

# Role of aquaporin activity in regulating deep and shallow root hydraulic conductance during extreme drought

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## Abstract

**Key message** Deep root hydraulic conductance is upregulated during severe drought and is associated with upregulation in aquaporin activity.

**Abstract** In 2011, Texas experienced the worst single-year drought in its recorded history and, based on tree-ring data, likely its worst in the past millennium. In the Edwards Plateau of Texas, rainfall was 58 % lower and the mean daily maximum temperatures were  $>5$  °C higher than long-term means in June through September, resulting in extensive tree mortality. To better understand the balance of deep and shallow root functioning for water supply, we measured root hydraulic conductance ( $K_R$ ) in deep ( $\sim 20$  m) and shallow (5–10 cm) roots of *Quercus fusiformis* at four time points in the field in

2011. Deep roots of *Q. fusiformis* obtained water from a perennial underground (18–20 m) stream that was present even during the drought. As the drought progressed, deep root  $K_R$  increased 2.6-fold from early season values and shallow root  $K_R$  decreased by 50 % between April and September. Inhibitor studies revealed that aquaporin contribution to  $K_R$  increased in deep roots and decreased in shallow roots as the drought progressed. Deep root aquaporin activity was upregulated during peak drought, likely driven by increased summer evaporative demand and the need to compensate for declining shallow root  $K_R$ . A whole-tree hydraulic transport model predicted that trees with greater proportions of deep roots would have as much as five times greater transpiration during drought periods and could sustain transpiration during droughts without experiencing total hydraulic failure. Our results suggest that trees shift their dependence on deep roots versus shallow roots during drought periods, and that upregulation of aquaporin activity accounts for at least part of this increase.

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## Introduction

For many trees, deep roots represent an integral component of plant water relations. Although most root biomass lies in the top 2 m of soil (Schenk and Jackson 2002), deep roots can supply a considerable proportion of the total nutrients and water required for daily transpiration (Nepstad et al. 1994; McCulley et al. 2004). The contribution of deep roots to total water uptake may be particularly high during drought. In addition to their direct importance for water uptake, deep roots also help maintain shallow root

functioning during drought through hydraulic redistribution (HR), the movement of water from wetter, deeper layers into shallow soil layers through roots (Burgess et al. 1998; Domec et al. 2004; Bleby et al. 2010). In spite of its importance for tree water relations in arid and semiarid regions, shallow, especially, deep root functioning is poorly understood compared to the rest of the xylem pathway. For example, little is known about root hydraulic conductance in situ, in part because of the difficulty in accessing roots, and virtually no work has assessed in situ root hydraulic conductance during severe drought. The 2011 drought in central Texas, Oklahoma, and eastern New Mexico was the most severe 1-year drought in the region since meteorological record keeping began in 1895, and these areas are predicted to become even drier over the next 50–100 years (Seager et al. 2007; Diffenbaugh et al. 2011). An estimated 300 million trees died in Texas between 2011 and 2013 due to the drought (Texas Forest Service 2012). The 2011 Texas drought provided a unique opportunity to examine the impacts of severe drought on deep and shallow root functioning.

Several recent studies have increased our theoretical understanding of deep root functioning in trees. Peñuelas and Filella (2003) found that *Pinus nigra* can have deep roots (~8 m) that access perennial water sources and those roots provide water to neighboring, shallowly rooted plants via HR. In *Quercus fusiformis*, deep roots can provide five times more water than shallow roots during drought (Bleby et al. 2010). Deep roots of *Q. fusiformis*, *Sideroxylon* (formerly *Bumelia*) *lanuginosum* and *Juniperus asheii* have larger vessels and tracheids and greater hydraulic conductance than shallow roots (McElrone et al. 2004). Further, trees in central Texas display diurnal and seasonal variation in deep root (~20 m) hydraulic conductivity and aquaporin activity (McElrone et al. 2007). Aquaporin (membrane-bound channels, see Maurel et al. 2008 for review) activity can be upregulated in roots during periods of increased transpirational demand (Laur and Hacke 2013) and can either be upregulated (e.g., Vandeleur et al. 2009) or downregulated during periods of water stress, depending on the species and type of aquaporin (Aroca et al. 2012).

The goal of our study was to assess the shifting dependence on deep (~20 m) versus shallow (5–10 cm) roots for water uptake in *Q. fusiformis* over the course of a growing season, and to examine the contribution of aquaporins to this hydraulic regulation. A severe drought during the study period also provided a unique opportunity to evaluate the effects of drought on root hydraulic functioning. To understand the impact of drought severity on root function and whole-plant water status, we used a whole-tree water use model that incorporated deep and shallow root distributions and functioning along with soil and plant water potentials.

