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## Research paper: part of a special section on adaptations of forest ecosystems to air pollution and climate change

# Associations between growth, wood anatomy, carbon isotope discrimination and mortality in a *Quercus robur* forest

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Observations of forest mortality are increasing globally, but relatively little is known regarding the underlying mechanisms driving these events. Tree rings carry physiological signatures that may be used as a tool for retrospective analyses. We capitalized on a local soil water drainage event in 1982 that resulted in increased mortality within a stand of oak trees (*Quercus robur*), to examine the underlying physiological patterns associated with survival and death in response to soil water limitations. Pre-dawn water potentials showed more negative values for trees in the process of dying compared with those that survived. We used tree rings formed over the 123 years prior to mortality to estimate productivity from basal area increment (BAI, mm<sup>2</sup>), multiple xylem hydraulic parameters via anatomical measurements and crown-level gas exchange via carbon isotope discrimination ( $\Delta$ , ‰). Oaks that died had significantly higher BAI values than trees that survived until the drainage event, after which the BAI of trees that died declined dramatically. Hydraulic diameter and conductivity of vessels in trees that died were higher than in surviving trees until the last 5 years prior to mortality, at which time both groups had similar values. Trees that died had consistently lower  $\Delta$  values than trees that survived. Therefore, tree mortality in this stand was associated with physiological differences prior to the onset of soil water reduction. We propose that trees that died may have been hydraulically underbuilt for dry conditions, which predisposes them to severe hydraulic constraints and subsequent mortality. Measurements of above-ground/below-ground dry mass partitioning will be critical to future tests of this hypothesis. Based on these results, it is probable that pedunculate oak trees will experience greater future mortality if climate changes cause more severe droughts than the trees have experienced previously.

**Keywords:** climate change, dendroecology, die-off, human impact, water stress.

## Introduction

Forest mortality appears to be rising globally, may be driven by changing climate and has significant climate-feedback potential (Adams et al. 2010, Allen et al. 2010). Improving our understanding of the survival and mortality mechanisms is critical if we are to accurately predict future distribution, productivity and survival of forests and associated carbon sequestration (Bonan 2008). Many questions remain regarding mortality mechanisms, in part because there are limited observations of trees that actually died (McDowell and Sevanto 2010, Sala et al. 2010). The lack of mechanistic studies has

forced climate models to include untested assumptions regarding how plants survive or die during climate extremes, resulting in diverging estimates of the net impact on atmospheric CO<sub>2</sub> (Friedlingstein et al. 2006, Sitch et al. 2008).

Some of the most informative studies of woody vegetation mortality have utilized dendroecological analyses to understand historical growth patterns associated with survival and death (reviewed by McDowell et al. 2008). Tree rings provide a powerful archive because they record signatures of growth and physiology that can be tracked back in time. Trees often exhibit reduced wood growth and greater sensitivity to climate

in the years prior to mortality (reviewed by McDowell et al. 2008). Comparison of tree-ring growth and carbon isotope ratios ( $\delta^{13}\text{C}$ ) between live and dead ponderosa pines (*Pinus ponderosa*) showed that trees that die have limited ranges of gas exchange, due to either chronic water stress or failure to adequately regulate stomatal conductance (McDowell et al. 2010). This failure to regulate gas exchange may predispose trees to succumb to attack from insects or pathogens, and is consistent with the 'mortality spiral' concept, in which trees that die are exposed to a long-term stressor such as poor edaphic location, a short-term stressor such as drought and a proximal stressor such as a pathogen (Manion 1991).

Pedunculate oak (*Quercus robur* L.) forests are experiencing widespread mortality in Europe. High mortality rates have resulted in uneven age-class proportions and have reduced seedling regeneration, resulting in sustainability concerns. The proportion of older, mature stands is declining due to unexplained mortality and many of the remaining trees have severe crown defoliation, which has led to further mortality via increased sanitary harvests (Čater 2003). Incidences of oak mortality have occurred repeatedly during the past three centuries but have become more pronounced in recent decades in Europe (Thomas et al. 2002) and North America (Allen et al. 2010). Although the cause of mortality is currently unknown, pathogen attack and mortality in Europe have been associated with changes in water availability (Hämmerli and Stadler 1989, Levanič 1993, Cochard et al. 1996, Triboulot et al. 1996, Tyree and Cochard 1996, Čater 2003, Čater and Batič 2006), weather extremes (Näveke and Meyer 1990, Siwecki and Ufnalski 1995) and forest management choices (Harapin and Androić 1996). Many studies have also explained periodic oak decline as a consequence of climate extremes and pathogens (Führer 1992, Donaubaauer 1995) or by the occurrence of *Phytophthora* fungi, although the primary pathogenicity remains unknown (Jung et al. 2000).

In this study, we examined oak mortality using dendroecological techniques as a tool to retrospectively investigate how trees that die differ from surviving trees. We measured annual ring widths to quantify wood growth,  $\delta^{13}\text{C}$  of rings to calculate carbon isotope discrimination ( $\Delta$ ) as an index of gas exchange and xylem anatomy as an index of hydraulic characteristics. We capitalized on a soil drainage event in 1982 that, in combination with severe drought in the early 2000s, led to oak mortality. Our primary objective was to examine evidence regarding potential physiological mechanisms responsible for oak mortality in response to reduced water availability.

## Materials and methods

Pedunculate oak is an important tree species of lowland and floodplain forests throughout Europe. It covers the largest area of all European oaks (Trinajstić 1996), stretching from the coastal

Atlantic region to the inner-continental region of Scandinavia and eastern Europe (Krahl-Urban 1959, Leibundgut 1991). Oak forests are important ecosystems in Europe as sources of high-quality wood and, since they grow in lowlands, are also very important wildlife habitats and biodiversity hot spots.

### Sampling location

The study site was located in the forest district of Maribor, Slovenia (46°12.872'N, 15°34.895'E). The stand is ~120 ha in area and is located on flat topography at 287 m elevation. This area experiences a continental climate with 1097 mm year<sup>-1</sup> precipitation, 60% of which falls during the period when oak foliage is present (April–September). Climate data were obtained from a long-term meteorological site located ~30 km from the study site. The average yearly mean, maximum and minimum temperatures are 9.9, 15.1 and 4.9 °C, respectively; July and August are the warmest months (average maximum 25.5 °C) and January and February are the coolest months (average minimum -4.2 °C). The soil is deep, seasonally saturated and strongly gleyed (amphigleys) on alluvial loams. The plant community is classified as *Quercus-roboris carpinetum*, with the overstorey dominated by *Q. robur* with a slight mixture of artificially planted Norway spruce (*Picea abies*) and an understorey of hornbeam (*Carpinus betulus*). The stand is dominated by even-aged oaks that were ~123 years old in the year of sampling (2004). Stand density is 250–300 trees ha<sup>-1</sup>. According to the local forestry service, large-scale drainage manipulations happened on nearby farmland in 1982, and soon afterwards a decrease in the groundwater table in the nearby forest was observed. There has been no evidence of the presence of *Phytophthora* or other pathogenic fungi or attacking insects at this site (D. Jurc, personal communication).

