Research paper

Above- and belowground controls on water use by trees of different wood types in an eastern US deciduous forest

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Stomata control tree transpiration by sensing and integrating environmental signals originating in the atmosphere and soil, and co-occurring species may differ in inherent stomatal sensitivity to these above- and belowground signals and in the types of signals to which they respond. Stomatal responsiveness to environmental signals is likely to differ across species having different types of wood (e.g., ring-porous, diffuse-porous and coniferous) because each wood type differs in the structure, size and spatial distribution of its xylem conduits as well as in the scaling of hydraulic properties with stem diameter. The objective of this study was to evaluate the impact of variation in soil water availability and atmospheric evaporative demand on stomatal regulation of transpiration in seven co-occurring temperate deciduous forest species representing three wood types. We measured whole-tree sap flux and soil and atmospheric variables in a mixed deciduous forest in central Pennsylvania over the course of a growing season characterized by severe drought and large fluctuations in atmospheric vapor pressure deficit (D). The relative sensitivity of sap flux to soil drying was ~2.2–2.3 times greater in the diffuse-porous and coniferous species than in the ring-porous species. Stomata of the ring-porous oaks were only about half as responsive to increased D as those of trees of the other two wood types. These differences in responsiveness to changes in the below- and aboveground environment implied that regulation of leaf water potential in the ring-porous oaks was less stringent than that in the diffuse-porous angiosperms or the conifers. The results suggest that increases in the frequency or intensity of summer droughts in the study region could have multiple consequences for forest function, including altered successional time courses or climax species composition and cumulative effects on whole-tree architecture, resulting in a structural and physiological legacy that would restrict the ability of trees to respond rapidly to more favorable growth conditions.

Keywords: critical zone observatory, drought, sap flow, stomata, transpiration, vapor pressure deficit.

Introduction

Stomata control tree transpiration by sensing and integrating environmental signals originating above- and belowground. Thus, stomata are not autonomous in their responses to atmospheric variables such as the vapor pressure deficit (D) and light. Operating ranges of stomatal aperture and the apparent sensitivity of stomatal conductance to changes in the aerial environment are partly determined by the properties of the xylem flow pathway upstream from the guard cells. Stomata respond to both hydraulic and chemical signals transmitted through the xylem, the former consisting of the changes in xylem water potential or pressure that influence leaf water potential, and the latter consisting of changes in fluxes and concentrations of plant growth regulators, and certain ions (Davies et al. 2005, Woodruff et al. 2010). The water potential

of the soil matrix (Ψ_s) surrounding growing root tips is a major determinant of xylem sap chemistry (Davies et al. 2005). Hydraulic signals sensed in transpiring leaves are modulated by transpiration itself, Ψ_s and the overall tree hydraulic architecture: the integration of xylem properties from roots to leaves, and tree allometric features such as the ratio of leaf area to sapwood area that ultimately determine the whole-plant leaf area-specific hydraulic conductance (K_{plant}). Stomatal conductance is closely coordinated with dynamic variation in K_{plant} resulting from the formation and reversal of xylem embolism (Domec et al. 2004, 2006) and capacitive discharge of water into the transpiration stream (Andrade et al. 1998, Meinzer 2002) in the short term, and shedding and regrowth of leaves and xylem development and transition to heartwood over the longer term (Whitehead 1998, Bucci et al. 2005). Capacitance influences apparent K_{plant} perceived by stomata through its transient buffering of changes in xylem tension associated with increasing transpiration (Meinzer et al. 2003, 2008). Co-occurring species may differ in inherent stomatal sensitivity to signals originating above- and belowground and in the types of signals to which they respond, but integration of signals and responses across species-specific hydraulic architecture may lead to convergent patterns of stomatal regulation when stomatal conductance is scaled by appropriate variables (Meinzer et al. 1997, Bucci et al. 2004).

The three major wood types—ring-porous, diffuse-porous and coniferous—differ in the structure, size and spatial distribution of xylem conduits. The tracheids of conifers are smaller in diameter and shorter than the vessels of ring- and diffuse-porous trees. The structure of the pit pairs and pit membranes that allow water to pass from conduit to conduit also differs between tracheid- and vessel-bearing species. Because tracheids are substantially shorter than vessels, water moving through conifer wood must pass through more inter-conduit pit membranes per unit length of stem than in vessel-bearing trees. Nevertheless, at a given conduit diameter the sapwood area-based hydraulic conductivity of conifer wood is as high as or higher than that of vessel-bearing species because of the unique structure of the more porous torus–margo pit membrane of conifers (Pittermann et al. 2005, McCulloh et al. 2010). The three major wood types also show differences in the scaling of wood hydraulic properties with stem diameter (McCulloh et al. 2010). Despite these documented differences in xylem conduit structural and hydraulic properties and their scaling with stem diameter and axial position, their functional consequences for the regulation of whole-tree water transport of trees of one wood type versus another are not well understood.

The objective of this study was to evaluate the impact of variation in soil water availability and atmospheric evaporative demand on stomatal regulation of transpiration in seven co-occurring eastern US deciduous forest species representing the three wood types described above. Previous work in the region suggests that ring-porous species, particularly *Quercus* species, have lower leaf water potential thresholds for stomatal closure, exhibit more anisohydric as opposed to isohydric regulation of leaf water potential, and are more deeply rooted than diffuse-porous and coniferous species in the region (Abrams 1990, Abrams et al. 1990, Kloeppel et al. 1993, Cavender-Bares and Bazzaz 2000, Ewers et al. 2007). These traits may contribute to overall lower responsiveness of transpiration to soil drying in ring-porous oaks compared with conifers and diffuse-porous species (Stoy et al. 2006, Ford et al. 2011). Other work suggests that the sensitivity of stomatal conductance to D (Oren et al. 1999) is lower in ring-porous species than in coniferous and diffuse-porous species (Oren and Pataki 2001, Hölscher et al. 2005, Ford et al. 2011), but one study conducted on irrigated urban trees concluded that stomatal sensitivity to D was greater in ring-porous than in diffuse-porous species (Bush et al. 2008) and another found no difference in sensitivity (Litvak et al. 2012).

