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RESEARCH PAPER

# Below-ground hydraulic constraints during drought-induced decline in Scots pine

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## Abstract

• ***Key message*** **Below-crown hydraulic resistance, a proxy for below-ground hydraulic resistance, increased during drought in Scots pine, but larger increases were not associated to drought-induced defoliation. Accounting for variable below-ground hydraulic conductance in response to drought may be needed for accurate predictions of forest water fluxes and drought responses in xeric forests.**

• ***Context*** Hydraulic deterioration is an important trigger of drought-induced tree mortality. However, the role of below-ground hydraulic constraints remains largely unknown.

• ***Aims*** We investigated the association between drought-induced defoliation and seasonal dynamics of below-crown hydraulic resistance (a proxy for below-ground hydraulic resistance), associated to variations in water supply and demand in a field population of Scots pine (*Pinus sylvestris* L.)

• ***Methods*** Below-crown hydraulic resistance ($r_{bc}$) of defoliated and non-defoliated pines was obtained from the relationship between maximum leaf-specific sap flow rates and maximum stem pressure difference estimated from xylem radius variations. The percent contribution of $r_{bc}$ to whole-tree hydraulic resistance ($\%r_{bc}$) was calculated by comparing stem water potential variations with the water potential difference between the leaves and the soil.

• ***Results*** $r_{bc}$ and $\%r_{bc}$ increased with drought in both defoliated and non-defoliated pines. However, non-defoliated trees showed larger increases in $r_{bc}$ between spring and summer. The difference between defoliation classes is unexplained by differences in root embolism, and it is possibly related to seasonal changes in other properties of the roots and the soil-root interface.

• ***Conclusion*** Our results highlight the importance of increasing below-ground hydraulic constraints during summer drought but do not clearly link drought-induced defoliation with severe below-ground hydraulic impairment in Scots pine.

**Keywords** Drought · Forest mortality · Hydraulic resistance · Rhizosphere · Roots · Xylem pressure

## 1 Introduction

Drought is a major driver of the composition, function and dynamics of forests worldwide (Allen et al. 2015). Anthropogenic global warming has amplified drought and heat stress conditions in the last decades (Trenberth et al. 2014), increasing background mortality rates and triggering widespread tree die-off episodes after extreme droughts (Allen et al. 2010; Young et al. 2017), which may cause profound changes in ecosystem structure and function (Anderegg et al. 2013). Over a decade of research on the physiological mechanisms of drought-induced tree mortality, McDowell et al. (2008), McDowell (2011) and Sala et al. (2010) has revealed that drought-exposed trees rarely die of a single cause (hydraulic failure, carbon starvation or phloem impairment) (Mencuccini et al. 2015; Sevanto et al. 2014), precluding

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robust predictions of vegetation mortality. Although hydraulic traits may be good predictors of drought vulnerability (Anderegg et al. 2015, 2016) and hydraulic failure is usually involved in drought-induced tree death processes (Adams et al. 2017), lethal physiological thresholds have proved difficult to determine. These thresholds are organ-specific (Bartlett et al. 2016), and it is still unclear which part of the plant's hydraulic pathway is more vulnerable to hydraulic failure during extreme drought under field conditions (Anderegg et al. 2014; Bartlett et al. 2016).

Our understanding of hydraulic failure during extreme drought is largely based on processes occurring in above-ground organs, mostly stems. For example, a recent synthesis of experiments on physiological mechanisms of drought-induced mortality only included stem hydraulics (Adams et al. 2017). However, below-ground hydraulic processes may have a strong influence on whole-tree hydraulic dynamics and tree survival because (1) spatial patterns of die-off are often associated with variability in soil conditions (Lloret et al. 2004; Peterman et al. 2012; Vilà-Cabrera et al. 2013; but see Dorman et al. 2015) or rooting depth patterns across species (Nardini et al. 2016), (2) roots are usually more vulnerable to embolism than stems (Hacke et al. 2000; Martínez-Vilalta et al. 2002; Johnson et al. 2016) and (3) incorporating topographically mediated soil moisture often improves prediction of drought-induced tree mortality (Tai et al. 2016).

Below-ground hydraulic resistance is typically around half of whole-tree hydraulic resistance (Irvine and Grace 1997; Running 1980), but its response to evaporative demand and water supply is complex and remains poorly studied. Increasing evaporative demand can reduce below-ground (Martínez-Vilalta et al. 2007) and fine-root hydraulic resistances (McElrone et al. 2007) of trees. On the other hand, below-ground hydraulic resistance can become the main bottleneck during edaphic drought as a result of root embolism (Domec et al. 2009) or reductions in the hydraulic conductance of the soil-root interface, for example following hydraulic disconnection from the soil (Sperry et al. 2002). This latter process has been suggested to occur in dying pines under extreme drought (Plaut et al. 2012). Nevertheless, it is not known whether roots and the rhizosphere constitute the main hydraulic bottleneck during drought-induced mortality processes.

Drought-induced mortality in Scots pine (*Pinus sylvestris* L.) is typically preceded by long-term growth declines lasting decades and by years of canopy defoliation (Galiano et al. 2011; Hereş et al. 2012). This defoliated stage is associated with severely limited gas exchange and depleted carbohydrate reserves (Aguadé et al. 2015a, b; Galiano et al. 2011; Poyatos et al. 2013). Compared to non-defoliated pines, defoliated individuals show a steeper decline of whole-plant hydraulic conductance, slightly lower predawn leaf water potentials (Poyatos et al. 2013; Salmon et al. 2015) and steeper vulnerability to embolism in roots (Aguadé et al. 2015a). This collective evidence suggests that impaired below-ground functioning may be associated to the mortality process.

Assuming steady-state conditions, xylem radius variations, combined with sap flow and leaf water potential measurements, can be used to estimate the seasonal dynamics of hydraulic conductance in different components of the plant's hydraulic pathway (Martínez-Vilalta et al. 2007). Here, we investigated the association between defoliation and drought responses of below-ground hydraulic resistance in a Scots pine population affected by drought-induced mortality (Hereş et al. 2012; Martínez-Vilalta and Piñol 2002). Xylem radius variations were measured below the living crown, assuming that bole resistance does not vary seasonally and that the dynamics of below-crown hydraulic resistance ($r_{\mathrm{bc}}$) are dominated by the below-ground component (Domec et al. 2009). Our main objective was to estimate $r_{\mathrm{bc}}$ and its contribution to whole-tree hydraulic resistance and to explore their seasonal dynamics in declining Scots pines. We hypothesised that (1) both $r_{\mathrm{bc}}$ and its contribution to whole-tree hydraulic resistance ($\%r_{\mathrm{bc}}$) will increase in summer in all trees studied due to the higher vulnerability to embolism of roots and the increase in resistance at the soil-root interface, and (2) the summer increase in $r_{\mathrm{bc}}$ will be more pronounced in defoliated pines.

Part of the results in this paper belongs to the PhD thesis by David Aguadé (Aguadé Vidal 2016).

