, soil drying progressively reduced  $\Psi_x$ ,  $\Psi_s$  and cell osmotic potential, which  
319 explained the rather constant pattern of cell turgor pressure, which was below the turgor threshold  
320 for cell wall yielding ( $\Psi_s^P < \Gamma$ , Fig. 3D), thus impeding irreversible stem growth (Fig. 3E). Morning stem  
321 shrinkage and afternoon stem swelling (Fig. 3B, 3E) were caused by the radial water flow between  
322 xylem and storage compartments ( $f_s$ , Fig. 3C, 3F).

323 Daily  $F_{STEM}$  was directly related to  $\Psi_{PD}$  in every surveyed stem ( $P < 0.01$ , Fig. 4A). Averaged among the  
324 six stems,  $F_{STEM}$  decreased from  $7.50 \pm 0.83 \text{ kg day}^{-1}$  during the wet period ( $\Psi_{PD} = -1.0 \text{ MPa}$ ) to  $1.46 \pm$   
325  $0.81 \text{ kg day}^{-1}$  at the end of the dry period ( $\Psi_{PD} = -3.3 \text{ MPa}$ ,  $P < 0.001$ ). The decrease in daily stem  
326 water release from the storage to the xylem compartment (daily sum of negative  $f_s$ , hereafter daily  
327  $|f_s|$ , in  $\text{kg day}^{-1}$ ) with  $\Psi_{PD}$  was significant in only half of the surveyed trees ( $P < 0.05$ , Fig. 4B) and  
328 became non-significant when pooling trees across a range of  $\Psi_{PD}$  from -1.0 to -3.3 MPa ( $P = 0.15$ ).  
329 Considering the dry period only, daily  $|f_s|$  significantly decreased with  $\Psi_{PD}$  ( $P < 0.001$ ) from  $0.13 \pm$   
330  $0.03$  to  $0.10 \pm 0.03 \text{ kg day}^{-1}$  for a corresponding reduction in  $\Psi_{PD}$  of 1.3 MPa (from -2.0 to -3.3 MPa).  
331 Across the year, daily sap flow was reduced to a greater extent than daily  $|f_s|$  as the soil dried out.

Hence, the daily contribution of  $|f_s|$  to  $F_{STEM}$  increased with drought severity ( $P < 0.001$ ) from  $2.0 \pm 0.9 \%$  to  $5.1 \pm 0.9 \%$  when  $\Psi_{PD}$  decreased from  $-1.0$  to  $-3.3$  MPa (Fig. 4C).

Stem hydraulic capacitance decreased with drought stress. Mean  $C_s$  on a storage volume basis was  $61.69 \pm 6.30 \text{ kg MPa}^{-1} \text{ m}^{-3}$  under wet conditions (Table 2) and reached lowest values of  $24.93 \pm 4.14 \text{ kg MPa}^{-1} \text{ m}^{-3}$  at the end of the summer drought (Table 3). Fig. 5A illustrates the mean cumulative release of water from storage compartments and the change in  $C_s$  at the tree level obtained in the dry period and extrapolated to a wider range of  $\Psi_{PD}$ . Hydraulic capacitance obtained from model calibration during the wet period ( $C_s = 314.51 \pm 38.83 \text{ g MPa}^{-1} \text{ tree}^{-1}$ ) was underestimated by the  $C_s$  curve extrapolated from the dry period. Similarly, Fig. 5B displays mean  $R_x$  and  $K_x$  curves obtained in the dry period. The vulnerability curve of root-to-leaf hydraulic conductance obtained from the dry period showed a mean  $\Psi_{50}$  value of  $-2.67 \pm 0.23$  MPa (Table 3). The  $R_x$  value obtained from direct model calibration under wet conditions ( $5.01 \pm 0.45 \times 10^{-4} \text{ MPa h g}^{-1}$ , Table 2) was overestimated by the  $R_x$  curve extrapolated from summer drought.

Two rain events were used to independently estimate  $C_s$  from stem water refilling. In 2006, 24 mm of rainfall were registered during the night of DOY 228 to 229, whereas in 2007, a stronger rainfall of 75.5 mm occurred in the night of DOY 261 to 262. The corresponding mean increase in  $\Psi_{PD}$  was 2.6 and 2.9 MPa for 2006 and 2007, respectively. Stem water refilling and  $C_s$  on a tree basis were exponentially related to stem size across the 23 trees examined ( $P < 0.001$ ; Fig. 6). Average stem water refilling and  $C_s$  among the five trees monitored in both approaches were  $1134 \pm 149 \text{ g tree}^{-1}$  and  $414 \pm 45 \text{ g MPa}^{-1} \text{ tree}^{-1}$ , respectively.

## 4 Discussion

### 4.1 The importance of a variable hydraulic capacitance in tree modelling

Hydraulic capacitance is commonly measured as the slope of the initial and nearly linear portion of the curve between the cumulative water release and water potential (Meinzer *et al.*, 2003, 2008; Barnard *et al.*, 2011; McCulloh *et al.*, 2014). These estimates of  $C_s$  are therefore obtained within the

range of  $\Psi_x$  when plants are not subjected to drought stress. Notwithstanding,  $C_s$  varies seasonally as water storage refilling is limited by soil drying (Verbeeck *et al.*, 2007; Scholz *et al.*, 2008; Steppe *et al.*, 2008b; Kocher *et al.*, 2013; Matheny *et al.*, 2015). Progressive decoupling between daily capacitive water discharge and daily water refilling leads to water reserves depletion, and accordingly, a net reduction in stem diameter is commonly observed during the dry season (Fig. 3E; Zweifel *et al.*, 2000; Lempereur *et al.*, 2015). Progressive depletion of water reservoirs should be incorporated in process-based hydraulic models by the implementation of a direct relationship between  $C_s$  and soil water availability. However, to the best of our knowledge, no mechanistic model has yet considered drought-induced variability in  $C_s$  to model tree hydraulic functioning. Furthermore, and unexpectedly, considering the variation of  $C_s$  with  $\Psi_{PD}$  was also found to result in more accurate simulations of  $\Psi_x$  and  $\Delta D$  during the wet period. In contrast to previous studies using this model in which  $C_s$  was assumed constant under well-watered regimes and stable soil water content (e.g. Steppe *et al.*, 2006, 2008a; De Schepper & Steppe, 2010), considering a variable  $C_s$  was here necessary to simulate the increase in turgor that yielded irreversible cell wall extension. Assuming a constant  $C_s$  in our study resulted in a progressive reduction of maximum daily turgor pressure that impeded stem growth (simulations not shown) as  $\Psi_{PD}$  slightly declined during this “wet” period (Fig. 3A). Consequently, under both wet and dry conditions, assumptions of constant  $C_s$  may partially explain why tree models fail to reproduce variations in diameter and  $\Psi_x$  in environments where soil water availability fluctuates. We suggest that to better predict and understand tree hydraulic functioning and the risk of hydraulic failure in dry regions, it is necessary to take into account the dynamic nature of  $C_s$ .