Based on the work cited above, we hypothesized that the ability of roots to continue extracting water during soil drying and the sensitivity of crown conductance (G_c) to changes in D would differ among wood types. To address this hypothesis, we measured sap flux in trees at the Susquehanna Shale Hills Critical Zone Observatory (http://www.czo.psu.edu/) over the course of a growing season characterized by three pronounced soil drying/rewetting cycles and large diurnal and seasonal fluctuations in atmospheric vapor pressure deficit.

Materials and methods

Site and species description

Measurements were carried out between May and October 2010 at the Susquehanna/Shale Hills Critical Zone Observatory (SSHO) near State College, Pennsylvania, USA (Lat. 40°39′N, Long. 77°54′ W, elev. 256–310 m). Vegetation is characterized by mixed-deciduous temperate forests dominated by *Quercus* and *Carya* on the hill slopes and *Tsuga canadensis* (L.) Carr. near the ephemeral, first-order stream. The mean annual temperature is 10 °C and the mean annual precipitation is 107 cm. Soil depth ranges from >2 m in the valley floor to <0.3 m on the ridge tops (Lin et al. 2006). Soil texture is primarily silt loam to silty clay loam on the hill slopes and silty clay loam to clay on the valley floor. A total of five soil series are associated with the catchment, distinguished primarily based on soil depth, landscape position and depth to redox features (Lin et al. 2006).

Sap flux measurements

Four individuals of each of the seven study species (Table 1) were selected for the measurement of sap flow. There was no single location where species occurrences overlapped sufficiently

Table 1. The wood type, the mean diameter ± SD of all individuals of each species in the stand, the mean diameter ± SD of trees selected for sap flow measurements, species densities and their relative contributions to total stand basal area and height ± SD of sap-flow trees.

| Species	Wood type	Diameter (m)		Density (trees ha⁻¹)	Relative basal area	Height (m)
		Stand	Sap flow			Sap flow
Quercus prinus L.	Ring-porous	0.33 ± 0.08	0.44 ± 0.08	70.6	0.248	25.4 ± 4.6
Quercus alba L.	Ring-porous	0.35 ± 0.09	0.57 ± 0.06	35.7	0.140	28.8 ± 4.9
Quercus rubra L.	Ring-porous	0.42 ± 0.11	0.46 ± 0.08	36.3	0.205	24.3 ± 3.7
Liriodendron tulipifera L.	Diffuse-porous	0.38 ± 0.08	0.40 ± 0.03	1.6	0.008	30.3 ± 3.0
Acer saccharum Marsh.	Diffuse-porous	0.30 ± 0.06	0.29 ± 0.03	15.4	0.044	21.9 ± 3.0
Pinus virginiana Mill.	Coniferous	0.31 ± 0.06	0.25 ± 0.03	12.2	0.039	20.5 ± 4.9
Tsuga canadensis (L.) Carr.	Coniferous	0.35 ± 0.08	0.42 ± 0.06	20.8	0.082	23.9 ± 5.2
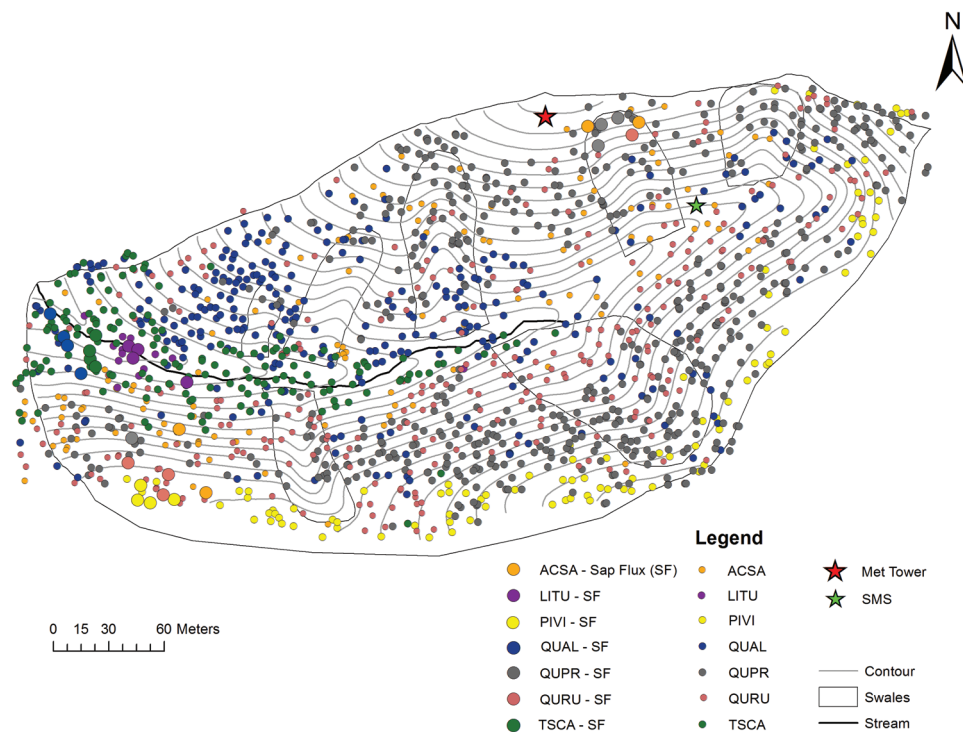
Figure 1. Map of the study site showing locations of trees in which sap flow was measured, locations from which soil water content and water potential data were obtained (SMS), and the location of the instrument tower from which relative humidity, air temperature and solar radiation data were obtained (Met Tower). Species abbreviations: ACSA, *Acer saccharum*; LITU, *Liriodendron tulipifera*; PIVI, *Pinus virginiana*; QUAL, *Quercus alba*; QUPR, *Quercus prinus*; QURU, *Quercus rubra*; TSCA, *Tsuga canadensis*.

to place all individuals at a distance within the maximum allowable length of the sap flow probe cables, so three locations for data logging were selected in order to characterize the behavior of all seven species. Individuals within the maximum cable length at each data logging location were selected on the basis of having a dominant or sub-dominant canopy position and a trunk diameter similar to or greater than the mean for other individuals of the species in the stand. The approximate locations of the data logging stations and individual trees are shown in Figure 1.

