

Interspecific Variation in the Timing and Magnitude of Hydraulic Redistribution in a Forest With Distinct Water Sources

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Abstract

Aims Trees regulate water availability among their rooting strata through a nocturnal, passive transference of water known as hydraulic redistribution (HR). This study investigates differences in HR and groundwater use among common canopy species in longleaf pine (*Pinus palustris* Mill., Pinaceae) woodlands and explores environmental factors influencing HR. Methods HR was estimated by sap flux of lateral roots and main stems of three mature canopy species (*P. palustris*, *Quercus laevis* Walter., Fagaceae and *Quercus margarettae* Ashe., Fagaceae). We used $\delta^{18}\text{O}$ and δD of xylem water, soil water, and groundwater to determine water source. Finally, we related HR to environmental factors (Temperature, VWC, VPD) to better understand controls of HR dynamics. Results *Pinus palustris* had higher water use than either *Quercus* species, and also redistributed significantly more water as a nocturnal subsidy. HR fluxes were inversely related with mean daily temperature and independent of shallow soil moisture. Stable isotope mixing models, based on $\delta^{18}\text{O}$ and δD , indicated that all species have access to groundwater, but utilized shallow soil water in differing amounts when available. Conclusions In systems with strong water potential gradients among soil strata, any species with access to a groundwater source is likely capable of HR; however, the magnitude of HR varies significantly by species, even among closely related taxa.

Introduction

Hydraulic redistribution (HR) is a physical process wherein water is transferred across the soil profile through a plant's roots (Caldwell et al. 1998). HR occurs primarily at night, when stomata are closed and the vapor pressure deficit (VPD) that typically drives transpiration is low, resulting in a greater water potential gradient among soil layers than between soil and the atmosphere (Oliveira et al. 2005b). HR has been observed and quantified in a variety of ecosystems across the world and has been shown to increase water and nutrient availability in shallow soils (Priyadarshini et al. 2015). Soil water availability ultimately affects nutrient uptake and whole plant carbon assimilation. Species with access to deeper groundwater may exhibit increased whole-plant drought resistance, and HR likely provides other physiological advantages (Nadezhdina et al. 2010, Neumann and Cardon 2012, David et al. 2013). For example, consistent water availability maintained via HR increases the lifespan of shallow fine roots, ensuring these species can take advantage of rainfall pulses following drought (Bauerle et al. 2008). Additionally, HR can increase diffusion rates of nutrients within the soil, which facilitates nutrient uptake and biotic soil processes during periods of low precipitation (Nippert and Knapp 2007, Scott et al. 2008b). Despite the importance of HR in forest ecosystems, relatively little is understood about interspecific variation in the timing and magnitude of HR in forests with distinct water resources

Measurements of nocturnal sap flux among co-occurring species in different forests systems suggest that rooting profile and water transport capacity influence HR (Scholz et al. 2008, Neumann and Cardon 2012). Rooting profile here is a multi-dimensional term referring to a tree's gross rooting depth, rooting distribution, and root morphology across that distribution. There is a great deal of evidence for environmentally controlled plasticity in rooting morphology, but broad differences in rooting profiles

remain among distally related taxa (Callaway, 1990; Canadell, J., et al., 1996; Jackson, R. B., et al., 1996; Hipondoka & Versfeld, 2006). Morphological differences in pine and oak rooting systems have been observed at coarse and fine scales, at all stages of growth (Comas et al., 2002; Curt et al., 2005; Kono[^]pka et al., 2005). When species with distinct rooting profiles are co-occurring in an ecosystem, the ability to transport water as nocturnal HR will differ among them. In the Cerrado savannas, tree species that rely exclusively on either shallow or deeper soil water have a poor capacity for HR in contrast to those that bridge across multiple soil strata (Scholz et al. 2008). In the coastal plain sandhills of the southeastern US, *Pinus* and *Quercus* species are known to redistribute groundwater, but the reported magnitudes of redistribution are inconsistent (Espeleta et al. 2004, Domec et al. 2010). These inconsistencies in magnitude could be the result of differences in measurement techniques among studies, such as psychrometry and sap flux, or due to differences in site-specific hydrology. Despite this, HR has been observed more frequently in *Pinus* species than co-occurring hardwoods of the southeastern US, potentially due to higher plasticity in their rooting morphology, but the mechanism remains unclear (Espeleta et al. 2004). These interspecific variations become especially important when accounting for HR as a function of an ecosystem's hydrologic budget. Redistributed water is often overlooked when assessing specific responses to water deficits, but can represent significant additions to shallow soil and whole-plant transpiration (Domec et al. 2010).

It has been hypothesized that species more readily redistributing water through their rooting profile should have an advantage mitigating water stress within shallow lateral roots during periods of drought (Emerman and Dawson 1996, Amenu and Kumar 2008); however, this has yet to be shown in an empirical study. In addition to considering differences in rooting morphology, it is necessary to understand the degree to which tree species are capable of drawing from deeper groundwater to supplement low moisture content of shallow soils (Ehleringer and Dawson 1992). Water from different soil strata can be used as a source for both HR and transpiration demand (Scott et al. 2008a). HR of deep groundwater has been observed up to a depth of 20m across arid regions of central Texas (Bleby et al. 2010). If a species does not have access to deeper groundwater, it may show a reduced HR capacity, or it may transfer water laterally from wetter soils to drier soils, rather than mixing soil water between strata (Scholz et al. 2008). For example, in the Amazon Basin, despite a water table more than 100m deep, HR can still occur across shallow soil and non-saturated soil layers between 6–11m (Oliveira et al. 2005a). This indicates HR may only be limited by the heterogeneity of water availability across a plant's rooting profile, rather than depth to saturated soils (Kembel and Cahill 2005).

Understanding how environmental factors that influence tree transpiration also influence HR could facilitate a mechanistic understanding of HR, thereby providing a framework for predicting HR dynamics from commonly measured variables (Richards and Caldwell 1987a, Burgess et al. 1998). It is predicted that the highest rates of HR occur during periods of low shallow soil moisture (Neumann and Cardon 2012). This assumes water potential differences among soil strata are the primary driver of HR, and not the strength of the gradient among soil, plant, and atmosphere (Emerman and Dawson 1996). However, it has also been hypothesized that extremely low soil moisture may hinder HR capacity due to the high rate of fine root mortality (Wang et al. 2011). High nocturnal VPD has also been linked to nocturnal

transpiration in many plant species (Dawson et al. 2007), which may depress HR capacity as the atmosphere competes as a strong driver for water loss through stomata. To eliminate the confounding effect of nocturnal transpiration on HR, VPD must consistently reach zero at night, reducing the competition between atmosphere and soil water gradients (Dawson et al. 2007).

Using a xeric longleaf pine (Family-Pinaceae, *Pinus palustris* Mill.) woodland, we explore the capacity, interspecific variation, and water source of HR among dominant overstory conifer (*P. palustris*) and ring-porous hardwood trees (*Quercus laevis* Walter and *Q. margarettae* Ashe; Fagaceae). Our first objective was to quantify the volume and rate of water replacement during nocturnal HR for each of our study species. For this study, we constrain our use of HR and related terminology to the movement of water solely within plant tissues, not across the root-to-soil interface. We expected *P. palustris* to have a higher capacity for HR per unit area than either *Quercus* species, due to its heavy carbon investment in belowground rooting biomass (Brockway and Outcalt 2000). The second objective was to identify the water resources accessible to these tree species by determining the source of HR water. Longleaf pine woodlands of southwest Georgia have high annual precipitation, high daily VPD and consistently low nocturnal VPD (Ford et al. 2008). The xeric woodland where this study occurred has deep, sandy, excessively well-drained soils resting atop a thinly confined karst-limestone aquifer (Bosch et al. 2003, Williams and Kuniansky 2016). We expected species with access to deeper ground water to show no relationship between shallow soil moisture and transpiration within their main stem. Finally, to determine if environmental factors could predict observed patterns in HR, we correlated the magnitude of observed HR to temperature, VPD, and soil moisture.

