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

Rafael POYATOS^{1,2,3}, David AGUADÉ^{1,3,4} and Jordi MARTÍNEZ-VILALTA^{1,3}

¹ CREAf, E08193 Bellaterra (Cerdanyola del Vallès), Catalonia, Spain

² Laboratory of Plant Ecology, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, 9000 Gent, Belgium

³ Universitat Autònoma de Barcelona, E08193 Bellaterra (Cerdanyola del Vallès), Catalonia, Spain

⁴ IRTA, Fruit Production Programme, E08140, Caldes de Montbui, Catalonia, Spain

Correspondence to: Rafael Poyatos (r.poyatos@creaf.uab.es)

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Keywords: drought, forest mortality, hydraulic resistance, rhizosphere, roots, xylem pressure.

Contributions: RP conceived the study, performed measurements, analysed data and wrote the paper; DA performed measurements, analysed data and wrote the paper; JMV conceived the study, performed measurements and revised the paper.

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Data availability:

The datasets generated and/or analyzed during the current study are available in Zenodo repository (Poyatos et al, 2018). Datasets not peer-reviewed. Poyatos R, Aguadé D, Martínez-Vilalta J. (2018) Below-ground hydraulic constraints during drought-induced decline in Scots pine. V1.0.0. Zenodo. [Dataset].

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

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.

Abstract

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

Aim: 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 belowground hydraulic impairment in Scots pine.

Keywords: drought, forest mortality, hydraulic resistance, rhizosphere, roots, xylem pressure.

25 **Introduction**

1 Drought is a major driver of the composition, function and dynamics of forests worldwide (Allen
2 et al. 2015). Anthropogenic global warming has amplified drought and heat stress conditions in the
3 last decades (Trenberth et al. 2014), increasing background mortality rates and triggering
4 widespread tree die-off episodes after extreme droughts (Allen et al. 2010; Young et al. 2017),
5 which may cause profound changes in ecosystem structure and function (Anderegg et al. 2013a).
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7 Over a decade of research on the physiological mechanisms of drought-induced tree mortality
8 (McDowell et al. 2008; McDowell 2011; Sala et al. 2010) has revealed that drought-exposed trees
9 rarely die of a single cause (hydraulic failure, carbon starvation or phloem impairment; Mencuccini
10 et al. 2015; Sevanto et al. 2014), precluding robust predictions of vegetation mortality. Although
11 hydraulic traits may be good predictors of drought vulnerability (Anderegg et al. 2015; Anderegg et
12 al. 2016) and hydraulic failure is usually involved in drought-induced tree death processes (Adams
13 et al. 2017), lethal physiological thresholds have proved difficult to determine. These thresholds are
14 organ-specific (Bartlett et al. 2016) and it is still unclear which part of the plant's hydraulic pathway
15 is more vulnerable to hydraulic failure during extreme drought under field conditions (Anderegg et
16 al. 2014; Bartlett et al. 2016).
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19 Our understanding of hydraulic failure during extreme drought is largely based on processes
20 occurring in aboveground organs, mostly stems. For example, a recent synthesis of experiments on
21 physiological mechanisms of drought-induced mortality only included stem hydraulics (Adams et
22 al. 2017). However, below-ground hydraulic processes may have a strong influence on whole-tree
23 hydraulic dynamics and tree survival because (1) spatial patterns of die-off are often associated with
24 variability in soil conditions (Lloret et al. 2004; Peterman et al. 2012; Vilà-Cabrera et al. 2013; but
25 see Dorman et al. 2015) or rooting depth patterns across species (Nardini et al. 2016); (2) roots are
26 usually more vulnerable to embolism than stems (Hacke et al. 2000; Martínez-Vilalta et al. 2002;
27 Johnson et al. 2016); and (3) incorporating topographically-mediated soil moisture often improves
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50 prediction of drought-induced tree mortality (Tai et al, 2016).

1 Below-ground hydraulic resistance is typically around half of whole-tree hydraulic resistance
2 (Irvine and Grace 1997; Running 1980) but its response to evaporative demand and water supply is
3 complex and remains poorly studied. Increasing evaporative demand can reduce below-ground
4 (Martínez-Vilalta et al. 2007) and fine-root hydraulic resistances (McElrone et al. 2007) of trees. On
5 the other hand, below-ground hydraulic resistance can become the main bottleneck during edaphic
6 drought as a result of root embolism (Domec et al. 2009) or reductions in the hydraulic conductance
7 of the soil-root interface, for example following hydraulic disconnection from the soil (Sperry et al.
8 2002). This latter process has been suggested to occur in dying pines under extreme drought (Plaut
9 et al. 2012). Nevertheless, it is not known whether roots and the rhizosphere constitute the main
10 hydraulic bottleneck during drought-induced mortality processes.
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13 Drought-induced mortality in Scots pine (*Pinus sylvestris* L.) is typically preceded by long-term
14 growth declines lasting decades and by years of canopy defoliation (Galiano et al. 2011, Heres et al.
15 2012). This defoliated stage is associated with severely limited gas exchange and depleted
16 carbohydrate reserves (Aguadé et al. 2015a,b; Galiano et al. 2011; Poyatos et al. 2013). Compared
17 to non-defoliated pines, defoliated individuals show a steeper decline of whole-plant hydraulic
18 conductance, slightly lower predawn leaf water potentials (Poyatos et al. 2013; Salmon et al. 2015)
19 and steeper vulnerability to embolism in roots (Aguadé et al. 2015a). This collective evidence
20 suggests that impaired below-ground functioning may be associated to the mortality process.
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23 Assuming steady-state conditions, xylem radius variations, combined with sap flow and leaf
24 water potential measurements can be used to estimate the seasonal dynamics of hydraulic
25 conductance in different components of the plant's hydraulic pathway (Martínez-Vilalta et al.
26 2007). Here, we investigated the association between defoliation and drought responses of below-
27 ground hydraulic resistance in a Scots pine population affected by drought-induced mortality (Heres
28 et al. 2012; Martínez-Vilalta and Piñol 2002). Xylem radius variations were measured below the
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75 living crown, assuming that bole resistance does not vary seasonally and that the dynamics of
1 below-crown hydraulic resistance (r_{bc}) are dominated by the below-ground component (Domec et
2 al. 2009). Our main objective was to estimate r_{bc} and its contribution to whole-tree hydraulic
3 resistance, and to explore their seasonal dynamics in declining Scots pines. We hypothesised that
4 (1) both r_{bc} and its contribution to whole-tree hydraulic resistance (% r_{bc}) will increase in summer in
5 all trees studied due to the higher vulnerability to embolism of roots and the increase in resistance at
6 the soil-root interface, (2) the summer increase in r_{bc} will be more pronounced in defoliated pines.
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80 Part of the results in this paper belong to the PhD thesis by David Aguadé (Aguadé 2016).
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20 Materials and methods