Sap flow was measured with heat dissipation probes (Granier 1985) consisting of a heated and a reference sensor

installed in the trunk at ~1.5 m above the soil surface. Two 20-mm-long probes were installed in the sapwood on opposite sides of the trunk of each of the 12 *Quercus* individuals selected for measurement. Sapwood depth was >20 mm in all of these individuals. In the remaining four species, variable length heat dissipation probes with a heated and a reference sensor, each 10 mm in length at the probe tip (James et al. 2002), were installed at distances of 1.5, 3 and 5 cm from the outer sapwood boundary. The sensors were coated with thermally conductive silicone heat sink compound before insertion. All probes were shielded from potential sunflecks by reflective

insulation. Signals from the sap flow probes were scanned every minute and 10-min means were recorded by data loggers equipped with 32-channel multiplexers. The voltage output from the probe thermocouples was converted to a temperature difference between the heated and reference sensors and was used to calculate sap flux (J_s; g m^{-2} s^{-1}) as described in James et al. (2002). To account for the impact of nocturnal sap flux on the maximum temperature difference between the heated and reference sensors (ΔT_{max}), we attempted to apply the procedure described by Oishi et al. (2008) to estimate ΔT_{max}. However, none of the nights during the study period met their criterion of having a 2-h interval during which the average D was <0.05 kPa. The minimum D on most nights was >0.15 kPa. Therefore, we devised an alternative procedure that consisted of plotting ΔT of individual sensors against night-time D (Figure 2). Linear regressions fitted to the data ($P < 0.05$) allowed ΔT_{max} to be estimated from the extrapolation of the regressions to $D = 0$. When the slope of the regression was not significantly different from zero, ΔT_{max} was taken to be the maximum recorded ΔT during the 24-h period under consideration. This procedure revealed that ΔT_{max} for a given probe was not constant, but fluctuated throughout the season (Figure 2), making it necessary to estimate values of ΔT_{max} for each probe on a daily basis.

Measurements recorded to a single sensor depth, 2 cm in the oaks and 1.5 cm in the remaining species, were used as an index to compare the relative responsiveness of sap flow to changes in above- and belowground environmental variables. Whole-tree sap flow and stand-level transpiration were not estimated. Values of sap flux represent the means of the two probes in each oak tree and one probe in each individual of the other species. Although probes were installed in four individuals of each of the study species, sensor failure and other technical difficulties resulted in a final sample size of two to four individuals per species.

Environmental variables

Air temperature, relative humidity and photosynthetically active radiation (PAR) were obtained from a data set collected at 10-min intervals from sensors mounted near the top of an instrument tower at the top of a ridge on the north edge of the catchment (Figure 1). For further details see http://www.czo.psu.edu/. Vapor pressure deficit (D) was calculated from air temperature and relative humidity. Soil volumetric water content (θ) at 0.1, 0.3 and 0.5 m was measured at 10-min intervals with ECH2O 20-cm probes (Decagon Devices, Pullman, WA, USA). Values reported here represent the means of nine sensors: three depths at each of three locations near the point indicated in Figure 1. Soil water potential (Ψ_s) was recorded at 10-min intervals with an MPS-1 sensor (Decagon Devices) installed at 15 cm depth (Figure 1). This sensor has a specified range of −0.01 to −0.5 MPa.
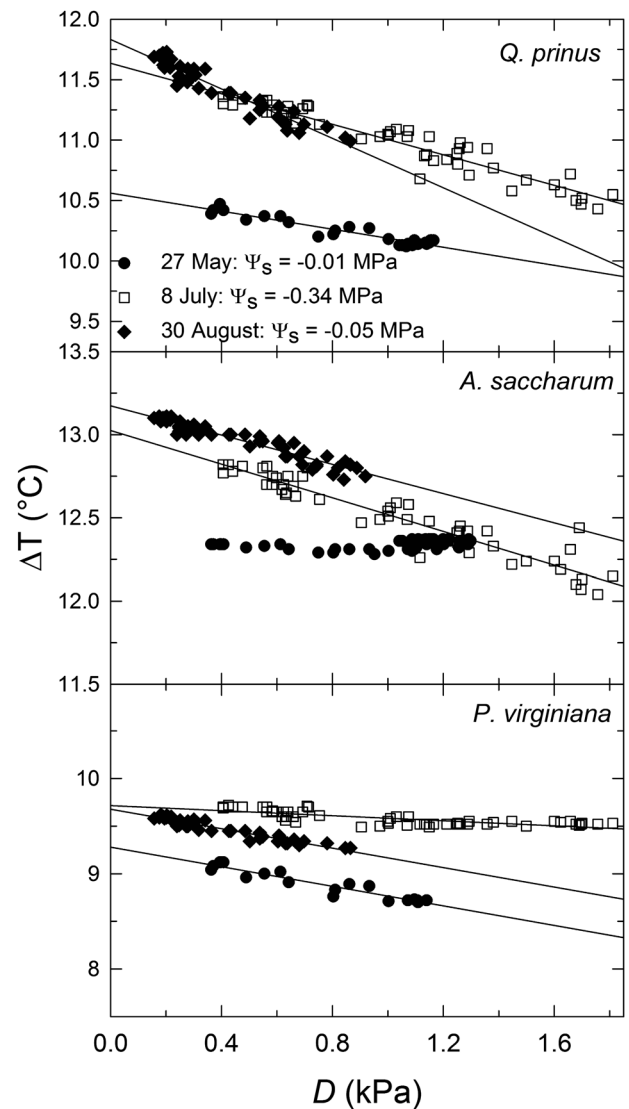

Figure 2. Night-time temperature differentials (ΔT) between the heated and reference sensors of individual sap flow probes in relation to the vapor pressure deficit (D). The set of symbols in each panel represents the relationship for the same probe at the levels of soil water potential (Ψ_s) shown. Regression lines indicate a significant relationship ($P < 0.05$) between ΔT and D. See the text for details.