Methods

-Site Description-

We conducted this study at the Jones Center at Ichauway, a privately-owned research site of 11,400 ha in the Dougherty Plain physiological district of southwest Georgia, USA. The climate is classified as humid subtropical, with ~ 1310 mm mean annual precipitation and 19°C mean annual temperature. This region sits atop the Upper Floridan Aquifer (UFA) at one of its closest points to the surface with a minimal confining unit. The UFA is an open, water-filled karst cavern system, which feeds directly into many of the surrounding river and stream systems and relies on soil filtration for recharge (Williams and Kuniansky 2016).

Our site was located in xeric soils (Goebel et al. 1997) immediately adjacent to the Ichawaynochaway Creek. The site occupies a flat plateau, approximately 10 m above the creek, with porous, excessively well-drained sandy soil that supports low volumetric water content (mean < 6% VWC). We selected four individuals from three dominant canopy tree species: *P. palustris* (mean DBH 32.15cm), *Q. laevis* (mean DBH 28.01cm), and *Q. margarettae* (mean DBH 27.75cm), with *P. palustris* occurring across a broad range of soil types and the latter two species primarily associated with xeric sites. During the study period (June-November), 615 mm of rainfall were recorded at a nearby USGS rain gauge (USGS gauge

#02355350). There was a period of low rainfall beginning in mid-September until the end of the study in November, less than 10% of the total recorded precipitation was observed in that time period. Mean daily temperature ranged from 8 C° – 28 C°, with a mean of 23.9 C°. We recorded soil moisture at 20 cm depth (EC-5cm, METER, Pullman, Washington, USA) in 15-minute intervals throughout the duration of the study.

-Sap flux-

We constructed heat ratio method (HRM) sap flux sensors following the Burgess et., al (2001) methodology. The HRM utilizes an upstream and downstream thermocouple with a central heat pulse to allow for bi-directional sap flux measurements. The accuracy of these sensors excels at low to medium flow conditions (Steppe et al. 2010). These two properties make the HRM ideal for capturing flow during nocturnal HR and contrasting against diurnal water uptake. For each tree, we installed two HRM sensors in opposing lateral roots 30cm from the stem base and an additional sensor in each primary stem at a height of 1.3m to estimate total tree transpiration. Aluminum templates were used to install HRM sensors in a -1cm, 0cm, 1cm configuration for both root and main stem sensors (Burgess et al. 2001).

Dataloggers (CR1000, Campbell Scientific, Logan, Utah, USA) recorded thermocouple temperature for 80 s after a 6 s heat pulse, every 30 minutes. To determine sap velocity ($V_s \text{ cm sec}^{-1}$), we first calculated heat pulse velocity ($V_h \text{ cm sec}^{-1}$) from thermocouples and applied to the equation:

$$V_h = \left(\frac{k}{x} \right) * \ln \left(\frac{v_1}{v_2} \right) * 3600$$

where k is the thermal diffusivity constant ($\text{Wm}\cdot\text{K}^{-1}$), x is distance between heating element and sensor, and v_1 and v_2 denote the thermocouple temperatures after the heat pulse (Burgess et al. 2001). To calculate k , we weighed and oven dried sapwood samples from each species to obtain density and water content. From there, we solved equations for the true calculation of k (Table 2.1) as outlined in Burgess et al. (2001).

The distance (x) between heat pulse generation and thermocouple measurement is critical to calculating the heat pulse velocity, so error due to probe misalignment is a serious concern when using the HRM (Ren et al. 2017). To address this, we performed a two-week laboratory trial under zero flow conditions using four sensors in excised stem segments of each study species. We established these conditions by sealing both ends and the sensor installation points with silicone gel. The error due to probe misalignment during installation was calculated at less than 1% among all species. We applied this empirically derived error rate to the corrected heat pulse equations from Burgess et al. (2001):

$$V_c = 4kt * \ln \left(\frac{v_1}{v_2} \right) - \frac{(x_1^2 - x_2^2)}{2t(x_1 - x_2)} * 3600$$

From the corrected heat pulse velocity ($V_c \text{ cm sec}^{-1}$), V_s can be calculated once wounding effects are taken into account, based on sensor needle size and spacing. x_1 is assumed to be the correctly aligned distance between the thermocouple and heat pulse, while x_2 is the incorrectly aligned distance based on the two-week laboratory trial. The t variable connotes the time (sec) between heat pulses. Afterwards, the final sap velocity was calculated using the corrected heat pulse value:

$$V_s = \frac{V_c \rho_b (c_w + m_c c_s)}{\rho_s c_s}$$

where ρ_b (g cm^{-3}) denotes the density of wood, c_w and c_s ($\text{J g}^{-1} \text{ } ^\circ\text{C}$) denote the specific heat capacity of the wood and sap respectively, m_c denotes the water content of sapwood (ml g^{-1}), and ρ_s (g cm^{-3}) denotes the density of water (Burgess et al. 2001). Positive sap velocity indicated uptake due to transpiration, while negative sap velocity indicated reverse sap flux, which we attribute to nocturnal HR transport out of lateral roots.

-Conductive Sapwood Area-

For each study species, we estimated conductive sapwood area (CSA) via active staining of stem and root tissues (Reyes-García et al. 2012). Before peak transpiration (~ 9 am), a 1-cm diameter hole was drilled into each tree at a height of 1.3m, or at 30cm from the tree base for root samples. We drilled these holes either to a depth of half the stem diameter or 15cm (whichever was lower), and gravity-fed crystal violet dye into the drill hole. We sampled from trees adjacent to the sample flow site with a DBH range for *P. palustris* ($n = 17$) between 5.3 cm to 28.2 cm, *Q. laevis* ($n = 13$) between 4.8 cm to 31.4 cm, and *Q. margarettae* ($n = 10$) between 4.0 and 35.5 cm. After 24 hours, wood bore samples 3 cm above the drilled hole, and the length of stained tissue were measured. We calculated conductive sapwood area from the stained tissue via:

$$A_{sw} = \pi * \left(\left(\frac{D_t}{2} \right) - L_b \right)^2 - \left(\left(\frac{D_t}{2} \right) - L_{sw} - L_b \right)^2$$

where A_{sw} is the CSA, D_t is the total diameter, L_b is the bark depth, and L_{sw} is the measured sapwood depth from the crystal violet stain.

-Stable Isotope Analysis-

We analyzed precipitation water, soil water, and water from plant xylem for analysis of $\delta^{18}\text{O}$ and δD isotope ratios in August and October 2017 to capture variations under different soil moisture conditions. We obtained soil water samples from 20 and 100 cm depths, and wood samples from the three study tree species ($n = 10$ per species). Deep soil pits were infeasible due to the risk of collapse in sandy soil, but a well water pump was available close to the site to collect deeper samples from $> 10\text{m}$ underground ($n = 10$). Plant water samples were taken from the terminal ends of the lowest branches. We collected

precipitation immediately prior to the October 2017 collection. After collection, we wrapped sample vials in paraffin wax to prevent evaporation and immediately put them on ice for transport to the laboratory.

We cryogenically extracted plant and soil water from each sample at 155°C for 25 minutes, verified to be within 99% of water extracted (Werner and Brand 2001). We then sent the samples to a stable isotope mass spectrometry lab, the UGA Center for Applied Isotope Studies, to obtain $\delta^{18}\text{O}$ and δD ratios. $\delta^{18}\text{O}$ and δD were analyzed by sources (20 cm soil, 100 cm soil, and ground water) and mixtures (*P. palustris*, *Q. laevis*, and *Q. margarettae*).

-Environmental Drivers of HR-

To examine the relationship between stem and root sap flux and environmental factors, we obtained data from an array of environmental variables recorded within our study site using a HOBO weather station (ONSET, Bourne, Massachusetts). Our measured variables were 20 cm soil volumetric water content (VWC), mean daily vapor pressure deficit (VPD), maximum daily VPD, solar radiation, and mean nightly air temperature (Fig. 5).

-Data Analyses-

We correlated CSA to DBH and created species specific allometric equations for *P. palustris* ($y = 1.22e^{-0.044x}$; $R^2 = 0.89$), *Q. laevis* ($y = 1.403e^{-0.073x}$; $R^2 = 0.97$), and *Q. margarettae* ($y = 1.0874e^{-0.052x}$, $R^2 = 0.97$). These equations were applied to a standard diameter for the lateral roots (5 cm) and main stem (25cm) to obtain CSA estimates for each species. Flow volumes were calculated using the velocity at each HRM sensor and multiplied by the species-specific CSA. We summed diurnal and nocturnal flow events separately to obtain hourly sap volumes to test for the effect of species on mean transpiration (diurnal sap flow) and HR (nocturnal sap flow) over the course of this study. We used these data in a mixed-effects repeated measures ANOVA with species as a fixed effect, time as the fixed repeated factor, and individual tree as the random effect.

