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Original Article

Stomatal acclimation to vapour pressure deficit doubles transpiration of small tree seedlings with warming

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ABSTRACT

Future climate change is expected to increase temperature (*T*) and atmospheric vapour pressure deficit (VPD) in many regions, but the effect of persistent warming on plant stomatal behaviour is highly uncertain. We investigated the effect of experimental warming of 1.9–5.1 °C and increased VPD of 0.5–1.3 kPa on transpiration and stomatal conductance (g_s) of tree seedlings in the temperate forest understory (Duke Forest, North Carolina, USA). We observed peaked responses of transpiration to VPD in all seedlings, and the optimum VPD for transpiration (D_{opt}) shifted proportionally with increasing chamber VPD. Warming increased mean water use of *Carya* by 140% and *Quercus* by 150%, but had no significant effect on water use of *Acer*. Increased water use of ring-porous species was attributed to (1) higher air *T* and (2) stomatal acclimation to VPD resulting in higher g_s and more sensitive stomata, and thereby less efficient water use. Stomatal acclimation maintained homeostasis of leaf *T* and carbon gain despite increased VPD, revealing that short-term stomatal responses to VPD may not be representative of long-term exposure. Acclimation responses differ from expectations of decreasing g_s with increasing VPD and may necessitate revision of current models based on this assumption.

Key-words: humidity; sap flux; stomata; stomatal conductance; temperature; warming experiment.

INTRODUCTION

Stomatal control of the exchange of carbon and water vapour between vegetation and the atmosphere plays an important role in the global carbon and water cycles. Each year, more than half of the precipitation that falls on land is transpired through plant stomata (Chahine 1992; Jackson *et al.* 2000). Global evapotranspiration increased by about 1 mm yr^{-1} as air temperature (*T*) rose in the 1980s and 1990s (Jung *et al.* 2010; Zhang *et al.* 2010; Zeng *et al.* 2012). General circulation models predict that warming will increase evapotranspiration (Manabe & Wetherald 1987; Gregory *et al.* 1997; Manabe *et al.* 2004) because of an acceleration of the hydrologic cycle (Milly *et al.* 2002; Bosilovich *et al.* 2005), because higher *T* increases the water-holding capacity of the atmosphere. Yet increases in global evapotranspiration have stalled since 1998 (Jung *et al.* 2010; Zeng *et al.* 2012), even as *T* has continued to increase (IPCC 2014), casting doubt on the extent to which *T* alone can predict evapotranspiration, particularly in the future. The disconnect between rising *T* and evapotranspiration since 1998 may relate to the differing effects of *T* and vapour pressure deficit (VPD) on stomata. Increases in *T* nearly always cause increases in VPD in natural ecosystems (Hardwick *et al.* 2015), and if relative humidity does not change markedly in the future (e.g. Trenberth *et al.* 2005), warming will create widespread regional increases in atmospheric VPD. Increases in *T* often cause plants to open their stomata (Bunce 1985; Lu *et al.* 2000; Kudoyarova *et al.* 2011; Sadras *et al.* 2012; Way *et al.* 2012, but see Maenpaa *et al.* 2011), but this is confounded by increases in VPD, which tend to trigger stomatal closure (Monteith 1995; Oren *et al.* 1999b). The extent to which stomatal closure is more strongly driven by *T* or VPD may, in turn, also be influenced by acclimation processes that modify stomatal responses to *T* and VPD, further complicating plant responses to climate change.

Acclimation is commonly interpreted as an adaptive response that allows organisms to maintain homeostasis during environmental (or other) change. For stomatal responses to VPD, however, maintaining homeostasis of water relations is at odds with maintaining homeostasis of carbon relations. Stomatal closure upon exposure to drier air occurs within minutes (Lange *et al.* 1971), but short-term responses may not be representative of longer-term exposure. To maintain carbon gain despite increases in VPD, we would expect long-term exposure to high VPD to have less of an effect on stomatal conductance (g_s) than is observed under short-term exposure to high VPD (Fig. 1). Stomatal acclimation maintaining homeostasis of carbon relations could occur either through (1) increases in conductance, for example, g_{sref}, the g_s at VPD = 1 kPa, or (2) decreases in stomatal sensitivity to VPD, that is, the slope (–*m*) of the logarithmic decrease of g_s with increasing VPD (Oren *et al.* 1999b). Besides sustaining high rates of assimilation, this also allows plants to cool leaves (Nobel 1974) and maintains leaf *T* within an optimal range (Mahan & Upchurch 1988), but it would result in increased plant water

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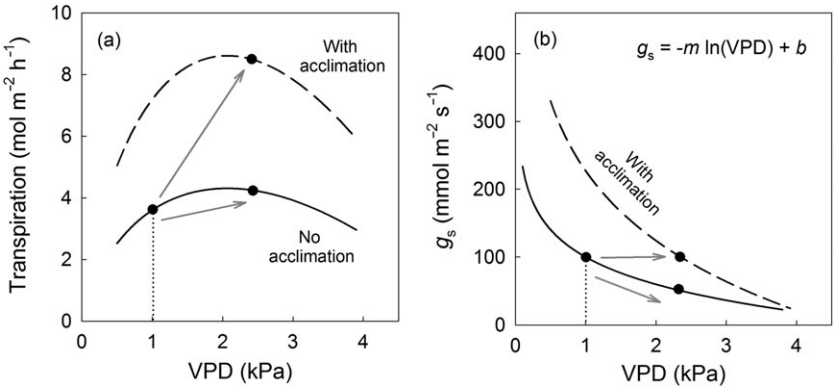

Figure 1. Theoretical effect of stomatal acclimation to vapour pressure deficit (VPD) on (a) transpiration and (b) stomatal conductance that maintains homeostasis of carbon relations over long time scales (i.e. weeks to months). Black circles and arrows depict the change from ambient conditions (highlighted by a dotted vertical line) to a warming scenario accompanied by an increase in atmospheric VPD of 1.3 kPa. Without acclimation, there would be a small increase in transpiration and a decrease in g_s upon exposure to high VPD. With acclimation, there would be a doubling of transpiration and no change in g_s after sustained exposure to high VPD.

deficits (Fig. 1) unless there is also a corresponding change in plant hydraulics.

If natural selection has prioritized homeostasis of plant water status, there may be little or no acclimation of stomatal responses to VPD (Cunningham 2005). Or, depending on the shape of the relationship between stomatal conductance and VPD, homeostasis of water status might require a decrease in conductance and/or higher stomatal sensitivity to VPD to stabilize water loss over prolonged exposure to high VPD. Acclimation responses in this direction could explain the cessation of the rising global evapotranspiration trend since 1998 (Jung *et al.* 2010), since a mechanism involving widespread stomatal closure is required to decrease transpiration at the global scale.

There are several reports documenting stomatal acclimation to VPD that maintains homeostasis of carbon relations (Wang & Kellomaki 1997; Kutsch *et al.* 2001; Herbst *et al.* 2008), where higher conductance and/or lower stomatal sensitivity to VPD led to increased water loss over timescales of weeks to months. Other studies document stomatal acclimation to VPD that maintains homeostasis of water status (Nejad & van Meeteren 2008; Sermons *et al.* 2012; Will *et al.* 2013; Carins Murphy *et al.* 2014), and the strength of the acclimation response can depend on growth T (Sermons *et al.* 2012). Despite these reports, ecosystem and global models still rely on expectations based on the well-known relationship between g_s and VPD described by Oren *et al.* (1999b), where reductions in g_s as a function of higher VPD at higher T are expected (de Boeck *et al.* 2012; Duursma *et al.* 2013) without accounting for any acclimation response.

Whether acclimation acts to maintain homeostasis of carbon or water status may depend on the water-use strategy of a plant. Species with an isohydric strategy of water use tend to regulate stomata in a manner that maintains homeostasis of plant water status (Tardieu & Simonneau 1998; McDowell *et al.* 2008), and therefore may be expected to exhibit little stomatal acclimation to VPD. In contrast, species with an anisohydric strategy of water use tend to maintain relatively high g_s despite increasing plant water deficits (Tardieu & Simonneau 1998; McDowell *et al.* 2008; Hoffmann *et al.* 2011), and therefore may be expected to exhibit considerable acclimatization of the stomatal response to VPD.

We used miniature sap flow gauges to measure transpiration of small tree seedlings in a temperate oak-hickory forest understory (Duke Forest, North Carolina, USA) under experimental warming of 1.9–5.1 °C. This level of warming closely matches expectations for T increase in the southeastern United States by 2100 (2–5 °C, IPCC 2014). Sap flux measurements were used to estimate g_s for whole tree seedlings of three common deciduous genera with differing stomatal behaviour: maple (*Acer rubrum* L.), hickory (*Carya tomentosa* (Lam.) Nutt.), and oak (*Quercus alba* L., *Quercus rubra* L.). *Acer rubrum* has diffuse-porous wood and is more isohydric than *C. tomentosa* and *Quercus* spp., exhibiting large declines in photosynthetic rate because of stomatal closure during drought (Abrams & Mostoller 1995; Abrams 1998). In contrast, leaves of *C. tomentosa* and *Quercus* spp. have more gradual stomatal closure during drought, allowing photosynthesis to continue at lower leaf water potentials than in *A. rubrum* (Hoffmann *et al.* 2011). We hypothesized that species differences in stomatal sensitivity and acclimation to VPD can affect the transpiration response to experimental warming. Specifically, we tested the hypotheses that: (1) acclimation of stomata in response to prolonged exposure to high VPD will counteract the short-term responses to VPD, (2) this acclimation will allow plants to sustain high g_s despite high VPD under experimental warming, and (3) the combination of high VPD and high g_s under prolonged warming will result in substantial increases in transpiration.

MATERIALS AND METHODS

Experimental study site

This study was conducted at a warming experiment in a *c.* 80-year-old oak-hickory forest stand of Duke Forest (36° 2′ 11″ N, 79° 4′ 39″ W, 130 m a.s.l.) in the piedmont region near Hillsborough, North Carolina, USA (Lynch 2006). Mean annual T in Duke Forest is 15.5 °C, and mean annual precipitation

is 1140 mm. Total precipitation was 884 mm in the study year 2010, 22% below average, and there was a moderate drought (Palmer Drought Index = −2 to − 2.9) during the growing season (NDMC, USDA, & NOAA 2012).

The experimental warming site consists of 15 plots in the forest understory: nine are heated chambers, three are unheated control chambers, and three are control plots that lack chambers but are the same size as the chambers. The octagonal, open-top chambers have a volume of 21.7 m^3 (5 m in diameter, 1.2 m wall height) and are located on a north-facing slope (6–15% slope). The chambers are heated by forced air blown over hydronic radiators fed by a closed-loop mixture of hot water and propylene glycol (antifreeze). To control for effects of wind, unheated chambers had the same forced air delivery system without the radiators. Heat delivery to the chambers began in January 2010, and chambers are heated year-round, both day and night. The experiment uses a regression design of chamber heating, where each chamber is heated to a target of 1.5–5.5 °C above ambient T with 0.5 °C increments between chambers. Maintaining a precise target T over long time periods is difficult, and the magnitude of warming varied by a small amount over time (but were not statistically different from target T, Fig. S1a). The T of control chambers was about 0.5–0.9 °C above ambient temperature (Fig. S1a), so all warming treatments were calculated relative to control chambers to account for this small chamber effect. Further details of the warming experiment can be found in Pelini *et al.* (2011).