Crown conductance and stomatal sensitivity to vapor pressure deficit

Crown conductance (G_c; mmol m^{-2} s^{-1}) was calculated as J_s/D with J_s expressed on a unit sapwood area basis (because estimates of total crown leaf area were not available) and D expressed as a mole fraction taking atmospheric pressure into account. This approach assumes that J_s is a proxy for transpirational flux and is therefore not appreciably affected by hydraulic capacitance between the point of sap flow measurement and the leaves. To minimize the impact of capacitance on the relationship between J_s and D, the values of G_c recorded between 09:00 and 16:30 h when PAR was >1000 µmol m^{-2} s^{-1} were used for the analyses of stomatal

sensitivity to D. This procedure also minimized the impact of low irradiance on G_c.

The sensitivity of G_c to D was analyzed using the approach described by Oren et al. (1999) in which reference G_c ($G_{c\,ref}$) is G_c at $D = 1$ kPa, and stomatal sensitivity to D is expressed as the slope of the nearly linear portion of the relationship between G_c and D ($-dG_c/dln\,D$). To estimate $G_{c\,ref}$ and $-dG_c/dln\,D$ for a given day, a series of 10-min data points for G_c and D were used to generate a plot of G_c versus ln D, permitting a regression to be fit during the portion of the day when the relationship was essentially linear (i.e., eliminating early morning and late afternoon values to attain relatively constant irradiance). The intercept of the regression at $D = 1$ kPa provided an estimate of $G_{c\,ref}$ and the slope was an estimate of $-dG_c/dln\,D$. Thus, this procedure necessarily yields single daily values of $G_{c\,ref}$ and $-dG_c/dln\,D$. Values of $G_{c\,ref}$ and $-dG_c/dln\,D$ reported here represent the mean data for all replicate individuals of a given species on a given day. Analyses were restricted to days when the mean PAR was $>\sim 350$ µmol m^{-2} s^{-1} to reduce the impact of low irradiance and variable cloudiness on G_c. To obtain a range of $G_{c\,ref}$ for each species, data collected over a range of Ψ_s ($\sim$0 to -0.35 MPa) between 27 May and 30 August were included in the analysis.

Data analyses

Data were pooled by wood type (Panshin and De Zeeuw 1970) for final analyses of below- and aboveground controls on tree water use because there is sufficient evidence that wood type exerts a great influence on tree hydraulic architecture and thus probably on soil water extraction and stomatal sensitivity to D. The impact of soil drying on tree transpiration was analyzed by plotting total daily sap flux (the means of two to four individuals per species) against θ separately for each of the three soil drying cycles. Days with mean PAR < 350 µmol m^{-2} s^{-1} were excluded from the analysis to minimize the confounding influence of low irradiance on total daily sap flux. The significance ($P < 0.05$) of relationships between sap flux and θ was assessed with one-way analysis of variance with sap flux as the response variable and θ as the independent variable. To assess the differences in sensitivity of sap flux to soil drying, sap flux was normalized with respect to its maximum value in a given soil drying cycle. Linear regressions were fitted to the combined data for the second and third drying cycles to obtain an overall slope describing the sensitivity of sap flux to declining θ in each species. Species means were then used to obtain an overall mean slope within each wood type. Data from the first soil drying cycle were not included in the sensitivity analysis because of the absence of measurements for *Liriodendron tulipifera* L. Differences among wood types in the mean sensitivity of sap flux to soil drying were assessed using Tukey's post hoc honestly significant difference (HSD) test. Data were also pooled by location (N ridge, S ridge, catchment base; Figure 1) to test for

the potential impact of slope position and measurement location on the relative sensitivity to soil drying (Tukey's HSD test).

For analyses of the sensitivity of G_c to D, linear regressions were fitted to data pooled by wood type. This procedure resulted in the data for the diffuse-porous and coniferous species being pooled because the slopes of their relationships between $-dG_c/dln\,D$ and $G_{c\,ref}$ did not differ significantly.

Results

Seasonal climate and soil moisture

The spring and summer of 2010 were characterized by a series of pronounced soil drying/rewetting cycles and large fluctuations in daily maximum D (Figure 3). Three major drying/rewetting cycles were identified for the analysis of responses of tree sap flux (shaded areas, Figure 3). The total precipitation between the beginning of the first drying cycle (May 27) and the end of the third drying cycle (September 15) was 92 mm, <30% of the mean rainfall for this period. Drought-breaking precipitation events occurred, but were typically insufficient to fully restore soil water storage in the upper 50 cm, which resulted in a seasonal minimum soil water content being
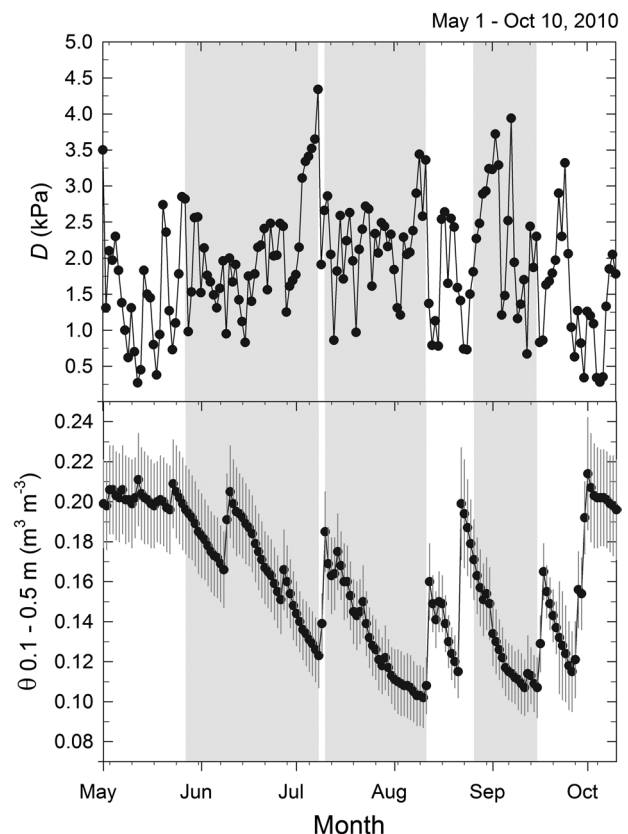

Figure 3. Seasonal courses of daily maximum atmospheric vapor pressure deficit (D) and the mean soil volumetric water content (θ) between 0.1 and 0.5 m depth. Values of θ are the means (±SE) of three locations. Shaded portions represent three soil drying cycles over which tree responses were analyzed.

reached in early August. The maximum soil water storage in the upper 50 cm was not fully restored until early October. The lowest soil water contents attained in the upper 50 cm corresponded to minimum values of Ψ_s of ~– 0.35 MPa.