We imposed stepwise regression based on minimum AIC to determine the environmental variables which explained a significant portion of HR variation. We analyzed the factors chosen above and their interactions against mean cumulative diurnal uptake and mean cumulative nocturnal HR for each species in a multivariate ANOVA. We plotted the predicted relationship from these tests against the observed data to determine how well these two factors could predict HR outflow.

$\delta^{18}\text{O}$ and δD ratios from soil sources and plant mixtures were plotted against the local meteoric water line (LMWL) for southwest GA, but were heavily fractionated (Jian Wang and Bojie 2019). Many of the plant sample values lay outside the boundary of the soil sources. To understand these outlying mixtures, we analyzed the source $\delta^{18}\text{O}$ and δD contributions to the plant mixture ratios using a Bayesian stable isotope mixing model (SIMM) in R (iterations = 10000, burn rate = 1000, using statistical mean and standard deviation) (Parnell et al. 2013, Jian Wang and Bojie 2019).

Results

-Lateral Root Sap Movement-

P. palustris had the highest mean nocturnal HR in lateral roots ($p < 0.001$) when compared to either *Quercus* species (Fig. 1). Over the course of the study, mean (\pm SE) lateral root uptake for *P. palustris* (6.2 cm mean diameter) was 407.2 ± 21.8 ml day⁻¹ (Fig. 2). Less than 20% of uptake occurred during the latter half of the study, between September and November (Fig. 1). The opposite trend was found for *P. palustris*' mean nocturnal root outflow, estimated at 198.1 ± 18.1 ml day⁻¹, 48% of root uptake. Over 50% of nocturnal HR in lateral roots occurred during the last two months of the study, between October and November (Fig. 1).

Q. margarettae showed significantly higher mean HR than *Q. laevis* within the lateral roots ($p = 0.003$). Mean lateral root uptake for *Q. margarettae* (6.1 cm mean diameter) was 141.5 ± 5.68 ml day⁻¹ (Fig. 2) with a nocturnal root HR of 89.1 ± 9.7 ml, 53% of root uptake. Mean lateral root uptake for *Q. laevis* (6.1 cm mean diameter) was 81.6 ± 2.656 ml day⁻¹ (Fig. 2) with a root HR of 17.2 ± 1.72 ml day⁻¹, 21% of root uptake. The majority of HR (> 60%) for both *Quercus* species occurred between October and November (Fig. 1).

-Main Stem Sap Movement-

P. palustris exhibited consistent fluctuations in stem uptake over the 158-day study. Mean diurnal uptake for the mean stem diameter (32.15 cm) was 15.9 ± 0.459 L, significantly higher than either *Quercus* species ($p < 0.001$). Mean main stem-mediated HR was $10.2 \pm .680$ L, or 64% of uptake (Fig. 2). More than 60% of stem-mediated HR occurred between October and November (Fig. 1). *Q. laevis* exhibited a lower mean HR in the main stem than the other species ($p < 0.001$). Mean uptake for the main stem diameter (mean = 28.75 cm) was 8.09 ± 0.301 L day⁻¹. Mean main stem HR was 1.99 ± 0.219 L day⁻¹, or 25% of uptake (Fig. 2). *Q. margarettae* had significantly greater mean daily stem-mediated HR than *Q. laevis* ($p < 0.001$), but significantly lower HR than *P. palustris* ($p < 0.001$). *Quercus laevis* exhibited an increased stem uptake during the last two months, October through November, which accounted for over 40% of total water use during the study (Fig. 1). Mean uptake for the main stem (mean diameter 27.40 cm) was 5.53 ± 0.395 L day⁻¹. HR within the main stem was less consistent and accounted for a smaller proportion of daily uptake than was observed in lateral roots. Mean daily main stem HR was 2.17 ± 0.223 L, 39% of uptake (Fig. 2). Similar to *Q. laevis*, the majority of HR (> 60%) within the main stem occurred between October through November (Fig. 1).

-Stable Isotope Analysis-

Source water usage was similar among species during a period of high VWC in August, but differed among species during an extended period of low VWC in October (Fig. 3). In August, *P. palustris* received an estimated mean (\pm SE) source contribution of $4.75 \pm 1.24\%$ of water from 20 cm soil layer, $6.95 \pm 2.05\%$ of water from the 100 cm deep soil layer, and $88.3 \pm 2.59\%$ of water from groundwater (Fig. 3).

These proportions were closely matched by *Q. laevis* ($5.61 \pm 1.41\%$, $7.67 \pm 2.10\%$, and $86.7 \pm 2.71\%$ respectively) and *Q. margarettae* in August ($5.92 \pm 1.23\%$, $6.85 \pm 1.53\%$, and $87.2 \pm 1.91\%$, respectively) (Fig. 3). The October samples show different water sources within all three study species. *P. palustris* was estimated to source $32.4 \pm 6.0\%$ of xylem water from 20 cm soil layer, $38.8 \pm 7.22\%$ of xylem water from the 100 cm deep soil layer, and $28.8 \pm 5.38\%$ of xylem water from groundwater in October (Fig. 3). These proportions were similar in *Q. laevis* ($35.2 \pm 6.41\%$, $40.7 \pm 7.5\%$, and $24.1 \pm 4.87\%$ respectively), but differed substantially in *Q. margarettae* ($68.8 \pm 6.42\%$, $20.4 \pm 5.69\%$, and $10.8 \pm 2.76\%$, respectively). (Fig. 3).

-Environmental Drivers of HR-

For all species, mean daily uptake had a moderate to strong positive relationship with mean daily VPD (mean *P. palustris* $R^2 = 0.56$; *Q. laevis* $R^2 = 0.73$; *Q. margarettae* $R^2 = 0.49$). When relating HR to environmental factors, all three species showed a moderate to strong relationship between HR and the combination of nightly air temperature, mean daily VPD (kPa), and their interactive effects (*P. palustris* $R^2 = 0.71$; *Q. laevis* $R^2 = 0.85$; *Q. margarettae* $R^2 = 0.90$). Mean nightly temperature (C°) explained 64% of the variation in HR for *P. palustris*, 68% for *Q. laevis*, and 78% for *Q. margarettae* (Table 2). This was an inverse relationship with HR, increasing HR as mean temperature decreased. By contrast, mean daily VPD (kPa) only explained 2–14% of the daily variation in HR among all three species (Table 2). Daily VPD (kPa) had a positive relationship with HR. Most surprisingly, hourly soil VWC did not explain enough variation in mean nocturnal HR to be included in any regression models (Table 2).

Discussion

Based on our estimates, HR in the lateral roots for *P. palustris* equaled almost half of the volume used for transpiration (Fig. 2). The magnitude of HR in the lateral roots was significantly higher in *P. palustris* and *Q. margarettae* than what was found in *Q. laevis*. We found that all three species were capable of water uptake from multiple soil strata, and that the reliance upon these strata was not constant throughout the study period (Fig. 3). The environmental factors we considered (VPD, soil moisture, and temperature) explained much less variation in HR than predicted. Temperature had the strongest impact on HR volumes, showing increased prevalence and magnitude of HR as temperatures cooled during the months of October and November (Table 2). Temperature and VPD have a strong co-variation and are typically not used as separate factors when assessing relationships with transpiration. However, for HR, these factors appear to have differing influence, suggesting a de-coupling of temperature and VPD as it relates to nocturnal HR fluxes. Additionally, temperature at our site was variable during peak HR, while hourly VPD reached zero for 87% of the nights during this study.