Because  $C_s$  is commonly measured under moist conditions, estimates of  $C_s$  on a storage volume basis in early summer ( $62 \text{ kg m}^{-3} \text{ MPa}^{-1}$ , Table 2) might be more appropriate for literature comparison. Hydraulic capacitance of *Q. ilex* at this time of year was consistent with the reported range for evergreen sclerophyll species ( $6\text{-}102 \text{ kg m}^{-3} \text{ MPa}^{-1}$ , Richards *et al.* 2014) and lower than the ones for tropical species ( $70\text{-}420 \text{ kg m}^{-3} \text{ MPa}^{-1}$ , Meinzer *et al.* 2003, 2009) and conifers ( $107\text{-}886 \text{ kg m}^{-3} \text{ MPa}^{-1}$ , Barnard *et al.* 2011, McCulloh *et al.* 2014). Nevertheless, neglecting the xylem tissues as capacitive



water sources might lead to underestimation of the overall hydraulic capacitance of *Q. ilex* stems in our study (see below). Hydraulic capacitance decreased across the season from 62 to 25 kg m<sup>-3</sup> MPa<sup>-1</sup> (from DOY 176 to 235, respectively). The 60% reduction in  $C_s$  illustrates the importance of implementing variable  $C_s$  in mechanistic models to accurately simulate tree water status and diameter variations. Our modelling approach further allowed estimation of the daily contribution of internal water storage to total daily sap flow ( $|f_s| / F_{STEM}$ ) as a function of  $\Psi_{PD}$ . The average  $|f_s| / F_{STEM}$  ratio ranged between 2% in early summer and 5% at the end of summer drought when  $\Psi_{PD}$  reached mean values of  $-3.3$  MPa (Fig. 4C). This ratio is at the lower end of observations made in other species that may reach up to 19% when only elastic tissues are considered (see Table 3 in Betsch *et al.*, 2011). The low reliance on stored water to maintain transpiration rates in *Q. ilex* might be related to the wood features of this species with relatively high wood density and small vessel size (Limousin *et al.*, 2010a). These characteristics make trees more cavitation-resistant to xylem tension, an adaptation which seems to be related to a limited capacity to reduce xylem tension via radial water release (Meinzer *et al.*, 2008, 2009; Richards *et al.*, 2014). This trade-off also explains that  $C_s$  of *Q. ilex* is at the lower end of reported values (see Fig. 5 in Meinzer *et al.* 2009 for comparison). Despite low  $|f_s| / F_{STEM}$  ratios, the reliance on stored water increased with drought stress due to a more pronounced reduction in daily  $F_{STEM}$  compared to daily  $|f_s|$ . A similar pattern was also noticed in *Quercus robur* L., which used stored water primarily when subjected to drought stress (Matheny *et al.*, 2015). These observations suggest greater relevance of stem water reservoirs to maintain transpiration rates and tree hydraulic integrity in future drier climates (see Tyree & Ewers 1991; and Bréda *et al.* 2006).

## 4.2 Model limitations

Two limitations related to the model structure and assumptions were detected during this study. First, accurate simulations of diameter variations during the wet period were restricted to time frames of 3-4 days. Beyond this short time interval, diameter simulations progressively deviated from measured values. During dry conditions, however, the length of the model period was not an issue

409 because irreversible stem growth was prevented by soil water limitation (Lempereur *et al.*, 2015).  
 410 Any diameter variation during summer drought could be uniquely ascribed to radial water flow  
 411 causing elastic stem shrinkage and swelling. In contrast, during the wet period when irreversible  
 412 growth occurred, the carbon status of the plant is a factor involved in growth, for example via  
 413 osmotic regulation of cell turgor and elongation (Lockhart, 1965; Daudet *et al.*, 2005). The lack of  
 414 carbon-related equations in our water-based model describing sugar transport and/or carbon  
 415 allocation to growth could explain inaccurate simulations when the modelled period extended  
 416 beyond 3-4 days. Accordingly, the generally prescribed time frame for this model ranges from one  
 417 day to two weeks (Steppe *et al.*, 2006, 2008b). More sophisticated models integrating water and  
 418 carbon transport processes should be further developed to simulate irreversible stem growth on a  
 419 seasonal basis (De Schepper & Steppe, 2010; Mencuccini *et al.*, 2015; Steppe *et al.*, 2015a).

420 Second, the model is theoretically designed to estimate  $C_s$  only for outer tissues and we ignore the  
 421 amount of water released by the xylem compartment. The contribution of the xylem to whole stem  
 422 diameter variations is expected to be minor (Zweifel *et al.*, 2000; Steppe & Lemeur, 2004; Steppe *et al.*, 2006), and our model therefore assumes the xylem as a rigid compartment and outer tissues –  
 423 namely cambium, phloem, and phelloderm – as an elastic storage compartment responsible for diel  
 424 shrinkage and swelling. Nevertheless, the amount of water released for a given change in volume is  
 425 higher in the xylem than in the outer tissues; about 3.5 times higher in the case of savanna trees,  
 426 which results in a greater capacitance of the xylem compartment (Scholz *et al.*, 2007, 2008). Higher  
 427 capacitance of the xylem can be ascribed to both elastic living parenchyma and the capacitive effect  
 428 of cavitated conduits (Tyree & Ewers, 1991; Hölttä *et al.*, 2009; Richards *et al.*, 2014). Thus, water  
 429 release from the xylem could be important in large trees with a high proportion of sapwood and  
 430 lumen volume (Waring *et al.*, 1979; Hölttä *et al.*, 2009; Betsch *et al.*, 2011) or with large and  
 431 numerous sapwood parenchyma rays such as *Q. ilex*. Models considering the capacitive effect of  
 432 cavitation (Hölttä *et al.*, 2009) and the xylem as an elastic compartment with a distinct elastic bulk  
 433 modulus and hydraulic capacitance (Perämäki *et al.*, 2005) would be necessary to further disentangle  
 434

the contribution of elastic outer tissues and xylem parenchyma, and inelastic cavitated conduits to the overall capacitive discharge of water to the transpiration stream.

#### **4.3 Simulated stem water release curves, root-to-leaf vulnerability curves and model validation**

Simulations performed under dry conditions misestimated both  $C_s$  and  $R_x$  when extrapolated to a wider range of  $\Psi_{PD}$  during wet conditions, and values obtained for well-watered conditions were also necessary to realistically describe the  $C_s$  and  $R_x$  curves over the entire  $\Psi_{PD}$  range (Fig. 5). This observation denotes that any extrapolation beyond the surveyed range of  $\Psi_{PD}$  must be taken with caution. More frequent measurements of  $\Psi_{LEAF}$  (or  $\Psi_x$ ) would be necessary to re-calibrate model parameters at a higher temporal resolution (Steppe *et al.*, 2008b) and obtain a more accurate evolution of  $C_s$  and  $R_x$  across a wider range of  $\Psi_{PD}$ .

