Wood anatomical responses of oak saplings exposed to air warming and soil drought

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Abstract

Water is vital for plant performance and survival. Its scarcity, induced by a seasonal decline in soil water availability or an increase of evaporative demand, can cause failures of the water conducting system. An adequate tolerance to drought and the ability to acclimate to changing hydraulic conditions are important features for the survival of long-lived woody plants in dry environments.

In this study we examine secondary growth and xylem anatomical acclimation of 6 year old saplings of three European oaks species (*Quercus robur*, *Q. petraea*, *Q. pubescens*) during the third consecutive year of exposure to soil drought and/or air warming (from 2007 to 2009). Intra-annual pinning was applied to mark the development of the formation of the annual ring 2009. Vessel size, parenchyma cell density and fiber size produced at different time of the growing season 2009 were compared between drought and warming treatments and species. Drought reduced secondary growth and induced changes in xylem structure while air warming had little effect on wood anatomical traits. Results indicate that drought-exposed saplings adjust their xylem structure to improve resistance and repairing abilities after cavitation. All species show a significant radial growth reduction, a reduced vessel size with diminished conductivity and a slightly increased density of parenchyma cells. Comparisons between species fostered our understanding of the relationship between the inter-specific xylem hydraulic plasticity and the ecological behavior to drought. The stronger changes observed for *Q. robur* and *Q. petraea* indicate a lower drought tolerance than *Q. pubescens*.

Introduction

Survival and performance of long-lived woody plants strongly depends on their ability to adjust their metabolic processes to the continuous and highly variable environmental conditions. From a broad perspective, there are two main strategies to cope with changes: species may adapt through genetic changes via the process of evolution, or acclimate by exploiting their range of phenotypic and physiological plasticity (Lambers et al., 2008). However, due to the

unprecedented rate of climate change and relative little time for altering the genetic constitution, responses of long-lived plants have mainly to rely to their acclimation capacity (Sultan, 2000; Valladares et al., 2007). Acclimation processes, i.e., positive structural or physiological response to new conditions, usually take place over the course of weeks to years, and thus differ from the short-term physiological regulation.

Among the environmental factors expected to vary with global climate change, soil water scarcity has been identified as the major factor affecting plant growth, development and productivity (IPCC, 2007). Drought is in fact an important stress factor and can lead to physiological and structural responses to maintain balanced water relations (Maherali and DeLucia, 2000; Arend and Fromm, 2007; McDowell et al., 2008; Eilmann et al., 2009; Martinez-Vilalta et al., 2009). To fix carbon and to grow, woody plants have to collect and transport water from the soil up to the leaves where photosynthesis occurs (Hopkins and Hüner, 2008). Water evaporating from the leaf generates a negative tension which is transmitted downwards through continuous water columns to the root-soil interface (Zimmermann, 1983). This hydraulic pathway is vulnerable to breakage through the induction of cavitation, when conduits are under exceedingly high tension (Tyree and Zimmermann, 2002; Lambers et al., 2008).

To cope with drought induced hydraulic damages, trees have developed an array of mechanisms occurring at different time scales (Maseda and Fernandez, 2006). Stomatal closure is the fastest physiological process to limit transpiration preventing a critical decrease in water potential (Tyree and Ewers, 1991; Sperry et al., 2008; Zweifel et al., 2009). Xylem embolism under severe drought can also be avoided through long-term acclimation processes occurring through changes on the structural level. On one hand trees can limit their need for water by reducing transpiring leaf area (Rigling et al., 2010), or by increasing the accessibility to the water source by expanding the roots (Klein et al., 2011). On the other hand however, trees can also adjust their water transport system by making it more tolerant to cavitation, which usually occurs at the cost of efficiency and performance (Sperry et al., 2008; McDowell, 2011). Tolerance to cavitation can be attained by i) increasing prevention against cavitation through a reinforced cell wall structure (Hacke et al., 2001; Domec et al., 2008); ii) increasing

the capacity to actively refilling embolized conduits using starch related energy stored in parenchyma cells (Bucci et al., 2003; Zwieniecki et al., 2004; Salleo et al., 2009; Zwieniecki and Holbrook, 2009;); or by iii) increasing redundancy in water pathways (Ewers et al., 2007; Schenk et al., 2008). All the changes occurring at the xylem structural level are ultimately permanently and chronologically stored inside the anatomical tree-ring structure and can thus be retrospectively analyzed (Fonti et al., 2010).