P. palustris exhibited higher volumes of HR through lateral roots and main stem than either *Quercus* species, despite similarities in numbers of lateral roots and sap velocities (Dupuy et al. 2005a). The higher cross-sectional sapwood area (CSA) in *P. palustris* contributed to greater water use and HR as compared to either *Quercus* species. This was seen primarily in the main stem, which had more than

double the mean daily transpiration of either *Quercus* species (Fig. 2). The lateral roots of *Q. margarettae* had significantly higher water use than *Q. laevis*, suggesting it may be more reliant on its lateral root system for water acquisition than the main tap root. Their lateral roots seem to be able to access water from a variety of soil strata, an idea supported by water source estimates during August and October 2017 (Fig. 3). *Q. laevis* by contrast had significantly lower mean daily water uptake within the lateral roots, but a higher overall uptake in the main stem (Fig. 2). If *Q. laevis* was more reliant on a deep-water source, it would agree with the isotope data indicating that it relied less on shallow soil water than *Q. margarettae* during times of higher shallow soil moisture availability (Fig. 3).

The $\delta^{18}\text{O}$ and δD within xylem tissue indicate all three species alter their reliance upon different soil strata for water uptake (Fig. 3), likely in response to changes in water potential gradients (Dawson and Pate 1996). During the August collection, shallow soil VWC was at a low for the month ($\sim 3\%$); however, in October the collection occurred immediately after the largest rain event of the month when shallow-soil VWC was recorded at 6.4%, which is quite high for this site. This suggests these tree species, like many other plants, utilize soil water from the source of highest water potential, or strongest gradient between soil and atmosphere. Of all species, *Q. margarettae* showed the strongest shift between groundwater and shallow water sources, indicating that it may devote more resources to root biomass in the upper 20cm of the soil profile, and still maintain a tap root deep enough to capitalize on groundwater (Fig. 3). All three species preferentially uptake shallow soil water when available, but the isotope data suggest some portion of water is always sourced from groundwater (Fig. 3).

We predicted that *P. palustris* would be better able to access ground water and thus, rely more heavily upon this resource than either *Quercus* species due to early-life root development and larger tap-root morphology (Barnett, 2002). If these species show a strong morphological difference, it would be expected to display itself among their hydrological niche differentiation (Nippert and Holdo 2015), preferentially using the groundwater resource that other species have difficulty accessing. However, the inference from our water partitioning data suggest that all three of these species acquired water from similar sources. Shallow soil VWC (i.e., 20cm) was not correlated with diurnal sap flow, indicating the decoupling between shallow soil water and transpiration that occurs if species have access to groundwater. Moreover, the two *Quercus* species showed the greatest difference between water sources during both August and October potentially indicating hydrologic differentiation is occurring more strongly between the more closely related species.

A factor potentially influencing the source contributions of water in our study was the effect of HR itself on the isotopic ratios of shallow soil water via mixing of distinct isotope N members. We hypothesize, due to the high volume of HR in this system, that there may be a dampening effect which artificially lowers the $\delta^{18}\text{O}$ and δD of shallow soil water. This would affect the baseline comparisons that were used in our model, causing less discrimination among soil strata, and appearing as a more “well mixed” system. Tree species would be observed to rely on more varied sources of soil water than in reality. Several previous studies have used labeled δD tracers to confirm the presence of HR in shallow soils across a variety of ecosystems (Brooks et al. 2002, Leffler et al. 2005, Priyadarshini et al. 2016). This

methodology acknowledges the vertical mixing of soil water that occurs due to HR, made apparent by deuterated water's extreme δD . It is unclear to what degree HR may confound the natural $\delta^{18}O$ and δD gradients that would otherwise be present. Future studies may need to rely on distinctly labeled water tracers, injected across the rooting profile, to determine tree water sources without the influence of HR mixing.

The large proportion of negative flow events recorded in the main stem of all three species lends more evidence to the stem-mediated HR hypothesis (Burgess and Bleby 2010). Because these three species all have a tap root with a pattern of access to deeper soil strata, it is likely that water transported via HR must first travel within the main stem before exiting through the lateral roots. Stem-mediated HR as observed in this study is useful in relating total daily transpiration to HR but could only be used as a conservative estimate if lateral root data were not available. As has been shown in other studies, roots provide the most accurate estimates of HR, as stem-mediated HR is only a portion of total tree HR and the magnitude is very sensitive to changes in both sensor height and azimuth (Burgess and Bleby 2006, Nadezhdina et al. 2009). Our stem-mediated HR estimates match well with the magnitude of HR in the lateral roots, averaging between three to seven times greater than major individual lateral roots. These numbers agree well with the number of major lateral roots estimated in these species by past research (Drexhage et al. 1999, Dupuy et al. 2005b).

The consistency of HR changed throughout the study with higher magnitudes during October and November (Fig. 1). Temperature had an inverse relationship with HR (Table 2), with each species having an increase in HR during cooler periods. Colder months typically result in lower mean carbon assimilation for both *Quercus* and *Pinus* species (Law et al. 1999). If leaves are senescing or closing their stomata more frequently, it may allow for daytime HR to occur as VPD is no longer a driving force on xylem water transport (Leffler et al. 2005, Gou and Miller 2014). Mean daily VPD was a weak predictor of mean daily HR for each species, which agrees with our current understanding, since HR movement primarily occurs at night, when VPD is typically zero at our study site (Table 2).

Soil moisture (VWC) was a poor predictor of HR within the lateral roots of all study species. The relationship between soil moisture and HR has been clearly documented in prior research, but this specific system does not show as strong of a link (Scott et al. 2008a, Neumann and Cardon 2012). A difference of 7% VWC between the wettest and driest conditions may not be large enough to have an impact on HR's water movement. Rather, it may be that the difference between soil water content between strata is very large in the *P. palustris* woodlands due to excessively well-drained shallow soils sitting atop deeper, saturated soils and water-filled karst topography. According to the isotopic data, all three tree species appear to have access to deeper groundwater, which may have created a soil water potential gradient between shallow and deeper soils to drive HR regardless of fluctuations in shallow soil moisture conditions (Gou and Miller 2014).

Our results suggest a substantial amount of water is transported during nocturnal HR and sourced from deeper groundwater. This deeper stratum is more saturated than shallow soils and linked to it by the

rooting profile of all the three tree species studied here, but the highest magnitudes recorded in *P. palustris*. This connection allows the difference in soil moisture to drive HR upwards, redistributing both water and soil nutrients (Nadezhdina et al. 2010). This reasoning is often used to suggest that HR is able to act as a community level water subsidy for all taxa unable to otherwise access deeper groundwater (Muler et al. 2018). If this is the case, its role to facilitate mixed-tree grass systems needs to be quantified and incorporated into our current view of ecosystem water dynamics. Further research focusing on understory plant communities will highlight this potential role of HR in adding water subsidies to woodland ecosystems.

Declarations

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References

- Amenu, G. G., and P. Kumar. 2008. A model for hydraulic redistribution incorporating coupled soil-root moisture transport. *Hydrology and Earth System Sciences* **12**:55-74.
- Bauerle, T. L., J. H. Richards, D. R. Smart, and D. M. Eissenstat. 2008. Importance of internal hydraulic redistribution for prolonging the lifespan of roots in dry soil. *Plant Cell and Environment* **31**:177-186.
- Bleby, T. M., A. J. Mcelrone, and R. B. Jackson. 2010. Water uptake and hydraulic redistribution across large woody root systems to 20 m depth. *Plant, Cell & Environment* **33**:2132-2148.
- Bosch, D. D., R. R. Lowrance, J. M. Sheridan, and R. G. Williams. 2003. Ground Water Storage Effect on Streamflow for a Southeastern Coastal Plain Watershed. *Ground Water* **41**:903-912.
- Brockway, D. G., and K. W. Outcalt. 2000. Restoring longleaf pine wiregrass ecosystems:: Hexazinone application enhances effects of prescribed fire. *Forest Ecology and Management* **137**:121-138.
- Brooks, J. R., F. C. Meinzer, R. Coulombe, and J. Gregg. 2002. Hydraulic redistribution of soil water during summer drought in two contrasting Pacific Northwest coniferous forests. *Tree Physiology* **22**:1107-1117.
- Burgess, S. S. O., M. A. Adams, N. C. Turner, C. R. Beverly, C. K. Ong, A. A. H. Khan, and T. M. Bleby. 2001. An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. *Tree Physiology* **21**:589-598.

