

Increased vapor pressure deficit due to higher temperature leads to greater transpiration and faster mortality during drought for tree seedlings common to the forest–grassland ecotone

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Summary

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Key words: drought, mortality, seedlings, transpiration, vapor pressure deficit (VPD), water potential.

- Tree species growing along the forest–grassland ecotone are near the moisture limit of their range. Small increases in temperature can increase vapor pressure deficit (VPD) which may increase tree water use and potentially hasten mortality during severe drought.
- We tested a 40% increase in VPD due to an increase in growing temperature from 30 to 33°C (constant dewpoint 21°C) on seedlings of 10 tree species common to the forest–grassland ecotone in the southern Great Plains, USA.
- Measurement at 33 vs 30°C during reciprocal leaf gas exchange measurements, that is, measurement of all seedlings at both growing temperatures, increased transpiration for seedlings grown at 30°C by 40% and 20% for seedlings grown at 33°C. Higher initial transpiration of seedlings in the 33°C growing temperature treatment resulted in more negative xylem water potentials and fewer days until transpiration decreased after watering was withheld. The seedlings grown at 33°C died 13% (average 2 d) sooner than seedlings grown at 30°C during terminal drought.
- If temperature and severity of droughts increase in the future, the forest–grassland ecotone could shift because low seedling survival rate may not sufficiently support forest regeneration and migration.

Introduction

Increased temperatures alone are assumed to allow a northward migration of plant species and increased heat stress may push the southern extent of less heat-tolerant species northward (Davis, 1989; Peters & Lovejoy, 1992). This north/south movement in response to changing climate is often identified as the route of large-scale change in the composition of North American ecosystems. However, the precipitation gradient that stretches from the wetter east coast of the North America to the drier Front Range of the Rocky Mountains could also be a front for vegetative change, especially as climate change alters precipitation patterns and evapotranspiration. In the center of this precipitation gradient is an ecotone between a tree-dominated landscape in the east and a grassland-dominated landscape in the west (Sims & Risser, 2000).

Forest–grassland ecotones are important globally and occur wherever grassland and forest ecosystems abut. In these transitional zones, many tree species live at the limit of their soil moisture or temperature requirements and vegetation dynamics in ecotones are particularly responsive to global climate change (Goldblum & Rigg, 2010). Changing between tree- and grass-dominated biomes as forest–grassland ecotones shift fundamentally alters carbon, water and nutrient cycling. In North America,

the largest forest–grassland ecotone occurs throughout the middle of the continent, from southern Canada to Texas, and is where many eastern tree species reach the western extreme of their range. For instance, in the state of Oklahoma, USA, over 80 species of trees reach the western edge of their range (Little, 2002) as the average total annual precipitation decreases from 145 to 80 cm across a span of *c.* 300 km.

By the end of the 21st century, an increase of 2.5–4.0°C in mean annual temperature is predicted for the south-central USA (Meehl *et al.*, 2007). Over that time, little change in total precipitation (Meehl *et al.*, 2007) and a decrease in summer precipitation (Diffenbaugh *et al.*, 2011) are predicted for this region. In addition there will probably be longer periods of drought and an increase in intensity of rainfall events which lead to greater water stress in mesic ecosystems (Knapp *et al.*, 2008). Increased temperature and more intense periods of soil moisture deficit may cause widespread changes in the range and dominance of tree species (Iverson *et al.*, 2008).

The direct effects of water availability and temperature are major drivers of plant species distribution along the ecotone (Sims & Risser, 2000). However, temperature also can modulate plant water use through its effects on vapor pressure deficit (VPD), such that higher temperatures may exacerbate the effects of drought (McDowell *et al.*, 2008; Allen *et al.*, 2010; Williams

et al., 2013). At a given temperature, VPD is the difference between the saturation vapor pressure and the actual vapor pressure. Because saturation vapor pressure increases exponentially with temperature, VPD increases exponentially when temperature increases and absolute humidity remains constant. Vapor pressure deficit is the driving force for transpiration in plants, that is, transpiration can be approximated as $VPD \times$ stomatal conductance. A 3°C increase in temperature can increase VPD by 45%. For instance, a temperature increase from 30 to 33°C at a constant dewpoint of 21°C increases VPD from 1.75 to 2.54 kPa.

Higher VPD probably will exacerbate physiological stress on plants during drought by either increasing plant water loss (anohyric species) or reducing carbon uptake (isohyric species; McDowell *et al.*, 2008). A comprehensive tree ring analysis in the southwestern USA indicated elevated VPD was a primary driver of recent tree mortality events (Williams *et al.*, 2013). However, we are not aware of any studies that have experimentally tested, under controlled conditions, the effects of increased VPD due to higher temperature on tree survival during extreme drought. While higher VPD increases the physical process of diffusion, plants may regulate water loss through stomatal closure or other physiological responses. Therefore, we do not know the extent to which higher VPD due to higher temperature will translate to plant water loss and ultimately mortality.