Quercus robur, *Quercus petraea* and *Quercus pubescens* are three of the most important broad leaved species in Europe, growing mainly on sites with irregular soil water supply. Among them, however, in recent decades *Q. robur* is experiencing increased mortality in Europe (Thomas et al., 2002) which have been often associated with changes in water availability (Cochard et al., 1996; Tyree and Cochard, 1996; Siwecki and Ufnalski, 1998; Cater, 2003; Cater and Batic, 2006; Allen et al., 2010; Bergman et al., 2011). Although these oak species have similar wood structure, characterized by a clear ring porosity, there are some differences in their ecological behavior especially to drought. *Q. robur* is not exclusively restricted to drought prone sites, occupying also hydromorphic soils experiencing periodic flooding. This ecological difference indicates a higher requirement for water in *Q. robur* than in *Q. petraea* and *Q. pubescens*, which is well reflected by lower water use efficiency (Ponton et al., 2002), higher sensitivity to decreasing soil moisture (Vivin et al., 1993) and lower stomatal responsiveness to decreasing air humidity (Gieger and Thomas, 2005). In contrast, *Q. pubescens* is commonly considered to be more tolerant to drought and heat, which is reflected in its main distribution area located in the warmer and dryer climates of the Mediterranean area in southern Europe.

We hypothesize that inter-specific differences in drought-tolerance might be related to a different capacity to acclimate to a safer hydraulic architecture when subjected to water stress. In this study, we analyzed i) xylem growth responses and ii) inter-specific acclimation differences in hydraulic-relevant xylem anatomical features in saplings of three oak species (*Q. robur*, *Q. petraea* and *Q. pubescens*) differing in their tolerance to drought. Saplings were experimentally exposed to soil drought and air warming during three consecutive growing seasons (2007 to 2009) and compared with control sapling growing with regular water supply

and ambient air temperature. Analyses consider differences in annual and intra-annual ring formation including growth and anatomical characteristics of vessels, parenchyma and fibers cells.

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Materials and methods

Experimental design and plant material

The 48 oak trees analyzed in this study are part of the interdisciplinary “*Querco*” experiment studying provenance-specific responses of *Q. robur*, *Q. petraea* and *Q. pubescens* to drought and air warming. The origin of the provenances and the experimental design are described in detail by [Arend et al. \(2011\)](#). In brief, four provenances from each oak species were selected from natural forest stands in Switzerland and Italy, covering a wide range of environmental conditions. Three-year old saplings from each provenance were grown for three years (2007 - 2009) in 16 lysimeter-based open-top chambers of the model ecosystem facility of the Swiss Federal Research Institute WSL, Birmensdorf, Switzerland (47°21'54"N, 8°27'5"E, 450 m a.s.l.). Each open top chamber had movable glass roofs closing automatically at the onset of rain fall. Three treatments were applied: drought and re-watering (D), air warming (AW) and their combination (AWD) as well as control (CO). The treatments were obtained by adjusting the aperture of the movable glass side walls of the open-top chambers (for temperature) and interrupting the irrigation (for drought). Air temperature outside and inside the chambers were measured at 120 cm height with shaded EL-USB-2 data loggers (Lascar Electronics Ltd., UK) and development of soil drought was controlled by weekly measurements of volumetric soil water content at 62 cm depth (TDR 100; Campbell Scientific Inc, USA).