- Burgess, S. S. O., and T. M. Bleby. 2006. Redistribution of soil water by lateral roots mediated by stem tissues. *Journal of Experimental Botany* **57**:3283-3291.
- Caldwell, M. M., T. E. Dawson, and J. H. Richards. 1998. Hydraulic lift: Consequences of water efflux from the roots of plants. *Oecologia* **113**:151-161.
- Canadell, J., R. B. Jackson, J. B. Ehleringer, H. A. Mooney, O. E. Sala and E. D. Schulze (1996). "Maximum rooting depth of vegetation types at the global scale." *Oecologia* **108**(4): 583-595.
- Comas, L., T. Bouma, and David Eissenstat. "Linking root traits to potential growth rate in six temperate tree species." *Oecologia* **132**.1 (2002): 34-43.
- Curt, Thomas, et al. "Plasticity in growth, biomass allocation and root morphology in beech seedlings as induced by irradiance and herbaceous competition." *Annals of Forest science* **62**.1 (2005): 51-60.
- David, T. S., C. A. Pinto, N. Nadezhdina, C. Kurz-Besson, M. O. Henriques, T. Quilhó, J. Cermak, M. M. Chaves, J. S. Pereira, and J. S. David. 2013. Root functioning, tree water use and hydraulic redistribution in *Quercus suber* trees: A modeling approach based on root sap flow. *Forest Ecology and Management* **307**:136-146.
- Dawson, T. E., S. S. O. Burgess, K. P. Tu, R. S. Oliveira, L. S. Santiago, J. B. Fisher, K. A. Simonin, and A. R. Ambrose. 2007. Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiology* **27**:561-575.
- Dawson, T. E., and J. S. Pate. 1996. Seasonal water uptake and movement in root systems of Australian phreatophytic plants of dimorphic root morphology: A stable isotope investigation. *Oecologia* **107**:13-20.
- Domec, J. C., J. S. King, A. Noormets, E. Treasure, M. J. Gavazzi, G. Sun, and S. G. McNulty. 2010. Hydraulic redistribution of soil water by roots affects whole-stand evapotranspiration and net ecosystem carbon exchange. *New Phytol* **187**:171-183.
- Drexhage, M., M. Chauviere, F. Colin, and C. N. N. Nielsen. 1999. Development of structural root architecture and allometry of *Quercus petraea*. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **29**:600-608.
- Dupuy, L., T. Fourcaud, and A. Stokes. 2005a. A Numerical Investigation into the Influence of Soil Type and Root Architecture on Tree Anchorage. *Plant and Soil* **278**:119-134.
- Dupuy, L., T. Fourcaud, A. Stokes, and F. Danjon. 2005b. A density-based approach for the modelling of root architecture: application to Maritime pine (*Pinus pinaster* Ait.) root systems. *Journal of Theoretical Biology* **236**:323-334.

- Ehleringer, J., and T. Dawson. 1992. Water uptake by plants: perspectives from stable isotope composition. *Plant, Cell & Environment* **15**:1073-1082.
- Emerman, S. H., and T. E. Dawson. 1996. Hydraulic lift and its influence on the water content of the rhizosphere: An example from sugar maple, *Acer saccharum*. *Oecologia* **108**:273-278.
- Espeleta, J. F., J. B. West, and L. A. Donovan. 2004. Species-specific patterns of hydraulic lift in co-occurring adult trees and grasses in a sandhill community. *Oecologia* **138**:341-349.
- Ford, C. R., R. J. Mitchell, and R. O. Teskey. 2008. Water table depth affects productivity, water use, and the response to nitrogen addition in a savanna system. *Canadian Journal of Forest Research* **38**:2118-2127.
- Goebel, P., B. Palik, L. Kirkman, and L. West. 1997. Field guide: landscape ecosystem types of Ichauway. Newton, GA.
- Gou, S., and G. Miller. 2014. A groundwater-soil-plant-atmosphere continuum approach for modelling water stress, uptake, and hydraulic redistribution in phreatophytic vegetation. *Ecohydrology* **7**:1029-1041.
- Jackson, R. B., J. Canadell, J. R. Ehleringer, H. A. Mooney, O. E. Sala and E. D. Schulze (1996). "A global analysis of root distributions for terrestrial biomes." *Oecologia* **108**(3): 389-411.
- Jian Wang, N. L., and F. Bojie. 2019. Inter-comparison of stable isotope mixing models for determining plant water source partitioning. *Science of the Total Environment*:685-693.
- Kembel, S. W., and J. F. Cahill. 2005. Plant phenotypic plasticity belowground: A phylogenetic perspective on root foraging trade-offs. *Am Nat*:216-230.
- Kono[^]pk, Bohdan, et al. "Comparison of fine root dynamics in Scots pine and Pedunculate oak in sandy soil." *Plant and Soil* 276.1-2 (2005): 33-45.
- Law, B. E., M. G. Ryan, and P. M. Anthoni. 1999. Seasonal and annual respiration of a ponderosa pine ecosystem. *Global Change Biology* **5**:169-182.
- Leffler, A. J., M. S. Peek, R. J. Ryel, C. Y. Ivans, and M. M. Caldwell. 2005. Hydraulic redistribution through the root systems of senesced plants. *Ecology* **86**:633-642.
- Muler, A. L., E. J. B. van Etten, W. D. Stock, K. Howard, and R. H. Froend. 2018. Can hydraulically redistributed water assist surrounding seedlings during summer drought? *Oecologia*.
- Nadezhdina, N., T. S. David, J. S. David, M. I. Ferreira, M. Dohnal, M. Tesař, K. Gartner, E. Leitgeb, V. Nadezhdin, J. Cermak, M. S. Jimenez, and D. Morales. 2010. Trees never rest: the multiple facets of hydraulic redistribution. *Ecohydrology* **3**:431-444.
- Nadezhdina, N., K. Steppe, D. J. W. De Pauw, R. Bequet, J. Cermak, and R. Ceulemans. 2009. Stem-mediated hydraulic redistribution in large roots on opposing sides of a Douglas-fir tree following localized

irrigation. *New Phytologist* **184**:932-943.

Neumann, R. B., and Z. G. Cardon. 2012. The magnitude of hydraulic redistribution by plant roots: a review and synthesis of empirical and modeling studies. *New Phytol* **194**:337-352.

Nippert, J. B., and R. M. Holdo. 2015. Challenging the maximum rooting depth paradigm in grasslands and savannas. *Functional Ecology* **29**:739-745.

Nippert, J. B., and A. K. Knapp. 2007. Soil water partitioning contributes to species coexistence in tallgrass prairie. *Oikos* **116**:1017-1029.

Oliveira, R. S., T. E. Dawson, S. S. Burgess, and D. C. Nepstad. 2005a. Hydraulic redistribution in three Amazonian trees. *Oecologia* **145**:354-363.

Oliveira, R. S., T. E. Dawson, S. S. O. Burgess, and D. C. Nepstad. 2005b. Hydraulic redistribution in three Amazonian trees. *Oecologia* **145**:354-363.

Parnell, A. C., D. L. Phillips, S. Bearhop, B. X. Semmens, E. J. Ward, J. W. Moore, A. L. Jackson, J. Grey, D. J. Kelly, and R. Inger. 2013. Bayesian stable isotope mixing models. *Environmetrics* **24**:387-399.

Priyadarshini, K., H. H. Prins, S. de Bie, I. M. Heitkönig, S. Woodborne, G. Gort, K. Kirkman, F. Ludwig, T. E. Dawson, and H. de Kroon. 2016. Seasonality of hydraulic redistribution by trees to grasses and changes in their water-source use that change tree–grass interactions. *Ecohydrology* **9**:218-228.

Ren, R., G. Liu, M. Wen, R. Horton, B. Li, and B. Si. 2017. The effects of probe misalignment on sap flux density measurements and in situ probe spacing correction methods. *Agricultural and Forest Meteorology* **232**:176-185.

Reyes-García, C., J. L. Andrade, J. L. Simá, R. Us-Santamaría, and P. C. Jackson. 2012. Sapwood to heartwood ratio affects whole-tree water use in dry forest legume and non-legume trees. *Trees* **26**:1317-1330.