The objective of this study was to determine the effects of increased temperature and associated increase in VPD on the leaf gas exchange, xylem water potential and survival of seedlings during terminal drought for 10 native Oklahoma tree species that occur along the forest–grassland ecotone. We hypothesized that a 3°C increase in temperature and subsequent increase in VPD increases water use and leads to more rapid mortality during drought. Greater susceptibility of tree seedlings to drought-induced mortality under a future climate scenario may lead to forest retreat along grassland/forest ecotones. By testing seedlings from 10 species, we were able to determine the consistency of response across a wide range of taxa for the life stage that is crucial for regeneration following disturbance and for migration of species into new areas.

Materials and Methods

Seedlings of 10 tree species were selected that are native to Oklahoma, USA along the grassland/forest ecotone. All seedlings were purchased as 1-yr-old, bare-root stock. The 10 species were hackberry (*Celtis occidentalis* L.), eastern redbud (*Cercis canadensis* L.), common persimmon (*Diospyros virginiana* L.), eastern redcedar (*Juniperus virginiana* L.), red mulberry (*Morus rubra* L.), sycamore (*Platanus occidentalis* L.), American plum (*Prunus americana* Marsh.), bur oak (*Quercus macrocarpa* Michx), post oak (*Quercus stellata* Wangenh.) and Shumard oak (*Quercus shumardii* Buckley). All species except post oak came from the Oklahoma Department of Agriculture, Food, and Forestry Division nursery in Goldsby (OK, USA). The post oak seedlings were purchased from the Senter's Nursery in Whitehouse (TX, USA). Depending on species, seedling height ranged from 15 to 100 cm.

In late March 2009, before bud break, the seedlings were planted in 7.6 l pots with an outside diameter at the top of 21.6 cm and a depth of 21.6 cm. Five 1.25 cm diameter holes were drilled in the bottom for water drainage. The pots were filled with a mix of 50% local soil and 50% medium-grained sand to promote drainage. The local soil was a loam (52% sand, 38% silt, 10% clay) and had a pH of 7.2. Once planted, the seedlings were placed under a rain out shelter covered with clear plastic, watered at least every other day and fertilized with a liquid complete fertilizer (Miracle-Gro® LiquaFeed®; The Scotts Miracle-Gro Company, Marysville, OH, USA) three times a week with *c.* 0.08 g N, 0.06 g P, 0.04 g K, 0.0004 g Mn and 0.0004 g Zn applied per pot at each application.

Seedlings were moved into four growth chambers that were 2.49 wide \times 1.37 deep \times 2.35 m tall (interior dimensions) (model PGW 36; Conviron, Winnipeg, MB, Canada). The light sources within the chambers were a combination of fluorescent and incandescent light bulbs that were capable of producing up to 960 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR). For this experiment, the light bank was adjusted *c.* 1 m from the tops of the seedlings to allow for adequate ventilation and to maintain seedling temperatures at chamber set points. At this height, PAR was *c.* 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the tops of the seedlings. When first moved to the chambers, baseline conditions were temperature 30°C and relative humidity 59% for the period when the lights were on (day-time, 14 h d^{-1}) and temperature 25°C and 79% relative humidity when the lights were off (night-time, 10 h d^{-1}). These settings gave a constant day-time and night-time dewpoint of 21°C with a day-time VPD of 1.74 kPa and a night-time VPD of 0.66 kPa. Temperature and relative humidity were recorded by a WatchDog A150 data logger (Spectrum Technologies Inc., Plainfield, IL, USA) every 5 min throughout each experimental period.

For each of two experimental periods, two trees from each species were moved into each of the four growth chambers (eight total of each species per experimental period). Experimental Period 1 was conducted from 26 July to 13 August 2009 and Period 2 was conducted from 17 August to 4 September 2009. Seasonal changes in seedling physiology between Periods 1 and 2 were minimal as natural leaf senescence occurs mainly in October and November in Oklahoma, USA. The two seedlings that were placed in a given chamber were paired based on size. Before each period, plants were maintained in the chambers under baseline conditions (described above) for at least 7 d to acclimate and watered every other day 2–4 h before the chamber lights turned on. Preliminary measurement of leaf gas exchange (net photosynthetic rate, leaf conductance and leaf transpiration) was conducted on fully expanded leaves, using a LI-6400 portable photosynthesis system with the 6400-02B blue-red LED Light Source (Li-Cor Inc., Lincoln, NE, USA) 2–4 h after the chamber lights turned on. Throughout the study, conditions in the leaf chamber were set to keep a constant airflow of 500 $\mu\text{mol s}^{-1}$, a reference CO_2 concentration of 400 $\mu\text{mol mol}^{-1}$, and a PAR of 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Throughout the study, temperature and relative humidity in the cuvette were controlled to exactly match the set points of the chamber (temperature \pm 0.2°C, relative humidity \pm 0.2%).

