HIGHLIGHTED STUDENT RESEARCH

Soil microbial communities buffer physiological responses to drought stress in three hardwood species

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Abstract Trees possess myriad adaptations for coping with drought stress, but the extent to which their drought responses are influenced by interactions with soil microbes is poorly understood. To explore the role of microbes in mediating tree responses to drought stress, we exposed saplings of three species (Acer saccharum, Liriodendron tulipifera, and Quercus alba) to a four week experimental drought in mesocosms. Half of the pots were inoculated with a live soil slurry (i.e., a microbial inoculum derived from soils beneath the canopies of mature A. saccharum, L. tulipifera or Q. alba stands), while the other half of the pots received a sterile soil slurry. Soil microbes ameliorated drought stress in L. tulipifera by minimizing reductions in leaf water potential and by reducing photosynthetic declines. In A. saccharum, soil microbes reduced drought stress by lessening declines in leaf water potential, though these changes did not buffer the trees from declining photosynthetic rates. In Q. alba, soil microbes had no effects on leaf physiological parameters during drought stress. In all species, microbes had no significant effects on dynamic C allocation during drought stress, suggesting that microbial effects on plant physiology were unrelated to source–sink dynamics. Collectively, our results suggest that soil microbes have the potential to alter key parameters that are used to diagnose drought sensitivity (i.e., isohydry or anisohydry). To the extent that our results reflect dynamics occurring in forests, a revised perspective on plant hydraulic strategies that considers root-microbe interactions may lead to improved predictions of forest vulnerability to drought.

Keywords Gas exchange · Drought stress · Inoculation · Root-microbe interaction · Isohydry

Introduction

Temperate forests are the primary sink for atmospheric CO₂ in North America (Pan et al. 2011; Xiao et al. 2011) and play an important role in regulating global carbon (C) and water fluxes (Bonan 2008). Given that these forests are predicted to experience increasing drought stress in the coming decades (Dai 2013), there is a need to better understand the mechanisms by which trees cope with drought. Most trees respond quickly to drought through numerous concerted morphological and physiological changes to avoid a failure of both C and hydraulic systems (Anderegg et al. 2012; McDowell 2011; Sala et al. 2012). Aboveground, trees may alter stomatal behavior, hydraulic properties, and leaf biomass to conserve water, while belowground, hydraulic traits, molecular responses, and root morphology can be adjusted to cope with drought stress (Aranda et al. 2012; Aroca and Ruiz-Lozano 2012; Brunner et al. 2015). While most theories of plant water relations are based on known physiological trade-offs between maintaining sufficient C supply and safe levels of xylem tension (McDowell et al. 2008), theories that consider the role of biotic interactions (e.g., between plants and soil microbes) are exceedingly rare.

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Soil microbes are known to influence how tree species respond to drought stress—both positively and negatively. For example, mycorrhizal fungi can benefit drought-stressed trees by increasing access to water in soil microsites (Augé et al. 2014; Li et al. 2014; Kivilin et al. 2013; Worchel et al. 2013), increasing stomatal conductance (Augé et al. 2014), synthesizing osmolytes (Rapparini and Peñuelas 2014), and enhancing nutrient uptake (Ebel et al. 1997). Free-living microbes can also ameliorate drought stress by producing compounds that trigger hormone production (e.g., abscisic acid; ABA) to mediate stomatal closure (Loewenstein and Pallardy 1998; Yang et al. 2009; Fan et al. 2015) or by regulating aquaporin expression (Brunner et al. 2015)—processes that enhance water uptake and conductance. However, microbes can also exacerbate drought by acting as pathogens (Aguadé et al. 2015). Given the number of water use strategies used by different plants, as well as the number of ways in which microbes can impact drought sensitivity, there is a need to develop a framework for considering microbial effects on plant water relations.

Tree species’ water use is often characterized along a continuum from isohydry to anisohydry (Klein 2014; Martínez-Vilalta et al. 2014; McDowell 2011; Tardieu and Simonneau 1998). This framework is based on the idea that leaf-level dynamics—photosynthesis (A), stomatal conductance (gs), and leaf water potential (ΨL)—often respond in coordinated ways as drought stress is exacerbated. Isohydric species close stomata as soil water declines, reducing C fixation but maintaining safe levels of xylem tension. In contrast, anisohydric species keep stomata open and maintain high levels of C fixation as drought progresses, but experience enhanced xylem tension that can increase the likelihood of cavitation. However, it has recently become apparent that the physiological and hydraulic function of drought-stressed trees may deviate from the predictions of the isohydry-anisohydry framework—highlighting the need to investigate the external factors that may alter tree drought responses (Garcia-Forner et al. 2016). Considering that soil microbes have been shown to impact stomatal closure, photosynthetic rates, leaf water potential and xylem tension (Fan et al. 2015; Friesen et al. 2011; Kim et al. 2012; Kivilin et al. 2013; Rapparini and Peñuelas 2014), an important but untested hypothesis is whether microbes can alter where a tree lies along the isohydric-anisohydric spectrum.