For intra-annual growth and wood anatomical analyses, the following Swiss provenances were chosen for each species: *Q. robur* Tägerwil (47°38' N, 9°08' E; 510 m a.s.l.; mesic site), *Q. petraea* Corcelles (46°51' N, 6°41' E; 550 m a.s.l.; mesic site) and *Q. pubescens* Leuk (46°18' N, 7°38' E; 720 m a.s.l.; dry site). Four saplings per provenance and treatment, in total 48 saplings, have been sampled. The dendrometric characteristics of the selected saplings at the end of the experiment are summarized in Table 1.

Differences in diurnal temperature relative to the control CO were +1.16, +0.96, +2.04 °C for AW, D and AWD, with peaks during the summer months (June, July, August) of up to 3°C for AWD (Fig. 1c). The drought treatments in 2009 (D and AWD) lasted from April to August, but were interrupted by short rewatering at July 1st and at August 21st, when predawn leaf water potential in AWD was reaching values around -3 MPa and -4 MPa, respectively. Pre-drawn leaf water potential was biweekly measured for each treatment on 1 leaf per tree with a pressure chamber (M 600, Mosler Tech Support, Berlin, Germany). The seasonal profiles of the leaf water potential show that both drought periods lasted for 1.5 months with progressively increasing intensity (Fig. 1d). Drought level was reaching minima of -3.65 and -4.00 MPa in D and AWD, while it never reaches value below -0.5 MPa in CO and AW.

Leaf net photosynthesis (A_{net}) was measured on recently expanded mature leaves using a portable gas-exchange system (Li-6400; Li-cor Inc., Lincoln, NE, USA). Measurements were taken before both re-watering events on three leaves per tree (Table 2).

Pinning, preparation of microsections and ring-width development

To monitor the dynamic of annual ring formation we applied the pinning method (Seo et al., 2007). The pinning consists in causing wound-reaction marks in the annual ring by using a pin briefly inserted at known dates into the stem cambium and to retrospectively use these marks in wood to reconstruct the position of the ring formation at pinning dates. Eleven pinning dates (P1 to P11) were selected in order to cover the whole 2009 growing season. The pinning dates performed throughout the season are indicated in Fig. 1b. Pinnings were performed in a spiral along the stem from 20 cm height upwards every 5 cm distance and pinning points marked on the bark. At the end of the growing season, the trees were cut and stems collected for the preparation of micro-sections at pinning positions. Thin micro-sections (thickness of 20 µm) of pinning marks and on a cross-section free of pinning reaction at 10 cm stem height (see example in Fig. 1a) were obtained using a sliding microtome (Reichert, Germany), stained with safranin (1%) and astrablue (2%), dehydrated with ethanol (70, 95 and 100%) and xylol, and fixed on microscope slides with Canada balsam.

Images of the pinning microsections (P1 to P11) were taken using a digital video camera (ColorView III, Soft Imaging System, Germany) connected to a transmitted light microscope (Olympus BX41, Japan) with a 100x magnification and a resolution of 300 dpi. Since the field of view through the objective was not large enough to cover the entire tangential width of the ring surface, adjacent images from the same annual ring were merged together (Adobe Photoshop Elements 2.0).

The intra-annual increment over the season was measured on pinning images as the distance between the previous tree-ring border and the last formed cell at time of the pinning (last cells with a secondary wall below the wound, (Seo et al., 2007)). Measurement of intra-annual ring-width increments were made on both sides of the pin entry and averaged. The relative increment, giving the percentage of the entire growth ring formed until the pinning date, was calculated as a proportion of the measured intra-annual increment on the total ring width.