### Materials

We selected 10 trees within 50 m of each other, of which 5 appeared healthy and the other 5 showed clear signs of ensuing mortality according to International Co-operative Programme on Forests methodology (Anonymous 2006) and would die in the year of sampling. Typical signs of dying oaks are dry branches, partially dead crowns and pale green to yellowish leaves at the end of branches. Approximately 35% of the trees were dead or dying. All sampled trees were dominants (class 2 after Assman's classification; Kramer 1988) without visible damage of the stem or crown. Sample trees were located far from skidding trails.

### Pre-dawn water potential

Pre-dawn water potential ( $\Psi_{pd}$ ) was measured on all 10 trees on 26 May 2004 between 04:00 and 04:30, prior to felling, to estimate soil water availability in the root zone. Three leaves per tree were extracted using shears mounted on a long telescopic arm. All leaves were located on the south to southeast side of the upper third of the crown, between 20 and 25 m

above ground.  $\Psi_{pd}$  for each tree was measured within a 5-min time window using a Scholander-type pressure chamber (Plant Moisture Stress, Skye, UK). All values were corrected for the hydrostatic gradient ( $0.01 \text{ MPa m}^{-1}$ ) based on branch height above the ground. We assumed that night-time transpiration was negligible and therefore  $\Psi_{pd}$  is representative of soil moisture availability in the rooting zone.

### Tree-ring sampling

All 10 trees were felled and one stem disc per tree was taken 4.5–5.5 m from the ground surface. Stem discs were air-dried and sanded to a high polish in the laboratory. Tree-ring widths (TRWs) were measured on three directions per stem disc with a LINTAB measuring table (precision  $0.01 \text{ mm}$ ). Tree-ring series were visually and statistically cross-dated, cross-checked, verified and synchronized against each other. For visual comparison, we used the dendrochronological program PAST-32, which allows the user to move tree-ring sequences left and right in search of the perfect, synchronized position. For statistical verification, we used two well-established statistical parameters in dendrochronology—the  $t$  value after Baillie and Pilcher ( $t_{BP}$ ) (Baillie and Pilcher 1973) and the so-called 'Gleichläufigkeit' coefficient (GLK%) (Eckstein and Bauch 1969).  $t_{BP}$  is a measure of the similarity between two TRW sequences and is based on the correlation between two samples. If the value of  $t_{BP}$  is  $>4.0$  and the overlap of two samples at least 40 years, then dendrochronologists conclude that the two samples are correctly cross-dated and in synchronized position. GLK% is similar to  $t_{BP}$ ; however, similarity between two tree-ring sequences is measured in percentages. Its range is between 0% (no match) and 100% (perfect match); the higher the value of GLK%, the better the match. One potential problem in TRW measurement in dying trees is missing or partially missing rings. Because oak is a ring-porous species that almost begins annual growth before buds open, missing rings are extremely rare. We did not identify missing rings in any of our sample trees.

Before further analysis, measurements on different radii of the stem discs were averaged, so only one average chronology for each tree was used for the construction of chronologies of dying and surviving trees. We converted ring-width measurements to basal area increment (BAI) using tree-specific cross-sectional radii (inside bark) and assuming that the average value for three radii represents the TRW of each ring. TRW and BAI data are presented for all trees from 1880 to 2003 (year of the last fully formed tree ring).

We identified years of particularly low or high growth, known as pointer or marker years, for anatomical and isotopic analyses. Our objective in sampling pointer years was not to examine correlations with climate per se because our sample size was too low to adequately examine climate relationships. Rather, our objective was to quantify variation in anatomical and isotopic patterns across the widest possible range of

climate to maximize our inference relative to the drought period in the early 2000s. An important characteristic of pointer years is their presence in at least 80% of the population of sampled trees (Schweingruber et al. 1990). We defined pointer years as years where at least four out of five trees react per treatment (surviving/dying) exhibited an increase or decrease in growth compared with the previous year.

### Ring anatomy

We analysed tree rings from two groups of trees (surviving/dying) and three types of tree ring (narrow/wide/1999–2003 rings). In addition to the narrow and wide pointer years, rings for the period 1999–2003 were selected to quantify the anatomical and isotopic features in the last 5 years before tree mortality. We were not able to analyse the rings from years 2002 and 2003 from one dying tree due to completely decomposed wood. Sample preparation for anatomical analysis comprised the sawing and fine sanding of wood surfaces. Sanding with fine sanding papers (grades 180–300–600) resulted in a surface quality satisfactory for automated image analysis. To enhance contrast and differentiate vessels from other tissues, we pressed a fine magnesium carbonate powder into the vessels while the remaining material was removed from the surface. Images were taken with an Olympus binocular SZ-60, equipped with a PixeLINK A662 digital camera with 1.3 million pixels. Each image was captured with a calibration scale. For image analysis, we used AnalySiS Pro v3.2 from Soft Imaging System GmbH, Germany. First, the colour image was transformed into black and white. Calibration ( $100 \mu\text{m}$ ) was performed in both horizontal and vertical directions of the image so as to avoid any potential distortion of the image.

We utilized numerous anatomical measurements to examine the hydraulic architecture of the xylem of trees that died and survived (Pockman and Sperry 2000, Hacke and Sperry 2001). For each ring, we measured the earlywood area between two of the transverse parenchyma rays ( $A_T$ ), total earlywood vessel surface area ( $A_V$ ), the number of earlywood vessels ( $N_V$ ), and the minimum and maximum diameter of each earlywood vessel to calculate an average diameter ( $D_V$ ). We calculated the earlywood-vessel density as a ratio between the number of earlywood vessels and the total surface area of earlywood within each image ( $N_V/A_T$ ). We also calculated the ratio between total earlywood vessel area and earlywood area ( $A_V/A_T$ ), and vessel hydraulic diameter expressed as a hydraulically weighted distribution of  $\Sigma r^5$  divided by  $\Sigma r^4$  ( $D_h$ , where  $r$  is radius; Pockman and Sperry 2000).

Vessel density and lumen areas within the sapwood were used to estimate potential saturated specific conductivity ( $k_{sp}$ ;  $\text{kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ ) according to the Hagen–Poiseuille law:

$$k_{sp} = \frac{\pi/8n(\Sigma r^4)}{A_s}, \quad (1)$$

where  $r$  is the radius of a circle having the same area as the corresponding vessel,  $A_s$  is sapwood area and  $\eta$  is dynamic viscosity of xylem water (Tyree and Zimmermann 2002).  $k_{sp}$  is an estimate of maximum potential conductivity because it assumes no differences in vessel length or friction due to bordered pits, vessel tapering or xylem cavitation.

### Carbon isotope composition

We sampled 15 tree rings from each disc such that the following groups of rings were represented for trees that survived and died: (i) the last 5 years before mortality (1999–2003), (ii) five narrow marker rings associated with relatively little growth and (iii) five wide marker rings associated with relatively high growth. These are the same rings as used for anatomical measurements. Samples for isotopic analyses were removed prior to sanding to avoid surface contamination. Tree rings, including both earlywood and latewood, were extracted using a sharp chisel and razor blade. Cellulose was extracted from finely chopped tree rings as described by Leavitt and Danzer (1993). Cellulose from each ring was analysed on a Eurovector Elemental Analyser coupled to a Micromass Isoprime isotope ratio mass spectrometer operated in continuous flow mode at Los Alamos National Laboratory's Stable Isotope Lab in Los Alamos (NM, USA). Nitrous oxide was removed by gas chromatography and corrections for  $^{17}\text{O}$  (Craig 1954) were carried out for all runs. In all, 148 tree rings were run and overall precision for  $\delta^{13}\text{C}$  was 0.05‰ ( $n = 103$  internal standards). The stable carbon isotope ratio was calculated using the following equation:

$$\delta^{13}\text{C}(\text{‰}) = \left( \frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{standard}}} - 1 \right) \times 1000, \quad (2)$$

where the  $^{13}\text{C}/^{12}\text{C}_{\text{standard}}$  is PeeDee Belmrite (Farquhar et al. 1989).