Following the preliminary gas exchange measurements, one tree of each species in each chamber was assigned to the well-watered treatment, while the other of that species was assigned to the terminal drought treatment. Also, two of the four chambers were set to a day-time temperature of 33°C with a relative humidity of 49% (VPD of 2.56 kPa) and night-time temperature of 28°C with a relative humidity of 66% (VPD of 1.29 kPa). These growing temperature and humidity conditions maintained a constant day-time dewpoint of 21°C for all chambers, but a different VPD due to the different temperatures, that is, 1.74 vs 2.56 kPa. Once the growing temperature was changed in the two chambers, water was withheld from the trees assigned to the terminal drought treatment until they died. Death, determined by stem desiccation as indicated by a loss of flexibility and browning of inner bark, was generally rapid and obvious. Depending on the species, stem desiccation coincided with either leaf drop or shriveling of existing leaves. With the exception of eastern redcedar, all species used in the experiment can resprout following topkill. Once the experimental period was over, nonwatered trees were placed back under the rainout shelter and watered to check for resprouting until the end of the following spring. In addition to the initial gas exchange measurements, gas exchange measurements were taken 2, 4, 7, 9 and 11 d after treatment imposition. Except when the leaf abscised, measurements were conducted on the same leaf for a given individual. For the nonwatered trees, gas exchange measurements continued until intercellular CO₂ concentration exceeded the reference CO₂ concentration. Gas exchange on the watered trees was measured one interval longer than the nonwatered tree in the same chamber.

Four days (Period 1) or 3 d (Period 2) after applying treatments, leaf gas exchange was measured on all seedlings at both the ambient condition within their assigned growth chamber and also at the environmental conditions of the reciprocal treatment. After measurement in ambient conditions, the environmental conditions of the chamber were changed and the reciprocal measurements were taken between 1 and 3 h of resetting the chamber. Concurrent with the gas exchange measurements under ambient conditions, xylem water potential was measured on excised leaves for each living seedling using a pressure chamber (Model 600; PMS Instrument Co, Albany, OR, USA). Following the reciprocal measurements, the chambers were returned to their previous set points.

For a given chamber, the reciprocal temperature treatment was used during the second experimental period and chamber served as the blocking variable. This procedure statistically removed the chamber effects and minimized possible chamber bias. The experiment was a split-plot design. Temperature treatments served as the whole-plot factor ($n=4$). The factorial combination of species ($n=16$) and watering treatments ($n=80$) served as the split-plot factors. The number of days until seedlings died was recorded. These data were log transformed and then analyzed using the Proc Mixed procedure (SAS v9.2; SAS Inc., Cary, NC, USA) with a model testing for differences in the fixed effects of temperature, species, watering as well as their interactions. Water potential data were tested using the Proc Mixed with a similar model. The ratio of

reciprocal gas exchange measurements was generated by dividing the higher VPD growth condition measurement (33°C) by the lower VPD growth condition measurement (30°C) for each tree. This ratio was analyzed using Proc Mixed to test for differences between growth conditions, watering treatment, species and any interactions. After this analysis, a *t*-test was used to determine whether the ratio was different from one, that is, a ratio of one indicates measurements did not differ among reciprocal environments. Different *t*-tests were conducted for each factor that contained significant differences based on the Proc Mixed analysis.

The repeated transpiration measurements (day 0, 2, 4, 7, 9 and 11) for the terminal drought trees were analyzed using Proc Mixed. To account for inherent variation between trees, the data were relativized using pretreatment data; that is, for each individual tree, each measurement was divided by the initial measurement. To further account for changes in gas exchange over time not due to the dry-down treatment, this ratio was then divided by a similar ratio for the well watered trees from the same chamber. These values representing changes in relative transpiration rate of the nonwatered trees were tested for differences among temperature treatments and species as well as interactions with measurement date. The variance for the time series was managed using a heterogeneous variance model.

Average actual temperatures were within 0.5°C of the set points (Table 1). While the average relative humidity differed from set points between 2.4% and 9.7%, dewpoints were similar within day-time and night-time periods for the two temperature set points; that is, actual was higher than set points during the day and lower than set points during the night for both treatments. There was a +0.2°C difference for dewpoint from the set point of 21°C for night-time and +1.4 and +1.7°C difference for day-time dewpoints in the high- and low-temperature treatments, respectively. The net effect was that actual VPDs were slightly lower than the set point during the day and slightly higher during the night. The actual day-time VPD was 40% higher in the 33°C set point than in the 30°C set point which is slightly lower than the 45% difference that was desired.

Results

When measured after the fourth (Period 1) or third day (Period 2) after imposing treatments, the ratio of net photosynthesis measured at 33°C divided by that measured at 30°C from the same seedling was calculated to determine the short-term effects of temperature. This ratio decreased by 12% in response to the 45% increase in VPD associated with the higher measurement temperature (ratio significantly different from 1; $P < 0.0001$). Differences in the ratio for seedlings growing at 33°C and seedlings growing at 30°C were not different (growing temperature; $P = 0.20$; Fig. 1a, Supporting Information Table S1). Likewise, short-term effects of measurement temperature and VPD on net photosynthesis were consistent, that is, not statistically significant, for the water stress treatments, species, and all interactions ($P > 0.10$).