In addition to physiological alterations to gas exchange and xylem tension, trees can alleviate drought stress by altering the allocation of recent photosynthates (Blessing et al. 2015; Ruehr et al. 2009). While drought-stressed trees generally increase fine root allocation at the expense of leaf biomass and stem growth, the presence of soil microbes can alter these patterns by increasing or even decreasing belowground C allocation (Brunner et al. 2015; Hasibeder et al. 2015; Song et al. 2012). However, how drought and soil microbes interact to influence tree C allocation patterns is poorly understood. As allocation patterns can be a valuable indicator of tree stress responses and have important implications for C turnover times and inputs to the soil C cycle, it is critical to consider the responses of trees to the interactive effects of drought and microbial communities.

In this study, we sought to assess the role of soil microbes on the drought responses of three tree species that differ in their hydraulic strategies (e.g., across the isohydric to anisohydric spectrum). We hypothesized that inoculation with native soil microbes would buffer trees against drought stress, with the strongest effects occurring in the most drought-sensitive (i.e., more isohydric) species. By inoculating drought-stressed and well-watered trees with native soil microbial communities, monitoring leaf water potential and gas exchange, and quantifying C allocation dynamics by pulse-labeling trees with 13CO2, we sought to acquire a holistic view of the coordinated changes in tree water status, leaf-level fluxes, and C allocation as an experimental drought progressed. Our main objectives were to quantify the magnitude and direction of microbial effects on tree physiology and C allocation, and to discern if those effects relate to tree hydraulic strategies or are idiosyncratic and species-specific.

Methods

Tree species and planting

Three tree species, all canopy dominant in the central hardwoods region, were chosen for this study: *Acer saccharum* (sugar maple), *Liriodendron tulipifera* (tulip poplar), and *Quercus alba* (white oak). These species are known to differ in their drought tolerance and hydraulic strategies, with field studies of mature trees indicating *L. tulipifera* as strongly isohydric, *Q. alba* as anisohydric and *A. saccharum* as more isohydric than anisohydric (Brzostek et al. 2014; Roman et al. 2015). *Acer saccharum* bare root saplings were purchased from Cold Stream Farm (Free Soil, MI), while the two other species were obtained from Valonia Nursery (Indiana DNR, Vallonia, IN). Tree saplings were planted in a greenhouse 6–7 months prior to the start of the experiment. All trees were 2–4 years in age at time of planting and ranged from 0.5 m to 1.5 m in height (see Supplemental Table 1 for dry biomass in all treatments and tissues). *Liriodendron tulipifera* saplings were planted in 20 cm × 46 cm (9.6 L volume) pots, whereas the other species were planted in 20 cm × 32 cm (7.6 L volume) pots. *L. tulipifera* saplings were considerably larger than the other species, and a larger pot size was used to keep the ratio of root to pot size consistent among species. Soils were a local...
mesic Typic Paleudalf cut with 50% coarse building sand by volume to facilitate improved drainage. The sand/soil mixture was homogenized with a soil mixer and sterilized via a steamer cart coupled to a steam aerator. Two small hyphal ingrowth bags (30 µm mesh) were installed ≈10 cm below the soil surface in each pot at the time of planting. All trees were planted during one month period in late winter and received one-time fertilizer amendment at leaf out of 200 ppm 20-20-20 Peters Liquid Soluble Fertilizer at the time of planting. All trees were watered as needed and maintained between 10 and 15% volumetric water content (VWC) until the start of the experiment.

Inoculation

Half of the trees were inoculated with one-time addition of 200 mL 1:4 soil:water slurry that was filtered through a 4 mm sieve to remove large soil particles and plant matter. This inoculation protocol ensured that an ecologically relevant density of microbes was added (Bever et al. 2009; Middleton and Bever 2012). Soils used to make the slurry were collected from beneath the canopies of the three species in a nearby forest (Griffy Woods, Bloomington, IN; 39°11′35″N, 86°30′07″W). Thus, three different slurries were used—one for each species. The other half of the trees received thrice-autoclaved slurry as a control (hereafter referred to as “uninoculated” trees).

Experimental drought

Following three-month “incubation” after the addition of the microbial inoculum, half of the pots were subjected to a four week experimental drought during mid-summer. In the drought treatment, watering was not immediately ceased; rather, watering frequency and volume were decreased over the first two weeks to simulate seasonal declines in soil moisture common to the region (Supplemental Fig. 1). Due to differences in water use and pot size, the watering frequency reduction was not equally applied across species. Rather, VWC was kept similar in all pots. An equal number of trees were designated as controls and maintained at around 50% water holding capacity for the duration of the study. VWC was measured every 2 days for all species using a HydroSense II moisture probe (Campbell Scientific, Logan, UT). During the course of the experiment, greenhouse daily high temperatures ranged from 22 to 31 °C and relative humidity was maintained around 50%.

