**Research paper**

# Conifers but not angiosperms exhibit vulnerability segmentation between leaves and branches in a temperate forest

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Vulnerability segmentation (VS), an important mechanism for protecting plants from drought, hypothesizes that the distal organs of a plant should be more susceptible to embolism than the basal organs. However, experimental studies testing the VS hypothesis for trees are limited and have reached inconsistent conclusions. Here, we tested the VS hypothesis with three angiosperms and four conifers co-existing in a temperate forest in northeastern China. The results showed that the difference in vulnerability to cavitation between leaves and branches ($P50_{leaf-branch}$) was positive for the conifers but negative for the angiosperms, implying that the conifers rather than the angiosperms exhibited VS. The conifers had lower leaf hydraulic safety margins and more embolism-resistant branches than the angiosperms. Although the angiosperms did not display VS, they took a hydraulic compensatory strategy (e.g., great leaf and branch hydraulic conductivities) to maintain the water supply of their leaves. In addition, we found a significant trade-off between the sapwood-specific hydraulic conductivity ($K_{SS}$) and xylem pressure inducing 50% loss of hydraulic conductivity ($P50_{branch}$) across all species. Both $K_{SS}$ and $P50_{branch}$ increased with the area-based light-saturated photosynthetic rate ($A_{area}$), suggesting that increased embolism resistance of branches comes at the cost of reduced hydraulic efficiency, which in turn constrains the photosynthesis. $A_{area}$ was negatively correlated with $P50_{leaf-branch}$, further indicating that the conifers had strong VS and were associated with a conservative strategy. Conversely, the angiosperms displayed an acquisitive strategy, tending to have higher $A_{area}$, leaf and branch hydraulic conductivities, but lower embolism resistance. These differentiations in the functional traits between the angiosperms and conifers provide potential mechanisms for their co-existence in this temperate forest community.

*Keywords*: hydraulic conductivity, photosynthesis, plant strategy, vulnerability to cavitation.

## Introduction

Drought results in shifts of species composition and forest structure, and even widespread tree mortality under ongoing climate change (Breshears et al. 2005). All forests are vulnerable to drought-induced hydraulic dysfunction regardless of their precipitation regimes (Choat et al. 2012). Therefore, it is critical to understand the susceptibility and adaptability of tree species to drought in diverse environments (Mueller et al. 2005).

Drought-induced embolism reduces xylem water transport efficiency and ultimately influences plant photosynthesis and growth (Brodribb and Field 2000, Brodribb et al. 2005). Plants have evolved a variety of hydraulic strategies to survive drought,

of which an important one is the segmentation between proximal and distal organs (Pivovaroff et al. 2014, Zhu et al. 2016). Zimmermann (1983) first proposed the hydraulic segmentation hypothesis stating that distal organs (e.g., leaves) have lower hydraulic conductance than branches or main stems. It was developed further by Tyree and Ewers (1991) as the vulnerability segmentation (VS) hypothesis, which predicts that distal portions are less resistant to embolism than basal portions. Such a hydraulic design maintains the water status of basal organs with massive carbon investment at the cost of sacrificing easily replaceable distal organs (Bucci et al. 2012, Johnson et al. 2012, Pivovaroff et al. 2014).

Most previous studies testing the VS hypothesis have focused on the difference in vulnerability to cavitation between leaves and branches ($P50_{leaf-branch}$), and defined VS species as ones with $P50_{leaf-branch} > 0$ (Peguero-Pina et al. 2015, Johnson et al. 2016, Zhu et al. 2016). However, it is unclear whether a positive $P50_{leaf-branch}$ value is realized by rendering leaves more vulnerable or branches more resistant to drought-induced cavitation, which may have different influences on tree growth and productivity. Greater hydraulic vulnerability of leaves implies that they may frequently suffer loss of hydraulic conductance, which might incur substantial metabolic costs for refilling the embolized conduits (Johnson et al. 2009, Nardini et al. 2011). However, increased resistance to embolism in branches often reduces hydraulic efficiency, and in turn constrains photosynthesis (Bucci et al. 2006). Therefore, revealing the relative role of leaf vs. branch in the context of VS is important for understanding the strategic balance between photosynthesis and risk of embolism (Santiago et al. 2004).

Although the VS hypothesis was proposed 30 years ago (Zimmermann 1983, Tyree and Ewers 1991), experimental studies testing the hypothesis for trees are limited and have also reached inconsistent conclusions (Chen et al. 2009, Villagra et al. 2013, Peguero-Pina et al. 2015, Johnson et al. 2016). For example, Johnson et al. (2016) provided strong support for the VS hypothesis based on studying the leaf-to-branch VS of four angiosperms and four conifer tree species from North America and France. By contrast, Villagra et al. (2013) studied five species in the semideciduous Atlantic Forest in Argentina and found no difference in vulnerability to embolism between leaf and branch. Recently, Zhang et al. (2017) tested the VS hypothesis in three sympatric tree species with different leaf phenology in a Chinese savanna and found that the evergreen and winter-deciduous species exhibited VS, whereas the drought-deciduous species did not. Additionally, Zhu et al. (2016) compiled branch and leaf hydraulic trait data of 69 broadleaved woody species worldwide and suggested that VS varied with climate conditions, i.e., tree species from arid areas often exhibited VS, whereas those from humid regions did not; however, this global data set contained 11 temperate angiosperm species that were mainly from Europe, and North and South Americas. To our knowledge, no studies have tested the VS hypothesis in the temperate forests in Asia—one of the three regions with large stretches of temperate forest in the world (i.e., northeastern North America, Europe and eastern Asia).

