Vegetation-zonation patterns across a temperate mountain cloud forest ecotone are not explained by variation in hydraulic functioning or water relations

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Many studies have demonstrated linkages between the occurrence of fog and ecophysiological functioning in cloud forests, but few have investigated hydraulic functioning as a determining factor that explains sharp changes in vegetation. The objective of this study was to compare the plant water status during cloud-immersed and non-immersed conditions and hydraulic vulnerability in branches and roots of species across a temperate, mountain fog ecotone. Because cloud forests are often dark, cool and very moist, we expected cloud forest species to have less drought-tolerant characteristics (i.e., lower $P_{e}$ and $P_{50}$—the pressures required to induce a 12 and 50% loss in hydraulic conductivity, respectively) relative to non-cloud forest species in adjacent (lower elevation) forests. Additionally, due to the ability of cloud forest species to absorb cloud-fog water, we predicted greater improvements in hydraulic functioning during fog in cloud forest species relative to non-cloud forest species. Across the cloud forest ecotone, most species measured were very resistant to losses in conductivity with branch $P_{50}$ values from $-4.5$ to $-6.0$ MPa, hydraulic safety margins ($\Psi_{min} - P_{50}$) $>1.5$ MPa and low calculated hydraulic conductivity losses. Roots had greater vulnerabilities, with $P_{50}$ values ranging from $-1.4$ to $-2.5$ MPa, leading to greater predicted losses in conductivity ($\sim20\%$). Calculated values suggested strong losses of midday leaf hydraulic conductance in three of the four species, supporting the hydraulic segmentation hypothesis. In both cloud forest and hardwood species, $\Psi_{s}$ were greater on foggy days than sunny days, demonstrating the importance of fog periods to plant water balance across fog regimes. Thus, frequent fog did not result in systemic changes in hydraulic functioning or vulnerability to embolism across our temperate cloud forest ecotone. Finally, roots functioned with lower hydraulic conductivity than branches, suggesting that they may serve as more sensitive indicators of hydraulic functioning in these mesic, foggy ecosystems.

**Keywords:** embolism, fog, hydraulic conductivity, southern Appalachians, vulnerability.

Introduction

Cloud forest ecosystems are characterized by frequent fog, and occur in both tropical and temperate regions around the world (Weathers 1999). Because of the distinct climate conditions, these ecosystems often harbor unique and endemic flora and fauna rarely found outside of the cloud forest environment. While many researchers have examined the ecology and community structure in cloud forests, comparisons of the explanatory processes that lead to sharp (often discontinuous) vegetation-zonation across cloud forest ecotones are few. The significant changes in microclimate include lower air and leaf temperatures, higher ambient humidity levels and a significant moisture input coming from intercepted fog water (Gu et al. 2002, Letts and Mulligan 2005, Min 2005) in cloud forests compared with lower-elevation, non-cloud forest environments. This sharp alteration in microclimate can exert strong environmental influence on...
physiological functioning in plant species by reducing transpiration, increasing water-use efficiency, reducing variation in leaf temperatures, enhancing stomatal opening and, ultimately, improving carbon gain (Dawson 1998, Johnson and Smith 2005, Reinhardt and Smith 2008, Berry et al. 2014). However, little is known about the differential impacts of cloud fog on water balance and transport in cloud forest compared with non-cloud forest species, or how this ecophysiological variation explains observed vegetation-zonation patterns, particularly for temperate cloud forest communities.

One ecophysiological factor that could explain vegetation-zonation patterns across cloud forest ecotones is variation in hydraulic conductivity and resistance to hydraulic dysfunction of plant organs. Maintaining a continuous water pathway from roots to leaves is critical for preserving a favorable water status leading to greater carbon capture and the avoidance of reduced growth and mortality. Reductions in hydraulic conductivity (k) likely occur in many plant species through xylem embolism (e.g., Tyree and Ewers 1989). There is a wide variation in how species manage hydraulic conductivity, with species operating along a spectrum from ‘safe’ (prevention of embolism) to ‘risky’ (typically experience some amount of embolism). In cloud forest zones, greater water inputs conceivably could result in plant hydraulic systems more representative of mesic environments, such as greater vulnerability to embolism and greater losses in hydraulic conductivity during dry periods (e.g., droughts). Indeed, mortality associated with major droughts in 2005 and 2010 was attributed to embolism in Amazon rain forest tree species (Doughty et al. 2015).