## 2 Materials and methods

### 2.1 Study site

Measurements were conducted in Tillar Valley within the Poblet Forest Natural Reserve (Prades Mountains, Northeastern Iberian Peninsula). The climate is Mediterranean, with a mean annual rainfall of 664 mm (spring and autumn being the rainiest seasons and with a marked summer dry period) and moderately warm temperatures (11.3 °C on average) (Poyatos et al. 2013). The substrate is fractured schist, and soils are rocky Xerochrepts, relatively shallow (~ 40 cm deep), with a clay loam texture (Barba et al. 2016). The study area has a predominantly northern aspect and steep slopes (35° on average).

The forest studied (41° 19′ 58.05″ N, 1° 0′ 52.26″ E; 1015 m asl) consists of a mixed holm oak (*Quercus ilex* L.)—Scots pine stand; the canopy is dominated by pines whereas oaks dominate the understorey (Poyatos et al. 2013). As a consequence of several drought-induced mortality episodes since the 1990s (Hereş et al. 2012; Martínez-Vilalta and Piñol 2002), Scots pine average standing mortality and crown defoliation are currently 12% and 52%, respectively. However, in some parts of the forest, standing mortality is > 20% and cumulative mortality is as high as 50% in the last

20 years (J. Martínez-Vilalta, unpublished). The Scots pine population studied is more than 150 years old and has remained largely unmanaged for at least 30 years (Hereş et al. 2012). No major insect infestation episode was detected that could explain the forest decline in the area (Mariano Rojo, Catalan Forest Service, personal communication).

## 2.2 Experimental design

Between 2010 and 2013, several physiological variables were measured in defoliated and non-defoliated Scots pine trees growing together in the same population (Aguadé et al. 2015a, b; Poyatos et al. 2013; Salmon et al. 2015). In addition, we also measured above-canopy meteorology and soil moisture in the top 30 cm using a frequency domain reflectometer (cf. Poyatos et al. 2013, for additional details). Defoliation was visually estimated relative to a completely healthy tree in the same population. A tree was considered as non-defoliated if the percentage of green needles was > = 80% and defoliated if the percentage of green needles was < = 50%. Here, we use the complete set of monitored trees to show the general pattern in the seasonal dynamics of sap flow for defoliated and non-defoliated trees, but we then focus on a subset of four defoliated and four non-defoliated co-occurring Scots pine trees in which sap flow, xylem radius variations and leaf water potentials were measured concurrently between August 2011 and November 2012 (Table 3 in the Appendix).

## 2.3 Sap flow measurements

Measurements of sap flow density were conducted at 15-min intervals using constant heat dissipation sensors (Granier 1985). The probes were inserted radially at breast height into the xylem after removing the bark and covered with a reflective material to avoid solar radiation. Two sensors (north- and south-facing side of the trunk) were placed in each tree and averaged to account for an azimuthal variation of sap flow within the trunk. Data processing of heat dissipation sensor output included correction for natural temperature gradients in the stem, and zero flow determination was based only on nights with low and stable evaporative demand (Poyatos et al. 2013). Sap flow density calculation in the outer sapwood followed the original calibration (Granier 1985) and was then integrated over the entire sapwood using radial profiles of sap flow (six depths) measured in three trees using the heat field deformation method (Nadezhdina 2018), during at least 7 days per tree. This sap flow per unit sapwood area was expressed on a leaf area basis ($J_L$) after estimation of tree leaf area using site-specific allometries for Scots pine and accounting for seasonal variations of leaf area (see Poyatos et al. 2013 for additional methodological details).

## 2.4 Water potential measurements

Predawn ($\Psi_{PD}$, MPa; just before sunrise, 0300–0500 hours, solar time) and midday ($\Psi_{MD}$, MPa; 1100–1300 hours, solar time) leaf water potentials were measured once per month in June, July, August and November in 2012. On each sampling time, a sun-exposed twig from each tree was excised using a pruning pole and stored immediately inside a plastic bag with a moist paper towel to avoid water loss until measurement time, typically within 2 h of sampling. Leaf water potentials were measured using a pressure chamber (PMS Instruments, Corvallis, OR, USA).

## 2.5 Xylem radius variations

Xylem radius variations were measured throughout the study period using point dendrometers consisting of linear variable displacement transducers (LVDTs; DG 2.5 Solartron Metrology, West Sussex, UK). LVDTs were mounted on metal frames following the design by Sevanto et al. (2005b) with some modifications. Briefly, the frame was held around the bole, just below the living tree crown, by two metal plates that were screwed ca. 20 cm above the measuring point. The frame was made of aluminium, except for the two rods parallel to the direction of the measured radius changes, which were made of Invar, a nickel-iron alloy with a low thermal expansion coefficient. Each frame held two LVDT sensors, placed ca. 30 mm apart from each other: one measured over bark radius changes (not used here) and the other one measured xylem radius changes (i.e. the sensor measuring tip was in contact with the xylem, since bark, phloem and cambium were removed before the installation). We used xylem radius changes because they have been successfully used to estimate stem water potentials in Scots pine (Irvine and Grace 1997; Martínez-Vilalta et al. 2007) and because they are more suitable to monitor changes in water potential than whole-stem radius changes (Offenthaler et al. 2001). LVDT measurements were taken every 30 s, and the average values were stored every 15 min in a datalogger (CR1000, Campbell Scientific, Inc., Logan, USA). Since natural expansion and contraction of the metal frame and sapwood occurs due to changes in air temperature, frame and sapwood temperatures were measured using thermocouples. We used these temperatures to account for thermal expansion of the frame and the sapwood using the linear expansion coefficients for the Invar rods ($1.2 \times 10^{-6}$ K$^{-1}$) and for Scots pine wood ($7.9 \times 10^{-6}$ K$^{-1}$; Sevanto et al. 2005a).

## 2.6 Estimation of stem water potential

We used within-day xylem radius variations to estimate changes in below-crown stem water potential. We first calculated the difference between the maximum stem radius within a day, which corresponds to the minimum shrinkage, and the instantaneous measurements of the xylem radius. This below-crown xylem radius difference ($\Delta R_{bc}$, mm) quantifies the instantaneous shrinkage compared to maximum swelling conditions within a day. We then used $\Delta R_{bc}$ to calculate the corresponding water pressure difference ($\Delta P_{bc}$, MPa) by dividing $\Delta R_{bc}$ by the sapwood depth ($R_{bc,sw}$, mm) and multiplying it by the radial modulus of elasticity of wood ($E_r$, MPa) following the equation (Irvine and Grace 1997)

$$\Delta P_{bc} = E_r \frac{\Delta R_{bc}}{R_{bc,sw}} \tag{1}$$

We can assume that the osmotic potential in xylem sap is very low and relatively constant over time, and therefore, water pressure in the xylem can be used to estimate xylem water potential (Irvine and Grace 1997). $\Delta P_{bc}$ is thus equivalent to the difference between water potential at the LVDT location (under the living crown) and the soil, assuming that the xylem water potential under conditions of maximum swelling was in equilibrium with the soil.

