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RESEARCH ARTICLE

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No role for xylem embolism or carbohydrate shortage in temperate trees during the severe 2015 drought

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Abstract

- Temperate forests are predicted to experience an increased frequency and intensity of climate change-induced summer droughts and heat waves in the near future. Yet, while previous studies clearly showed a high drought sensitivity of different temperate tree species, the vulnerability of the physiological integrity of these trees remains unclear.
- 2. Here, we assessed the sensitivity of six temperate tree species to severe water limitation during three consecutive growing seasons, including the exceptional 2015 central European summer drought and heat wave. Specifically, we assessed stem increment growth, sap flow, water potentials, hydraulic vulnerability, and nonstructural carbohydrate contents in leaves and branches to determine how mature temperate trees responded to this exceptional weather event and how the observed responses relate to variation in xylem embolism and carbohydrate economy.
- 3. We found that the trees' predawn water potentials reached their minimum values during the 2015 summer drought and most species reduced their sap flow by up to 80%. Also, increment growth was strongly impaired with the onset of the drought in all species. Despite the strong responses in the trees' growth and water relations, all species exhibited minimum midday shoot water potentials well away from values associated with severe embolism (P_{50}). In addition, we detected no distinct decrease in nonstructural carbohydrate contents in leaves, bark, and stems throughout the drought event.
- 4. Synthesis. This study shows that mature individuals of six common central European forest tree species strongly reacted to a severe summer drought by reducing their water consumption and stopping growth. We found, however, no indications for xylem embolism or carbohydrate depletion in these trees. This suggests, that xylem embolism formation and carbohydrate reserve depletion are not routine in temperate trees during seasonal strong drought and reveals a low vulnerability of the physiological integrity of temperate trees during drought events as we describe here.

KEYWORDS

carbon starvation, drought, ecophysiology, heat, hydraulic safety, mature trees, water limitation

1 | INTRODUCTION

Current climate models project an increased frequency and intensity of heat waves and drought events globally (Trenberth, 2011) and for central Europe in particular (Fischer et al., 2014; Orth, Zscheischler, & Seneviratne, 2016). The consequences of the increased frequency and duration of heat waves and drought events for terrestrial forest ecosystems are thought to be manifold (Reichstein et al., 2013). Among others, heat waves and drought events can disrupt the physiological integrity of trees which in turn affects tree growth and can even cause large-scale tree mortality leading to forest dieback with severe consequences for ecosystem goods and services (Allen et al., 2010; Carnicer, Coll, & Ninyerola, 2011; Williams, Allen, & Macalady, 2013).

The physiological susceptibility of trees in the temperate forest biome to heat and drought is surprisingly poorly understood. Those studies that have assessed the water relations of temperate trees during naturally occurring drought indicate that mature trees respond very sensitively to drought (Brinkmann, Eugster, Zweifel, Buchmann, & Kahmen, 2016; Hoffmann, Marchin, & Change, 2011; Hölscher, Koch, Korn, & Leuschner, 2005; Köcher, Gebauer, Horna, & Leuschner, 2009; Meinzer et al., 2013). For example, Brinkmann et al. (2016) have shown that trees strongly reduced their sap flow for several weeks in response to low soil moisture. Also, during the 2003 centennial heatwave in central Europe, various temperate tree species revealed a dramatic decline in sap flow for extended periods of time (Leuzinger, Zotz, Asshoff, & Körner, 2005). While these previous studies clearly showed the drought sensitivity of different temperate tree species, it remains unclear to what extent this drought response indicates a high vulnerability of these trees with respect to their physiological integrity.

Besides other factors, xylem embolism and carbohydrate depletion are thought to be among the main processes that can compromise the physiological integrity of trees during drought (Martínez-Vilalta, Lloret, & Breshears, 2012; McDowell, 2011; Mencuccini, Minunno, Salmon, Martínez-Vilalta, & Hölttä, 2015; Zeppel, Anderegg, & Adams, 2012). Xylem embolism is the result of a disruption of the water column due to very high tensions in the xylem conduits (Sperry, 2000). High levels of embolism are assumed to impair water supply to the foliage and ultimately lead to tissue desiccation. While some studies suggest that xylem embolism may play an important role during exceptional and devastating drought events (Anderegg et al., 2016), it is still discussed whether xylem embolism is a common phenomenon in mature trees under nonlethal drought events (Cochard & Delzon, 2013; Klein et al., 2016). The depletion of nonstructural carbohydrate pools has been suggested to result from the extended closure of stomata during drought leading to reduced photosynthesis and eventually a shortage of carbohydrate metabolites in different tree tissues (Hartmann, 2015). However, tree carbohydrate reserve pools were often only measured towards the end of drought periods (Adams et al., 2017; Galiano, Martinez-Vilalta, & Lloret, 2011) or observed in seedlings under additional shading treatment (Hartmann, McDowell, & Trumbore, 2015; Hartmann, Ziegler, & Trumbore, 2013;

Hartmann, Ziegler, Kolle, & Trumbore, 2013; Maguire & Kobe, 2015). Hence, most of the information on the vulnerability of temperate trees to xylem embolism and carbohydrate depletion was obtained in experimental work or from very few scattered measurements throughout or at the end of a given period of time. Evidence is therefore needed on the physiological integrity of mature temperate trees during naturally occurring drought events, in particular with respect to xylem embolism and carbohydrate depletion.

In the months July, August and September 2015, most parts of central Europe were hit by an exceptional heat wave and dry spell. The 2015 summer precipitation amount was among the lowest quantities since 1901 and soil moisture was even lower than during the centennial heat wave of 2003 (Orth et al., 2016). The 2015 summer drought thus exposed forests to weather conditions that are expected to regularly occur during central European summers by the end of this century. The impacts of the 2015 heat wave and dry spell on agriculture and human health were dramatic, with substantial losses in yield and an estimated number of 800 human fatalities that were attributed to the heat wave in Switzerland alone (Federal Office for the Environment (FOEN), 2016). We took advantage of this exceptional climatic event and tested (a) how mature individuals of six temperate tree species responded in their water relations and growth to the severe water limitation during the 2015 summer drought. We also determined (b) the physiological integrity of these trees with respect to xylem embolism and levels of carbohydrate reserves.