Anatomical analysis

Cellular anatomical characteristics of wood tissue (vessels, parenchyma and fibers) were measured on the pinning-free samples (P0) to avoid potential bias due to the wound reactions. Information about intra-annual increment has been reported into the new images to distinguish between timing of formation. Anatomical measurements have been grouped into three periods, i.e., during first dry period (Period 1, from P1 to P7), from rewatering to second dry period (Period 2, from P8 to P9) and after second rewatering (Period 3, from P10 to P11). Measurements of cell anatomical characteristics were performed using image analysis. Images were taken with the same devices as for the pinning images, but with different magnification, depending on the features to be measured (40x for vessels, 100x for parenchyma and 200x for fibers).

Vessels anatomical measurements have been performed on the ring cross-section surface covered by a 30° stem circular angle using the image analysis software (Image Pro Plus, Media Cybernetics, Silver Spring, MD, USA). Vessel lumina were automatically recognized using the function for “bright object recognition” using filters to exclude vessel lumina smaller than 200 μm^2 . Manual correction was performed by object miss-recognition. For each object

the vessel lumen area, vessel mean radius, and the radial position in the ring was measured. Sums for mean vessel area (MVA) and for conductivity (Cond, calculated according to the Hagen–Poiseuille law as the sum of the forth power of each vessel mean radius) per circular angle (i.e., divided by the measured stem circular angle) were finally calculated for each species, treatment, and period of growth (Period 1 to 3).

The characterization of parenchyma cells was then performed by counting the number of parenchyma cells (excluding rays cells) on a radial band (minimal width 106 μm , maximal width 504 μm) of the ring and finally density of parenchyma cells per surface (PD) was calculated for each species, treatment, and period of growth. Counting and relative radial position of each cell in the ring was performed using the manual measurement tool of the software Image Pro Plus.

Finally, to evaluate the effect of drought on fiber cells (Fig. 1a), per each tree the radial diameter of a sequence of 14 fiber cells along 5 radial files (7 before and 7 after the first rewetting on July 1st) have been measured using the Image Pro Plus manual measurement tool. Results have been grouped and averaged per species and treatment.

Results

Growth

The three consecutive experimental growing seasons under different climate treatments have significantly affected the whole tree growth of the saplings (Table 1). Differences to the control (CO) were particularly strong for both drought treatments (D and AWD). All growth related parameters except root length (i.e., sapling height, sapling stem diameter, and leave surface area) showed a reduction between 31% and 54% depending on the parameter and the species considered. In general *Q. robur* was the most sensitive species to drought, while *Q. petraea* and *Q. pubescens* showed proportionally less growth reduction. In contrast to drought, air warming (AW) alone seemed to slightly promote growth in comparison to the control saplings although there was no consistent warming effect among species and analyzed

parameters. The observed growth responses to drought were well reflected by measurements of net photosynthesis (A_N , Table 2) which were strongly reduced in both drought treatments in a species-dependent manner with *Q. robur* being more sensitive than *Q. petraea* and *Q. pubescens*.

We observed increasing differences in radial growth of the annual ring 2009 between the treatments in coincidence of the pinning P5 (May 18th) and P6 (June 2nd) (Fig. 2), when the leaf water potential of drought-exposed saplings (D and AWD) dropped below $< -1\text{MPa}$ (Fig. 1d). The radial growth of the drought-exposed saplings in all species continued to be strongly reduced in comparison to the control and the not-drought-limited treatment (AW), even after rewatering. Over the entire growing season 2009 the final reduction in ring width in comparison to the control was 59 % (Qro), 66% (Qpe) and 60% (Qpu) for D and 74%, 57% and 77% for AWD. In some drought exposed saplings we observed an earlier stop in radial growth (no growth increment in Period 3). The reduction was stronger for *Q. robur* and less pronounced for *Q. petraea* than *Q. pubescens*. In contrast air warming (AW) slightly modified radial growth with changes of +19% (Qro), +1% (Qpe) and -10% (Qpu) in comparison to the control saplings.