In this study, we measured deep and shallow root hydraulic conductance ( $K_R$ ) and the contribution of aquaporins to  $K_R$  at four sampling periods during the 2011 drought in central Texas. For shallow roots, we hypothesized that  $K_R$  and aquaporin contribution to  $K_R$  would decrease under drought stress. For deep roots, we hypothesized that  $K_R$  and aquaporin contribution to  $K_R$  would increase under drought stress and be highest in the summer because of increased transpirational demand. We also modeled the impacts of drought and access to deepwater sources on overall plant hydraulic status. We predicted that trees with a greater proportion of deep roots would have higher transpiration and greater resistance to hydraulic failure.

## Methods

### Study site and climate data

An established research site (Powell's cave, see Jackson et al. 1999; McElrone et al. 2004, 2007; Bleby et al. 2010) in central Texas was used to access deep (~20 m) and shallow roots (<10 cm) of the dominant tree species, *Q. fusiformis* Small (Texas live oak). The deep roots were accessed via an underground cave system, which contains a perennial underground stream. Several tree species in this region have deep roots capable of accessing this permanent water source (e.g., *Q. fusiformis*, *Sideroxylon* (formerly *Bumelia*) *lanuginosum*, Jackson et al. 1999; McElrone et al. 2004). Shallow *Q. fusiformis* roots were sampled by excavating roots from soil in close proximity to a parent tree.

Monthly temperature and precipitation data were obtained from a nearby weather station managed by the National Climatic Data Center (Station: GHCN:USC00415822, Menard, TX USA). Tree-ring reconstructions of Palmer Drought Severity Index (PDSI) are from Cook et al. (2004), gridpoint number 165. PDSI data from 1895 onward are from NOAA National Climatic Center.

### Root sampling

We sampled roots at four different times in 2011: (1) Jan 23–Feb 12, (2) Apr 13–26, (3) Jun 10–23, and (4) Sep 4–7. Although several species can access the permanent underground stream at Powell's Cave (McElrone et al. 2004, 2007), the roots of *Q. fusiformis* and *S. lanuginosum*, the two main species, are easily distinguished from other species; this visual distinction has been confirmed with molecular analysis (Jackson et al. 1999; McElrone et al. 2004, 2007). The deep roots of *S. lanuginosum* are white, unbranched, and fragile, whereas the deep roots of *Q. fusiformis* are dark reddish-brown, highly branched, and more woody. Deep roots were sampled at points along a

~ 1 km section of the underground stream. We attempted to maximize the distance between selected fine root samples to avoid pseudoreplication. During each sampling period, 13–19 individual roots were measured. During the first sampling period, we severed the sampled deep roots and made the root hydraulic measurements in a water bath in the cave at a constant temperature (21 °C). For the three subsequent sampling periods, deep roots were severed and root hydraulic measurements were made at the cave entrance in a small water bath using ice cubes to maintain the same constant temperature (21 °C). Travel time from the stream's edge to the cave surface was ~ 20 min.

For the shallow roots, we excavated an individual root of appropriate diameter (mean diameter =  $1.8 \pm 0.4$  cm) and traced the root back to its parent tree. The shallow roots were severed, left in the soil, and measured for root hydraulic conductance ( $K$ ) by perfusing water toward the root tip. This approach was necessary to avoid damaging the fine roots between the point where the root was severed and the root tip. After we measured  $K$ , we attempted to excavate the root segment to measure length; however, root segments were often damaged during this effort. Consequently, we weighed  $K$  by the cross-sectional area of the root segment ( $K_R$ ). We measured 12–33 shallow roots per sampling period, and these roots were sampled from approximately 50 different trees.

#### Root hydraulic conductance

We measured  $K_R$  in deep and shallow roots of *Q. fusiformis* using a high-pressure flow meter (HPFM; HPFM2, Dynamax Inc., Houston, TX, USA). The HPFM perfuses degassed water through root segments by applying pressure to a water-filled bladder contained within the unit. Using the HPFM, we measured the flow rate of water through root tissue by constructing transient curves (Tyree et al. 1995; Bogeat-Triboulot et al. 2002), which measure the flow rate under increasing pressure applied by a nitrogen gas cylinder. The applied pressure gradually rises from 0 to 500 kPa over the course of ~ 1 min, and the flow rate at each pressure value is logged every 2 s using the Dynamax software. Flow rates measured at increasing pressure are less vulnerable to flow rate reductions caused by the plant's wound response than flow rates measured at constant pressure. Once the transient curve was constructed, root hydraulic conductance ( $K_R$ ) was calculated using the formula:

$$K_R = (Q_v/P)(1/A)$$

where  $Q_v$  is the volumetric flow rate ( $\text{kg s}^{-1}$ );  $P$  is the applied pressure (MPa); and  $A$  ( $\text{m}^2$ ) is the surface area of a cylinder calculated from the radius of the root segment. The value of  $Q_v/P$  was quantified using linear regression and the Dynamax software. To remove any air emboli

present in the vessels before  $K_R$  measurement, we flushed the root segments with degassed water using the same technique that we used to make the transient curve. More specifically, we perfused water at steadily increasing pressure from 0 to 500 kPa over the course of ~ 1 min, then repeated this method and estimated  $K_R$  from the second curve.

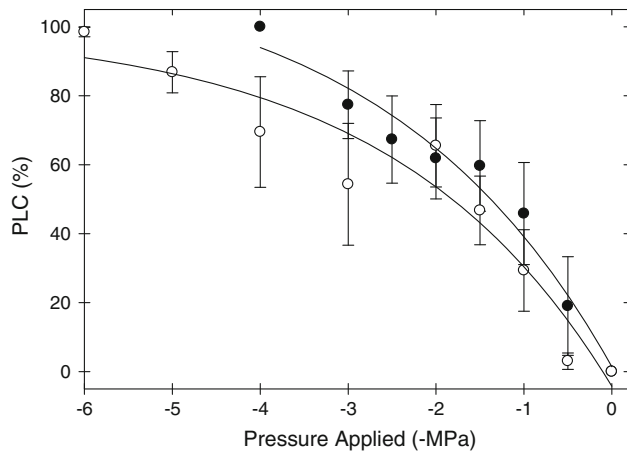
#### Aquaporin contribution to root hydraulic conductance

We quantified aquaporin contribution to  $K_R$  using hydroxyl radicals (\*OH) to inhibit aquaporin function. Henzler et al. (2004) and Ye and Steudle (2006) showed that hydroxyl radicals effectively inhibit aquaporins and are less toxic to sample tissues than other aquaporin inhibitors (e.g.,  $\text{HgCl}_2$ ). We produced hydroxyl radicals using the Fenton reaction. Specifically, we mixed equal parts 0.6 mM  $\text{H}_2\text{O}_2$  and 3 mM  $\text{FeSO}_4$ , then perfused ~ 10 mL of this solution through the root over a period of 30 min to inhibit aquaporin activity. After inhibition, we once again quantified flow rate by constructing a transient curve with the HPFM. We estimated aquaporin contribution to  $K_R$  as the difference between initial  $K_R$  and  $K_R$  after aquaporin inhibition, divided by the initial  $K_R$ . This approach was used in a previous study assessing aquaporin function on deep roots at this site (McElrone et al. 2007).

We compared  $K_R$  using a nested two-way analysis of variance (ANOVA) where root depth and time (root depth) were fixed effects. Pairwise comparisons between the initial  $K_R$  and  $K_R$  after aquaporin inhibition were performed separately for deep roots and shallow roots using paired  $t$  tests. All values were considered significant at  $p < 0.05$  and marginally significant at  $p < 0.10$ .