2 | MATERIALS AND METHODS

2.1 | Study site and study species

We conducted our study from 2014 to 2016 in a diverse mixed forest 15 km south of Basel, Switzerland (47°28'N, 7°30'E) equipped with a canopy crane (Swiss Canopy Crane facility; Pepin & Körner, 2002). The forest is located at an elevation of 550 m a.s.l. and has a rocky rendzina-type soil based upon calcareous bedrock at *c*. 1 m depth. The forest contains a mix of evergreen and deciduous tree species, dominated by *Fagus sylvatica* L. and *Quercus petraea* (Matuschka) Liebl. Other frequent species are *Abies alba* Mill., *Larix decidua* Mill., *Picea abies* (L.) Karst, *Pinus sylvestris* L., and *Carpinus betulus* L. (Pepin & Körner, 2002). The trees are about 130 years old and between 35 and 40 m tall. We performed measurements on four mature individuals of the species *C. betulus*, *F. sylvatica*, *L. decidua*, *P. abies*, *P. sylvestris*, and *Q. petraea* during the growing seasons of 2014, 2015, and 2016 (1 May to 31 October), resulting in a total of 24 study trees. *L. decidua* was only investigated in 2015 and 2016.

The climate at the site is temperate-humid with mild winters and moderately warm summers (mean January and July temperatures of 2.1°C and 19.2°C, respectively). Total mean annual precipitation of the region is c. 900 mm with two-thirds of precipitation generally falling during the growing season (15 April to 31 October). We measured air temperature, relative humidity, and precipitation during all three

years with a weather station (Davis Vantage Pro 2, Scientific Sales Inc., Lawrenceville, NJ, USA) at ten-minute intervals. We also recorded soil water potential (Ψ_{soil}) at 20 cm depth with dielectric sensors (MPS-2, Decagon Devices, Pullman, WA, USA) on ten-minute intervals. We employed 20 Ψ_{soil} sensors in 2014 and 12 in 2015 and 2016.

To put our three-year sampling campaign into the long-term climatic context of the site, we analysed precipitation and temperature data from 1900 until present for the weather station Basel-Binningen, which is located at 8 km from the research site. The data were provided by the Federal Office of Meteorology and Climatology (Zurich, Switzerland). Furthermore, we extracted the Standardized Precipitation Evapotranspiration Index (SPEI) at our site for July and August since 1955 from the Global SPEI database (Vicente-Serrano, Beguería, & López-Moreno, 2010).

2.2 | Sap flow measurements

To determine the transpiration response of the six species to drought, we measured the sap flow of four individuals of each species from April 2014 to October 2015 using Granier-type heat dissipation probes (SFS2-M, UP GmbH, Ibbenbüren, Germany) installed at the NE and SW sides of the stems. At the two insertion points of the sensor needles (at c. 1.5 m stem height), the bark was carefully peeled off. Aluminium sleeves were inserted 20 mm deep into the sapwood with a 10-cm vertical distance from each other. Then, the greased sensor needles were inserted into the sleeves and sealed with Teroson MS 930[©] sealing adhesive from the outside. Sensors were protected from weather influences and solar radiation by a radiation shield made of thick bubble warp aluminium foil. During operation, the upper needle constantly heated the sapwood and measured temperature with a copper-constantan thermocouple, while the lower needle measured the ambient sapwood temperature. Sensor voltage values were recorded every 10 min with a sensor node (Channel Node, Decentlab GmbH, Dübendorf, Switzerland), wirelessly transmitted to a data logger (Base Station, Decentlab GmbH) and then broadcast to a server via cellular network.

Data evaluation was done in compliance with the method of Granier (1985; 1987) and the considerations of Peters et al. (2018). We calculated the temperature difference between the two needles (ΔT) of each sap flow device from voltage values. In order to precisely estimate no-flux conditions during night-time and account for night-time transpiration, no-flux conditions were defined as the maximum ΔT (ΔT_{max}) during the night within a five-day period. In case of a drift over time in ΔT_{max} , we applied a linear regression through ΔT_{max} over five days and set ΔT_{max} to the regression values if it was below the regression line. If the individual trees were found to exhibit a sapwood depth shorter than the length of the sensor needles, we performed a correction of ΔT to determine sapwood ΔT excluding the fraction of the probes that was inserted into nonconducting heartwood (Clearwater, Meinzer, Andrade, Goldstein, & Holbrook, 1999):

$$\Delta T_{\rm SW} = \frac{\Delta T - b \Delta T_{\rm max}}{a}$$

where *a* and *b* are the fractions of the sensor needle in sapwood and inactive heartwood, respectively, and ΔT_{sw} is the temperature difference between the sapwood proportions of the needles. Total sap flow density *u* was calculated (Granier, 1985; 1987) by

$$u = 119 * 10^{-6} * K^{1.231}$$

where K is a dimensionless parameter:

$$K = \frac{\Delta T_{\max} - \Delta T}{\Delta T}$$

Since increment growth and a concomitant shift of maximum sap flow velocities would render the sensor signal over the years, we corrected sap flow of each sensor per individual by the difference between the two years to account for year-to-year differences: We screened for days with similar environmental conditions (Ψ_{soil} , VPD, PAR) in both summers of the two growing seasons and divided the mean daily maximum sap flow of 2014 on these days by the respective mean of the values of the corresponding days in 2015. All maximum daily sap flow values of 2015 were then multiplied with the resulting conversion factor.