Scholz, F. G., S. J. Bucci, G. Goldstein, M. Z. Moreira, F. C. Meinzer, J. C. Domec, R. Villalobos-Vega, A. C. Franco, and F. Miralles-Wilhelm. 2008. Biophysical and life-history determinants of hydraulic lift in Neotropical savanna trees. *Functional Ecology* **22**:773-786.

Scott, R. L., W. L. Cable, and K. R. Hultine. 2008a. The ecohydrologic significance of hydraulic redistribution in a semiarid savanna. *Water Resources Research* **44**.

Scott, R. L., W. L. Cable, and K. R. Hultine. 2008b. The ecohydrologic significance of hydraulic redistribution in a semiarid savanna. *Water Resources Research* **44**:n/a-n/a.

Steppe, K., D. J. W. De Pauw, T. M. Doody, and R. O. Teskey. 2010. A comparison of sap flux density using thermal dissipation, heat pulse velocity and heat field deformation methods. *Agricultural and Forest*

Meteorology **150**:1046-1056.

Wang, G. L., C. Alo, R. Mei, and S. S. Sun. 2011. Droughts, hydraulic redistribution, and their impact on vegetation composition in the Amazon forest. *Plant Ecology* **212**:663-673.

Werner, R. A., and W. A. Brand. 2001. Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry* **15**:501-519.

Williams, L. J., and E. L. Kuniansky. 2016. Revised hydrogeologic framework of the Floridan aquifer system in Florida and parts of Georgia, Alabama, and South Carolina. United States Department of the Interior, United States Geological Survey.

Tables

Table 1

Sap velocity constants for each study species. (k = thermal diffusivity ($W(m \cdot K)^{-1}$), ρ_b = density of wood, m_c = water content of sapwood ($ml g^{-1}$), c_w = specific heat capacity of wood ($J g^{-1} C^\circ$))

	<i>P. palustris</i>	<i>Q. margarettae</i>	<i>Q. laevis</i>
k ($W(m \cdot K)^{-1}$)	0.081	0.059	0.048
ρ_b ($g cm^{-3}$)	0.42	0.77	0.71
m_c ($ml g^{-1}$)	0.38	0.31	0.29
c_w ($J g^{-1} \text{ }^\circ C$)	4.65	3.60	3.47

Table 2: Species models for the multiple regression analysis of mean daily HR. Factors were selected via a stepwise regression based on minimum AIC. R^2 values are shown for the model as well as the contributions for individual factors.

Species	AIC & Model Components	R ²	Prob > F
<i>P. palustris</i>	Minimum AIC = 931.44	R ² = 0.71	< 0.001
	Temperature	R ² = 0.64	< 0.001
	Mean daily VPD	R ² = 0.023	< 0.001
<i>Q. laevis</i>	Minimum AIC = 942.35	R ² = 0.85	< 0.001
	Temperature	R ² = 0.64	< 0.001
	Mean Daily VPD	R ² = 0.13	< 0.001
	Temperature * Mean Daily VPD	R ² = 0.021	0.004
<i>Q. margarettae</i>	Minimum AIC = 758.49	R ² = 0.90	< 0.001
	Temperature	R ² = 0.78	< 0.001
	Mean Daily VPD	R ² = 0.14	0.0015

Figures

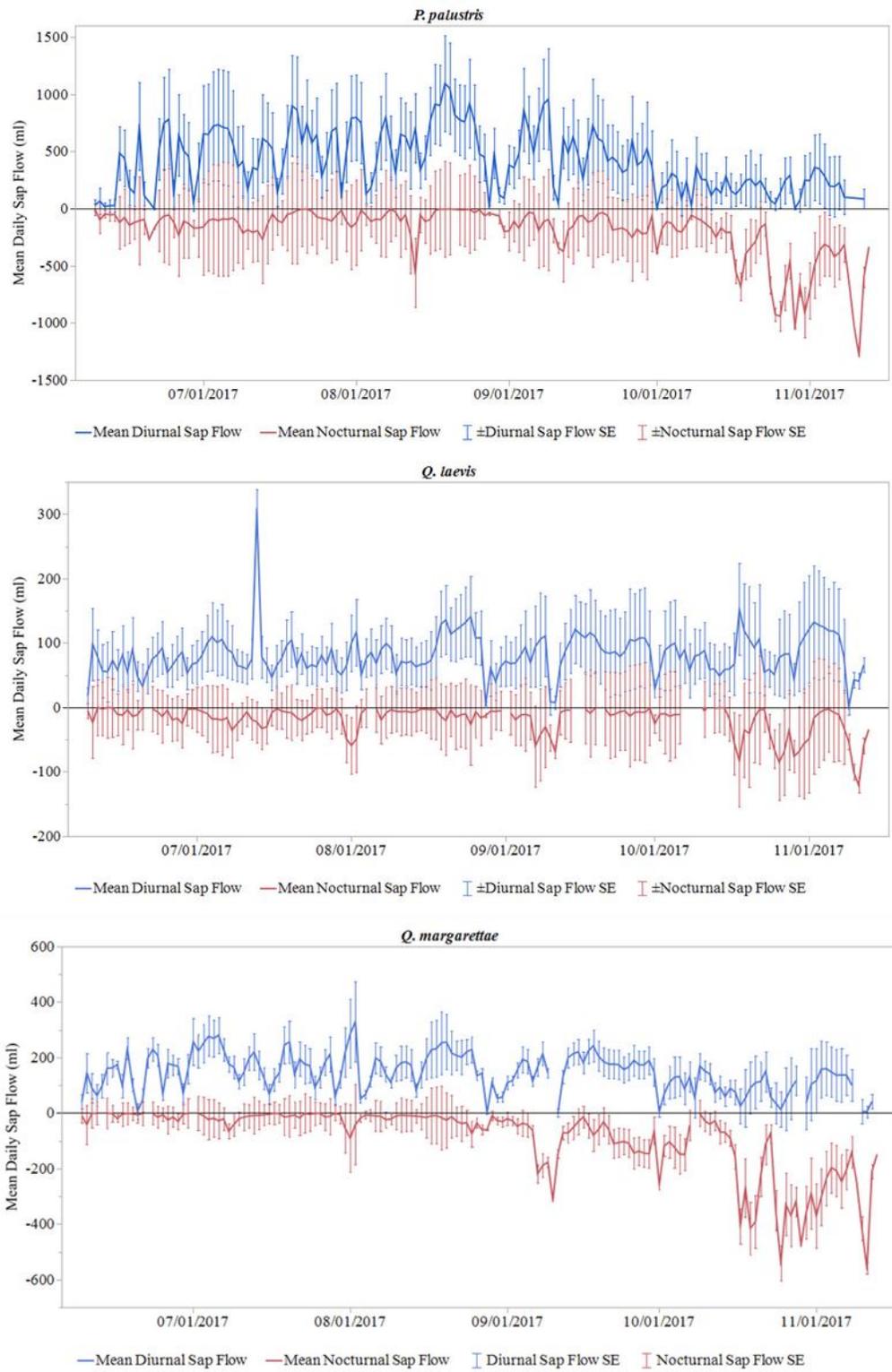


Figure 1

Mean daily uptake and HR in lateral roots among all three study species over the study period. A large increase in HR is seen during the months of October and November, coinciding with cooler temperatures and drier soil conditions.