Estimates of stem water refilling following first heavy rains at the end of the drought period and the vulnerability curves obtained by measurements at the organ scale were compared to model simulations. At the end of summer drought, the modelled cumulative water release averaged 683 g tree<sup>-1</sup> at a  $\Psi_{PD}$  of -3.33 MPa (Fig. 5A), which is 60% of that estimated from the refilling calculations (1134 g tree<sup>-1</sup> for a corresponding increase in  $\Psi_{PD}$  of 2.75 MPa, Fig. 6A). Likewise, mean  $C_s$  modelled across the range of surveyed conditions (from 103 to 314 g MPa<sup>-1</sup> tree<sup>-1</sup>, Fig. 5A) was lower than that estimated following heavy rains (414 g MPa<sup>-1</sup> tree<sup>-1</sup>, Fig. 6B). Inaccurate assumptions to estimate stem storage capacity and  $C_s$  using both approaches may account for this difference. First,  $C_s$  could be overestimated from water refilling calculations because the corresponding water potential increase was measured several days apart from the refilling event, and was therefore slightly underestimated. Besides, water refilling of branches and leaves incorrectly attributed to the stem may overestimate the actual stem storage capacity. Second,  $C_s$  might be underestimated from model simulations due to the water release from xylem tissues (Scholz *et al.*, 2008; Hölttä *et al.*, 2009) undetected by our model. Models that integrate the hydraulic capacitance of the xylem compartment, continuous *in vivo* measurements of wood water content using frequency domain

reflectometry (Matheny *et al.*, 2015), or destructive sampling to obtain stem moisture release curves with psychrometers (Meinzer *et al.*, 2003) might be complementary approaches to more accurately estimate the overall hydraulic capacitance of the stem. Likewise, direct measurements of  $\Psi_x$  would result in more accurate estimates of  $C_s$  than the simulated here, in which  $\Psi_x$  was inferred from  $\Psi_{\text{LEAF}}$  measurements.

On the other hand, variation in  $R_x$  as a function of drought stress affected to a lesser extent the model performance. The simulated vulnerability curve along the root-to-leaf continuum exhibited reasonable agreement with measurements made at the organ scale at the same site. The mean simulated  $\Psi_{50}$  was -2.67 MPa (Table 3). Estimates of  $\Psi_{50}$  in excised branches and roots of *Q. ilex* trees located at the same site averaged -3.88 and -2.39 MPa, respectively, using the air injection technique after flushing native embolism (Limousin *et al.*, 2010a), and -4.7 MPa in branches using the bench drying technique (Martin-StPaul *et al.* 2014). Vulnerability to drought in the root-to-leaf continuum is determined to the greatest extent by the most vulnerable node along this hydraulic pathway (Baert *et al.*, 2015). In this particular case, roots might be the major constrain to water flow along the root-to-leaf continuum, followed by stems and branches (Tyree & Ewers, 1991; Sperry & Love, 2015). Therefore, the integrated  $\Psi_{50}$  (-2.67 MPa) might primarily reflect the hydraulic vulnerability of roots. This alternative approach to generate integrated root-to-leaf vulnerability curves might be useful to describe the hydraulic functioning of the whole tree while avoiding to separately measure multiple hydraulic resistances (Baert *et al.*, 2015), which might be controversial due to the strong variability in hydraulic conductance ascribed to methodological issues (Cochard & Delzon, 2013; Martin-StPaul *et al.*, 2014).

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## References

- Baert A, De Schepper V, Steppe K (2015) Variable hydraulic resistances and their impact on plant drought response modelling. *Tree Physiology*, **35**, 439–449.
- Barnard DM, Meinzer FC, Lachenbruch B, McCulloh KA, Johnson DM, Woodruff DR (2011) Climate-related trends in sapwood biophysical properties in two conifers: avoidance of hydraulic dysfunction through coordinated adjustments in xylem efficiency, safety and capacitance. *Plant, Cell & Environment*, **34**, 643–654.
- Betsch P, Bonal D, Breda N, Montpied P, Peiffer M, Tuzet A, Granier A (2011) Drought effects on water relations in beech: The contribution of exchangeable water reservoirs. *Agricultural and Forest Meteorology*, **151**, 531–543.
- Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science*, **63**, 625–644.
- Brodersen CR, McElrone AJ (2013) Maintenance of xylem network transport capacity: a review of embolism repair in vascular plants. *Frontiers in Plant Science*, **4**, 108.
- Campbell GS (1974) A simple method for determining unsaturated conductivity from moisture retention data. *Soil Science*, **117**, 311–314.

509 Choat B, Jansen S, Brodribb TJ et al. (2012) Global convergence in the vulnerability of forests to  
510 drought. *Nature*, **491**, 752–5.

511 Cochard H, Delzon S (2013) Hydraulic failure and repair are not routine in trees. *Annals of Forest  
512 Science*, **70**, 659–661.

513 Daudet F-A, Améglio T, Cochard H, Archilla O, Lacointe A (2005) Experimental analysis of the role of  
514 water and carbon in tree stem diameter variations. *Journal of Experimental Botany*, **56**, 135–44.

515 Epila J, De Baerdemaeker NJF, Vergeynst LL, Maes WH, Beeckman H, Steppe K (2017) Capacitive  
516 water release and internal leaf water relocation delay drought-induced cavitation in African  
517 *Maesopsis eminii*. *Tree Physiology*, DOI: 10.1093/treephys/tpw128.

518 Goldstein G, Andrade JL, Meinzer FC, Holbrook NM, Cavelier J, Jackson P, Celis A (1998) Stem water  
519 storage and diurnal patterns of water use in tropical forest canopy trees. *Plant, Cell &  
520 Environment*, **21**, 397–406.

521 Granier A (1985) Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des  
522 arbres. *Annals of Forest Science*, **42**, 193–200.

523 Hölttä T, Cochard H, Nikimaa E, Mencuccini M (2009) Capacitive effect of cavitation in xylem  
524 conduits: results from a dynamic model. *Plant, Cell & Environment*, **32**, 10–21.

525 Kocher P, Horna V, Leuschner C (2013) Stem water storage in five coexisting temperate broad-leaved  
526 tree species: significance, temporal dynamics and dependence on tree functional traits. *Tree  
527 Physiology*, **33**, 817–832.

528 Lempereur M, Martin-StPaul NK, Damesin C, Joffre R, Ourcival J-M, Rocheteau A, Rambal S (2015)  
529 Growth duration is a better predictor of stem increment than carbon supply in a Mediterranean  
530 oak forest: implications for assessing forest productivity under climate change. *New Phytologist*,  
531 **207**, 579–590.

532 Limousin JM, Rambal S, Ourcival JM, Rocheteau A, Joffre R, Rodriguez-Cortina R (2009) Long-term

533 transpiration change with rainfall decline in a Mediterranean *Quercus ilex* forest. *Global Change*  
534 *Biology*, **15**, 2163–2175.

535 Limousin JM, Longepierre D, Huc R, Rambal S (2010a) Change in hydraulic traits of Mediterranean  
536 *Quercus ilex* subjected to long-term throughfall exclusion. *Tree Physiology*, **30**, 1026–1036.

537 Limousin JM, Misson L, Lavoie AV, Martin NK, Rambal S (2010b) Do photosynthetic limitations of  
538 evergreen *Quercus ilex* leaves change with long-term increased drought severity? *Plant, Cell &*  
539 *Environment*, **33**, 863–875.

540 Lockhart JA (1965) An analysis of irreversible plant cell elongation. *Journal of Theoretical Biology*, **8**,  
541 264–275.

542 Martin-StPaul NK, Longepierre D, Huc R et al. (2014) How reliable are methods to assess xylem  
543 vulnerability to cavitation? The issue of “open vessel” artifact in oaks. *Tree Physiology*, **34**, 894–  
544 905.