The ratio of transpiration measured at 33°C divided by that measured at 30°C was greater than one, indicating greater transpiration at the higher temperature and VPD set point. The

Table 1 Set points and actual environmental measurements from the chambers used to control temperature and VPD during the experiment where numbers following the \pm refer to the standard deviation of the four chamber means based on measurements conducted every 5 min

	Set T (°C)	Act. T (°C)	Set RH (%)	Act RH (%)	Dewpoint (°C)	Set VPD (kPa)	Act VPD (kPa)
Cool-day	30.0	30.5 \pm 1.0	59	61.4 \pm 3.0	22.4 \pm 1.3	1.74	1.69
Cool-night	25.0	25.2 \pm 0.7	79	69.3 \pm 7.5	21.2 \pm 1.0	0.66	0.98
Warm-day	33.0	33.3 \pm 1.0	49	53.6 \pm 1.6	22.7 \pm 1.4	2.56	2.36
Warm-night	28.0	28.0 \pm 1.0	66	61.3 \pm 9.7	21.2 \pm 1.0	1.29	1.46

T, temperature; RH, relative humidity; VPD, vapor pressure deficit.

short-term effects of altering temperature during measurement were larger for seedlings growing at 30°C than those growing at 33°C (growing temperature; $P=0.04$). The ratio was 1.4 for seedlings growing at 30°C ($P<0.0001$) and 1.2 for seedlings growing at 33°C ($P<0.0001$; Fig. 1b, Table S2). The short-term

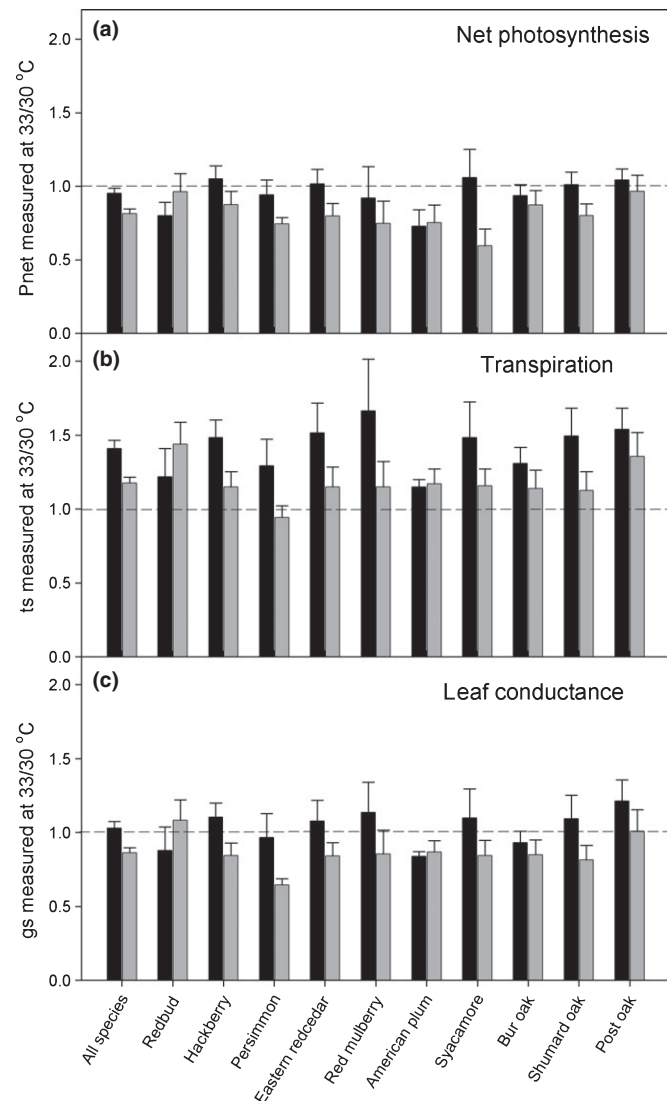


Fig. 1 The ratio of net photosynthesis (P_{net}), transpiration (t_s), and leaf conductance (g_s) measured at 33°C divided by that measured at 30°C for seedlings of 10 tree species. Seedlings were grown at either 33°C (grey bars) or 30°C (black bars) ($n=8$). Vertical bars represent $+1$ SE.

effects of temperature and VPD on transpiration were consistent among species, water stress treatments, and for their interactions ($P>0.10$). These increases in transpiration were $<45\%$ increase in VPD in part because stomatal conductance was lower in the higher temperature treatment. On average, stomatal conductance decreased by 14% for seedlings growing at 33°C ($P<0.0001$; Fig. 1c, Table S3) which experienced smaller increases in transpiration than for seedlings grown at 30°C. By contrast, stomatal conductance was not significantly affected by measurement temperature; that is, 30 vs 33°C, for seedlings growing at 30°C (3% increase) ($P=0.49$) (growing temperature effect; $P=0.05$). Aside from the growing temperature effects, the effects of species, watering treatment and their interactions were not significant ($P>0.12$).