To allow an easy comparison of seasonal sap flow patterns across the six species, we only considered relative daily maximum sap flow values which were calculated for each sensor by dividing the daily maximum absolute sap flow by the 95th percentile of maximum sap flow values throughout the 2014 and 2015 growing seasons. We calculated the mean sap flow values of both sensors per tree and then averaged these values to obtain mean values for each species (n = 4per species).

2.3 | Water potential measurements

To evaluate the water status of the trees, we measured midday (Ψ_{midday}) and predawn $(\Psi_{predawn})$ shoot water potentials. Ψ_{midday} was assessed around noon at an irregular interval throughout the two growing seasons (10 and 17 measurement campaigns in 2014 and 2015, respectively). $\Psi_{predawn}$ was assessed shortly before dawn on nine days during the 2015 growing season. Ψ_{midday} and $\Psi_{predawn}$ were measured with a Scholander pressure bomb (Model 1000, PMS Instruments, Albany, OR, USA) on three *c*. 15 cm long terminal shoots per tree with two to four leaves (broad-leaved) from the upper part of the sunlit crown. To obtain values for a species, we first averaged the three shoot measurements per tree and then averaged the means of the individual trees, resulting in a total number of four replicates per species.

2.4 | Stem diameter variations and modelling of Ψ

To determine the seasonal increment growth and the water deficit of the trees, we installed automated point dendrometers (ZN11-T-WP, Natkon, Oetwil am See, Switzerland) to assess diurnal and seasonal stem diameter variations (SDV). On each tree, one dendrometer was installed on the north-east facing side of the stem at *c*. 2 m of height

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at the beginning of the study (April 2014). From diurnal SDV, we calculated tree water deficit (TWD) as described in Dietrich, Zweifel, and Kahmen (2018). In brief, TWD is a measure for water loss in the nonconducting stem tissue that is expressed in the shrinking of the stem when transpiration of the tree exceeds water uptake of its roots. TWD equals zero when the tree is fully hydrated and increases when the tree is exposed to progressive soil drying and cannot refill its nonconducting tissue during night-time.

TWD has been shown to correlate with Ψ (Dietrich, Zweifel, et al., 2018; Drew, Richards, Downes, Cook, & Baker, 2011). We thus employed TWD to model the seasonal variability of Ψ_{midday} and $\Psi_{predawn}$ at a daily resolution for the 2014–2016 growing seasons. To do so, we employed linear functions that we empirically obtained for each of the six species from the 27 and 9 observations of Ψ_{midday} and $\Psi_{predawn}$, respectively, and their corresponding TWD values (Dietrich, Zweifel, et al., 2018). We then used these functions for each species to model Ψ_{midday} and $\Psi_{predawn}$ for each day of the study period on a species level.

SDV were also used to calculate the daily radial increment for all 24 trees. We considered growth to only occur during periods of effective diameter increases, and assumed no growth during periods of stem shrinkage (Zweifel, Haeni, Buchmann, & Eugster, 2016). Hence, during times of radial shrinkage, stem diameter was set to the last maximum measured before shrinkage for the calculation of daily increment growth. All individuals of a species were pooled to obtain a single mean stepwise-increasing increment growth curve per species. The stem increment data from all three years were standardized on the total increment growth from 2014 to 2016 and expressed as % growth of three years.

2.5 | PLC curves, P_{50}/P_{88} and hydraulic safety margin

A branch segment of about 35 cm length and 1 cm of diameter was collected from the sunlit crown of as many individual trees per species that were safely accessible from the crane before dusk in October 2015. The branch segments were directly wrapped into moist paper towels and stored in plastic bags at 4°C. Branch segments were sent to the Caviplace lab at INRA Bordeaux within a week, where they were stored at 4°C prior to measurements. Samples were then recut to 28 cm long segments under tap water. Centrifuge measurements were performed within three weeks using the Cavitron technique (Cochard, 2002; Cochard et al., 2005). For Q. petraea, we collected branch segments of 1.2 m and performed maximum vessel length estimations on additional stems by injecting air at 2 bars and cutting the apical end of the water-immersed stem section until air bubbles emerged. This procedure revealed that Q. petraea stems have a maximum vessel length of c. 50 cm, which confirms that vulnerability curves in this species cannot be adequately constructed using the 27 cm diameter rotor, where a significant proportion of open-cut vessels surpass the centre of the plant segment or even permeate through its whole length. Therefore, all samples were recut under water at 1 m and measured with a large rotor Cavitron. The stem segments were spun at different speeds, thereby creating water potentials from moderate to very negative values within the segments. The hydraulic conductance of the stem segments at each generated water potential was measured, and based on the initial conductance of the segment the loss of conductance at the generated water potentials was calculated according to Wang, Burlett, Feng, and Tyree (2014). Since measurements with the large rotor can only be conducted on very straight branch segments, only two specimens of *Q. petraea* turned out to be adequately measurable.

We fitted a logistic function through each set of data points per individual and extracted the pressure values at 50% loss of conductivity (P_{50}) and 88% loss of conductivity (P_{88}) from the resulting percent loss of conductivity (PLC) curve. Both the P_{50} and P_{88} were then averaged per species. We calculated hydraulic safety margins ($\Delta\Psi$) for the six species by subtracting the mean P_{50} or P_{88} value from the minimum Ψ_{midday} measured and modelled during the three growing seasons (Ψ_{min}) for each species. In order to account for the proposed higher sensitivity of conifers over angiosperm trees to hydraulic failure (Choat, 2013; Delzon & Cochard, 2014), we calculated $\Delta\Psi$ with P_{50} values for the three conifer species ($\Delta\Psi_{50}$), and with P_{88} for the three angiosperm species ($\Delta\Psi_{88}$).