We did not measure $E_r$ for the trees in our study. A literature survey showed that values of $E_r$ for Scots pine are variable across trees and populations, with reported average values ranging between 150 and 750 MPa (Irvine and Grace 1997; Mencuccini et al. 1997; Perämäki et al. 2001). Given this variability and the fact that applying literature values of $E_r$ to estimate xylem pressure dynamics can be problematic (Sevanto et al. 2008), we applied a value of $E_r$ (180 MPa) so that the maximum percentage of below-crown resistance to whole-tree hydraulic resistance ($\%r_{bc}$) observed during summer (see next section) was 100%. This assumption is justified on the grounds that, under extreme drought conditions, it is expected that most of the hydraulic resistance is found below ground (Domec et al. 2009; Duursma et al. 2008; Sperry et al. 2002). While this assumption should not affect the relative values of $\%r_{bc}$ over time or between trees, the absolute $\%r_{bc}$ values reported here are subject to high uncertainty and have to be considered with caution.

Sapwood radius was measured below the living crown, at each LVDT location. We extracted wood cores with a Pressler borer, put them in paper bags and stored them in a portable cooler. Samples were then coloured with bromocresol green in the laboratory to distinguish between sapwood and heartwood and measure their length.

## 2.7 Below-crown hydraulic resistance

We used the maximum daily values of sap flow per unit leaf area ($J_{L,max}$, kg m$^{-2}$ s$^{-1}$) and stem pressure difference ($\Delta P_{bc,max}$, MPa) to calculate the below-crown hydraulic resistance ($r_{bc}$, MPa kg$^{-1}$ m$^2$ s)

$$r_{bc} = \frac{\Delta P_{bc,max}}{J_{L,max}} \tag{2}$$

The percent contribution of below-crown resistance to whole-tree hydraulic resistance ($\%r_{bc}$) was calculated by comparing the water pressure difference ($\Delta P_{bc}$) measured with LVDTs and the leaf water potential difference ($\Delta\psi$; $\psi_{MD}$ minus $\psi_{PD}$) when both measurements were taken on the same day (Irvine and Grace 1997)

$$\%r_{bc} = \frac{\Delta P_{bc}}{\Delta\psi} \cdot 100 \tag{3}$$

We assumed that pines reached equilibrium with the soil during night, and as a consequence, $\psi_{PD}$ is an estimate of soil water potential (Irvine et al. 2004), which corresponded to the maximum stem radius within a day. Two pieces of evidence suggest that this assumption was not critical in our case (comparison of temporal dynamics and drought responses between defoliation classes). First, we quantified night-time sap flow per unit leaf area ($J_{L,night}$, global radiation $< 5$ W m$^{-2}$) for the winter and spring periods only and expressed it as a percentage of daytime flow; the calculations for summer were not used because daytime flows were very low and dividing two low flows sometimes yielded unreasonable values. Median values of percentage $J_{L,night}$ were 10% for defoliated and 6% for non-defoliated trees and did not differ between classes ($P = 0.182$). Second, we modelled $r_{bc}$ as a function of $J_{L,night}$ interacting with defoliation class, and no effect of either $J_{L,night}$ ($P = 0.454$) or its interaction with defoliation class ($P = 0.389$) was detected.

## 2.8 Data analysis

The analyses of $\Delta P_{bc}$, $r_{bc}$ and $J_L$ need sap flow and xylem radius variations data measured simultaneously. However, due to technical problems mostly related to power failures due to the remote location of the sampling site, these data were not always available. We selected representative periods with maximum data availability within three different seasons of year 2012 showing contrasted characteristics of soil water content: winter (from day 53 to day 78), spring (days 145–164) and summer (days 224–248). Note that for these periods, we did not always have usable data from the four trees per defoliation class.

Linear mixed-effects models were fitted to test for statistical differences between defoliation classes (defoliated or non-defoliated) and season (winter, spring or summer) on $r_{bc}$, $\%r_{bc}$, $\psi_{PD}$, $\psi_{MD}$ and $\Delta\psi$. Measurements of $\psi$ in June and August were considered as spring and summer seasons, respectively. Winter measurements of $\psi$ were not available, and thus, models for $\%r_{bc}$ and $\psi$ did not include a winter season. To test the combined effects of vapour pressure deficit (VPD), soil water content (SWC) and defoliation class on $\Delta P_{bc}$ and $r_{bc}$, we fitted similar linear mixed-effects models including a quadratic term in the case of the relationships with VPD or log-transforming SWC, in order to account for non-linear responses. In all mixed-effects models, tree identity was included as a random factor. $r_{bc}$ and $\Delta P_{bc}$ were log transformed, and $\%r_{bc}$ was square root transformed to achieve normality prior to all analyses. In all cases, we started by fitting the most complex, biologically plausible model and then this, and all the alternative models resulting from different combinations of explanatory variables, were ranked from lowest to highest Akaike information criterion corrected (AICc) for small sample sizes. When multiple models minimising AICc lied within 2 AICc units, likelihood ratio tests were performed to select the best-performing model. All statistical analyses were carried out using the R Statistical software, version 3.3.1 (R Core Team 2016), using the lme function to fit mixed-effects models (Pinheiro et al. 2018), and the dredge function (MuMIn package) to compare models (Bartoń 2017).

# 3 Results

## 3.1 Seasonal course of sap flow and environmental conditions

The values of sap flow per unit leaf area ($J_L$) measured in all defoliated and non-defoliated Scots pine trees from our whole study population were higher in defoliated trees relative to non-defoliated ones throughout the study period, especially in late spring and early summer (Fig. 1). With the beginning of summer drought, $J_L$ strongly decreased in both defoliated and non-defoliated pines, though this response was more acute in the former. Winter, spring and summer periods selected in Fig. 1 and used in subsequent analyses are representative of different values of $J_L$ and environmental conditions in year 2012. Summer drought conditions in 2012 were intense in terms of both evaporative demand and soil water supply, with maximum VPD of 3.86 kPa and minimum SWC of 0.08 $m^3\ m^{-3}$ (Fig. 2c, f). In fact, 2012 was the second driest growing season since 1951 in the study area (133.9 mm of rainfall between May and October).

## 3.2 Seasonal courses of sap flow, leaf water potentials and stem pressure difference in selected trees

Sap flow per unit leaf area ($J_L$) for the selected trees ($N = 4$; see Section 2) has the same patterns as the full set of measured trees (Fig. 1), with higher $J_L$ in defoliated trees over all periods, and an acute reduction of $J_L$ in both defoliation classes in summer (Figs. 1 and 2g–i). The temporal dynamics of $\Delta P_{bc}$ generally followed the course of $J_L$, and $\Delta P_{bc}$ was higher in defoliated pines over winter and spring (Fig. 2j, k). However, defoliated and non-defoliated Scots pines showed similar $\Delta P_{bc}$ values in summer (Fig. 2l).

Midday leaf water potential ($\Psi_{PD}$) was similar between defoliation classes but significantly different between seasons, with lower values in summer (Fig. 3, Table 1). In contrast, $\Psi_{MD}$ showed differences between defoliation classes, with lower values for defoliated trees, but no seasonal effect (Fig. 3, Table 1). On the other hand, the interaction between season and defoliation was significant for $\Delta\psi$, indicating that the water potential difference was highest for defoliated trees in spring and similarly low for all the other combinations of season and defoliation class (Fig. 3, Table 1).