We present carbon isotope discrimination ( $\Delta$ ) as a measure of canopy gas exchange rather than  $\delta^{13}\text{C}$  because  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  became progressively depleted during the last century due to fossil fuel burning (Keeling et al. 1989), thus shifting the isotopic record within tree rings. Because  $\Delta$  accounts for changing atmospheric  $\delta^{13}\text{C}$ , we were able to directly quantify tree physiology without confounding by atmospheric  $\delta^{13}\text{C}$ .  $\Delta$  was calculated using the following equation (Farquhar et al. 1989):

$$\Delta = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{sample}}}{1 + \delta^{13}\text{C}_{\text{plant}}}. \quad (3)$$

$\delta^{13}\text{C}_{\text{air}}$  for the studied years was obtained from Moana Loa, Hawaii (Keeling et al. 1989).

### Statistical analysis

We used paired  $t$ -tests to determine differences between treatments within specific blocks of the chronology. This was

necessitated by change in patterns over time and our objective was simply to assess treatment differences, i.e., in the last few years prior to mortality, not temporal trends. Repeated-measures analysis was not feasible due to low sample sizes; however, we reduced the degrees of freedom associated with repeated sampling by averaging all rings within each tree for each group, i.e., the main group (dying/surviving) and sub-group (wide/narrow/1999–2003). This reduced the sample size for any given group and sub-group from 25 (5 trees  $\times$  5 rings) to 5 (5 trees, with ring values averaged within each tree). Homogeneity and heteroscedasticity of the variances were tested using the Fligner–Killeen test; none of the parameter sets failed either assumption (see Crawley 2007). All statistical analyses were performed in the R 2.10 program.

### Results

Precipitation and air temperature were significantly different in 1999–2003 compared with the long-term mean (1961–98, Figure 1). Total precipitation and mean temperature during the growing season (April–September) in 1999–2003 were 586 mm and 17.5 °C, respectively, compared with the

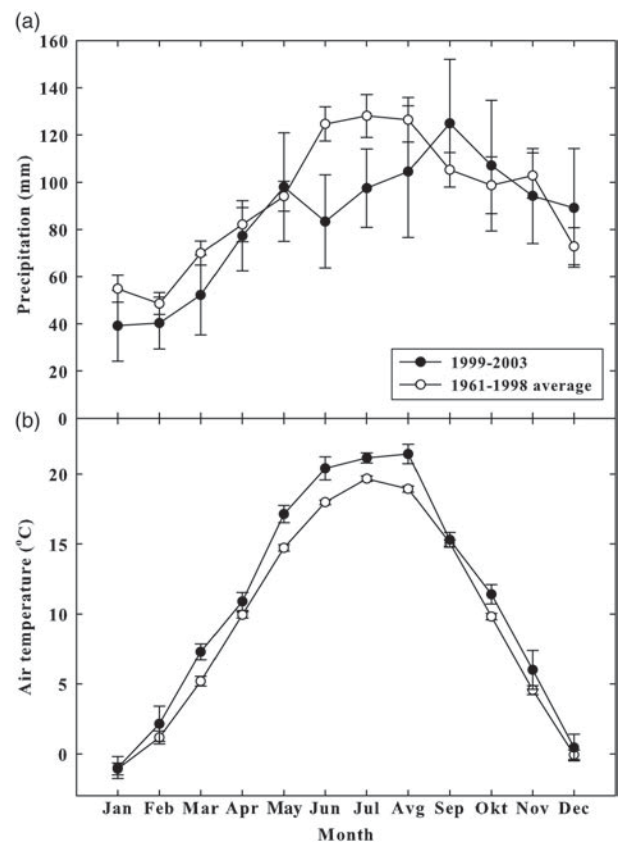


Figure 1. Precipitation (a) and temperature (b) data of a nearby meteorological station. Monthly averages for 1999–2003 (filled symbols) and 1961–98 (open symbols) are shown. Bars represent standard error.

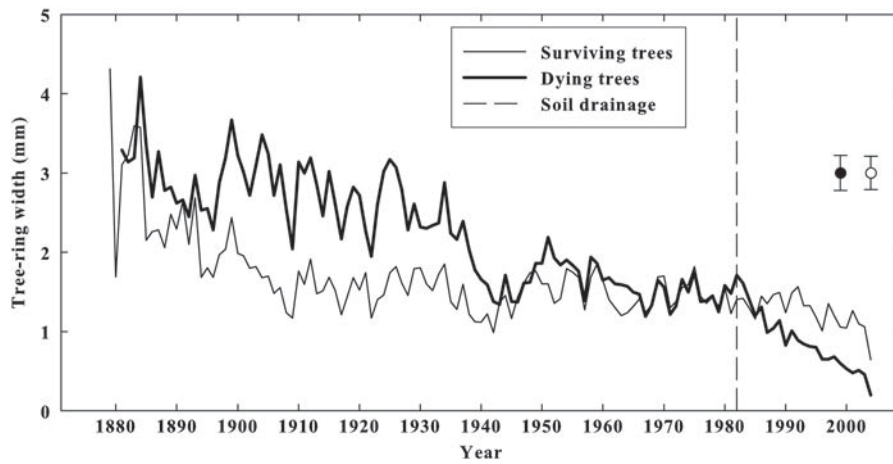


Figure 2. Tree-ring widths of trees that died (thick line) and survived (thin line) between 1880 and 2003. The dashed vertical line marks the year 1982 in which soil drainage was carried out. Error bars are omitted for clarity. The average standard error over the entire period was 0.22 mm for dying trees and 0.21 mm for surviving trees. These are shown for reference.

long-term mean of 660 mm and 16.1 °C. Pre-dawn water potential was more negative in dying than in surviving trees (−0.87 versus −0.54 MPa, respectively;  $P < 0.001$ ).

The dying trees grew significantly better than the surviving trees until 1982 (Figure 2). Average TRW values before 1982 were significantly higher in trees that died than in those that survived (2.3 and 1.7 mm, respectively,  $P < 0.001$ ). After 1982, TRW of dying trees rapidly declined to an average of 0.87 mm while TRW of surviving trees remained unchanged ( $P < 0.001$  between surviving and dying trees after 1982).

BAI exhibited similar yet more pronounced trends than ring width (Figure 3). Before 1982, BAI of dying trees was nearly twice that of surviving trees (average 412.1 versus 240.8 mm<sup>2</sup>, respectively;  $P < 0.001$ ). After 1982, BAI of dying trees

experienced a severe decline that did not occur for surviving trees (average 331.8 versus 383.9 mm<sup>2</sup>, respectively;  $P < 0.05$ ).