545 Matheny AM, Bohrer G, Garrity SR, Morin TH, Howard CJ, Vogel CS (2015) Observations of stem  
546 water storage in trees of opposing hydraulic strategies. *Ecosphere*, **6**, 165.

547 McCulloh KA, Johnson DM, Meinzer FC, Woodruff DR (2014) The dynamic pipeline: hydraulic  
548 capacitance and xylem hydraulic safety in four tall conifer species. *Plant, Cell & Environment*,  
549 **37**, 1171–1183.

550 Meinzer FC, James SA, Goldstein G, Woodruff D (2003) Whole-tree water transport scales with  
551 sapwood capacitance in tropical forest canopy trees. *Plant, Cell & Environment*, **26**, 1147–1155.

552 Meinzer FC, Woodruff DR, Domec J-C, Goldstein G, Campanello PI, Gatti MG, Villalobos-Vega R (2008)  
553 Coordination of leaf and stem water transport properties in tropical forest trees. *Oecologia*,  
554 **156**, 31–41.

555 Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR (2009) Xylem hydraulic safety  
556 margins in woody plants: coordination of stomatal control of xylem tension with hydraulic

557 capacitance. *Functional Ecology*, **23**, 922–930.

558 Mencuccini M, Minunno F, Salmon Y, Martínez-Vilalta J, Hölttä T (2015) Coordination of physiological  
559 traits involved in drought-induced mortality of woody plants. *New Phytologist*, **208**, 396–409.

560 Mouillot F, Rambal S, Lavorel S (2001) A generic process-based Simulator for mediterranean  
561 landscApes (SIERRA): design and validation exercises. *Forest Ecology and Management*, **147**,  
562 75–97.

563 De Pauw DJW, Steppe K, De Baets B (2008) Identifiability analysis and improvement of a tree water  
564 flow and storage model. *Mathematical Biosciences*, **211**, 314–332.

565 Perämäki M, Vesala T, Nikinmaa E (2005) Modeling the dynamics of pressure propagation and  
566 diameter variation in tree sapwood. *Tree Physiology*, **25**, 1091–1099.

567 Rambal S, Ourcival J-M, Joffre R, Mouillot F, Nouvellon Y, Reichstein M, Rocheteau A (2003) Drought  
568 controls over conductance and assimilation of a Mediterranean evergreen ecosystem: scaling  
569 from leaf to canopy. *Global Change Biology*, **9**, 1813–1824.

570 Rambal S, Lempereur M, Limousin JM, Martin-StPaul NK, Ourcival JM, Rodríguez-Calcerrada J (2014)  
571 How drought severity constrains gross primary production (GPP) and its partitioning among  
572 carbon pools in a *Quercus ilex* coppice? *Biogeosciences*, **11**, 6855–6869.

573 Richards AE, Wright IJ, Lenz TI, Zanne AE (2014) Sapwood capacitance is greater in evergreen  
574 sclerophyll species growing in high compared to low-rainfall environments. *Functional Ecology*,  
575 **28**, 734–744.

576 Rodríguez-Calcerrada J, Li M, López R et al. (2017) Drought-induced shoot dieback starts with massive  
577 root xylem embolism and variable depletion of nonstructural carbohydrates in seedlings of two  
578 tree species. *New Phytologist*, **213**, 597–610.

579 Ruffault J, Martin-StPaul NK, Rambal S, Mouillot F (2013) Differential regional responses in drought  
580 length, intensity and timing to recent climate changes in a Mediterranean forested ecosystem.



581           *Climatic Change*, **117**, 103–117.

582   Sack L, Cowan PD, Jaikumar N, Holbrook NM (2003) The “hydrology” of leaves: co-ordination of  
583           structure and function in temperate woody species. *Plant, Cell and Environment*, **26**, 1343–  
584           1356.

585   De Schepper V, Steppe K (2010) Development and verification of a water and sugar transport model  
586           using measured stem diameter variations. *Journal of Experimental Botany*, **61**, 2083–2099.

587   Scholz FC, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F (2007) Biophysical  
588           properties and functional significance of stem water storage tissues in Neotropical savanna  
589           trees. *Plant, Cell & Environment*, **30**, 236–248.

590   Scholz FC, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F (2008) Temporal  
591           dynamics of stem expansion and contraction in savanna trees: withdrawal and recharge of  
592           stored water. *Tree Physiology*, **28**, 469–480.

593   Sperry JS, Love DM (2015) What plant hydraulics can tell us about responses to climate-change  
594           droughts. *New Phytologist*, **207**, 14–27.

595   Sperry JS, Adler FR, Campbell GS, Comstock JP (1998) Limitation of plant water use by rhizosphere  
596           and xylem conductance: Results from a model. *Plant, Cell & Environment*, **21**, 347–359.

597   Steppe K, Lemeur R (2004) An experimental system for analysis of the dynamic sap-flow  
598           characteristics in young trees: results of a beech tree. *Functional Plant Biology*, **31**, 83–92.

599   Steppe K, De Pauw DJW, Lemeur R, Vanrolleghem A (2006) A mathematical model linking tree sap  
600           flow dynamics to daily stem diameter fluctuations and radial stem growth. *Tree Physiology*, **26**,  
601           257–273.

602   Steppe K, De Pauw DJW, Lemeur R (2008a) A step towards new irrigation scheduling strategies using  
603           plant-based measurements and mathematical modelling. *Irrigation Science*, **26**, 505–517.

604   Steppe K, De Pauw DJW, Lemeur R (2008b) Validation of a dynamic stem diameter variation model

605 and the resulting seasonal changes in calibrated parameter values. *Ecological Modelling*, **218**,  
606 247–259.

607 Steppe K, Cochard H, Lacointe A, Améglio T (2012) Could rapid diameter changes be facilitated by a  
608 variable hydraulic conductance? *Plant, Cell & Environment*, **35**, 150–157.

609 Steppe K, Sterck F, Deslauriers A (2015a) Diel growth dynamics in tree stems: linking anatomy and  
610 ecophysiology. *Trends in Plant Science*, **20**, 335–343.

611 Steppe K, Vandegehuchte MW, Tognetti R, Mencuccini M (2015b) Sap flow as a key trait in the  
612 understanding of plant hydraulic functioning. *Tree Physiology*, **35**, 341–345.

613 De Swaef T, De Schepper V, Vandegehuchte MW, Steppe K (2015) Stem diameter variations as a  
614 versatile research tool in ecophysiology. *Tree Physiology*, **35**, 1047–1061.

615 Tyree M, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. *New*  
616 *Phytologist*, **119**, 345–360.

617 Verbeeck H, Steppe K, Nadezhkina N et al. (2007) Atmospheric drivers of storage water use in Scots  
618 pine. *Biogeosciences*, **4**, 657–671.

619 Waring RH, Whitehead D, Jarvis PG (1979) The contribution of stored water to transpiration in Scots  
620 pine. *Plant, Cell & Environment*, **2**, 309–317.

621 Winsor CP (1932) The Gompertz curve as a growth curve. *Proceedings of the National Academy of*  
622 *Sciences*, **18**, 1–8.

623 Zweifel R, Item H, Häslér R (2000) Stem radius changes and their relation to stored water in stems of  
624 young Norway spruce trees. *Trees - Structure and Function*, **15**, 50–57.

