

Title: The role of environmental gradients in the ontogenetic shift of wood traits from seedlings to adults in two temperate tree species.

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Key words: ontogeny, wood anatomical traits, functional traits, sugar maple, *Acer saccharum*, yellow birch, *Betula alleghaniensis*, hydraulic efficiency, hydraulic safety, light, water availability

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ABSTRACT

Tree development, or ontogeny, involves concurrent changes in plant size and environmental conditions, both of which can influence wood structure and function. Disentangling the factors underlying ontogenetic variation in wood traits remains a major challenge. This is important because wood underpins hydraulic efficiency, hydraulic safety, and mechanical support - functions critical for tree performance. In this study, we sampled twigs from a fixed distance from the apex from sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*) to quantify how wood traits, light availability, and water availability change across seedling, sapling, and adult developmental stages.

Across both species, wood structure shifted toward greater hydraulic safety with ontogeny, demonstrated through decreased vessel diameter (D_h), increased vessel number (VN) and increased vessel reinforcement ($((t/b)^2)$) in adult trees. Despite this shift, hydraulic efficiency (K_s) was maintained across developmental stages, indicating that increases in VN and lumen fraction (F) compensated for reductions in efficiency associated with smaller D_h . Wood trait covariation was structured by a hydraulic efficiency–safety trade-off, however traits such as F varied more independently, enabling compensations such that this trade-off did not constrain tissue-level hydraulic function.

Contrary to expectations, we did not detect differences in water availability across developmental stages. Although light availability increased from seedlings to adults, it explained little variation in wood traits, while height was associated with only a subset of traits in sugar maple and none in yellow birch. The shift toward smaller D_h and maintained K_s in adult apices

contrasts with expectations of increasing transport efficiency in taller trees but is consistent with selection for resistance to freeze–thaw embolism in temperate environments.

INTRODUCTION

Trees must maintain essential functions such as water transport and mechanical support throughout development while simultaneously experiencing changes in both environmental conditions and plant size (Niinemets, 2010; Pratt & Jacobsen, 2017). As individuals grow from seedlings to adult trees, they undergo shifts in light and moisture availability. At the same time, increasing height imposes intrinsic hydraulic constraints through increasing water transport distances, resulting in increasingly negative xylem water potentials (Niinemets, 2010; Ryan and Yoder, 1997). Together, these changes alter both the external demands placed on the hydraulic system and the structural requirements needed to maintain water flow. However, because environmental conditions and tree height change concurrently across ontogeny, their relative roles in shaping wood traits are difficult to disentangle.

Wood structure needs to balance multiple and often competing functions (Pratt & Jacobsen, 2017). In angiosperms, hydraulic function is governed by xylem conduits known as vessels, while mechanical support is provided by fibers. Vessels must efficiently transport water from roots to leaves to support photosynthesis (hydraulic efficiency) while simultaneously maintaining resistance to hydraulic failure (hydraulic safety; Tyree et al., 1994; Zimmermann, 1983). In contrast, the main function of fibers is to provide structural support to reinforce the trunk and branches (Pratt et al., 2007; Tyree & Zimmermann, 2002). Wood traits associated with vessels and fibres provide a direct link between xylem structure and its hydraulic and mechanical support functions.

Wood traits are structured by trade-offs and reflect the need to balance multiple functions within limited space and resources. A classic framework for understanding wood trait variation is the hydraulic efficiency-safety trade-off. Hydraulic efficiency is strongly influenced by vessel diameter, which influences the rate of flow through conduits to the fourth power (Appendix 1; Tyree and Ewers, 1991). However, larger vessels increase vulnerability to embolism where, under conditions like drought and freeze-thaw events, gas bubbles expand to fill vessels with air and disrupt water transport (Sperry et al., 1994; Tyree & Sperry, 1989). In contrast, narrower conduits increase hydraulic safety but reduce efficiency. At the same time, allocation of xylem tissue can involve trade-offs between hydraulic and mechanical support functions, such that increasing conductive area may reduce mechanical support (Chave et al., 2009; Pratt & Jacobsen, 2017; Zanne et al., 2010). As a result of trade-offs in wood function, wood traits do not vary independently and are instead coordinated along major functional axes. However, the extent to which these trade-offs constrain hydraulic function at the tissue level, and how this changes across ontogeny, remains unclear (Burgess et al., 2006; Gleason et al., 2016).

Among the drivers of wood trait variation, ontogeny is widely recognized as a primary influence on wood traits due to changes in tree height (Hietz et al., 2017; Olson et al., 2014; Soriano et al., 2020). As trees grow taller, increasing path length and gravitational resistances require more negative xylem pressures to sustain water transport (Appendix 1; Koch et al., 2004; Ryan & Yoder, 1997; Tyree & Ewers, 1991). Increased resistances are associated with predictable organism-level patterns of xylem adjustment where conduit diameters widen axially from the tips to the base of trees (Anfodillo et al., 2013; Olson et al., 2014). This widening pattern compensates for accumulating path length resistance with height by increasing hydraulic efficiency, such that taller trees have wider conduits on average (Olson et al., 2018). While

height is an important driver of wood traits, the effect of ontogenetic environmental gradients on wood traits is under explored.

Concurrent with increases in height, trees undergo shifts in environmental conditions across ontogeny (Cavender-Bares & Bazzaz, 2000; Niinemets, 2010; Poorter, 2007). Seedlings typically establish in shaded understories where light is limited, while adult trees grow into the canopy and experience substantially higher light availability (Poorter, 2007; Thomas & Bazzaz, 1999). In addition, seedlings have shallow root systems and thus rely solely on surface soil moisture, while adults have deeper root systems that can access more stable water sources (Cavender-Bares & Bazzaz, 2000; Dawson, 1996; Thomas & Bazzaz, 1999). Sunlight and water availability gradients are expected to influence wood traits because they directly influence hydraulic demand: light drives transpiration and increases tension on the water column, while water availability determines the ability to sustain flow (Zimmermann, 1983). Thus, environmental changes across ontogeny could shift the balance among hydraulic efficiency, hydraulic safety, and mechanical support, potentially altering wood traits. However, whether these environmental gradients have an independent effect on wood traits beyond the dominant effects of plant height remains unclear. Resolving this is important for determining whether wood strategies are solely constrained by intrinsic size-related factors or whether they retain flexibility to local environmental conditions across development.

A limitation in exploring the effects of environment on ontogenetic shifts in wood traits is that much of the variation attributed to ontogeny reflects axial patterns associated with tree height. Since vessel diameters widen predictably from the tip to the base of trees, environmental influences are obscured unless sampling is standardized to remove this pattern (Anfodillo et al., 2013; Hietz et al., 2017; Olson et al., 2014). As a result, it remains unclear whether

environmental gradients across ontogeny play an independent and meaningful role in shaping wood function.

To address this gap, we quantified wood traits, light availability, and water availability across seedling, sapling, and adult developmental stages in two co-occurring temperate angiosperms, sugar maple (*Acer saccharum* Marsh.) and yellow birch (*Betula alleghaniensis* Britt.). By standardizing sampling at a fixed distance from the shoot apex, we minimized confounding effects of wood trait variation associated with tree height. We examined how trait values and multivariate trait coordination differed across developmental stages and investigated whether these differences were driven by shifts in light availability, water availability, or additional effects of tree height. Specifically, we aimed to answer the following questions 1) How do wood traits shift with ontogeny, and 2) Do ontogenetic changes in light and water availability influence wood trait values and their coordination?

METHODS

Study Area

From late-May to mid-August 2024, we collected branch samples and environmental data from sugar maple (*A. saccharum*; n = 60) and yellow birch (*B. alleghaniensis*; n = 60) from Kenauk Nature Reserve (45.65° N, 74.90° W), a northern temperate forest in southwestern, Quebec (Canada). This region has an average annual temperature of 5.3°C, with temperatures ranging from an average minimum of -16.1°C in January to an average maximum of 25.1°C in July. The average annual precipitation is 1204 mm (1991 – 2010 temperature and precipitation averages; Montebello station, Environment & Climate Change Canada). The forest type sampled consisted of mixed, uneven-aged hardwood stands dominated by sugar maple, alongside yellow birch, American beech (*Fagus grandifolia* Ehrh.), American hophornbeam (*Ostrya virginiana* (Mill) K. Koch), and American basswood (*Tilia americana* L.). All samples were collected within a radius of 10 km from each other to minimize site differences across the samples.

Study Species

Sugar maple (*Acer saccharum* Marsh.) and yellow birch (*Betula alleghaniensis* Britt.) are two diffuse-porous hardwood species widely distributed throughout eastern North America, from Nova Scotia west to Manitoba, and from the Appalachian Mountains north into central Ontario and Quebec. Both species are abundant and often co-occur on well-drained, nutrient-rich soils (Post et al., 1969). They occupy cool and moist climates characterized by moderate growing season temperatures (15°C - 25°C) and cold winters, tolerating cold extremes as low as -40°C and -45°C for yellow birch and sugar maple, respectively (Dansereau and Pageau, 1996; Godman et al., 1990). Both species establish in the shaded understory and persist across all stages of succession (Nolet et al., 2008). Sugar maple is highly shade tolerant, with seedlings

often withstanding prolonged periods of suppression before responding to increased light availability (Godman et al., 1990). Yellow birch is moderately shade tolerant and typically regenerates in canopy gaps (Erdmann, 1990; Logan, 1965). Seedlings of both species are sensitive to drought due to their shallow root systems, while mature individuals develop roots that permit access to deeper water sources (Erdmann, 1990; Godman et al., 1990). Sugar maple can reach up to 37 m tall and 400 years old, and yellow birch up to 35 m tall and 300 years old (Godman, 1990). Their transition from shaded, shallow-rooted seedlings to canopy-dominant trees with deep roots makes sugar maple and yellow birch good species for examining ontogenetic shifts in wood traits across environmental gradients.

Branch Sampling

To capture ontogenetic variation in wood traits and environment, we sampled trees to represent three developmental stages that we classified by height: seedlings (0.7 – 2.0 m), saplings (2.5 – 7.5 m), and adults (> 8.0 m). We sampled 20 individuals per developmental stage for each species, for a total of 120 trees. We sampled wood of the main stem at a fixed distance of 70 cm from the tree apex (Figure 1). When there were multiple co-dominant stems in a tree, we sampled a vertical twig near the center of the crown. Sampling from a fixed distance from the apex controls for the known pattern of vessel widening from the apex to base of trees (Olson et al., 2014; Soriano et al., 2020). Cutting at 70 cm captured two or more years of growth which was necessary to capture the 2023 growth year. Twig samples were stored at room temperature and kept dry until they were processed in the lab.