There were distinct differences in the xylem anatomy of dying and surviving trees. Across all anatomical characteristics, dying trees consistently exhibited greater variability than surviving trees (for all anatomical results and statistics, see Table 1).  $D_h$  of earlywood vessels was consistently higher in dying trees than surviving trees until the last 5 years prior to mortality, during which time these differences disappeared due to a large decline in  $D_h$  of the dying trees and stable  $D_h$  in the surviving trees (Figure 4).  $k_{sp}$  was also higher in trees that died prior to 1999, after which it became similar across both groups of trees (Figure 5).  $N_v/A_T$  was always lower in trees that died than in those that survived until 1999, at which time

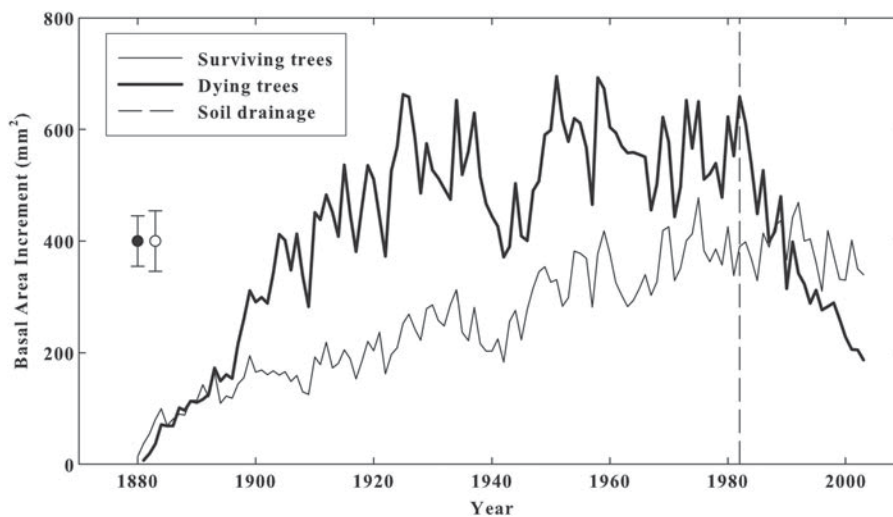


Figure 3. BAI of trees that died (thick line) or survived (thin line) between 1880 and 2003. The dashed vertical line marks the year 1982 in which soil drainage was carried out. Error bars are omitted for clarity. The average standard error over the entire period was 54.07 mm<sup>2</sup> for dying trees and 45.64 mm<sup>2</sup> for surviving trees. These are shown for reference.

Table 1. Comparison of different anatomical features of trees that died or survived during years of low growth, high growth, all years before 1999 and just prior to mortality (1999–2003).

	Narrow tree rings		Wide tree rings		Pre-1999		1999–2003	
	Dead	Live	Dead	Live	Dead	Live	Dead	Live
$D_v$	0.28 (0.006)	0.26 (0.004)*	0.27 (0.008)	0.26 (0.004)	0.28 (0.005)	0.26 (0.003)*	0.24 (0.022)	0.26 (0.012)
$D_h$	159.8 (3.42)	149.3 (3.53)*	157.4 (1.97)	149.0 (2.58)*	158.6 (1.9)	149.1 (2.1)**	136.2 (10.61)	149.0 (6.61)
$A_v$	0.98 (0.15)	1.09 (0.14)	1.00 (0.13)	0.89 (0.16)	0.99 (0.09)	0.99 (0.10)	0.55 (0.16)	0.61 (0.21)
$N_v$	15.00 (1.80)	18.80 (2.61)	16.56 (1.74)	16.16 (3.14)	15.78 (1.21)	17.48 (1.98)	11.47 (1.84)	10.64 (3.23)
$N_v/A_T$	6.53 (0.25)	7.26 (0.37)	6.61 (0.17)	7.51 (0.30)*	6.57 (0.14)	7.39 (0.23)**	10.42 (1.35)	7.69 (0.78)
$A_v/A_T$	42.10 (0.79)	41.28 (2.34)	39.60 (1.09)	41.58 (1.77)	40.85 (0.76)	41.43 (1.38)	43.86 (2.84)	41.15 (1.13)
$k_{sp}$	1308 (67)	1119 (93)	1184 (66)	1100 (67)	1246 (49)	1110 (54)*	1044 (190)	1097 (86)

All values are for earlywood. Values are: average vessel diameter ( $D_v$ , mm), hydraulic diameter ( $D^5/D^4$ ,  $D_h$ ,  $\mu\text{m}$ ), vessel area ( $A_v$ ,  $\text{mm}^2$ ), number of earlywood vessels ( $N_v$ ), vessel density ( $N_v/A_T$ ,  $\text{mm}^{-2}$ ), lumen to total area ratio ( $A_v/A_T$ , %) and hydraulic conductance calculated from Poiseuille's law ( $k_{sp}$ ;  $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ). Values in parentheses are standard error.

\*Significance at 0.05; \*\*significance at 0.01; \*\*\*significance at 0.001.

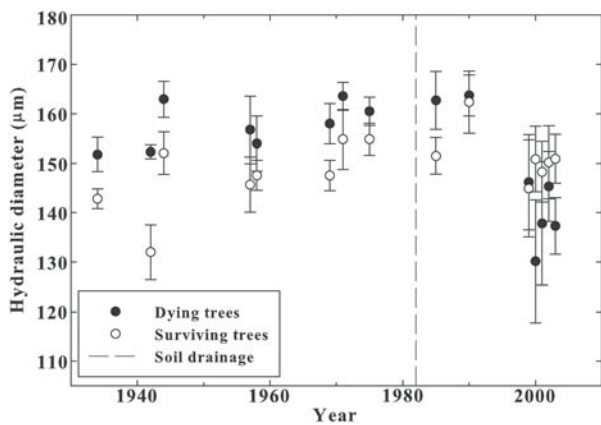


Figure 4. Hydraulic diameter of earlywood vessels of dying (filled symbols) and surviving trees (open symbols). Bars represent standard error.

$N_v/A_T$  was higher in those that died (Figure 6a).  $A_v/A_T$  was statistically similar for both groups, but with larger variability in dying trees (Figure 6b).

Differences in anatomy also emerged for years of low growth versus high growth (narrow and wide tree rings) prior to 1999. In years of low growth, trees that died had larger vessel diameters ( $D_v$  and  $D_h$ ), higher  $k_{sp}$  and lower  $N_v/A_T$  (Table 1). This pattern was retained in years of high growth for  $D_h$  and  $N_v/A_T$  (Table 1). Across all anatomical parameters, the years 1999–2003 induced particularly large responses in trees that died (Table 1, Figures 4–6).