2.6 | Nonstructural carbohydrates (NSC)

NSC (i.e., starch, sucrose, fructose, and glucose) were quantified in leaves, bark and xylem of sun-exposed 3- to 4-year-old branches in each of the four individuals of the six species (one branch per individual). Samples were collected throughout the summer of 2015. For chemical analysis in the laboratory, we used a modified protocol after Wong (1990): 8-12 mg of the dried (24 hr at 75°C) and finely ground plant tissue was extracted with 2 ml of distilled water in glass vials which were covered with marbles and boiled over steam at 100°C for 30 min. To degrade sucrose and convert fructose to glucose, an aliquot of 200 µl of the solution was treated with invertase, an isomerase from baker's yeast. Then, the glucose in the solution was converted to gluconate-6-phosphate using glucose-hexokinase. The total amount of gluconate-6-phosphate (equal to glucose concentration) was determined with a 96-well multiplate photometer (Multiscan EX, Thermo Fisher Scientific, Waltham, MA, USA) at 340 nm. For the determination of starch concentrations, a fungal amyloglucosidase from Aspergillus niger was added to an aliquot of 500 μ l of the remaining extract. The solution was put in a water bath at 49°C for 12 hr to progressively digest starch to glucose. Total glucose concentration in the solution was then determined with a photometer as described above. Starch concentration was calculated as the difference between the glucose concentrations with and without degradation of starch. Enzymes were purchased from Sigma-Aldrich (St. Louis, MO, USA), and solutions of glucose, fructose and sucrose as well as pure starch and a homogenized plant powder (Orchard leaves, Leco, St. Joseph, MI, USA) were used as standards and control of reproducibility.

2.7 | Statistical analyses

Statistical analyses and data visualization were done using R, version 3.4.1 (R Core Team, 2015), with its packages zoo (Zeileis & Grothendieck, 2005), xts (Ryan & Ulrich, 2014), data.table (Dowle, Srinivasan, Short, & Lianoglou, 2015), caTools (Tuszynski, 2014), scales (Wickham, 2015), gridExtra (Auguie, 2015), and ggplot2 (Wickham, 2009). We assumed a *p* < 0.05 as the level of significance for all statistical tests. For the regression analyses between Ψ_{midday} and Ψ_{soil} , we used the *nls*() command with the function

$$\psi_{\text{midday}} = a * \left(1 - e^{\frac{\psi \text{soil}}{b}} \right)$$

The parameters *a* and *b* thereby determine the saturation value and curvature of the function, respectively. Relationships between sap flow and Ψ_{soil} were assessed with a linear regression model per species (*Im*() function). We used a repeated-measures ANCOVA to test for species effects on sap flow and growth.

3 | RESULTS

3.1 | Environmental conditions

For central Europe, the 2015 summer had the lowest rainfall since 1901, and soil moisture was even lower than during the centennial heat wave of 2003 (Orth et al., 2016). For our research site, the year 2015 was the second warmest since climate recordings began in 1900 at a nearby climate station. These temperature extremes are represented by both the mean annual and the mean July/August temperature (Figures 1 and 2). In addition, the summer (i.e., July to September) of 2015 was among the 10% of summers that exhibited the lowest rainfall amounts of the past 100 years with c. 45% less precipitation in the months July-September compared to the long-term mean (Figures 1 and 2). The extreme air temperatures in July. August. and the beginning of September 2015 also caused VPD during this time to be substantially higher than in the same period in 2014 or 2016 (Figure 1). High summer VPD in combination with low seasonal precipitation resulted in substantial soil drying, where Ψ_{soil} at -20 cm progressively decreased to -1.3 MPa in July, August, and September 2015 and remained low throughout these months (Figure 1). The extent of soil drying during the summer of 2015 becomes particularly apparent when compared to the same months in 2014 and 2016. While long-term soil moisture records do not exist for our research site, regional hydrological models suggest that soil moisture in northwestern Switzerland in the 2015 summer was within the lowest five percentiles compared to the record available since 1979 (Orth et al., 2016). Differences in Standardized Precipitation Evapotranspiration Index (SPEI) also clearly show the severely dry climate conditions in 2015 when compared to the record, and the overall decreasing trend of SPEI towards drier summers at our study site (Figure 2). The heat and drought of 2015, however, did not lead to any apparent signs of reduced health or mortality in the study trees.



FIGURE 1 Environmental data for the growing seasons 2014, 2015, and 2016. Dashed lines around mean daily temperature are minimum and maximum temperatures of the respective day. All variables except Ψ_{soil} were measured with a weather station at the top of the canopy crane at 40 m above-ground. Ψ_{soil} was calculated as the mean of the daily maxima of 20 (2014) and 12 (2015 and 2016) sensors at a depth of 20 cm ± SD



FIGURE 2 Frequency distribution of annual precipitation, mean annual temperature, mean July and August temperature since 1900 at Binningen (Canton Basel County) in the vicinity of the study site, and mean July/August Standardized Precipitation Evapotranspiration Index (SPEI) of the region since 1955. The inlet graph shows SPEI values across years. The decreasing linear regression (±95% CI) is significant at *p* < 0.05. Arrows indicate the values of the three study years and the record summer of 2003 [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Seasonal patterns of Ψ_{predawn} and Ψ_{midday}

Modelled $\Psi_{\rm predawn}$ values of the six species were in good agreement with measured values ($R^2 = 0.76$ across all species; Supporting Information Figure S1). Modelled seasonal variation in $\Psi_{predawn}$ differed substantially among the six species and among years (Figure 3). Importantly, however, all species reached the most negative $\varPsi_{\rm predawn}$ of the three years during the 2015 summer drought (down to -2.1 MPa in P. abies) and exhibited the least negative minima in 2014. Also, $\Psi_{\rm predawn}$ remained low throughout July, August, and September 2015, indicating that the 2015 summer drought severely impacted water availability of the trees for an extended amount of time. We observed substantial differences in $\Psi_{\rm predawn}$ during the 2015 summer drought among the six species, where $\Psi_{\rm predawn}$ was particularly negative in C. betulus, F. sylvatica and P. abies, while P. sylvestris, L. decidua, and Q. petraea showed less negative values. Variation in $\Psi_{\rm predawn}$ among years ranged between 0.5 and 1.5 MPa, and was higher in P. abies, C. betulus, and F. sylvatica (above 1 MPa), while Q. petraea, P. sylvestris, and L. decidua revealed comparably low amounts of variation (below 1 MPa).