Sensors were installed in each chamber to measure air T (two probes per chamber at 22 cm above ground level), soil T (two sensors buried 2 cm and two sensors buried 6 cm below the surface), relative humidity (HS-2000 V capacitive polymer sensor; Precon, Memphis, TN, USA), soil moisture (Model CS616 TDR probes, Campbell Scientific Inc., Logan, UT, USA), and photosynthetically active radiation (PAR; Model SQ110; Apogee Instruments Inc., Logan, UT, USA). These measurements were recorded every 10 min by automated dataloggers (CR1000; Campbell Scientific Inc.). A duplicate set of sensors were installed within 1 m of a focal *A. rubrum* seedling in each chamber to measure T and relative humidity (CS215-L sensor; Campbell Scientific Inc.), soil water content (Model CS616 TDR probes, Campbell Scientific Inc.), and PAR (Model SQ110; Apogee Instruments Inc.) every 10 min. Environmental data were averaged over all available sensors in an experimental chamber for data analysis. Relative extractable soil water content (REW) was calculated for each chamber according to the equation:

$$\mathrm{REW} = (\theta - \theta_{min})/(\theta_{max} - \theta_{min})$$

where θ is the hourly soil water content, $\theta_{\min}$ is minimum soil water content, and $\theta_{\max}$ is the mean maximum volumetric soil water content over the study period. Readings from three saturating rainfall events were averaged to determine $\theta_{\max}$. Each reading was taken 2 h after the rainfall event to account for soil macropore drainage and thus avoid overestimating $\theta_{\max}$.

Many warming experiments unintentionally reduce light availability and soil moisture (Kennedy 1995), but PAR and REW was not affected by warming in our experiment (Fig. S1e,f). Our warming treatment heated the air inside chambers by 1.9–5.1 °C above controls (Fig. S1a), while the soil was indirectly warmed by up to 3.0 °C above controls (Fig. S1b). Warming decreased relative humidity by 5–14% and increased VPD by 0.5–1.3 kPa inside the experimental chambers (Fig. S1c,d).

Sap flux gauge design and deployment in the field

Miniature sap flux gauges were constructed according to the design by Clearwater *et al.* (2009) with several modifications. Similar sensor designs have been successfully used to measure sap flux in a variety of plants and plant tissues, including flowering stems (Roddy & Dawson 2012), fruit pedicels (Clearwater *et al.* 2012), epiphytes (Gotsch *et al.* 2015) and shrubs (Skelton *et al.* 2013). Each gauge had a small external heater (560 Ω, film-type resistor filed to flatten the external side; filing did not affect the resistance) and two T sensors (fine-wire thermocouples) at equal distances (6 mm) above and below the heater (Clearwater *et al.* 2009). These were affixed with cyanoacrylate adhesive to a cork block (32 mm long, 7 mm wide, 4.5 mm high). The heating element was connected in series with a voltage regulator delivering 11.5 V from a 12-V battery and controlled by a CR1000 datalogger to deliver a 6-second heat pulse every 10 min. This procedure dissipated 0.24 W from the heating element and caused no perceptible damage to the stem. The two thermocouples were connected to a multiplexer (AM16/32B, Campbell Scientific Inc.) via thermocouple extension wire (Omega Engineering Inc., Stamford, CT, USA). Temperature of the thermocouples was measured immediately prior to each heat pulse and at 3-s intervals thereafter for sap flux calculations.

The four study species (*A. rubrum*, *C. tomentosa*, *Q. alba*, *Q. rubra*) are common in Duke Forest, enabling the sampling of naturally occurring tree seedlings that were present at the site before the chambers were installed. One seedling per species per chamber was sampled, if present, plus two seedlings per species were measured in chamberless control plots. Seedlings ranged from 9 to 84 cm in height with small-diameter stems (1.8–6.6 mm). Gauges were installed on the surface of the main stem on 25 May–9 June, firmly secured with laboratory film (Parafilm M, Alcan Packaging, Neeah, WI, USA), and insulated with foam rubber pipe insulation, bubble wrap and foil.

Sap flux calculation

The heat ratio method (Marshall 1958) allows for the determination of low flow rates in plant stems (Clearwater *et al.* 2009). Sap flux was quantified at 10-minute intervals by applying a heat pulse and measuring T differentials (°C) in the top and bottom thermocouples (δT_1 and δT_2, respectively). These were measured over the interval from 60 to 90 s after a heat pulse, which has been found to cover the period of greatest stability in T differentials (Clearwater

et al. 2009). Whole-plant sap flux (F, g h^{-1}) was calculated using the equation:

$$F = B^* \ln\left(\frac{\delta T_1}{\delta T_2}\right)$$

where B is an empirical coefficient that is a function of species and sapwood area and which is obtained from laboratory calibrations. The calibration was performed to account for the thermal properties of stem anatomy (e.g. bark thickness, sapwood area, volumetric stem water content) and for effects of using an external heat pulse (Marshall 1958; Clearwater *et al.* 2009). We calculated B as the slope of the relationship between $\ln(\delta T_1/\delta T_2)$ and actual sap flow (F) in a series of calibration experiments in the laboratory.

For these calibrations, stems of non-study plants ($n = 10$–12 per species) were collected adjacent to the warming site in Duke Forest. Stems were cut in the field and submersed in water for transport to the laboratory. Stem ends were recut under water in the laboratory, and stems were flushed with deionized water at 200 kPa for 20 min to remove emboli. A sap flux gauge was installed on the stem and insulated as described earlier. The calibration was conducted by supplying water at a range of pressures, beginning at 200 kPa and reducing the applied pressure in approximately 10–20-kPa increments until the flux reached zero. After flux stabilized at each pressure, $\ln(\delta T_1/\delta T_2)$ (i.e. the gauge signal) was recorded and the sap flow rate (F, g h^{-1}) was measured volumetrically. Each calibration curve contained 10–25 points.

To investigate potential sources of variation in B, sapwood area was measured on stem segments by perfusing stems with methylene blue solution to determine the cross-sectional area of active xylem (A_S). The B was positively correlated to sapwood area (A_S) for each species (Fig. S2), so we estimated B for each study plant based on its A_S (Table S1). Species-specific allometric quadratic equations (Table S2) were used to relate stem diameter to A_S, as determined from non-experimental plants ($n = 11$–17 per species) at the study site.

Sap flux data management

Significant offsets were detected in the sap flux measurements in the field (i.e. nighttime measurements were significantly different from zero) and were caused by small errors in the positioning of the thermocouples on the gauge (Clearwater *et al.* 2009). There was also seasonal drift in the zero offset of the gauges, possibly because of small fluctuations in stem diameter and gauge—bark contact throughout the growing season. It was therefore necessary to re-zero sap flux measurements on a daily basis using mean F from the previous night (23:00 to 4:00). This procedure required the assumption of negligible nighttime transpiration rates (E_{night}), which we tested by enclosing all study seedlings inside plastic bags to prevent transpiration and comparing the difference between the nighttime sap flux on bagged (22–23 October 2010) versus unbagged (21, 24 October 2010) nights. Maximum E_{night} per unit leaf area was less than 0.7 mol H_2O m^{-2} h^{-1} for all plants, and mean E_{night} was not significantly different from zero (*Ar*: 0.02 ± 0.04 SE; *Ct*: 0.14 ± 0.21; *Qa*: 0.01 ± 0.04; *Qr*: 0.01 ± 0.07). VPD can be positively correlated with E_{night} (Caird *et al.* 2007), but this relationship existed only for *A. rubrum* (*Ar*: $r^2 = 0.53$, $p = 0.041$; *Ct*: $r^2 = 0.01$, $p = 0.884$; *Qa*: $r^2 = 0.06$, $p = 0.505$; *Qr*: $r^2 = 0.26$, $p = 0.136$). To account for this effect, the nightly zero offset of *A. rubrum* seedlings heated by 2 °C or higher was increased by adding estimated E_{night} based on the T–E_{night} relationship. These adjustments ranged from 0.007 to 0.025 mmol H_2O s^{-1}.

Sap flux measurements from June to September 2010 were obtained at 10-min intervals but were converted to hourly averages for subsequent analyses. Despite care to insulate the gauges, sap flux was highly variable during rain events (possibly due to wetting of gauges), so all measurements during rain events were discarded. Midday transpiration (J_{mid}, mol m^{-2} h^{-1}, 1200 to 1400 h) and whole-tree daily water flux (E_{tree}, mol m^{-2} day^{-1}, 0700 to 1800 h) were calculated on a leaf area basis. The A_L of each study plant was estimated monthly by counting number of leaves and using a species-specific allometric equation (Table S2) relating leaf number and stem height (H, cm) or diameter to A_L, as determined from non-experimental plants ($n = 15$–29 per species).

Whole-plant stomatal conductance

Stomatal conductance of whole seedlings (g_s, mmol H_2O m^{-2} s^{-1}) was estimated from

$$g_s = \left(\frac{D_L{}^* A_L}{J^* P_{atm}} - \frac{1}{g_b}\right)^{-1}$$

where D_L is leaf-to-air vapour pressure deficit (kPa), A_L is leaf area (m^2), J is transpiration rate (mmol H_2O s^{-1}), P_{atm} is atmospheric pressure (kPa), and g_b is boundary layer conductance (mmol m^2 s^{-1}). To determine leaf T, which is necessary for calculating D_L, abaxial leaf T was measured on two leaves per plant with thermocouples attached to the leaf with surgical tape.

For six to eight plants per species, g_b was estimated by measuring the rate of mass loss of water from two water-saturated filter paper leaf replicas placed among the foliage, following the method of Roberts *et al.* (1990). Abaxial leaf surface T was measured on one replica with a thermocouple and recorded by a CR1000 datalogger, while mass loss from the other leaf replica was measured at 5-min intervals. Values of g_b were calculated using the relationship:

$$g_b = (E^* P_{atm})/D_L$$

where E (mmol m^2 s^{-1}) is the evaporation rate of water from the leaf replica. We found that g_b ranged from 762 to 1969 mmol m^2 s^{-1} (Table S1).

To provide independent assessment of g_s, leaf-level g_s was measured monthly using an AP4 porometer (Delta-*T*, Cambridge, UK). Stomatal conductance was measured on two leaves per plant at 2-h intervals from 0900 to 1700 h on 14 June, 21 July and 29 August and at midday on 24 September 2010. By comparing sap flux-based g_s and porometric g_s, we found that seven of 40 gauges did not function properly and

exhibited extremely low g_s throughout the summer, possibly because of gauge attachment near a non-conducting stem segment or poor contact between the gauge and the stem surface. We therefore removed these seedlings from analyses, leaving the following sample sizes: *A. rubrum* ($n = 10$), *C. tomentosa* ($n = 11$) and *Quercus* spp. ($n = 12$). The two *Quercus* spp. were pooled for analysis because of low sample sizes (*Q. alba*: 7; *Q. rubra*: 5).