In addition, each component of the hydraulic pathway from roots to leaves often operates with a different hydraulic vulnerability to embolism. More distal portions (roots and leaves) are typically more vulnerable, acting as ‘safety valves’ (Tyree and Ewers 1991, Alder et al. 1996, Domec et al. 2010). This strategy, known as the hydraulic vulnerability segmentation hypothesis (Tyree and Ewers 1991), seems to occur, to varying degrees, across the plant kingdom. Conifers, in particular, seem to operate with larger root-to-branch and leaf-to-branch safety margins, and experience minimal embolism in branches and more frequent embolism in roots. Many angiosperms, on the other hand, likely experience branch embolism on a daily basis (Meinzer et al. 2008, Johnson et al. 2012) with narrow or even negative root-to-branch and leaf-to-branch safety margins (Johnson et al. 2012). In the context of our study, it might be expected that tree species below cloud forest zones (occupying relatively drier and warmer microsites) are more hydraulically segmented compared with cloud forest tree species, in order to avoid irreparable damage and mortality during dry and sunny periods. However, the dynamics between phylogeny (conifer vs angiosperm) and environment (mesic vs xeric) have yet to be studied in this context. Increasingly, research is demonstrating linkages between hydraulic functioning, physiological water management strategies and species distributions, which provides an excellent framework for understanding sharp vegetation ecotones, such as the transition to cloud forest zones in this study.

Fog in the southern Appalachian Mountains occurs, generally, above 1500 m above sea level (a.s.l.) on 60–75% of summer days (Berry and Smith 2012) and is considered a major contributor to the persistence of the threatened spruce–fir forests (Cogbill and White 1991). In this ecosystem, fog has been shown to improve daily carbon gain and water status in local cloud forest species by reducing transpiration, allowing greater daily stomatal conductance and by acting as an important water subsidy through foliar uptake (Reinhardt and Smith 2008, Berry and Smith 2013, 2014). In these mountains, there is a sharp vegetation ecotone between cloud forest (spruce–fir forests; red spruce—Picea rubens Sarg., Fraser fir—Abies fraseri Pursh) and Catawba rhododendron—Rhododendron catawbiense Michx.) and non-cloud forest (northern hardwood; sugar maple—Acer saccharum Marsh, yellow birch—Betula alleghaniensis Brit and American beech—Fagus grandifolia Ehrh.), that co-occurs with the average altitude of cloud bases (~1500 m). The existence of this boundary and the sharp change in climate from one of high rainfall, milder temperatures and large moisture inputs from fog to a dryer and warmer climate may lead to alternative physiological strategies to maintain hydraulic function. Species adapted to wetter climates (such as cloud forests) may be more sensitive to drops in vapor pressure deficit (VPD) on dry, sunny days and thus more vulnerable to embolism during these periods. In tropical montane cloud forests, Metrosideros polymorpha Gaudich. grown in fog environments were more susceptible to xylem cavitation than non-fog environment trees (Santiago et al. 2000). Additionally, Drimys brasiliensis Miers, a common tropical cloud forest species in Brazil, operates with a very narrow hydraulic safety margin suggesting frequent loss of hydraulic conductivity (Oliveira et al. 2014). In contrast, the temperate chaparral species Arctostaphylos crustacea Eastw. did not show variation in hydraulic traits across a fog ecotone leading to the suggestion that individuals living in the coastal fog environment were hydraulically ‘over built’ (Jacobsen and Pratt 2013). Thus an understanding of how cloud fog affects hydraulic functioning and how vulnerable cloud forest species are to changing climate is far from being resolved and requires further examination.