Surviving trees had a significantly higher  $\Delta$  than dying trees ( $P < 0.001$ ) (Figure 7). In only 2 years (1957 and 1958) was this pattern reversed, although the differences in those years were not statistically significant ( $P = 0.14$ ). Analysis by ring type showed that surviving oaks had higher  $\Delta$  than dying trees in two of the three sub-groups of rings studied—narrow ( $P < 0.01$ ), and the last 5 years prior to death (period 1999–2003,  $P < 0.01$ ). No statistically

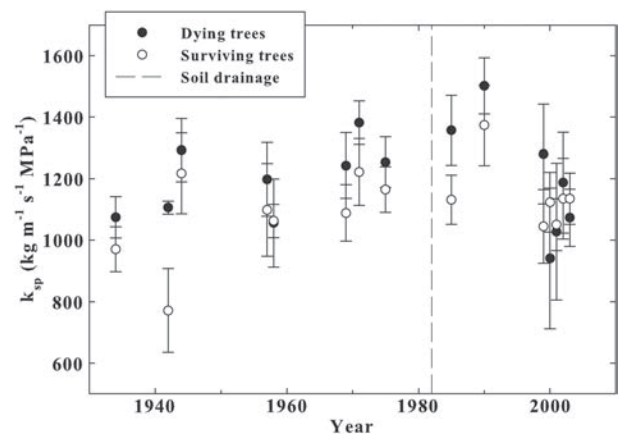


Figure 5. Potential saturated hydraulic conductance calculated of earlywood from Eq. (1) of dying (filled symbols) and surviving (open symbols) trees. Bars represent standard error.

significant differences were found in wide rings of dying and surviving oaks.

## Discussion

We investigated the physiological patterns recorded in tree rings associated with mortality in a pedunculate oak stand exposed to declining groundwater availability followed by a severe drought (Figure 1). Water availability, growth (Figures 2 and 3), xylem hydraulic anatomy (Figures 4–6) and carbon isotope discrimination (Figure 7) revealed that the population of oaks that died was physiologically different from those that survived. In particular, trees that died showed higher variability in all investigated parameters, including large shifts associated with drought. In contrast, trees that survived exhibited stability for all investigated parameters, even during drought (Figures 2–7). Periods of dry climate have caused widespread oak mortality throughout Europe—Austria, Finland, Germany, Slovakia, Slovenia and Spain (Donaubauer 1995, Čater 2003, Čater and

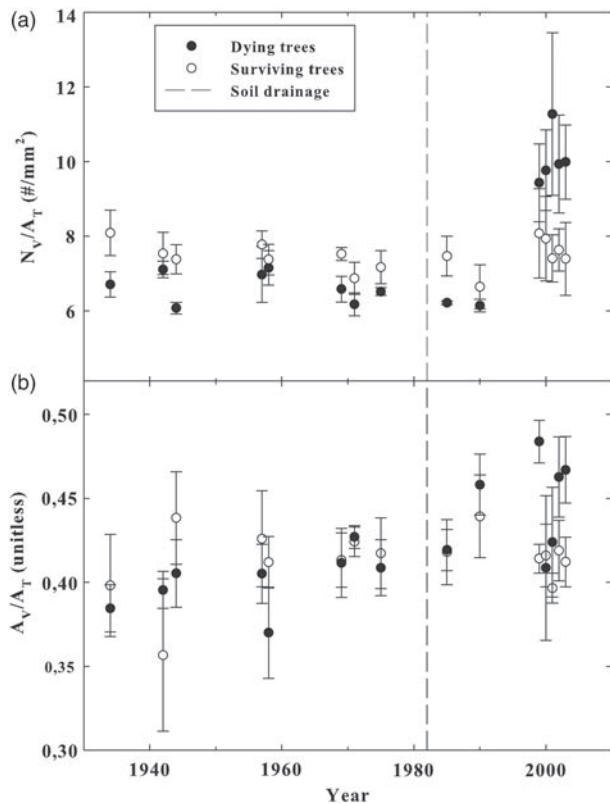


Figure 6. Number of vessels per unit earlywood area (a) and total vessel-lumen area per unit earlywood area (b) of dying (filled symbols) and surviving (open symbols) trees. Bars represent standard error.

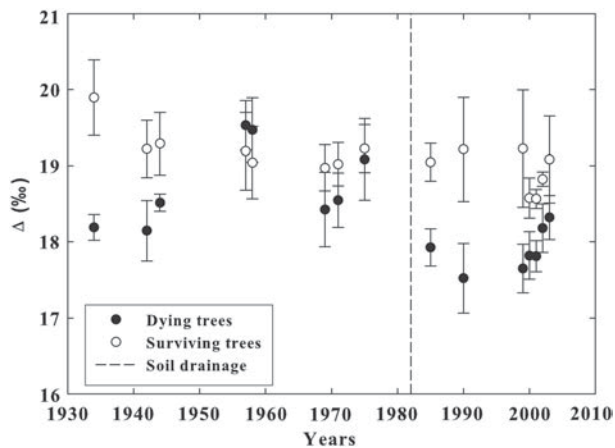


Figure 7. Carbon isotope discrimination of trees that died (filled symbols) and survived (open symbols). Bars represent standard error.

Batič 2006, Helama et al. 2009). There is also widespread mortality of the oak genus (*Quercus*) in North America that has been linked to water stress and pathogens (reviewed by Allen et al. 2010). In our study, it appears that the conditions and resulting growth patterns that oaks experience early in life may set them on a trajectory for survival or mortality when the climate and soil water conditions they have been acclimated to

change to conditions that they have not previously experienced. This may have consequences if climate continues to dry and warm, as is currently predicted (Seager et al. 2007, Allison et al. 2009).

It appears that a physiological mechanism underlies the vulnerability of oaks to soil water drainage and drought because of the clear differences between these two populations throughout the prior 123 years (Figures 2–7) and because there is no evidence of pathogen attack, such as *Phytophthora* fungi, at this site (D. Jurc, personal communication). According to official reports from the Slovene Environmental Agency (ARSO), there were no late frosts after 1990 that could drive differential embolism in these trees. Potential physiological mechanisms include (but are not limited to) carbon starvation, hydraulic failure or an interaction of both carbon starvation and hydraulic failure (McDowell et al. 2008). Carbon starvation is the failure to maintain metabolism and fend off pests due to limited carbohydrate availability, whereas hydraulic failure is defined as irreversible desiccation when embolism is not repaired. These two processes could interact with each other (McDowell and Sevanto 2010) and may be exacerbated by other physiological constraints associated with drought, such as limitations on phloem transport (Sala et al. 2010). In the remaining discussion, we examine our results in the context of these hypotheses along with other potential mechanisms.

The  $\Psi_{pd}$  observations in our study provide only a snapshot into the water availability to the trees that died and those that survived; however, they are consistent with lower water availability to trees that died, as has been observed for dying pedunculate oaks in Germany (Thomas et al. 2002). This observation may be representative of the long-term patterns since 1982 as represented by the shifts in  $D_n$ , which is consistent with changes in water availability (Figure 4; Pockman and Sperry 2000, McDowell et al. 2002). While this interpretation is logical, an additional explanation is that lower  $\Psi_{pd}$  in trees that died may have been associated with the process of dying that the oaks were undergoing at the time of sampling. Progressive embolism of the xylem during the final years prior to mortality could feed back to pre-dawn water potential by reducing hydraulic conductance (Sperry 1993). Lower  $\Psi_{pd}$  reduces stomatal conductance and hence photosynthesis, and will result in embolism in pedunculate oak if xylem water potential exceeds  $-2.2$  MPa (Tyree and Cochard 1996). Likewise, while moderate constraints on carbon assimilation due to drought increase root growth (Gower et al. 1992, Litton et al. 2007, Hummel et al. 2010), severe periods of carbon starvation should reduce the root growth due to lack of available photosynthate (Gibson et al. 2009). Observations of native embolism, hydraulic conductance, carbon uptake and root growth are needed within experimental water manipulation to test these alternatives.