Hourly estimates of g_s from sap flux measurements generally agreed with porometric g_s, but tended to underestimate g_s of *Quercus* spp. (Fig. S3). In *Quercus*, the slope of the relationship between sap flux-based g_s and porometric g_s was significantly different from 1 ($m = 1.176$, $t_{165} = 11.953$, $p = 0.001$) and the intercept was significantly below 0 ($b = -1.325$, $t_{165} = 5.146$, $p < 0.001$), but not for *Acer* or *Carya* (slope: *Ar*: $m = 0.985$, $t_{146} = 0.103$, $p = 0.749$; *Ct*: $m = 0.965$, $t_{160} = 0.460$, $p = 0.499$; intercept: *Ar*: $b = -0.086$, $t_{146} = 0.448$, $p = 0.655$; *Ct*: $b = -0.105$, $t_{160} = 0.459$, $p = 0.647$).

Leaf water potential

Midday leaf water potentials (Ψ_L) were measured monthly on 14 June, 21 July, 29 August and 24 September 2010. One leaf per species per chamber was collected from non-study plants, and measured with a pressure chamber (Model 1000, PMS Instruments, Corvallis, OR, USA). Leaves were not collected from *Q. rubra* because of low seedling density in the chambers.

Statistical analyses

Unheated chambers had a minimal impact on plant water relations, relative to chamberless controls. There was no difference between transpiration of plants in unheated chambers and chamberless controls in *Acer* ($t_3 \leq 1.43$, $p \geq 0.290$) or *Quercus* ($t_4 \leq 1.05$, $p \geq 0.372$). Transpiration of *Carya* seedlings was lower in the control chambers relative to the chamberless control plots (0.5 versus 1.5 mol m^{-2} s^{-1}, respectively: $t_4 = 3.59$, $p = 0.037$) in late August and September, but was unaffected earlier in the growing season. We therefore restricted analyses of warming effects on plant water relations to summer (JJA) days and hereafter do not differentiate between plants in control chambers and chamberless control plots. Coverage of the sap flux dataset (i.e. days with $n = 5$–12 seedlings per genus) was 74–79 days (>80%) of JJA, because of data losses from sap flux sensor malfunctions, gaps in chamber environmental data and rain events.

We fit both linear and quadratic models to determine the effect of chamber T and VPD on midday transpiration (J_{mid}) for each seedling using the 'lm' function in the R STATISTICAL PACKAGE (R 3.1.1, R Core Team 2014). We tested for individual as well as combined effects of T and VPD on transpiration (i.e. T, VPD, T*VPD, T + VPD), thus testing a total of eight models. We chose the best model using Akaike's Information Criterion (AIC, Akaike 1974), which is a likelihood-based method that compares the fit of models with a penalty for the number of parameters. When relevant, we determined the optimal VPD for transpiration (D_{opt}), that is, VPD at maximum J_{mid}, of the second-order polynomials and tested for treatment-induced shifts in D_{opt} using least-square regression.

Stomatal response to VPD was quantified for each seedling using the relationship described by Oren *et al.* (1999b):

$$g_s = -m \cdot \ln D_L + g_{sref}$$

where $-m$ is the sensitivity of g_s to D_L and g_{sref} is a reference conductance at $D_L = 1$ kPa. Midday g_s data was pooled from June–August, when mean monthly REW was similar (June: 0.41, July: 0.35 and August: 0.34).

Differences in daily water flux, midday Ψ_L, stomatal sensitivity and g_{sref} due to the experimental treatment were analyzed using least-square regression for each genus separately, as appropriate for the experimental design. Standardized major axis (SMA) regression was used to characterize the relationship between sap flux-based and porometric g_s and between leaf water potential and g_s using SMATR 2.0 (Warton *et al.* 2006). SMA was used because it is not possible to functionally assign either variable as dependent. Before analyses, daily water flux and g_s were ln-transformed to achieve normality. Means were considered significantly different at $p \leq 0.05$.

RESULTS

The effect of warming on plant water relations was dependent on genus. Warming increased daily water flux of *Carya* and *Quercus*, but not *Acer* (Fig. 2a). This effect was consistent over time, with significant increases occurring on 61% of summer days for *Carya* ($r^2 \geq 0.36$, $p \leq 0.049$), 70% for *Quercus* ($r^2 \geq 0.42$, $p \leq 0.045$), and 0% for *Acer* ($r^2 \leq 0.56$, $p \geq 0.062$). Daily water flux of *Carya* and *Quercus* seedlings heated by 5 °C ranged from 0 to 7 times greater than that of controls (Fig. 2b). The largest warming-induced increases in transpiration of *Quercus* occurred on cooler summer days (ambient $T < 30$ °C; $r^2 = 0.24$, $p < 0.001$, Fig. 2b). Increased evaporative cooling of leaves with warming was observed for all three genera (*Ar*: $r^2 = 0.61$, $p = 0.008$; *Ct*: $r^2 = 0.49$, $p = 0.016$; *Qu*: $r^2 = 0.72$, $p < 0.001$, Fig. 2c), which prevented leaf T of warmed seedlings from exceeding 41 °C (Fig. S4).

The shape of the relationship between air T and transpiration varied among seedlings, with peaked (quadratic) responses found in 26 seedlings and positive linear relationships found in seven seedlings (Table 1). Linear responses of transpiration to air T were observed in control and heated individuals and were most common in *Acer* (*Ar*: 4, *Ct*: 2, *Qu*: 1; Fig. 3a–c). Transpiration rate was more closely related to changes in VPD than T (or the combined effect of T and VPD, i.e. T*VPD or T + VPD) over the summer (Table 1), however. For all seedlings, a peaked response of transpiration to VPD was observed (Table 1, Fig. 3d–f). The optimum VPD for transpiration (D_{opt}) was not constant within a genus, but rather was positively correlated to chamber VPD (*Ar*: $r^2 = 0.50$, $p = 0.033$; *Ct*: $r^2 = 0.80$, $p < 0.001$; *Qu*: $r^2 = 0.81$, $p < 0.001$, Fig. 4).

Midday leaf water potential (Ψ_L) was affected by the warming treatment (Fig. 5a), even with no detectable change in soil water content (Fig. S1e). Midday Ψ_L was negatively correlated with chamber VPD in *Acer* and *Quercus* in July

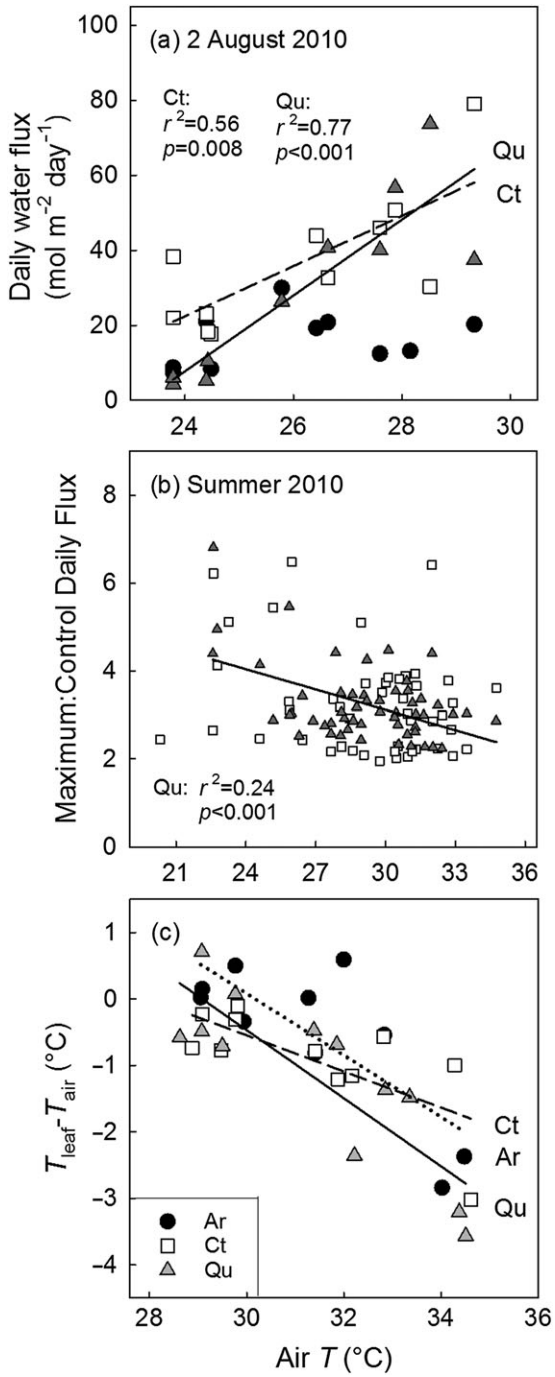

Figure 2. (a) Effect of chamber air T on daily water flux on 2 August 2010 for all species. Daytime was defined as 7:00 to 18:00. Daily flux was ln-transformed for analysis, but actual flux values are shown for clarity. (b) Effect of ambient air T on the magnitude of the increase in daily flux between control seedlings and seedlings heated by 5 °C; only summer days (June–August 2010) when warming significantly affected daily water flux were included. The line shows the relationship for *Quercus* spp; this relationship was not significant for *Carya* ($r^2 = 0.06$, $p = 0.091$). Warming did not affect daily water flux of *Acer*. (c) Effect of chamber air T on evaporative leaf cooling, which is represented as the difference between leaf T and air T. Values are summer means for each seedling; lines represent significant species relationships (*Ar*: $r^2 = 0.61$, $p = 0.008$; *Ct*: $r^2 = 0.49$, $p = 0.016$; *Qu*: $r^2 = 0.72$, $p < 0.001$). Species codes: *Acer rubrum* (*Ar*), *Carya tomentosa* (*Ct*), *Quercus* spp. (*Qu*).

(*Ar*: $r^2 = 0.37$, $p = 0.022$; *Ct*: $r^2 = 0.02$, $p = 0.746$; *Qu*: $r^2 = 0.32$, $p = 0.036$, Fig. 5a), but not in any other month ($r^2 \leq 0.12$, $p \geq 0.220$). Soil water content was highest during the July sampling and declined sharply in August because of a moderate drought (Fig. 5c). As a result of drought, mean Ψ_L decreased from −1.3 MPa in June to −2.9 MPa in September ($F_{3,143} = 69.827$, $p < 0.001$; Fig. 5c). Species differences in Ψ_L also existed, as *A. rubrum* had higher mean and minimum Ψ_L than *Q. alba* ($F_{2,143} = 15.437$, $p < 0.001$, Fig. 5c).