21 85 Study site

22 Measurements were conducted in Tollar Valley within the Poblet Forest Natural Reserve (Prades
23 Mountains, northeast Iberian Peninsula). The climate is Mediterranean, with a mean annual rainfall
24 of 664 mm (spring and autumn being the雨iest seasons and with a marked summer dry period),
25 and moderately warm temperatures (11.3°C on average) (Poyatos et al. 2013). The substrate is
26 fractured schist and soils are rocky Xerocrepts, relatively shallow (~40 cm deep), with a clay loam
27 texture (Barba et al. 2016). The study area has a predominantly northern aspect and steep slopes
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42 The forest studied (41°19'58.05"N, 1°0'52.26"E; 1015 m asl) consists of a mixed holm oak
43 (*Quercus ilex* L.) - Scots pine stand; the canopy is dominated by pines whereas oaks dominate the
44 understorey (Poyatos et al. 2013). As a consequence of several drought-induced mortality episodes
45 since the 1990s (Hereş et al. 2012; Martínez-Vilalta and Piñol 2002), Scots pine average standing
46 mortality and crown defoliation are currently 12% and 52%, respectively. However, in some parts
47 of the forest, standing mortality is > 20% and cumulative mortality is as high as 50% in the last 20
48 years (J. Martínez-Vilalta, unpublished). The Scots pine population studied is more than 150 years
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100 old and has remained largely unmanaged for at least 30 years (Heres et al. 2012). No major insect
1 infestation episode was detected that could explain the forest decline in the area (Mariano Rojo,
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3 Catalan Forest Service, pers com.).
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8 *Experimental design*
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10 Between 2010 and 2013, several physiological variables were measured in defoliated and non-
11 defoliated Scots pine trees growing together in the same population (Aguadé et al. 2015a,b; Poyatos
12 et al. 2013; Salmon et al. 2015). In addition, we also measured above-canopy meteorology and soil
13 moisture in the top 30 cm using a frequency-domain reflectometer (cf. Poyatos et al. 2013, for
14 additional details). Defoliation was visually estimated relative to a completely healthy tree in the
15 same population. A tree was considered as non-defoliated if the percentage of green needles was \geq
16 80% and defoliated if the percentage of green needles was \leq 50%. Here, we use the complete set
17 of monitored trees to show the general pattern in the seasonal dynamics of sap flow for defoliated
18 and non-defoliated trees, but we then focus on a subset of four defoliated and four non-defoliated
19 co-occurring Scots pine trees in which sap flow, xylem radius variations and leaf water potentials
20 were measured concurrently between August 2011 and November 2012 (**Table 3**).
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41 *Sap flow measurements*
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44 Measurements of sap flow density were conducted at 15-min intervals using constant heat
45 dissipation sensors (Granier 1985). The probes were inserted radially at breast height into the xylem
46 after removing the bark, and covered with a reflective material to avoid solar radiation. Two sensors
47 (north- and south-facing side of the trunk) were placed in each tree and averaged to account for
48 azimuthal variation of sap flow within the trunk. Data processing of heat dissipation sensors output
49 included correction for natural temperature gradients in the stem and zero flow determination was
50 based only on nights with low and stable evaporative demand (Poyatos et al. 2013). Sap flow
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125 density calculation in the outer sapwood followed the original calibration (Granier 1985) and was
1 then integrated over the entire sapwood using radial profiles of sap flow (6 depths) measured in
2 three trees using the heat field deformation method (Nadezhina et al. 2018), during at least seven
3 days per tree. This sap flow per unit sapwood area was expressed on a leaf area basis (J_L) after
4 estimation of tree leaf area using site-specific allometries for Scots pine and accounting for seasonal
5 variations of leaf area (see Poyatos et al. 2013 for additional methodological details).
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16 *Water potential measurements*
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18 Predawn (Ψ_{PD} , MPa; just before sunrise, 03:00-05:00 h, solar time) and midday (Ψ_{MD} , MPa;
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20 11:00-13:00 h, solar time) leaf water potentials were measured once per month in June, July, August
21 and November in 2012. On each sampling time, a sun-exposed twig from each tree was excised
22 using a pruning pole and stored immediately inside a plastic bag with a moist paper towel to avoid
23 water loss until measurement time, typically within 2 hours of sampling. Leaf water potentials were
24 measured using a pressure chamber (PMS Instruments, Corvallis, OR, USA).
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3640 *Xylem radius variations*
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38 Xylem radius variations were measured throughout the study period using point dendrometers
39 consisting of linear variable displacement transducers (LVDTs; DG 2.5 Solartron Metrology, West
40 Sussex, UK). LVDTs were mounted on metal frames following the design by Sevanto et al. (2005b)
41 with some modifications. Briefly, the frame was held around the bole, just below the living tree
42 crown, by two metal plates that were screwed ca. 20 cm above the measuring point. The frame was
43 made of aluminium, except for the two rods parallel to the direction of the measured radius changes,
44 which were made of Invar, a nickel-iron alloy with a low thermal expansion coefficient. Each frame
45 held two LVDT sensors, placed ca. 30 mm apart from each other, one measured over bark radius
46 changes (not used here) and the other one measured xylem radius changes (i.e., the sensor
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150 measuring tip was in contact with the xylem, since bark, phloem and cambium were removed
1 before the installation). We used xylem radius changes because they have been successfully used to
2 estimate stem water potentials in Scots pine (Irvine et al. 1997; Martínez-Vilalta et al. 2007) and
3 because they are more suitable to monitor changes in water potential than whole-stem radius
4 changes (Offenthaler et al. 2001). LVDTs measurements were taken every 30 s and the averages
5 stored every 15 min in a datalogger (CR1000, Campbell Scientific Inc., Logan, US). Since natural
6 expansion and contraction of the metal frame and sapwood occurs due to changes in air
7 temperature, frame and sapwood temperatures were measured using thermocouples. We used these
8 temperatures to account for thermal expansion of the frame and the sapwood using the linear
9 expansion coefficients for the Invar rods ($1.2 \times 10^{-6} \text{ K}^{-1}$) and for Scots pine wood ($7.9 \times 10^{-6} \text{ K}^{-1}$,
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2360 Sevanto et al. 2005a).

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28 *Estimation of stem water potential*
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30 We used within-day xylem radius variations to estimate changes in below-crown stem water
31 potential. We first calculated the difference between the maximum stem radius within a day, which
32 corresponds to the minimum shrinkage, and the instantaneous measurements of the xylem radius.
33 34
35 This below-crown xylem radius difference (ΔR_{bc} , mm) quantifies the instantaneous shrinkage
36 compared to maximum swelling conditions within a day. We then used ΔR_{bc} to calculate the
37 corresponding water pressure difference (ΔP_{bc} , MPa), by dividing ΔR_{bc} by the sapwood depth
38 ($R_{bc,sw}$, mm), and multiplying it by the radial modulus of elasticity of wood (E_r , MPa) following the
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4770 equation (Irvine and Grace 1997):
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$$\Delta P_{bc} = E_r \frac{\Delta R_{bc}}{R_{bc,sw}} \quad \text{Equation 1}$$

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53 We can assume that the osmotic potential in xylem sap is very low and relatively constant over
54 time, and therefore water pressure in the xylem can be used to estimate xylem water potential
55 (Irvine and Grace 1997). ΔP_{bc} is thus equivalent to the difference between water potential at the
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175 LVDT location (under the living crown) and the soil, assuming that the xylem water potential under
1 conditions of maximum swelling was in equilibrium with the soil.
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3 We did not measure E_r for the trees in our study. A literature survey showed that values of E_r for
4 Scots pine are variable across trees and populations, with reported average values ranging between
5 150 and 750 MPa (Irvine and Grace 1997; Mencuccini et al. 1997; Perämäki et al. 2001). Given this
6 variability and the fact that applying literature values of E_r to estimate xylem pressure dynamics can
7 be problematic (Sevanto et al. 2008), we applied a value of E_r (180 MPa) so that the maximum
8 percentage of below-crown resistance to whole-tree hydraulic resistance ($\%r_{bc}$) observed during
9 summer; see next section) was 100%. This assumption is justified on the grounds that, under
10 extreme drought conditions, it is expected that most of the hydraulic resistance is found below-
11 ground (Domec et al. 2009; Duursma et al. 2008; Sperry et al. 2002). While this assumption should
12 not affect the relative values of $\%r_{bc}$ over time or between trees, the absolute $\%r_{bc}$ values reported
13 here are subject to high uncertainty and have to be considered with caution.
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15 Sapwood radius was measured below the living crown, at each LVDT location. We extracted
16 wood cores with a Pressler borer, put them in paper bags and stored them in a portable cooler.
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18 Samples were then coloured with bromocresol green in the laboratory to distinguish between
19 sapwood and heartwood and measure their length.
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$$r_{bc} = \frac{\Delta P_{bc,max}}{J_{L,max}} \quad \text{Equation 2}$$