Wood anatomy and conductivity

Results obtained from wood anatomical measurements displayed a very large variability among saplings and treatments. Thus it was important to standardize the results relative to tree size (i.e., for vessels anatomical measurements) or to surface or to the ring portion considered (i.e., for the quantification of the parenchyma cells). In general, responses observed at the anatomical level reflected the differences observed in whole tree growth, whereby differences relative to the control became apparent only for the drought-exposed saplings (D and AWD). The distribution of vessel size classes for total vessel area and conductivity (Fig. 3 and 4) clearly indicated that drought-exposed seedlings produced fewer vessels in general, and in particular the larger vessels were smaller. The average vessel lumen area of the five largest measured vessels in *Q. petraea* was reduced by 52% in both drought treatments compared to the five largest vessels of the control. This reduction of the five largest vessels was less pronounced in the other two species, but was still 18% and 17%

for *Q. robur* and *Q. pubescens*, respectively. However, In terms of average size changes in total vessel area, the droughts treatments (D and AWD) causes a strong reduction for all the species (ranging from -44% to -68%), except for the drought treatment in *Q. pubescens* which by reduces only 15% (Fig. 3). Similar results are observed for the total potential conductivity, the reduction of drought exposed saplings (D) in comparison to the control (CO) was -64% for *Q. robur*, and -68% for *Q. petraea*, while for *Q. pubescens* it remained almost unchanged (-15%, Fig. 4). Moreover, due to the limited growth recovery after drought, the number of vessels produced after rewatering was also strongly reduced, especially for the Period 3.

Differences in response to drought were also observed in the density of parenchyma cells (Fig. 5), which tended to increase in drought-exposed trees (two factors ANOVA, $P < 0.1$), except for *Q. pubescens*. In general, in all the species the density of parenchyma cells tended to increase after the drought periods (Period 2 and 3 compared to 1). No differences between species were observed in the density over the total measured ring surface.

A clear anatomical mark of drought was observed in the size of the fiber cells. The radial diameter of fiber cells produced under drought was significantly smaller than those produced in the control and air warming treatment or those formed after the rewatering (Fig. 6). After rewatering, usually the fiber diameter (around 4-5 μ m) recovered quickly to almost the diameter of the control trees (> 10 μ m).

Discussion

Xylem structural adjustments to drought

Plant strategies to control water status and resist drought are numerous (Maseda and Fernandez, 2006). A key component in drought tolerance of woody plants is to avoid the detrimental effect of embolism (Maherali et al., 2004). This can be achieved by resistance, redundancy and repair, which usually occur at conduction and efficiency costs (Pratt et al., 2008). The experiment conducted over three consecutive growing seasons clearly indicates that repeated periods of water shortage have induced significant anatomical adjustments in the xylem structure of the saplings of all the three oak species towards resistance and repair,

which also had important impact on the growth performance. All drought-exposed saplings
275 have shown a significant radial growth reduction, a reduced vessels size with diminished
conductivity and a slightly increased density of parenchyma cells.

Safety against the formation of embolism can be achieved through the avoidance of critical
water potential or by building up a xylem tissue which is resistant to low water potential. The
safety margin, i.e., the range between water potential at stomatal closure and cavitation, can
280 only be increased through structural modifications that reduce plant water losses with more
efficient roots or less transpiring leaf area (Limousin et al., 2009) and/or by improving the
xylem resistance to drought-induced embolism (Beikircher and Mayr, 2008). Since cavitation
resistance mainly depends on xylem structure as arrangement, size, wall reinforcement and
pit properties of conduits (Choat et al., 2008; Comstock and Sperry, 2000; Hacke and Jansen,
285 2009; Hacke and Sperry, 2001; Hacke et al., 2006; Sperry et al., 2006), xylem anatomical
traits can be used to indirectly assess hydraulic safety. Many authors demonstrated the impact
of drought on oak vessel characteristics (Barij et al., 2007; Campelo et al., 2010; Corcuera et
al., 2004a; Corcuera et al., 2004b; Corcuera et al., 2006; De Micco et al., 2008; Eilmann et
al., 2009; Galle et al., 2010; Levanic et al., 2011; Thomas et al., 2006), which are confirmed
290 by the present study. Adjustments were usually oriented at reducing the size of the larger
earlywood vessels which are more vulnerable to cavitation (Sperry, 2003). Since the 2009
earlywood vessels in oak have been build before June (Fonti and Garcia-González, 2008),
i.e., before the negative leaf water potential starts to decrease, the size adjustments are
brought back to the drought occurred in the previous growing seasons (2007 and 2008). Size
295 differences to the control, which have been observed in the very few latewood vessels (formed
after the first rewatering period 2 and 3), indicated that wood structural adjustments
compensated for loss in conductivity, but were only possible in very limited magnitude during
the year of stress (Arend and Fromm, 2007; Galle et al., 2010).