The range of xylem water potential for well-watered trees in the 30°C growing temperature treatment was -0.46 MPa for red mulberry to -0.86 MPa for American plum and -0.43 MPa for red mulberry to -1.00 MPa for bur oak in the 33°C growing temperature treatment. The range for the nonwatered trees was -0.60 MPa for post oak to -1.25 MPa for American plum for the 30°C growing temperature treatment and -0.78 MPa for post oak to -1.65 MPa for American plum for the 33°C growing temperature treatment (species effects; $P<0.0001$; Table S4). Across species, water potentials were more negative for the non-watered compared to the watered trees ($P<0.0001$), and this effect was consistent for the various species (species \times growing temperature; $P=0.45$; Fig. 2). There was a small difference between the temperature regimes for the well-watered trees (-0.07 MPa) and larger differences between the growing temperatures for the nonwatered trees (-0.33 MPa; water regime \times growing temperature interaction; $P=0.002$; Fig. 2). The interaction between species, watering treatment and growing temperature was not significant ($P=0.99$).

The transpiration rate of nonwatered seedlings relative to the pre-treatment measurement declined as the seedlings became water stressed. Values of $<20\%$ of the pretreatment measurement were reached between 4 and 20 d depending on the species. When analyzed as a repeated measures, relative transpiration of seedlings in the 33°C growing temperature treatment tended to decrease more rapidly than those from the 30°C treatment (growing temperature \times date interaction; $P=0.02$; Fig. 3, Table S5). Relative transpiration declined with date (date; $P<0.0001$) and the rate of decline differed among species (date \times species; $P=0.02$). However, the overall effect of growing temperature was not significant ($P=0.10$) because all trees started with values

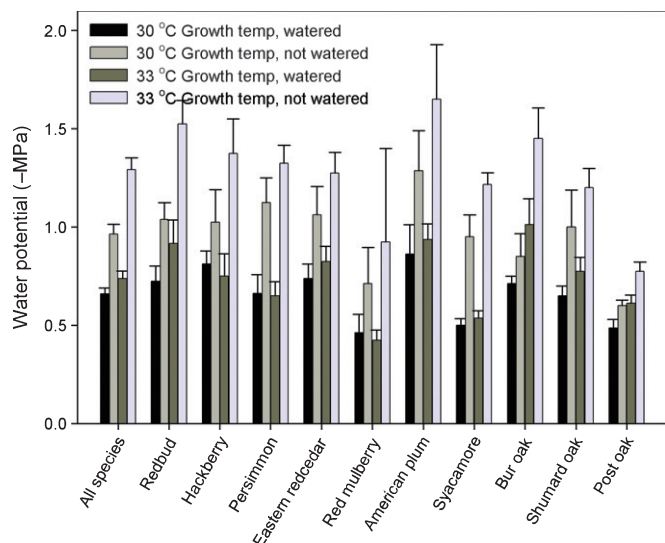


Fig. 2 The average water potential (–MPa) for seedlings of 10 tree species measured 3 or 4 d after imposing treatments. Seedlings were grown at either 33°C or 30°C ($n = 4$). Vertical bars represent + SE.

of 1.0 and decreased to near 0 regardless of growing temperature. When day 4 (midpoint of dry down for most species) was analyzed separately, the 33°C growing temperature treatment seedlings had lower relative transpiration rates than the 30°C growing temperature treatment seedlings ($P = 0.07$).

The number of days until seedling mortality of the nonwatered seedlings varied greatly between species, with a range of 5.9 (red mulberry) to 24 d for (eastern redcedar) (species effect $P < 0.0001$). The mean days until mortality for the 33°C growing temperature treatment seedlings was almost 2 d sooner than for the 30°C growing temperature treatment seedlings (30° seedling mean of 14.7 d, 33°C seedling mean of 12.8 d) (growing temperature effect; $P = 0.05$; Fig. 4, Table S6). Overall the effects of growing temperature were consistent across species (species \times growing temperature; $P = 0.44$). Only one well-watered seedling (redbud) died during this study.

Discussion

Our results support the hypothesis that an increase in temperature-modulated VPD increases transpiration and results in more rapid mortality during terminal drought. The reciprocal measurements caused an immediate, short-term increase in transpiration due to higher temperature and resultant VPD. Higher transpiration led seedlings to have more negative xylem water potentials and to exhibit quicker reductions in leaf gas exchange when watering was withheld. The net effect was that seedlings grown at a temperature +3°C died 13% sooner. The importance and novelty of our findings is that they indicate the effect of a 3°C temperature increase on the physical process of diffusion (increased VPD) can accelerate mortality during drought even though plants counteract with physiological changes to reduce water. Our highly controlled growth chamber study agrees with a region-wide tree ring analysis of Williams *et al.* (2013) indicating

that increased VPD associated with higher temperatures intensify the effects of drought (McDowell *et al.*, 2008; Allen *et al.*, 2010).

Our study demonstrated susceptibility to higher VPD and ‘climate change drought’ of seedlings from a broad range of species with results generally indicating a consistent trend for tree species along the forest–grassland ecotone. More rapid mortality due to global change drought, that is, prolonged drought under higher temperatures, has been reported for trees in the southwestern and intermountain west of the United States (Allen & Breshears, 1998; Breshears *et al.*, 2005, 2009; Adams *et al.*, 2009; van Mantgem *et al.*, 2009; Anderegg *et al.*, 2012). Based on our findings, seedling survival might decrease and might lead to changes in tree distribution in the Midwest and Great Plains of the USA under global change drought scenarios.