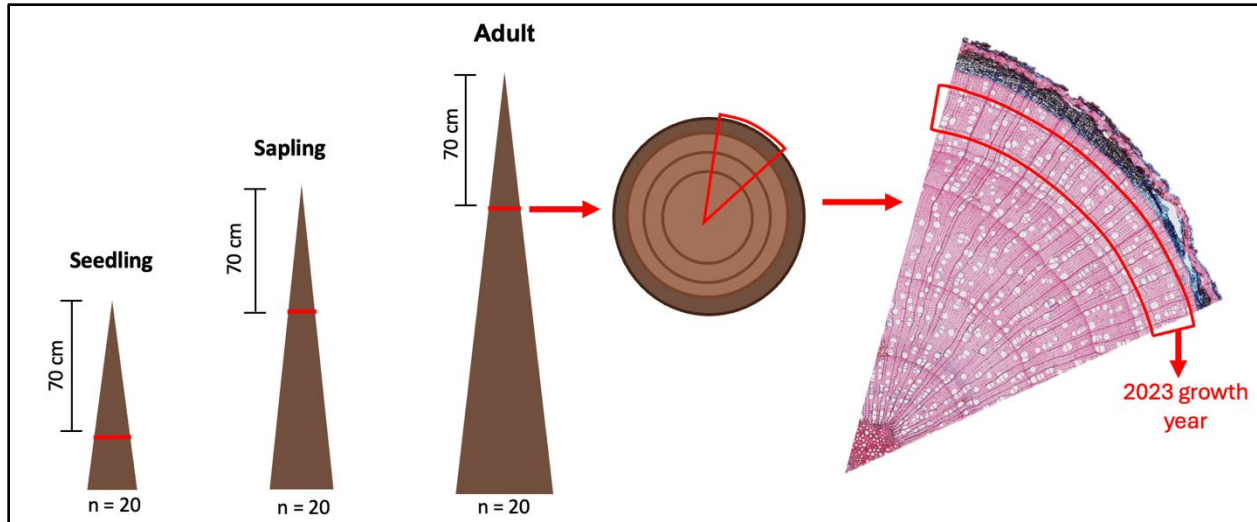


Figure 1. Sampling design for wood anatomical trait measurements across developmental stages. Samples were taken at a fixed distance of 70 cm from the tree apex to standardize for the pattern of tip-to-base vessel widening in trees (Olson et al., 2014). Cross sections were prepared from apical twigs, and wood traits were quantified from a one-eighth segment of the most recent complete growth ring (2023), indicated in red.

Environmental Measurements

To capture the ontogenetic variation in light environment, we estimated light availability using hemispherical photography. Photographs were taken with a 180° lens (4.5mm F2.8 EX DC Circular Fisheye, SIGMA) mounted to a camera (EOS Rebel T7i, Canon). The lens was positioned vertically facing the sky and directly above the foliage of the main stem. For adults and tall saplings, the lens was positioned above the tree crown using a telescopic pole (HV-245, Hastings). All photographs were taken under diffuse light conditions (before sunrise or under overcast skies) to prevent direct sunlight from saturating the image and being misclassified as canopy gaps. Hemispherical photographs were analyzed using Gap Light Analyzer v. 2.0 to estimate percent total transmitted irradiance (from hereon, irradiance), defined as the proportion of potential above-canopy radiation that reaches the tree after transmission through the canopy (Frazer et al., 1999).

To capture ontogenetic variation in water availability, we measured predawn leaf water potential (Y_{PD}) with a Scholander pressure chamber (Scholander et al., 1965). During predawn (between 0100 and 0500 hours), leaf Y acts as a proxy for soil Y because before sunrise, stomata are closed, transpiration ceases, and the Y continuum from the soil to the leaves is at equilibrium (Hinckley et al., 1978; Jordan & Ritchie, 1971). To take Y_{PD} measurements, we collected fully expanded upper canopy leaves and placed them into the pressure chamber within 15 seconds from excision (Rodriguez-Dominguez et al., 2022). With the leaf in place, we gradually increased the pressure in the chamber with nitrogen gas until a drop of water extruded from the petiole, indicating that the pressure in the chamber was equal to the Y of the leaf (Figure 2). Measurements were taken on 3 leaves per tree.

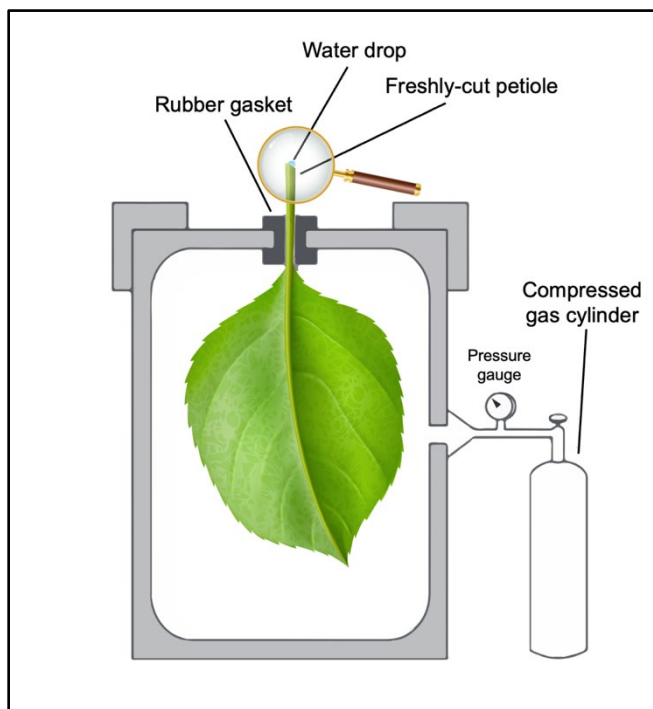


Figure 2. Schematic of a Scholander pressure chamber used to measure predawn leaf water potential (Ψ_{PD}). Sampled leaves were placed inside the chamber with the freshly cut petiole protruding from the rubber gasket. The chamber was sealed, and nitrogen gas was added until a drop of water appeared at the surface of the petiole, indicating the pressure in the chamber was equal to the pressure of the leaf. All measurements were taken between 0100 and 0500 hours (Pérez-Harguindeguy et al., 2013).

Covariates

I measured the following covariates for each tree sampled: age, height, stem diameter, age of the sampled twig (from hereon, twig age), microtopography, slope angle, soil organic layer thickness (OLT), and volumetric soil water content (VWC). Tree age was approximated by counting annual growth rings. For seedlings and saplings, rings were counted from cross-sections of the main stem cut at the base of the tree. For adults, rings were counted from increment cores taken at 1.37 m above ground (breast height). Tree height was calculated with trigonometry based on vertical angles to the top and base of the tree, measured with a clinometer (PM-5, Suunto). Stem diameter was measured at ground height for seedlings, at both ground height and breast height for saplings, and at breast height for adults. Twig age was determined by counting rings in cross sections cut at 70 cm from the twig apex. Microtopography was classified categorically based on whether each tree was positioned in a hollow, hummock, flat, or midslope position. Slope angle was estimated by measuring the angles to the highest and lowest elevation points within 3 m of the tree and averaging these values. Both OLT and VWC were measured at three points located 30 cm from the tree stem, spaced 60° apart, and averaged. Organic layer thickness (OLT) was measured by exposing the organic layer with a soil knife and measuring it's thickness with a measuring tape. Volumetric water content (VWC) was measured using a 5 cm soil moisture probe (TEROS 11 + 12, METER Group).

Wood Trait Measurements

To quantify wood traits, we cut 12 μm -thick cross sections of twig samples at the 70 cm mark using a rotary microtome (HistoCore MULTICUT, Leica). Cut sections were stained with a solution of Safranin and AstraBlue (1% and 0.5% in distilled water, respectively). This combination of stains distinguishes between lignified and non-lignified tissues; safranin stains lignified tissue (xylem, secondary cell walls, etc.) a reddish pink, and AstraBlue stains non-lignified tissues (parenchyma cells, phloem, etc.) blue. Stained sections were washed with distilled water and then gradually dehydrated in ethanol (50%, 75%, 99% ethanol solutions), before being permanently fixed to a microscope slide with Eukitt UV (following von Arx et al., 2016). Slides were then photographed with a scanning microscope (Axioscan 7, ZEISS) and uploaded to ROXAS v. 4.0 (von Arx & Carrer, 2014) where images were analyzed to quantify six traits that represent hydraulic efficiency, hydraulic safety, and mechanical support (Table 1) for the 2023 growth year (Figure 1). We analyzed the 2023 growth year because it provides the most recent complete year of growth that can be paired to the environmental variables measured in 2024. Due to limits in computer processing power, image analysis was limited to a randomly selected one-eighth of the 2023 growth ring within the area of best image quality (Figure 1).

Table 1. Wood anatomical traits and their abbreviation, units, and associated physiological functions.

Trait	Abbreviation	Units	Description
Theoretical maximum xylem specific conductivity	K _s	m ² × s ⁻¹ × MPa ⁻¹	A proxy for hydraulic efficiency. Theoretical maximum conductivity (K _h) calculated from the Hagen-Poiseuille equation of water transport through pipes: $K_h = (\frac{\pi\rho}{128\eta} \sum d^4)$, where d is vessel diameter, and ρ and η are the density and viscosity of water, respectively (Tyree & Ewers, 1991). K _h is divided by sapwood area to obtain a relative measurement (K _s).
Hydraulically weighted vessel diameter	D _h	mm	↑ Hydraulic efficiency and ↓ safety. Calculated from the Hagen-Poiseuille equation of water transport through pipes: $D_h = ((\sum d^4)/n)^{1/4}$. Where d is the diameter of each of the n conduits. Large vessels promote greater efficiency of water flow because K _h increases to the 4 th power of vessel diameter (Tyree et al., 1994). Large vessels are more vulnerable to freeze-thaw induced cavitation (Davis et al., 1999), and in some cases drought-induced cavitation (Hacke et al., 2017).
Vessel number	VN	vessel # × mm ⁻²	↑ Hydraulic safety. Greater VN provides increased redundancy in the event of vessel failure from either drought or freeze-thaw-induced cavitation (Ewers et al., 2007).
Lumen fraction	F	%	↑ Hydraulic efficiency. The fraction of sapwood area occupied by vessel lumens.
Vessel reinforcement index	$(t/b)^2$	unitless (mm × mm ⁻¹)	↑ Hydraulic safety. Vessel reinforcement against collapse from tension (implosion). Calculated from t , the thickness of adjoining vessel cell walls, and b , maximum vessel lumen diameter (Hacke et al., 2001). Greater $(t/b)^2$ values are associated with greater resistance to drought-induced cavitation ($R^2=0.81$ in Hacke et al., 2001, $R^2 = 0.95$ in Jacobsen et al., 2005).
Fiber cell wall thickness	FWT	mm	↑ Mechanical support and ↑ hydraulic safety. Thicker cell walls provide greater structural support and are associated with reduced risk of drought-induced cavitation ($R^2 = 0.89$; Jacobsen et al., 2005).