Stomatal behaviour was not constant within *Quercus*, but rather acclimated to changes in VPD of the growth environment. Temperature strongly influenced the stomatal VPD response of *Quercus* (Fig. 6c), as warming increased the slope ($r^2 = 0.51$, $p = 0.010$) and intercept ($r^2 = 0.47$, $p = 0.013$) of the g_s–D_L relationship (Table S3). Warming-induced increases in g_s of *Quercus* were confirmed through independent measurement with a porometer ($r^2 = 0.11$, $p = 0.023$, Fig. S5). In contrast, warming did not significantly affect stomatal behaviour of *Acer* or *Carya*, as neither the slope (–m) nor the intercept (g_{sref}) of the g_s–D_L relationship varied significantly with chamber VPD (*Ar*: $r^2 = 0.05$, $p = 0.553$; $r^2 = 0.01$, $p = 0.744$, *Ct*: $r^2 = 0.19$, $p = 0.176$; $r^2 = 0.18$, $p = 0.191$, respectively; Fig. 6a,b, Fig. S5). Although there was no significant stomatal response to VPD in *Carya*, it is worth noting that the highest g_s in *Carya* was observed under the hottest treatment (+5 °C, Fig. 6b). Similarly, the highest stomatal sensitivities were observed in the two ring-porous trees that were heated by 5 °C (Fig. 7). Across all seedlings, the sensitivity of g_s to D_L was tightly correlated ($r^2 = 0.94$, $p < 0.001$) to the reference conductance (at $D_L = 1$ kPa) with a slope of 0.61 (Fig. 7, Table S3). This slope is not significantly different (0.56–0.68, 95% confidence interval) from the expected value of 0.6 (Oren *et al.* 1999).

DISCUSSION

Experimental warming of 5 °C increased mean water flux of *Carya* by 140% and of *Quercus* by 150% (Fig. 2), following our hypothesis and expectations based on the physical properties of a warmer atmosphere. This increase is double the effect measured in previous experiments; most studies have found that warming of 1.6–5 °C increased transpiration by 11–74% (Kellomaki & Wang 1998; Van Herk *et al.* 2011; Way *et al.* 2012; Duan *et al.* 2014; Wieser *et al.* 2015; Juice *et al.* 2016). Transpiration of Norway spruce trees was 265% higher than control trees with soil warming of 5 °C, but was attributed to higher water availability in heated plots because of earlier snowmelt (Bergh & Linder 1999). The dramatic increase in transpiration in our study was not caused by differences in soil water availability among chambers (Fig. S1e), but was driven by local changes in T and VPD. Warming of 5.1 °C was associated with increases in VPD of 1.3 kPa (Fig. S1d), causing an 'atmospheric drought' that increased daily water use of ring-porous seedlings by 0–7 times that of controls (Fig. 2b). The treatment effect was large enough to decrease midday Ψ_L in July by about 1–2 MPa, even in *Acer* (Fig. 5a), which only had small increases in transpiration (Fig. 3a,d) that did not significantly affect water use with warming (Fig. 2). These results suggest that deciduous tree species will experience more

Table 1. Akaike's Information Criterion from six different models predicting the effect of air temperature (T, in °C) and VPD (in kPa) on midday transpiration (J_{mid}) of four species in Duke Forest, NC in 2010. Mean summer (JJA) air T and VPD are given for all chambers, and chamber treatments relative to controls are in parentheses. The best model for each seedling is in bold, and total seedlings for each model are provided

Species	Air T (°C)	Air VPD (kPa)	T	$T + T^2$	VPD	VPD + (VPD)^2	T(VPD)	T(VPD) + T(VPD)^2
Acer rubrum	29.1	1.3	68.1	70.1	16.9	**−6.8**	25.3	−6.0
Acer rubrum	29.1	1.3	34.3	35.6	24.5	6.9	24.6	**4.8**
Acer rubrum	29.8 (+0.3)	1.5 (+0.1)	310.6	309.0	316.8	**292.2**	316.8	296.0
Acer rubrum	29.9 (+0.4)	1.5 (+0.1)	146.4	147.9	148.0	136.4	149.1	**135.3**
Acer rubrum	31.3 (+1.9)	1.9 (+0.5)	221.0	208.9	227.5	**198.6**	227.7	200.4
Acer rubrum	31.4 (+1.9)	1.9 (+0.5)	238.3	231.7	246.3	**215.8**	247.5	219.8
Acer rubrum	31.9 (+2.5)	2.0 (+0.6)	270.2	267.8	274.7	241.6	275.0	**240.5**
Acer rubrum	33.1 (+3.7)	2.3 (+0.9)	125.5	112.8	125.0	**77.8**	124.9	83.0
Acer rubrum	34.0 (+4.6)	2.6 (+1.2)	54.3	37.1	66.2	**4.1**	68.6	15.7
Acer rubrum	34.5 (+5.1)	2.7 (+1.3)	249.8	251.8	255.0	242.5	254.5	**242.1**
Carya tomentosa	29.1	1.3	85.9	73.3	98.4	**62.7**	101.9	68.0
Carya tomentosa	29.1	1.3	133.5	**115.4**	139.2	121.6	141.7	122.6
Carya tomentosa	29.5 (+0)	1.4 (+0)	41.4	28.0	51.8	**−11.5**	55.6	−9.9
Carya tomentosa	29.8 (+0.3)	1.5 (+0.1)	253.0	247.7	250.6	**243.1**	251.5	246.4
Carya tomentosa	29.9 (+0.4)	1.5 (+0.1)	188.2	176.2	197.1	**153.5**	197.4	154.4
Carya tomentosa	31.3 (+1.9)	1.9 (+0.5)	184.7	171.6	204.1	143.0	204.2	**140.0**
Carya tomentosa	31.9 (+2.5)	2.0 (+0.6)	238.3	228.5	250.5	207.7	250.4	**204.0**
Carya tomentosa	32.2 (+2.7)	2.1 (+0.7)	197.2	180.9	207.7	161.9	207.7	**161.6**
Carya tomentosa	33.1 (+3.7)	2.3 (+0.9)	168.1	168.9	141.9	**128.3**	141.9	128.7
Carya tomentosa	34.3 (+4.9)	2.7 (+1.3)	**230.4**	232.1	246.6	239.8	244.1	235.1
Carya tomentosa	34.5 (+5.1)	2.7 (+1.3)	193.6	185.4	194.0	**182.9**	195.8	184.2
Quercus alba	29.1	1.3	10.4	12.0	3.3	−34.6	5.7	**−35.6**
Quercus alba	29.1	1.3	57.1	54.8	50.5	**17.9**	54.2	20.5
Quercus rubra	29.1	1.3	124.6	122.3	129.6	**70.1**	131.8	77.1
Quercus alba	29.5 (+0)	1.4 (+0)	38.9	34.1	38.2	**−69.2**	43.8	−55.5
Quercus alba	29.8 (+0.3)	1.5 (+0.1)	124.3	119.5	126.4	**117.4**	126.5	118.9
Quercus alba	31.4 (+1.9)	1.9 (+0.5)	92.0	**71.2**	95.2	76.3	96.4	78.6
Quercus rubra	31.9 (+2.5)	2.0 (+0.6)	299.9	292.9	310.8	268.9	310.9	**265.9**
Quercus rubra	32.2 (+2.7)	2.1 (+0.7)	217.3	211.8	222.3	**178.9**	224.0	182.4
Quercus. alba	33.1 (+3.7)	2.3 (+0.9)	175.6	161.3	142.3	**99.7**	142.3	105.7
Quercus rubra	33.4 (+4.0)	2.5 (+1.1)	343.9	336.9	350.4	**328.9**	350.4	330.1
Quercus alba	34.3 (+4.9)	2.7 (+1.3)	332.2	322.5	333.0	**321.3**	333.2	321.9
Quercus rubra	34.5 (+5.1)	2.7 (+1.3)	206.5	194.5	216.6	163.6	216.7	**163.5**
Total seedlings per model			1	2	0	20	0	10

VPD, vapour pressure deficit.

frequent drought stress under warming scenarios accompanied by increases in VPD.

The large increase in water use of *Quercus* with warming can be attributed to two causes: (1) higher air T and (2) stomatal acclimation to VPD that maintained homeostasis of carbon relations (Fig. 8) and/or leaf T (Fig. 2c, S4). Despite increases in VPD of up to 1.3 kPa, warming *increased* g_s of *Quercus* and did not significantly decrease g_s of the other two genera (Fig. 6; Table S3). Stomatal acclimation occurred in the same direction in a beech forest in Germany and led to lower annual water use efficiency (Kutsch *et al.* 2001). This response allows sustained high rates of assimilation and carbon gain despite increased VPD, but may be simply a consequence of maintaining leaf T within an optimal range. Increased transpiration with warming allows plants to cool leaves (Nobel 1974; Mahan & Upchurch 1988), and similar leaf T were observed with warming in all three study species (Fig. 2c, S4), despite differences in transpiration rates (Fig. 2a). Avoidance of high leaf T (>41 °C, Fig. S4) through evaporative cooling may become increasingly important for preventing thermal damage as the climate warms.

Acclimation responses differ from expectations based on the well-known relationship between g_s and VPD (Oren *et al.* 1999b). Stomatal responses to VPD are generally modelled using only a single value of $-m$ and g_{sref}, and increasing VPD is assumed to *decrease* g_s (de Boeck *et al.* 2012; Duursma *et al.* 2013). Using these assumptions, de Boeck (2012) predicted that warming of 3 °C should increase water loss from plant canopies by 43–61%, only about a third of the mean response measured in ring-porous species here. To improve climate model predictions of warming effects on transpiration, models should allow for acclimation of stomata to VPD in certain plant functional types. For *Quercus* and other ring-porous species such as *Carya*, using single parameters of $-m$ and g_{sref} would drastically underestimate transpiration with warming. Rather, modellers should allow for shifts in stomatal parameters under varying climates. For species that do not show acclimation, such as *A. rubrum* and perhaps other isohydric species, use of single parameters of $-m$ and g_{sref} is appropriate for predicting the effect of long-term exposure to VPD on transpiration.