In this study, we tested the VS hypothesis with three angiosperm and four conifer tree species co-occurring in a temperate forest in northeastern China. These sympatric species provide an opportunity to examine whether VS varies with plant functional types that have contrasting eco-physiological traits (e.g., stomatal regulation, resistance to cavitation, xylem embolism refilling, xylem anatomical traits; Carnicer et al. 2013). Specifically, we addressed the following questions: (i) Whether the VS hypothesis is valid for both the conifers and angiosperms? (ii) If the conifers and angiosperms exhibit divergent hydraulic safety strategies (e.g., VS vs. non-VS), what are the potential mechanisms responsible for this difference? And (iii) what are the economic consequences induced by such different safety strategies? Answering these questions would be helpful for understanding the hydraulically adaptive strategies of trees and revealing potential mechanisms for species co-existence in temperate forest communities.

## Materials and methods

### Study site and species selection

Our study was carried out in a temperate mixed forest at the Maoershan Forest Ecosystem Research Station, northeastern China (45°20′ N, 127°30′ E; 400 m above sea level). This site is significantly influenced by a continental monsoon climate with a humid, warm summer and a dry, cold winter. The annual precipitation varies from 600 to 800 mm, of which ~62% falls during the growing season (June to September). Mean annual temperature is 3.1 °C; January and July are the coldest and warmest months with mean temperatures of −18.5 °C and 22.0 °C, respectively. Only 120–140 days are frost-free. Within the forest stand, three angiosperm (*Betula platyphylla* Suk., *Populus davidiana* Dode, *Tilia amurensis* Rupr.) and four conifer tree species (*Pinus koraiensis* Sieb. et Zucc., *Larix gmelinii* Rupr., *Picea koraiensis* Nakai, *Pinus sylvestris* var. *mongholica* Litv.) were selected (Table 1). For each species, four healthy trees were sampled for the measurements, which were performed in August 2015.

### Leaf hydraulic conductance and vulnerability

The timed rehydration method was used to measure leaf hydraulic conductance ($K_{leaf}$; Brodribb and Holbrook 2003). Branches from four individuals of each species were collected at predawn, re-cut under water and transported to the laboratory immediately. The branches were dehydrated for different periods of time to obtain various leaf water potentials, and then were placed in black plastic bags for at least 1 h to allow equilibration of the leaf water potential. The initial leaf water potential ($\Psi_O$) and leaf water potential after rehydration for $t$ seconds ($\Psi_f$) were measured. The $K_{leaf}$ was calculated as

$$K_{leaf} = C_{leaf} \times \ln(\psi_O/\psi_f)/t \qquad (1)$$

where $C_{leaf}$ is the leaf capacitance, which was calculated from the pressure–volume curves based on the slope of the relationship between relative water content and leaf water potential. Four leaves of each species (individual leaves for the angiosperms and small terminal shoots for the conifers) were used to construct pressure–volume curves. Small branch samples were cut at predawn, transferred to the laboratory immediately and rehydrated in deionized water until the leaf water potential was

Table 1. Hydraulics and economic traits for the seven tree species. The numbers are means ($n = 4$). DBH, diameter at breast height (cm); SWC, soil gravimetric water content (%); $K_{leaf}$, leaf hydraulic conductance (mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$); $C_{leaf}$, leaf capacitance (mol m$^{-2}$ MPa$^{-1}$); $K_{SS}$, sapwood-specific hydraulic conductivity of branch (kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$); $P50_{leaf}$, leaf water potential inducing 50% loss of hydraulic conductance ($-$MPa); $P50_{branch}$, xylem pressure inducing 50% loss of hydraulic conductivity of branch ($-$MPa); $A_{area}$, light-saturated photosynthetic rate per leaf area (µmol m$^{-2}$ s$^{-1}$); $N$, leaf nitrogen concentration (mg g$^{-1}$); SLA, specific leaf area (m$^2$ kg$^{-1}$).