625 Zweifel R, Steppe K, Sterck FJ (2007) Stomatal regulation by microclimate and tree water relations:  
626 interpreting ecophysiological field data with a hydraulic plant model. *Journal of Experimental*  
627 *Botany*, **58**, 2113–2131.

628 **Table 1.** Diameter at breast height (DBH), tree height, accumulated diameter increment and mean  
629 daily sap flow during 2009 for the six monitored *Quercus ilex* trees.

	DBH	Height	Annual diameter increment	Mean sap flow
	(cm)	(m)	(mm year <sup>-1</sup> )	(kg day <sup>-1</sup> )
TREE1	9.55	5.3	0.25	2.55
TREE2	11.05	5.0	0.16	3.00
TREE3	10.70	5.0	0.51	5.87
TREE4	13.20	5.6	1.21	7.32
TREE5	10.05	5.2	-0.10	2.90
TREE6	12.25	4.5	1.15	3.00

630 Tree DBH measured at the beginning of the 2009 growing season

631

**Table 2.** Hydraulic resistance to radial flow between the xylem and the storage compartment ( $R_s$ ), hydraulic resistance of the root-to-leaf segment ( $R_x$ ), and stem hydraulic capacitance ( $C_s$ ) obtained from the mechanistic water flow and storage model describing the hydraulic functioning of *Quercus ilex* trees during the wet season when stem growth occurred.

	$R_s$	$R_x$	$C_s$
	(MPa h g <sup>-1</sup> )	(MPa h g <sup>-1</sup> )	(kg MPa <sup>-1</sup> m <sup>-3</sup> )
	10 <sup>-2</sup>	10 <sup>-4</sup>	
TREE1	0.97	4.59 [4.59 - 4.59]	64.02 [54.66 – 72.22]
TREE2	1.41	5.72 [5.72 -5.72]	50.06 [45.03 – 54.79]
TREE3	0.93	4.59 [4.59 -4.59]	86.07 [73.33 – 98.28]
TREE4	0.53	3.65 [3.62 -3.69]	61.38 [54.98 – 66.94]
TREE5 <sup>1</sup>	NA	NA	NA
TREE6	1.15	6.49 [6.38 – 6.62]	46.91 [41.67 – 52.69]
mean	1.00	5.01	61.69
(SE)	(0.13)	(0.45)	(6.30)

$R_s$  was assumed constant whereas  $R_x$  and  $C_s$  were allowed to vary with predawn water potential (Eqn. 1 and Eqn. 4, respectively). The modelled period lasted 3-4 days and included DOY176, when leaf water potential was measured. Mean values of  $R_x$  and  $C_s$  on a storage volume basis are shown; minimum and maximum values for this period are indicated in square brackets.

<sup>1</sup> TREE5 was not modelled due to an inconsistent dendrometer signal.

**Table 3.** Calibrated parameters used in the mechanistic water flow and storage model describing the hydraulic functioning of *Quercus ilex* trees during summer drought when stem growth was impeded. The hydraulic resistance ( $R_x$ ) and hydraulic capacitance ( $C_s$ ) were allowed to vary with predawn water potential ( $\Psi_{PD}$ ). Estimates of  $C_s$  and the  $\Psi_{50}$  from the vulnerability curve were obtained from model simulations.

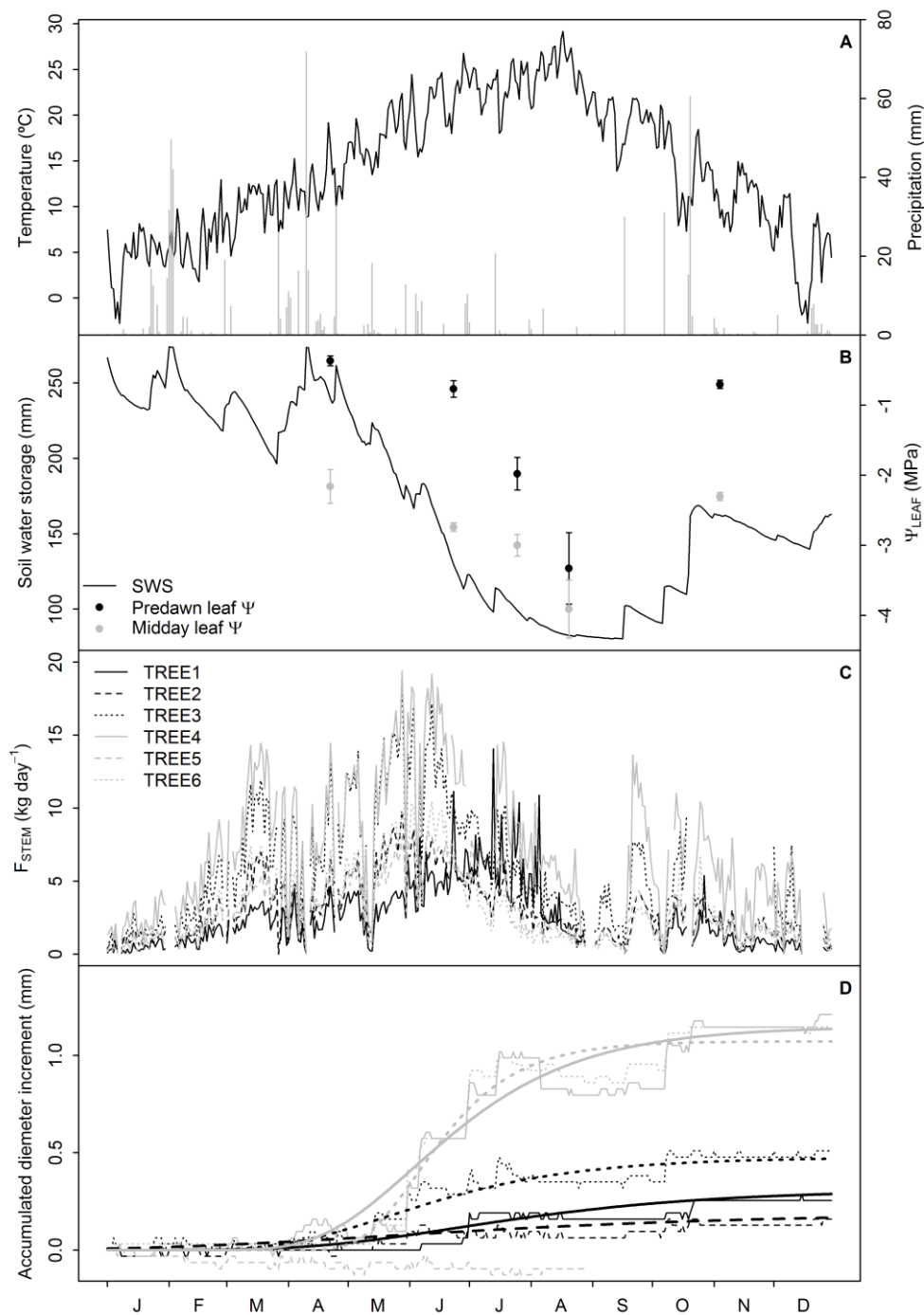
	Calibrated parameters <sup>1</sup>					C <sub>s</sub> <sup>2</sup>		Ψ <sub>50</sub> <sup>3</sup>
	R <sub>s</sub>	c <sub>1</sub>	c <sub>2</sub>	r <sub>1</sub>	r <sub>2</sub>	DOY208	DOY234	
	(MPa h g <sup>-1</sup> )	(g <sup>-1</sup> )	(MPa g <sup>-1</sup> )	(MPa h g <sup>-1</sup> )	(MPa <sup>-2</sup> )	(kg MPa <sup>-1</sup> m <sup>-3</sup> )		
	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-3</sup>	10 <sup>-3</sup>	10 <sup>-1</sup>			
TREE1	1.95	-3.07	2.68	0.99	1.29	26.52	18.48	-2.32
TREE2	1.73	-2.54	2.01	1.68	0.67	28.91	19.84	-3.21
TREE3	2.30	-2.33	2.78	1.26	1.53	31.75	23.34	-2.13
TREE4	0.53	-0.73	1.11	0.77	0.91	56.30	45.30	-2.76
TREE5	1.31	-2.99	1.20	3.15	1.55	37.43	22.66	-2.12
TREE6	1.88	-2.28	2.34	3.67	0.58	28.04	19.96	-3.46
mean	1.62	-2.32	2.02	1.92	1.09	34.82	24.93	-2.67
(SE)	(0.25)	(0.35)	(0.29)	(0.49)	(0.17)	(4.57)	(4.14)	(0.23)