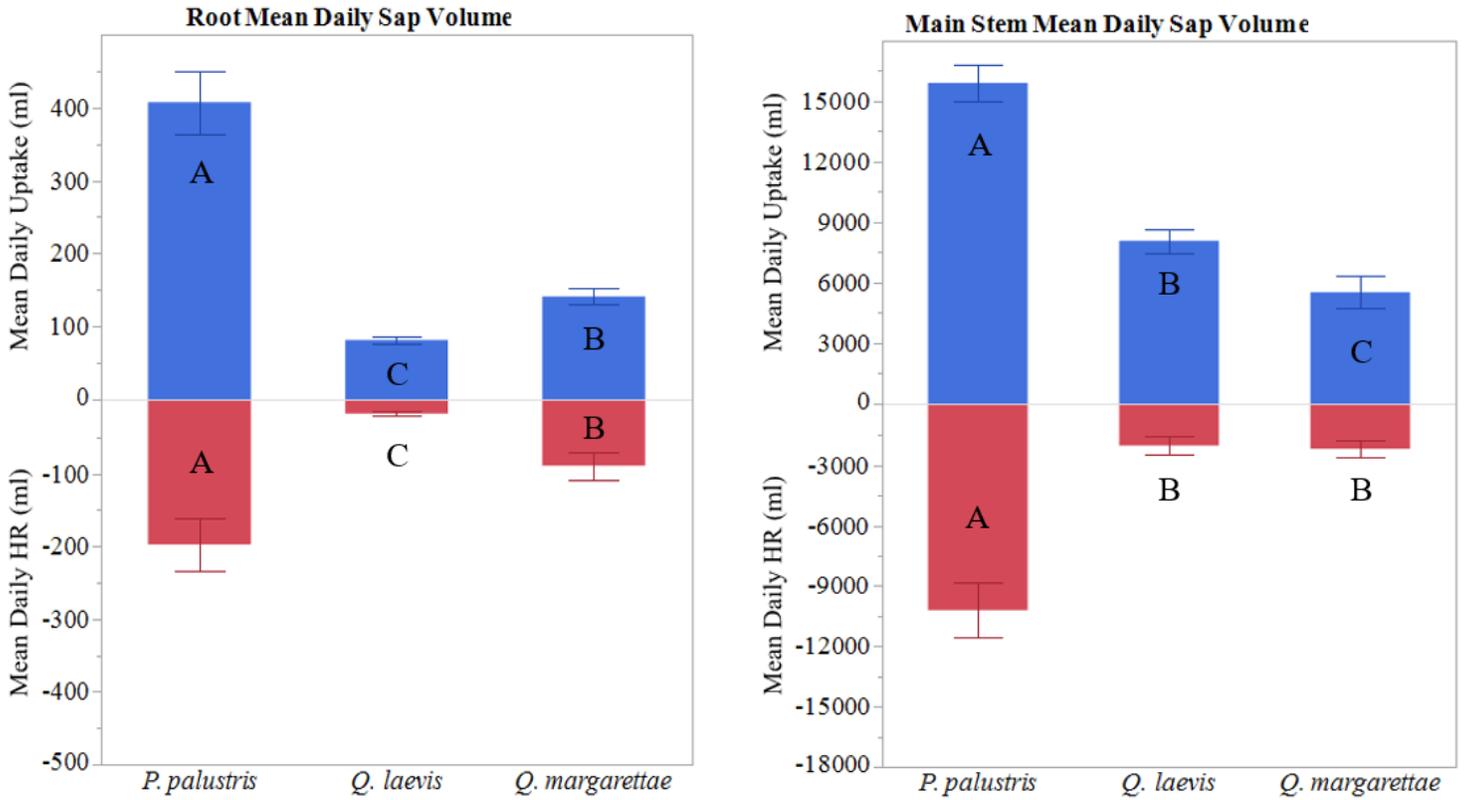
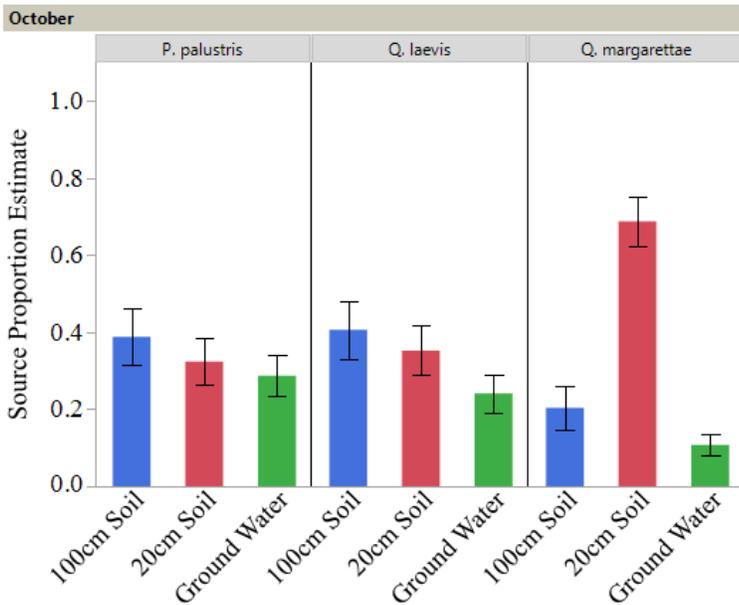
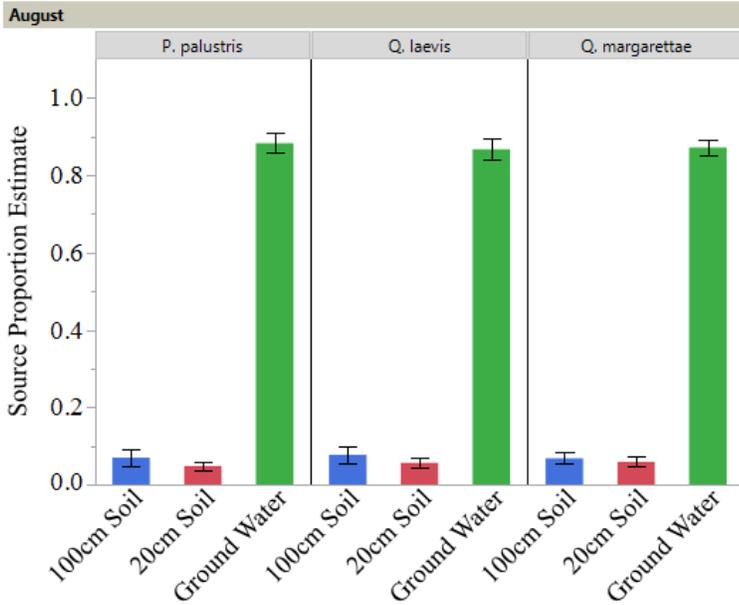


Figure 2

Comparison of mean diurnal uptake and nocturnal HR (ml) among species for lateral roots and main stems, relativized for a 25cm DBH stem. Error bars represent the 95% confidence interval around the mean and connecting letters indicate significant differences in uptake or HR among species based on Tukey HSD post hoc ($\alpha = 0.05$).

Graph Builder



Each error bar is constructed using \pm statistics.se.

P. palustris

Q. laevis

Q. margarettae

Figure 3

Comparison of estimated source contributions among *P. palustris*, *Q. laevis*, and *Q. margarettae* xylem water samples during August and October 2017. Sources are: ground water, 20 cm soil water, and 100 cm soil water. Error bars are constructed using standard error of the mean.