| Species | Symbol | DBH | SWC | $K_{leaf}$ | $C_{leaf}$ | $K_{SS}$ | $P50_{leaf}$ | $P50_{branch}$ | $A_{area}$ | $N$ | SLA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Angiosperm | | | | | | | | | | | |
| Betula platyphylla | △ | 24.5 | 38.7 | 29.3 | 0.9 | 5.0 | 1.4 | 1.3 | 15.7 | 15.5 | 14.2 |
| Populous davidiana | ○ | 31.2 | 43.9 | 15.3 | 0.3 | 5.1 | 2.1 | 1.9 | 15.5 | 16.3 | 12.4 |
| Tilia amurensis | □ | 25.8 | 40.3 | 39.6 | 0.7 | 6.0 | 1.1 | 0.9 | 17.4 | 22.5 | 12.9 |
| Conifer | | | | | | | | | | | |
| Pinus koraiensis | ● | 28.2 | 40.9 | 21.3 | 1.4 | 1.8 | 1.1 | 2.1 | 10.4 | 9.1 | 6.5 |
| Larix gmelinii | ▲ | 29.8 | 28.3 | 24.0 | 1.9 | 2.4 | 1.6 | 2.1 | 4.6 | 18.8 | 13.9 |
| Picea koraiensis | ■ | 30.1 | 45.8 | 16.7 | 1.0 | 2.0 | 1.3 | 2.0 | 4.8 | 9.9 | 4.8 |
| Pinus sylvestris var. mongolica | ◆ | 26.3 | 33.9 | 23.6 | 1.9 | 1.3 | 1.3 | 2.0 | 7.7 | 10.4 | 4.9 |

greater than $-0.1$ MPa. As the leaf/shoot dehydrated slowly in the laboratory, the leaf water potential and fresh mass were measured periodically. The leaf turgor loss point (TLP) was calculated following Tyree and Hammel (1972). If $\Psi_O$ was higher than TLP, $C_{leaf}$ before TLP was used to calculate $K_{leaf}$; if $\Psi_f$ was lower than TLP, $C_{leaf}$ after TLP was used; if $\Psi_O$ and $\Psi_f$ spanned TLP, the mean value of $C_{leaf}$ pre- and post-TLP was used (McCulloh et al. 2014). The leaf vulnerability curve was generated by plotting $K_{leaf}$ against $\Psi_O$, which was used to estimate the leaf water potential inducing 50% loss of $K_{leaf}$ ($P50_{leaf}$) (Figure S1).

### Branch hydraulic conductivity and vulnerability

One branch (~1.5 m long) from each of four individuals per species was harvested at predawn, re-cut under water and transported to the laboratory immediately. The air infiltration method was used to measure the maximum vessel length for the angiosperms (Zimmermann and Jeje 1981). The maximum vessel lengths for *B. platyphylla*, *P. davidiana* and *T. amurensis* were $21.05 \pm 1.22$, $18.7 \pm 2.29$ and $23.02 \pm 0.80$ cm, respectively. Branch samples with a length of 30 cm (5.8–7.6 mm in diameter without bark) were used for measuring the hydraulic conductivity. The branches were first flushed for 20 min with deionized and degassed water under a 100 kPa pressure to remove native embolisms (Tyree and Sperry 1989), and then the maximum flow rate was induced by a hydrostatic pressure of 6–9 kPa. The maximum hydraulic conductivity of branch ($K_{hmax}$) was calculated as the ratio of the maximum flow rate and the corresponding pressure. The sapwood-specific ($K_{SS}$) and leaf-specific ($K_{SL}$) hydraulic conductivities of branches were calculated as $K_{hmax}$ divided by the sapwood area and the total distal leaf area, respectively.

Vulnerability curves in branches were constructed by the air injection method (Sperry and Saliendra 1994). Specifically, after $K_{hmax}$ was measured, the branch was inserted into an air injection chamber with both ends protruding. Cavitation was induced by applying successively increasing pressures to the branch; the hydraulic conductivity ($k_h$) was measured after each pressurization treatment. The percentage loss in hydraulic conductivity at a given applied pressure (PLC) was calculated as

$$PLC = 100 \times \left(1 - \frac{k_h}{K_{hmax}}\right) \qquad (2)$$

Vulnerability curves for each species were generated by a sigmoid function (Figure S2; Pammenter and Vander Willigen 1998).

$$PLC = \frac{100}{1 + \exp(a(\psi - b))} \qquad (3)$$

where $\Psi$ is the injection pressure, $a$ is the maximum slope of the curve and $b$ is the xylem pressure inducing 50% loss in hydraulic conductivity ($P50_{branch}$).

### Leaf water potential

The midday leaf water potential ($\Psi_{midday}$) was measured between 12:00 and 14:00 h on the same day as the hydraulic conductivity measurement. Three leaves per individual for each species were collected; they were immediately sealed in plastic bags with moist paper towels inside and kept in a cooler before measuring. Leaf water potential was measured with a pressure chamber (Model 1505D; PMS Instrument Company, Albany, OR, USA). Leaf hydraulic safety margin (HSM) was defined as the difference between $\Psi_{midday}$ and $P50_{leaf}$.

### Leaf gas exchange rate

The leaf-area-based light-saturated photosynthetic rate ($A_{area}$) and stomatal conductance ($g_s$) were measured with a portable $CO_2$ analyzer (Li-6400; Li-Cor Inc., Lincoln, NE, USA) between 08:30 and 10:30 h on sunny days; the photosynthetic photon flux density and $CO_2$ concentration in the chamber were 1500 µmol m$^{-2}$ s$^{-1}$ and 400 µmol mol$^{-1}$, respectively. Afterwards, the leaves were oven-dried at 70 °C for 48 h and ground to powder for determining leaf nitrogen concentration ($N$). Instantaneous photosynthetic water-use efficiency (WUE$_i$) was calculated as $A_{area}/g_s$.