Gas exchange and \(\Psi_L\) data collection

Measurements of assimilation (A) and stomatal conductance \((g_s)\) were recorded biweekly using a Li-6400 XT (Li-Cor Inc., Lincoln, NE) at the following chamber conditions: 400 µmol mol\(^{-1}\) CO\(_2\) concentration, 1000 µmol m\(^{-2}\) s\(^{-1}\) photosynthetic photon flux density, 25 °C leaf temperature, and flow rates set to 500 µmol s\(^{-1}\). VPD was kept between 1.7 and 2.3 kPa in the chamber at the time of each measurement. Each measurement was made in less than 2 min to avoid increases in chamber humidity. \(\Psi_L\) was measured weekly using a Model 610 Scholander-type pressure chamber (PMS Instrument Company, Corvallis, OR). One leaf from each tree was covered with aluminum foil for 15 min prior to measurement to allow equilibration of leaf water potential with xylem water potential. Gas exchange measurements were performed every 3 days on all trees \((n = 48)\), while a subset of trees \((n = 36)\) were sampled for \(\Psi_L\) five times throughout the experiment to minimize the disturbance caused by destructive sampling of leaves. All measurements were made in the mid-morning or early afternoon (9:00 am–1:00 pm).

Pulse-labeling

A custom-made chamber was designed to track the fate of recently fixed C through the plant-soil system. A transparent Tedlar bag (Plastic Film Enterprises, Royal Oak, MI) was fit over a PVC frame to make a rectangular 45.7 cm × 137.1 cm × 76.2 cm chamber that was open on the bottom. The chamber bag was equipped with an inflow valve, outflow valve, and a septum for \(^{13}\)CO\(_2\) injection. The bag was sealed to the PVC frame and weather stripping was applied to the bottom of the chamber to ensure a gas-tight seal with the baseplate. The baseplate was a 50.8 cm × 142.2 cm PVC plate 1.3 cm thick, with four slits cut on the long side to allow for insertion of tree stems. Once the chamber was placed on top of the baseplate and trees, the slits were sealed with wooden pieces and putty. A PVC riser system was also constructed that allowed for the chamber height to be adjustable. Two small, battery powered fans were installed on the baseplate to ensure rapid circulation of injected \(^{13}\)CO\(_2\). A tank of pure N\(_2\) (Airgas, Radnor, PA) was attached to one valve, and a Li-6262 CO\(_2\) analyzer (Li-Cor Inc., Lincoln, NE) was attached to the other.

Labeling occurred on sunny days between 11 am and 2 pm. Both valves were opened to allow N\(_2\) to flow into the chamber and chamber air to flow into the CO\(_2\) analyzer. The N\(_2\) tank was turned on until chamber CO\(_2\) concentration (measured via outflow gas) reached ≈300 µmol mol\(^{-1}\). The valves were then closed, and 95 mL of 99% \(^{13}\)CO\(_2\) (Cambridge Isotope Laboratories Inc., Tewksbury, MA) was injected, which increased CO\(_2\) concentration in the chamber to ≈500 µmol mol\(^{-1}\). These values were chosen to minimize physiological stresses from anomalously low or high CO\(_2\) concentration in the chamber during labeling. Trees were allowed to take up the label for 4 h.
Harvesting procedure

All trees were harvested eight hours after the cessation of labeling. This period was chosen so as to capture the peak of belowground $^{13}$C flux, as determined from time series data of $^{13}$C in soil respiration following the labeling (data not shown). After the eight hour period, trees were harvested from the pots with care taken not to disturb the root system. Biomass was immediately separated into leaf, stem, fine root (first and second order roots) and coarse root (third and higher root orders) pools. Fine and coarse roots were defined based on the absorptive/transport classification of McCormack et al. (2015). Roots were thoroughly washed, and all tissues were dried at 60 °C for 48 h. Following drying, tissues were weighed and a subset of each tissue was ground to a fine powder with a ball mill (SPEx Sample Prep, Metuchen, NJ). 1.25 mg of each ground tissue was packaged in tin capsules for isotopic and elemental analysis. Fine root subsamples from each tree were stained for mycorrhizal fungi using a modified Trypan blue staining method and visually assessed for colonization using light microscopy (Brundrett et al. 1996).