## 3.3 Seasonal course of below-crown hydraulic resistance and contribution to whole-tree resistance

Below-crown hydraulic resistance ($r_{bc}$) increased from winter to summer for both defoliation classes (Fig. 4a–c, Table 2). Although defoliated and non-defoliated Scots pine trees presented similar levels of $r_{bc}$ in winter and spring, the summer increase in $r_{bc}$ was significantly larger for non-defoliated Scots pine trees (Fig. 4, Table 2). The percent contribution of below-crown hydraulic resistance to whole-tree hydraulic resistance ($\%r_{bc}$) increased in summer for both defoliated and non-defoliated Scots pine trees relative to spring (Fig. 4d, e, Table 2), but we did not find any effect of defoliation class on the seasonal dynamics of $\%r_{bc}$ (Fig. 4d, e, Table 2).

## 3.4 Responses of stem pressure difference and below-crown hydraulic resistance to VPD and SWC

Soil water content interacted with VPD and defoliation class to determine $\Delta P_{bc}$ (Table 4 and Fig. 6 in the Appendix). The values of $\Delta P_{bc}$ were lower and less variable for non-defoliated pines. For defoliated pines, variation with environmental demand and soil water supply was more evident: high values of $\Delta P_{bc}$ were observed at high VPD and SWC (Fig. 6 in the Appendix). In contrast, we did not detect VPD effects on $r_{bc}$, which did decline with SWC, especially for non-defoliated pines (Fig. 5, Table 3 in the Appendix). Therefore, $r_{bc}$ in non-defoliated pines tended to be more sensitive to the depletion of soil water content (Table 4 in the Appendix), leading to higher $r_{bc}$ at low levels of SWC, compared to defoliated Scots pines (Fig. 5).

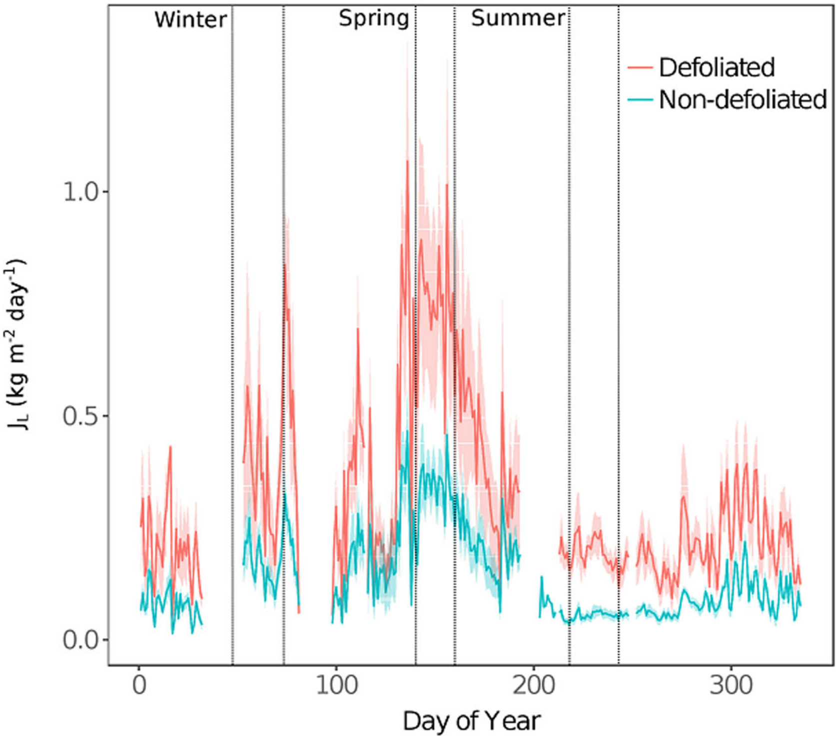


**Fig. 1** Seasonal course of daily-averaged sap flow per unit leaf area ($J_L$) over year 2012 in defoliated (red) and non-defoliated (blue) Scots pine trees. Error bars indicate + 1 SE. Periods selected for the present paper, representative of different seasons, are indicated by vertical bars

## 4 Discussion

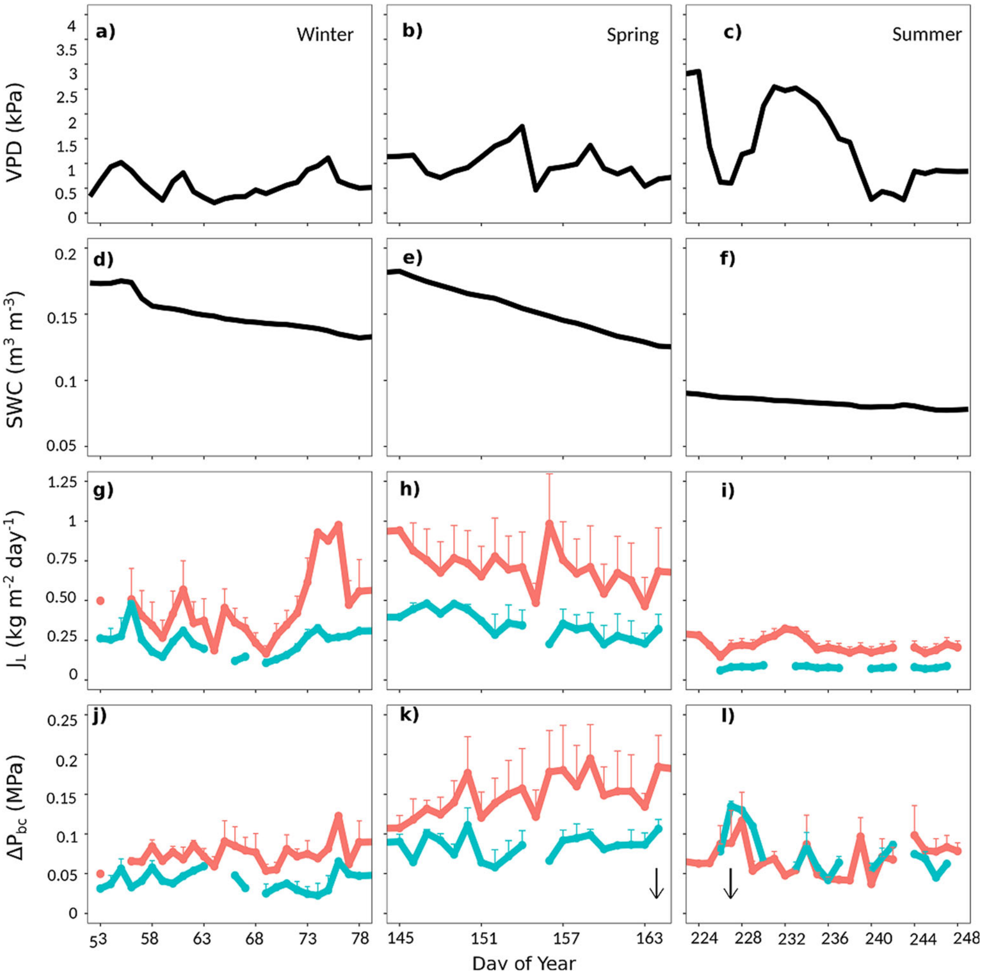
Our results are consistent with whole-plant responses of Scots pine suffering drought-induced decline and support an important role of below-ground hydraulic constraints during seasonal drought. The eight intensively measured trees in this study showed sap flow responses (Figs. 1 and 2) largely consistent with the patterns observed for the full set of sampled trees in 2012 and in the previous 2 years (Poyatos et al. 2013). As for leaf water potentials, lower $\psi_{\mathrm{MD}}$ was observed in defoliated trees but $\psi_{\mathrm{PD}}$ was similar between defoliation classes. Here, adding xylem radius variations to sap flow and leaf water potential measurements has allowed us to dig deeper into the factors driving these whole-tree drought responses, by describing the dynamic patterns of below-crown hydraulic resistance ($r_{\mathrm{bc}}$) associated to seasonal drought and defoliation. This approach for estimating $r_{\mathrm{bc}}$ is not new (Irvine and Grace 1997; Martínez-Vilalta et al. 2007) but has been used surprisingly little in the literature. It is based on several assumptions (overnight equilibration of plant and soil water potentials, constant modulus of elasticity and osmotic potential of xylem sap), and it is certainly not free of potential methodological issues (for instance, in our case, the high uncertainty in the estimation of the modulus of elasticity). However, it remains one of the easiest ways of obtaining reliable time series of below-ground (or below-crown) plant hydraulic resistance without having to resort to modelled estimates (Johnson et al. 2018).