Repairing, by the refilling of embolized conduits, is a process that requires energy that
300 depends on the conversion of starch to sugar for aquaporin regulation and for the creation of
an osmotic gradient within embolized conduits (Bucci et al., 2003; Salleo et al., 2009;
Zwieniecki and Holbrook, 2009). In the entire 2009 annual ring, we observed an increase in

the density of parenchyma cells containing starch in drought-exposed saplings (period P1 to P3) supporting the hypothesis of increased energy storage during drought to repair embolized vessels as soon as the conditions improve.

It is well known that a major direct effect of decreased water availability is diminished photosynthetic carbon fixation due to stomatal closure. Reduced growth has also been suggested as an acclimative feature for plant survival under stress (Rigling et al., 2002; Rigling et al., 2003; Weber et al., 2007), because it allows plants do redirect assimilates and energy (otherwise used for shoot growth) for example to maintain respiration or to stimulate root growth and other protective adjustments against stress (McDowell, 2011). This supports the theory of carbon allocation that attributes the lowest priority to the stem growth under stress conditions (Waring, 1986). In this study it has been shown that water deficiency reduces photosynthetic carbon fixation and wood growth in stems. Furthermore water deficiency induced the formation of smaller fiber cells and an earlier stop of growth, especially if the drought occurs late in the growing season. Cell division and expansion are in fact directly inhibited by water stress (Abe and Nakai, 1999; Abe et al., 2003; Arend and Fromm 2007, Rossi et al., 2009) and in an extreme situation, plants might escape dehydration by shortening the length of the growing season (Eilmann et al., 2010).

Intra-specific phenotypic plasticity and ecological behavior

Differences between species for anatomical properties were first assessed in the absence of drought. *Q. robur* was different from the other species showing higher growth and a vessel size distribution characterized by larger and more numerous vessels. Under drought (D, AWD), *Q. robur* displayed a higher range of vessel phenotypic plasticity reducing size to almost a comparable level of the other two species. This might explain the larger ecological range of this species. On mesic sites, *Q. robur* might be more competitive because it can fully exploit the more efficient water conducting system. This assumption is supported by the increased radial growth achieved under air warming conditions (AW) observed for only *Q. robur*. In contrast, the hydraulic safer species (*Q. petraea* and *Q. pubescens*) might be excluded from humid places because of the limited efficiency in water transport and growth performance (Maseda and Fernandez, 2006). On drought prone sites it might be that *Q. robur*

is approaching the critical threshold to survive under water scarcity due to the lower capacity of performing net photosynthesis under water scarcity as shown in the present study. Between the two other oak species which are considered to be more competitive under extreme drought, there was no clear difference in drought tolerance with respect to photosynthesis. Clear differences are however observed with respect to xylem anatomical plasticity. To further distinguish between these two species, a more extreme drought treatment would have likely been necessary. Our results support the hypothesis that *Q. robur* is hydraulically underbuilt for dry conditions, which predisposes the species to severe hydraulic constraints and subsequent mortality (Levanic et al., 2011). *Q. pubescens*, in comparison to *Q. petraea*, seems to be more drought tolerant since a strong reduction in conductivity is achieved only in the combined effect of drought and air warming (AWD).