Modelled midday values for Ψ were also in good agreement with measured values ($R^2 = 0.71$ across all species; Supporting Information Figure S1). Similar to $\Psi_{
m predawn}, \, \Psi_{
m midday}$ differed substantially among the six species and years. $\varPsi_{\mathrm{midday}}$ showed Pearson correlation coefficients close to 1 with daily minimum $\varPsi_{\rm soil}$ at 20 cm depth in all species (Table 1). The most negative $\Psi_{\rm middav}$ values for each species were reached during the 2015 summer drought (Figure 3). C. betulus, F. sylvatica, and P. abies reached their most negative Ψ_{midday} towards the end of the 2015 summer drought, while Q. petraea, L. decidua, and P. sylvestris exhibited $\varPsi_{\rm middav}$ values that were consistently low throughout the 2015 summer drought. During 2015, Q. petraea reached the most negative $\Psi_{\rm midday}$ values of all species followed by L. decidua, F. sylvatica, P. abies, C. betulus, and P. sylvestris. In P. sylvestris, midday values turned out to vary only slightly throughout the 2015 summer drought, and unlike for the other species, no pronounced extreme values were observed (Figure 3).

To test if the Ψ_{midday} values during the 2015 summer drought were saturating at minimum values, we plotted Ψ_{midday} over Ψ_{soil} for each species (Figure 4). All of the species showed Ψ_{midday} to level off with decreasing Ψ_{soil} . This indicates that the species were able to maintain a minimum Ψ_{midday} despite the continuous decrease of Ψ_{soil} during the investigated drought period.

3.3 | Hydraulic integrity

 P_{50} and P_{88} values varied significantly among the six species (Table 2, Supporting Information Figure S2). *Q. petraea* was the most embolism-resistant species (most negative P_{50} and P_{88} values), while *P. sylvestris* and *L. decidua* showed the least negative values for P_{50} and P_{88} .

 $\Psi_{\rm midday}$ of the different species was at least c. 1 MPa higher than the measured P_{50} values throughout the whole study period (Figure 4). This indicates that the investigated species experienced no or little embolism (at most 10% loss of conductance in branches), even during the 2015 summer drought (cf. Supporting Information Figure S2). To determine the actual susceptibility of the investigated species to strong xylem embolism during the 2015 summer drought, we estimated the hydraulic safety margin (HSM, $\Delta\Psi$) for each species as the difference between the minimum $\Psi_{\rm midday}$ ($\Psi_{\rm min}$) of a respective species that was reached during the 2015 summer drought and the P_{50} (conifers) or P_{88} (angiosperms) value. HSMs ranged between 1.2 MPa in *L. decidua* and 3.9 MPa in *Q. petraea* (Table 2). *C. betulus* showed a wide HSM similar to *Q. petraea*, while *F. sylvatica* exhibited a HSM resembling more those of the coniferous species, which generally exhibited safety margins between 1 and 2 MPa (Table 2).

3.4 | Sap flow

For all species, daily maximum sap flow remained consistently high throughout the 2014 growing season (Figure 5). Values decreased only towards the very end of that growing season, which can be



FIGURE 3 Modelled predawn and midday Ψ_{shoot} of the six investigated species during the summers of 2014–2016. The values are derived from the relationship between predawn and midday Ψ_{shoot} and tree water deficit measured at the base of the stem on 9 (predawn) and 28 (midday) different days in the growing seasons of 2014 and 2015 (predawn only in 2015)

attributed to leaf senescence. In contrast, daily maximum relative sap flow decreased strongly in most species with the onset of the 2015 summer drought (around DOY 180) and stayed low until the end of the season (Figure 5). *C. betulus, F. sylvatica, P. abies,* and *P. sylvestris* reduced their maximum daily sap flow by up to 80% during the 2015 drought. *L. decidua* showed a reduction of about 60%.

The only exception was *Q. petraea*, which only weakly responded to the 2015 summer drought. Differences in sap flow among species and years were found to be statistically significant (Table 3). However, it is important to notice that differences among species only relate to differing responses of sap flow relative to the species-specific maximum and not to differences in absolute sap flow.

TABLE 1 Pearson correlation coefficients for the relationship of Ψ_{midday} with daily minimum Ψ_{soil} in the different species

| Species | Pearson correlation coef. |
|------------------|---------------------------------|
| Carpinus betulus | 0.87 |
| Fagus sylvatica | 0.79 |
| Quercus petraea | 0.74 |
| Larix decidua | 0.85 |
| Picea abies | 0.86 |
| Pinus sylvestris | 0.89 |
| | |

All species revealed their highest sap flow at a VPD of around 0.5–0.8 kPa (Supporting Information Figure S3). The coniferous species generally showed a steeper decline of sap flow with increasing VPD beyond 0.8 kPa than the angiosperm species, of which *Q. petraea* showed the weakest response (Supporting Information Figure S3). *P. abies* and *P. sylvestris* reached sap flow values close to zero at a VPD of *c*. 3 kPa. All of the species except *Q. petraea* showed a steep decline of sap flow with decreasing Ψ_{soil} at –20 cm soil depth, with *P. sylvestris*, *P. abies*, and *F. sylvatica* revealing the highest sensitivity (Supporting Information Figure S3).