### 4.1 Drought increases below-crown hydraulic resistance and its contribution to whole-tree resistance

As hypothesised, below-crown hydraulic resistance ($r_{\mathrm{bc}}$) increased in both defoliated and non-defoliated Scots pine trees as drought progressed (Fig. 4). In our study, $r_{\mathrm{bc}}$ includes the whole trunk, the root system and the rhizosphere, but we can assume that the trunk will probably contribute little to variations in below-crown hydraulic resistance, compared to the roots and the rhizosphere (Domec et al. 2009; Johnson et al. 2016; McCulloh et al. 2014). We observed increases in $r_{\mathrm{bc}}$ with declining soil water content, which were pronounced under very dry soils (Fig. 5), consistent with the decrease in root hydraulic conductance observed in other conifer species (Domec et al. 2009). In contrast, we did not find any effect of VPD on $r_{\mathrm{bc}}$. An increase in below-ground conductance (i.e. a decrease in resistance) with increasing evaporative demand was reported for a mesic Scots pine population (Martínez-Vilalta et al. 2007). Nevertheless, in the xeric population studied here, low values of SWC concur with high VPDs and, therefore, the effect of dry soils appears to override any influence of evaporative demand on $r_{\mathrm{bc}}$.

The increase in $r_{\mathrm{bc}}$ with seasonal drought can be related to several xylem and rhizosphere processes (Newman 1969; Sperry et al. 2002). Minimum $\psi_{\mathrm{PD}}$ values observed in our study corresponded to root embolism levels < 50% (cf. on-site root vulnerability curves reported in Aguadé et al. 2015a) and, thus, were unlikely to be the only (or main)

**Fig. 2** Seasonal course of (a–c) daily vapour pressure deficit (VPD), (d–f) soil water content (SWC), (g–i) daily-averaged sap flow per unit leaf area ($J_L$) and (j–l) stem pressure difference ($\Delta P_{bc}$) for the three seasons studied in 2012. $J_L$ and $\Delta P_{bc}$ are given for defoliated (red) and non-defoliated (blue) Scots pine trees. Error bars indicate + 1 SE. Arrows in the lower panel indicate sampling dates of leaf water potentials



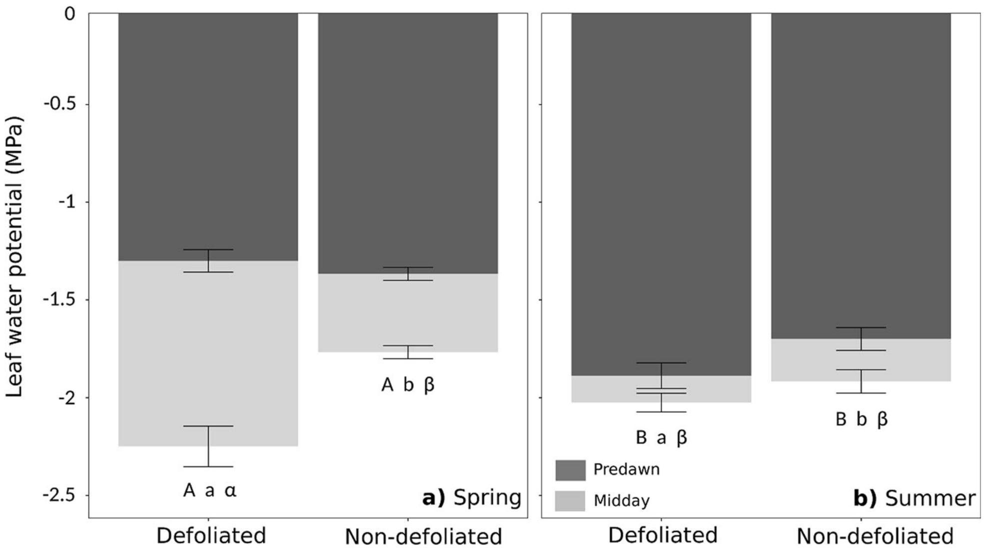


**Fig. 3** Daily averages of predawn ($\psi_{PD}$; dark grey) and midday ($\psi_{MD}$; light grey) leaf water potential measurements in defoliated and non-defoliated Scots pine trees in (a) spring and (b) summer of 2012. Different uppercase letters indicate significant differences ($P < 0.05$) between predawn measurements, different lowercase letters indicate significant differences between midday measurements and different Greek letters indicate significant differences in the water potential difference between predawn and midday ($\Delta\psi$) across defoliation classes and seasons. Error bars indicate ± 1 SE

**Table 1** Summary of the linear mixed-effects models with predawn ($\psi_{\mathrm{PD}}$), midday ($\psi_{\mathrm{MD}}$) leaf water potentials and their difference ($\Delta\psi$) as response variables

| Parameter | $\psi_{\mathrm{PD}}$ $R^2(m) = 0.82$ | $\psi_{\mathrm{MD}}$ $R^2(m) = 0.50$ | $\Delta\psi$ $R^2(m) = 0.87$ |
|---|---|---|---|
| Intercept | $-1.33 \pm 0.05$*** | $-2.14 \pm 0.06$*** | $-0.95 \pm 0.08$*** |
| Non-defoliated | ni | $0.30 \pm 0.09$* | $0.55 \pm 0.12$** |
| Summer | $-0.48 \pm 0.07$*** | ni | $0.81 \pm 0.11$*** |
| Non-defoliated: summer | ni | ni | $-0.63 \pm 0.17$* |

The coefficients indicate the difference between each level of a given variable and the corresponding reference level ('defoliated' for defoliation class and 'spring' for season). The values are the coefficient estimates ± 1 SE. Only marginal $R^2$, $R^2(m)$, is reported as the variance associated to the 'tree' random effect was estimated as near zero