Growth differences between trees that died and those that survived were pronounced (Figure 2), and were particularly striking when scaled from TRWs to BAI (Figure 3). Trees that died grew significantly more quickly early in life, resulting in larger-diameter stems and thus greater BAI in subsequent years. Potential causes of faster growth in the 1880s and early 1900s include better water access, higher light environments, genetic differences in inherent growth rates or earlier establishment and hence a competitive advantage. Regardless of the cause, a clear predictor of mortality was high growth rates prior to the soil water drainage in 1982. Larger basal areas of trees that eventually died are likely to have supported (and been supported by) larger leaf areas than in trees that survived. Although a large leaf area should be an advantage during periods of relatively high moisture availability, it may also predispose trees to severe water stress per unit leaf area when water availability declines, due to higher transpirational demand (i.e., McDowell et al. 2006). Measurements of leaf area in trees that die and survive are difficult (if not impossible) retrospectively. A precipitation manipulation demonstrated that droughted *Quercus ilex* reduced leaf area more than any other physiological parameter (Limousin et al. 2010). This result as well as the isotopic results (Figure 7, described below) led us to speculate that trees that died had a larger leaf area, and hence a higher transpiration requirement, than trees that survived.

Growth of trees that died was severely reduced after 1982, and was more affected during the drought conditions of 1999–2003 than that of trees that survived (Figures 2 and 3). Trees that died formed only one row of earlywood vessels and no latewood between 1999 and 2003, whereas trees that survived continued to produce multiple rows of earlywood vessels as well as latewood. In the ring-porous oaks, earlywood is formed using photosynthate from carbohydrates assimilated the previous year, whereas latewood is formed using current year photosynthate. Thus, photosynthate availability for stemwood growth was particularly low during the drought in the trees that died. Other investigations of oak mortality in non-manipulated, climate-associated events have shown that both particularly high and particularly low growth can be associated with mortality (Jenkins and Pallardy 1995, Haavika et al. 2008, Wyckoff and Bowers 2010); however, in all cases that we are aware of, including oaks and all other woody plants, growth has been particularly low in the years immediately preceding death (reviewed by McDowell et al. 2008). Low growth prior to mortality is consistent with carbon starvation as a factor in mortality, but does not exclude other mechanisms.

The greater  $D_h$  of trees that died prior to 1982 promoted high  $k_{sp}$  (Figures 4 and 5).  $D_h$  and  $k_{sp}$  are static estimates of potential water transport, yet they are indicative of greater transport capacity in part because the conducting tissue of oaks is almost entirely located in the current annual ring, with the older rings used for carbohydrate storage (Kozlowski and

Pallardy 1997). The trees that died had significantly greater basal area (Figures 2 and 3); therefore, the potential conductance of the stems was much greater than for trees that survived. A consequence of large-diameter vessels is greater vulnerability to embolism (Tyree and Zimmermann 2002); thus trees that eventually died may have experienced greater risk of hydraulic failure. Native embolism is relatively high in *Quercus* (Cochard and Tyree 1990, Sperry et al. 1994, Tognetti et al. 1996), but this may be compensated for by refilling when soil water is available (Meinzer et al. 2009). A decline in ground-water depth, coupled with the severe drought in the early 2000s, may have limited such refilling, leading to progressive embolism, reduced conductance and either (or both) hydraulic failure or carbon starvation.

Trees that were in the process of dying in the 2000s had similar  $k_{sp}$  to that of trees that survived, but lower ring widths (Figures 2 and 5). Because all water transport in this species is in the current annual ring, this indicates that the transport capacity in the final years before death was severely diminished, similar to other species that must produce sufficient xylem to transport water (i.e., Sperry et al. 1994, Brodribb et al. 2010). In other, more drought-tolerant species of oak, earlywood vessels have been observed to be the primary anatomical parameter that declines during drought in trees that survive (García-González and Eckstein 2003, Corcuera et al. 2004, Fonti and García-González 2004). It appears that the trees that were dying in this study were unable to sufficiently reduce vessel area, diameter and  $k_{sp}$ , but rather compensated by increasing the number of vessels per unit earlywood area ( $N_v/A_T$ , Figure 6a) and, to a lesser extent, the ratio of vessel area to unit earlywood area ( $A_v/A_T$ , Figure 6b). It appears that  $N_v/A_T$  is more plastic than vessel diameter in pedunculate oaks that are dying, and may buffer the decline in hydraulic conductance. Despite this apparent homeostatic response, it was clearly inadequate to prevent mortality.

Trees that died had consistently lower  $\Delta$  than those that survived (Figure 7). Lower stomatal conductance per unit leaf area often drives reduced  $\Delta$  because of the increased resistance of  $\text{CO}_2$  transport from the atmosphere to the sub-stomatal pore (Farquhar et al. 1989). If other factors are equal, plants with higher leaf area have lower stomatal conductance per unit leaf area than those with lower leaf area (Mencuccini and Comstock 1999), a pattern that is exacerbated in field conditions during drought (McDowell et al. 2006). Alternative explanations can be associated with nutrient availability and mesophyll conductance. Trees that died may have had higher nutrient availability, which would promote relatively higher photosynthetic rates and hence lower  $\text{CO}_2$  within the stomatal pore (Farquhar et al. 1989). Likewise, lower mesophyll conductance, such as that associated with thicker leaves, would have a similar effect to lower stomatal conductance on resistance to  $\text{CO}_2$  transport to the sites of photosynthesis (Dickson and Tomlinson



1996, Warren 2008). Future work investigating the spatial distribution of nutrient availability in relation to mortality is warranted to investigate this hypothesis.

Taking all the results together (Figures 1–7, Table 1), we propose that oak trees that died had a larger biomass to support relative to the supply of water, leading to vulnerability to mortality when drought conditions achieved a severity that was unprecedented in the history of the individual trees. Higher leaf area in the trees that died is likely, given their larger basal area and BAI (Figure 3),  $D_h$  (Figure 4) and  $\Delta$  (Figure 7). High leaf area relative to water availability (or root foraging area) should promote reduced carbon uptake per unit leaf area during drought (McDowell et al. 2006). Additionally, higher sapwood and foliar biomass may exacerbate carbon limitations through higher maintenance respiration costs (Ryan et al. 2004, Atkin and Macherel 2009). In addition to higher biomass to support, trees that died may have been predisposed to mortality by lower root biomass or a lower ratio of root area to leaf area (Sperry et al. 1998). Previous studies of oak mortality have shown particularly low root biomass in oaks (*Q. petraea*) that were in the process of dying (Thomas and Harmann 1996, Thomas et al. 2002). Lower root biomass could be driven by microsite differences early in life, such as higher nutrient availability (consistent with higher photosynthetic capacity and lower  $\Delta$ , Figure 7) or better water availability (Gower et al. 1992). During periods of drought stress, however, lower root biomass or a lower ratio of root area to leaf area would predispose them to hydraulic failure and carbon starvation due to reduced capacity to forage for water to supply transpirational demands. Trees that survived, in contrast, showed stability in all investigated parameters throughout periods of wet and dry climate, suggesting that greater allocation to root area over leaf area early in life allowed greater water foraging capacity relative to transpirational demand during drought.