Isotopic analysis

The $\delta^{13}$C of plant tissue was determined at the Stable Isotope Research Facility (Dept. of Geological Sciences, Indiana University), using a Costech ECS 4010 Elemental Analyzer (Costech Analytical Technologies, Valencia, CA) as an inlet to a ThermoFinnigan DELTA plus XP isotope ratio mass spectrometer (Thermo Fischer Scientific, San Jose, CA). Elemental C and nitrogen (N) content were simultaneously determined using a thermal conductivity detector in the elemental analyzer. Carbon isotopic ratios of samples were converted to the Vienna Pee Dee Belemnite (VPDB) international scale by comparison to Acetanilide 1 and EDTA-1 which have been calibrated to the primary C isotopic standards NBS 19, L-SVEC, USGS40, and USGS41 using offline techniques (Schimmelmann et al. 2009). To quantify the incorporation of labeled $^{13}$C into various plant tissues, the following calculation for atom % was used:

\[
\text{atom}\% = \frac{100 \cdot 0.0111802 \cdot (\frac{\delta}{1000} + 1)}{1 + 0.0111802 \cdot (\frac{\delta}{1000} + 1)},
\]

where $\delta$ is the isotopic ratio of the tissue (%) and 0.0111802 is the standard value for the isotopic ratio of VPDB. Excess $^{13}$C for each tissue was then calculated, representing the total amount of $^{13}$C added by the pulse-labeling procedure:

\[
\text{excess} \, ^{13}\text{C} = \frac{\text{atom}\%_L - \text{atom}\%_{UL}}{100} \times B \times \frac{\%C}{100},
\]

where atom %$_L$ is the atom % in labeled tissues, atom %$_{UL}$ is the atom % of unlabeled tissues, $B$ is the dry weight biomass of the tissue in mg, and $\%C$ is the percentage of C in the tissue. Whole plant uptake of the $^{13}$C label was calculated as the sum of the excess $^{13}$C in all tissues:

\[
\text{total} \, ^{13}\text{C} = \text{excess} \, ^{13}\text{C}_{\text{leaf}} + \text{excess} \, ^{13}\text{C}_{\text{stem}} + \text{excess} \, ^{13}\text{C}_{\text{fine root}} + \text{excess} \, ^{13}\text{C}_{\text{coarse root}}.
\]

Proportional allocation of $^{13}$C for each tree was calculated as a ratio of excess $^{13}$C in one tissue (excess $^{13}$C$_{\text{tissue}}$) to the excess $^{13}$C in the whole tree (excess $^{13}$C$_{\text{tree}}$):

\[
\text{proportional} \, ^{13}\text{C} \, \text{allocation} = \frac{\text{excess} \, ^{13}\text{C}_{\text{tissue}}}{\text{total} \, ^{13}\text{C}},
\]

Data analysis

Data to compare treatment effects were binned into the last two weeks of the experimental period as this was the time in which VWC, $A$, $g_s$, and $\Psi_L$ diverged from control treatments, and therefore represented the maximum effect of the drought treatment. Results were insensitive to alterations in this binning method by ±1 week. To quantify the effects of drought on individual trees, we calculated the average $A$, $g_s$, and $\Psi_L$ during that time period. Differences among species and treatment groups in response to drought were analyzed using ANOVA, with two-way interaction effects and Tukey’s HSD for pairwise comparisons as needed. Among species differences in gas exchange and $\Psi_L$ (e.g., Supplemental Fig. 2) were determined via ANCOVA (with Tukey’s HSD to discern differences in slope) with VWC as the covariate. All statistical analyses were conducted using R 3.0.2 (cran.us.r-project.org).

Results

Pot conditions

At the start of the drought treatment, all pots were relatively well-watered (≈13% VWC, Supplemental Fig. 1). Once the drought treatment began, however, control and drought treatment VWC quickly diverged, with drought pots experiencing a ≈66% decline in VWC within 2 weeks compared to starting conditions. This level of soil moisture (≈5% VWC) was maintained for the remaining 2 weeks of the study. Control pots remained well-watered throughout the course of the experiment despite minor fluctuations between 10 and 15% VWC.

There was no appreciable evidence of any mycorrhizal structures in stained root subsamples. In addition, no hyphae were visible in the fungal ingrowth bags included
in our pots. This indicates that the observed effects of inoculation were most likely due to free-living soil microbes, not mycorrhizal fungi.

Effects of inoculation

Microbial inoculation primarily increased $g_s$ and $\Psi_L$, although there were minimal alterations to $A$ in one case (Fig. 1). In *A. saccharum*, inoculation increased $g_s$ and $\Psi_L$ in the drought treatment. In *L. tulipifera*, inoculation increased $g_s$ and $\Psi_L$ in drought-stressed trees and was the only species to alter assimilation rates in response to inoculation, as the presence of soil microbes slightly increased $A$ in drought-stressed trees. *Q. alba* was entirely insensitive to microbial inoculation.

Furthermore, there were significant drought by inoculation interactions for *A. saccharum* $\Psi_L$, and for *L. tulipifera* $A$ and $\Psi_L$, suggesting that the effects of inoculation were exacerbated in drought-stressed trees. Specifically, inoculation significantly increased *L. tulipifera* $A$ and *A. saccharum* and *L. tulipifera* $\Psi_L$ in the drought treatment, while inoculation effects in the control treatment were either small or nonexistent (Table 1).