3.5 | Nonstructural carbohydrates

NSC contents in leaves, bark, and xylem differed substantially among species and organs but showed no trend of declining values during the 2015 summer drought. In leaves, NSC values ranged from 15 mg/g dry wt in C. betulus to around 5 mg/g in P. sylvestris (Figure 6). In all species except Q. petraea, we observed slightly but nonsignificantly declining NSC values at the beginning of the 2015 summer drought, but either stable or increasing NSC contents throughout the twomonth drought period. In bark tissue, NSC contents were in the same range as foliar NSC contents and showed similar temporal patterns. We found that xylem NSC contents were either stable throughout the 2015 summer drought or declined slightly in the early summer but increased before the end of the drought (Figure 6). Independent of the seasonal dynamics and drought, a clear difference in NSC contents was found in branch xylem between angiosperm and conifer species. While the angiosperms had high xylem NSC contents (with a high proportion of starch) of 8 to 20 mg/g, the conifers only had low contents of around 2 mg/g (Figure 6). We found no significant differences in NSC within species and tissues throughout the 2015 summer drought. Most species, however, showed significant differences in NSC among tissues (Figure 6).



FIGURE 4 P_{50} values (horizontal lines) ± SD (cf. Table 2) and the relationship between modelled (open symbols) and measured (filled symbols) midday Ψ_{shoot} , and Ψ_{soil} in the considered species during the 2014, 2015, and 2016 summers (1 June to 30 September). P_{50} values were obtained from PLC curves deriving from centrifuge measurements on branches of a variable number of trees per species [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 P_{50} and P_{88} values (±*SD*) measured on branches from the sun-exposed canopy of the investigated species with the Cavitron technique and hydraulic safety margins ($\Delta \Psi$) calculated as the difference between the P_{50}/P_{88} value and the most negative measured/ modelled Ψ of the respective species. $\Delta \Psi$ values given in brackets are regarded as less meaningful for the respective species than those given without brackets (cf. Choat, 2013). For the assessment of P_{50}/P_{88} values, one branch per tree was harvested before dusk during October 2015, stored at 4°C and sent to the Cavitron laboratory in Bordeaux within one week. Pressures at 50% and 88% loss of conductance, respectively, were extracted from the resulting vulnerability curves. The number of replicates varied among species from five (*Fagus sylvatica, Larix decidua, Picea abies*) over four (*Carpinus betulus*) and three (*Pinus sylvestris*) to two (*Quercus petraea*)

| Species | P ₅₀ (MPa) | P ₈₈ (MPa) | ΔΨ ₅₀ (MPa) | ΔΨ ₈₈ (MPa) |
|------------------|-----------------------|-----------------------|------------------------|------------------------|
| Carpinus betulus | -4.7 ± 0.28 | -5.8 ± 0.34 | (2.6) | 3.7 |
| Fagus sylvatica | -3.8 ± 0.28 | -4.5 ± 0.35 | (1.5) | 2.2 |
| Quercus petraea | -5.0 ± 0.35 | -6.5 ± 0.02 | (2.3) | 3.9 |
| Larix decidua | -3.6 ± 0.40 | -4.4 ± 0.37 | 1.2 | (1.9) |
| Picea abies | -4.0 ± 0.37 | -4.8 ± 0.40 | 1.7 | (2.5) |
| Pinus sylvestris | -3.5 ± 0.42 | -4.6 ± 1.3 | 1.7 | (2.8) |

3.6 | Stem increment growth

Relative stem increment differed substantially among species and years (Figure 7, Table 3). Interestingly, the 2015 summer drought had no consistent effect on total annual stem increment compared to 2014 or 2016. The onset of growth was similar among years in all species around DOY 120. Stem increment growth continued throughout the growing seasons 2014 and 2016 with the end of seasonal stem increment being variable among species and years. Importantly, however, stem growth in 2015 ceased sharply in all six species with the onset of the summer drought around DOY 180 and did not or only negligibly resume in the 2015 growing season (Figure 7). We found significant effects on growth of DOY, year, and the interaction of species with both DOY and year (Table 3).

4 | DISCUSSION

The goal of this study was to assess the physiological integrity of mature temperate trees, in particular with respect to xylem embolism and carbohydrate reserves during the severe 2015 central European summer drought. For this purpose, we continuously determined the water relations and nonstructural carbohydrate (NSC) contents of 24 individuals of six common European temperate tree species. Our data indicate that the midday water potential of apical shoots $(\Psi_{
m middav})$ approached minimum values and that the trees responded by strongly reducing their sap flow (likely through stomatal closure) for several weeks during the drought event. Also, radial stem growth of all six species ceased sharply with the onset of the drought event and hardly resumed in that growing season. These findings indicate a high physiological sensitivity of all investigated temperate tree species to the 2015 summer drought. Importantly, however, minimum $\Psi_{\rm midday}$ of all species were far from values that would cause pronounced xylem embolism in the branches. Also, NSC reserves in leaves, bark, or xylem remained rather constant and did not indicate a depletion of carbohydrate reserves in any species throughout the drought period. In summary, our data indicate a high physiological sensitivity but nevertheless a low vulnerability of the hydraulic system and the carbon household of six major temperate tree species during the severe 2015 summer drought.

4.1 $\mid \Psi$, sap flow, and growth during the 2015 drought

Low Ψ_{soil} and $\Psi_{predawn}$ indicate that the high July, August, and September 2015 temperatures and the low 2015 growing season precipitation substantially reduced water availability in the soil and that the forest experienced indeed an exceptional drought in the months July, August, and September of 2015. In fact, $\Psi_{predawn}$ measured in the six species during the 2015 summer drought were in a similar low range as those observed in a previous study at the same site during the record-breaking 2003 centennial summer drought (Leuzinger et al., 2005).