## Conclusions

Oak trees exposed to reduced water availability due to soil drainage after ~100 years of growth experienced divergent patterns of survival and mortality during a subsequent severe drought. Trees that survived exhibited relatively stable patterns of growth, hydraulic anatomy and carbon isotope discrimination before and after the soil water drainage and during drought. In contrast, dying trees had high growth rates and large earlywood vessel diameters and conductivity, lower discrimination and greater variability in all parameters throughout their ~123-year life. We interpret these results as indicative that the divergent patterns of survival and mortality within this stand were contingent on physiological adaptations prior to drought, with microsite or genetic conditions favourable for fast growth early in life predisposing trees to vulnerability to

drought later in life. Dying trees exhibited patterns consistent with high biomass relative to root area; this hypothesis remains to be tested. We conclude that *Q. robur* trees may exhibit greater rates of mortality, particularly on sites of prior fast growth, if predictions of future increased drought severity and frequency are accurate.

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## References

- Adams, H.D., A.K. Macalady, D.D. Breshears, C.D. Allen, N.L. Stephenson, S.R. Saleska, T.E. Huxman and N.G. McDowell. 2010. Climate-induced tree mortality: earth system consequences. *EOS* 91:153–154.
- Allen, C.D., A.K. Macalady, H. Chenchouni et al. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For. Ecol. Manage.* 259:660–684.
- Allison, I., N.L. Bindoff, R.A. Bindshadler et al. 2009. The Copenhagen Diagnosis, 2009: updating the world on the latest climate science. The University of New South Wales Climate Change Research Centre (CCRC), Sydney, Australia, p 60.
- Anonymous. 2006. Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. UNECE, p 61.
- Atkin, O.K. and D. Macherel. 2009. The crucial role of plant mitochondria in orchestrating drought tolerance. *Ann. Bot.* 103:581–597.
- Baillie, M.G.L. and J.R. Pilcher. 1973. A simple crossdating program for tree-ring research. *Tree Ring Bull. Publ.* 1974, 33:7–14.
- Bonan, G.B. 2008. Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science* 320:1444–1449.
- Brodribb, T.J., D.J.M.S. Bowman, S. Nichols, S. Delzon and R. Burlett. 2010. Xylem function and growth rate interact to determine recovery rates after exposure to extreme water deficit. *New Phytol.* 188:533–542.
- Cochard, H., N. Breda and A. Granier. 1996. Whole tree hydraulic conductance and water loss regulation in *Quercus* during drought: evidence for stomatal control of embolism? *Ann. For. Sci.* 53:197–206.
- Cochard, H. and M.T. Tyree. 1990. Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation and seasonal changes in embolism. *Tree Physiol.* 6:393–407.
- Corcuera, L., J.J. Camarero and G.-P. Eustaquio. 2004. Effects of a severe drought on growth and wood anatomical properties of *Quercus faginea*. *IAWA J.* 25:185–204.
- Craig, H. 1954. Carbon-13 variations in *Sequoia* rings and the atmosphere. *Science* 119:141–144.
- Crawley, M.J. 2007. *The R book*. John Wiley & Sons, Ltd, Chichester, p 292, 942 p.