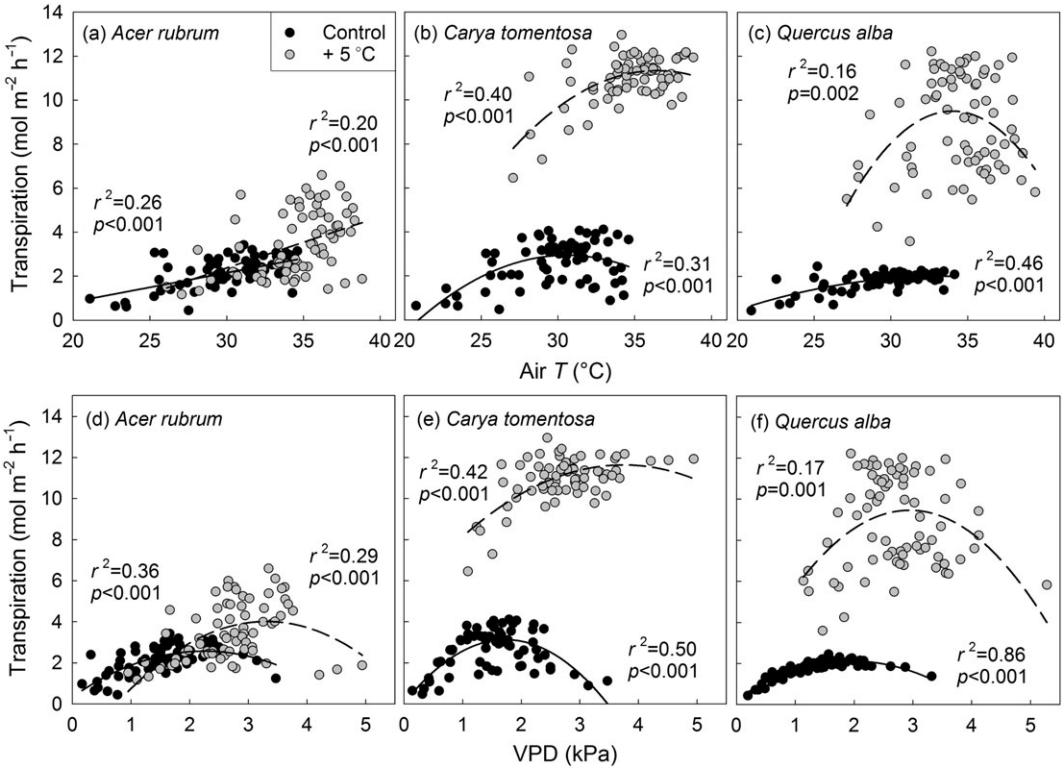

Figure 3. Comparison of the relationship between air *T* and midday transpiration rate for a control seedling (black circles) and a seedling heated by 5 °C (grey circles) of (a) *A. rubrum*, (b) *C. tomentosa* and (c) *Q. alba*. The relationship between vapour pressure deficit (VPD) and transpiration rate is shown for the same seedlings of (d) *A. rubrum*, (e) *C. tomentosa* and (f) *Q. alba*. Values are daily midday means from June–August 2010.

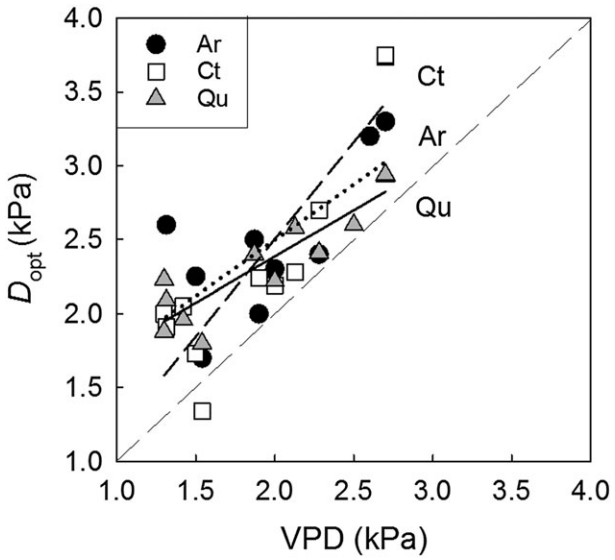

Figure 4. Effect of mean chamber vapour pressure deficit (VPD) on the optimum VPD for midday transpiration (D_{opt}) for all species in summer (JJA) of 2010. Values are estimated from second-order polynomial fits of the relationship depicted in Fig. 3b. One *Acer* control individual is omitted due to undeterminable D_{opt}. The lines represent the least-square fit to data for each genus (*Ar*: $r^2 = 0.50$, $p = 0.033$; *Ct*: $r^2 = 0.80$, $p < 0.001$; *Qu*: $r^2 = 0.81$, $p < 0.001$). The dotted line represents the 1:1 line. Species codes are as in Fig. 2.

The peaked response of transpiration to vapour pressure deficit

Mechanistically, stomata do not respond directly to air humidity or VPD (Lange *et al.* 1971; Mott & Parkhurst 1991; Monteith 1995), but instead appear to respond to changes in water potential of the leaf, which are controlled by changes in transpiration rate (Buckley 2005; Shope *et al.* 2008; Pettijohn *et al.* 2009; Peak & Mott 2011). Transpiration of tree seedlings was more closely related to atmospheric VPD than *T* (Fig. 3, Table 1). At low VPD, transpiration increases approximately linearly with VPD, but transpiration saturates with increasing VPD due to decreasing g_s (Monteith 1995). Above this D_{opt}, transpiration can decrease with increasing VPD (Fig. 3). This 'apparent feed-forward response' is not always observed, particularly under laboratory conditions (Farquhar 1978; Monteith 1995; Franks *et al.* 1997). Even under field conditions, numerous studies have not reported peaked responses of transpiration to VPD (Oren *et al.* 1999a; Oren & Pataki 2001; Van Herk *et al.* 2011; Pfautsch & Adams 2013; Skelton *et al.* 2013; Wieser *et al.* 2015). It is more commonly observed in field studies under drought conditions where *T* covaries with VPD (Pataki *et al.* 2000; Pataki & Oren 2003; Macfarlane *et al.* 2004; Whitley *et al.* 2008). Similarly, our study examined transpiration responses during drought, as soil REW declined below 0.2 in mid-July and late August. By September, several

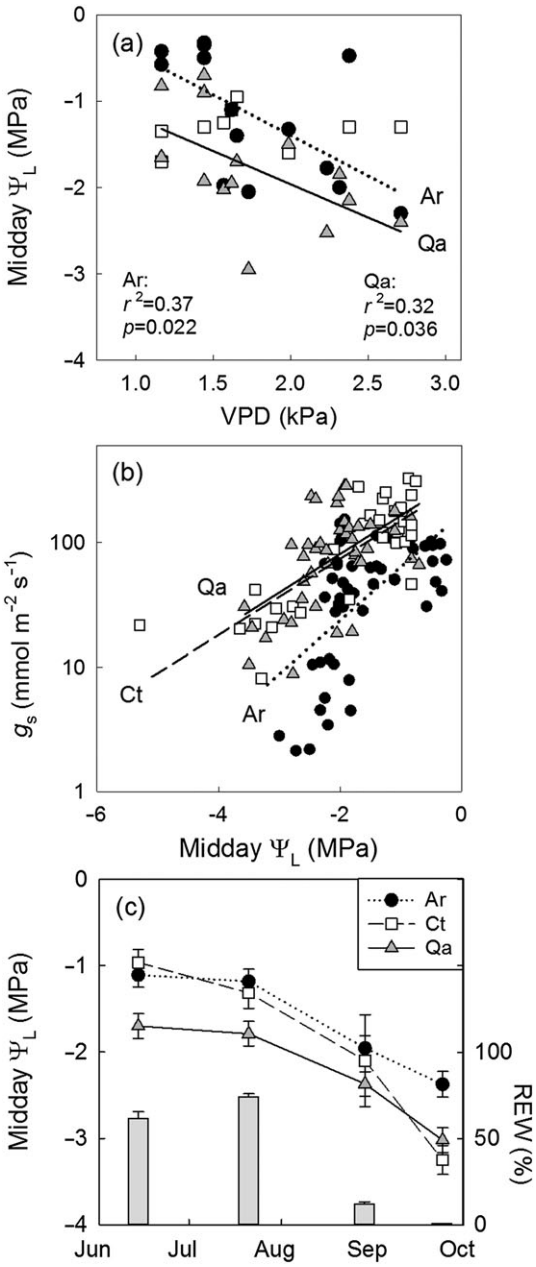

Figure 5. (a) Effect of warming on midday leaf water potential on 21 July 2010. Midday Ψ_L was correlated to chamber vapour pressure deficit (VPD) in *Ar* and *Qa*, but not in *Ct* ($r^2 = 0.02$, $p = 0.746$). (b) Relationship between Ψ_L and stomatal conductance measured with a porometer at midday in *Ar* ($r^2 = 0.35$, $p < 0.001$), *Ct* ($r^2 = 0.65$, $p < 0.001$), and *Qa* ($r^2 = 0.30$, $p < 0.001$). (c) Seasonal changes in midday Ψ_L and relative extractable soil water content in 2010. There were significant effects of species ($F_{2,143} = 15.437$, $p < 0.001$) and date ($F_{3,143} = 69.827$, $p < 0.001$) on Ψ_L, and the interaction between factors was also significant ($F_{6,143} = 2.511$, $p = 0.025$). Values are means across chambers ($n = 9$–14 leaves). Species codes: *Acer rubrum* (*Ar*), *Carya tomentosa* (*Ct*), *Quercus alba* (*Qa*).

A. rubrum seedlings had dropped all or a portion of their leaves, which is a drought avoidance strategy in this species (Marchin *et al.* 2010). Drought severity may influence the VPD at which maximum transpiration occurs (i.e. D_{opt}), which has been reported to vary from 1.2 kPa under moderate drought (Pataki & Oren 2003) to 3 kPa under severe drought (Whitley *et al.* 2008). Our results suggest that *T* and VPD alone, without concurrent decreases in soil water content, can drive similarly large shifts in D_{opt} among individuals of a single species (1.3–3.8 kPa, Fig. 4).

The peaked response of transpiration to VPD has typically been explained by hydraulic mechanisms (Farquhar 1978; Macfarlane *et al.* 2004; Buckley 2005), where declines in g_s and transpiration at high VPD are thought to be caused by decreases in whole-plant hydraulic conductance as a result of xylem cavitation under high evaporative demand (Oren *et al.* 1999b; Dewar 2002; Buckley 2005; Ocheltree *et al.* 2014). Although we did not measure cavitation of xylem, it is likely that some loss of conductivity occurred in our study seedlings during drought. In late August, midday Ψ_L had declined to nearly −3 MPa (Fig. 5c). Xylem vulnerability curves indicate that the air entry pressure (i.e. the threshold xylem pressure at which loss of conductivity begins to increase rapidly) in mature branches is –0.9 MPa for *A. rubrum* and is −0.3 MPa for *Q. alba* (Maherali *et al.* 2006; Meinzer *et al.* 2009), and if seedlings are similarly vulnerable, this threshold was likely surpassed in late summer.

The peaked response of transpiration to VPD could also be caused by declines in photosynthetic rate past the optimum *T* for photosynthesis (T_{opt}), resulting in decreases in g_s that are in addition to the direct effect of VPD on g_s (Duursma *et al.* 2014). Stomatal conductance can decrease at leaf *T* above the T_{opt}, possibly mediated by increased photorespiration at high leaf *T* (Day 2000; Pons & Welschen 2003). Temperate deciduous trees usually increase T_{opt} in response to warming (Sendall *et al.* 2015), which would correspond with our observation of increasing D_{opt} with increasing VPD in all species (Fig. 4).