The growing temperatures used in this study are relevant to current growing conditions along the forest–grassland ecotone in Oklahoma, USA. Along the ecotone, average July and August daily high temperatures are 34°C and average daily mean temperatures are 28 and 27°C, respectively. Average dewpoints during July and August are 20°C (Oklahoma Climatological Survey; http://www.mesonet.org/index.php/weather/mesonet_averages_maps). Likewise, the long periods without rainfall do occur along the forest–grassland ecotone. During the 5 yr between 2008 and 2012, McCalester, OK (34°54′N, 95°46′W) experienced seven periods during June to September with 14 d or longer without rainfall (Oklahoma Climatological Survey; http://www.mesonet.org/index.php/weather/station_monthly_summaries), the longest of which was 25 d. In one period between July and August 2011, there was a stretch of 37 d when it rained a total of 0.25 cm.

We kept dewpoint constant between temperature treatments which resulted in a large increase in VPD. As saturation vapor pressure increases with increasing temperature, dewpoint may increase (Anderson *et al.*, 2010), especially in more humid or maritime climates. However, future changes in climate are expected to result in periods of higher VPD. The central USA is predicted to have higher summer temperature, lower summer precipitation and reduced absolute humidity (Diffenbaugh *et al.*, 2011) which cause a more extreme VPD increase than we assumed. In addition, increased atmospheric CO₂ may reduce relative humidity due to decreased plant transpiration (Cao *et al.*, 2010).

The generally consistent response of 10 different species compensates for the relatively small sample size ($n = 4$) for temperature treatments and the fact that each species \times treatment combination was only represented by four individuals. More importantly, it indicates the robustness of the finding that increased VPD due to 3°C temperature increase can accelerate water use and hasten mortality of tree seedlings common to the forest–grassland ecotone. Although species responses to treatments varied in magnitude and in a few cases the direction of response, an advantage of including species as a factor is that tests for treatment effects are more powerful and interpretation more straightforward if there are no species \times temperature treatment interactions, as was the case in our study. Certainly species will vary in their response to drought, temperature and VPD which may alter forest composition under