*ni* not included in the model

*$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$

mechanism explaining increases in $r_{\mathrm{bc}}$ with drought, particularly in non-defoliated trees. Increased hydraulic resistance of the soil-root interface can be also caused by root shrinkage or suberisation (Brunner et al. 2015). Hydraulic disconnection from the soil reduces water loss from drying soils (North and Nobel 1997) and has been reported in pines under extreme drought, associated with anomalous $\psi_{\mathrm{PD}}$ (e.g. more negative than $\psi_{\mathrm{MD}}$ or soil $\psi$) (Plaut et al. 2012; Pangle et al. 2015) or with reduced sap flow responses to precipitation pulses (Plaut et al. 2013). In our study, $\psi_{\mathrm{PD}}$ was always less negative than $\psi_{\mathrm{MD}}$, but Scots pine sap flow recovered only partially after autumn rains (Fig. 1; see also Poyatos et al. 2013), which could indicate hydraulic isolation from the soil. Root hydraulic conductance during drought can also be constrained by suppressed root elongation rates below $\psi_{\mathrm{PD}} < -1$ MPa, as observed in other pine species (Zou et al. 2000), by decreased fine root production (Olesinski et al. 2011) or by fine root mortality (Gaul et al. 2008). The latter, in particular, could lead to the observed decrease in $r_{\mathrm{bc}}$ during drought.

We also showed that the percentage contribution of below-crown to whole-tree hydraulic resistance (%$r_{\mathrm{bc}}$) increases with severe drought in Scots pine (Fig. 4).

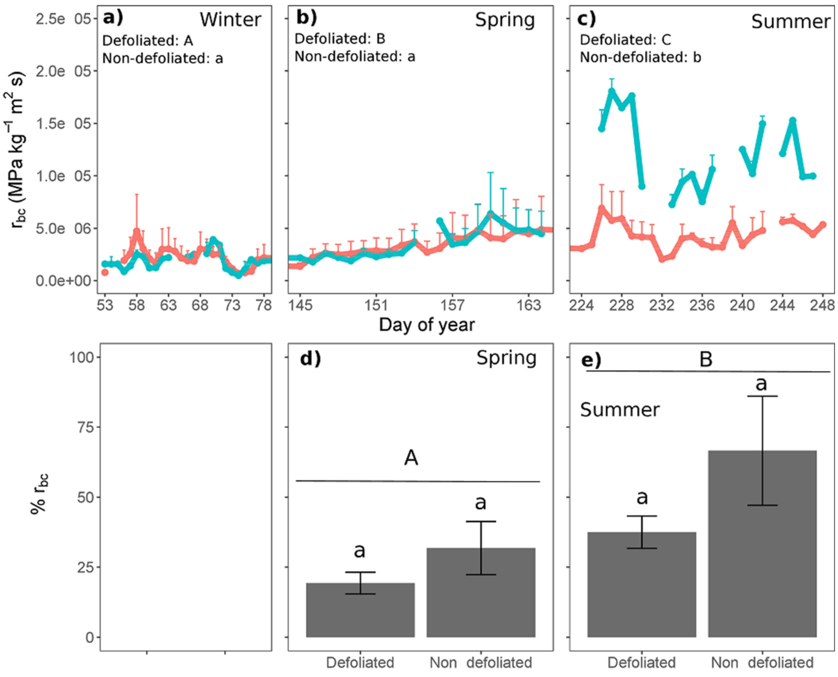


**Fig. 4** (a–c) Seasonal course of daily averages of below-crown hydraulic resistance ($r_{\mathrm{bc}}$) in defoliated (red) and non-defoliated (blue) Scots pine trees over the three seasons studied in 2012. Different letters indicate significant differences ($P < 0.05$) between seasons within a given defoliation class. (d, e) Percent contribution of below-crown resistance to whole-tree hydraulic resistance (%$r_{\mathrm{bc}}$). Different uppercase letters indicate significant differences ($P < 0.05$) between seasons, and different lowercase letters indicate significant differences between defoliation classes within a given season. Error bars indicate ± 1 SE in all panels. Note that we did not measure leaf water potentials in winter, so we could not estimate %$r_{\mathrm{bc}}$ for this season

**Table 2** Summary of the linear mixed-effects models of below-crown hydraulic resistance ($r_{bc}$) and the percent contribution of below-crown hydraulic resistance to whole-tree hydraulic resistance ($\%r_{bc}$) as a function of defoliation class and season

| Parameter | Log($r_{bc}$) $R^2(m,c) = 0.28, 0.89$ | sqrt($\%r_{bc}$) $R^2(m,c) = 0.51, 0.82$ |
|---|---|---|
| Intercept | $-1.62 \pm 0.47$*** | $4.84 \pm 0.56$** |
| Non-defoliated | $-0.41 \pm 0.67$ (ns) | ni |
| Spring | $0.33 \pm 0.09$*** | |
| Summer | $1.28 \pm 0.09$*** | $2.16 \pm 0.54$* |
| Non-defoliated: spring | $-0.04 \pm 0.14$ (ns) | |
| Non-defoliated: summer | $0.60 \pm 0.14$*** | ni |

The coefficients indicate the difference between each level of a given variable and its reference level (‘defoliated’ for defoliation class and ‘winter’ for season, except for $\%r_{bc}$ where the reference season was ‘spring’). The values are the coefficient estimates ± 1 SE. $R^2$ $(m,c)$ refers to marginal and conditional $R^2$ values, respectively

*ns* no significant differences, *ni* not included in the model

**$0.001 < P < 0.01$; ***$P < 0.001$

Although the absolute values of $\%r_{bc}$ should be taken with caution because of the use of approximated values for the radial modulus of elasticity (see Section 2), our results imply that constraints on below-ground water transport increase with drought comparatively more than above-ground limitations (Domec et al. 2009). Given that Scots pine needles are even more vulnerable than roots (Aguadé et al. 2015a; Salmon et al. 2015), the higher vulnerability of roots compared to branches (Aguadé et al. 2015a) only partially explains this drought-driven increase in $\%r_{bc}$, which may be more associated to rhizosphere changes outlined in the previous paragraph.

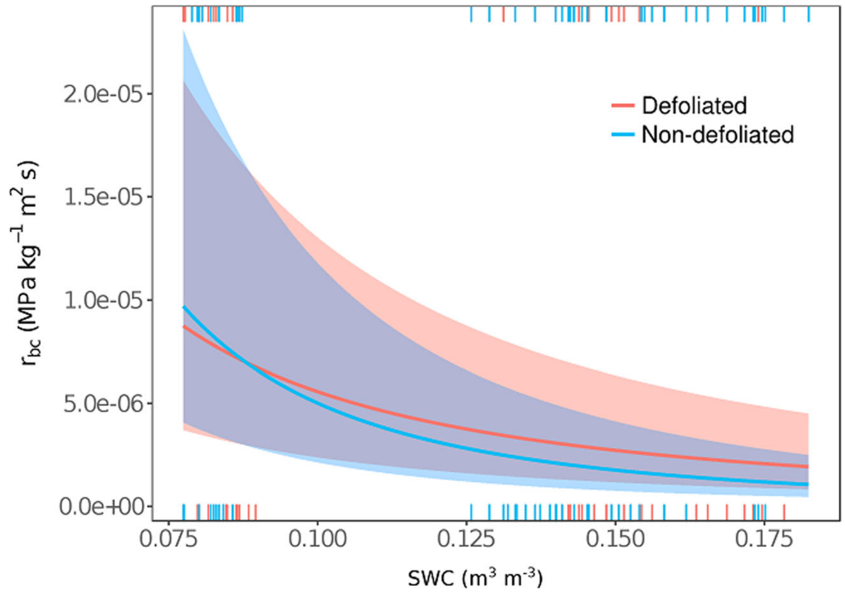
## 4.2 Defoliation is not associated with increased below-crown hydraulic resistance