Data Analysis

All statistical analyses were conducted in R (R Core Team, 2025) and were performed separately for each species. All measured covariates were tested during preliminary analyses. Their inclusion did not improve model fit or alter the interpretation of results, so they were not included in final models

To determine if wood trait values shifted with ontogeny, we performed analysis of variances (ANOVAs) on each of the six wood traits as a function of developmental stage using the *aov()* function. To meet assumptions of normality and homoscedasticity, vessel reinforcement ($(t/b)^2$) values were $\log_{10}$ -transformed. When ANOVA results were significant, we assessed pairwise differences among developmental stages with post-hoc Tukey tests using the *TukeyHSD()* function. Significant differences in mean trait values between at least two developmental stages were taken as evidence of an ontogenetic shift in that wood trait.

To assess if wood trait coordination changed with ontogeny, we conducted principal component analyses (PCA) for each developmental stage and compared the strength of trait coordination with relative eigenvalue variance (Vrel). Using the *pca()* function in the *vegan* package, PCAs were used to visualize the structure of trait coordination for each developmental stage and identify the primary axes of trait variation. All variables were standardized (z-scored) prior to analysis. To complement this visual assessment, we quantified the strength of trait covariation within each stage with relative eigenvalue variance (Vrel), using the *integration.Vrel()* function in the *geomorph* package (Adams et al., 2024; Conaway & Adams, 2022). Vrel measures the dispersion of eigenvalues from a correlation matrix and provides an effect size representing the magnitude of trait covariation. Differences in covariation strength among developmental stages were assessed using pairwise comparisons of Vrel with the

compare.ZVrel() function. Patterns of trait coordination were further explored by examining pairwise Pearson correlations among traits within each developmental stage using the *cor()* function.

Before determining if ontogenetic shifts in mean wood trait were driven by changes in water or light availability, we first tested the assumption that irradiance and Y_{PD} increased across developmental stages using ANOVA and post-hoc Tukey tests. To meet assumptions of normality and within group homoscedasticity, irradiance values were $\log_{10}$ -transformed. Since the assumption of homoscedasticity was still not met for irradiance in sugar maple, we instead fit generalized least squares (GLS) models using the *gls()* function in the *nlme* package, allowing residual variance to differ among developmental stages with a *varIdent()* variance structure (Pineiro et al., 2024). If irradiance or Y_{PD} values differed significantly among stages and were lowest in seedlings and highest in adults, we concluded that light or water availability increased across ontogeny.

To test whether increases in light or water availability explained ontogenetic shifts in wood traits, we fit linear models of each trait as a function of Y_{PD} or irradiance with the *lm()* function. Since irradiance covaried significantly with tree height (sugar maple: $R^2 = 0.39$, $P < 0.001$, yellow birch: $R^2 = 0.39$, $P < 0.001$), we fit multiple regression models including both $\log_{10}$ -transformed irradiance and $\log_{10}$ -transformed height as predictors to disentangle their independent effects. Using the *vif()* function from the *car* package, we confirmed that variance inflation factor (VIF) values were < 3 , indicating that multicollinearity between predictors was low (Fox & Weisberg, 2019; Zuur et al., 2010). We used the *varpart()* function in the *vegan* package to partition the explained variance (R^2) into the proportion uniquely explained by each of irradiance and height, and the proportion explained by their shared covariance. Ontogenetic

shifts in each trait were attributed to changes in light or water availability when 1) irradiance or Y_{PD} increased across developmental stages and 2) irradiance or Y_{PD} explained a large portion of variation in that wood trait.

Since irradiance increased with height (sugar maple: $R^2 = 0.39$, $P < 0.001$, yellow birch: $R^2 = 0.39$, $P < 0.001$), height could obscure a relationship between irradiance and wood traits. Thus, we also performed linear models within developmental stages to assess the effect of irradiance on the traits, irrespective of tree height. Within stages, the correlation between height and irradiance was weak ($|R^2| \leq 0.3$), allowing the effect of irradiance on wood traits to be evaluated independently of height.

To test whether light and water availability explained shifts in ontogenetic wood trait coordination, we conducted redundancy analyses (RDA) using the *rda()* function in the *vegan* package (Oksanen et al., 2025). RDA models included matrices of six wood traits as the response variable and $\log_{10}$ -transformed irradiance, Y_{PD} , and $\log_{10}$ -transformed height as predictors. All variables were standardized (z-scored) prior to analyses. Tree height was used as a continuous measure of ontogeny rather than developmental stage, as the latter integrates variation in both irradiance and height; two factors we are trying to disentangle. Including both height and irradiance in the same model allowed their shared and unique contributions on trait coordination to be disentangled. The significance of the overall model and individual predictors was tested using permutation tests (999 permutations) with the *anova.cca()* function. We evaluated the contribution of each predictor using marginal tests (*anova.cca(..., by = "margin")*), which quantifies the variation in trait covariation uniquely explained by each predictor after accounting for its shared variance with others. Ontogenetic shifts in the strength of trait coordination were attributed to light and water availability if 1) irradiance or Y_{PD} increased across developmental

stages and 2) irradiance or Y_{PD} explained a large proportion of trait covariation, after accounting for tree height.

RESULTS

Do Trait Values Shift Across Ontogeny?

In sugar maple, four out of six traits, lumen fraction (F), vessel number (VN), vessel diameter (D_h), and vessel reinforcement index $((t/b)^2)$, differed significantly among developmental stages (one-way ANOVA, $P \leq 0.03$; Table 2; Figure 3), whereas fiber cell wall thickness (FWT) and theoretical maximum conductivity (K_s) did not differ (one-way ANOVA, $P > 0.05$). Of the four traits that differed with stage, F , VN, and $(t/b)^2$ demonstrated a monotonic shift across ontogeny, with seedlings differing from adults and saplings exhibiting intermediate values; F , VN, and $(t/b)^2$ increased by 62.8%, 131.1%, and 20.4 %, respectively. In D_h , seedlings and saplings did not differ, but adult vessels were 19% smaller than both (Tukey HSD, $P \leq 0.02$).

In yellow birch, all six wood traits differed among developmental stages (one-way ANOVA, $P < 0.05$; Table 2; Figure 3). Fiber wall thickness (FWT), F , and VN showed monotonic trends, with seedlings differing from adults and saplings exhibiting intermediate values. Specifically, FWT decreased by 10% across ontogeny, while F and VN increased by 40% and 58%, respectively (Tukey HSD, $P \leq 0.03$). In contrast, K_s , $(t/b)^2$, and D_h exhibited non monotonic patterns. Specifically, K_s increased by 55% from seedlings to saplings (Tukey HSD, $P = 0.04$), but then declined in adults, resulting in no significant difference between adults and the other two stages. Vessel reinforcement index $((t/b)^2)$ was highest in adults but differed significantly only from saplings (+16%; Tukey HSD, $P = 0.03$), not seedlings. Lastly, D_h did not

differ between seedlings and either saplings or adults but was significantly lower in adults than saplings (-19%; Tukey HSD, $P = 0.03$).

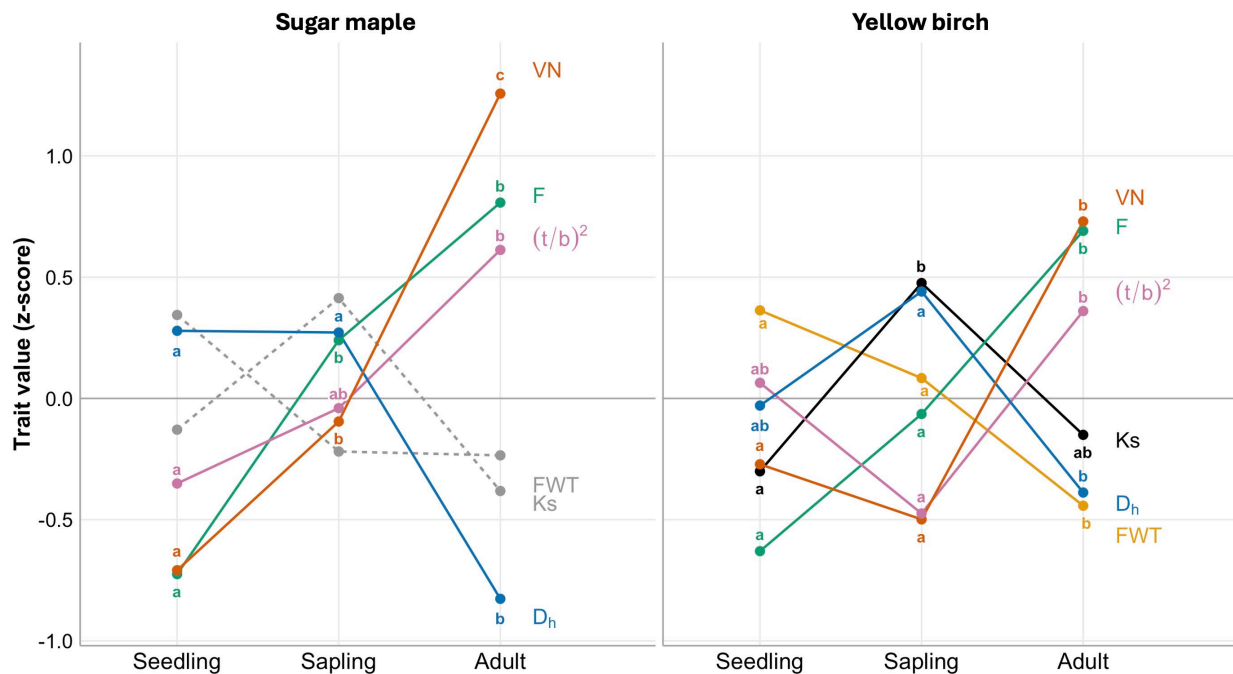


Figure 3. Mean wood trait trajectories of sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*) across seedling, sapling, and adult developmental stages. Values represent z-scored trait means ($n = 13 - 20$) and each line represents one trait. Pairwise significance among developmental stages is indicated with Tukey letters (a, b, c; $P < 0.05$). Non-significant differences are represented by grey and dashed lines. VN = vessel number (vessel # · mm^{-2}), F = lumen fraction (%), $(t/b)^2$ = vessel reinforcement index, FWT = fiber cell wall thickness (μm), K_s = theoretical maximum xylem-specific conductivity ($\text{m}^2 \cdot \text{s}^{-1} \cdot \text{MPa}^{-1}$), D_h = hydraulically weighted vessel diameter (μm).