Inoculation with native soil microbes also lessened the extent to which $\Psi_L$ responded to VWC in both *A. saccharum* and *L. tulipifera* (Fig. 2, $p \leq 0.05$). However, microbial inoculation failed to buffer *Q. alba* against declines in $\Psi_L$.

Species differences in gas exchange and $\Psi_L$ dynamics

Over the 5 weeks of drought stress, significant reductions in $A$, $g_s$, and $\Psi_L$ occurred for *L. tulipifera* and *Q. alba* as soil dried (Supplemental Fig. 2, $p \leq 0.05$). In *A. saccharum*, however, only $\Psi_L$ significantly declined with VWC, while $A$ and $g_s$ were insensitive to changing soil water content. In addition to the insensitivity of these physiological
Table 1  Gas exchange and leaf water potential data for all species and treatments averaged over the last two weeks of the experimental period

<table>
<thead>
<tr>
<th>Species</th>
<th>Drought treatment</th>
<th>Inoculation treatment</th>
<th>$\text{Mean } A \pm \text{SE } \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$</th>
<th>$p$ value</th>
<th>Effect size (%)</th>
<th>$\text{D × I } p$ value</th>
<th>$\text{Mean } g_s \pm \text{SE } \text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$</th>
<th>$p$ value</th>
<th>Effect size (%)</th>
<th>$\text{D × I } p$ value</th>
<th>$\text{Mean } \Psi_L \pm \text{SE } \text{MPa}$</th>
<th>$p$ value</th>
<th>Effect size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. saccharum</td>
<td>Control</td>
<td>Uninoculated</td>
<td>2.43 ± 0.52</td>
<td>0.039 ± 0.0084</td>
<td></td>
<td></td>
<td>−0.33 ± 0.02</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inoculated</td>
<td>3.56 ± 0.64</td>
<td>0.096 ± 0.0158</td>
<td>0.0042</td>
<td>147.9</td>
<td>−0.33 ± 0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−0.90 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drought stress</td>
<td>Uninoculated</td>
<td>2.42 ± 0.52</td>
<td>0.032 ± 0.0083</td>
<td></td>
<td></td>
<td>−0.90 ± 0.05</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Inoculated</td>
<td>2.53 ± 0.70</td>
<td>0.070 ± 0.0145</td>
<td>0.03811</td>
<td>117.4</td>
<td>0.4669</td>
<td>−0.53 ± 0.08</td>
<td>0.0041</td>
<td>−40.7</td>
<td>0.0020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. tulipifera</td>
<td>Control</td>
<td>Uninoculated</td>
<td>4.00 ± 0.29</td>
<td>0.094 ± 0.0180</td>
<td></td>
<td></td>
<td>−0.63 ± 0.03</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Inoculated</td>
<td>3.04 ± 0.41</td>
<td>0.110 ± 0.0132</td>
<td>0.4712</td>
<td>17.1</td>
<td>0.9889</td>
<td>−0.53 ± 0.02</td>
<td>0.0296</td>
<td>−15.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drought stress</td>
<td>Uninoculated</td>
<td>−0.13 ± 0.23</td>
<td>0.009 ± 0.0034</td>
<td></td>
<td></td>
<td>−1.90 ± 0.14</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Inoculated</td>
<td>0.75 ± 0.30</td>
<td>0.077 ± 0.0218</td>
<td>0.0230</td>
<td>735.6</td>
<td>0.1461</td>
<td>−1.08 ± 0.08</td>
<td>0.0005</td>
<td>−42.9</td>
<td>0.0003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q. alba</td>
<td>Control</td>
<td>Uninoculated</td>
<td>4.54 ± 0.41</td>
<td>0.077 ± 0.0078</td>
<td></td>
<td></td>
<td>−0.37 ± 0.05</td>
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<tr>
<td></td>
<td></td>
<td>Inoculated</td>
<td>4.96 ± 0.79</td>
<td>0.077 ± 0.0136</td>
<td>0.9889</td>
<td>−0.3</td>
<td>−0.35 ± 0.04</td>
<td>0.804</td>
<td>−4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drought stress</td>
<td>Uninoculated</td>
<td>1.26 ± 0.34</td>
<td>0.014 ± 0.0011</td>
<td></td>
<td></td>
<td>−0.98 ± 0.16</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inoculated</td>
<td>0.70 ± 0.44</td>
<td>0.024 ± 0.0066</td>
<td>0.2186</td>
<td>76.9</td>
<td>0.5769</td>
<td>−0.88 ± 0.14</td>
<td>0.6459</td>
<td>−10.2</td>
<td>0.7101</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$p$ values and effect sizes represent relationships between inoculated and uninoculated trees within the same drought stress treatment, while the $p$ values of the drought × inoculation interaction within a species are reported in their own column (titled, “D × I $p$ value”)

Significant differences ($\alpha = 0.05$) are in bold ($n = 4$ for each species × treatment combination)
parameters in *A. saccharum* to drought stress, declines of these parameters in *Q. alba* (considered a drought-tolerant species) were statistically indistinguishable from those in *L. tulipifera* (usually regarded as a drought-sensitive species).