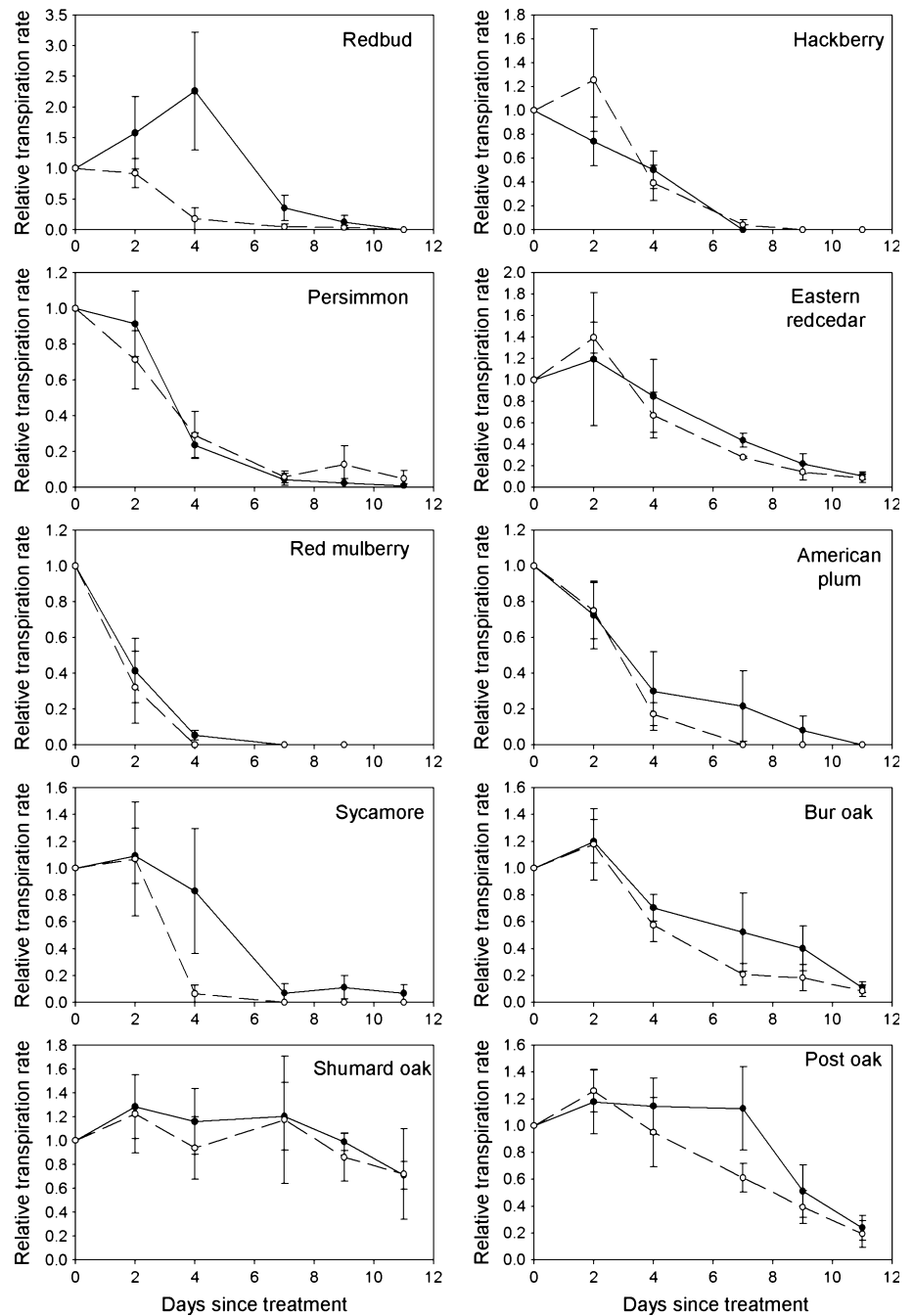


Fig. 3 Change in transpiration over time for 10 species of tree seedlings. Measurements were relativized based on measurements before imposing treatments. After the pre-measurement for all seedlings at 30°C, seedlings were measured at their growing temperature of 30°C (closed circles) or 33°C (open circles). Only seedlings from which water was withheld are included in this analysis ($n = 4$). Vertical bars represent \pm SE.

future climate conditions. Our design sacrificed numbers of individuals for each species for the inclusion of 10 different species. As such, limited sample size did not permit us to tease apart meaningful differences among species. Given that we included 10 species in our study, however, we can make the general inference that seedlings of species common to the forest–grassland ecotone can be expected to use water more quickly and die sooner with an increase in temperature-modulated VPD. Given that our objective was to understand potential shifts of the forest–grassland ecotone, making a statement about forests in general was more important than fine tuning species responses.

Species differences in leaf gas exchange rates, tree size and leaf area all affect the rate of water use and timing of water stress, but the statistical analyses accounted for the main effect of species to focus on the treatment effects. Given the number of trees (160), more intensive measurements of light response curves or $A-C_i$ curves were not practical. Rather, our approach was to measure gas exchange under nonlimiting light conditions to serve as an index of gas exchange potential at particular points in time. Given that foliage developed in full-sun conditions and was measured under light-saturating conditions in the cuvette, a light intensity \times treatment interaction on leaf gas exchange was unlikely.

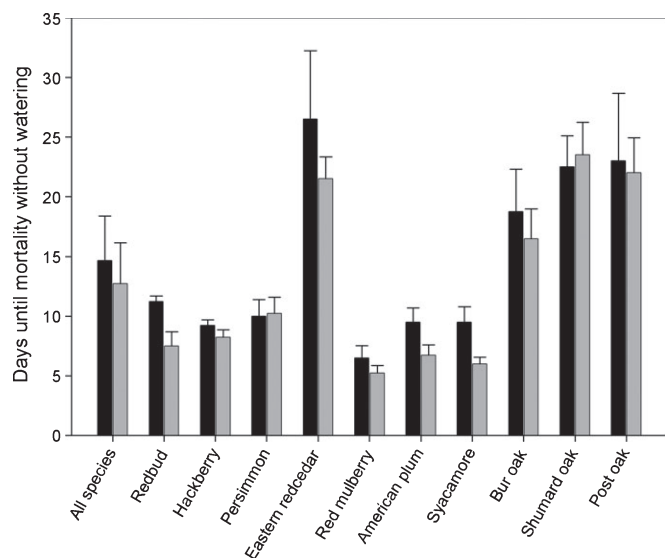


Fig. 4 Average days until death for seedlings of 10 tree species that were not watered. Seedlings were grown at either 33°C (grey bars) or 30°C (black bars) ($n = 4$). Vertical bars represent +1 SE.

Our experimental treatment – higher temperature and constant dewpoint – is very relevant as it reflects the most likely global change scenario where both temperature and VPD increase. An inevitable consequence of testing the effects of increased VPD due to increased temperature is that temperature and VPD are confounded. Although our results strongly point to higher VPD and accelerated transpiration leading to desiccation-induced mortality, we did not determine the proximal mechanism of death. The mortality mechanism could be complicated and variable among species (McDowell *et al.*, 2011). Mortality might occur due to hydraulic failure, carbon starvation (McDowell *et al.*, 2008), or water-stress impaired carbon metabolism and transport (Sala *et al.*, 2010). While both carbon metabolism interference and hydraulic failure result directly from VPD-modulated water stress, confounding temperature with VPD is important in regards to carbon starvation because a 3°C increase in temperature can increase respiration by 23% (assuming a Q10 of 2.0). However, we do not think that carbon starvation played a role in seedling mortality in our study for a number of reasons. Net photosynthesis remained positive until a day or two before mortality (net photosynthesis was proportional to transpiration data presented in Fig. 3). This combined with the relatively short time until mortality – between 6 and 27 d after cessation of watering depending on the species – probably resulted in adequate nonstructural carbohydrate supply throughout the mortality process. In a study where carbon starvation was identified as the proximal cause of mortality, it took > 15 wk for saplings (average height 1.7 m) to die without watering (Adams *et al.*, 2009). In studies not related to drought, woody plant seedlings have been documented to survive long periods in the dark. *Picea mariana* seedlings survived 10 d of exposure to continuous darkness at 35°C (the longest treatment imposed; Zhang & Sutton, 1994) and physiologically active seedlings of tropical trees routinely survive up to 3 months of submergence in the dark