Table 2. Wood trait values of seedling, sapling, and adult developmental stages for sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*). Values are represented as means $\pm$ standard error. Significant *P* indicates differences among developmental stages for a given trait (ANOVA, $\alpha = 0.05$, $n = 12-20$). Pairwise significance is represented by letters in superscript (Tukey HSD, $\alpha = 0.05$). Significant results are bolded.

Species	Wood Trait	<i>P</i>	Developmental Stage (mean $\pm$ SE)		
			Seedling	Sapling	Adult
Sugar maple	FWT (μm)	0.156	1.40 $\pm$ 0.03	1.34 $\pm$ 0.02	1.34 $\pm$ 0.02
	Ks ($\text{m}^2 \cdot \text{s}^{-1} \cdot \text{MPa}^{-1}$)	0.081	5.4e-04 $\pm$ 6.2 x 10 ⁻⁵	6.8e-04 $\pm$ 6.5 x 10 ⁻⁵	4.7e-04 $\pm$ 5.0 x 10 ⁻⁵
	F (%)	<0.001	8.93 $\pm$ 0.67 ^a	12.46 $\pm$ 0.72 ^b	14.54 $\pm$ 0.80 ^b
	D _h (μm)	0.003	11.9 $\pm$ 0.5 ^a	11.9 $\pm$ 0.5 ^a	9.6 $\pm$ 0.4 ^b
	VN (vessel # $\cdot$ mm ⁻²)	<0.001	950 $\pm$ 65 ^a	1360 $\pm$ 100 ^b	2250 $\pm$ 160 ^c
	log ₁₀ (<i>t/b</i>) ² ($\mu\text{m} \cdot \mu\text{m}^{-1}$)	0.029	-1.03 $\pm$ 0.05 ^a	-0.96 $\pm$ 0.05 ^{ab}	-0.82 $\pm$ 0.05 ^b
Yellow birch	FWT (μm)	0.002	1.36 $\pm$ 0.06 ^a	1.31 $\pm$ 0.03 ^a	1.22 $\pm$ 0.01 ^b
	Ks ($\text{m}^2 \cdot \text{s}^{-1} \cdot \text{MPa}^{-1}$)	0.042	8.0e-04 $\pm$ 1.3 x 10 ^{-4a}	1.23e-03 $\pm$ 1.4 x 10 ^{-4b}	8.8e-04 $\pm$ 1.0 x 10 ^{-4ab}
	F (%)	<0.001	10.30 $\pm$ 0.49 ^a	12.04 $\pm$ 0.76 ^a	14.37 $\pm$ 0.56 ^b
	D _h (μm)	0.038	14.1 $\pm$ 1.1 ^{ab}	15.8 $\pm$ 0.6 ^a	12.8 $\pm$ 0.6 ^b
	VN (vessel # $\cdot$ mm ⁻²)	<0.001	870 $\pm$ 110 ^a	760 $\pm$ 90 ^a	1370 $\pm$ 97 ^b
	log ₁₀ (<i>t/b</i>) ² ($\mu\text{m} \cdot \mu\text{m}^{-1}$)	0.038	-1.19 $\pm$ 0.08 ^{ab}	-1.34 $\pm$ 0.05 ^a	-1.12 $\pm$ 0.05 ^b

Does Trait Coordination Shift Across Ontogeny?

In both species and all developmental stages, the primary axis of variation (PC1) consistently reflected a hydraulic efficiency-safety axis (Figure 4; Table S1 & S2). This axis captured 43% to 62% of the total trait variation, with D_h and K_s acting in one direction and VN and $(t/b)^2$ acting in the other direction. Individuals at one end of this axis were characterized by a low density of large vessels and high conductivity (hydraulically efficient), while individuals at the opposite end had a higher density of small, reinforced vessels (hydraulically safe). Secondary axes (PC2 and PC3) captured orthogonal dimensions of trait variation. PC2 explained 19 – 30% of variation and was mainly driven by F (Figure 4; Table A1 & A2). PC3 explained 8 – 14% of variation and was mainly driven by FWT (Table A1 & A2).

While trait coordination structure was largely conserved across ontogeny, the proportion of variance explained by PC1 increased with developmental stage in sugar maple from 41% in seedlings to 50% in saplings and 61% in adults, indicating a progressive strengthening of trait integration (Figure 4). Supporting this pattern were increasingly large V_{rel} values from seedlings to adults (seedling = 0.16 < sapling = 0.23 < adult = 0.34). Increasing trait integration across ontogeny was driven by strengthening correlations among hydraulic traits and a reorganization of fiber–hydraulic trait relationships (Figure 5). The positive correlation between D_h and K_s increased, while the negative correlations between D_h and both VN and $(t/b)^2$ became more negative. In addition, FWT became correlated with PC1 traits in adults, including a positive correlation with $(t/b)^2$ and a negative correlation with D_h (Figure 5).

In contrast to sugar maple, changes in trait coordination in yellow birch did not follow a clear directional trend toward increasing integration across ontogeny. The strength of trait integration was highest in seedlings (63%), followed by adults (54%) then saplings (49%), with

391 Vrel values showing a similar pattern (Figure 4; seedling Vrel = 0.33, sapling Vrel = 0.23, adult
 392 Vrel = 0.27). In seedlings, relatively high integration was driven by strong coordination among
 393 hydraulic traits and positive correlations among FWT and both D_h and K_s (Figure 5). However,
 394 these relationships weakened in saplings and only partially re-emerged in adults, with F and
 395 FWT remaining inconsistent.

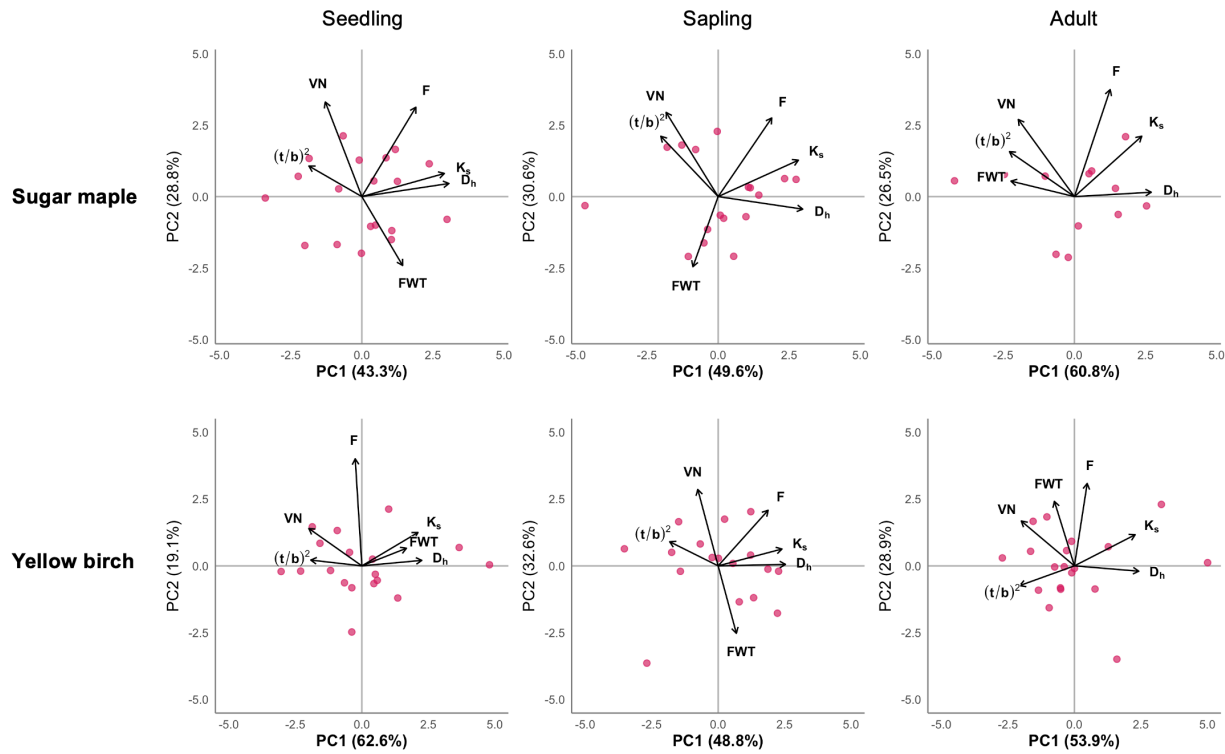


Figure 4. Principal component analyses (PCAs) of wood traits across seedling, sapling, and adult developmental stages in sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*). Points represent individual trees and arrows represent trait loadings. Arrow length reflects the strength of each trait's contribution to principal components (PC1 and PC2) and arrow direction shows the relationships among traits. The percentage of variance explained by each axis is shown in parentheses. The primary axis (PC1) represents a hydraulic efficiency–safety trade-off: traits associated with larger, more conductive vessels (Dh, Ks) contrast against those associated with greater hydraulic safety (VN, $(t/b)^2$). VN = vessel number (vessel # · mm⁻²), F = lumen fraction (%), $(t/b)^2$ = vessel reinforcement index, FWT = fiber cell wall thickness (μm), Ks = theoretical maximum xylem-specific conductivity (m² · s⁻¹ · MPa⁻¹), Dh = hydraulically weighted vessel diameter (μm).