### Leaf and wood structural traits

Leaf area was measured using a flatbed scanner and image analysis software. Leaf and wood volumes were measured with the water displacement method; then the samples were dried in an oven (70 °C for 48 h) to determine their dry mass. Leaf density was calculated as leaf dry mass divided by leaf volume. Specific leaf area (SLA) was calculated as leaf area divided by leaf dry mass. Wood density (WD) was calculated as wood dry mass divided by volume with the bark and pith removed.

### Data analysis

Vulnerability segmentation for each species was assessed by the difference between $P50_{leaf}$ and $P50_{branch}$ ($P50_{leaf-branch}$). If the $P50_{leaf-branch}$ was greater than zero, the species was considered to have VS. A two-way ANOVA procedure was used to test the differences in hydraulics and economic traits of the leaves and branches, with plant group and species as the fixed factors. Principal component analysis (PCA) was used to examine multivariate associations among the measured traits.

## Results

The seven tree species displayed large variations in both hydraulics and economic traits (Table 1). The ranges of $K_{leaf}$ and $K_{SS}$ were 15.3–39.6 mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ and 1.3–6.0 kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$, respectively. *Tilia amurensis* had the maximum $K_{leaf}$ and $K_{SS}$, whereas *P. davidiana* had the minimum $K_{leaf}$ and *P. sylvestris* had the minimum $K_{SS}$, respectively. $C_{leaf}$ ranged between 0.3 and 1.9 mol m$^{-2}$ MPa$^{-1}$, with the conifers showing higher $C_{leaf}$ than the angiosperms. $P50_{leaf}$ and $P50_{branch}$ varied from −2.1 to −1.1 MPa and from −2.1 to −0.9 MPa, respectively. $A_{area}$, $N$ and SLA varied from 4.6 to 17.4 μmol m$^{-2}$ s$^{-1}$, 9.1 to 22.5 mg g$^{-1}$ and 4.8 to 14.2 m$^2$ kg$^{-1}$, respectively.

$P50_{leaf-branch}$ was positive for the conifers, but negative for the angiosperms (Figure 1). The angiosperms had significantly higher $K_{leaf}$, $K_{SS}$ and $K_{SL}$ than the conifers ($P < 0.01$; Figure 2a–c). $P50_{leaf}$ did not differ significantly between the conifers and angiosperms, but $P50_{branch}$ for the former was more negative than that for the latter ($P < 0.001$; Figure 2d and e). The conifers had significantly lower $\Psi_{midday}$ and greater loss of $K_{leaf}$ at midday than the angiosperms ($P < 0.01$; Figure 2f and g). The angiosperms exhibited positive leaf HSM, while it was negative for the conifers (Figure 2h). In addition, the angiosperms had lower $C_{leaf}$ and WD, but higher $A_{area}$ and SLA than the conifers ($P < 0.001$; Figure 2i–l).

Several coordinated relationships were observed between hydraulics and economic traits across all species. The leaf HSM decreased with increasing $C_{leaf}$ ($P = 0.007$; Figure 3). $P50_{branch}$ was significantly negatively correlated with WD, but positively correlated with $K_{SS}$ ($P < 0.05$; Figure 4). $A_{area}$ was significantly positively related to $K_{SS}$ and $P50_{branch}$, but negatively related to $P50_{leaf-branch}$ ($P < 0.05$; Figure 5). Principal component analysis
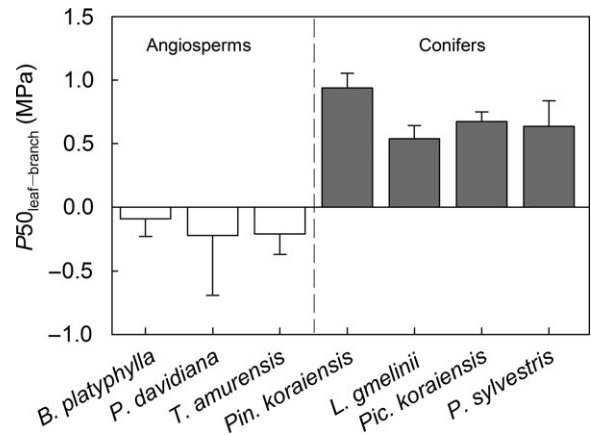


Figure 1. Comparisons of differences between leaf water potential inducing 50% loss of hydraulic conductance and xylem pressure inducing 50% loss of hydraulic conductivity of branch ($P50_{leaf-branch}$) for the three angiosperm and four conifer tree species. The error bars represent SE ($n = 4$).

axis 1 showed strong positive loadings for $K_{leaf}$, $K_{SS}$, $K_{SL}$, $A_{area}$, $N$, SLA and $WUE_i$, but negative loadings for $P50_{branch}$, $P50_{leaf-branch}$ and WD (Figure 6). Principal component analysis axis 2 had positive loading for $P50_{leaf}$.