<sup>1</sup> Five parameters were calibrated: the proportionality parameters  $c_1$  and  $c_2$  defining  $C_s$  (Eqn. 4), the proportionality parameters  $r_1$  and  $r_2$  defining  $R_x$  (Eqn. 1), and the radial hydraulic resistance between the xylem and the storage compartment ( $R_s$ ).

<sup>2</sup>  $C_s$  was estimated for two dates when  $\Psi_{PD}$  was measured (DOYs 208 and 234) and calculated on a storage volume basis.

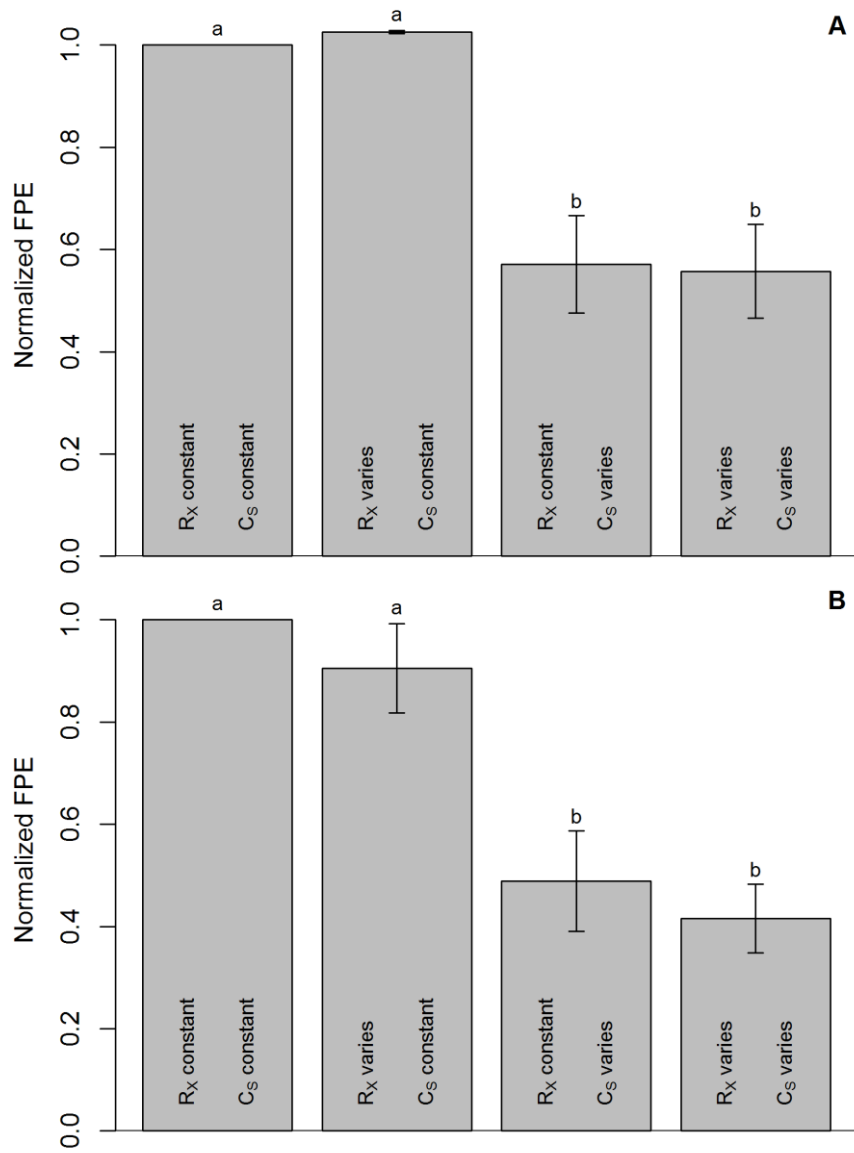
<sup>3</sup>  $\Psi_{50}$  is the  $\Psi_{PD}$  causing 50% loss of maximum hydraulic conductance along the integrated root-to-leaf continuum.

**Figure 1.** Daily air temperature and precipitation (A), soil water storage and predawn and midday leaf water potential (means  $\pm$  SE; B), daily sap flow (C), and daily stem diameter variations (D) during the year 2009. Ecophysiological measurements were performed on six *Quercus ilex* trees of the experimental site.