The objectives of this study were to quantify and compare hydraulic performance along with diurnal variation in leaf and branch water status in six tree species growing across a southeastern Appalachian cloud forest ecotone. This sharp ecotone results in a transition from predominantly broadleaf, deciduous angiosperms to gymnosperms and evergreen angiosperms. Despite this possibly confounding phylogenetic constraint (which our experimental design did not directly address), we aimed to determine whether plant water relations and hydraulic functioning were explanatory factors driving this sharp change in species
ranges, which co-occurs with sharp changes in hydroclimate. We measured branch and root vulnerability to embolism and water potential (Ψ) values for branches and leaves on clear (sunny) and foggy days for the three dominant species in the cloud forest zone and three dominant non-cloud forest species below the cloud forest zone from June to August in 2014. Because of the distinct vegetation ecotone that co-occurs with strong gradients in cloud-fog occurrence across this boundary, we expected a wide variation in hydraulic functioning in the tree species across this ecotone. We hypothesized that water status in cloud forest species (Picea, Abies, Rhododendron) would be improved on foggy days more so than in non-cloud forest species (Betula, Acer, Fagus) because these cloud forest species have been shown to absorb cloud-fog water directly through their leaves (Berry and Smith 2014). We also expected that species in the cloud forest zone would have lower $P_2$ and $P_{50}$ values (pressure required to induce a 12 and 50% loss in hydraulic conductivity, respectively) as these species experience less negative daily Ψ due to the cooler and more-moist environmental conditions provided by frequent fog occurrence. For species growing below the cloud forest zone, we expected more drought-tolerant characteristics such as greater embolism resistance and more negative Ψ during periods of greater atmospheric water demand (sunny vs foggy days).

Materials and methods

Field sites and study species

Field work was conducted at Roan Mountain (36°6′N, 82°7′W) and Grandfather Mountain (36°5′N, 81°50′W), NC, USA. These two nearby sites both harbor spruce–fir cloud forests on the peaks shifting to northern hardwood forests around 1600 m elevation in this specific zone. Southern Appalachian spruce–fir ecosystems are uniquely different from their more northern counterparts in that they experience greater frequency of fog and cloud cover, have greater annual precipitation (can exceed 2000 mm year$^{-1}$) and warmer temperatures (Reinhardt and Smith 2008, Berry and Smith 2012). Sites were established that had stand structure representative of both spruce–fir forests (higher elevation—1870 m a.s.l.) and northern hardwood forests (lower elevation—1578 m a.s.l.), and did not seem to have significant damage from pests (such as Balsam wooly adelgid in the spruce–fir zone). The three most dominant woody species from these communities were examined. In spruce–fir these were the canopy species $P$. rubens (red spruce) and A. fraseri (Fraser fir—endemic) and the shrub $R$. catabiens. These three species combined often make up over 85% of the woody basal area within these communities (Nicholas et al. 1992). In northern hardwood (below cloud base) zones, three representative species were chosen as common northern hardwood species that rarely occur in the spruce–fir zone: A. saccharum (sugar maple), B. alleghaniensis (yellow birch) and $F$. grandifolia (American beech).

The specific microclimate for 2014 within the spruce–fir zone at Roan Mountain (1875 m a.s.l.) had an average annual temperature of 5.28 °C with 1567 mm of rainfall (L. Baker Perry, unpublished data). The climate during the study period May–August 2014 was characterized by mild temperatures (average 12.6 °C) and consistent rainfall throughout the period (544 mm May–August). Data for temperature and rainfall from two nearby mountain peaks at similar elevations as the lower-elevation site (Sugar Mountain 1585 m a.s.l., Beech Mountain 1532 m a.s.l.) demonstrate the change in temperature and rainfall with elevation. During the same May–August period in 2014, the average temperatures were 14.5 and 15.3 °C, with 253 and 365 mm of rainfall at Sugar and Beech Mountain, respectively (www.averyweather.com). These values are warmer and with less rainfall. There is also strong, historical and recent evidence that the amount of rainfall and cloud immersion decline with elevation within the region (Smathers 1982, Berry and Smith 2012, 2013). Smathers (1982) demonstrated greater fog interception within the spruce–fir zone while Berry and Smith (2012) found warmer temperatures and greater VPD at a lower-elevation spruce–fir site.

Branch and root hydraulic conductivity and vulnerability

Branches and roots ∼30 cm long and ∼10 mm in diameter were collected (July–August 2013, 2014) in the field, bagged and kept in a cooler for transport back to the lab. Branches were collected by selecting mid-canopy, sun-exposed shoots while roots were harvested by tracing from the trunk base to a root of a similar diameter. More specifically, we took distal portions of branches, and removed ∼15 cm from the distal end to obtain segments that were uniform in diameter (∼10 mm). All samples collected were between 6 and 9 years old. Segments were cut under water to 20–25 cm length and tested for open vessels by following the air injection method of Ewers and Fisher (1989), which involves submerging one end of the segment and applying air pressure on the other end. If open vessels existed (through the observation of air bubbles in the submerged end), then the segments were discarded. If there were no open vessels, then the segments were kept and embolisms flushed with filtered, distilled water at pH = 2 in a vacuum chamber overnight. Segments were recut underwater, and maximum hydraulic conductivity was measured using a hydrostatic pressure head (∼50 cm) to induce flow through the branch or root segments. Tubing was attached to the distal end of the segment and the time it took for flow to reach gradations on a pipette was used to calculate volume flow rate. Hydraulic conductivity ($k_h$) was then calculated by dividing the volume flow rate of water by the hydrostatic pressure gradient and xylem area-specific conductivity ($k_s$) was calculated by dividing $k_h$ by the cross-sectional xylem area.