Nocturnal sap flux

Nocturnal sap flux (combined recharge and transpiration) ranged from 2 to 25% of the daily total and showed no consistent trend with soil water status. Species means across the season were 5% in *Quercus alba* L., 10% in *Quercus prinus* L., 13% in *Quercus rubra* L., 7% in *Acer saccharum* Marsh., 6% in *L. tulipifera*, 8% in *Pinus virginiana* Mill. and 14% in *T. canadensis*, and showed no trends with wood type. Substantial variation in ΔT_{max} values of some sensors (Figure 2) may have been attributable to the impact of soil water deficits on the hydration and therefore thermal properties of the sapwood. For example, ΔT_{max} of sensors in *Q. prinus* and *Q. rubra* was negatively correlated with Ψ_s ($P < 0.0001$).

Belowground controls on sap flux

The relative sensitivity of sap flux to soil drying cycles was similar ($P = 0.79$, Tukey's HSD test) across the three locations where sap flow was measured. Relative declines in sap flux between the wettest and driest soil conditions were 41, 43 and 49% for the catchment base, S ridge and N ridge locations, respectively. When total daily sap flux was plotted as a function of θ during each of the three soil drying/rewetting cycles, responses appeared to differ among wood types (Figure 4). In the ring-porous species, sap flux either showed no apparent relationship with declining θ during the first soil drying cycle (*Q. alba*), increased significantly ($P < 0.05$) with declining θ (*Q. rubra*) or decreased ($P < 0.05$) as θ declined (*Q. prinus*). In the second and third soil drying cycles, sap flux either declined significantly or remained relatively constant (e.g., *Q. rubra*, the second drying cycle) as θ declined. In all three oaks, maximum daily sap flux remained relatively constant seasonally. In contrast, sap flux of the diffuse-porous and coniferous species declined significantly ($P < 0.05$) with θ in each soil drying cycle. Moreover, there was a trend for maximum daily sap flux to decline in each successive soil drying cycle. The relative sensitivity of sap flux to soil drying was ~2.2–2.3 times greater in the diffuse-porous and coniferous species than in the ring-porous species ($P < 0.05$, Tukey's HSD test).

Aboveground controls on sap flux

Daily maximum D exceeded 3 kPa on 15 occasions during the study period (Figure 3). Soil drying cycles coincided with
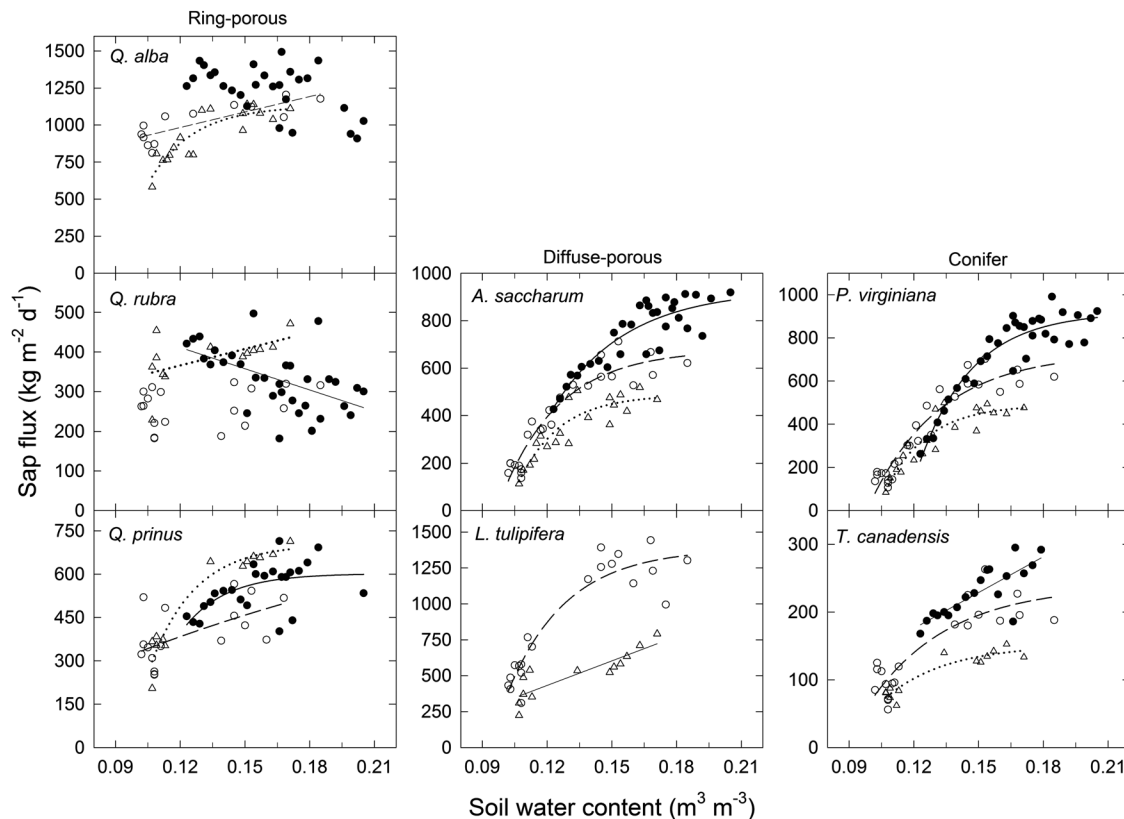