### 13C allocation and %N

Few shifts in proportional 13C allocation were observed in response to drought, inoculation, or the interaction between the two (Table 2, *p* ≤ 0.05). Inoculation did not significantly alter 13C allocation or %N in any tissue or treatment over the course of the drought (data not shown) while drought stress decreased proportional 13C allocation to *L. tulipifera* fine roots and increased proportional 13C allocation to *Q. alba* stems. Drought stress decreased tissue %N for *A. saccharum* leaves, *L. tulipifera* leaves, and in every *Q. alba* tissue (Table 2, *p* ≤ 0.05).

### Discussion

Given emerging evidence of microbial impacts on plant drought tolerance (Yang et al. 2009), we investigated how the presence or absence of soil microbes would impact tree physiological responses to drought stress. Consistent with our hypotheses, our results suggest that microbes have the capacity to alter tree physiology in isohydric trees during drought stress. However, microbial inoculation did not buffer assimilation from drought stress, instead causing increases in *g*s and ΨL. The two species that were most altered by soil microbes during drought, *A. saccharum* and *L. tulipifera*, also exhibited a more conservative hydraulic strategy (i.e., became more isohydric) in the presence of soil microbes. While the mechanisms underlying these effects are unclear, our results are consequential for diagnosing tree responses to drought stress as they indicate that hydraulic strategies can be altered by the presence of microbes. Thus, understanding how climate change will impact forests may depend not only on the physiological responses of trees, but also on how such changes impact soil microbial communities and their influence on adaptive plant responses (Friesen et al. 2011; Lau and Lennon 2012).

### Microbial effects on drought sensitivity

Numerous studies have reported that rhizosphere microbes can alleviate plant drought stress by enhancing photosynthetic capacity or growth (see Aroca and Ruiz-Lozano 2009 for a review). However, few studies have explored the effects of soil microbial communities on plant hydraulic and leaf-level gas exchange (Rolli et al. 2015; Sandhya et al. 2010). In this study, microbial inoculation caused widespread changes to tree physiological parameters that differed based on species and drought treatment—*A. saccharum* and *L. tulipifera* were highly sensitive to inoculation, whereas *Q. alba* was not. While previous work has shown that the effects of microbes on tree physiology may be contingent on species’ hydraulic dynamics (i.e., isohydry or anisohydry; Rincón et al. 2008), the microbial effects observed in this study did not intuitively track hydraulic strategies of the trees. Rincón et al. (2008), for example, found that the addition of soil microbes buffered *g*s and ΨL against drought stress in the anisohydric tree species only, whereas isohydric tree species were mostly unaffected. A possible explanation for this discrepancy relates to the type of microbial inoculum used. In the Rincón et al. (2008) study, trees were inoculated with one specific
Table 2 Isotopic and %N data for all species and treatments

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>Mean proportional allocation ± SE</th>
<th>p value</th>
<th>Effect size (%)</th>
<th>Mean %N ± SE</th>
<th>p value</th>
<th>Effect size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%13C in tissue / total %13C assimilated</td>
<td></td>
<td></td>
<td>Pre-drought</td>
<td>Post-drought</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre-drought</td>
<td>Post-drought</td>
<td></td>
<td>%N</td>
<td>Pre-drought</td>
<td>Post-drought</td>
</tr>
<tr>
<td>A. saccharum</td>
<td>Leaf</td>
<td>42.5 ± 2.7</td>
<td>47.5 ± 4.7</td>
<td>0.1776</td>
<td>10.5</td>
<td>2.23 ± 0.07</td>
<td>1.77 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>27.9 ± 2.2</td>
<td>22.0 ± 2.9</td>
<td>0.6866</td>
<td>-27.0</td>
<td>0.67 ± 0.03</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Coarse root</td>
<td>22.2 ± 2.1</td>
<td>21.4 ± 3.0</td>
<td>0.8298</td>
<td>-3.2</td>
<td>0.80 ± 0.06</td>
<td>0.87 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>Fine root</td>
<td>9.4 ± 1.3</td>
<td>8.9 ± 0.4</td>
<td>0.1898</td>
<td>-5.3</td>
<td>1.28 ± 0.08</td>
<td>1.21 ± 0.04</td>
</tr>
<tr>
<td>L. tulipifera</td>
<td>Leaf</td>
<td>42.8 ± 3.4</td>
<td>56.3 ± 4.8</td>
<td>0.1254</td>
<td>24.0</td>
<td>1.98 ± 0.09</td>
<td>1.50 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>28.2 ± 2.2</td>
<td>23.2 ± 3.4</td>
<td>0.3486</td>
<td>-21.8</td>
<td>0.67 ± 0.06</td>
<td>0.76 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Coarse root</td>
<td>16.4 ± 1.9</td>
<td>12.8 ± 3.7</td>
<td>0.3807</td>
<td>-28.1</td>
<td>1.05 ± 0.08</td>
<td>1.21 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Fine root</td>
<td>12.6 ± 1.2</td>
<td>7.7 ± 2.3</td>
<td>0.0117</td>
<td>-63.2</td>
<td>1.27 ± 0.14</td>
<td>1.38 ± 0.20</td>
</tr>
<tr>
<td>Q. alba</td>
<td>Leaf</td>
<td>52.6 ± 4.5</td>
<td>54.9 ± 2.2</td>
<td>0.2843</td>
<td>4.1</td>
<td>2.38 ± 0.11</td>
<td>2.07 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>13.9 ± 2.0</td>
<td>20.0 ± 1.8</td>
<td>0.0312</td>
<td>30.4</td>
<td>0.70 ± 0.03</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Coarse root</td>
<td>26.4 ± 2.7</td>
<td>21.3 ± 2.6</td>
<td>0.8815</td>
<td>-23.5</td>
<td>1.32 ± 0.07</td>
<td>0.77 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Fine root</td>
<td>7.1 ± 1.5</td>
<td>3.8 ± 0.5</td>
<td>0.5489</td>
<td>-88.7</td>
<td>1.28 ± 0.06</td>
<td>0.86 ± 0.04</td>
</tr>
</tbody>
</table>