Our results do not support the hypothesis that defoliated trees would show stronger increases in $r_{bc}$ during drought. On the contrary, non-defoliated trees showed larger increases in $r_{bc}$ between spring and summer (Fig. 4). These results are not entirely consistent with the differences in root vulnerability to embolism between defoliation classes reported in Aguadé et al. (2015a). In that study, defoliated trees showed a significantly steeper decline of root hydraulic conductance with decreasing water potentials but, at relatively high $\psi$ ($>-2$ MPa), roots of non-defoliated trees tended to show higher percent loss of conductivity (PLC). Assuming that $\psi_{PD}$ represents root water potential, the estimated variation in root PLC between spring (non-defoliated, $\psi_{PD} = -1.4$ MPa, PLC = ~ 38%; defoliated, $\psi_{PD} = -1.3$ MPa, PLC = ~ 36%) and summer (non-defoliated, $\psi_{PD} = -1.7$ MPa, PLC = ~ 40%; defoliated, $\psi_{PD} = -1.9$ MPa, PLC = ~ 42%) is at odds with the $r_{bc}$ dynamics observed here (Fig. 4).

Differences in $r_{bc}$ responses to plot-level soil moisture between defoliation classes could be related to more intense local soil water depletion by non-defoliated pines and associated increases in hydraulic resistances in the soil and the rhizosphere. This could occur because, during summer, tree-level sap flow of defoliated trees is 60–70% of that of non-defoliated trees (Poyatos et al. 2013). However, summer $\psi_{PD}$ values were similar between defoliation classes (Fig. 3), showing that the stronger increase in $r_{bc}$ with decreasing soil moisture observed in non-defoliated pines is unlikely to result from locally reduced soil moisture.

Aquaporins, the integral membrane proteins conducting water in and out of the cells (Maurel et al. 2008), could have

**Fig. 5** Modelled responses (and 95% confidence bands) of below-crown hydraulic resistance ($r_{bc}$) to soil water content (SWC) for defoliated and non-defoliated Scots pines, according to the linear mixed-effects model in Table 4. The rug depicts the location of the actual observations used in the model; when lines are placed at the top (bottom) of the plot, they denote positive (negative) residuals



also played a role in modifying extra-xylary components of root hydraulic conductance (e.g. Vandeleur et al. 2009). Upregulation of aquaporin activity has been reported to occur during extreme drought (Johnson et al. 2014), and it may have contributed to minimise the summer increase in $r_{bc}$ of defoliated pines, as there is evidence that defoliation may be related to increased aquaporin expression in leaves and roots (Liu et al. 2014).

### 4.3 Below-ground hydraulic constraints in the context of the whole-plant physiology of declining Scots pine

Increased $r_{bc}$ during drought was not related to defoliation, a result which is consistent with the lack of association between defoliation and root functioning in other drought-exposed Scots pine populations (Brunner et al. 2009). Therefore, below-ground hydraulics cannot explain the larger declines in whole-plant hydraulic conductance of defoliated pines during drought (Poyatos et al. 2013). Minimising drought-driven increases in $r_{bc}$ may have contributed to the higher gas exchange rates observed in defoliated pines during summer (Salmon et al. 2015). Likewise, we observed less variable $\Delta P_{bc}$ under varying soil water content in non-defoliated pines (Fig. 5), which is consistent with their more isohydric behaviour compared to defoliated pines (Salmon et al. 2015). Nevertheless, the fact that higher tree-level hydraulic sensitivity in defoliated pines is not explained by differences in below-ground hydraulics (Aguadé et al. 2015a; this study) does not imply that below-ground processes are irrelevant during drought-induced decline. At longer time scales, increased below-ground biomass allocation enhances survival of Scots pine saplings (Garcia-Forner et al. 2016; Matías et al. 2014). Deep rooting can buffer the impact of extreme drought (Nardini et al. 2016), and declines in modelled below-ground hydraulic conductance have been recently associated with increased mortality risk across species (Johnson et al. 2018).

Our results need to be interpreted together with the evidence supporting the role of carbon limitations in driving Scots pine drought-induced mortality (Aguadé et al. 2015a, b; Galiano et al. 2011; Garcia-Forner et al. 2016; Poyatos et al. 2013; Salmon et al. 2015). High root turnover to compensate for drought-related root mortality (Meier and Leuschner 2008) may aggravate carbon limitations, consistent with depleted root non-structural carbohydrate (NSC) reserves in defoliated pines measured in 2012 (Aguadé et al. 2015a, b). The need to maintain adequate NSC levels for a proper hydraulic and metabolic functioning of the plant (Dietze et al. 2014; Martínez-Vilalta et al. 2016) appears to dominate the physiological response of defoliated pines (Salmon et al. 2015), as prioritising carbon acquisition at the expense of water loss seems to promote survival under extreme drought in this species (Garcia-Forner et al. 2016).

## 5 Conclusions

We still know very little about the role of below-ground hydraulics on drought-induced tree mortality mechanisms, which mostly reflects the difficulty in measuring the relevant processes involved. We provide one of the few studies addressing the dynamics of below-ground hydraulics measured in drought-exposed trees in the field, without constraints on root development (i.e. by pots) and fully acclimated to prevailing climatic and below-ground conditions. This study has a number of limitations, including the low replication, the uncertainties associated to $E_r$ estimation and the assumption of steady state in the calculation of $r_{bc}$. This latter assumption may not hold during extreme drought when the stem may be shrinking because of the depletion of internal water storage. However, our results complement previous studies of the ecophysiology of Scots pine under extreme drought based on above-ground responses and contribute to guide further field-based research on tree functioning and survival under extreme drought. Our results may also serve to improve models, which frequently assume fixed contributions of root hydraulic conductance to whole-plant conductance (e.g. Sperry and Love 2015; Sperry et al. 2016).

The observed patterns in $r_{bc}$ and $\%r_{bc}$ highlight the dynamic nature of below-ground hydraulics in forests experiencing drought-induced decline processes, as already suggested by a modelling study using data from the same site (Sus et al. 2014). Our results suggest a partial buffering of below-ground hydraulic constraints during summer drought in trees experiencing drought-induced defoliation and are consistent with carbon limitations being involved in the process of drought-induced decline in Scots pine. We did not find direct evidence of extensive root hydraulic impairment associated with defoliation and hence with increasing mortality risk, as shown for some angiosperm species (Rodríguez-Calcerrada et al. 2016). More detailed measurements would be needed to disentangle the role of different components of below-ground hydraulics during drought-induced decline in situ, including changes in the functional balance of shallow and deep roots (Grossiord et al. 2016; Johnson et al. 2014) and the dynamics of hydraulic conductance in the xylem and the soil-root interface.