#### Model description

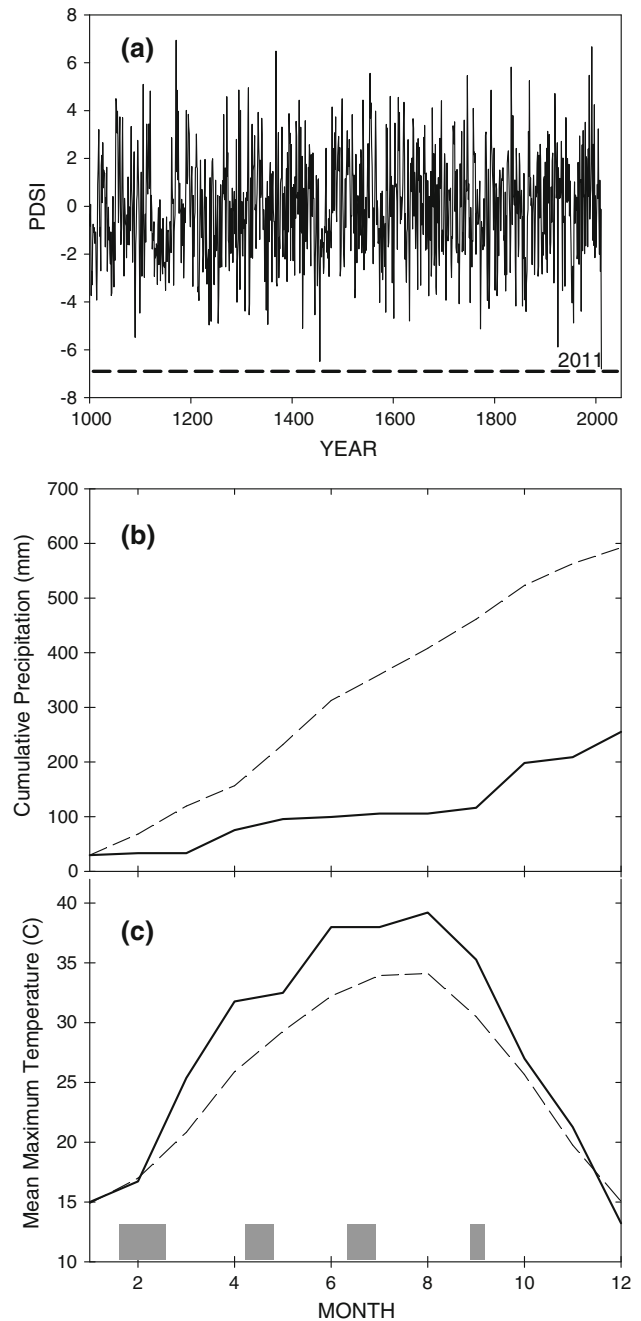
To estimate values of tree transpiration ( $E$ ) that would have occurred as soil dried, we used the hydraulic transport model of Sperry et al. (1998). The model predicts the upper boundary for steady-state water transport ( $E_{\text{crit}}$ ), which is a surrogate for plant hydraulic limits as a function of soil water potential. The range of soil water potentials from 0 to the value at which the plant reaches  $E_{\text{crit}}$  represents the plant's 'water use envelope'. Once  $E$  exceeds  $E_{\text{crit}}$ , water uptake ceases because of irreversible hydraulic dysfunction at some point along the soil–leaf hydraulic pathway (Sperry et al. 2002). Values of root and stem hydraulic vulnerability used for the model were from McElrone et al. (2004) and from our measurements using the method of Sperry and Saliendra (1994; see Fig. 1). Additionally, transpiration was measured using an LI-6400 (ambient temperature and humidity, saturating photosynthetically active radiation =  $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; LI-COR, Inc.,



**Fig. 1** Hydraulic vulnerability curves for roots (*closed symbols*) and shoots (*open symbols*) of *Q. fusiformis*. These curves were used to parameterize the model (see section “[Methods](#)”)

Lincoln, NE, USA) in these trees during the spring and summer of 2013 to further validate the model estimates of transpiration. Predawn and midday leaf water potentials were measured on the same days as transpiration (6 days in May, 4 days in July and 3 days in September) throughout the summer of 2013 and used as parameters in the model. Branch water potentials were measured using the bagged shoot method (e.g., Johnson et al. 2011) to assess the validity of the model.

Based on the hydraulic properties of soil and xylem, the model predicts the relationship between steady-state  $E$  and the driving force in the soil–plant continuum ( $E = K_{\text{plant}} (\Psi_{\text{soil}} - \Psi_{\text{L}})$ ), where  $K_{\text{plant}}$  is the leaf-specific hydraulic conductance of the whole plant, and  $\Psi_{\text{soil}} - \Psi_{\text{L}}$  is the water potential difference between soil and leaf. The drop in  $\Psi$  in the continuum is used to calculate the reduction in  $K_{\text{plant}}$  from the rhizosphere drying, root and stem embolism, and to determine  $E$ . The root components were assumed to comprise 50 % of  $K_{\text{plant}}$  and stems, branches and leaves were assumed to represent the other 50 % (Sperry et al. 1998). The water potential-dependent decrease in xylem hydraulic conductance was calculated from a Weibull function fit to the root and stem vulnerability curve data. The model further divides root and shoot systems into lateral and axial components. We used the same fitting parameters for axial and lateral components within the root and shoot systems. We partitioned the root system into two distinct modules: roots found from 0 to 50 cm soil depth and those found 10–20 m under ground. The shallow roots were assumed to be exposed to the drying soil during drought, but the deep roots were assumed to have access to water in streams. The soil water potentials for deep roots were assumed to be approximately zero and the water potentials for soil around shallow roots varied with the simulated drought. We ran the model by



**Fig. 2** Climatic data from the Texas Edwards Plateau region; **a** Historical Summer Palmer Drought Severity Index (data adapted from Cook et al. 2004). *Horizontal dashed line* indicates the 2011 PDSI of  $-6.8$ , **b** historical mean and 2011 cumulative precipitation and **c** historical mean and 2011 mean maximum monthly temperature. *Dashed lines* in panels **b** and **c** are historical means and *solid lines* are 2011 data. *Boxes* in panel **c** indicate sampling dates

modifying the proportion of shallow and deep root area by 1 % increments until we found the best fit between measured and modeled  $E$  data over the drying period.