Vulnerability curves were developed using the air injection method (Sperry and Salienstra 1994). Segments are pressurized using a pressure sleeve connected to a Scholander-type

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pressure chamber with external chamber attachment (Model 1505D-EXP, PMS Instrument Company, Albany, OR, USA). After measuring initial conductivity ($k_{h,max}$), segments were placed in the pressure sleeve and pressurized to either 0.5 MPa (roots) or 1.0 MPa (branches) for 2 min. Segments were removed from the pressure sleeve and $k_{h}$ was remeasured. Pressurization was increased progressively by 0.5 MPa (roots) or 1.0 MPa increments (branches) until there was at least 85% loss from initial conductivity. These measurements were used to calculate the percentage loss in hydraulic conductivity (PLC) using the following equation:

$$\text{PLC} = 100 \times \left(1 - \frac{k_h}{k_{h,max}}\right)$$

(1)

Vulnerability curves were constructed for branches and roots by plotting the PLC values (%) by the pressure applied (in MPa). For each hydraulic vulnerability curve, the $P_{50}$ and $P_e$ values were calculated as the amount of pressure necessary to induce a 50 and 12% loss of hydraulic conductivity, respectively. While the physiological meaning of these values can vary with species, they are useful parameters for assessing the relative susceptibility of plant organs to embolism.

**Leaf and branch water potentials and leaf hydraulic conductivity**

To quantify the seasonal variation in diurnal patterns of plant water status, native embolism, and $k_{h,\text{sat}}$ in situ, branch and leaf $\Psi$ were measured on one clear and one foggy day in June, July and August of 2014, with five replicates per species. These were conducted using a pressure chamber to immediately measure the pressure necessary to force water from xylem on individual leaves (broadleaves) or terminal shoots (conifers). Leaf $\Psi$ were measured at 08:00, 11:00, 14:00 and 17:00 h, while branch $\Psi$ was measured only at 14:00 h to estimate $\Psi_{\text{min}}$ (the minimum water potential experienced during the day). Dis-equilibrium can exist between branch and leaf water potentials and thus shoots were bagged and covered using plastic bags and aluminum foil before dawn to allow equilibration by midday (Berry et al. 2012). While microclimate at each site was not measured, previous research has demonstrated reduced solar radiation, VPD and temperature leading to reduced transpiration rates during fog events (Berry and Smith 2012).

To understand maximum losses to hydraulic functioning of leaves, midday leaf hydraulic conductance on clear days was calculated by dividing midday transpiration (from gas exchange measurements on clear days; K. Reinhardt, unpublished data) by the driving force of water, which is the difference between leaf and branch water potentials during 14:00 h measurements ($k_{h,\text{sat}} = E/\Delta\Psi$; Domec et al. 2009). Transpiration was obtained from light response curves taken on each species during midday periods on clear days using an LI-6400 portable photosynthesis

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**Table 1.** Maximum specific conductivity ($k_{h}$), $P_{50}$, $P_e$, branch safety margins and $\Psi_{\text{min}}$ across the growing season. Asterisks indicate significant differences between clear and fog days for that measurement period. Error values represent standard error. †Cloud forest species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Max $k_{h}$ (kg m$^{-2}$ s$^{-1}$ MPa$^{-1}$)</th>
<th>$P_{50}$ (MPa)</th>
<th>$P_e$ (MPa)</th>
<th>$P_{50,\text{sat}}$ (MPa)</th>
<th>$P_{50,\text{branch safety margin}}$</th>
<th>$\Psi_{\text{min}}$ (MPa)</th>
<th>$\Psi_{\text{midday}}$ (MPa)</th>
<th>$\Psi_{\text{midday}}$ (MPa)</th>
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<td>Acer saccharum</td>
<td>0.55 ± 0.19</td>
<td>−4.98 ± 0.31</td>
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<td>−7.9 ± 0.32</td>
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<td>Betula</td>
<td>0.54 ± 0.08</td>
<td>−3.17 ± 0.25</td>
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<td>−2.55 ± 0.25</td>
<td>−3.0 ± 0.17</td>
<td>−2.0 ± 0.10</td>
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<td>Fagus</td>
<td>0.84 ± 0.10</td>
<td>−4.44 ± 0.04</td>
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<td>Picea rubens</td>
<td>0.25 ± 0.06</td>
<td>−2.68 ± 0.28</td>
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machine (LI-COR Inc., Lincoln, NE, USA). All measurements of transpiration were on fully sun-exposed branches. For conifer species, silhouette leaf area was used for all measurements. Branch safety margins were calculated as the difference between the minimum branch water potential experienced during the study period (typically from clear days) and the $P_{50}$ and $P_e$ values derived from the constructed vulnerability curves. For example, the calculated $P_{50}$ safety margin for A. fraseri is $(-0.69 \text{ MPa}) - (-5.38 \text{ MPa}) = 4.69 \text{ MPa}$ (values from Table 1).