Figure 4. Relationships between total daily sap flux and mean soil water content between 0.1 and 0.5 m for species representing each wood type. Each soil drying/rewetting cycle (see Figure 2) is represented by a different symbol: filled circle June–July (solid line), open circle July–August (dashed line) and open triangle August–September (dotted line). Points are the means for three to four individuals. Lines indicate significant relationships ($P < 0.05$) for asymptotic or linear regressions. Note the differences in vertical axis scales.

trends of rising daily maximum D values with the highest values of D and the lowest values of $G_{c\,ref}$ being attained just prior to drought-breaking precipitation events (Figure 3). When stomatal sensitivity to D ($-dG_c/dln\,D$) was assessed as a function of $G_{c\,ref}$ (G_c at $D = 1$ kPa) using pooled data for all seven species, a linear relationship ($P < 0.0001$) with a slope of 0.62 was obtained (Figure 5, upper). When slopes for each wood type were analyzed separately, those of the diffuse-porous and coniferous species did not differ significantly, but the ring-porous species followed a significantly different trajectory ($P < 0.01$) from that of the diffuse-porous and coniferous species indicative of a lower stomatal responsiveness to D (Figure 5, lower). The slope of the decline in G_c with an incremental increase in D was 46% lower in the ring-porous than in the other species, and the r^2 values for the separate relationships were greater than that of the relationship based on pooled data. Daily time courses of sap flux of *Q. alba*, *A. saccharum* and *P. virginiana* in wet and dry soil (Figure 6) were
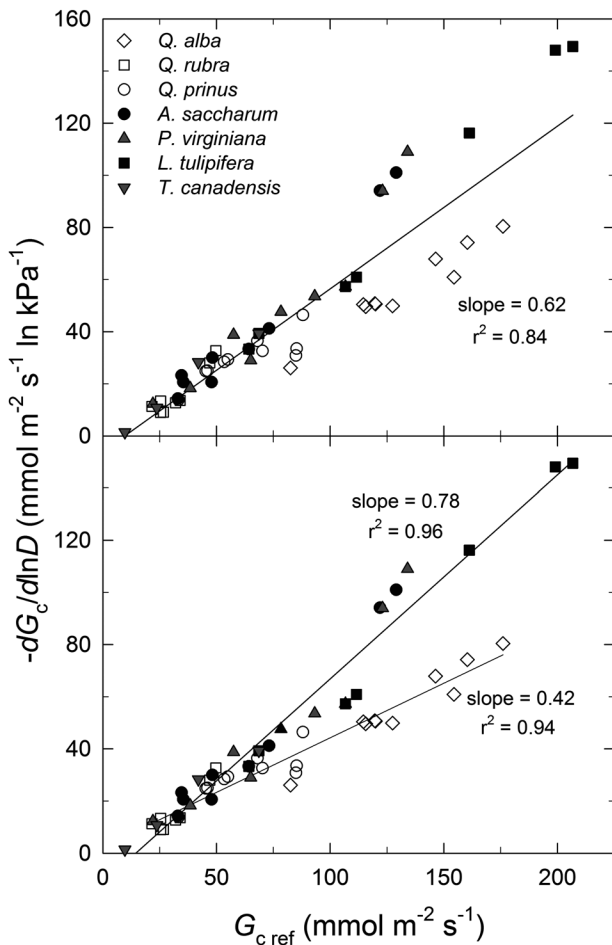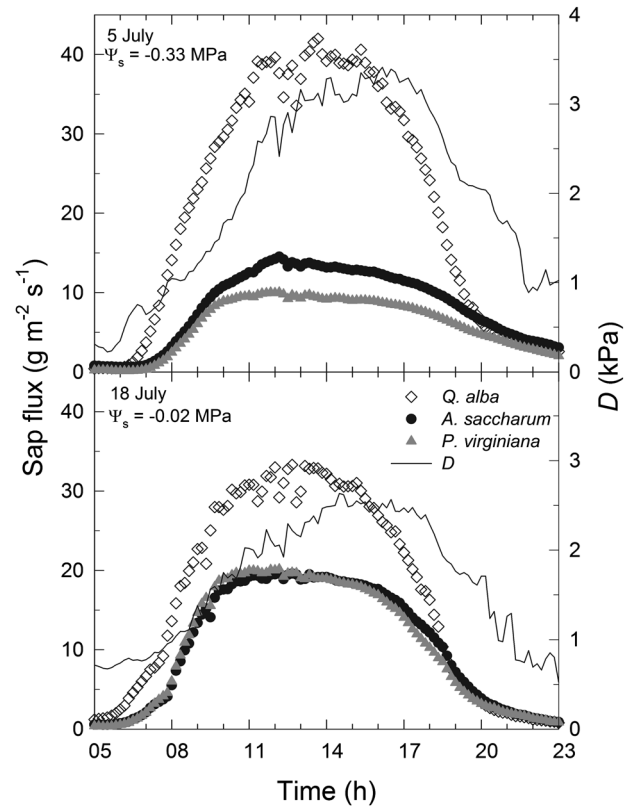

Figure 6. Daily courses of sap flux and vapor pressure deficit (D) at low and high soil water potential for a ring-porous, diffuse-porous and coniferous species. The mean responses of three to four individuals per species are shown.

Figure 5. The sensitivity of crown-level stomatal conductance to increasing vapor pressure deficit ($-dG_c/dln\,D$) as a function of reference crown conductance at $D = 1$ kPa ($G_{c\,ref}$). Each point represents the response of two to four individuals of a given species on a single day. Note that G_c is expressed per unit sapwood area (see Materials and methods).

consistent with tighter stomatal control of transpiration with increasing D in the diffuse-porous and coniferous species than in the oaks. In *A. saccharum* and *P. virginiana* sap flux typically reached a maximum value during the mid-morning and remained relatively constant until late afternoon, whereas in *Q. alba* sap flux continued to increase 2–3 h longer reaching a maximum at midday or later.