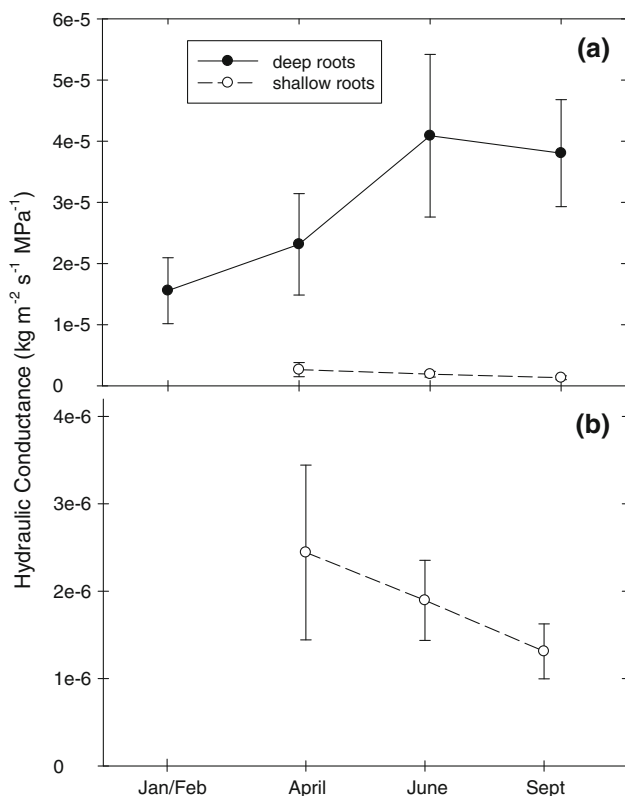
The water potential-dependent decrease in rhizosphere conductance was calculated from soil conductivity, soil

texture and  $\Psi_{\text{soil}}$  assuming a cylindrical rhizosphere sheath of 5 mm thickness from bulk soil to root surface (for details, see Sperry et al. 1998). Soils in the Edwards Plateau are typically shallow (<20 cm) and sit atop fractured limestone (Elliot and Veni 1994).

## Results

The 2011 summer in central Texas experienced the most severe drought (most negative Palmer Drought Severity Index) in over 1,000 years (Fig. 2a). Cumulative 2011 precipitation was only 43 % of mean annual precipitation (Fig. 2b). The precipitation between May and September, when over half of the precipitation typically occurs in this area, was 87 % below average. Mean maximum daily temperatures (averaged over a month) between June and September of 2011 were 4.1–5.8 °C greater than the long-term mean maximum daily temperatures (Fig. 2c).

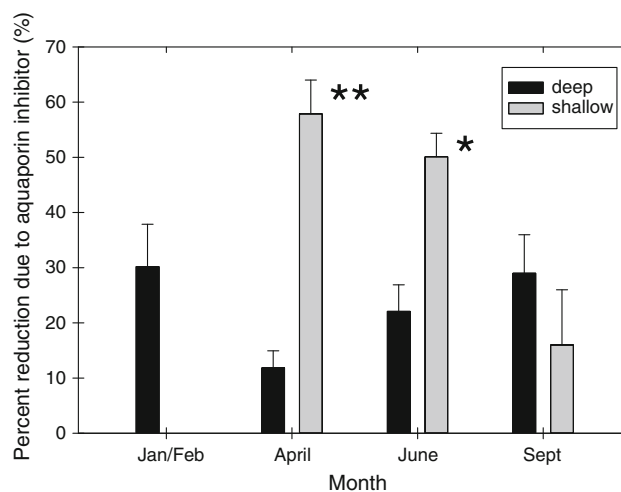
Between January and September of 2011, deep root hydraulic conductance ( $K_R$ ) increased 2.6-fold ( $p = 0.004$ ) and shallow root  $K_R$  decreased by 50 % between April and September (Fig. 3; marginally significant,  $p = 0.10$ ). At the peak of the drought in September, deep root  $K_R$  was 29-fold greater than shallow root  $K_R$  ( $p = 0.001$ ).