Figure 5. Correlation matrices among wood traits in sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*) across developmental stages. Values represent Pearson's correlation coefficients (r). Significance is indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, * $P < 0.001$). Significance of Pearson correlations was assessed using t-tests, with P -values adjusted for multiple comparisons using Benjamini–Hochberg correction. Non-significant correlations are displayed with reduced opacity. Color indicates the strength and direction of correlations. VN = vessel number (vessel # $\cdot$ mm⁻²), F = lumen fraction (%), $(t/b)^2$ = vessel reinforcement index, FWT = fiber cell wall thickness (μ m), K_s = theoretical maximum xylem-specific conductivity (m² $\cdot$ s⁻¹ $\cdot$ MPa⁻¹), D_h = hydraulically weighted vessel diameter (μ m).**

Does the Environment Shift Across Ontogeny?

To test my assumption that trees experience contrasting microenvironments across ontogeny, we compared irradiance and Y_{PD} among developmental stages. As expected, irradiance increased across ontogeny (Table 3; Figure 6). In sugar maple, the irradiance experienced by each developmental stages differed significantly from one another, increasing from 6% in seedlings to 8% in saplings to 39% in adults (Tukey HSD, $P \leq 0.03$). In yellow birch, seedlings and saplings experienced similar irradiance (6 and 10%, respectively, $P > 0.05$) and both stages experienced significantly lower irradiance than adults (37%; Tukey HSD, $P < 0.001$). Contrary to expectations, water availability measured as Y_{PD} did not differ among developmental stages in either species (Table 3; Figure 6).

Table 3. Irradiance and predawn leaf water potential values (Y_{PD}) of seedling, sapling, and adult developmental stages for sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*). Values are represented as means $\pm$ standard error. Significant P indicates differences among developmental stages for a given variable (one-way ANOVA, $n = 20$). Pairwise significance is represented by letters in superscript (Tukey HSD, $P < 0.05$). Significant results are bolded.

Species	Variable	P	Developmental Stage (mean $\pm$ SE)		
			Seedling	Sapling	Adult
Sugar maple	$\log_{10}$ Irradiance (%) [*]	<0.001	0.77 ± 0.04^a	0.89 ± 0.03^b	1.45 ± 0.09^c
	Y_{PD} (MPa)	0.612	0.27 ± 0.03	0.25 ± 0.02	0.34 ± 0.06
Yellow birch	$\log_{10}$ Irradiance (%)	<0.001	0.75 ± 0.05^a	0.88 ± 0.07^a	1.46 ± 0.07^b
	Y_{PD} (MPa)	0.657	0.47 ± 0.06	0.39 ± 0.03	0.45 ± 0.04

^{*}Analyzed using generalized least squares (GLS) models with stage-specific variance structures instead of ANOVA. Significance was assessed using likelihood ratio tests

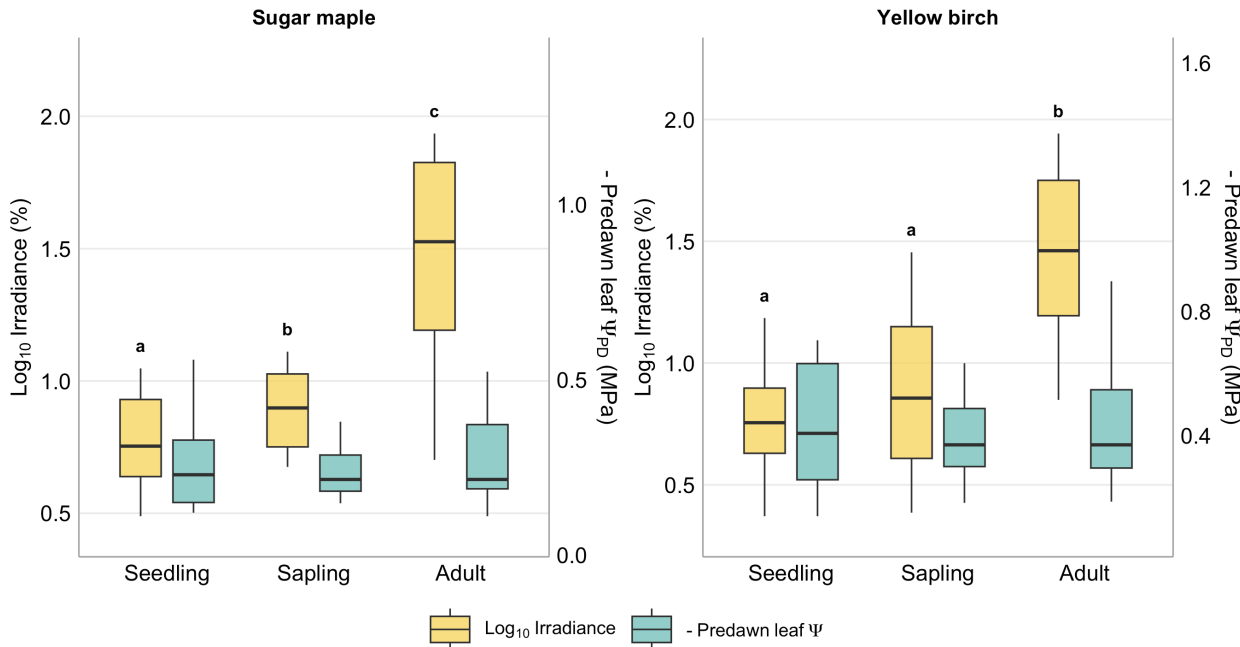


Figure 6. Boxplots of $\log_{10}$ -transformed irradiance (%) and predawn leaf water potential (Y_{PD}) across seedling, sapling, and adult developmental stages of sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*). Boxes represent the interquartile range, horizontal lines represent the median, and whiskers extend to the most extreme values. Letters (a, b, c) indicate significant differences among developmental stages (one-way ANOVA + Tukey HSD, $P < 0.05$, $n = 20$) within each species. The absence of letters indicates no significant difference. Predawn leaf water potential (Y_{PD}) values were multiplied by -1 for visualization; higher plotted values represent drier soil conditions.

Are Shifts in Traits Across Ontogeny Associated with Shifts in Environment?

To test whether increases in irradiance explained ontogenetic shifts in wood trait values, we regressed each trait as a function of irradiance and height. In both species and in all traits, the unique effect of irradiance explained a minimal amount of the total variance (Figure 7; Table 4). Even for traits where the model explained significant total variance (e.g., VN and F in sugar maple), the unique effect of irradiance was negligible, with $R^2 < 0.04$ in all traits (Table 4; $P > 0.05$). For the traits F and VN in sugar maple, the unique effect of height was moderate (26% and 27%, respectively), as was the shared effect of height and irradiance (17% and 29%, respectively; Table 4; Figure 7). In yellow birch, the unique effect of irradiance explained a small amount of variation in F ($R^2 = 0.07$, $P = 0.01$) and the unique effect of height was marginally significant ($R^2 = 0.04$, $P = 0.054$). Overall, the unique effect of irradiance only explained one trait across both species. Even within developmental stages, where the correlation between irradiance and height was weak ($|R^2| \leq 0.3$), irradiance did not explain variation in wood traits in either species ($R^2 < 0.05$, $P > 0.05$), indicating that even though irradiance increases across ontogeny, it does not drive shifts in wood trait variation.

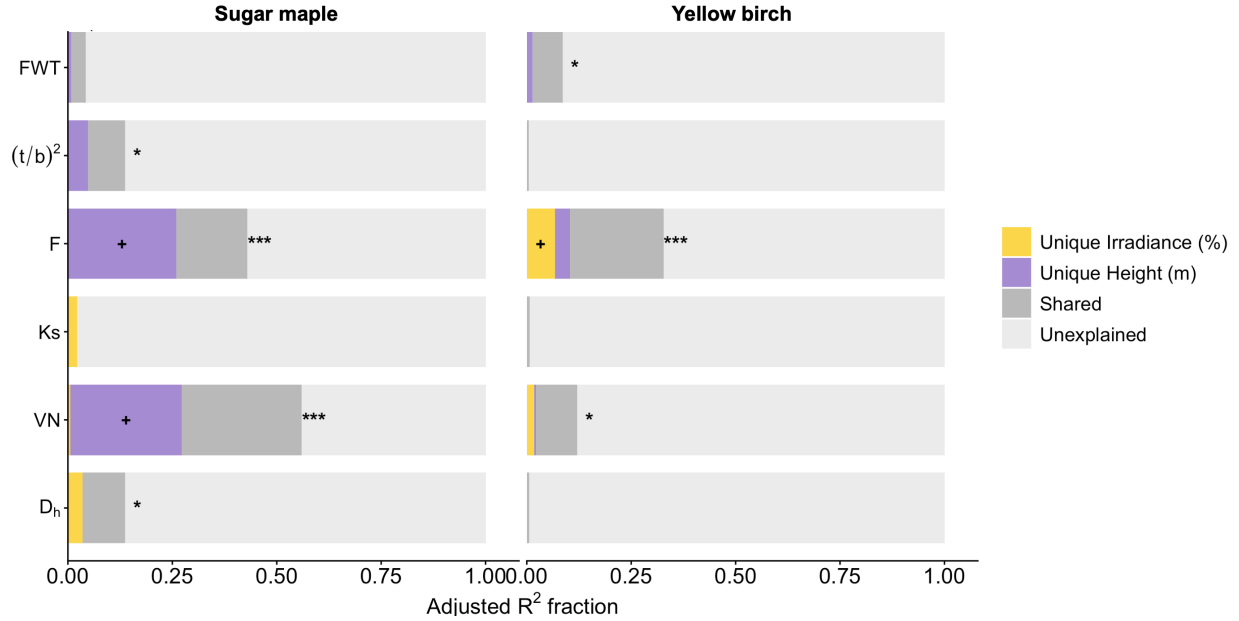


Figure 7. Variance partitioning of wood trait variation explained by $\log_{10}$ -transformed irradiance (%) and $\log_{10}$ -transformed tree height (m) of sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*). Bars show the proportion of adjusted R^2 attributed to the unique effects of irradiance (yellow), the unique effects of height (pink), their shared covariance (dark grey), and unexplained variance (grey). Plus symbols (+) denote significant positive unique effects of either irradiance or height ($P < 0.05$), while the absence of a symbol indicates non-significant unique effects. Asterisks denote the overall significance of the full model (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

To test if water availability affected trait variation, we regressed each trait as a function of Ψ_{PD} and none of the relationships were significant ($P > 0.05$). Predawn leaf water potential (Ψ_{PD}) values indicated that soil moisture was abundant in both species and all developmental stages (mean $\Psi_{PD} > -0.5$ MPa) and did not significantly differ among stages (Table 3; Figure 6).