at water temperatures near 30°C (Parolin, 2009). Another piece of evidence pointing towards desiccation rather than carbon starvation as the mechanism of death is that the rapid desiccation of attached foliage that corresponded to mortality in our study was similar to that identified by Kursar *et al.* (2009) as desiccation related mortality. Lastly, a 3°C increase in temperature probably did not have a large effect on rates of enzyme driven processes such as photosynthesis and respiration as mesic tree species readily acclimate to temperature changes which minimizes changes due to growing temperature in the range that we imposed (Teskey & Will, 1999; Will, 2000; Campbell *et al.*, 2007; Gunderson *et al.*, 2010).

During the reciprocal measurements, lower conductance moderated the effects of higher VPD on transpiration. For instance, VPD was increased by 45% during the reciprocal measurements, but transpiration only increased by 29%. This stomatal ‘dampening’ of the transpiration response was more pronounced in the seedlings grown at 33°C than those grown at 30°C, indicating greater stomatal sensitivity associated with the higher growing temperature treatment. Greater stomatal sensitivity to VPD has been reported for water-stressed trees (Saliendra *et al.*, 1995; Aasamaa & Söber, 2011) and for trees growing on xeric sites (Addington *et al.*, 2004). More negative xylem water potentials were measured concurrently with the reciprocal gas exchange measurements for seedlings in the nonwatered, higher temperature treatment.

The ability to moderate water loss through stomatal closure is an important adaptive mechanism to increased water stress, especially for isohydric species. Relative transpiration for most species, except post oak and Shumard oak, and to a lesser extent eastern redcedar and bur oak, decreased sharply by the fourth day from when watering was withheld and the decrease was greater for seedlings in the higher temperature treatment. The species that exhibited a rapid decline in relative transpiration also died in 10 or fewer days from when water was withheld as compared to between 17 and 25 d for the oaks and eastern redcedar. More gradual decreases in transpiration could be due to lower rates of leaf-level transpiration or to lower leaf area. We could not measure leaf area for individual seedlings as shriveling, senescence and abscission occurred during the mortality process. However, the oaks and eastern redcedar had lower initial transpiration rates (average 1.63 mmol m⁻² s⁻¹) than the other species (average 2.56 mmol m⁻² s⁻¹), suggesting lower leaf-level transpiration rates may have contributed to the longer survival of eastern redcedar and the oaks during drought.

The time from when watering is withheld until death is an intuitive metric to gauge the probability of seedlings surviving protracted drought (Adams *et al.*, 2009). In addition to leaf area and transpiration (water use), however, this metric is affected by plant available soil water which is a function of rooting volume, soil texture, and competition (Peterman *et al.*, 2012). In this study, we isolated transpiration differences by comparing pot-grown individuals of similar size within each chamber × species comparison. Overall, all species died relatively quickly compared to reports from arid environments (Adams *et al.*, 2009) in part due to the low water-holding capacity of the soil (76% sand) and

because our seedlings had less rooting volume and were of species that naturally grow in mesic environments. Experimenting with seedlings growing in their native condition that include the effects of competition is a more realistic test of the effects of increasing VPD, and time to mortality would probably be longer and more variable without watering or precipitation. Regardless, our results indicate that with all else being equal, higher VPD due to a 3°C increase in temperature accelerates water use and hastens mortality.

In Oklahoma and other places along the forest–grassland ecotone in the central USA, most tree species are at the margin of their range. While the location of the forest–grassland ecotone is a function of the interacting effects of climate, fire and grazing, (Sankey *et al.*, 2006), water availability plays a large, direct role in the distribution of trees in the Midwestern USA. Rice & Penfound (1959) studied tree mortality in response to the intense drought of 1956–1957 and concluded that the drought caused a partial shift from oak savanna to grassland, a shift that would have been completed had the drought continued for a few more years. A similar account of direct mortality of trees in Midwestern prairies and savannas was documented due to droughts in the 1930s (Albertson & Weaver, 1945). If temperature increases in the future, it could create an eastward range shift of the ecotone by eliminating tree seedlings such that species cannot replace themselves following disturbance. While we have no direct evidence that seedlings will be the limitation to forest persistence in a hotter, drier climate, seedlings are necessary for regeneration and are the most susceptible life stage to drought (Albertson & Weaver, 1945; Hanson & Weltzin, 2000). A combination of increased temperature and increased variability in precipitation predicted for the future in the south-central United States may ultimately lead to a shift in community composition and cause profound changes in ecosystem carbon, water and nutrient dynamics.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Tables S1 Statistical results for reciprocal measurements of net photosynthesis

Tables S2 Statistical results for reciprocal measurements of transpiration

Tables S3 Statistical results for reciprocal measurements of leaf conductance

Tables S4 Statistical results for xylem water potential measurements

Tables S5 Statistical results for relative transpiration measurements

Tables S6 Statistical results for days until mortality (log transformed)

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