## Discussion

### The angiosperms lack vulnerability segmentation

Our results showed that the values of $P50_{leaf-branch}$ for the three angiosperms were negative (Figure 1), suggesting that they lack VS. This finding contradicts that of Zhu et al. (2016) who found that all of the 11 temperate angiosperms species exhibited VS. Such discrepancy may be attributed to, besides the biogeographical heterogeneity, the divergence in soil water regimes. For example, the six *Nothofagus* species in their study were from Argentina and Chile where a large proportion of rainfall falls in winter (non-growing season; Bucci et al. 2012, Zhu et al. 2016). Our site has a slightly lower annual precipitation than the *Nothofagus*-dominated sites (600–800 mm vs. 671–1000 mm; Bucci et al. 2012), but more than 60% of it falls in the growing season. Melt water from snow also provides an important source of soil water at the beginning of the growing season in our study area. These circumstances lead to the soil water content at our site being ~1.8 times higher than that at the site of Bucci et al. (2012) during the study period (38.8% vs. 14.1%; Table 1). Meanwhile, the means of $K_{leaf}$ and $K_{SS}$ for our three angiosperms are 1.3 and 4.7 times higher than those for the six *Nothofagus* species (Bucci et al. 2012), respectively, implying that the abundant soil water at our site may not act as a selective pressure to enhance leaf-to-branch VS for the angiosperms (Villagra et al. 2013). In addition, deciduousness is a strategy of plants to avoid drought or freezing temperatures (Villagra et al. 2013, Zanne et al. 2014). The angiosperms in the present study are
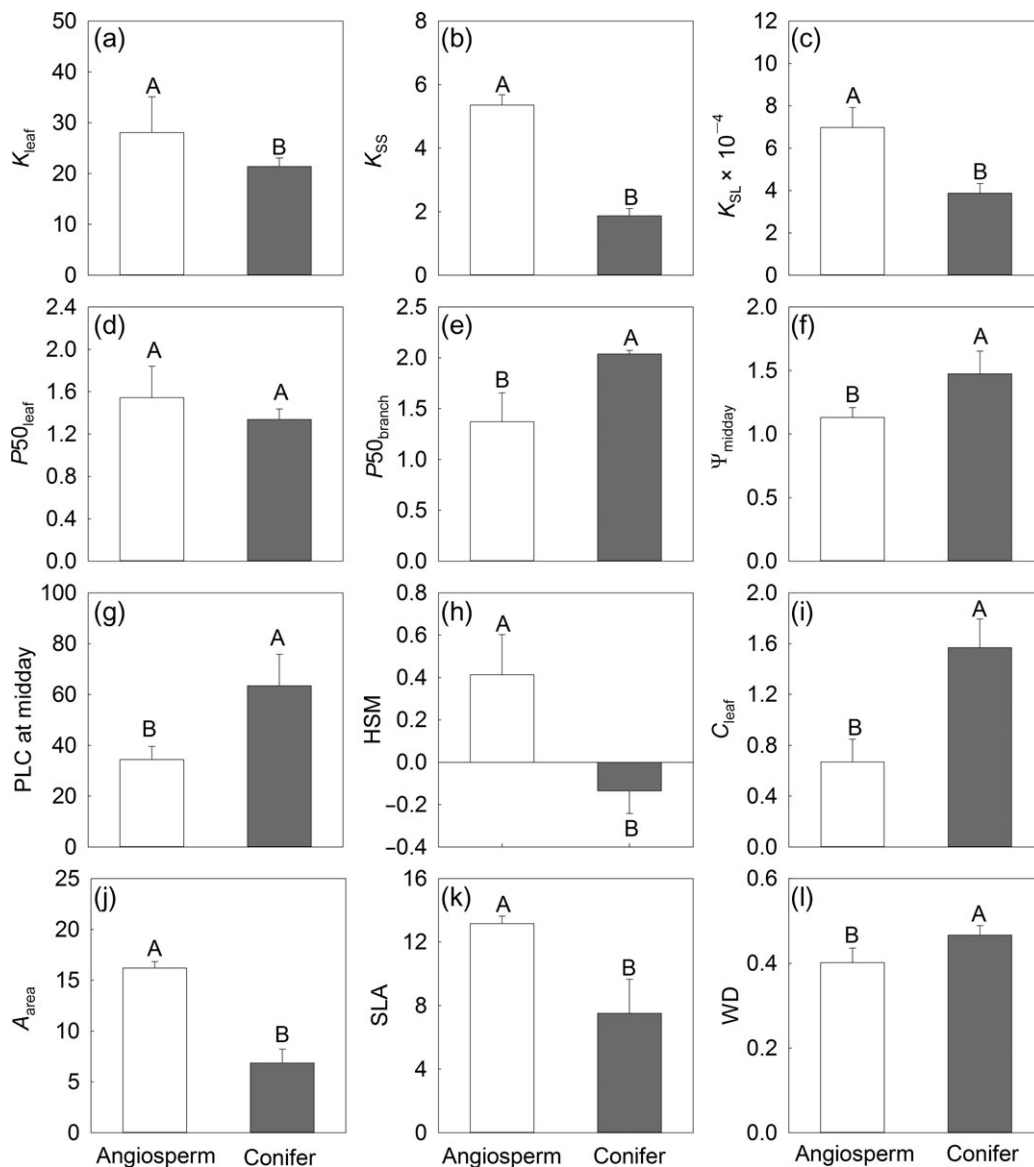
Figure 2. Comparisons of leaf hydraulic conductance ($K_{leaf}$, mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$, a), sapwood-specific hydraulic conductivity of branch ($K_{SS}$, kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$, b), leaf-specific hydraulic conductivity of branch ($K_{SL}$, kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$, c), leaf water potential inducing 50% loss of $K_{leaf}$ ($P50_{leaf}$, −MPa, d), xylem pressure inducing 50% loss of $K_{SS}$ ($P50_{branch}$, −MPa, e), midday leaf water potential ($\Psi_{midday}$, −MPa, f), percent loss of $K_{leaf}$ at $\Psi_{midday}$ (PLC at midday, %, g), leaf hydraulic safety margin (HSM, MPa, h), leaf capacitance ($C_{leaf}$, mol m$^{-2}$ MPa$^{-1}$, i), light-saturated photosynthetic rate per leaf area ($A_{area}$, μmol m$^{-2}$ s$^{-1}$, j), specific leaf area (SLA, m$^2$ kg$^{-1}$, k) and wood density (WD, g cm$^{-3}$, l) between the angiosperms and conifers. Different capital letters above the bars (means ± SE, $n = 4$) indicate significant differences between the angiosperms and conifers ($\alpha = 0.05$).