**Data analysis**

Hydraulic vulnerability curves were generated using a three-parameter sigmoid function fit to the data in SigmaPlot version 12.5 (Systat Software, Inc., Chicago, IL, USA). Equations from these curves were used to determine the values for $P_{50}$ and $P_e$, which were determined as different if the 95% confidence intervals did not overlap. Branch and leaf $\Psi$ were averaged by species and time, and assessed for differences between groups using analysis of variance and post hoc tests for individual differences (JMP version 7, SAS Institute, Cary, NC, USA). All datasets were tested for normality and equal variance.

**Results**

Branch vulnerability curves indicated few differences in hydraulic functioning for species on each side of the cloud forest ecotone (Figure 1, Table 1). Five of the six species were very resistant to cavitation, with $P_{50}$ values less than $-4.5 \text{ MPa}$ (Table 1), with only one species, B. alleghaniensis (a non-cloud forest species) exhibiting moderate resistance ($P_{50} = -2.58 \pm 0.44 \text{ MPa}$). The non-cloud forest species, B. alleghaniensis and F. grandifolia, had lower $P_{50}$ and $P_e$ values than all three cloud forest species. However, the third non-cloud forest species, A. saccharum, had $P_e$ and $P_{50}$ values that were greater than cloud forest species (Table 1). Roots of cloud forest species were more vulnerable to embolism than branches, with $P_{50}$ values ranging from $-1.39$ to $-2.58 \text{ MPa}$ (Figure 2). The differences between $P_{50}$ values of roots and branches for each species were 2.5 MPa or greater for all three cloud forest species. Attempts were made to construct vulnerability curves for roots of non-cloud forest species but segments without open vessels (segments up to 40 cm) could not be obtained, indicating that the root vessel length of non-cloud forest species was longer than vessels in branches. Additionally, midday hydraulic conductance of leaves was calculated from transpiration fluxes and differences in leaf and branch water potentials. Maximum declines (on sunny days) in leaf hydraulic conductance appeared to be large for most of the species examined. Calculated midday $k_{\text{leaf}}$ values were very low relative to literature values for Acer, Betula and Abies, but not in Picea, suggesting large losses of leaf hydraulic conductivity (Table 2).

Calculations of $P_{50}$ and $P_e$ safety margins, the difference between $\Psi_{\text{min}}$ in the field and each of these values, revealed large values for most species, except B. alleghaniensis (Figure 3, Table 1). Minimum daily branch water potentials were typically similar within groups of species and day type (e.g., $\Psi_{\text{min}}$ of A. fraseri on clear days ranged from $-0.59 \pm 0.05$ to $-0.65 \pm 0.13 \text{ MPa}$) across the season and thus values were

![Figure 1. Vulnerability curves for branches of three cloud forest species (top row) and three non-cloud forest species (bottom row). Vertical lines represent the minimum branch water potential ($\Psi_{\text{min}}$) experienced during this study on clear (solid lines) and fog (dashed lines) days. No vertical lines exist for R. catawbiense or F. grandifolia because branch water potentials were not measured in this study. Bars represent standard error.](http://treephys.oxfordjournals.org/)

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pooled across months (Figure 4). In *A. fraseri*, *P. rubens* and *A. saccharum* $\Psi_{\text{min}}$ for the season never reached the $P_e$ point and often had as much as a 3.0 MPa difference between $\Psi_{\text{min}}$ and $P_e$ (Figure 3, Table 1). In *B. alleghaniensis*, minimum seasonal branch $\Psi$ suggested ~20% loss of branch conductivity on clear days and ~10% on fog days.