Discussion

As hypothesized, there were substantial differences in stomatal regulation of sap flux in response to changes in soil water availability and atmospheric evaporative demand among trees having different wood types. As a group, the three ring-porous species studied behaved similarly and their water use was considerably less responsive to soil drying than that of either the two diffuse-porous or two coniferous species. Stomata of the ring-porous species were also less responsive to increased D than those of the diffuse-porous and coniferous trees. These differences in responsiveness to changes in the below- and aboveground environment imply that regulation of leaf water potential in the ring-porous oaks was less stringent than in the other species (see below). Regardless of whether differences

in this suite of functional traits are associated with differences in wood anatomy per se, they enabled the ring-porous oaks to more effectively exploit soil water resources over a broader range of soil water availability and atmospheric dryness than either the diffuse-porous angiosperms or the tracheid-bearing conifers. These differences in environmental and physiological controls on water use among the species studied are consistent with the behavior observed at the stand level (Stoy et al. 2006, Ford et al. 2011) and with their distributions across broad geographical and local topographical gradients of water availability.

Stomatal response to evaporative demand

Although $G_{c\,ref}$ was expressed on a unit sapwood area basis in the present study, its range of values was similar to that reported for diverse tree species when expressed on the more commonly used unit leaf area basis (e.g., Oren et al. 1999, Oren and Pataki 2001, Ewers et al., 2007, 2008). Normalizing fluxes by sapwood rather than leaf area should have no effect on apparent stomatal sensitivity to D, provided that calculations are based on data collected when the impact of capacitance on sap flux is minimal and stomatal conductance is not appreciably limited by light. Similar constraints apply when using sap flow data normalized by leaf area to calculate crown-level stomatal conductance.

Variable rather than fixed values of $G_{c\,ref}$ within species were likely associated in part with changes in soil-to-leaf hydraulic conductance as soil water was depleted and recharged. The exact locations of changes in the conductance of the soil-to-leaf hydraulic pathway were not determined, but earlier work on field-grown trees has shown associations between variation in maximum stomatal conductance and embolism in shallow roots during seasonal cycles of soil drying (Domec et al. 2004, 2006). Changes in root/soil interfacial resistances may also have been involved (Pettijohn et al. 2009). Non-hydraulic or chemical signals such as root-derived abscisic acid may also have influenced stomatal responses to soil drying in some of the species studied. In split root system experiments conducted on saplings of *A. saccharum* and the three oak species studied here, Auge and Moore (2002) observed a marked stomatal response to non-hydraulic root signals in *A. saccharum* when half of the root system was dried and slight stomatal responses in *Q. alba*, *Q. prinus* and *Q. rubra*. Regardless of the nature of the signals perceived by stomata during soil drying in the present study, repeated soil drying/recovery cycles led to reductions in $G_{c\,ref}$ in the diffuse-porous and coniferous species, but not in the ring-porous oak species by the end of the measurement period (data not shown).

The slope of the relationship between $G_{c\,ref}$ and the sensitivity of G_c to increasing D fitted to pooled data for all seven species (0.62) was not significantly different from the theoretical value of 0.60 predicted for the situation in which stomata limit transpiration with increasing D such that leaf water potential does not fall below a species-specific threshold value (Oren et al. 1999). Under these circumstances, transpiration would reach a plateau value rather than continue to increase with D. However, the trajectory of the relationship for the three oak species diverged substantially from that of the remaining species at values of $G_{c\,ref} > \sim$50 mmol m^{-2} s^{-1} (Figure 5, lower), yielding a significantly lower slope for the oaks (0.42) than for the diffuse-porous and coniferous species (0.78). Both slopes were significantly different from the theoretical value of 0.6 ($P < 0.01$) predicted by Oren et al. (1999). However, forcing the regression for the diffuse-porous and coniferous species through the origin, which is a reasonable expectation of the y-intercept, resulted in a slope of 0.68, closer to the theoretical value associated with homeostasis of the minimum leaf water potential. Forcing the regression for the oaks through the origin increased its slope slightly to 0.44. The greater stomatal sensitivity to D in the diffuse-porous and coniferous species than in the ring-porous oaks was consistent with previous observations (Oren and Pataki 2001, Hölscher et al. 2005, Bush et al. 2008, Ford et al. 2011). However, here we have applied the model of Oren et al. (1999) to document consistent groupings of differential stomatal responsiveness to D across a broad range of both D and soil water availability. These differences in stomatal regulation were evident in daily time courses of sap flux, which reached a plateau by mid-morning in the diffuse-porous and coniferous species despite increasing D, whereas sap flux of the oaks continued to increase until midday or later despite increasing D (Figure 6). Although leaf water potential was not measured in the present study, the patterns in Figures 5 and 6 are suggestive of isohydric behavior (maintenance of a relatively constant minimum leaf water potential; see Tardieu and Simonneau 1998) in the diffuse-porous and coniferous species and rather extreme anisohydric behavior in the oaks (Ogle and Reynolds 2002, Ewers et al. 2005). Previous work has shown that *Quercus* species in eastern USA exhibit strong anisohydric behavior in response to seasonal drought in contrast to isohydric regulation of leaf water status in coniferous species and genera such as *Acer* (Abrams et al. 1990, Kloeppel et al. 1993, Cavender-Bares and Bazzaz 2000, Ewers et al. 2007).

Impact of soil water deficits on tree water use

The maximum sap flux of the study species under moist soil conditions was similar to that reported for the same species growing under comparable conditions in other locations (e.g., Wullschleger et al. 2001, Pataki and Oren 2003). Although some of the study species occupied different slope positions within the catchment, analyses of responsiveness of sap flux to soil drying across slope positions indicated that location did not play a major role in determining the observed differences in behavior (see Results). For example, individuals of *Q. prinus*

and *Q. rubra* were located near the north and south ridge tops, where soil depths were most shallow, and the *Q. alba* individuals were located at the base of the south ridge, yet the regulation of transpiration during soil drying cycles appeared to be relatively consistent among the three species (Figure 4). The individuals of *L. tulipifera* were located at the base of the south ridge near the stream, but their behavior resembled that of *A. saccharum* individuals located near both the ridge tops. Similarly, the behavior of the *P. virginiana* individuals near the crest of the south ridge was consistent with that of *T. canadensis* near the base of the ridge.