all winter-deciduous species that shed leaves as the dry and cold season is approaching, which may preclude them from developing segmented hydraulic architecture.

The angiosperms may have compensatory hydraulic strategies to maintain the water supply and functionality of their leaves. First, the angiosperms had higher leaf and branch hydraulic conductivities than the conifers (Figure 2a–c), which might facilitate their leaves to acquire sufficient water and maintain less negative $\Psi_{midday}$ (Figure 2f; Bucci et al. 2012, Gleason et al. 2012). Second, the angiosperms had lower WD than the conifers (Figure 2l), implying that they may have higher sapwood

capacitance in their branches (Scholz et al. 2007), which could alleviate potential embolism by the transient release of stored water into the transpiration stream (Meinzer et al. 2009). This mechanism may play a role in maintaining plant water balance and extending carbon gain on a daily basis (Carnicer et al. 2013, Villagra et al. 2013, McCulloh et al. 2014, Zhu et al. 2016).

### The conifers exhibit vulnerability segmentation

Unlike the angiosperms, the conifers displayed VS evidenced by the positive values of $P50_{leaf-branch}$ (Figure 1). One probable
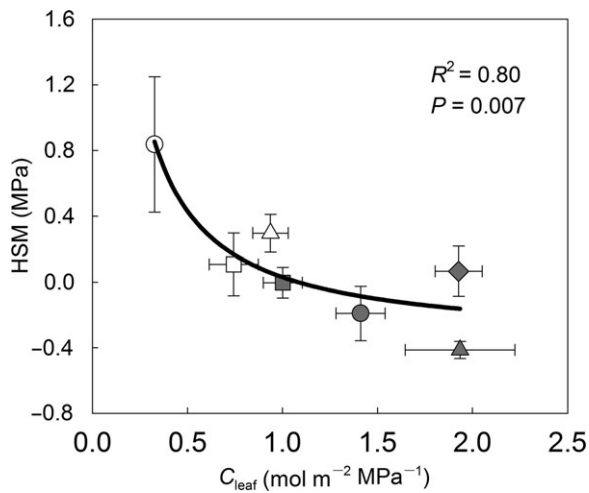
Figure 3. Relationship between leaf hydraulic safety margin (HSM) and leaf capacitance ($C_{leaf}$). Each symbol represents the mean value ($\pm$SE, $n = 4$) for a single species. Symbols as in Table 1. White and gray symbols represent angiosperms and conifers, respectively. The solid line represents the regression fitted to the data: $y = 0.40/x - 0.37$.

reason is that the conifers had lower leaf hydraulic safety margins than the angiosperms (Figure 2h), which supports previous results that species with greater leaf to branch segmentation tend to have greater leaf hydraulic dysfunction (Johnson et al. 2016). Although the $P50_{leaf}$ of our conifers and angiosperms was comparable (Figure 2d), the conifer leaves operated below $P50_{leaf}$ (lower leaf HSM; Figure 2h) and thus lost more $K_{leaf}$ at $\Psi_{midday}$ than the angiosperm leaves (63% vs. 34%; Figure 2g). However, the lower leaf HSM in the conifers might be compensated by their higher leaf capacitances (Figure 3), in agreement with the trade-offs that have frequently been reported for stems (Meinzer et al. 2008, 2009). The larger daily declines of $K_{leaf}$ in the conifers may be easier to reverse because transfusion tissue in the needles can release solutes into adjacent tracheids to prompt refilling (Canny 1993). Another reason why the conifers displayed VS may be associated with their more embolism-resistant branches than the angiosperms (Figure 2e). Anatomically, conifers with narrower tracheids are more resistant to embolism than angiosperms, which have wider vessels (Hacke et al. 2001, Chen et al. 2009). In addition, the conifers had greater WD than the angiosperms, which precludes them from having large volumes of living parenchyma cells that drive embolism repair, and consequently requires them to construct more safety xylem to resist potential embolism in their branches (Figure 4a; Santiago et al. 2004, Johnson et al. 2012, 2016).