 $\Psi_{\rm midday}$ was also low and reached values of -1.9 MPa (*P. sylvestris*) to -2.8 MPa (*Q. petraea*). These values are similar to minimum $\Psi_{\rm midday}$ found in temperate tree species during natural and experimental soil drying (Backes & Leuschner, 2000; Köcher et al., 2009; Lu, Biron, Granier, & Cochard, 1996; Maier-Maercker, 1998). Importantly, plotting $\Psi_{\rm midday}$ against $\Psi_{\rm soil}$ throughout the 2015 summer drought, revealed that $\Psi_{\rm midday}$ levelled off at species-specific minimum values (Figures 3 and 4) and that transpiration was substantially down-regulated to avoid even more negative $\Psi_{\rm midday}$ (Jones & Sutherland, 1991; Lemoine, Cochard, & Granier, 2002). It remains, however, unclear to which degree the stabilization of $\Psi_{\rm midday}$ in the six species was caused by a regulation of stomatal conductance and cuticular transpiration or other changes in the conductivity of the hydraulic pathway such as changes in root conductivity, petiole embolism, or a decline of leaf hydraulic conductivity.

Our data indicate that the 2015 summer drought pushed the study tree species into a range of $\Psi_{\rm midday}$ values which were strongly regulated to avoid a further rapid decrease. However, far more negative $\Psi_{\rm midday}$ have already been shown in temperate trees, suggesting that under extreme conditions when $\Psi_{\rm predawn}$ further decreases, the physiological regulation of $\Psi_{\rm midday}$ cannot keep up with



FIGURE 5 Relative daily maximum sap flow of the six investigated species from 1 June to 30 September in 2014 and 2015 (*n* = 4 individuals per species ± *SD*). Species means were fitted with a locally fitted nonparametric regression (LOESS). The sap flow of *Larix decidua* was only measured in 2015

progressive soil drying (Blackman et al., 2016; Breshears et al., 2009; Hoffmann et al., 2011; Martínez-Vilalta, Poyatos, Aguadé, Retana, & Mencuccini, 2014; Meinzer et al., 2016). Interestingly, all species showing Ψ_{midday} to level off with declining Ψ_{soil} suggests that the physiological reaction to drought is rather uniform among these temperate tree species (Figure 4, Supporting Information Figure S3). Thus, a distinct classification of species into an iso- or anisohydric

behaviour (Martínez-Vilalta et al., 2014) is not suitable in this case. Anyway, the isohydry/anisohydry concept has not proven useful when comparing drought susceptibility among species (Martínez-Vilalta & Garcia-Forner, 2016).

Corroborating the stabilization of $\mathcal{\Psi}_{\rm midday}$ at low $\mathcal{\Psi}_{\rm soil}$, we found that the species examined substantially reduced their sap flow throughout the 2015 summer drought, which is likely a measure

TABLE 3 Results of repeated measures ANCOVA on sap flow and radial growth of the six species

| Response variable | Factor | df | F-value | p |
|-------------------|--------------|----|---------|--------|
| Sap flow | Species | 5 | 4.35 | 0.005 |
| | DOY | 1 | 207.44 | <0.001 |
| | Year | 1 | 17.43 | <0.001 |
| | Species:DOY | 5 | 5.25 | 0.001 |
| | Species:year | 4 | 14.88 | <0.001 |
| Radial growth | Species | 5 | 1.922 | 0.14 |
| | DOY | 1 | 1584.51 | <0.001 |
| | Year | 1 | 1794.6 | <0.001 |
| | Species:DOY | 5 | 18.38 | <0.001 |
| | Species:year | 5 | 7.428 | <0.001 |

of the trees to stabilize Ψ_{middav} . Except for Q. petraea, sap flow was downregulated in all other species by up to 80% for several weeks. This suggests, that the trees' main moisture reservoirs in the soil were depleted but that the trees had yet access to some residual moisture sources-possibly in deep soil layers-to maintain some minimal gas exchange levels. Nevertheless, the gas exchange of the species was severely impacted for an extended period of time. Similar downregulation of sap flow in response to declining soil moisture (cf. Supporting Information Figure S3) has previously been described for a variety of temperate tree species (Brinkmann et al., 2016; Hölscher et al., 2005; Irvine, Perks, Magnani, & Grace, 1998; Leuzinger et al., 2005; Pataki, Oren, & Smith, 2000). However, in most cases, the respective drought periods were shorter, lasting only a few days to weeks. Only during the extreme heat drought of 2003, Leuzinger et al. (2005) showed that sap flow and stomatal conductance were decreased up to 50% and 80%, respectively, for several weeks, a situation similar to the 2015 summer drought investigated here.

Quite unexpectedly, we did not find a consistent effect of the 2015 summer drought on total annual stem radial growth compared to that of 2014 and 2016. The likely reason for the missing direct drought effect on annual growth is that spring growth in 2015 was substantial and exceeded that of the other two years (Figure 7). However, the increment growth of 2015 more or less ceased in all species with the onset of the drought, while radial growth in 2014 and 2016 extended well beyond DOY 180 in most species.

It is an ongoing debate whether stem growth can occur during very negative Ψ and at times when there is a water deficit in the trunk (Zweifel et al., 2016). Our data suggest that growth is strongly impaired during drought, which lines up with previous findings (Buell, Buell, Small, & Monk, 1961; Leuzinger et al., 2005; Pflug et al., 2015; Pichler & Oberhuber, 2007). Our data also show that weather effects on annual tree growth depend not only on the intensity and duration of the event but also on its timing and the environmental conditions that prevailed before (e.g., Zielis et al., 2014; D'Orangeville et al., 2018). In this regard, the mesic conditions in 2014 could have favoured a strong growth in 2015. It is, however,

important to note here that we only investigated basal increment growth. Above-ground leaf flushes and branch growth as well as below-ground root growth remain unknown in this study.