Mechanisms of stomatal acclimation

Stomatal closure in angiosperms appears to be primarily mediated by the phytohormone abscisic acid (ABA) in plant leaves (Mittelheuser & Van Steveninck 1969; Acharya & Assmann 2009; Brodribb & McAdam 2011; McAdam *et al.* 2011; McAdam *et al.* 2016). A recent study has discovered that angiosperms activate extremely rapid (i.e. within 20 min) *de novo* biosynthesis of ABA in response to a sudden doubling of VPD (McAdam *et al.* 2016). This closes stomata and is mediated by upregulation of a single gene in the ABA biosynthetic pathway (McAdam *et al.* 2016). Under natural changes in VPD, the seasonal dynamics of stomatal behaviour were predictable functions of ABA concentration in a cycad (McAdam & Brodribb 2015). In immature leaves produced during the dry season, high foliar ABA concentrations ensure that stomata remain closed until the wet season, when foliar ABA declines and stomata open (McAdam & Brodribb 2015). These findings clearly point to a central role of foliar ABA biosynthesis in mechanistic models of stomatal behaviour (Buckley 2016).

In this study, acclimation was observed such that *Quercus* seedlings exhibited higher g_s and stomatal sensitivity with increased *T* and VPD (Fig. 6; Table S3). Such behaviour could have resulted from one or a combination of three causes. First,

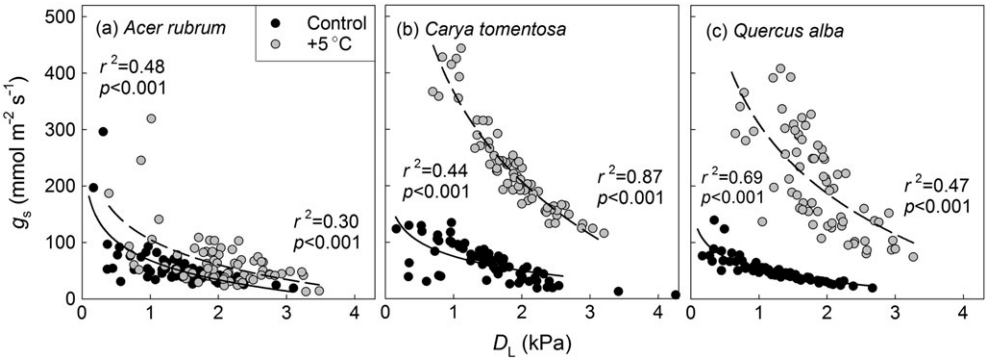

Figure 6. Comparison of the relationship between vapour pressure deficit and midday stomatal conductance for a control seedling (black circles) and a seedling heated by 5 °C (grey circles) of (a) *A. rubrum*, (b) *C. tomentosa* and (c) *Q. alba*. Values are midday means from June–August 2010.

ABA biosynthesis under high VPD could remain the same, but the stomatal complex sensitivity to ABA could change such that guard cells remain open at ABA concentrations in which they previously would have closed. Second, ABA biosynthesis itself may become less sensitive to VPD. Differences in ABA sensitivity have been observed among species (Brodribb & McAdam 2011; McAdam & Brodribb 2015) and, if possible within a single species, could drive stomatal acclimation. Third, plants could maintain a high level of leaf hydration at high VPD through changes in plant architecture. This mechanism has been observed in a subtropical rainforest tree. When leaves expanded under high VPD, leaves were one-third the size with only marginally greater vein and stomatal densities (Carins Murphy *et al.* 2014), which allowed for greater water supply to stomata. We did not observe any drastic changes in leaf size during our experiment, however, suggesting that acclimation was more likely related to changes in ABA biosynthesis.

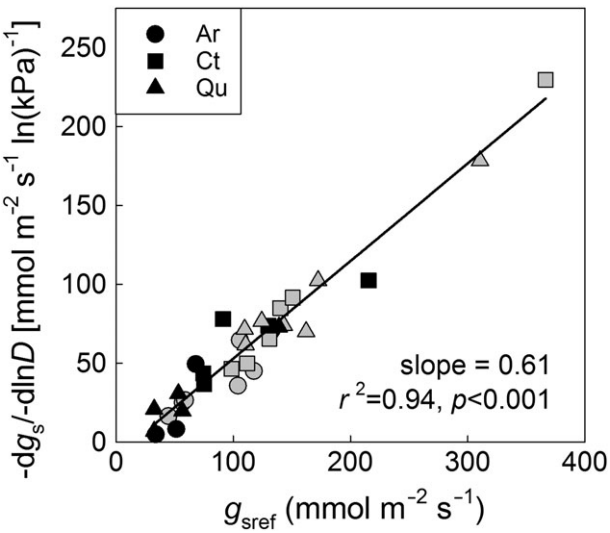

Figure 7. The sensitivity of midday stomatal conductance to increasing vapour pressure deficit at the leaf surface ($-dg_s/d\ln D_L$) as a function of mean g_s at 1 kPa (g_{sref}) in all species. The two axes represent the slope and intercept of the relationship that is depicted in Fig. 6. Control seedlings are shown in black, heated seedlings are shown in grey, and the line represents the least-square fit to data. Species codes are as in Fig. 2.

Implications of stomatal acclimation for a warmer world

The response of global transpiration to future warming is dependent on many abiotic factors, such as water availability and wind speed, and biotic factors, such as species composition and species-specific stomatal responses to VPD, that will likely vary among ecosystems. It will also depend on regional changes in T and VPD, which are currently highly uncertain. When increases in VPD accompany climate warming, our results suggest that transpiration of some plant functional types (e.g. ring-porous trees) will increase because of acclimation of stomata (Fig. 8) over longer time-scales (i.e. weeks to months). Because this study measured stomatal responses of seedlings, it is unclear if the transpiration response of mature trees to warming would be as large, particularly given that seedlings generally have higher g_s and lower water use efficiency than mature trees (Bond 2000; Cavender-Bares & Bazzaz 2000).

Species differences in stomatal acclimation can affect the transpiration response to warming. Transpiration did not increase significantly with warming in *A. rubrum* (Fig. 2) because of its isohydric response to water deficit. Stomatal conductance of *A. rubrum* was generally lower and significant decreases in g_s occurred earlier in the summer (Marchin, unpublished data), which narrowed the range of Ψ_L relative to the ring-porous species (Fig. 5b). Stomatal control of transpiration tends to be less conservative in ring-porous oaks (Meinzer *et al.* 2013), as evidenced here by increased water use (Fig. 2), stomatal acclimation to VPD (Fig. 6), higher rates of g_s (Fig. 5b), and lower Ψ_L under warming (Fig. 5c). It is worth investigating further if simple plant traits, such as wood anatomy (diffuse versus ring-porous) or wood density, could be used as reliable indicators of species transpiration responses to warming.

Theoretically, stomatal acclimation could lead to increased carbon uptake and a competitive advantage of ring-porous over diffuse-porous species under certain warming scenarios. In deciduous forests of the eastern US, however, red maple has replaced white oak throughout the 19th and 20th centuries and is expected to increase in dominance during the next century (Abrams 1998; Abrams 2003). The spread of red maple has been attributed to its shade tolerance (Abrams 1998) and its effective stomatal control of water

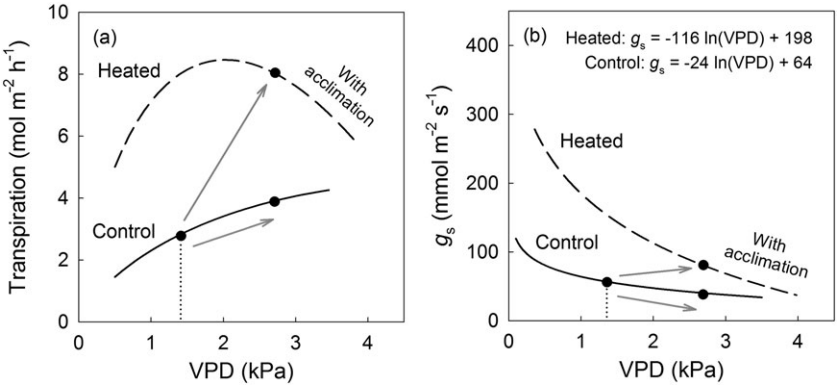

Figure 8. The effect of stomatal acclimation to vapour pressure deficit (VPD) on (a) transpiration and (b) stomatal conductance. Responses of control *Quercus* seedlings are shown with solid lines, while responses of *Quercus* seedlings heated by 5 °C are shown with dashed lines. Black circles and arrows depict the change from ambient conditions (highlighted by a dotted vertical line) to heated conditions. Stomatal acclimation doubled transpiration and increased g_s.

loss (Kloeppel *et al.* 1993). The current decline of white oak is mainly attributed to decreasing fire frequencies (Abrams 2003) since the beginning of the Holocene epoch. Future changes in seedling growth and survival are highly uncertain and will depend on many factors, including regional changes in T and VPD.

Stomatal acclimation to VPD could potentially have a large impact on the global carbon and water cycle. It has already been shown that stomatal acclimation has measurable effects on carbon and water fluxes of forests at the landscape-scale (Kutsch *et al.* 2001; Herbst *et al.* 2008). Some model simulations have predicted that future changes in g_s may affect annual transpiration flux considerably (e.g. 60 W m^{-2}, de Boer *et al.* 2011), and plant responses can also affect various aspects of ecosystem water cycling, including soil moisture (Harte & Shaw 1995; Zavaleta *et al.* 2003) and streamflow (Wigley & Jones 1985). Understanding the complex web of interactions and feedbacks between plant hydraulic properties (i.e. g_s, leaf water potential, transpiration) and T and VPD is vital for improving predictions of climate change effects on global carbon and water cycles.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

REFERENCES

Abrams M.D. (1998) The red maple paradox. *Bioscience* **48**, 355–364.

Abrams M.D. (2003) Where has all the white oak gone? *Bioscience* **53**, 927–939.

Abrams M.D. & Mostoller S.A. (1995) Gas exchange, leaf structure and nitrogen in contrasting successional tree species growing in open and understory sites during a drought. *Tree Physiology* **15**, 361–370.

Acharya B.R. & Assmann S.M. (2009) Hormone interactions in stomatal function. *Plant Molecular Biology* **69**, 451–462.

Akaike H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**, 716–723.

Bergh J. & Linder S. (1999) Effects of soil warming during spring on photosynthetic recovery in boreal Norway spruce stands. *Global Change Biology* **5**, 245–253.

de Boeck H.J., Kimball B.A., Miglietta F. & Nijs I. (2012) Quantification of excess water loss in plant canopies warmed with infrared heating. *Global Change Biology* **18**, 2860–2868.

de Boer H.J., Lammertsma E.I., Wagner-Cremer F., Dilcher D.L., Wassen M.J. & Dekker S.C. (2011) Climate forcing due to optimization of maximal leaf conductance in subtropical vegetation under rising CO_2. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 4041–4046.

Bond B.J. (2000) Age-related changes in photosynthesis of woody plants. *Trends in Plant Science* **5**, 349–353.

Bosilovich M.G., Schubert S.D. & Walker G.K. (2005) Global changes of the water cycle intensity. *Journal of Climate* **18**, 1591–1608.

Brodribb T.J. & McAdam S.A.M. (2011) Passive origins of stomatal control in vascular plants. *Science* **331**, 582–585.