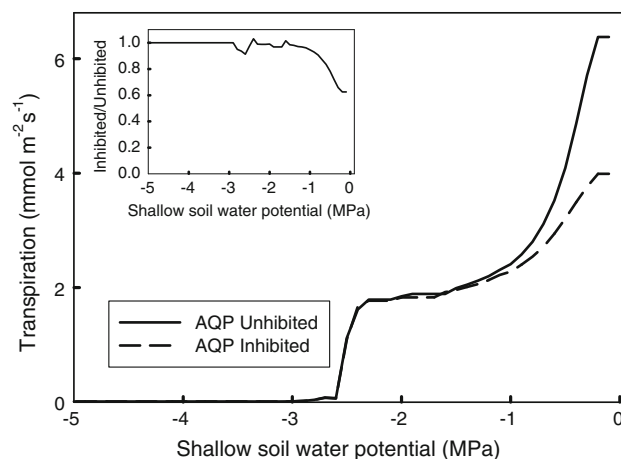
**Fig. 3** 2011 *Quercus fusiformis* deep and shallow (a) and shallow-only (b, notice y-axis scale changed from panel a) root hydraulic conductance

Shallow root aquaporin activity was much greater than deep root aquaporin activity in April and June ( $p$  values = 0.0001 and 0.001, respectively) (Fig. 4). Observed patterns in aquaporin activity from spring to autumn were opposite in deep and shallow roots. Between April and September, aquaporin activity decreased in shallow roots ( $p = 0.002$ ) but increased in deep roots ( $p = 0.01$ ). Using the model, we predicted that transpiration would be greater, especially for trees in moist soils, when aquaporins were active instead of inhibited (Fig. 5).

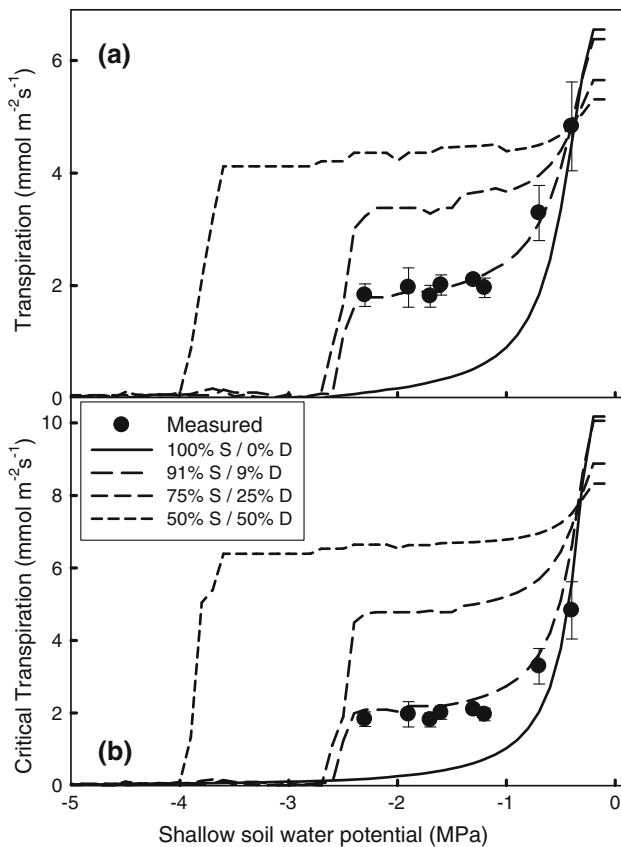
Based on field measurements of  $E$ , we were able to solve for the estimated deep:shallow root area percentages as 9 % deep: 91 % shallow. The model also predicted that



**Fig. 4** The percent reduction in hydraulic conductance after treatment with aquaporin inhibitor in deep and shallow roots. Asterisks indicate significant differences between deep and shallow roots; \*\* $p < 0.0001$ , \* $p < 0.001$



**Fig. 5** Modeled transpiration versus soil water potential in *Q. fusiformis* with aquaporins either inhibited or uninhibited. The inset panel shows the ratio of tree water use without and with AQP activity



**Fig. 6** Modeled transpiration versus soil water potential in *Q. fusiformis* with varying deep and shallow root distributions. Panel **a** is the predicted transpiration over a range of soil water potentials and panel **b** is the upper value for transpiration that would result in critical hydraulic failure. Solid symbols are measured values

trees with 9 % deep root area could continue to transpire with soil water potentials as low as  $-2.5$  MPa, whereas trees with no deep roots would not be able to transpire when soil water potentials fell below  $-1$  MPa. Results from the transport model predicted that trees would have twofold to threefold greater transpiration with 50 % of their root area in deep streams as compared to trees with 9 % deep root area or 0 % deep root area (Fig. 6a,b). The model also predicted that the measured trees would experience complete hydraulic failure if transpiration was to continue when shallow soil water potentials fell below  $-2.5$  MPa. Trees with greater deep:shallow root area ratios would not be able to transpire beyond 4 MPa, due to hydraulic failure of above-ground portions of the trees. Based on measured predawn leaf water potentials of adjacent plants (shallowly rooted *Juniperus ashei* =  $-5.5$  MPa—should be indicative of shallow soil water potential) and midday branch water potentials of *Q. fusiformis* ( $-2.2$  MPa), the model appeared to accurately capture the hydraulic failure of these trees at low soil water potentials.