Consistent with univariate results, multivariate analyses showed no effect of irradiance or Y_{PD} on wood trait coordination (Figure 8). Redundancy analyses (RDA) including irradiance, height, and Y_{PD} as predictors showed overall model significance for both sugar maple ($P = 0.001$) and yellow birch ($P = 0.013$; Figure 8; Table A3), indicating that the shared covariance of

485 predictors explained significant trait covariation. However, irradiance alone did not explain
486 variation in trait covariation (marginal tests: sugar maple, $P = 0.379$; yellow birch, $P = 0.123$),
487 nor did Ψ_{PD} (marginal tests: sugar maple, $P = 0.706$; yellow birch, $P = 0.651$). In contrast, tree
488 height alone explained a significant but small amount of variation in sugar maple ($R^2 = 11.4\%$; P
489 $= 0.001$), but not in yellow birch ($P = 0.469$). Overall, these results suggest that ontogenetic
490 shifts in wood traits in these species were not driven by shifts in irradiance or water availability.

Table 4. Variance partitioning results showing the proportion of variation (R^2) in each wood trait explained by irradiance and tree height in sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*). Total adjusted R^2 (Total R^2) represents the total explained variance from models including both predictors. R^2 irradiance and R^2 Height represent the unique contributions of $\log_{10}$ -transformed irradiance (%) and $\log_{10}$ -transformed tree height (m), respectively, after accounting for their shared covariance. The variance jointly explained by irradiance and height is represented by R^2 Shared. P -values indicate the significance of regression coefficients for irradiance and height from linear models ($n = 60$ for each species). Significant independent effects are bolded.

Species	Trait	Total R^2	R^2 Irradiance	R^2 Height	R^2 Shared	P Irradiance	P Height
Sugar maple	FWT (μm)	0.025	0.018	0.008	0.036	0.718	0.250
	Ks ($\text{m}^2 \cdot \text{s}^{-1} \cdot \text{MPa}^{-1}$)	0.001	0.022	0.001	0.020	0.162	0.333
	F (%)	0.417	0.012	0.259	0.170	0.871	<0.001
	D _h (μm)	0.136	0.035	0	0.101	0.096	0.329
	VN (vessel # $\cdot \text{mm}^{-2}$)	0.559	0.006	0.267	0.287	0.212	<0.001
	$\log_{10}(t/b)^2$ ($\mu\text{m} \cdot \mu\text{m}^{-1}$)	0.126	0.011	0.048	0.088	0.509	0.066
Yellow birch	FWT (μm)	0.081	0.005	0.014	0.072	0.400	0.185
	Ks ($\text{m}^2 \cdot \text{s}^{-1} \cdot \text{MPa}^{-1}$)	0.025	0.019	0.013	0.007	0.930	0.572
	F (%)	0.327	0.068	0.036	0.224	0.014	0.054
	D _h (μm)	0.016	0.004	0.018	0.006	0.374	0.882
	VN (vessel # $\cdot \text{mm}^{-2}$)	0.120	0.018	0.004	0.098	0.155	0.269
	$\log_{10}(t/b)^2$ ($\mu\text{m} \cdot \mu\text{m}^{-1}$)	0.027	0.012	0.019	0.005	0.540	0.957

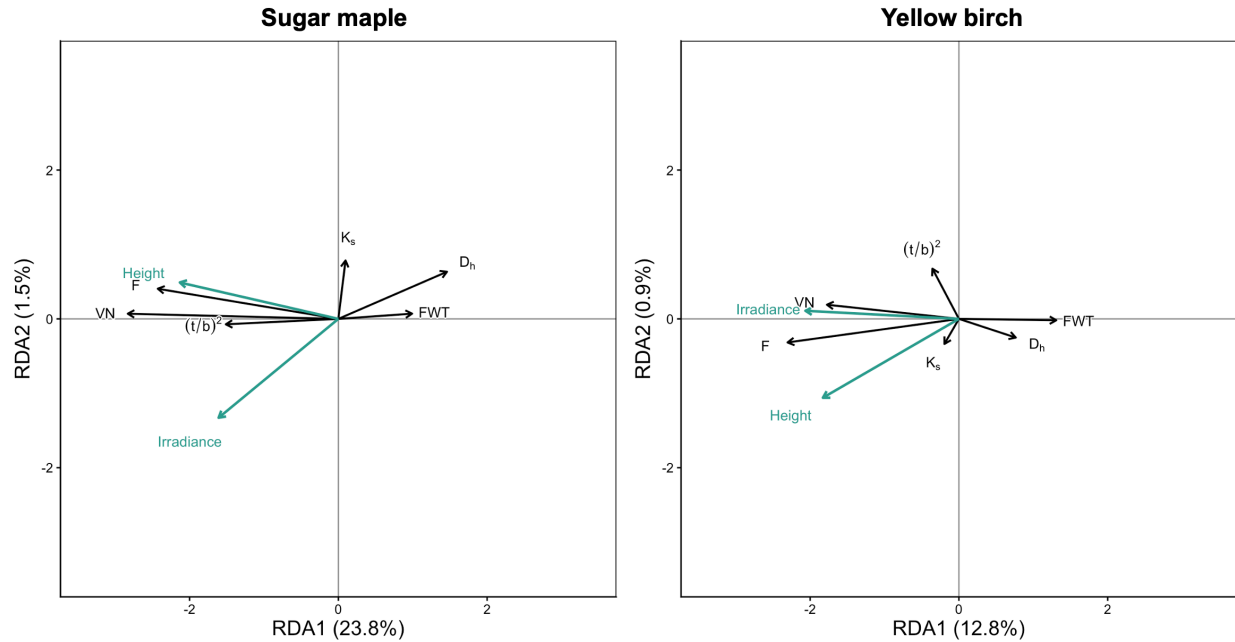


Figure 8. Redundancy analyses (RDA) of wood traits as a function of $\log_{10}$ -transformed irradiance (%) and, $\log_{10}$ -transformed height (m), and predawn leaf water potential (Y_{PD} ; MPa) for sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*). Arrows represent loadings for wood traits (black), of $\log_{10}$ -transformed irradiance (blue), and $\log_{10}$ -transformed height (blue). Y_{PD} explained no variation and is not shown. Arrow length reflects the strength of each variable's contribution of axes of variation (RDA1 and RDA2) and arrow direction shows the relationships among traits and predictor variables. The percentage of variance explained by RDA1 and RDA2 is shown in parentheses. In both species, only RDA1 is significant ($P < 0.05$).

DISCUSSION

Ontogenetic Shifts in Environment

Across ontogeny, irradiance increased in both sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*), whereas no differences in water availability were detected among developmental stages. As expected for these shade-tolerant species, irradiance increased across ontogeny, with relatively small differences between seedlings and saplings that remained in the understory, and a pronounced increase from saplings to adults, corresponding with the transition of adults into the sun-exposed upper canopy (Figure 6) . In contrast, we did not detect difference in water availability because sampling occurred during a wet summer. The summer of 2024 experienced high precipitation and predawn leaf water potential (Y_{PD}) values indicated abundant water availability across all developmental stages and species (mean $Y_{PD} > -0.5$ MPa; Figure 6).

Since seedlings and adult trees typically access different water sources, with seedlings relying solely on surface soil moisture and adults accessing deeper reserves, differences in water availability detected by Y_{PD} should only emerge under conditions where surface soils are dry (Cavender-Bares & Bazzaz, 2000; Dawson, 1996). The absence of such differences in this study do not imply that water availability is unimportant in shaping wood traits in these species. Rather, hydraulic traits may be under selection during drought years where soil Y reach critical thresholds. To better assess the role of water limitation in driving ontogenetic shifts in wood traits, long term observational studies or experimental studies are needed to evaluate these relationships under drier conditions.

Ontogenetic Shifts Toward Hydraulic Safety While Efficiency is Maintained

Both species exhibited coordinated trait shifts towards a hydraulically safer strategy across ontogeny. This was demonstrated by decreases in vessel diameter (D_h), increases in vessel

reinforcement ($((t/b)^2)$), and increases vessel number (VN) from seedlings to adults (Figure 3). Smaller-diameter vessels are less vulnerable to embolism through two mechanisms. First, during freeze-thaw cycles, small vessels form smaller air bubbles that are more likely to dissolve upon thawing instead of expanding to form embolisms (Ewers, 1985; Mayr & Sperry, 2010; Pittermann & Sperry, 2006). Second, wider conduits are more vulnerable to air-seeding through pit membranes under drought (Hacke & Sperry, 2001; Wheeler et al., 2005). Furthermore, greater $(t/b)^2$ enhances xylem safety by reducing cell wall deformation under tension, which can lower the likelihood of air-seeding through pit membranes under drought (Hacke et al., 2001; Jacobsen et al., 2005; Lens et al., 2011). Lastly, increased VN enhances safety by increasing vessel redundancy, reducing the impact of individual vessel failure on overall hydraulic conductivity (Ewers et al., 2007). Together, shifts in these three traits indicate that the wood of apical branches in adult trees is more resistant to freezing and drought stress compared to seedlings and saplings.

Importantly, the shift towards hydraulically safer wood structure across ontogeny did not correspond with a decrease in hydraulic efficiency. Given that hydraulic conductivity scales with the fourth power of vessel diameter, even small reductions in D_h leads to large reduction in hydraulic efficiency (Appendix 1; Tyree et al., 1994; Zimmermann, 1983). However, the concurrent increases in both VN and F represent compensatory adjustments to maintain efficiency across developmental stages, as reflected by the lack of a decline in K_s when comparing seedlings with adults of both species. These results contrast the classic expectation of a trade-off with between hydraulic efficiency and safety (Zimmermann, 1983), and instead support growing evidence that the loss of efficiency associated with reductions in D_h can be offset by adjustments in other wood traits (Burgess et al., 2006; Gleason et al., 2016; Zanne et

al., 2010). Thus, while safety and efficiency may trade off at the level of individual vessels and among species, it did not constrain hydraulic function at the tissue scale in sugar maple and yellow birch (Hacke et al., 2006).