Buckley T.N. (2005) The control of stomata by water balance. *New Phytologist* **168**, 275–291.

Buckley T.N. (2016) Stomatal responses to humidity: has the 'black box' finally been opened? *Plant, Cell & Environment* **39**, 482–484.

Bunce J.A. (1985) Effect of boundary-layer conductance on the response of stomata to humidity. *Plant, Cell & Environment* **8**, 55–57.

Caird M.A., Richards J.H. & Donovan L.A. (2007) Nighttime stomatal conductance and transpiration in C_3 and C_4 plants. *Plant Physiology* **143**, 4–10.

Carins Murphy M.R., Jordan G.J. & Brodribb T.J. (2014) Acclimation to humidity modifies the link between leaf size and the density of veins and stomata. *Plant, Cell & Environment* **37**, 124–131.

Cavender-Bares J. & Bazzaz F.A. (2000) Changes in drought response strategies with ontogeny in *Quercus rubra*: implications for scaling from seedlings to mature trees. *Oecologia* **124**, 8–18.

Chahine M.T. (1992) The hydrologic cycle and its influence on climate. *Nature* **359**, 373–380.

Clearwater M.J., Luo Z.W., Mazzeo M. & Dichio B. (2009) An external heat pulse method for measurement of sap flow through fruit pedicels, leaf petioles and other small-diameter stems. *Plant, Cell & Environment* **32**, 1652–1663.

Clearwater M.J., Luo Z., Ong S.E.C., Blattmann P. & Thorp T.G. (2012) Vascular functioning and the water balance of ripening kiwifruit (*Actinidia chinensis*) berries. *Journal of Experimental Botany* **63**, 1835–1847.

Cunningham S.C. (2005) Photosynthetic responses to vapour pressure deficit in temperate and tropical evergreen rainforest trees of Australia. *Oecologia* **142**, 521–528.

Day M.E. (2000) Influence of temperature and leaf-to-air vapor pressure deficit on net photosynthesis and stomatal conductance in red spruce (*Picea rubens*). *Tree Physiology* **20**, 57–63.

Dewar R.C. (2002) The Ball-Berry-Leuning and Tardieu-Davies stomatal models: synthesis and extension within a spatially aggregated picture of guard cell function. *Plant, Cell & Environment* **25**, 1383–1398.

Duan H., Duursma R.A., Huang G., Smith R.A., Choat B., O'Grady A.P. & Tissue D.T. (2014) Elevated CO_2 does not ameliorate the negative effects of elevated temperature on drought-induced mortality in *Eucalyptus radiata* seedlings. *Plant, Cell & Environment* **37**, 1598–1613.

Duursma R.A., Payton P., Bange M.P., Broughton K.J., Smith R.A., Medlyn B.E. & Tissue D.T. (2013) Near-optimal response of instantaneous transpiration efficiency to vapour pressure deficit, temperature and CO_2 in cotton (*Gossypium hirsutum* L.) *Agricultural and Forest Meteorology* **168**, 168–176.

Duursma R.A., Barton C.V.M., Lin Y.-S., Medlyn B.E., Eamus D., Tissue D.T., … McMurtrie R.E. (2014) The peaked response of transpiration rate to vapour pressure deficit in field conditions can be explained by the temperature optimum of photosynthesis. *Agricultural and Forest Meteorology* **189**, 2–10.

Farquhar G.D. (1978) Feedforward responses of stomata to humidity. *Australian Journal of Plant Physiology* **5**, 787–800.

Franks P.J., Cowan I.R. & Farquhar G.D. (1997) The apparent feedforward response of stomata to air vapour pressure deficit: Information revealed by different experimental procedures with two rainforest trees. *Plant, Cell & Environment* **20**, 142–145.

Gotsch S.G., Nadkarni N., Darby A., Glunk A., Dix M., Davidson K. & Dawson T.E. (2015) Life in the treetops: ecophysiological strategies of canopy epiphytes in a tropical montane cloud forest. *Ecological Monographs* **85**, 393–412.

Gregory J.M., Mitchell J.F.B. & Brady A.J. (1997) Summer drought in northern midlatitudes in a time-dependent CO_2 climate experiment. *Journal of Climate* **10**, 662–686.

Hardwick S.R., Toumi R., Pfeifer M., Turner E.C., Nilus R. & Ewers R.M. (2015) The relationship between leaf area index and microclimate in tropical forest and oil palm plantation: Forest disturbance drives changes in microclimate. *Agricultural and Forest Meteorology* **201**, 187–195.

Harte J. & Shaw R. (1995) Shifting dominance within a montane vegetation community - Results of a climate-warming experiment. *Science* **267**, 876–880.

Herbst M., Rosier P.T.W., Morecroft M.D. & Gowing D.J. (2008) Comparative measurements of transpiration and canopy conductance in two mixed deciduous woodlands differing in structure and species composition. *Tree Physiology* **28**, 959–970.

Hoffmann W.A., Marchin R.M., Abit P.P. & Lau O.L. (2011) Hydraulic failure and tree dieback are associated with high wood density in a temperate forest under extreme drought. *Global Change Biology* **17**, 2731–2742.

IPCC (2014) Summary for policymakers. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Field CB B.V., Dokken D.J., Mach K.J., Mastrandrea M.D., Bilir T.E., Chatterjee M., et al.), pp. 1–32. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Jackson R.B., Sperry J.S. & Dawson T.E. (2000) Root water uptake and transport: using physiological processes in global predictions. *Trends in Plant Science* **5**, 482–488.

Juice S.M., Templer P.H., Phillips N.G., Ellison A.M. & Pelini S.L. (2016) Ecosystem warming increases sap flow rates of northern red oak trees. *Ecosphere* **7**(3), e01221.

Jung M., Reichstein M., Ciais P., Seneviratne S.I., Sheffield J., Goulden M.L., … Zhang K. (2010) Recent decline in the global land evapotranspiration trend due to limited moisture supply. *Nature* **467**, 951–954.

Kellomaki S. & Wang K.Y. (1998) Sap flow in Scots pines growing under conditions of year-round carbon dioxide enrichment and temperature elevation. *Plant, Cell & Environment* **21**, 969–981.

Kennedy A.D. (1995) Simulated climate change: are passive greenhouses a valid microcosm for testing the biological effects of environmental perturbations? *Global Change Biology* **1**, 29–42.

Kloeppel B.D., Abrams M.D. & Kubiske M.E. (1993) Seasonal ecophysiology and leaf morphology of four successional Pennsylvania barrens species in open versus understory environments. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **23**, 181–189.

Kudoyarova G., Veselova S., Hartung W., Farhutdinov R., Veselov D. & Sharipova G. (2011) Involvement of root ABA and hydraulic conductivity in the control of water relations in wheat plants exposed to increased evaporative demand. *Planta* **233**, 87–94.

Kutsch W.L., Herbst M., Vanselow R., Hummelshoj P., Jensen N.O. & Kappen L. (2001) Stomatal acclimation influences water and carbon fluxes of a beech canopy in northern Germany. *Basic and Applied Ecology* **2**, 265–281.

Lange O.L., Losch R., Schulze E.D. & Kappen L. (1971) Responses of stomata to changes in humidity. *Planta* **100**, 76–86.

Lu Z.M., Quinones M.A. & Zeiger E. (2000) Temperature dependence of guard cell respiration and stomatal conductance co-segregate in an F_2 population of Pima cotton. *Australian Journal of Plant Physiology* **27**, 457–462.

Lynch I.P. (2006) The Duke Forest at 75: A Resource for All Seasons. Office of the Duke Forest.

Macfarlane C., White D.A. & Adams M.A. (2004) The apparent feed-forward response to vapour pressure deficit of stomata in droughted, field-grown *Eucalyptus globulus* Labill. *Plant, Cell & Environment* **27**, 1268–1280.

Maenpaa M., Riikonen J., Kontunen-Soppela S., Rousi M. & Oksanen E. (2011) Vertical profiles reveal impact of ozone and temperature on carbon assimilation of *Betula pendula* and *Populus tremula*. *Tree Physiology* **31**, 808–818.

Mahan J.R. & Upchurch D.R. (1988) Maintenance of constant leaf temperature by plants. I. Hypothesis-limited homeothermy. *Environmental and Experimental Botany* **28**, 351–357.

Maherali H., Moura C.F., Caldeira M.C., Willson C.J. & Jackson R.B. (2006) Functional coordination between leaf gas exchange and vulnerability to xylem cavitation in temperate forest trees. *Plant, Cell and Environment* **29**, 571–583.

Manabe S. & Wetherald R.T. (1987) Large-scale changes of soil wetness induced by an increase in atmospheric carbon dioxide. *Journal of the Atmospheric Sciences* **44**, 1211–1235.

Manabe S., Milly P.C.D. & Wetherald R. (2004) Simulated long-term changes in river discharge and soil moisture due to global warming. *Hydrological Sciences Journal-Journal Des Sciences Hydrologiques* **49**, 625–642.

Marchin R., Zeng H.N. & Hoffmann W. (2010) Drought-deciduous behavior reduces nutrient losses from temperate deciduous trees under severe drought. *Oecologia* **163**, 845–854.

Marshall D.C. (1958) Measurement of sap flow in conifers by heat transport. *Plant Physiology* **33**, 385–396.

McAdam S.A.M. & Brodribb T.J. (2015) Hormonal dynamics contributes to divergence in seasonal stomatal behaviour in a monsoonal plant community. *Plant, Cell & Environment* **38**, 423–432.

McAdam S.A.M., Brodribb T.J., Ross J.J. & Jordan G.J. (2011) Augmentation of abscisic acid (ABA) levels by drought does not induce short-term stomatal sensitivity to CO_2 in two divergent conifer species. *Journal of Experimental Botany* **62**, 195–203.

McAdam S.A.M., Sussmilch F.C. & Brodribb T.J. (2016) Stomatal responses to vapour pressure deficit are regulated by high speed gene expression in angiosperms. *Plant, Cell & Environment* **39**, 485–491.

McDowell N., Pockman W.T., Allen C.D., Breshears D.D., Cobb N., Kolb T., … Yepez E.A. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178**, 719–739.

Meinzer F.C., Johnson D.M., Lachenbruch B., McCulloh K.A. & Woodruff D.R. (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Functional Ecology* **23**, 922–930.

Meinzer F.C., Woodruff D.R., Eissenstat D.M., Lin H.S., Adams T.S. & McCulloh K.A. (2013) Above- and belowground controls on water use by trees of different wood types in an eastern US deciduous forest. *Tree Physiology* **33**, 345–356.

Milly P.C.D., Wetherald R.T., Dunne K.A. & Delworth T.L. (2002) Increasing risk of great floods in a changing climate. *Nature* **415**, 514–517.

Mittelheuser C.J. & Van Steveninck R.F.M. (1969) Stomatal closure and inhibition of transpiration induced by (RS)-abscisic acid. *Nature* **221**, 281–282.

Monteith J.L. (1995) A reinterpretation of stomatal responses to humidity. *Plant, Cell & Environment* **18**, 357–364.