However, differences in tracheid- vs vessel-bearing structures and repairing capacity between conifers and angiosperms did not lead to a significant difference in the cavitation resistance of leaves (Figure 2d). A possible explanation is that the vulnerability in outside-xylem pathways in the coniferous needles weakens the embolism resistance at the whole-leaf level (Bouche et al. 2015). For example, Bouche et al. (2015) found that the $P50$
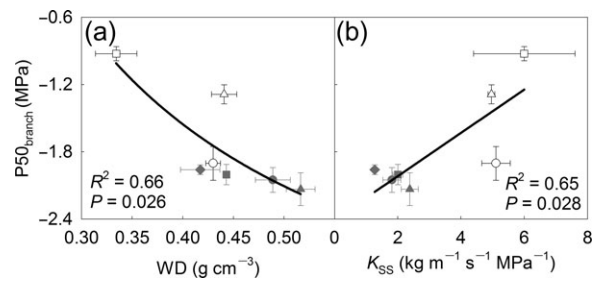


Figure 4. Relationships between xylem pressure inducing 50% loss of hydraulic conductivity ($P50_{branch}$) and (a) wood density (WD) and (b) sapwood-specific hydraulic conductivity of branch ($K_{SS}$). Each symbol represents the mean value ($\pm$SE, $n = 4$) for a single species. Symbols as in Table 1. White and gray symbols represent angiosperms and conifers, respectively. The solid lines represent the regressions fitted to the data: (a) $y = 1.1/x - 4.31$; (b) $y = 0.19x - 2.4$.

of the xylem tracheids of *Pinus pinaster* needles was similar to that of the branches (−3.62 vs. −3.88 MPa); but the $P50$ at the whole-leaf level by integrating the outside-xylem pathways in the needles increased significantly (−1.71 MPa) and was less negative than $P50_{branch}$. This greater vulnerability in extra-xylem pathways preferentially partitions low potentials to the mesophyll, which can prevent strong tensions in the leaf xylem and possibly delay the onset of xylem embolism or collapse (Scoffoni et al. 2014, 2017).

It is worth noting that conifers, with narrower tracheid diameters in their branches, tend to be more resistant to freezing-induced embolism than angiosperms, which have wider vessels (Pittermann and Sperry 2003, 2006), because the bubbles formed in tracheids are very small during the sap freezing process and are easily redissolved during thawing (Feild and Brodribb 2001). The tolerance to freezing at the branch level in conifers permits the maintenance of hydraulic capacity and enables leaves to be retained throughout the winter (Sakai et al. 1981, Zimmermann 1983). Therefore, besides conifers' needles protecting their branches, the branches, being resistant to freeze–thaw-induced embolism, may in turn protect the needles during the dry and cold winter period (Sakai et al. 1981, Pittermann and Sperry 2006).

### *Economic consequences of divergent hydraulic safety strategies*

Hydraulic dysfunction has important implications for tree survival and productivity, because photosynthesis and growth depend on an efficient supply of water to the sites of evaporation in the leaves (Brodribb and Holbrook 2007). Pooling all data of both angiosperms and conifers, we found that the hydraulic performance mediated the leaf economic traits. The conifers construct more embolism-resistant branches (Figure 2e), which incur greater construction costs and reduce the branch hydraulic efficiency (Figure 4; Hacke et al. 2001, Jacobsen et al. 2007), and consequently lead to a trade-off between $K_{SS}$ and $P50_{branch}$
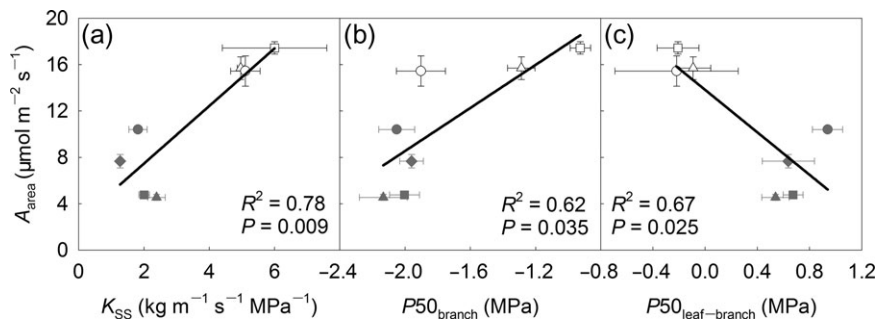
Figure 5. Relationships between light-saturated photosynthetic rate per leaf area ($A_{area}$) and (a) sapwood-specific hydraulic conductivity of branch ($K_{SS}$), (b) xylem pressure inducing 50% loss of hydraulic conductivity ($P50_{branch}$) and (c) the difference in $P50$ between leaves and branches ($P50_{leaf-branch}$). Each symbol represents the mean value ($\pm$SE, $n = 4$) for a single species. Symbols as in Table 1. White and gray symbols represent angiosperms and conifers, respectively. The solid lines represent the regressions fitted to the data: (a) $y = 2.48x + 2.51$; (b) $y = 9.29x + 27.13$; (c) $y = -9.16x + 13.83$.
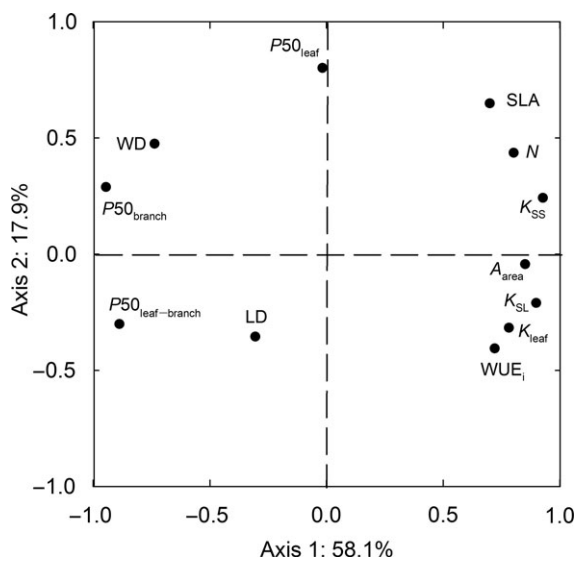