The percent contribution of below-crown resistance to whole-tree hydraulic resistance ($\%r_{bc}$) was calculated by comparing the water pressure difference (ΔP_{bc}) measured with LVDTs and the

200 leaf water potential difference ($\Delta\psi$; ψ_{MD} minus ψ_{PD}) when both measurements were taken on the
1 same day (Irvine and Grace 1997):
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$$\%r_{bc} = \frac{\Delta P_{bc}}{\Delta\psi} \cdot 100 \quad \text{Equation 3}$$

6 We assumed that pines reached equilibrium with the soil during night and, as a consequence, ψ_{PD}
7 is an estimate of soil water potential (Irvine et al. 2004), which corresponded to the maximum stem
8 radius within a day. Two pieces of evidence suggest that this assumption was not critical in our case
9 (comparison of temporal dynamics and drought responses between defoliation classes). First, we
10 quantified night-time sap flow per unit leaf area ($J_{L,night}$, global radiation $< 5 \text{ W m}^{-2}$) for the winter
11 and spring periods only and expressed it as a percentage of daytime flow; the calculations for
12 summer were not used because daytime flows were very low and dividing two low flows sometimes
13 yielded unreasonable values. Median values of percentage $J_{L,night}$ were 10% for defoliated and 6%
14 for non-defoliated trees and did not differ between classes ($P = 0.182$). Second, we modelled r_{bc} as a
15 function of $J_{L,night}$ interacting with defoliation class, and no effect of either $J_{L,night}$ ($P = 0.454$) or its
16 interaction with defoliation class ($P = 0.389$) was detected.
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3615 Data analysis

37 The analyses of ΔP_{bc} , r_{bc} , and J_L need sap flow and xylem radius variations data measured
38 simultaneously. However, due to technical problems mostly related to power failures due to the
39 remote location of the sampling site, these data were not always available. We selected
40 representative periods with maximum data availability within three different seasons of year 2012
41 showing contrasted characteristics of soil water content: winter (from day 53 to day 78), spring
42 (days 145–164) and summer (days 224–248). Note that for these periods we did not always have
43 usable data from the four trees per defoliation class.
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59 Linear mixed-effects models were fitted to test for statistical differences between defoliation
60 classes (defoliated or non-defoliated) and season (winter, spring or summer) on r_{bc} , $\%r_{bc}$, ψ_{PD} , ψ_{MD}
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225 and $\Delta\psi$. Measurements of ψ in June and August were considered as spring and summer seasons,
1 respectively. Winter measurements of ψ were not available and, thus, models for $\%r_{bc}$ and ψ did not
2 include a winter season. To test the combined effects of vapour pressure deficit (VPD), soil water
3 content (SWC) and defoliation class on ΔP_{bc} and r_{bc} we fitted similar linear mixed-effects models
4 including a quadratic term in the case of the relationships with VPD or log-transforming SWC, in
5 order to account for nonlinear responses. In all mixed models, tree identity was included as a
6 random factor. r_{bc} and ΔP_{bc} were log-transformed and $\%r_{bc}$ square root transformed to achieve
7 normality prior to all analyses. In all cases, we started by fitting the most complex, biologically
8 plausible model and then this, and all the alternative models resulting from different combinations
9 of explanatory variables, were ranked from lowest to highest AICc (Akaike information criterion
10 corrected for small sample sizes). When multiple models minimising AICc lied within 2 AICc units,
11 likelihood ratio tests were performed to select the best-performing model. All statistical analyses
12 were carried out using the R Statistical software version 3.3.1 (R Core Team, 2016), using the “lme”
13 function to fit mixed-effects models (Pinheiro et al. 2018), and the “dredge” function (“MuMIn”
14 package) to compare models (Bartoń 2017).

3. Results

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
1 pine trees from our whole study population were higher in defoliated trees relative to non-defoliated
2 ones throughout the study period, especially in late spring and early summer (**Fig. 1**). With the
3 beginning of summer drought, J_L strongly decreased in both defoliated and non-defoliated pines,
4 though this response was more acute in the former. Winter, spring and summer periods selected in
5 **Fig. 1** and used in subsequent analyses are representative of different values of J_L and
6 environmental conditions in year 2012. Summer drought conditions in 2012 were intense in terms
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250 of both evaporative demand and soil water supply, with maximum VPD of 3.86 kPa and minimum
1 SWC of 0.08 m³ m⁻³ (**Fig. 2c, f**). In fact, 2012 was the second driest growing season since 1951 in
2 the study area (133.9 mm of rainfall between May and October).
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8 *Seasonal courses of sap flow, leaf water potentials and stem pressure difference in selected trees*
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11 Sap flow per unit leaf area (J_L) for the selected trees ($N = 4$; see Materials and Methods) the same
12 patterns as the full set of measured trees (**Fig. 1**), with higher J_L in defoliated trees over all periods,
13 and an acute reduction of J_L in both defoliation classes in summer (**Fig. 1, 2g, h, i**). The temporal
14 dynamics of ΔP_{bc} generally followed the course of J_L , and ΔP_{bc} was higher in defoliated pines over
15 winter and spring (**Fig. 2j, k**). However, defoliated and non-defoliated Scots pines showed similar
16 ΔP_{bc} values in summer (**Fig. 2l**).
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19 Midday leaf water potential (Ψ_{PD}) were similar between defoliation classes but significantly
20 different between seasons, with lower values in summer (**Fig. 3, Table 1**). In contrast, Ψ_{MD} showed
21 differences between defoliation classes, with lower values for defoliated trees, but no seasonal
22 effect (**Fig. 3, Table 1**). On the other hand, the interaction between season and defoliation was
23 significant for $\Delta\psi$, indicating that the water potential difference was highest for defoliated trees in
24 spring, and similarly low for all the other combinations of season and defoliation class (**Fig. 3,**
25 **Table 1**).
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28 *Seasonal course of below-crown hydraulic resistance and contribution to whole-tree resistance*
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30 Below-crown hydraulic resistance (r_{bc}) increased from winter to summer for both defoliation
31 classes (**Fig. 4a-c, Table 2**). Although defoliated and non-defoliated Scots pine trees presented
32 similar levels of r_{bc} in winter and spring, the summer increase in r_{bc} was significantly larger for non-
33 defoliated Scots pine trees (**Fig. 4, Table 2**). The percent contribution of below-crown hydraulic
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275 resistance to whole-tree hydraulic resistance ($\%r_{bc}$) increased in summer for both defoliated and
1 non-defoliated Scots pine trees relative to spring (**Fig. 4d, e, Table 2**) but we did not find any effect
2 of defoliation class on the seasonal dynamics of $\%r_{bc}$ (**Fig. 4d, e, Table 2**).
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8 *Responses of stem pressure difference and below-crown hydraulic resistance to VPD and SWC*
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10 280 Soil water content interacted with VPD and defoliation class to determine ΔP_{bc} (**Table 4, Fig. 6**).
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12 The values of ΔP_{bc} were lower and less variable for non-defoliated pines. For defoliated pines,
13 variation with environmental demand and soil water supply was more evident: high values of ΔP_{bc}
14 were observed at high VPD and SWC (**Fig. 6**). In contrast, we did not detect VPD effects on r_{bc} ,
15 which did decline with SWC, especially for non-defoliated pines (**Fig. 5, Table 3**). Therefore, r_{bc} in
16 non-defoliated pines tended to be more sensitive to the depletion of soil water content (**Table 4**),
17 leading to higher r_{bc} at low levels of SWC, compared to defoliated Scots pines (**Fig. 5**).
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30 **Discussion**
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32 Our results are consistent with whole-plant responses of Scots pine suffering drought-induced
33 decline and support an important role of below-ground hydraulic constraints during seasonal
34 drought. The eight intensively measured trees in this study showed sap flow responses (**Fig. 1, Fig.**
35 **2**) largely consistent with the patterns observed for the full set of sampled trees in 2012 and in the
36 previous two years (Poyatos et al. 2013). As for leaf water potentials, lower ψ_{MD} were observed in
37 defoliated trees but ψ_{PD} were similar between defoliation classes. Here, adding xylem radius
38 variations to sap flow and leaf water potential measurements, has allowed us to dig deeper into the
39 factors driving these whole-tree drought responses, by describing the dynamic patterns of below-
40 crown hydraulic resistance (r_{bc}) associated to seasonal drought and defoliation. This approach for
41 estimating r_{bc} is not new (Irvine and Grace 1997; Martínez-Vilalta et al. 2007) but has been used
42 surprisingly little in the literature. It is based on several assumptions (overnight equilibration of
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300 plant and soil water potentials, constant modulus of elasticity and osmotic potential of xylem sap)
1 and it is certainly not free of potential methodological issues (for instance, in our case, the high
2 uncertainty in the estimation of the modulus of elasticity). However, it remains one of the easiest
3 ways of obtaining reliable time series of belowground (or below-crown) plant hydraulic resistance
4 without having to resort to modelled estimates (Johnson et al. 2018).
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Drought increases below-crown hydraulic resistance and its contribution to whole-tree resistance