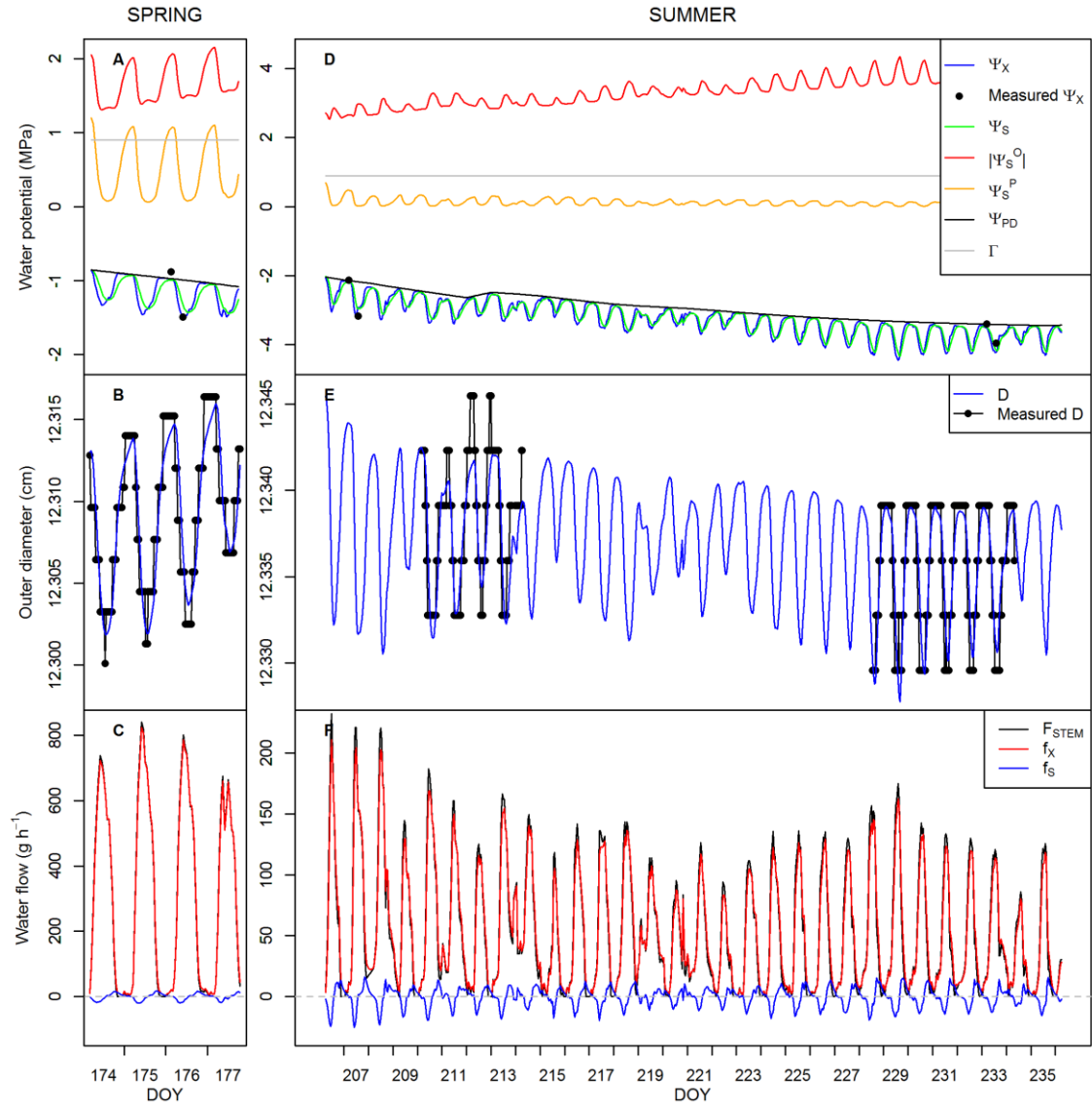
Curves of accumulated diameter increment were smoothed according to the Gompertz's equation (dashed lines). Diel variations in diameter are not shown for clarity.

**Figure 2.** Normalized final prediction error (FPE) of four tested models with constant or variable hydraulic resistance ( $R_x$ ) and capacitance ( $C_s$ ) to compare model performance. Models were used to simulate hydraulic functioning of *Quercus ilex* trees under wet (A) and dry (B) conditions.



The FPE evaluates the accuracy of simulated diameter variations ( $\Delta D$ ) and xylem water potential ( $\Psi_x$ ) and penalizes over-parameterized models. Low FPE values indicate better model performance. Values of FPE were normalized relative to the model with constant  $R_x$  and  $C_s$  to remove inter-stem variability associated to the number of observations. Normalized FPE was averaged among six trees. Different letters show significant differences ( $P < 0.05$ ).

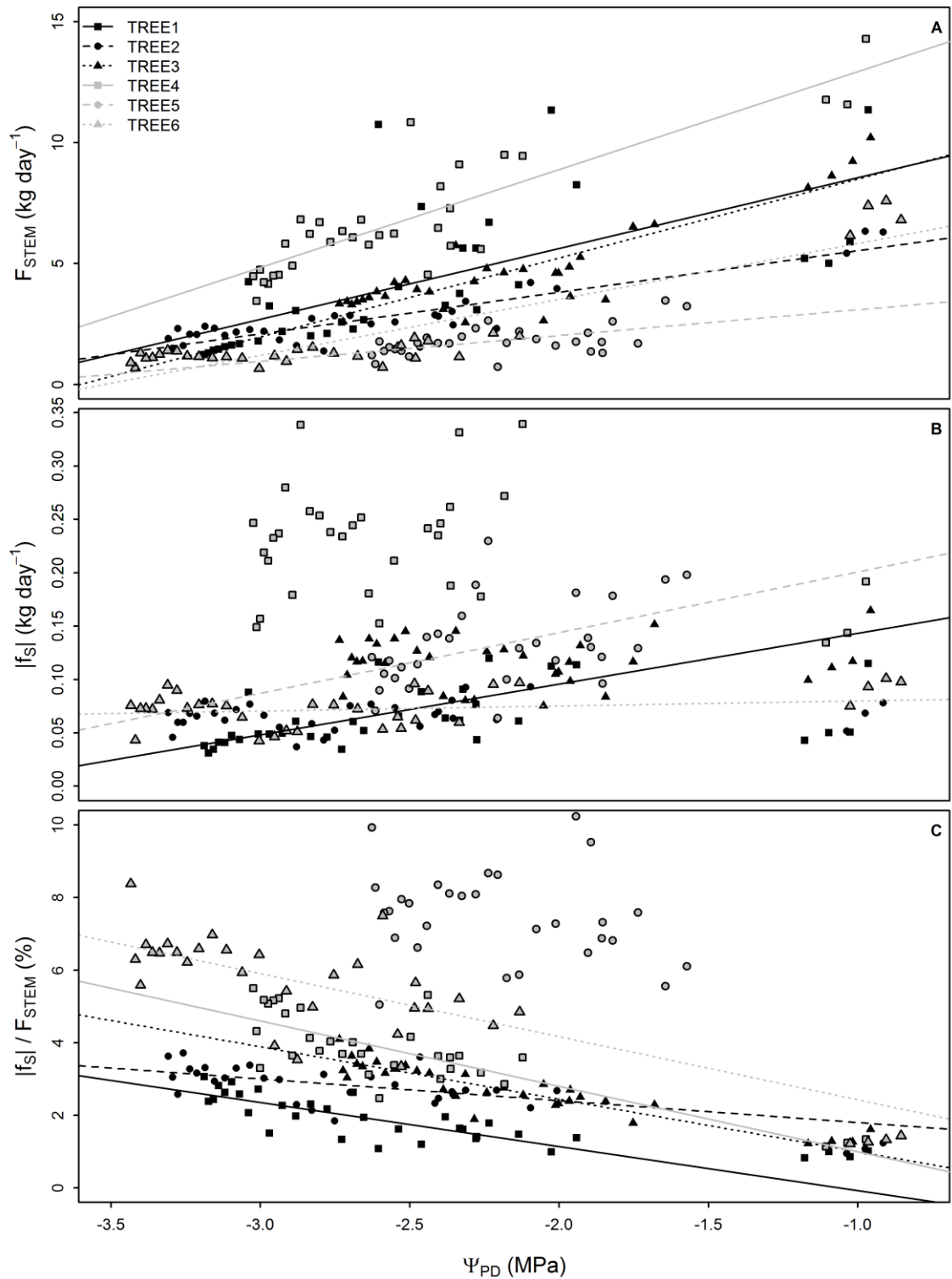
**Figure 3.** Measured and simulated water potentials (A, D), diameter variations (B, E) and water flows (C, F) in *Quercus ilex* trees during the wet (A-C) and the dry (D-F) season. Predawn water potential ( $\Psi_{PD}$ ) and sap flow ( $F_{STEM}$ ) (continuous black lines) were used as model inputs, whereas diameter variations and xylem water potential ( $\Psi_X$ ) (black dots) were used for calibration purposes.