Conclusions

In this work we assessed and compared major strategies of xylem hydraulic adjustments of three common oak species with differing drought tolerance growing under controlled experimental conditions during three consecutive years. We observed that acclimation to water scarcity of saplings exposed to drought and air warming is primarily oriented in increasing the safety against cavitation by developing a higher proportion of small and hydraulically safer xylem vessels. All oak species showed a relative increase in parenchyma cell density to augment the capacity for actively repair cavitation. These adjustments imply reductions in growth performance and in the duration of the growing season.

Comparison between the species fostered our understanding of the relationship between the inter-specific xylem hydraulic plasticity and the ecological behavior to drought. Through a broader range of phenotypic plasticity, principally characterized by the ability of producing larger vessel, *Q. robur* is more competitive under favorable growing conditions but at risk under severe exposition to drought. Although the results obtained from the present study performed on saplings seems to supply coherent results with observations of the ecological distributions of mature trees, future studies are needed to prove whether adult trees show similar growth responses.

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Fig. 1: Overview of the growing year 2009. a) Microscope image of a cross-section of the 2009 annual ring of a tree (*Q. robur*) subjected to drought and air warming (ring width = 575 μm). EV=earlywood vessel, LV=latewood vessel, P=parenchyma cell, F=fiber. **b)** Growth levels reached at pinning dates are indicated with dotted lines. After end of June an abrupt increase in the fiber size due to the rewatering is apparent. **c)** Temperature difference between the treatments inside the chamber and the measurement performed outside the chamber **d)** mean predawn leaf water potential measured for each treatment. CO=Control, AW=Air warming, D=Drought, AWD=Drought and Air warming. Periods of growth are indicated on the x-axis. Periods 1, 2 and 3 correspond to the time span with growth before and including first drought, before and including second drought and after the second drought respectively.

Fig. 2: Development of ring width at the pinning dates grouped by species and treatment. CO=Control, AW=Air warming, D=Drought, AWD=Drought and Air warming. Mean $\pm$ SD; n=4.

Fig. 3: Total vessel lumen area grouped by treatment and species. Standardization is performed by dividing total vessel area by the angular sector considered. Bars indicate results for vessel lumen area grouped in classes of 2000 μm^2 . Periods correspond to the periods of growth with cells formed before and including first drought (Period 1, light grey), before and including second drought (Period 2, grey) and after the second drought (Period 3, dark grey), respectively. Values in the boxes indicate the percentage reduction of the average total vessel lumen area in comparison to the control.

Fig. 4: Total conductivity grouped by treatment and species. Standardization is performed by dividing total conductivity by the angular sector considered. Bars indicate results for conductivity grouped in classes of $2000 \mu\text{m}^2$. Periods correspond to the periods of growth with cells formed before and including first drought (Period 1, light grey), before and including second drought (Period 2, grey) and after the second drought (Period 3, dark grey), respectively. Values in the boxes indicate the percentage reduction of the average total conductivity in comparison to the control.

Fig. 5: Density of parenchyma cells [number/ μm^2], grouped by species, treatment and period of growth. Periods correspond to the periods of growth with vessels cells before and including first drought (Period 1, light grey), before and including second drought (Period 2, grey) and after the second drought (Period 3, dark grey), and for the whole ring (Total, black), respectively. Mean $\pm$ SD, n=4.

Fig. 6: Radial diameter [μm] of fiber cells measured along radial file. Cell position -7 to -1 are cells built before the end of first drought and 1 to 7 are cells build after the subsequent re-watering on July 1st. Results are shown grouped by species and treatment. CO=Control, AW=Air warming, D=Drought, AWD=Drought and Air warming. Mean $\pm$ SD.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 6

Figure 7