As hypothesised, below-crown hydraulic resistance (r_{bc}) increased in both defoliated and non-defoliated Scots pine trees as drought progressed (Fig. 4). In our study, r_{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_{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_{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_{bc} .

The increase in r_{bc} with seasonal drought can be related to several xylem and rhizosphere processes (Newman 1969; Sperry et al. 2002). Minimum ψ_{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) mechanism explaining increases in r_{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 (Brunnner et al. 2015).

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325 Hydraulic disconnection from the soil reduces water loss from drying soils (North & Nobel 1997)

1 and has been reported in pines under extreme drought, associated with anomalous ψ_{PD} (e.g., more
2 negative than ψ_{MD} or soil ψ ; Plaut et al. 2012; Pangle et al. 2015) or with reduced sap flow
3 responses to precipitation pulses (Plaut et al. 2013). In our study, ψ_{PD} was always less negative than
4 ψ_{MD} , but Scots pine sap flow recovered only partially after autumn rains (Fig. 1; see also Poyatos et
5 al. 2013), which could indicate hydraulic isolation from the soil. Root hydraulic conductance during
6 drought can also be constrained by suppressed root elongation rates below $\psi_{PD} < -1$ MPa, as
7 observed in other pine species (Zou et al. 2000), by decreased fine root production (Olesinski et al.
8 2011) or by fine root mortality (Gaul et al. 2008). The latter, in particular, could lead to the observed
9 decrease in r_{bc} during drought.
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2335 We also showed that the percentage contribution of below-crown to whole-tree hydraulic
24 resistance ($\%r_{bc}$) increases with severe drought in Scots pine (Fig. 4). Although the absolute values
25 of $\%r_{bc}$ should be taken with caution because of the use of approximated values for the radial
26 modulus of elasticity (see Materials and Methods), our results imply that constraints on below-
27 ground water transport increase with drought comparatively more than aboveground limitations
28 (Domec et al. 2009). Given that Scots pine needles are even more vulnerable than roots (Aguadé et
29 al. 2015a; Salmon et al. 2015), the higher vulnerability of roots compared to branches (Aguadé et al.
30 2015a) only partially explains this drought-driven increase in $\%r_{bc}$, which may be more associated
31 to rhizosphere changes outlined in the previous paragraph.
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35 *Defoliation is not associated with increased below-crown hydraulic resistance*
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37 Our results do not support the hypothesis that defoliated trees would show stronger increases in
38 r_{bc} during drought. On the contrary, non-defoliated trees showed larger increases in r_{bc} between
39 spring and summer (Fig. 4). These results are not entirely consistent with the differences in root
40 vulnerability to embolism between defoliation classes reported in Aguadé et al. (2015a). In that
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350 study, defoliated trees showed a significantly steeper decline of root hydraulic conductance with
1 decreasing water potentials but, at relatively high ψ (> -2 MPa), roots of non-defoliated trees tended
2 to show higher percent loss of conductivity (PLC). Assuming that ψ_{PD} represents root water
3 potential, the estimated variation in root PLC between spring (Non-defoliated, $\psi_{PD} = -1.4$ MPa, PLC
4 = ~38 %; defoliated, $\psi_{PD} = -1.3$ MPa, PLC = ~36 %) and summer (Non-defoliated, $\psi_{PD} = -1.7$ MPa,
5 PLC = ~40 %; defoliated, $\psi_{PD} = -1.9$ MPa, PLC = ~42 %) is at odds with the r_{bc} dynamics observed
6 here (**Fig. 4**).
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9 Differences in r_{bc} responses to plot-level soil moisture between defoliation classes could be
10 related to more intense local soil water depletion by non-defoliated pines and associated increases in
11 hydraulic resistances in the soil and the rhizosphere. This could occur because, during summer, tree-
12 level sap flow of defoliated trees is 60-70% of that of non-defoliated trees (Poyatos et al. 2013).
13 However, summer ψ_{PD} values were similar between defoliation classes (**Fig. 3**), showing that the
14 stronger increase in r_{bc} with decreasing soil moisture observed in non-defoliated pines is unlikely to
15 result from locally reduced soil moisture.
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18 Aquaporins, the integral membrane proteins conducting water in and out of the cells (Maurel et
19 al. 2008) could have also played a role in modifying extra-xylary components of root hydraulic
20 conductance (e.g. Vandeleur et al. 2009). Upregulation of aquaporin activity has been reported to
21 occur during extreme drought (Johnson et al. 2014) and it may have contributed to minimise the
22 summer increase in r_{bc} of defoliated pines, as there is evidence that defoliation may be related to
23 increased aquaporin expression in leaves and roots (Liu et al. 2014).
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26 *Below-ground hydraulic constraints in the context of the whole-plant physiology of declining Scots
27 pine*
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30 Increased r_{bc} during drought was not related to defoliation, a result which is consistent with the
31 lack of association between defoliation and root functioning in other drought-exposed Scots pine
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375 populations (Brunner et al. 2009). Therefore, below-ground hydraulics cannot explain the larger
1 declines in whole-plant hydraulic conductance of defoliated pines during drought (Poyatos et al.
2 2013). Minimising drought-driven increases in r_{bc} may have contributed to the higher gas exchange
3 rates observed in defoliated pines during summer (Salmon et al. 2015). Likewise, we observed less
4 variable ΔP_{bc} under varying soil water content in non-defoliated pines (Fig. 5), which is consistent
5 with their more isohydric behaviour compared to defoliated pines (Salmon et al. 2015).
6 Nevertheless, the fact that higher tree-level hydraulic sensitivity in defoliated pines is not explained
7 by differences in below-ground hydraulics (Aguadé et al. 2015a, this study) does not imply that
8 below-ground processes are irrelevant during drought-induced decline. At longer time scales,
9 increased below-ground biomass allocation enhances survival of Scots pine saplings (Garcia-Forner
10 et al. 2016, Matías et al. 2014). Deep rooting can buffer the impact of extreme drought (Nardini et
11 al. 2016) and declines in modelled below-ground hydraulic conductance have been recently
12 associated with increased mortality risk across species (Johnson et al. 2018).
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14 Our results need to be interpreted together with the evidence supporting the role of carbon
15 limitations in driving Scots pine drought-induced mortality (Aguadé et al. 2015a,b; Galiano et al.
16 2011; Garcia-Forner et al. 2016; Poyatos et al. 2013; Salmon et al. 2015). High root turnover, to
17 compensate for drought-related root mortality (Meier and Leuschner 2008) may aggravate carbon
18 limitations, consistent with depleted root NSC reserves in defoliated pines measured in 2012
19 (Aguadé et al. 2015a,b). The need to maintain adequate NSC levels for a proper hydraulic and
20 metabolic functioning of the plant (Dietze et al. 2014; Martínez-Vilalta et al. 2016) appears to
21 dominate the physiological response of defoliated pines (Salmon et al. 2015), as prioritising carbon
22 acquisition at the expense of water loss seems to promote survival under extreme drought in this
23 species (Garcia-Forner et al. 2016).
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25 **Conclusions**