Foggy days seemed to have varying effects on branch $\Psi$. $\Psi_{\text{min}}$ significantly improved in *P. rubens*, *B. alleghaniensis* and *A. saccharum* (all $P < 0.02$, Figure 4), while there were no differences ($P = 0.7$) in branch $\Psi_{\text{min}}$ during foggy vs clear days in

![Figure 2. Vulnerability curves for roots of the three cloud forest species. Vertical lines represent the minimum predawn branch water potential measured during the study on clear (solid lines) and fog (dashed lines) days. Bars represent standard error.](http://treephys.oxfordjournals.org/)

**Table 2.** Calculated minimum daily leaf hydraulic conductance ($k_{\text{leaf}}$) and leaf minimum daily water potential across the growing season for two species from the cloud forest zone and two from below the cloud forest zone. Values were calculated using midday transpiration rates and water potential values. Variation presented as standard error. For water potentials, asterisks represent significant differences between clear and foggy days at that measurement point.

<table>
<thead>
<tr>
<th>Species</th>
<th>Minimum $k_{\text{leaf}}$ (mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$)</th>
<th>Afternoon leaf transpiration (mmol m$^{-2}$ s$^{-1}$)</th>
<th>Leaf minimum daily water potential (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Abies fraseri</em></td>
<td>2.7 ± 0.4</td>
<td>0.90 ± 0.09</td>
<td>−1.10 ± 0.09</td>
</tr>
<tr>
<td><em>Picea rubens</em></td>
<td>19.2 ± 4.6</td>
<td>1.29 ± 0.32</td>
<td>−1.01 ± 0.09</td>
</tr>
<tr>
<td><em>Acer saccharum</em></td>
<td>4.7 ± 2.3</td>
<td>0.93 ± 0.27</td>
<td>−1.05 ± 0.07</td>
</tr>
<tr>
<td><em>Betula alleghaniensis</em></td>
<td>8.8 ± 1.8</td>
<td>2.42 ± 0.39</td>
<td>−1.17 ± 0.04</td>
</tr>
</tbody>
</table>
with decreasing leaf water potentials and increasing VPD (Schultz 2003). On clear days, leaf $\Psi$ of spruce and fir dropped quickly to between $-1.1$ and $-1.6$ MPa before rebounding as much as 1 MPa. On foggy days, the cloud forest conifers did not reach a $\Psi_{\text{min}}$ and then recover during the examination period; often the most negative values were the last measurements of the day at 17:00 h (Figure 5). As a result, the difference between $\Psi$ on clear and fog days during midday periods was greater for cloud forest species than northern hardwood species. However, on the same day types, daily leaf $\Psi_{\text{min}}$ were similar between cloud forest and non-cloud forest species ($P = 0.30$).

**Discussion**

Many recent studies have collectively provided observational evidence for linkages between plant hydraulic functioning and climate (Choat et al. 2012). However, few studies have thoroughly examined branch and root hydraulic conductivity as a trait that could influence the distribution of cloud forest communities (but see Santiago et al. 2000, Choat et al. 2007, Jacobsen and Pratt 2013). Our examination of hydraulic traits and diurnal variation in water status suggests that the species that exist only in the cloud forest (Abies, Picea, Rhododendron) are equally as resistant to cavitation and lose little branch hydraulic conductivity throughout the growing season compared with species that grow primarily below the cloud forest zone (Betula, Acer, Fagus) (Figure 1). This suggests that across the sharp southern Appalachian cloud forest ecotone, the differences in microclimate due to frequent fog do not significantly affect hydraulic conductivity or species-specific vulnerability to embolism, and that species across both sides of this ecotone exhibit drought-tolerant characteristics. Additionally, the presence of fog improved branch and leaf $\Psi_s$ in both cloud forest and non-cloud forest species, which implies that improvements in $\Psi_s$ are driven by common biophysical responses (reduced transpiration rates and foliar uptake; Dawson 1998, Laur and Hacke 2014) to low VPDs and moisture on leaf surfaces (Reinhardt and Smith 2008, Berry et al. 2014).