## Discussion

Previous studies have used deep root sap flow and isotopic labeling (Burgess et al. 2000; Peñuelas and Filella 2003; Doody and Benyon 2010; Bleby et al. 2010) to study the contribution of deep roots to hydraulic lift and total tree water use, but few studies have measured deep or shallow root hydraulic conductance ( $K_R$ ) in situ. In this study, we observed seasonal variation in deep and shallow root  $K_R$ , and in aquaporin contribution to  $K_R$ , during a severe and prolonged drought in central Texas in 2011. Our results suggest that trees increase their dependence on deep roots and decrease their use of shallow roots for water uptake during drought and that aquaporin activity plays a regulatory role in this shift.

In the current study, deep root hydraulic conductance of *Q. fusiformis* increased and shallow root  $K_R$  decreased over the course of a drought (Fig. 3a, b). It is worth noting that only the maximum root hydraulic conductance was measured in the current study. If measures of hydraulic conductance with native embolism would have been included, the differences in conductance between deep and shallow roots would have likely been even greater. Our results are consistent with previous research in this system, which found that deep roots of *Q. fusiformis* had greater sap velocities (and represented a greater contribution to trunk sap flow) during drought than after a rain event (Bleby et al. 2010). In contrast with our results, other studies in *Quercus* have reported an overall decrease in whole-plant hydraulic conductance during drought (*Quercus petraea*; Cochard et al. 1996). This was attributed to either a soil or root component of the hydraulic pathway, as no embolism was observed in stems. One potential reason for the differing results was that *Q. petraea* likely had no access to deep, persistent pools of water because its deepest roots were approximately 1.4 m (Bréda et al. 1993). Accordingly, Nardini and Pitt (1999) found no decrease in root  $K_R$  in saplings ( $\sim 1$  m tall) of *Quercus pubescens* over a growing season despite substantial embolism in the stems. Similarly, in a Mediterranean system, *Quercus ilex* appeared to have deeper roots than *Quercus suber* and was able to maintain a more favorable water status during drought (David et al. 2007). Although the trees at our site have access to deep, reliable pools of water, the shifting dependence on deep versus shallow roots for water uptake during drought is likely a common phenomenon in many trees.

Shallow root  $K_R$  decreased by 50 % between April and September (Fig. 3b), which may have been driven by a reduction in aquaporin activity or extensive hydraulic failure that led to root death. Although the decrease in aquaporin activity between April and September coincides well with the decrease in  $K_R$ , it is difficult to rule out fine

root death as a potential mechanism as it too would lead to a decrease in  $K_R$  and aquaporin activity. In support of the role of reduced aquaporin activity,  $K_R$  was still significantly different than zero even in September and inhibition still resulted in a minor reduction to  $K_R$  in September, suggesting that not all fine roots had died. Further, Bleby et al. (2010) reported a substantial increase in shallow root sap flow after rainfall events, indicating that some shallow roots of *Q. fusiformis* survive drought. Nevertheless, shallow root mortality is fairly common (see Eissenstat and Yanai 1997). A previous study on *Picea abies* found high fine root mortality in response to drought (Persson et al. 1995). Alder et al. (1996) found a 60 % loss of hydraulic conductance in roots of *Acer grandidentatum*, but a much smaller loss in stems, during a drought year. In general, distal organs (roots and leaves) may be more vulnerable to embolism than boles (e.g., Domec et al. 2009a, b, 2010, Johnson et al. 2011, McCulloh et al. 2014) and this may be an adaptation to protect the bole of the tree from hydraulic failure (Tyree and Ewers 1991).