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400 We still know very little about the role of below-ground hydraulics on drought-induced tree
1 mortality mechanisms, which mostly reflects the difficulty in measuring the relevant processes
2 involved. We provide one of the few studies addressing the dynamics of below-ground hydraulics
3 measured in drought-exposed trees in the field, without constraints on root development (i.e. by
4 pots) and fully acclimated to prevailing climatic and below-ground conditions. This study has a
5 number of limitations, including the low replication, and the uncertainties associated to E_r
6 estimation and the assumption of steady state in the calculation of r_{bc} . This latter assumption may
7 not hold during extreme drought when the stem may be shrinking because of the depletion of
8 internal water storage. However, our results complement previous studies of the ecophysiology of
9 Scots pine under extreme drought based on aboveground responses, and contribute to guide further
10 field-based research on tree functioning and survival under extreme drought. Our results may also
11 serve to improve models, which frequently assume fixed contributions of root hydraulic
12 conductance to whole-plant conductance (e.g., Sperry & Love 2015, Sperry et al 2016).
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15 The observed patterns in r_{bc} and $\%r_{bc}$ highlight the dynamic nature of below-ground hydraulics
16 in forests experiencing drought-induced decline processes, as already suggested by a modelling
17 study using data from the same site (Sus et al. 2014). Our results suggest a partial buffering of
18 below-ground hydraulic constraints during summer drought in trees experiencing drought-induced
19 defoliation and are consistent with carbon limitations being involved in the process of drought-
20 induced decline in Scots pine. We did not find direct evidence of extensive root hydraulic
21 impairment associated with defoliation, and hence with increasing mortality risk, as shown for some
22 Angiosperm species (Rodríguez-Calcerrada et al. 2016). More detailed measurements would be
23 needed to disentangle the role of different components of below-ground hydraulics during drought-
24 induced decline *in situ*, including changes in the functional balance of shallow and deep roots
25 (Grossiord et al. 2016; Johnson et al. 2014) and the dynamics of hydraulic conductance in the xylem
26 and the soil-root interface.
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1 **Tables**

2 Table 1. Summary of the linear mixed models with predawn (ψ_{PD}), midday (ψ_{MD}) leaf water
 3 potentials and their difference ($\Delta\psi$) as response variables. The coefficients indicate the difference
 4 between each level of a given variable and the corresponding reference level (“Defoliated” for
 5 defoliation class and “spring” for season). The values are the coefficient estimates $\pm 1SE$.
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 10 Abbreviations: * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$; ni, not included in the model.
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 13 Only marginal R^2 , $R^2(m)$, is reported as the variance associated to the “Tree” random effect was
 14
 15 estimated as near zero.
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18 Parameter	19 ψ_{PD}	20 ψ_{MD}	21 $\Delta\psi$
	$R^2(m) = 0.82$	$R^2(m) = 0.50$	$R^2(m) = 0.87$
24 Intercept	$-1.33 \pm 0.05***$	$-2.14 \pm 0.06***$	$-0.95 \pm 0.08***$
25 Non-defoliated	ni	$0.30 \pm 0.09*$	$0.55 \pm 0.12**$
26 Summer	$-0.48 \pm 0.07***$	ni	$0.81 \pm 0.11***$
27 Non-defoliated:Summer	ni	ni	$-0.63 \pm 0.17*$

Table 2. Summary of the linear mixed 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. 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 $\pm 1SE$. Abbreviations: ns, no significant differences; ** $0.001 < P < 0.01$; *** $P < 0.001$; ni, not included in the model. $R^2 (m, c)$ refer to marginal and conditional R^2 values, respectively.

Parameter	$\log(r_{bc})$	$\sqrt{\%r_{bc}}$
	$R^2 (m, c) = 0.28, 0.89$	$R^2 (m, c) = 0.51, 0.82$
Intercept	$-1.62 \pm 0.47***$	$4.84 \pm 0.56**$
Non-defoliated	-0.41 ± 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 ± 0.14 (ns)	
Non-defoliated:Summer	$0.60 \pm 0.14***$	ni