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**Data availability** The datasets generated and/or analyzed during the current study are available from the authors on reasonable request.

## Compliance with ethical standards

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

## Appendix

**Table 3** Main characteristics of the trees studied (defoliation class (defoliated and non-defoliated), diameter at breast height, height ($h$), basal area ($A_b$), sapwood area ($A_s$), maximum leaf area ($A_L$), leaf-to-sapwood area ratio ($A_L$:$A_S$))

| Tree | Class | Green leaves (%) | DBH (cm) | $h$ (m) | $A_b$ ($cm^2$) | $A_s$ ($cm^2$) | $A_L$ ($m^2$) | $A_L$:$A_S$ ($m^2$ $cm^{-2}$) |
|---|---|---|---|---|---|---|---|---|
| 364 | D | 40 | 40.1 | 11.5 | 1262.9 | 430.0 | 19.53 | 0.045 |
| 542* | D | 45 | 42.6 | 14.5 | 1425.3 | 496.0 | 19.46 | 0.039 |
| 544* | D | 45 | 28.4 | 16.1 | 633.5 | 174.2 | 8.53 | 0.049 |
| 693 | D | 35 | 42.8 | 11.7 | 1438.7 | 501.4 | 10.47 | 0.021 |
| 699* | D | 40 | 45.7 | 15.7 | 1640.3 | 583.3 | 15.23 | 0.026 |
| 704 | D | 50 | 48.0 | 11.2 | 1809.6 | 652.1 | 16.64 | 0.026 |
| 706 | D | 50 | 38.8 | 14.6 | 1182.4 | 397.3 | 20.89 | 0.053 |
| 707 | D | 45 | 24.9 | 11.5 | 487.0 | 114.7 | 6.69 | 0.058 |
| 714 | D | 50 | 40.9 | 14.9 | 1313.8 | 450.7 | 16.52 | 0.037 |
| 748* | D | 40 | 37.8 | 11.9 | 1122.2 | 372.8 | 22.25 | 0.060 |
| 561* | ND | 80 | 35.5 | 16.1 | 989.8 | 319.0 | 17.06 | 0.097 |
| 562* | ND | 80 | 38.3 | 18.0 | 1152.1 | 385.0 | 37.24 | 0.097 |
| 572* | ND | 100 | 44.7 | 17.3 | 1569.3 | 554.5 | 45.99 | 0.083 |
| 711 | ND | 90 | 46.5 | 15.2 | 1698.2 | 606.8 | 32.91 | 0.054 |
| 712 | ND | 80 | 59.4 | 14.4 | 2771.2 | 1042.8 | 93.33 | 0.089 |
| 713 | ND | 80 | 26.7 | 8.2 | 559.9 | 144.3 | 19.94 | 0.138 |
| 715 | ND | 100 | 45.8 | 14.1 | 1647.5 | 586.2 | 37.02 | 0.063 |
| 716 | ND | 80 | 43.8 | 14.9 | 1506.7 | 529.0 | 31.63 | 0.060 |
| 717 | ND | 100 | 41.7 | 15.2 | 1365.7 | 471.7 | 25.84 | 0.055 |
| 725* | ND | 100 | 41.0 | 14.4 | 1320.3 | 453.3 | 23.03 | 0.051 |

The subset of trees with measurements of xylem radius variations is marked with an asterisk (*)

*D* defoliated, *ND* non-defoliated, *DBH* diameter at breast height

**Table 4** Summary of the linear mixed-effects models of below-crown stem pressure difference ($\Delta P_{bc}$) and below-crown hydraulic resistance ($r_{bc}$) as a function of soil water content (SWC), vapour pressure deficit (VPD) and defoliation class

| | log($\Delta P_{bc}$)<br>$R^2(m,c) = 0.28, 0.81$ | log($r_{bc}$)<br>$R^2(m,c) = 0.29, 0.85$ |
|---|---|---|
| Intercept | $-3.02 \pm 0.56$*** | $-16.2 \pm 0.51$*** |
| Non-defoliated | $-2.33 \pm 0.59$** | $-1.99 \pm 0.74$* |
| Log(SWC) | $-0.15 \pm 0.23$ (ns) | $-1.77 \pm 0.13$*** |
| VPD | $1.94 \pm 0.42$*** | ni |
| VPD$^2$ | $-0.18 \pm 0.08$* | ni |
| Non-defoliated: log(SWC) | $-0.69 \pm 0.18$*** | $-0.81 \pm 0.20$*** |
| Log(SWC): VPD | $0.63 \pm 0.22$** | ni |

For factors, the coefficients indicate the difference between each level of a given variable and its reference level. In models, the reference defoliation class was 'defoliated'. The values depicted are estimates ± SE. $R^2$ $(m,c)$ refer to marginal and conditional $R^2$ values, respectively

*ns* no significant differences, *ni* not included in the model

*$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$

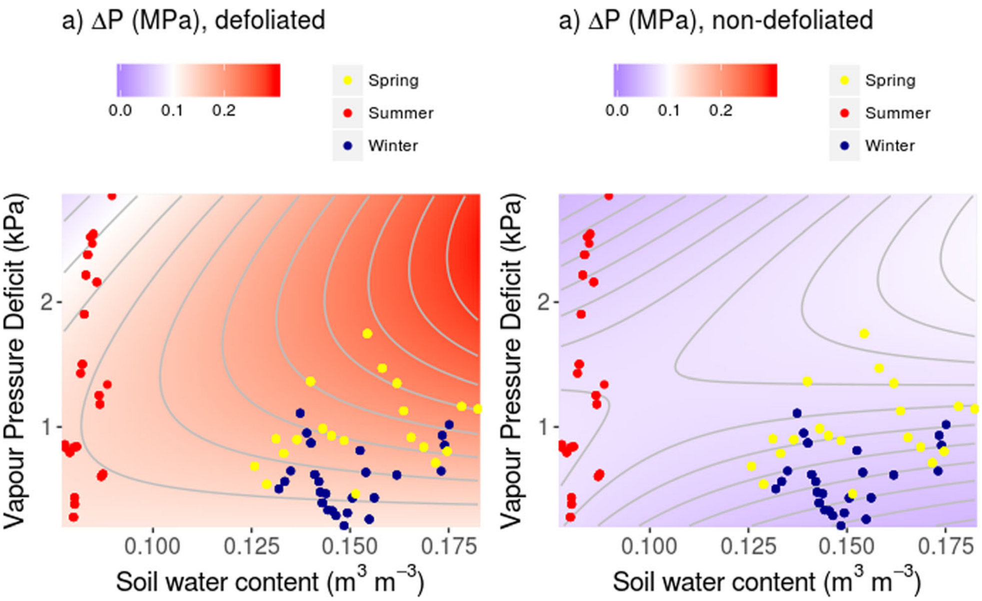

**Fig. 6** Modelled response surfaces of below-crown stem pressure difference ($\Delta P_{bc}$) as a function of vapour pressure deficit (VPD), soil water content (SWC) and defoliation class (Table 4). Dots represent the VPD and SWC values corresponding to the measured $\Delta P_{bc}$ values, coloured by season (cf. Section 2)

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