Figure 6. Principal component analysis for the 12 traits measured in this study for the first two axes. $K_{leaf}$, leaf hydraulic conductance (mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$); $K_{SS}$, sapwood-specific hydraulic conductivity of branch (kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$); $K_{SL}$, leaf-specific hydraulic conductivity of branch (kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$); $P50_{leaf}$, leaf water potential inducing 50% loss of hydraulic conductance ($-$MPa); $P50_{branch}$, xylem pressure inducing 50% loss of hydraulic conductivity of branch ($-$MPa); $P50_{leaf-branch}$, the difference in $P50_{leaf}$ and $P50_{branch}$ (MPa); $A_{area}$, light-saturated photosynthetic rate per leaf area (µmol m$^{-2}$ s$^{-1}$); N, leaf nitrogen concentration (mg g$^{-1}$); SLA, specific leaf area (m$^2$ kg$^{-1}$); WUE$_i$, instantaneous water-use efficiency (µmol mol$^{-1}$); LD, leaf density (g cm$^{-3}$); WD, wood density (g cm$^{-3}$).

(Hacke et al. 2006). The reduced branch hydraulic efficiency will constrain the photosynthetic capacity and growth because $K_{SS}$ and $P50_{branch}$ are positively associated with photosynthesis (Figure 5; Brodribb and Holbrook 2007). Evergreen conifers have a long season for carbon gain, which might compensate their lower photosynthetic rates to some extent (Eamus 1999). In addition, conifers have great leaf capacitances, which might allow their needles to function longer after becoming hydraulically detached from the plant, and prolong stomatal opening and

extend photosynthesis during drought (Figure 2i; Brodribb et al. 2005, 2014).

Further PCA analysis revealed that the angiosperms with higher rates of water transport and water-use efficiency were associated with higher $A_{area}$, SLA and leaf N, which are generally associated with a rapid growth (Figure 6; Santiago et al. 2004, Reich 2014); however, they were more vulnerable to cavitation in branches. These results imply that the angiosperms have an advantage in productive environments by maximizing resource acquisition at the cost of safe hydraulic structure (Brodribb and Field 2000). Conversely, the conifers, which displayed the contrasting traits of greater investment in the structure of leaves and branches (lower SLA and higher WD) and greater cavitation resistance of branches, have a competitive advantage in resource-limited habitats (Hacke et al. 2001, Reich 2014). In addition, $A_{area}$ was negatively correlated with $P50_{leaf-branch}$ (Figure 5c), suggesting that the conifers showed VS, which are associated with conservative strategies.

## Conclusions

The current study tested the vulnerability segmentation (VS) hypothesis with four conifer and three angiosperm tree species that coexist in a Chinese temperate forest. We found that VS in the temperate forest varied with plant functional type. The conifers exhibited a VS pattern that may be attributed to their suffering greater leaf hydraulic dysfunction and constructing more embolism-resistant branches. Conversely, the angiosperms did not display VS but were compensated by their higher leaf and branch hydraulic conductivities to maintain the water supply and functionality of their leaves. In addition, the angiosperms had higher rates of water transport and water-use efficiency that were associated with higher photosynthetic capacity, specific leaf area and leaf nitrogen concentration, but they were more vulnerable to cavitation in their branches, whereas the conifers showed contrasting traits with greater investment in the structure of their leaves and branches and greater xylem resistance to

cavitation. These differences in functional traits between the angiosperms and conifers may provide a basis for mechanistically understanding the co-existence of different functional types in temperate forest communities.

## Supplementary Data

We are grateful to Dr. Frederick Meinzer and two anonymous reviewers for their valuable comments on the manuscript. Supplementary Data for this article are available at *Tree Physiology* Online.

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## Conflict of interest

None declared.

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