Although seedlings are widely considered vulnerable to drought (Cavender-Bares & Bazzaz, 2000; Dawson, 1996; Donovan & Ehleringer, 1992), early developmental stages were characterized by lower investment in hydraulic safety traits in this study (Figure 3). This pattern is consistent with evidence that seedling survival in temperate forests is more strongly associated with size and rapid growth than with drought tolerance (Walters et al., 2023). Under this framework, drought is primarily avoided rather than tolerated, with larger seedlings accessing deeper and more reliable water sources than smaller individuals of the same age. Consequently, selection may favour rapid early growth, allowing seedlings to reach a size where water limitation is reduced. Investment in wood traits that support faster carbon gain and root growth may facilitate escape from the vulnerable seedling stage by accessing deeper soil water and avoiding dry surface soils. Achieving hydraulic conductivity (K_s) with larger D_h rather than increased VN may be consistent with this strategy; larger vessels contribute disproportionately to water transport, allowing high conductivity to be reached with fewer conduits and thus potentially lower carbon investment in cell walls (Tyree et al., 1994). This may support faster growth, facilitating more rapid increases in plant size and rooting depth.

Mechanical Support Traits and Sampling Considerations

While mechanical support in xylem is often reported to increase across ontogeny, this was not observed in this study. Adult trees often demonstrate greater investment in mechanical support to maintain stability with increasing height and biomass (González-Melo et al., 2023; Hietz et al., 2013; Rungwattana & Hietz, 2018; Spatz & Bruechert, 2000). However, we sampled

apical branches from a fixed distance from tree apex, where the distal wood biomass supported by the sampled tissue is standardized (Figure 1). With sampling design, ontogenetic differences in mechanical demand among developmental stages may be minimized. We found fiber wall thickness (FWT) was unchanged in sugar maple and decreased in yellow birch across ontogeny. The mechanisms driving these patterns of FWT in apical branches in these species may represent trade-offs with hydraulic traits or differences in mechanical loads associated with canopy position and branch architecture among small and large trees (Butler et al., 2012; Pratt & Jacobsen, 2017; Spatz & Bruechert, 2000), but further research is needed to elucidate these mechanisms.

Ontogenetic Shifts in Trait Coordination

Across both species and across developmental stages, wood trait covariation was structured along a primary axis (PC1) representing a trade-off between hydraulic efficiency and hydraulic safety. Although the strength and composition of trait loadings varied among developmental stages, PC1 consistently reflected hydraulic efficiency traits such as D_h and high K_s , to oppose traits associated with greater hydraulic safety, such as high VN and $(t/b)^2$. This is consistent with frequently reported patterns of wood anatomical structure (Sperry et al., 2008; Zanne et al., 2010) and indicates that the efficiency–safety trade-off remains a dominant axis structuring wood trait relationship across ontogeny.

Beyond the primary efficiency-safety axis, F was frequently associated with a second orthogonal axis whose composition varied among species and developmental stages, indicating that not all aspects of wood structure are constrained by the efficiency-safety trade-off. Since F reflects the allocation of cross-sectional area to conductive vs structural tissues, its relative independence suggests that trees retain flexibility in allocating conductive area, even when other

hydraulic traits are more tightly coordinated (Zanne et al., 2010). This flexibility in F from the hydraulic may explain its ability to act in a compensatory role in maintaining hydraulic efficiency (K_s) across ontogeny despite increases in hydraulic safety.

Changes in the strength of trait integration with ontogeny differed between species. In sugar maple, trait integration increased from seedlings to adult trees, consistent with the interpretation that increasing physiological and structural constraints associated with greater height, including longer hydraulic pathlengths and greater gravitational resistance, restrict the sets of viable trait combinations to maintain function (Bucci et al., 2004; Meinzer, 2003). However, this is not supported in yellow birch, where trait integration was slightly greater in seedlings despite both species experiencing similar constraints associated with the adult development stage, suggesting that the strength of trait coordination may depend on species-specific life history strategies.

Irradiance and Height Explain Limited Variation in Wood Traits

Despite clear increases in irradiance across developmental stages (~30%), irradiance explained little variation in wood traits (Figure 7; Table A3). Similarly, height explained variation in only a subset of traits, significantly influencing VN and F in sugar maple while showing no significant effects on traits in yellow birch (Figure 7). A substantial portion of explained variance was shared between height and irradiance, reflecting their covariance across ontogeny and limiting the ability to attribute wood trait variation to either factor. Overall, the independent effects of both variables were weak (Figure 7). Nevertheless, height explained more variation than irradiance, particularly in sugar maple, suggesting that some aspects of wood structure in this species responds to increasing tree height.

Maintenance of Hydraulic Conductivity with Increasing Height

Although height explained a limited fraction of wood trait variation, the traits that were associated with height provide insight into how trees shift hydraulic function as they increase in size. As trees grow taller, pathlength resistance increases, requiring more negative xylem pressures to sustain water flow to the canopy (Appendix 1; Koch et al., 2004; Ryan & Yoder, 1997). These increasingly negative xylem pressures elevate embolism risk. Hydraulic efficiency can partly offset this challenge by allowing greater hydraulic conductivity (K_s) for a given pressure gradient, reducing the additional tension required to sustain water transport. Increasing D_h is the most effective way to reduce resistance within individual vessels, and vessel diameter at comparable distal positions has been frequently observed to increase with tree height across species and biomes (Jamaluddinsyah et al., 2022; Olson et al., 2014, 2018; Prendin et al., 2018). However, despite increasing tree height, D_h did not increase across ontogeny in this study. Instead, D_h was lowest in adult trees while VN and F increased. These shifts suggest that increases in VN and F may have compensated for reductions in D_h , allowing hydraulic conductivity (K_s) to be maintained. While adjustments in these two traits do not reduce resistance within individual conduits, they increase the number and cross-sectional area of flow pathways, helping sustain water transport in taller trees.

Freeze-Thaw Constraints and the Shift Toward Hydraulic Safety

The shift toward smaller D_h in adult tree apices is consistent with selection pressure imposed by freeze-thaw embolism in temperate environments. Vulnerability to freeze-thaw induced embolism increases with D_h , as larger conduits form larger gas bubbles that are less likely to dissolve upon thawing (Ewers, 1985). Both sugar maple and yellow birch have been shown to form substantial winter embolism (Sperry et al., 1988, 1994), and this risk may be

greater in the upper canopy where branches are exposed to greater temperature fluctuations due to increased solar radiation (Niinemets, 2010; Sperry et al., 1988). Since irradiance did not explain ontogenetic wood trait shifts in this study, it is unlikely to be a direct driver of trait variation. Instead, canopy position associated with increasing height likely alters the thermal environment experienced by apical twigs, exposing them to greater temperature fluctuations and thus greater freeze-thaw conditions in the springtime when temperatures can fluctuate around the freezing mark between day and night. Under these conditions, selection for narrower conduits at the apex would reduce embolism risk, explaining the observed shift toward safer hydraulic safety in adult tree apices.

Conclusions

This study shows that ontogenetic shifts in wood anatomy in sugar maple and yellow birch are characterized by increased hydraulic safety in adult tree apices, achieved through reductions in vessel diameter (D_h), increases in vessel reinforcement $((t/b)^2)$, and increases in vessel number (VN). Despite substantial increases in irradiance across developmental stages, wood trait variation was not driven by irradiance in either species, and we found no evidence to suggest environmental gradients associated with ontogeny drive shifts in wood traits. Importantly, increased hydraulic safety did not result in reduced hydraulic efficiency (K_s), which was maintained through compensatory increases in VN and lumen fraction (F). These findings demonstrate that, when D_h decreases, anatomical adjustments can maintain conductivity, and the safety-efficiency trade-off can be avoided at the tissue level.

REFERENCES

- Anfodillo, T., Petit, G., & Crivellaro, A. (2013). Axial conduit widening in woody species: A still neglected anatomical pattern. *IAWA Journal*, 34(4), 352–364.
<https://doi.org/10.1163/22941932-000000030>
- Bucci, S. J., Goldstein, G., Meinzer, F. C., Scholz, F. G., Franco, A. C., & Bustamante, M. (2004). Functional convergence in hydraulic architecture and water relations of tropical savanna trees: From leaf to whole plant. *Tree Physiology*, 24(8), 891–899.
<https://doi.org/10.1093/treephys/24.8.891>
- Burgess, S. S. O., Pittermann, J., & Dawson, T. E. (2006). Hydraulic efficiency and safety of branch xylem increases with height in *Sequoia sempervirens* (D. Don) crowns. *Plant, Cell & Environment*, 29(2), 229–239. <https://doi.org/10.1111/j.1365-3040.2005.01415.x>
- Butler, D. W., Gleason, S. M., Davidson, I., Onoda, Y., & Westoby, M. (2012). Safety and streamlining of woody shoots in wind: An empirical study across 39 species in tropical Australia. *New Phytologist*, 193(1), 137–149. <https://doi.org/10.1111/j.1469-8137.2011.03887.x>
- Cavender-Bares, J., & Bazzaz, F. A. (2000). Changes in drought response strategies with ontogeny in *Quercus rubra*: Implications for scaling from seedlings to mature trees. *Oecologia*, 124(1), 8–18. <https://doi.org/10.1007/PL00008865>
- Chave, J., Coomes, D., Jansen, S., Lewis, S. L., Swenson, N. G., & Zanne, A. E. (2009). Towards a worldwide wood economics spectrum. *Ecology Letters*, 12(4), 351–366.
<https://doi.org/10.1111/j.1461-0248.2009.01285.x>