Based on the model used in the current study, *Q. fusiformis* was able to transpire during simulated moderate to extreme drought scenarios depending on the proportions of deep and shallow roots (Fig. 6). In individuals where gas exchange was measured, transpiration was able to continue until soil water potentials reached approximately  $-2.5$  MPa. These individuals were predicted to have 9 % deep and 91 % shallow root area. If the deep root area was increased to 50 % of the total root area, predicted transpiration could continue when soil water potential was near  $-4.0$  MPa. Below  $-4.0$  MPa transpiration was not possible, regardless of root distribution, because the above-ground portions of the trees had experienced total hydraulic failure. This was supported by branch water potential measurements that indicated that branches had lost 80–100 % of hydraulic conductance on dry days (where *Q. fusiformis* predawn leaf water potential was  $<-2$  MPa, midday branch water potentials were  $\sim -2.2$  MPa). However, it is possible that trees with access to deep pools of water can isolate themselves from drying soil through shallow root death. If this were the case, then they may be able to transpire at more negative water potentials than the model predicted.

The shift in deep root physiology with changing above-ground conditions is consistent with a previous study in *Q. fusiformis*. McElrone et al. (2007) observed diurnal cycling of deep root  $K_R$  and that shading reduced deep root  $K_R$ . These changes in  $K_R$  were also associated with changes in aquaporin activity. During the 2011 drought, *Q. fusiformis* deep root  $K_R$  increased 2.6-fold (Fig. 3a) and aquaporin activity also increased between April and September (Fig. 3) as the drought became particularly severe. Upregulation of root aquaporin activity during drought or during periods of

increased transpirational demand has been observed in other woody plants including grape vine (Vandeleur et al. 2009) and poplar (Laur and Hacke 2013). The upregulation of aquaporin activity is much more rapid than the production of new roots and is therefore a more effective method of increasing water transport over short time scales or if carbon is in short supply. The high aquaporin contribution to deep root  $K_R$  in January/February is somewhat counter-intuitive with the rest of the results and may have been driven by a sudden decrease in shallow soil water availability. Central Texas experienced a severe ice storm in December 2010, which froze the ground and may have temporarily reduced shallow root  $K_R$ . This reduction could have resulted in a corresponding need to increase deep root  $K_R$  through aquaporin upregulation. Annual cycles of increasing or decreasing aquaporin activity (e.g., McElrone et al. 2007) and associated root hydraulic conductance could be reflected in our results. Also, although hydroxyl radicals have been shown to inhibit aquaporins, they, along with other reactive oxygen species, have many different effects on plant physiology (for review see Apel and Hirt 2004), which should be taken into account when considering our results.

Water flow in the soil–plant–atmosphere continuum is determined by the whole-tree hydraulic resistance of soil and plant tissues, which characterizes the structural capacity of the whole plant at moving water (Tyree and Zimmermann 2002). Approximately, 50–60 % of whole-plant hydraulic resistances are located in the root system (Cruizat et al. 2002; Domec et al. 2009a, b), which shows the outstanding importance of this organ of water uptake within the flow path (Steudle and Peterson 1998). Changes in tree resistance related to drought have been linked to variation in gas exchange and transpiration (Meinzer et al. 1995, Hubbard et al. 2001). At low water stress, the increase in flow in resistance when aquaporins were inhibited increased the xylem component of root resistance proportionally more than the soil resistance. This larger influence of root resistance to the overall plant resistance at higher water potentials explained the larger difference in transpiration between aquaporins inhibited and uninhibited (Fig. 5). When soil was drying, soil resistance became a larger component of resistance (Sperry et al. 1998) and inhibiting aquaporin activity had a smaller effect on whole-tree resistance and transpiration (Fig. 5).

Drought intensity and frequency are predicted to increase in many arid and semiarid areas, including the south central USA, over the next decade (Seager et al. 2007; Diffenbaugh et al. 2011). Based on tree-ring reconstructions, the 2011 Texas drought was likely the most severe single-year drought there in the past millennium. Based on the PDSI, the 2011 Texas drought surpassed all of the major droughts of the twentieth century, including

the devastating dust bowl of the 1930s and the extreme drought of the 1950s. Millions of trees died because of the 2011 drought (Texas Forest Service 2012). Several recent droughts in other regions have also resulted in extensive tree mortality (Allen et al. 2009; Hoffmann et al. 2011; Nardini et al. 2013).

Recent research has made great strides toward understanding the mechanisms of drought-induced mortality, but root function during drought largely remains unstudied. In this study, we show that trees shift their dependence on deep roots over shallow roots during drought and that upregulation of aquaporin activity accounts for at least part of this increase. The modeling presented in this paper suggests that shallowly rooted species and those without access to deep pools of water are particularly susceptible to mortality with future droughts. These results should help us make better predictions about tree mortality under future climate change scenarios.

**Author contribution statement** DMJ contributed to data collection and analysis, and writing and editing the manuscript. MES contributed to data collection and analysis, and writing and editing the manuscript. JCD contributed to data collection and analysis, and writing and editing the manuscript. RBJ contributed to data analysis, and writing and editing the manuscript.

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

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