We also observed similar diurnal variation in leaf and shoot $\Psi$ measurements from species both in the cloud forest and non-cloud forest zone, and these patterns demonstrated similar and strong isohydric responses in both cloud forest and non-cloud forest species (Figure 5). All species had mean $\Psi_{\text{min}}$ values between $-1.0$ and $-1.2$ MPa on clear days. Because these values are well above branch $P_{50}$ values, the driver of this isohydric response may be the loss of hydraulic conductivity of distal organs such as leaves and roots leading to high leaf to air VPDs and thus strong stomatal responses (Domec et al. 2006, Reinhardt and Smith 2008, Woodruff et al. 2015). Indeed, calculated midday $k_{\text{leaf}}$ values were very low during afternoon periods in Abies, Betula and Acer (Table 2) on clear days (when maximal declines in $k_{\text{leaf}}$ might be expected relative to declines in $k_{\text{leaf}}$ on...
foggy days), while leaf transpiration remained high due to the pressure drop from shoot to leaf (Tables 1 and 2). Improvements in leaf $\Psi$ on clear days in this study along with previous research that demonstrates stomatal closure coupled with greater leaf $\Psi$ underscores the ability of species in this region to recover hydraulic function during the daytime (Berry and Smith 2012).

No matter which forest-type species were sampled from (conifer cloud forest or northern hardwood non-cloud forest), fog generally improved $\Psi$ of both branches and leaves throughout the day. These data are supported by several other studies that reported increases in $\Psi$ in response to fog (Johnson and Smith 2008, Reinhardt et al. 2009). On clear days, three of the four species reached a midday minimum $\Psi$ that was followed by increases in $\Psi$, whereas all species on fog days typically did not reach their lowest $\Psi$ values until the 17:00 h measurements (Figure 5). Although all species demonstrated improved $\Psi_{s}$ in response to fog, the two cloud forest conifers had unique patterns with a maintenance or rise of leaf $\Psi$ from 08:00 to 11:00 h measurements during fog periods. This shift during peak photosynthetic periods (prior to 'midday depression' e.g., Schulze et al. 1980, Tenhunen et al. 1982, Chaves et al. 1987) most likely is driven by the utilization of fog water through foliar water uptake and the simultaneous suppression of transpiration due to low leaf to air VPDs (Berry and Smith 2014, Berry et al. 2014). This could act as a mechanism that maximizes leaf water content during periods of greatest photosynthetic fluxes during the day, enhancing water-use efficiency (photosynthesis/transpiration). The improvements from fog in Betula and Acer demonstrate the importance of fog for water balance in both cloud forest and non-cloud forest species across the ecotone. Interestingly, A. fraseri, the only species in this study that is endemic to cloud forests, showed minimal gains in branch $\Psi_{s}$ from fog (Figure 4). Branch $\Psi_{s}$ in A. fraseri were not significantly different between the two day types, suggesting physiological water management strategies that did not differ in the two climates. The minimal difference between the two day types could be due to better management of $\Psi_{s}$ on clear days than other species from the region. All three other species had less negative midday branch $\Psi_{s}$ on fog days. While these differences were significant, it should be noted that these differences only resulted in significant changes to losses in branch hydraulic conductivity for B. alleghaniensis.

For species in the cloud forest zone, root $P_{50}$ values were 2.5–4.0 MPa greater and maximum $k_{s}$ values were 11–45 times greater (Table 1) than branches. This demonstrates the large differences in hydraulic functioning and highlights the potential importance of distal organs as key safety valves (Johnson et al. 2012, McCulloh et al. 2014). While we were unsuccessful in measuring hydraulic vulnerability of roots in tree species below the cloud forest zone, the fact that we could not obtain closed vessels in roots 40 cm long along with previous data of root hydraulic conductivity (Maherali et al. 2006, Johnson et al. 2012) suggest that the roots of these species are likely to be more vulnerable to cavitation than branches as well. The large

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**Figure 5.** Diurnal curves of leaf water potentials for two cloud forest (Abies, Picea) and two non-cloud forest species (Acer, Betula) across the summer growing season. Dashed lines represent curves on foggy days and solid lines are curves constructed on clear days. Bars represent standard error.
$P_{50}$ safety margins of branches (1.2–4.9 MPa, Figure 3), the greater daily loss of root conductivity relative to branches (up to 20%) and the low midday hydraulic conductivity of leaves support the hydraulic segmentation hypothesis where more distal organs (e.g., roots and leaves) have greater hydraulic vulnerabilities (Zimmermann 1983, Tyree and Ewers 1991). Non-repairable cavitation in roots could result in a large loss of investment in organs at the beginning of the hydraulic pathway and, thus, it may be more common for embolism repair to occur in roots with low $P_{50}$ values (Johnson et al. 2012). Additionally, roots represent a critical link between soil moisture availability and supply of water to the plant and more studies need to examine the hydraulic architecture and hydraulic transport through the entire system (Johnson et al. 2014). Additionally, in species and ecosystems where branches seem to lose very little hydraulic conductivity, measurement of roots and leaves may be a better proxy for explaining hydraulic strategies and stomatal responses to the environment (Domé et al. 2006).