The three soil drying cycles were approximately equal in intensity in terms of minimum θ and Ψ_s attained, but drought-breaking precipitation events during the growing season were insufficient to restore θ in the upper 0.5 m of the soil profile to the maximum values observed in early May. Periodic manual measurements at 0.8 m depth at several locations in the catchment indicated that θ decreased from ~0.3 m^3 m^{-3} on May 10 to 0.2 m^3 m^{-3} near the end of the first pronounced soil drying cycle on July 8, indicating substantial soil water extraction even at this depth (data not shown). At least three factors may have contributed to the relative insensitivity of water use in the oaks to soil drying. First, co-occurring tree species are known to differ in their ability to access progressively deeper sources of soil water during periods of seasonal drought (Dawson and Pate 1996, Meinzer et al. 1999, Warren et al. 2005). Thus, the greater rooting depth and the ability to tap deeper sources of soil water in the oaks than in the other species (Abrams 1990) may have partly decoupled their leaf water potential and water uptake from changes in the availability of water in the upper 0.5 m of soil (Lowenstein and Pallardy 1998). Second, anisohydric or isohydrodynamic (Franks et al. 2007) regulation of leaf water potential in the oaks (see above) may have stabilized their sap flux through the maintenance of a constant driving force for soil-to-leaf water transport despite declining Ψ_s. Several North American *Quercus* species tend to have lower leaf water potential thresholds for stomatal closure than many other angiosperm and coniferous species native to the region (Abrams 1990). The isohydric behavior in the diffuse-porous and coniferous species at the study site would invariably lead to reduced sap flux with declining Ψ_s. Stomatal conductance is closely coordinated with variation in leaf hydraulic capacity both within and among species (Brodribb et al. 2005, Nardini and Salleo 2005, Brodribb and Holbrook 2007; Woodruff et al. 2007, Domec et al. 2009) and there is considerable interspecific variation in the vulnerability of leaves to water stress-induced loss of their hydraulic conductance (Brodribb and Holbrook 2006, Johnson et al. 2009). Some tree species normally experience considerable loss of leaf hydraulic conductance diurnally followed by complete recovery overnight (Bucci et al. 2003, Brodribb and Holbrook 2004, Johnson et al. 2009, 2011), whereas others do not. For example, *Quercus garryana* Dougl. leaves showed no clear diurnal trend in

their hydraulic conductance while leaves of co-occurring *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus ponderosa* Dougl. ex Laws. experienced 50–60% loss of hydraulic conductance diurnally (Johnson et al. 2009). Leaf water potential thresholds for increased stomatal restriction of transpiration may thus be associated with the magnitude of loss of leaf hydraulic capacity. Finally, a greater resistance to drought-induced xylem embolism or a greater capacity for embolism repair in the oaks would maintain hydraulic integrity at increasing xylem tensions associated with their anisohydric behavior in response to drought (e.g., Taneda and Sperry 2008).

Seasonal trends in sap flux at both high and low θ (Figure 4) were likely attributable to a combination of causes. Among the oaks, for example, contrasting relationships between sap flux and θ during the first soil drying cycle may have been associated with differences in leaf phenology independent of soil water status. Trends in other species may have been associated with leaf shedding, a common response to drought that serves to partly or completely maintain whole-plant leaf area-specific hydraulic conductance (K_{plant}), thereby stabilizing leaf water potential as well as reducing whole-plant water use at a given level of evaporative demand (Bucci et al. 2005, Zeppel and Eamus 2008, Martínez-Vilalta et al. 2009). One of the species studied here, *L. tulipifera*, is reported to undergo premature leaf senescence and shedding in response to severe drought (Ford et al. 2011). However, if *D* increases concurrently with soil drying and leaf shedding, whole-plant water use may not decrease during periods of drought (Bucci et al. 2008). Seasonal accumulation of drought-induced xylem embolism in the absence of leaf shedding would also reduce sap flux because stomata coordinate transpiration with dynamic variation in K_{plant} (Pataki et al. 1998, Cochard et al. 2000, Hubbard et al. 2001). Even in the absence of leaf shedding or reduced K_{plant} associated with accumulated embolism, stomatal reopening in the diffuse-porous and coniferous species following soil recharge may have been delayed by sustained alterations in the export of chemical signals such as abscisic acid or cytokinins from roots in areas of soil that had not experienced complete recharge following precipitation events (Davies et al. 2005; Figures 2> and 4 in this study). Although stomata of *A. saccharum* saplings were reported to be responsive to root-derived non-hydraulic signals during soil drying in split root system experiments (Auge and Moore 2002), there are conflicting results with regard to the importance of chemical root signals in controlling stomatal behavior of woody species subjected to soil drying (e.g., Fuchs and Livingston 1996, Liang et al. 1996, Jarvis and Davies 1997, Lowenstein and Pallardy 1998, Perks et al. 2002).

Implications

The three oak species studied plus another ring-porous species, *Carya tomentosa* Nutt., comprise ~66% of the total tree

basal area in the catchment. Our results obtained during a growing season characterized by unusually prolonged and severe drought suggest that increases in the frequency or intensity of summer droughts in the study region could have multiple consequences for forest function across a broad range of scale. Although oaks are not thought to be climax species at most sites in the region (Abrams 1996), long-term changes in summer precipitation regimes could alter successional time courses or climax species composition resulting in increased dominance of ring-porous species, especially oaks. At the individual tree level, persistent drought is known to have a cumulative effect on whole-tree architecture with consequent impacts on functional traits such as leaf area-specific hydraulic conductance (Maherali and DeLucia 2001, Addington et al. 2006, Sterck et al. 2008). The gradual accumulation of a structural and physiological legacy in response to prior environmental conditions restricts the ability of trees to respond rapidly to more favorable growth conditions. Thus, after release from several years of episodic droughts, tree- and stand-level carbon and water fluxes would be expected to recover slowly as trees embark on new architectural trajectories.

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Conflict of interest

None declared.

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