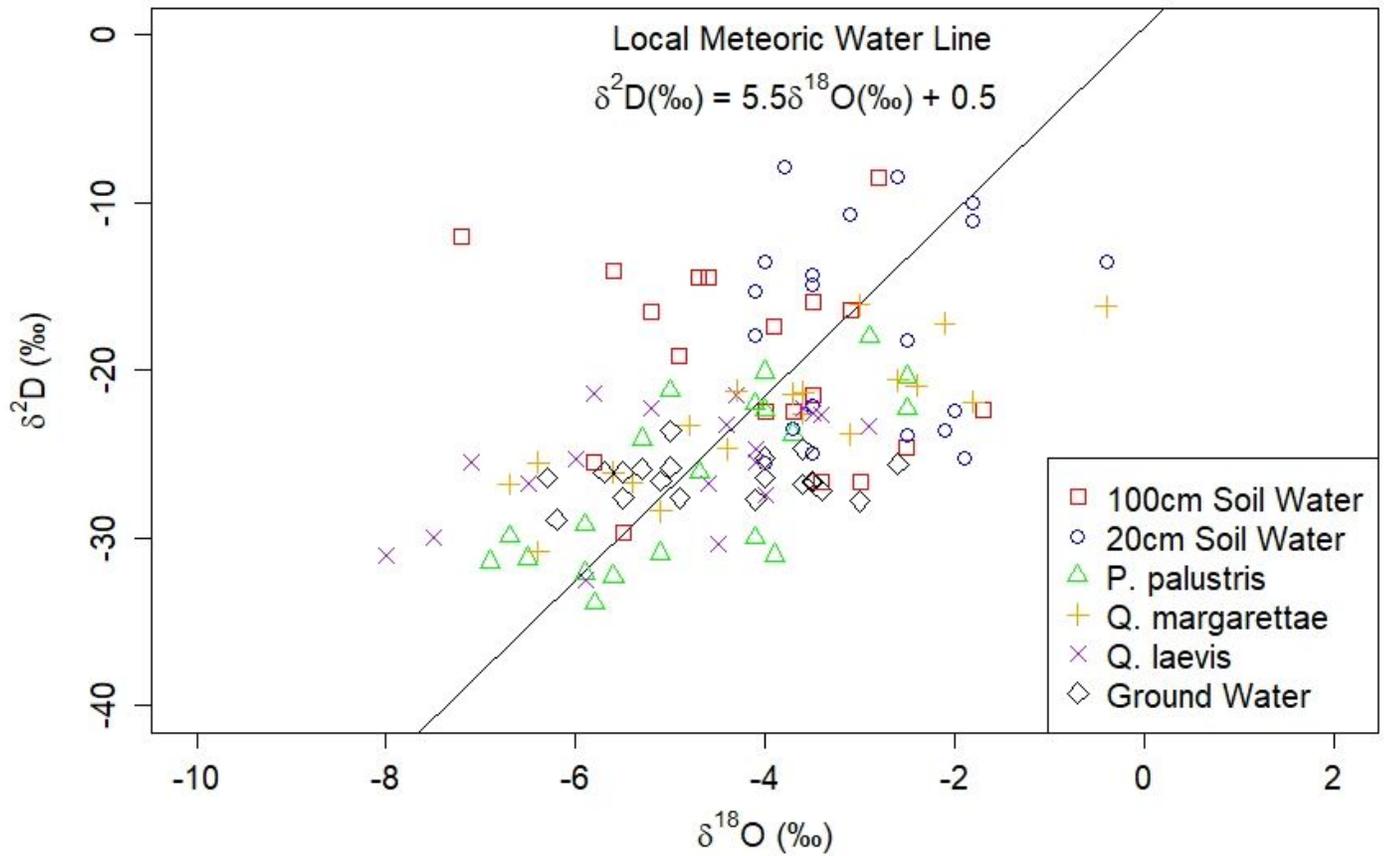
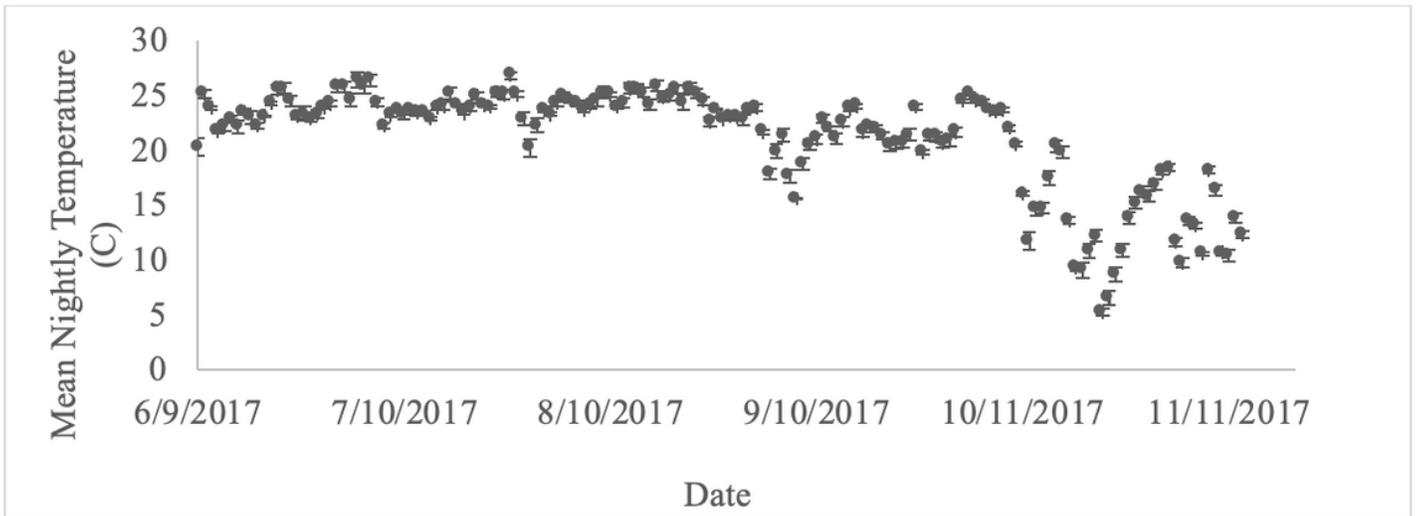
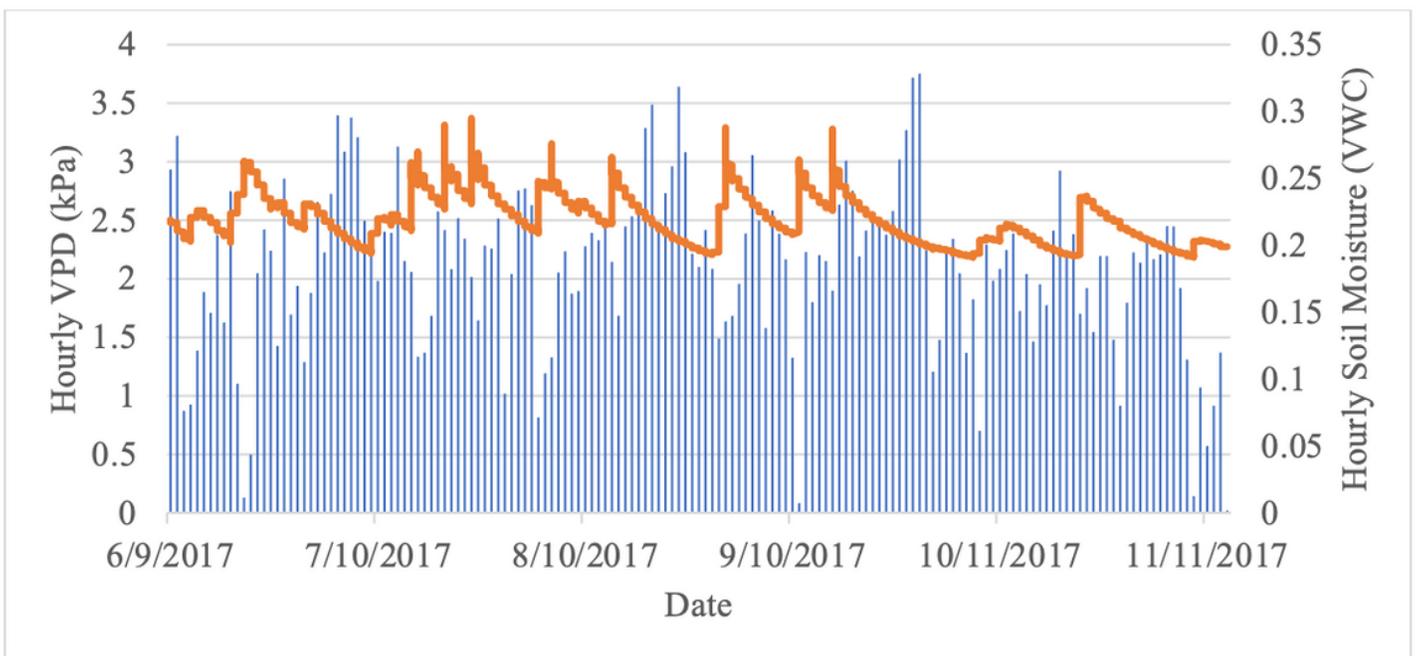


Figure 4

$\delta^{18}\text{O}$ and δD isotope ratios for source and xylem water samples plotted against the local meteoric water line (LMWL). The distribution of the points away from the LMWL indicates preferential fractionation. Several xylem water mixtures lie beyond the range of our source samples indicating further fractionation. The Bayesian stable isotope mixing model was constructed from these ratios.



(a)



(b)

Figure 5

Raw nightly air temperature (a), mean daily VPD (b; blue bars), and hourly soil moisture (b; orange line) dataset used throughout the study period. These data contributed to the construction of our stepwise regression model relating HR to external environmental factors.

Supplementary Files

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