Mott K.A. & Parkhurst D.F. (1991) Stomatal responses to humidity in air and helox. *Plant, Cell & Environment* **14**, 509–515.

NDMC, USDA & NOAA (2012) United States Drought Monitor.

Nejad A.R. & van Meeteren U. (2008) Dynamics of adaptation of stomatal behaviour to moderate or high relative air humidity in *Tradescantia virginiana*. *Journal of Experimental Botany* **59**, 289–301.

Nobel P. (1974) *Introduction to biophysical plant physiology*. WH Freeman & Company, San Francisco, CA.
Ocheltree T.W., Nippert J.B. & Prasad P.V.V. (2014) Stomatal responses to changes in vapor pressure deficit reflect tissue-specific differences in hydraulic conductance. *Plant, Cell & Environment* **37**, 132–139.
Oren R. & Pataki D.E. (2001) Transpiration in response to variation in microclimate and soil moisture in southeastern deciduous forests. *Oecologia* **127**, 549–559.
Oren R., Phillips N., Ewers B.E., Pataki D.E. & Megonigal J.P. (1999a) Sap-flux-scaled transpiration responses to light, vapor pressure deficit, and leaf area reduction in a flooded *Taxodium distichum* forest. *Tree Physiology* **19**, 337–347.
Oren R., Sperry J.S., Katul G.G., Pataki D.E., Ewers B.E., Phillips N. & Schafer K.V.R. (1999b) Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant, Cell & Environment* **22**, 1515–1526.
Pataki D.E. & Oren R. (2003) Species differences in stomatal control of water loss at the canopy scale in a mature bottomland deciduous forest. *Advances in Water Resources* **26**, 1267–1278.
Pataki D.E., Oren R. & Smith W.K. (2000) Sap flux of co-occurring species in a western subalpine forest during seasonal soil drought. *Ecology* **81**, 2557–2566.
Peak D. & Mott K.A. (2011) A new, vapour-phase mechanism for stomatal responses to humidity and temperature. *Plant, Cell & Environment* **34**, 162–178.
Pelini S.L., Bowles F.P., Ellison A.M., Gotelli N.J., Sanders N.J. & Dunn R.R. (2011) Heating up the forest: open-top chamber warming manipulation of arthropod communities at Harvard and Duke Forests. *Methods in Ecology and Evolution* **2**, 534–540.
Pettijohn J.C., Salvucci G.D., Phillips N.G. & Daley M.J. (2009) Mechanisms of moisture stress in a mid-latitude temperate forest: Implications for feedforward and feedback controls from an irrigation experiment. *Ecological Modelling* **220**, 968–978.
Pfautsch S. & Adams M.A. (2013) Water flux of *Eucalyptus regnans*: defying summer drought and a record heatwave in 2009. *Oecologia* **172**, 317–326.
Pons T.L. & Welschen R.A.M. (2003) Midday depression of net photosynthesis in the tropical rainforest tree *Eperua grandiflora*: contributions of stomatal and internal conductances, respiration and Rubisco functioning. *Tree Physiology* **23**, 937–947.
R Core Team (2014) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
Roberts J., Cabral O.M.R. & Deaguiar L.F. (1990) Stomatal and boundary-layer conductances in an Amazonian terra-firme rain forest. *Journal of Applied Ecology* **27**, 336–353.
Roddy A.B. & Dawson T.E. (2012) Determining the water dynamics of flowering using miniature sap flow sensors. *Acta Horticulturae* **951**, 47–53.
Sadras V.O., Montoro A., Moran M.A. & Aphalo P.J. (2012) Elevated temperature altered the reaction norms of stomatal conductance in field-grown grapevine. *Agricultural and Forest Meteorology* **165**, 35–42.
Sendall K.M., Reich P.B., Zhao C.M., Hou J.H., Wei X.R., Stefanski A., … Montgomery R.A. (2015) Acclimation of photosynthetic temperature optima of temperate and boreal tree species in response to experimental forest warming. *Global Change Biology* **21**, 1342–1357.
Sermons S.M., Seversike T.M., Sinclair T.R., Fiscus E.L. & Rufty T.W. (2012) Temperature influences the ability of tall fescue to control transpiration in response to atmospheric vapour pressure deficit. *Functional Plant Biology* **39**, 979–986.
Shope J.C., Peak D. & Mott K.A. (2008) Stomatal responses to humidity in isolated epidermes. *Plant, Cell & Environment* **31**, 1290–1298.
Skelton R.P., West A.G., Dawson T.E. & Leonard J.M. (2013) External heat-pulse method allows comparative sapflow measurements in diverse functional types in a Mediterranean-type shrubland in South Africa. *Functional Plant Biology* **40**, 1076–1087.
Tardieu F. & Simonneau T. (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany* **49**, 419–432.
Trenberth K.E., Fasullo J. & Smith L. (2005) Trends and variability in column-integrated atmospheric water vapor. *Climate Dynamics* **24**, 741–758.
Van Herk I.G., Gower S.T., Bronson D.R. & Tanner M.S. (2011) Effects of climate warming on canopy water dynamics of a boreal black spruce plantation. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **41**, 217–227.
Wang K.Y. & Kellomaki S. (1997) Stomatal conductance and transpiration in shoots of Scots pine after 4-year exposure to elevated CO_2 and temperature. *Canadian Journal of Botany-Revue Canadienne De Botanique* **75**, 552–561.
Warton D.I., Wright I.J., Falster D.S. & Westoby M. (2006) Bivariate line-fitting methods for allometry. *Biological Reviews* **81**, 259–291.
Way D.A., Domec J.-C. & Jackson R.B. (2012) Elevated growth temperatures alter hydraulic characteristics in trembling aspen (*Populus tremuloides*) seedlings: implications for tree drought tolerance. *Plant, Cell & Environment* **36**, 103–115.
Whitley R., Zeppel M., Armstrong N., Macinnis-Ng C., Yunusa I. & Eamus D. (2008) A modified Jarvis-Stewart model for predicting stand-scale transpiration of an Australian native forest. *Plant and Soil* **305**, 35–47.
Wieser G., Grams T.E.E., Matyssek R., Oberhuber W. & Gruber A. (2015) Soil warming increased whole-tree water use of *Pinus cembra* at the treeline in the Central Tyrolean Alps. *Tree Physiology* **35**, 279–288.
Wigley T.M.L. & Jones P.D. (1985) Influences of precipitation changes and direct CO_2 effects on streamflow. *Nature* **314**, 149–152.
Will R.E., Wilson S.M., Zou C.B. & Hennessey T.C. (2013) 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. *New Phytologist* **200**, 366–374.
Zavaleta E.S., Thomas B.D., Chiariello N.R., Asner G.P., Shaw M.R. & Field C. B. (2003) Plants reverse warming effect on ecosystem water balance. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 9892–9893.
Zeng Z., Piao S., Lin X., Yin G., Peng S., Ciais P. & Myneni R.B. (2012) Global evapotranspiration over the past three decades: estimation based on the water balance equation combined with empirical models. *Environmental Research Letters* **7**, 8.
Zhang K., Kimball J.S., Nemani R.R. & Running S.W. (2010) A continuous satellite-derived global record of land surface evapotranspiration from 1983 to 2006. *Water Resources Research* **46**, 21.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Summary of anatomical and physiological traits for tree seedlings used in this study. Stem diameter was directly measured, while sapwood area and leaf area were estimated using species-specific allometric equations (Table S2). The multiplier B was estimated from sap flux calibration curves boundary layer conductance (g_b) was estimated via mass loss of water from filter paper leaf replicas. Mean summer (June-August) air T and VPD are given for all chambers, and chamber treatments relative to controls are in parentheses

Table S2 Species-specific allometric equations used to estimate sapwood area (A_S, mm^2) and leaf area (A_L, m^2) of tree seedlings for calculation of transpiration and canopy conductance. Predictor variables are stem diameter (x, mm), leaf number (L), and stem height (H, cm). Equations were determined from non-experimental plants at the study site (A_S: $n = 11$–17 per species; A_L: $n = 15$–29 per species).

Table S3 Stomatal responses of seedlings were quantified using the function shown in Fig. 6: $g_s = -m \cdot \ln D_L + g_{sref}$, where g_s is midday stomatal conductance (mmol m^{-2} s^{-1}), m is in mmol m^{-2} s^{-1} ln(kPa)$^{-1}$, D_L is vapor pressure deficit at the leaf surface (kPa), and g_{sref} is estimated stomatal conductance at 1 kPa (mmol m^{-2} s^{-1}). Mean summer (June-August) air T and VPD are given for all chambers, and chamber treatments relative to controls are in parentheses. Significant relationships are indicated with stars: * $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$.

Figure S1 Mean midday (a) air temperature, (b) soil temperature, (c) relative humidity, (d) vapor pressure deficit, (e) relative extractable soil water content, and (f) photosynthetically active radiation inside the 12 experimental chambers in Duke Forest, NC in July 2010. Control chambers are shown as black

circles; heated chambers are shown as gray circles. Significant effects of warming on environmental variables ($p < 0.05$) are depicted with lines. In panel (a), air T is compared to the target T of each chamber. In panel (d), the line depicts the effect of T on the saturated vapor pressure of air, using the mean T in control chambers as the reference T.

Figure S2 Differences in B were related to stem sapwood area in (a) *Acer rubrum*, (b) *Carya tomentosa*, (c) *Quercus alba*, and (d) *Quercus rubra*. The B coefficient incorporates the thermal properties of stem anatomy (e.g. bark thickness, sapwood area, volumetric stem water content). Sampled stems ranged in diameter from 1.5–7 mm.

Figure S3 Comparison of stomatal conductance measured at the leaf-level with a porometer and estimated via whole-plant sap flux for (a) *Acer rubrum*, (b) *Carya tomentosa*, and (c) *Quercus* spp. Porometer measurements ($n = 2$ leaves per plant) were made on 14 June, 21 July, 29 August, and 24 September 2010, and sap flux measurements are hourly means of data collected every 10 minutes. The dotted line represents the 1:1 relationship. Relationships were analyzed using standardized major axis (SMA) regression, because neither variable can be functionally assigned as dependent.

Figure S4 Maximum midday leaf T observed in summer 2010 for all study seedlings. Chamber T was positively correlated to maximum leaf T for all species (*Ar*: $r^2 = 0.69$, $p = 0.017$; *Ct*: $r^2 = 0.88$, $p < 0.001$; *Qu*: $r^2 = 0.83$, $p < 0.001$), although only one curve is plotted for ease of visualization.

Figure S5 Effect of warming on midday stomatal conductance ($n = 2$ leaves) of (a) *Acer rubrum*, (b) *Carya tomentosa*, and (c) *Quercus* spp. Temperature did not significantly affect stomatal conductance in *Acer* ($r^2 = 0.001$, $p = 0.837$) or *Carya* ($r^2 = 0.01$, $p = 0.511$). Stomatal conductance was measured with a porometer on 14 June, 21 July, and 29 August 2010.