- Čater, M. 2003. Pedunculate oak (*Quercus robur* L.) crown defoliation—changes on permanent research plots. *Ekologia* 22:430–443.
- Čater, M. and F. Batič. 2006. Groundwater and light conditions as factors in the survival of pedunculate oak (*Quercus robur* L.) seedlings. *Eur. J. For. Res.* 125:419–426.
- Dickson, R.E. and S.T. Tomlinson. 1996. Oak growth, development and carbon metabolism in response to water stress. *Ann. For. Sci.* 53:181–196.
- Donaubauer, E. 1995. Recent oak decline and possible pathogens. *In* IUFRO-95 Papers and Abstracts. IUFRO XX World Congress, Tampere, Finland.
- Eckstein, D. and J. Bauch. 1969. Beitrag zur Rationalisierung eines dendrochronologischen Verfahrens und zur Analyse seiner Aussagesicherheit. *Forstw. Cbl.* 88:230–250.
- Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40:503–537.
- Fonti, P. and I. García-González. 2004. Suitability of chestnut earlywood vessel chronologies for ecological studies. *New Phytol.* 163:77–86.
- Friedlingstein, P., P. Cox, R. Betts et al. 2006. Climate-carbon cycle feedback analysis: results from the C4MIP model intercomparison. *J. Clim.* 19:3337–3353.
- Führer, E. 1992. Der Zusammenhang zwischen der Dürre und der Erkrankung der Traubeneichenbestände in Ungarn. *Forstw. Cbl.* 111:129–136.
- García-González, I. and D. Eckstein. 2003. Climatic signal of earlywood vessels of oak on a maritime site. *Tree Physiol.* 23:497–504.
- Gibon, Y., E.T. Pyl, R. Sulpice, J.E. Lunn, M. Höhne, M. Günther and M. Stitt. 2009. Adjustment of growth, starch turnover, protein content and central metabolism to a decrease of the carbon supply when *Arabidopsis* is grown in very short photoperiods. *Plant, Cell Environ.* 32:859–874.
- Gower, S.T., K.A. Vogt and C.C. Grier. 1992. Carbon dynamics of Rocky Mountain Douglas-fir: influence of water and nutrient availability. *Ecol. Monogr.* 62:43–65.
- Haavik, L.J., F.M. Stephen, M.K. Fierke, V.B. Salisbury, S.W. Leavitt and S.A. Billings. 2008. Dendrochronological parameters of northern red oak (*Quercus rubra* L. (Fagaceae)) infested with red oak borer (*Enaphalodes rufulus* (Haldeman) (Coleoptera: Cerambycidae)). *For. Ecol. Manage.* 255:1501–1509.
- Hacke, U.G. and J.S. Sperry. 2001. Functional and ecological xylem anatomy. *Perspect. Plant Ecol. Evol. Systematics* 4:97–115.
- Hämmerli, F. and B. Stadler. 1989. Eichenschäden, Eine Übersicht zur Situation in Europa und in der Schweiz. *Schweiz. Z. Forstwes.* 104:357–374.
- Harapin, M. and M. Androić. 1996. Sušenje i zaštita šuma hrasta lužnjaka. *In* Hrast lužnjak (*Quercus robur* L.) u Hrvatskoj. Ed. D. Klepac. Hrvatska akademija znanosti i umjetnosti, Zagreb, Vinkovci, p 559.
- Helama, S., A. Läänelaid, J. Raisio and H. Tuomenvirta. 2009. Oak decline in Helsinki portrayed by tree-rings, climate and soil data. *Plant Soil* 319:163–174.
- Hummel, I., F. Pantin, R. Sulpice et al. 2010. *Arabidopsis* plants acclimate to water deficit at low cost through changes of carbon usage: an integrated perspective using growth, metabolite, enzyme, and gene expression analysis. *Plant Physiol.* 154:357–372.
- Jenkins, M.A. and S.G. Pallardy. 1995. The influence of drought on red oak group species growth and mortality in the Missouri Ozarks. *Can. J. For. Res.* 25:1119–1127.
- Jung, T., H. Blaschke and W. Obwald. 2000. Involvement of soilborne *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. *Plant Pathol.* 49:706–718.
- Keeling, C.D., R.B. Bacastow, A.F. Carter, S.C. Piper, T.P. Whorf, M. Heimann, W.G. Mook and H. Roeloffzen. 1989. A three dimensional model of atmospheric CO<sub>2</sub> transport based on observed winds. 1. Analysis of observational data. *In* Geophysical Monograph. Ed. D.H. Peterson. American Geophysical Union, Washington, DC, pp 165–236.
- Kozłowski, T.T. and S.G. Pallardy. 1997. Physiology of woody plants, 2nd edn. Academic Press, London, 411 p.
- Krahl-Urban, J. 1959. Die Eichen. Forstliche Monographie der Traubeneiche und der Stieleiche. Paul Parey, Hamburg, Berlin, 288 p.
- Kramer, H. 1988. Waldwachstumslehre. Paul Parey, Hamburg, Berlin, 374p.
- Leavitt, S.W. and S.R. Danzer. 1993. Methods for batch processing small wood samples to holocellulose for stable-carbon isotope analysis. *Anal. Chem.* 65:87–89.
- Leibundgut, H. 1991. Unsere Waldbäume. Paul Haupt, Bern, Stuttgart, 172p.
- Levanič, T. 1993. Effects of hydromelioration on diameter growth and increment of black alder, ash and oak in Slovene Prekmurje. *Res. Rep. For. Wood Technol.* 42:7–65.
- Limousin, J.M., D. Longepierre, R. Huc and S. Rambal. 2010. Change in hydraulic traits of Mediterranean *Quercus ilex* subjected to long-term throughfall exclusion. *Tree Physiol.* 30:1026–1036.
- Litton, C.M., J.W. Raich and M.G. Ryan. 2007. Carbon allocation in forest ecosystems. *Global Change Biol.* 13:2089–2109.
- Manion, P.D. 1991. Tree disease concepts, 2nd edn. Prentice-Hall, Upper Saddle River, NJ, 416 p.
- McDowell, N.G. and S. Sevanto. 2010. The mechanisms of carbon starvation: how, when, or does it even occur at all? *New Phytol.* 186:264–266.
- McDowell, N.G., N. Phillips, C.K. Lurch, B.J. Bond and M.G. Ryan. 2002. Hydraulic limitation and compensation in large, old Douglas-fir trees. *Tree Physiol.* 22:763–774.
- McDowell, N.G., H.D. Adams, J.D. Bailey, M. Hess and T. Kolb. 2006. Homeostatic maintenance of Ponderosa pine gas exchange in response to stand density changes. *Ecol. Appl.* 16:1164–1182.
- McDowell, N.G., W.T. Pockman, A. Craig et al. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought. *New Phytol.* 178:719–739.
- McDowell, N.G., C.G. Allen and L. Marshall. 2010. Growth, carbon isotope discrimination, and mortality across a ponderosa pine elevation transect. *Global Change Biol.* 16:399–415.
- Meinzer, F.C., D.M. Johnson, B. Lachenbruch, K.A. McCulloh and D.R. Woodruff. 2009. Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Funct. Ecol.* 23:922–930.
- Mencuccini, M. and J.P. Comstock. 1999. Gas exchange of common bean (*Phaseolus vulgaris* L.) under well-watered conditions: interactions between hydraulic architecture and leaf size. *Aust. J. Plant Physiol.* 26:115–124.
- Näveke, S. and F.H. Meyer. 1990. Feinwurzelsysteme unterschiedlich geschädigter Eichen im Lappwald. *AFZ* 45:382–384.
- Pockman, W.T. and J. Sperry. 2000. Vulnerability to xylem cavitation and the distribution of Sonoran vegetation. *Am. J. Bot.* 87:1287–1299.
- Ryan, M.G., D. Binkley, J.H. Fownes, G.C.P. and R.S. Senock. 2004. An experimental test of the causes of forest growth decline with stand age. *Ecol. Monogr.* 74:393–414.
- Sala, A., F. Piper and G. Hoch. 2010. Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytol.* 186:274–281.
- Schweingruber, F.H., D. Eckstein, F. Serre-Bachet and O.U. Bräker. 1990. Identification, presentation and interpretation of event years and pointer years in dendrochronology. *Dendrochronologia* 8:9–38.
- Seager, R., M. Ting, I. Held et al. 2007. Model projections on an imminent transition to a more arid climate in southwestern North America. *Science* 316:1181–1184.
- Sitch, S., C. Huntingford, N. Gedney et al. 2008. Evaluation of the terrestrial carbon cycle, future plant geography and climate-carbon

- cycle feedbacks using five Dynamic Global Vegetation Models (DGVMs). *Global Change Biol.* 14:2015–2039.
- Siwecki, R. and K. Ufnalski. 1995. Oak stand decline and climate change. *In* IUFRO-95 Papers and Abstracts. IUFRO XX World Congress, Tampere, Finland.
- Sperry, J., F.R. Adler, G.S. Campbell and J.P. Comstock. 1998. Limitation of plant water use by rhizosphere and xylem conductance: results from a model. *Plant, Cell Environ.* 21:347–359.
- Sperry, J.S. 1993. Winter embolism and spring recovery in *Betula cordifolia*, *Fagus grandifolia*, *Abies balsamifera*, and *Picea rubens*. *In* *Water Transport in Plants Under Climatic Stress*. Cambridge University Press, Cambridge, UK, pp 86–98.
- Sperry, J.S., K.L. Nichols, J.E.M. Sullivan and S.E. Eastlack. 1994. Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology* 75:1736–1752.
- Thomas, F.M. and G. Harmann. 1996. Soil and tree water relations in mature oak stands of northern Germany differing in the degree of decline. *Ann. For. Sci.* 53:697–720.
- Thomas, F.M., R. Blank and G. Hartmann. 2002. Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. *For. Pathol.* 32:277–307.
- Tognetti, R., A. Giovannelli, A. Longobucco, F. Miglietta and A. Raschi. 1996. Water relations of oak species growing in the natural CO<sub>2</sub> spring of Rapolano (central Italy). *Ann. For. Sci.* 53:475–485.
- Triboulot, M.B., M.L. Fauveau, N. Breda, S. Label and E. Dreyer. 1996. Stomatal conductance and xylem-sap abscisic acid (ABA) in adult oak trees during a gradually imposed drought. *Ann. Sci. For.* 53:207–220.
- Trinajstić, I. 1996. Taksonomska problematika hrasta lužnjaka u Hrvatskoj. *In* *Hrast lužnjak (Quercus robur L.) u Hrvatskoj*. Ed. D. Klepac. Hrvatska akademija znanosti i umjetnosti, Zagreb, Vinkovci, pp 96–101.
- Tyree, M.T. and H. Cochard. 1996. Summer and winter embolism in oak: impact on water relations. *Ann. Sci. For.* 53:173–180.
- Tyree, M.T. and M.H. Zimmermann. 2002. *Xylem structure and the ascent of sap*. Springer series in wood science, 2nd edn. Springer, Berlin, New York, 283p.
- Warren, C.R. 2008. Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO<sub>2</sub> transfer. *J. Exp. Bot.* 59:1475–1487.
- Wyckoff, P.H. and R. Bowers. 2010. Response of the prairie–forest border to climate change: impacts of increasing drought may be mitigated by increasing CO<sub>2</sub>. *J. Ecol.* 98:197–208.