This figure displays an example of model simulation for a single tree (TREE6) when the hydraulic resistance and hydraulic capacitance were allowed to vary with predawn water potential (best model obtained). Under well-watered conditions, cell turgor ( $\Psi_S^P$ ) at night-time exceeded the critical value for wall-yielding ( $\Gamma$ ) (A) resulting in irreversible stem growth (B). During summer drought,  $\Psi_S^P$  never exceeded  $\Gamma$  (D) and irreversible stem growth was impeded (E). The storage compartment supplied water to the xylem to fulfill transpiration requirements during the morning (negative  $f_S$ ) and was refilled during the afternoon (positive  $f_S$ ) (C, F). Radial water exchange caused diel patterns of stem shrinkage and swelling. Note different scales for the same variables in wet and dry periods. Note that absolute values of osmotic water potential ( $\Psi_S^O$ ) are displayed for clarity.

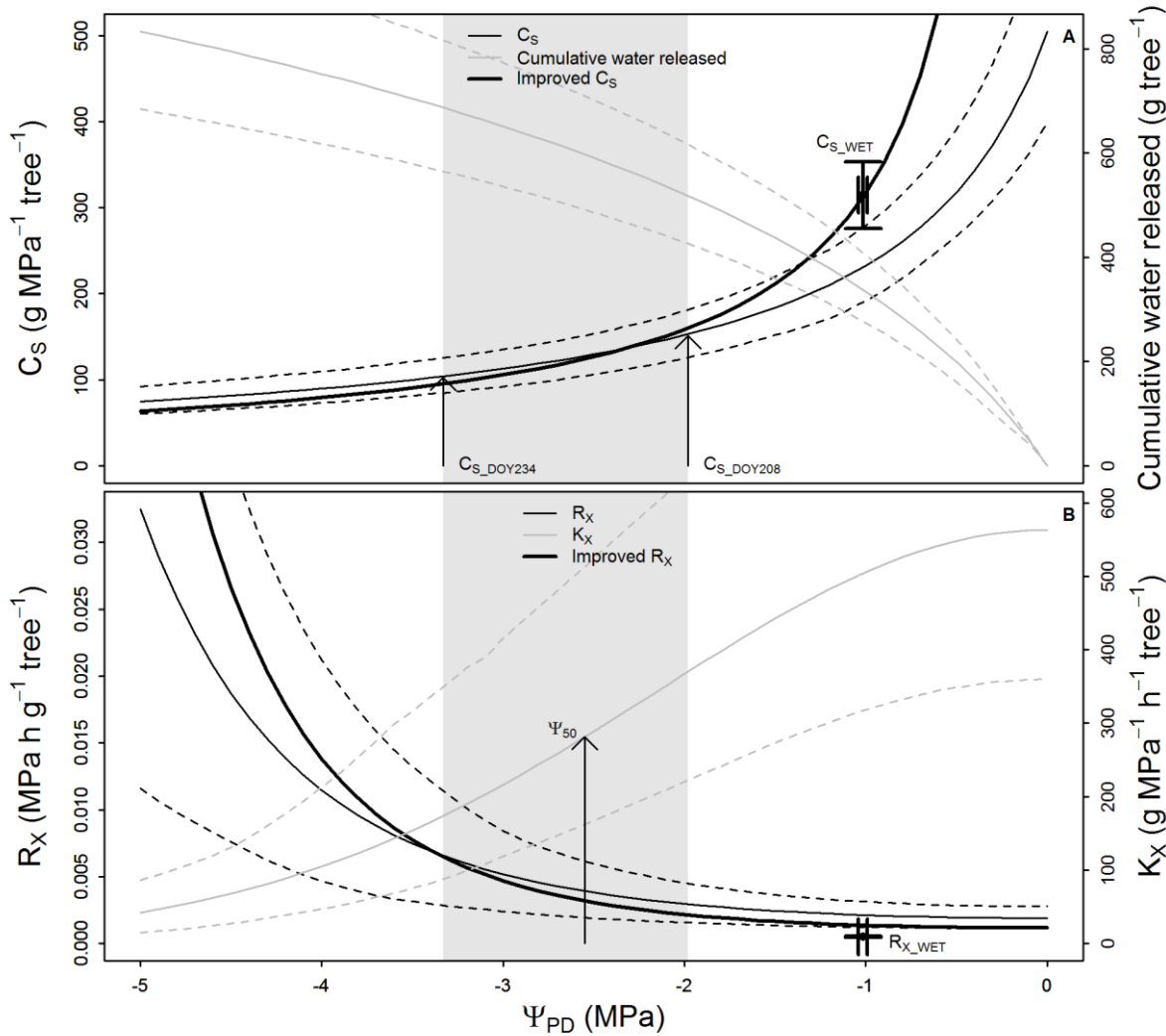


**Figure 4.** Variation in daily sap flow (A;  $F_{STEM}$ ), daily stem water release (B;  $|f_s|$ ), and the contribution of  $|f_s|$  to  $F_{STEM}$  (C) in *Quercus ilex* trees along a gradient of predawn water potential ( $\Psi_{PD}$ ).



Data from model simulations in early summer (wet period) and summer drought (dry period) are pooled. Only significant regressions are depicted ( $P < 0.05$ ).

**Figure 5.** Hydraulic capacitance ( $C_s$ ) and cumulative water released at the tree level (A), and hydraulic resistance ( $R_x$ ) and conductance ( $K_x$ ) in the root-to-leaf continuum (B) along a gradient of predawn water potential ( $\Psi_{PD}$ ).



Calibrated parameters obtained from six *Quercus ilex* trees during the dry period (Table 3) were used to estimate the mean (continuous line) and 95<sup>th</sup> and 5<sup>th</sup> percentile (dashed lines) curves of each variable. The calibrated parameters were obtained from a mechanistic model in which  $R_x$  (Eqn. 1) and  $C_s$  (Eqn. 4) were allowed to vary with  $\Psi_{PD}$ . Shaded areas indicate the modelled dry period, when average  $\Psi_{PD}$  ranged between -1.98 and -3.33 MPa. The curves of  $C_s$  and  $R_x$  adjusted for long-term (one-month) simulations of the dry period (in black) were improved by including values of the short-term (3-4 days) calibrations of the wet period (in red).

**Figure 6.** Stem water refilling (A) and hydraulic capacitance ( $C_s$ ) (B) at the tree level estimated after the first heavy rain following the dry season. Two rain events registered in 2006 and 2007 (open and closed circles, respectively) met the minimum requirements to estimate stem storage capacity. Stem water refilling and  $C_s$  were estimated for a total of 23 *Quercus ilex* trees ranging in diameter at breast height (DBH) from 4 to 12.1 cm. Stem water refilling and  $C_s$  were exponentially related to tree DBH (Stem water refilling =  $30.27 \times e^{(0.34 \times \text{DBH})}$ ;  $C_s = 15.05 \times e^{(0.31 \times \text{DBH})}$ ;  $P < 0.001$ ). Shaded areas indicate the range in DBH of trees additionally surveyed for modelling.