Figure captions

690 **Fig. 1.** Seasonal course of daily-averaged sap flow per unit leaf area (J_L) over year 2012 in
2 defoliated (red) and non-defoliated (blue) Scots pine trees. Error bars indicate $\pm 1\text{SE}$. Periods
3 selected for the present paper, representative of different seasons, are indicated by vertical bars.
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8 **Fig. 2.** Seasonal course of (a-c) daily vapour pressure deficit (VPD), (d-f) soil water content (SWC),
9 (g-i) daily-averaged sap flow per unit leaf area (J_L) and (j-l) stem pressure difference (ΔP_{bc}) for
10 three seasons studied in 2012. J_L and ΔP_{bc} are given for defoliated (red) and non-defoliated (blue)
11 Scots pine trees. Error bars indicate $\pm 1\text{SE}$. Arrows in the lower panel indicate sampling dates of
12 leaf water potentials.
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15 **Fig. 3.** Daily averages of predawn (ψ_{PD} ; dark grey) and midday (ψ_{MD} ; light grey) leaf water
16 potential measurements in defoliated and non-defoliated Scots pine trees in (a) spring and (b)
17 summer 2012. Different uppercase letters indicate significant differences ($P < 0.05$) between
18 predawn measurements, different lowercase letters indicate significant differences between midday
19 measurements and different Greek letters indicate significant differences in the water potential
20 difference between predawn and midday ($\Delta\psi$) across defoliation classes and seasons. Error bars
21 indicate $\pm 1\text{SE}$.
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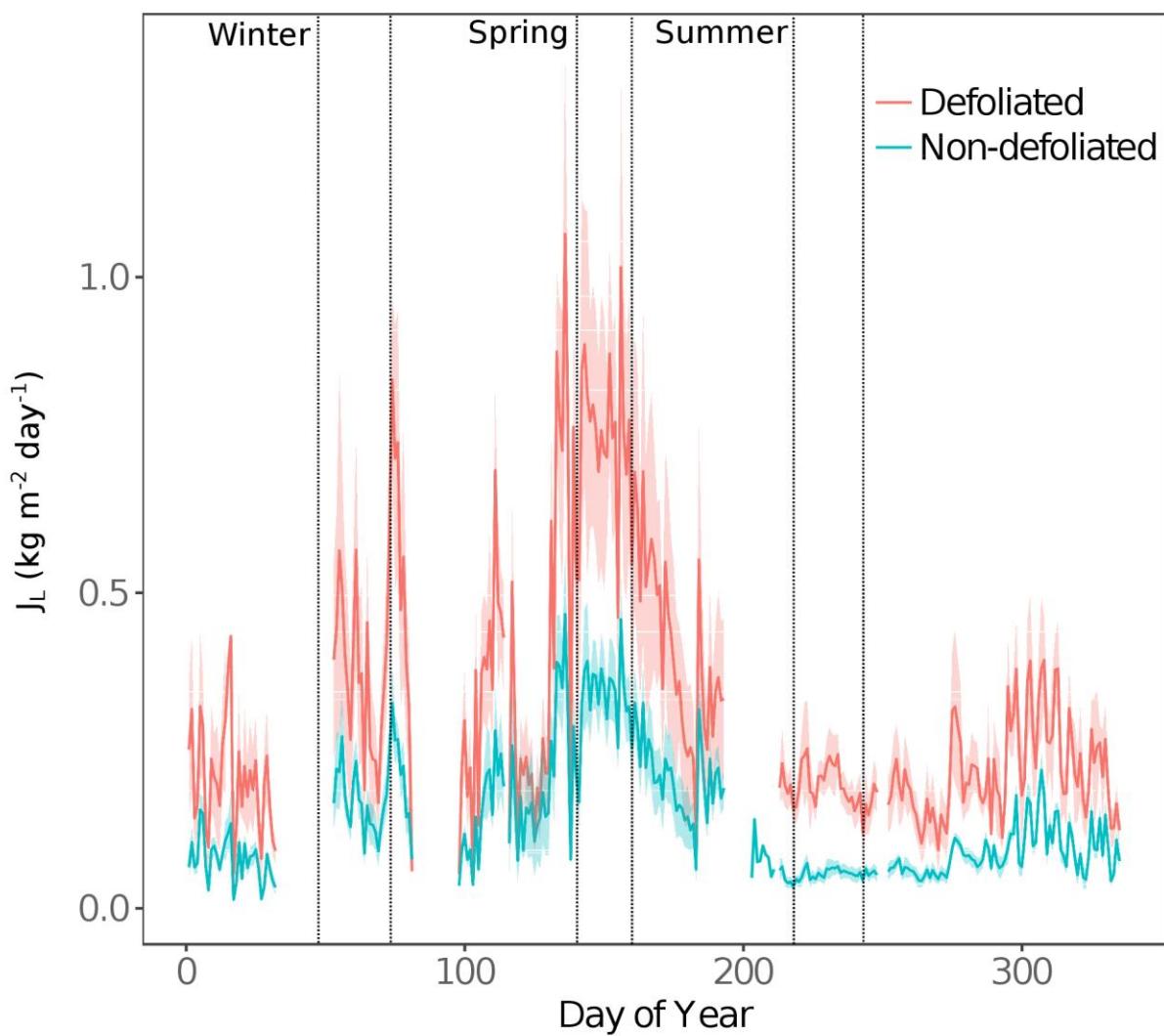
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24 **Fig. 4.** (a-c) Seasonal course of daily averages of below-crown hydraulic resistance (r_{bc}) in
25 defoliated (red) and non-defoliated (blue) Scots pine trees over the three seasons studied in 2012.
26 Different letters indicate significant differences ($P < 0.05$) between seasons within a given
27 defoliation class. (d-e) Percent contribution of below-crown resistance to whole-tree hydraulic
28 resistance ($\%r_{bc}$). Different uppercase letters indicate significant differences ($P < 0.05$) between
29 seasons, and different lowercase letters indicate significant differences between defoliation classes
30 within a given season. Error bars indicate $\pm 1\text{SE}$ in all panels. Note that we did not measure leaf
31 water potentials in winter, so we could not estimate $\%r_{bc}$ for this season.
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34 **Fig. 5.** Modelled responses (and 95% confidence bands) of below-crown hydraulic resistance (r_{bc})
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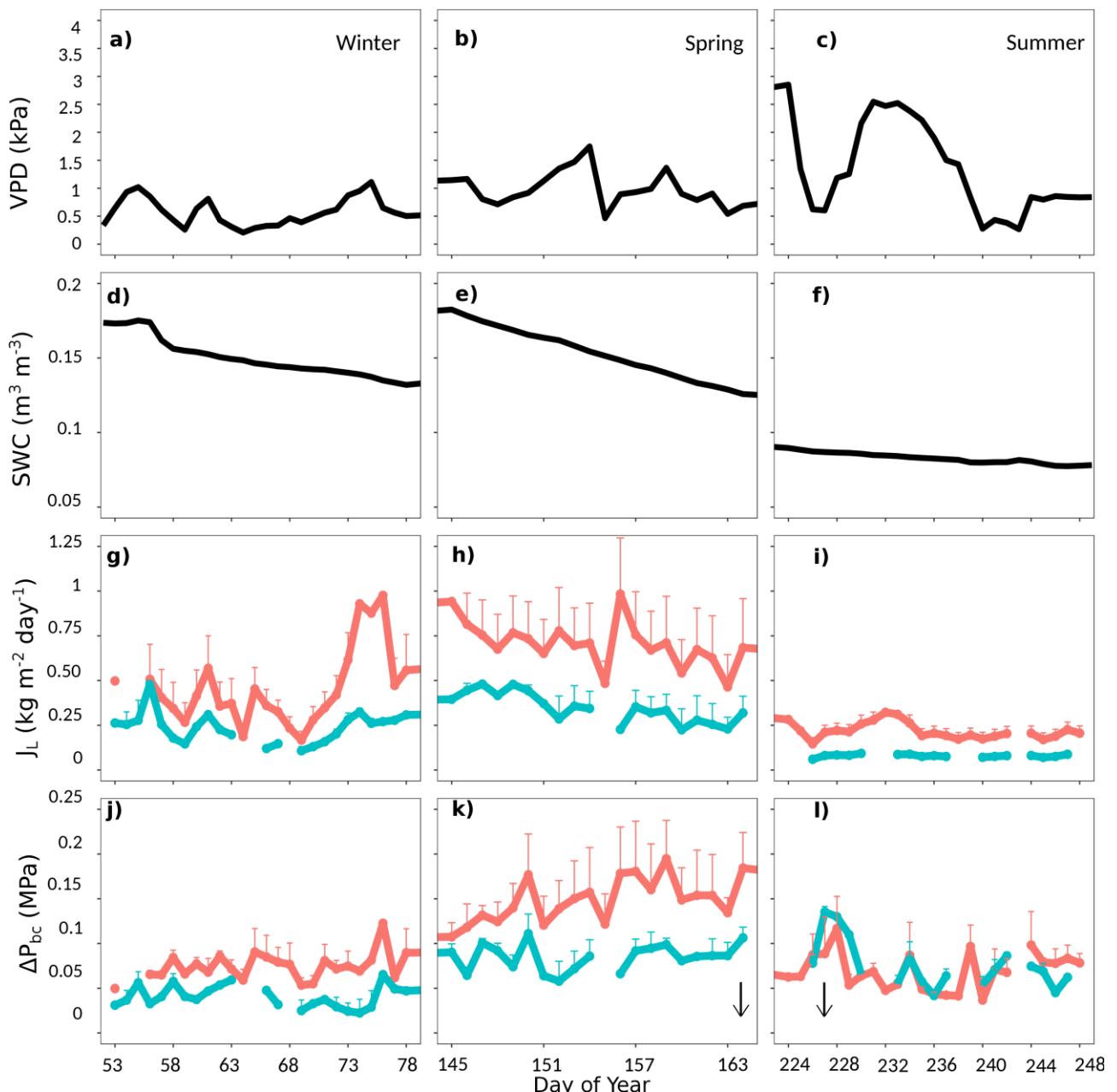
to soil water content (SWC) for defoliated and non-defoliated Scots pines, according to the linear
715 mixed model in [Table 4](#). The rug depicts the location of the actual observations used in the model;
2 when lines are placed at the top (bottom) of the plot, they denote positive (negative) residuals.
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Figures