Inoculation had no significant effect on either %13C allocation or %N so reported values combine both inoculated and uninoculated treatments

p values and effect sizes represent relationships between pre- and post-drought pulse-labeling data

Significant differences (α = 0.05) are in bold (n = 8 for each species × tissue combination)

rhizobacterium, whereas in our study we used slurry that contained a diverse assemblage of bacteria and fungi. While a field-derived microbial inoculum allowed us to evaluate plant responses to drought stress under more ecologically relevant conditions, the mechanisms underlying these patterns are difficult to disentangle, given (1) we did not characterize the microbial community or activity and (2) that the integrated responses of trees likely reflected multiple microbial effects. Due to the limitations of common microbial biomass measurements in a drought treatment (i.e., chloroform fumigation does not differentiate between live and dead cells) we chose not to measure microbial biomass, although techniques that can accurately assess microbial growth (such as substrate-induced respiration) could be used in future studies to quantify how microbial community dynamics during drought impact plant function.

Although we did not determine the mechanism by which the microbes altered plant responses to drought stress, we can rule out several putative mechanisms. First, soil microbes can alleviate the nutritional deficiencies that often accompany drought stress (Chapin 1991), as microbe-driven nutrient cycling may reduce the impacts of diffusive constraints on nutrient acquisition. In our study, we found no evidence that microbes affected tree nutritional status, as N concentration was unaffected by the inoculum in both aboveground and belowground tissues (data not shown). We can also reject the hypothesis that plant responses to drought stress were ameliorated through interactions with mycorrhizal fungi. Mycorrhizal fungi are known to improve drought tolerance by increasing the absorptive surface area (Augé 2001), promoting osmolyte synthesis (Rapparini and Peñuelas 2014) and by regulating aquaporins in roots (Lehto and Zwiazek 2011). In this study, the lack of mycorrhizal structures present on roots and the limited recovery of %13C in the hyphal ingrowth bags make it unlikely that mycorrhizal fungi affected plant water relations. A third possibility is that the presence of rhizobacteria altered root allocation or architecture—changes that could enhance water uptake (Mantelin and Touraine 2004). However, we found no changes in dynamic %13C allocation or biomass partitioning among root orders in inoculated versus uninoculated trees exposed to drought stress. Thus, none of the above mechanisms likely accounted for the observed microbial effects.

One possible explanation for the microbial effects relates to microbial signaling cascades that can alter root physiology and stomatal behavior. Under drought conditions, gs is strongly mediated by signaling compounds (Joseph and Phillips 2003; Loewenstein and Pallardy 1998; Matiru and Dakora 2005) and many bacterial taxa have been shown to produce compounds that can trigger an ABA response from roots to leaves (Yang et al. 2009). And while some microbes can enhance ABA signaling in plants (Dodd et al. 2010), others suppress the response, ultimately opening gs (Fan et al. 2015). Moreover, ABA can signal aquaporin expression in root cells, enhancing
physiological changes that can increase both $g_s$ and $\Psi_L$. In addition, there are numerous other bacterially produced phytohormones (e.g., cytokinins, indoleacetic acid, ethylene) that are also known to play a role in plant drought responses (Forni et al. 2016). As $g_s$ is a primary control on $\Psi_L$ (Ewers et al. 2007; Tardieu et al. 1996), a combination of microbial signaling and stomatal responses to those signals may explain, in part, the observed microbial effects on tree physiology. Given that the microbial effects were detectable within only a few weeks after one-time inoculation, our results suggest that plant water relations may be far more sensitive to the presence of microbes than has been appreciated. Whether plants show greater sensitivity to microbes that they are adapted to associate with (Lau and Lennon 2012), or whether their responses depend on the presence of select taxa or specific microbial assemblages, would be a fruitful area for future research.