Changes in the abiotic environment can alter plant physical properties that govern hydraulic conductivity. In relatively drier areas, which could be compared with non-cloud forest regions in this study, species tend to have lower hydraulic conductivity and more negative $P_{50}$ values (Choat et al. 2007, Poorter et al. 2010, Corcuera et al. 2012). In the present study, species from the non-cloud forest zone did not exhibit $P_{50}$ values distinctly different from species in the cloud forest zone. The non-cloud forest zone had both the species with the greatest (Betula) and lowest (Acer) $P_{50}$ values (Table 1, Figure 6). However, species from the cloud forest zone did have tightly clustered maximum branch-specific conductivities and $P_{50}$ values (Figure 6), supporting work suggesting that species exhibit less variability and greater clustering of physiological traits in low resource environments (Chapin et al. 1993). Similarly, Jacobsen and Pratt (2013) found that fog did not have a significant effect on mean hydraulic values in Arctostaphylos from the California maritime region. In addition, the cloud forest species in the current study had less negative $Ψ_{\min}$ resulting in greater safety margins, leading to the suggestion that the hydraulic architecture was ‘over built’ in this system. In the cloud forest ecotone of the southern Appalachians, we found large branch safety margins in cloud forest and non-cloud forest species, despite the sharp transition that occurs between the two. While branches may have large safety margins in this ecosystem, the vulnerability of roots, the low calculated leaf hydraulic conductance of Abies, Acer and Betula and the strong isohydric responses between −1.1 and −1.6 MPa suggest that hydraulic conductivity of roots and leaves might better explain species responses to environmental cues.

While the present study examined hydraulic functioning across a fog ecotone to determine the influence on explaining species distribution, it should be noted that extensive work in these cloud forests has demonstrated the influence of fog in several other ways. Due to the effects of frequent fog, both $A. fraseri$ and $P. rubens$ seem to have greater overall annual carbon gain potential because of low light compensation points, low specific leaf area and ability to leave stomata open later in the day on foggy days during the light-limited growing season (Johnson and Smith 2006, Reinhardt and Smith 2008, Berry and Smith 2013). Additionally, both of these species exhibited foliar water uptake in greenhouse studies, which improved $Ψ_{\min}$ and potentially leaf and branch hydraulics as well (Berry and Smith 2013, Berry et al. 2014, Laur and Hacke 2014). It is also possible that the high avoidance of branch cavitation could contribute to reduced frost-induced embolism during the winter periods but to date this has not been evaluated in these cloud forests.

This examination of hydraulic vulnerability and functioning in six species across a cloud forest ecotone suggests that variation in hydrological cycles from frequent fog does not result in strong changes to physiological functioning and plant water relations. Additionally, species from across this ecotone demonstrated physiological improvements during fog, suggesting that the improvements in water potentials during fog periods are largely a biophysical response. The strong avoidance of losses in branch hydraulic conductivity combined with the greater losses in root hydraulic conductivity and low midday leaf hydraulic conductances suggests that root and leaf vulnerability to water deficit may be more important drivers of physiological functioning in these mesic systems. To better understand the distribution of these forests and their association with frequent fog warrants future study to ascertain the interactions between hydraulic functioning, foliar uptake, plant water status and carbon gain across the entire plant from leaves to roots.

**Acknowledgments**

The authors are grateful to the staffs at Roan and Grandfather Mountains for assistance and guidance, particularly Jesse Pope and Mickey Short. Additionally, climate data for Roan and
Grandeir Mountains were graciously provided by L. Baker Perry. The authors would also like to thank Dave Cook and Alyssa Teat for extensive assistance in the field and Remi Wortemann for laboratory advice in constructing vulnerability curves.

Conflict of interest
None declared.

Funding
D.M.J. was funded by NSF (IOS-1146746) and a grant from USDA-AFRI (#2012-00857). K.R. was funded by Idaho State University.

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