20 **Fig. 1**
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45 **Fig. 2**
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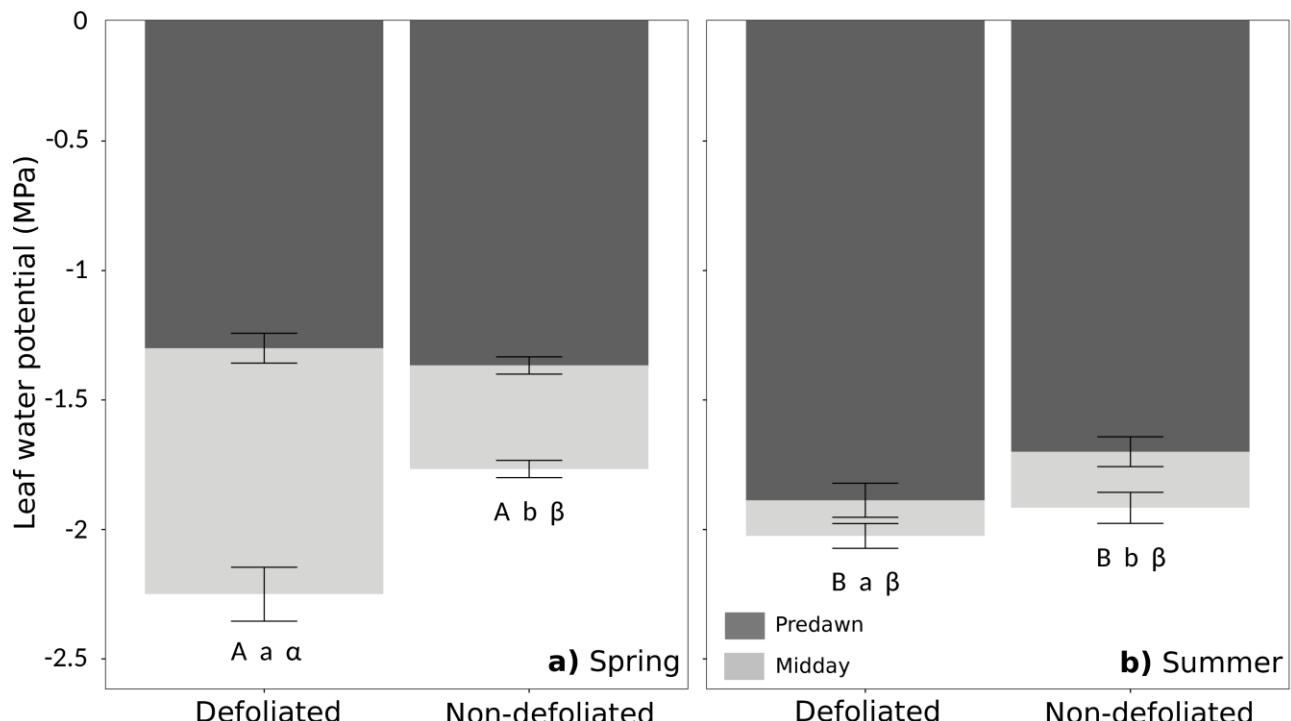


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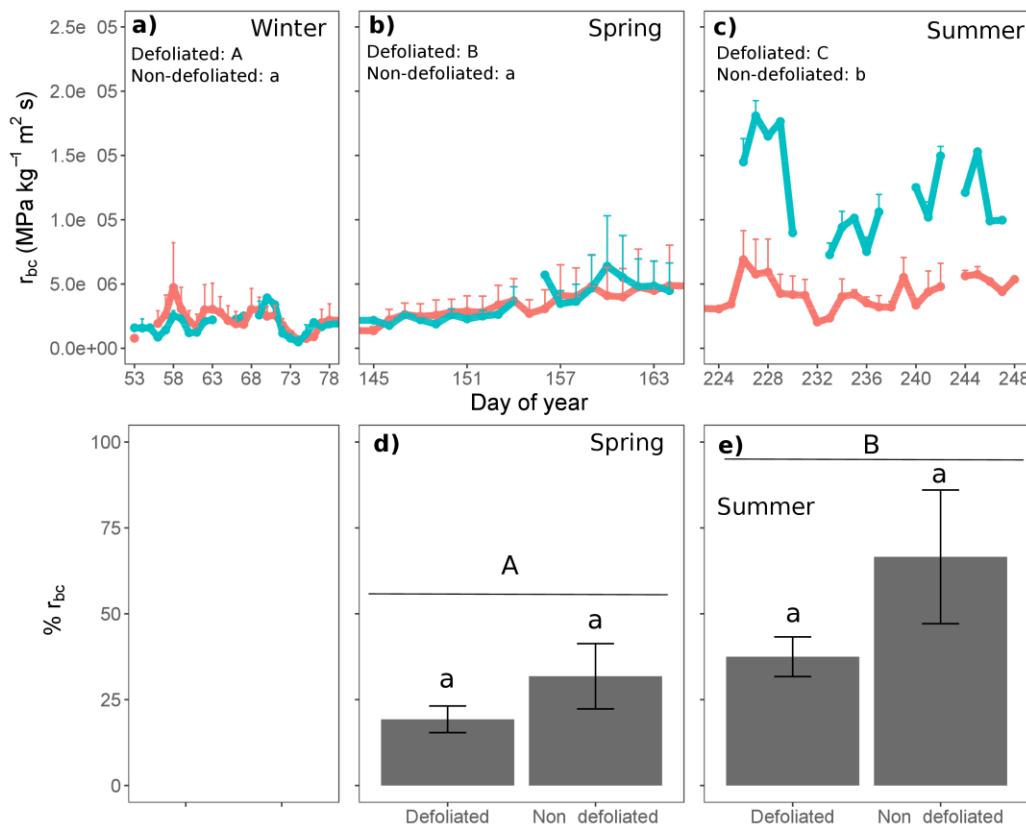