4.2 | Hydraulic integrity

All species were far from water potential values that seriously challenged the hydraulic system of the branches even at the low end of measured $\varPsi_{\rm soil}$ during the 2015 summer drought (cf. Figure 4, Table 2). At most, 10% of branch conductive capacity should have been affected by embolism during that period. The hydraulic safety margins that we found (Table 2) are within the range of values that Hoffmann et al. (2011) reported for temperate tree species of North America under moderate and strong drought. Although we cannot predict how the species we investigated here would behave during an even stronger drought than that of 2015, the fact that the $\Psi_{\rm midday}$ of all species levelled off with declining soil moisture suggests that it would take a stronger decline in $\Psi_{\rm soil}$ to induce substantially more negative $\varPsi_{\rm middav}$ values (combined with cuticular transpiration). By this means, the observed safety margins would be reduced only slightly for a range of further decreasing soil water availability until \varPsi_{midday} gets solely dependent on \varPsi_{soil} , and cuticular transpiration and stomatal leakiness. Our data, therefore, support the claims of Cochard and Delzon (2013) and Delzon and Cochard (2014) who stated that hydraulic failure is not routine in mature trees. Furthermore, our results are supported by a recent study on grapevine in which stomatal closure preceded embolism in leaves by days, and leaves were shed before a significant amount of embolism did accumulate in the stem (Hochberg et al., 2017; cf. Charrier et al., 2018). However, we acknowledge that when $\Psi_{\rm soil}$ gets extremely negative, the trees will not be able to significantly uncouple their $\Psi_{\rm predawn}/\Psi_{\rm midday}$ from the decrease in $\Psi_{\rm soil}$ as long as they do not take effective means to uncouple themselves from the soil matrix (cf. Cuneo, Knipfer, Brodersen, & McElrone, 2016). We conclude that the 2015 summer drought caused the tree species to significantly reduce their water loss but did not induce substantial amounts of xylem embolism in the branches of the trees. Therefore, xylem embolism is unlikely to disrupt the hydraulic integrity of the trees during the type of drought that we investigated here.

4.3 | Nonstructural carbohydrates

The NSC concentrations measured in leaves, bark, and xylem of the six species throughout the 2015 summer drought are in the same range as NSC concentrations measured in the same species (partly at the same location) in previous studies, when the trees were not exposed to drought (Bazot, Barthes, Blanot, & Fresneau, 2013; Hoch, Richter, & Körner, 2003; Körner, 2003). Given that NSC concentrations remained mostly constant throughout the 2015 summer drought and the fact that the concentrations that we measured are in the same range as concentrations of not drought-stressed trees measured in previous years, we conclude that the nonstructural carbohydrate reserves were not significantly impacted by the 2015



FIGURE 6 Nonstructural carbohydrate contents (divided into starch and sugar) of leaf, bark, and branch xylem in the six investigated species over the course of the 2015 summer drought (n = 4 individuals per species ± SD). A Tukey's Honest Significant Difference post hoc test (lower case letters) revealed no significant differences within tissues of the different species throughout the measurement period



FIGURE 7 Basal increment growth of the six species in 2014, 2015, and 2016 (n = 4 trees per species \pm SD). To obtain growth curves, we connected the maxima of continuously measured stem diameter variations. Therefore, only increases in diameter above the last measured radius maximum were considered as growth

summer drought in any of the six investigated species. It is, however, important to note that the cessation of basal increment growth (strong sink reduction) could have prevented decreasing NSC values in the leaves and branches (Anderegg, 2012).

Overall, the response of NSC tissue concentrations to a 12-week summer drought that we report here supports the increasing evidence from recent studies that NSC concentrations in trees are a very conservative and extremely resistant trait (cf. Weber et al., 2018) even under severe drought conditions. Previous studies investigating NSC concentrations in trees under drought revealed decreasing NSC concentrations only in potted seedlings or saplings (Adams et al., 2013; Hartmann, Ziegler, & Trumbore, 2013; Mitchell et al., 2012) or in trees that were heavily impacted or died due to extreme drought (Adams et al., 2017; Galiano et al., 2011). Other studies described only small or no differences in NSC concentrations between droughttreated and control trees (Gruber, Pirkebner, Florian, & Oberhuber, 2011; Klein, Hoch, Yakir, & Körner, 2014; Rowland et al., 2015), while Galvez, Landhäusser, and Tyree (2011), and Anderegg (2012) even reported an increase of NSC concentrations in Populus tremuloides under drought. The data we present here are thus supported by previous findings suggesting that a depletion of NSC is rather unlikely to affect the physiological integrity of temperate tree species during drought events such as we observed (e.g., Körner, 2003; Rosas, Galiano, Ogaya, Penuelas, & Martínez-Vilalta, 2013).

5 | CONCLUSIONS

This study reports the effects of a severe 12-week summer heat drought on the water and carbon relations of mature individuals of six temperate forest tree species. We observed that the 2015 summer drought dramatically affected the water relations and growth of the trees. However, our data do not suggest xylem embolism in branches or the depletion of carbohydrate reserves throughout the drought. Hence, our results indicate that xylem embolism and declining carbohydrate reserves do not compromise the physiological integrity of temperate trees during the type of severe drought that we investigated in this study. We would like to caution, however, that a sequence of reoccurring drought events in subsequent growing seasons or a different timing of the drought could possibly lead to stronger effects on the hydraulic system or the carbohydrate reserves of trees than we report here. Further, effects such as pest infestation or herbivory are likely to impact the vigour of temperate European tree species during intense droughts.

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AUTHORS' CONTRIBUTIONS

L.D. and A.K. designed the study. L.D. conducted the fieldwork and analysed the data. Measurements of hydraulic vulnerability were done in the laboratory of S.D. All authors jointly interpreted the data and wrote the manuscript.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: https://doi. org/10.5061/dryad.25b8k25 (Dietrich, Delzon, Hoch, & Kahmen, 2018).

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