In contrast to other studies, we did not find any evidence that trees shifted their allocation patterns in response to inoculation. This result is surprising given that belowground allocation is often increased during drought to support rhizosphere biota and water uptake (Jones et al. 2004; Sanaullah et al. 2012). However, given that we did not measure $^{13}$C fluxes to exudation or microbial biomass, it is possible that belowground allocation did increase with inoculation but was promptly released from roots to soil. Moreover, we observed no shifts in $^{13}$C allocation due to drought in general. While increasing C belowground (absolute or relative) in response to drought stress is a well-documented trend (Ruehr et al. 2009; Zang et al. 2014), our experimental setup could have hindered belowground allocation if rooting densities in the pots were already high. While it is possible that the larger pot size of the *L. tulipifera* saplings affected belowground C allocation, this seems unlikely given that none of the trees appeared to be pot-bound at the end of the experiment and all plots had similar ratios of root mass to pot size. In addition, while our chase period was optimized for maximum short-term belowground C allocation, the time frame for our chase period was relatively short compared to other pulse-labeling studies of drought-stressed trees (Ruehr et al. 2009; Zang et al. 2014). Nevertheless, if C allocation shifts were ongoing during our chase period, their effects were presumably small in magnitude.

**Consequences for defining hydraulic strategies**

The isohydr-d-anisohydr continuum is an emerging framework for understanding the physiological responses of trees to drought. To date, these distinctive patterns of stomatal behavior have been viewed as immutable traits of a species, independent from biotic or abiotic context. Our results challenge this view. In our study, species commonly thought of as isohydric (*L. tulipifera* and *A. saccharum*) became more isohydric with the addition of native soil microbes, indicating that hydraulic strategies may be more plastic than previously thought. Those microbes also changed $g_s$ and $\Psi_L$ without any changes to $A$ also suggests that predictable relationships between leaf hydraulics and C gain (McDowell et al. 2008; McDowell 2011) may need to be revisited. Thus, microbially triggered changes in stomatal closure may have limited impacts on C assimilation.

Our results indicate that in addition to soil environment, ontogeny may play a large role in determining tree responses to drought stress. The physiological responses of our saplings to soil drying differed from those reported for mature trees. *A. saccharum* was most resistant to declining soil water in our experiment, showing little change in $A$ and $g_s$ over the course of the drought manipulation. In contrast, *L. tulipifera* and *Q. alba* showed significant declines in all physiological parameters in response to drought stress and the magnitude of these declines were statistically indistinguishable between the two species. These observations are contrary to responses observed with mature trees, which indicate that *A. saccharum* and *L. tulipifera* are drought-sensitive, isohydric species, while *Q. alba* is a drought-tolerant, anisohydric species (Brzostek et al. 2014; Hoffmann et al. 2011; Roman et al. 2015). It has been suggested that environmental and ontogenetic factors play a large role in determining tree responses to drought stress (Cavender-Bares and Bazzaz 2000; Mediavilla and Escudero 2004). These factors, such as life stage, rooting depth, root architecture, the development of the hydraulic system, and biomass allocation patterns—all differ between greenhouse and field contexts. If these factors do alter a tree’s isohydric or anisohydric behavior, then a revised perspective on hydraulic strategies may be necessary. Understanding which environmental factors and biotic agents influence tree hydraulics, and the mechanisms behind these effects, are a profitable avenue of future research.

**Conclusions**

Our results call for a more context-dependent perspective on the isohydr-anisohydr continuum and suggest that investigations of tree physiology and hydraulic function should consider the role of soil microbes. While a mesocosm experiment may not accurately reflect the plant–microbe interactions occurring in a mature forest, we have shown that microbes can buffer trees against the negative impacts of drought. In addition, our results indicate that the hydraulic strategies of trees may be partially contingent upon their microbial community, highlighting the importance of understanding the coupled responses of trees and
microbes to environmental stress. Given the large impacts that increasing drought stress (Dai 2013) and species composition shifts (Vose et al. 2012) will have on forests the coming decades, understanding the biotic factors that influence a tree species’ response to drought will be crucial for predicting whole forest dynamics.

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Author contribution statement SAK and RPP conceived and designed the experiment. SAK performed the experiment and data analysis. SAK and RPP wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References


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Zang U, Goisser M, Grams TEE et al (2014) Fate of recently fixed carbon in European beech (Fagus sylvatica) saplings during drought and subsequent recovery. Tree Physiol 34:29–38