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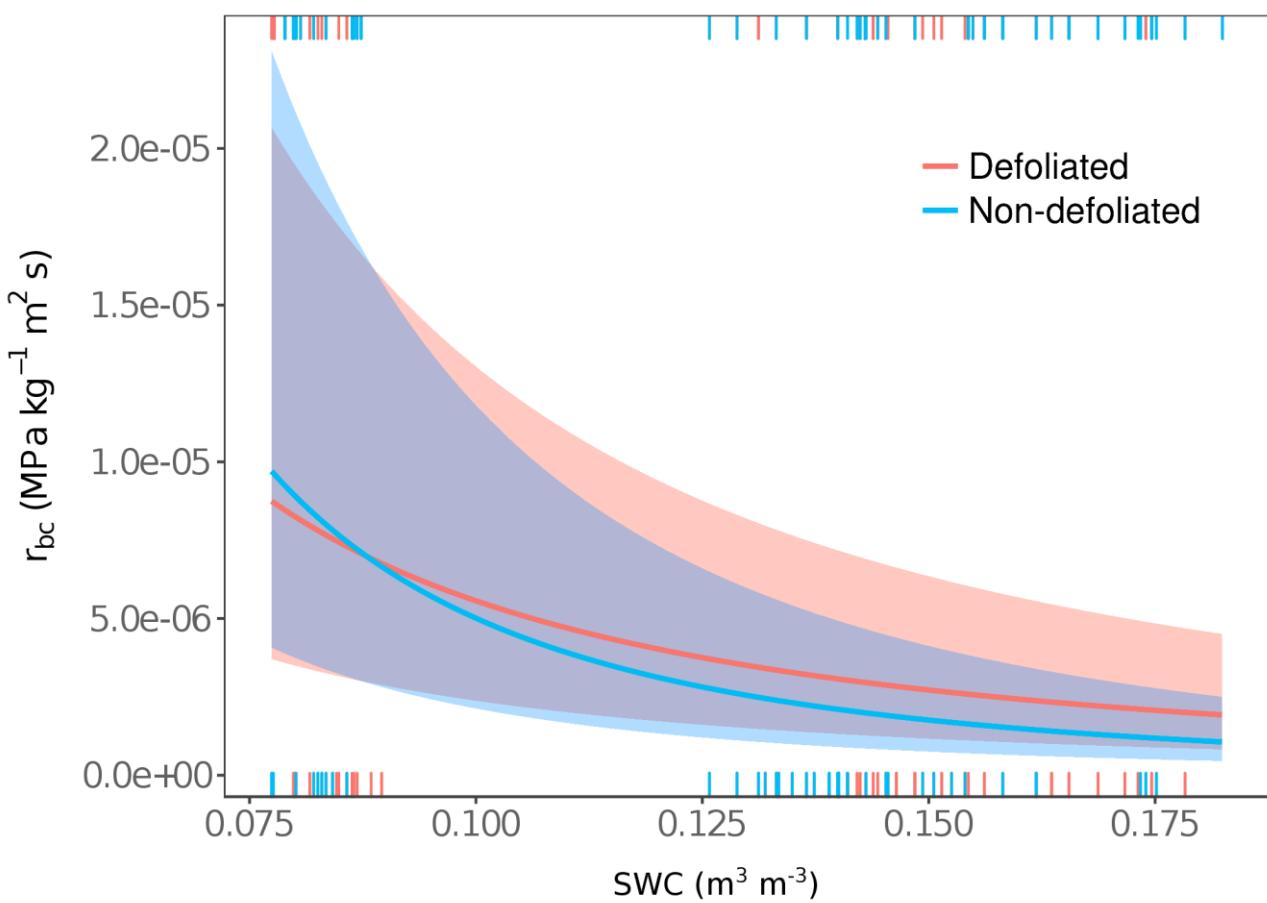


Fig. 5.

Appendix

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235 Table 3. Main characteristics of the trees studied. Defoliation class (D, defoliated; ND, non-
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4 defoliated), diameter at breast height (DBH), height (h), basal area (A_b), sapwood area (A_s),
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6 maximum leaf area (A_L), leaf-to-sapwood area ratio ($A_L:A_s$). The subset of trees with measurements
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8 of xylem radius variations are marked with an asterisk (*).
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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

1 **Table 4.** Summary of the linear mixed models of below-crown stem pressure difference (ΔP_{bc}) and
 2 below-crown hydraulic resistance (r_{bc}) as a function of soil water content (SWC), vapour pressure
 3 deficit (VPD) and defoliation class. For factors, the coefficients indicate the difference between
 4 each level of a given variable and its reference level. In models the reference defoliation class was
 5 “Defoliated”. The values depicted are estimates \pm SE. Abbreviations: ns, no significant differences;
 6 $*0.01 < P < 0.05$; $**0.001 < P < 0.01$; $***P < 0.001$; ni, not included in the model. R^2 (m, c) refer to
 7 marginal and conditional R^2 values, respectively.

	log(ΔP_{bc})	log(r_{bc})
	$R^2 (m, c) = 0.28, 0.81$	$R^2 (m, c) = 0.29, 0.85$
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)	$-0.15 \pm 0.23(\text{ns})$	$-1.77 \pm 0.13***$
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)

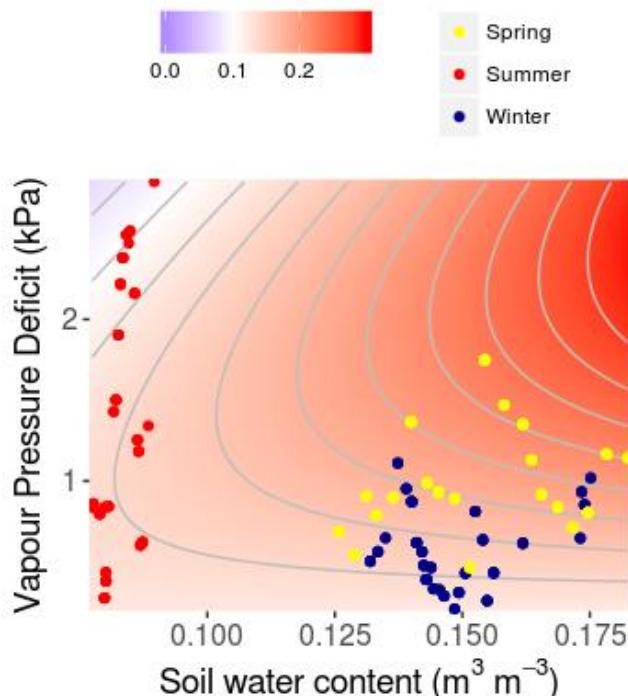
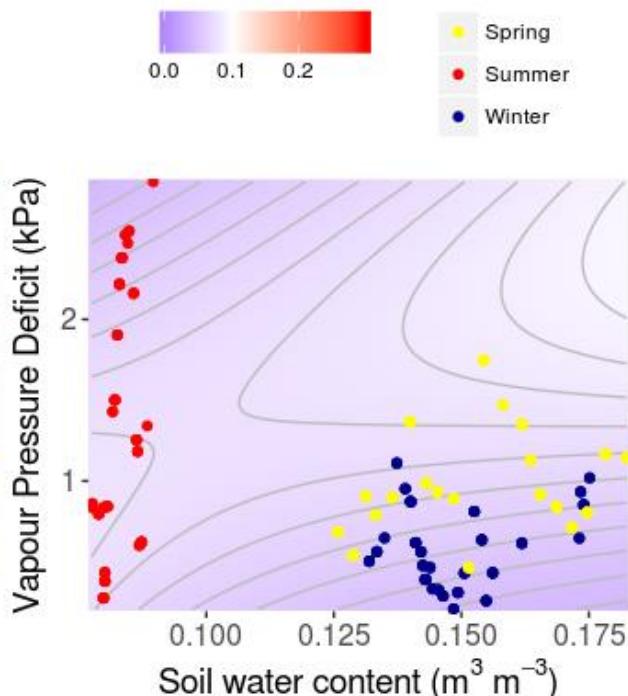
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a) ΔP (MPa), defoliateda) ΔP (MPa), non-defoliated

Figure 6. Modelled response surfaces of below-crown stem pressure difference (ΔP_{bc}) as a function of vapour pressure deficit (VPD), soil water content (SWC) and defoliation class (Table S2). Dots represent the VPD and SWC values corresponding to measured ΔP_{bc} values, coloured by season (cf. Methods).



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