491 Conaway, M. A., & Adams, D. C. (2022). An effect size for comparing the strength of
 492 morphological integration across studies. *Evolution*, 76(10), 2244–2259.
 493 <https://doi.org/10.1111/evo.14595>
 494 Davis, S. D., Sperry, J. S., & Hacke, U. G. (1999). The relationship between xylem conduit
 495 diameter and cavitation caused by freezing. *American Journal of Botany*, 86(10), 1367–
 496 1372. <https://doi.org/10.2307/2656919>
 497 Dawson, T. E. (1996). Determining water use by trees and forests from isotopic, energy balance
 498 and transpiration analyses: The roles of tree size and hydraulic lift. *Tree Physiology*,
 499 16(1–2), 263–272. <https://doi.org/10.1093/treephys/16.1-2.263>
 500 Donovan, L. A., & Ehleringer, J. R. (1992). Contrasting Water-Use Patterns Among Size and
 501 Life-History Classes of a Semi-Arid Shrub. *Functional Ecology*, 6(4), 482–488.
 502 <https://doi.org/10.2307/2389287>
 503 Ewers, F. W. (1985). Xylem' Structure and Water Conduction in Conifer Trees, Dicot Trees, and
 504 Llanas. *IAWA Journal*, 6(4), 309–317. <https://doi.org/10.1163/22941932-90000959>
 505 Ewers, F. W., Ewers, J. M., Jacobsen, A. L., & López-Portillo, J. (2007). *Vessel Redundancy:*
 506 *Modeling Safety In Numbers*. <https://doi.org/10.1163/22941932-90001650>
 507 Frazer, G. W., Canham, C. D., & Lertzman, K. P. (1999). *Gap Light Analyzer (GLA), Version 2.0:*
 508 *Imaging software to extract canopy structure and gap light transmission indices from*
 509 *true-colour fisheye photographs, users manual and program documentation*. Simon
 510 Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies,
 511 Millbrook, New York. [https://rem-](https://rem-main.rem.sfu.ca/downloads/Forestry/GLAV2UsersManual.pdf)
 512 [main.rem.sfu.ca/downloads/Forestry/GLAV2UsersManual.pdf](https://rem-main.rem.sfu.ca/downloads/Forestry/GLAV2UsersManual.pdf)

491 Gleason, S. M., Westoby, M., Jansen, S., Choat, B., Hacke, U. G., Pratt, R. B., Bhaskar, R.,
 492 Brodribb, T. J., Bucci, S. J., Cao, K.-F., Cochard, H., Delzon, S., Domec, J.-C., Fan, Z.-
 493 X., Feild, T. S., Jacobsen, A. L., Johnson, D. M., Lens, F., Maherali, H., ... Zanne, A. E.
 494 (2016). Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency
 495 across the world's woody plant species. *New Phytologist*, 209(1), 123–136.
 496 <https://doi.org/10.1111/nph.13646>
 497 González-Melo, A., Posada, J. M., Beauchêne, J., Lehnebach, R., Levionnois, S., Derroire, G., &
 498 Clair, B. (2023). The links between wood traits and species demography change during
 499 tree development in a lowland tropical rainforest. *AoB Plants*, 16(1), plad090.
 500 <https://doi.org/10.1093/aobpla/plad090>
 501 Hacke, U. G., & Sperry, J. S. (n.d.). *Functional and ecological xylem anatomy*.
 502 Hacke, U. G., Sperry, J. S., Pockman, W. T., Davis, S. D., & McCulloh, K. A. (2001). Trends in
 503 wood density and structure are linked to prevention of xylem implosion by negative
 504 pressure. *Oecologia*, 126(4), 457–461. <https://doi.org/10.1007/s004420100628>
 505 Hacke, U. G., Spicer, R., Schreiber, S. G., & Plavcová, L. (2017). An ecophysiological and
 506 developmental perspective on variation in vessel diameter. *Plant, Cell & Environment*,
 507 40(6), 831–845. <https://doi.org/10.1111/pce.12777>
 508 Hietz, P., Rosner, S., Hietz-Seifert, U., & Wright, S. J. (2017). Wood traits related to size and life
 509 history of trees in a Panamanian rainforest. *New Phytologist*, 213(1), 170–180.
 510 <https://doi.org/10.1111/nph.14123>
 511 Hietz, P., Valencia, R., & Joseph Wright, S. (2013). Strong radial variation in wood density
 512 follows a uniform pattern in two neotropical rain forests. *Functional Ecology*, 27(3),
 513 684–692. <https://doi.org/10.1111/1365-2435.12085>

491 Hinckley, T. M., Lassoie, J. P., & Running, S. W. (1978). Temporal and Spatial Variations in the
 492 Water Status of Forest Trees. *Forest Science*, 24(suppl_1), a0001-z0001.
 493 <https://doi.org/10.1093/forestscience/24.s1.a0001>
 494 Jacobsen, A. L., Ewers, F. W., Pratt, R. B., Paddock, W. A., & Davis, S. D. (2005). Do Xylem
 495 Fibers Affect Vessel Cavitation Resistance? *Plant Physiology*, 139(1), 546–556.
 496 <https://doi.org/10.1104/pp.104.058404>
 497 Jamaluddinsyah, J., Kotowska, M. M., Sulistijorini, S., Waite, P.-A., Brambach, F., Schuldt, B.,
 498 & Triadiati, T. (2022). Tree height effects on vascular anatomy of upper-canopy twigs
 499 across a wide range of tropical rainforest species. *Journal of Tropical Ecology*, 38(6),
 500 416–425. <https://doi.org/10.1017/S0266467422000335>
 501 Jordan, W. R., & Ritchie, J. T. (1971). Influence of Soil Water Stress on Evaporation, Root
 502 Absorption, and Internal Water Status of Cotton 1. *Plant Physiology*, 48(6), 783–788.
 503 <https://doi.org/10.1104/pp.48.6.783>
 504 Koch, G. W., Sillett, S. C., Jennings, G. M., & Davis, S. D. (2004). The limits to tree height.
 505 *Nature*, 428(6985), 851–854. <https://doi.org/10.1038/nature02417>
 506 Lens, F., Sperry, J. S., Christman, M. A., Choat, B., Rabaey, D., & Jansen, S. (2011). Testing
 507 hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in
 508 the genus *Acer*. *New Phytologist*, 190(3), 709–723. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.2010.03518.x)
 509 [8137.2010.03518.x](https://doi.org/10.1111/j.1469-8137.2010.03518.x)
 510 Mayr, S., & Sperry, J. S. (2010). Freeze–thaw-induced embolism in *Pinus contorta*: Centrifuge
 511 experiments validate the ‘thaw-expansion hypothesis’ but conflict with ultrasonic
 512 emission data. *New Phytologist*, 185(4), 1016–1024. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.2009.03133.x)
 513 [8137.2009.03133.x](https://doi.org/10.1111/j.1469-8137.2009.03133.x)

491 Meinzer, F. C. (2003). Functional convergence in plant responses to the environment. *Oecologia*,
 492 134(1), 1–11. <https://doi.org/10.1007/s00442-002-1088-0>

493 Niinemets, Ü. (2010). Responses of forest trees to single and multiple environmental stresses
 494 from seedlings to mature plants: Past stress history, stress interactions, tolerance and
 495 acclimation. *Forest Ecology and Management*, 260(10), 1623–1639.
 496 <https://doi.org/10.1016/j.foreco.2010.07.054>

497 Nolet, P., Delagrange, S., Bouffard, D., Doyon, F., & Forget, E. (2008). The successional status
 498 of sugar maple (*Acer saccharum*), revisited. *Annals of Forest Science*, 65(2), 1.
 499 <https://doi.org/10.1051/forest:2007091>

500 Olson, M. E., Anfodillo, T., Rosell, J. A., Petit, G., Crivellaro, A., Isnard, S., León-Gómez, C.,
 501 Alvarado-Cárdenas, L. O., & Castorena, M. (2014). Universal hydraulics of the flowering
 502 plants: Vessel diameter scales with stem length across angiosperm lineages, habits and
 503 climates. *Ecology Letters*, 17(8), 988–997. <https://doi.org/10.1111/ele.12302>

504 Olson, M. E., Soriano, D., Rosell, J. A., Anfodillo, T., Donoghue, M. J., Edwards, E. J., León-
 505 Gómez, C., Dawson, T., Camarero Martínez, J. J., Castorena, M., Echeverría, A.,
 506 Espinosa, C. I., Fajardo, A., Gazol, A., Isnard, S., Lima, R. S., Marcati, C. R., & Méndez-
 507 Alonzo, R. (2018). Plant height and hydraulic vulnerability to drought and cold.
 508 *Proceedings of the National Academy of Sciences*, 115(29), 7551–7556.
 509 <https://doi.org/10.1073/pnas.1721728115>

510 Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-
 511 Harte, M. S., Cornwell, W. K., Craine, J. M., Gurvich, D. E., Urcelay, C., Veneklaas, E.
 512 J., Reich, P. B., Poorter, L., Wright, I. J., Ray, P., Enrico, L., Pausas, J. G., Vos, A. C. de,
 513 ... Cornelissen, J. H. C. (2013). New handbook for standardised measurement of plant

functional traits worldwide. *Australian Journal of Botany*, 61(3), 167–234.

<https://doi.org/10.1071/BT12225>

Pittermann, J., & Sperry, J. S. (2006). Analysis of Freeze-Thaw Embolism in Conifers. The Interaction between Cavitation Pressure and Tracheid Size. *Plant Physiology*, 140(1), 374–382. <https://doi.org/10.1104/pp.105.067900>

Poorter, L. (2007). Are Species Adapted to Their Regeneration Niche, Adult Niche, or Both? *The American Naturalist*, 169(4), 433–442. <https://doi.org/10.1086/512045>

Post, B. W., Carmean, W. H., & Curtis, R. O. (1969). Birch soil-site requirements. In: Doolittle, W.T.; Bruns, P.E., Comps. 1969. *Birch Symposium Proceedings; 1969 August 19-21; Durham, NH. Upper Darby, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station: 95-101.*, 95–101.

Pratt, R. B., & Jacobsen, A. L. (2017). Conflicting demands on angiosperm xylem: Tradeoffs among storage, transport and biomechanics. *Plant, Cell & Environment*, 40(6), 897–913. <https://doi.org/10.1111/pce.12862>

Pratt, R. B., Jacobsen, A. L., Ewers, F. W., & Davis, S. D. (2007). Relationships among xylem transport, biomechanics and storage in stems and roots of nine Rhamnaceae species of the California chaparral. *New Phytologist*, 174(4), 787–798. <https://doi.org/10.1111/j.1469-8137.2007.02061.x>

Prendin, A. L., Mayr, S., Beikircher, B., von Arx, G., & Petit, G. (2018). Xylem anatomical adjustments prioritize hydraulic efficiency over safety as Norway spruce trees grow taller. *Tree Physiology*, 38(8), 1088–1097. <https://doi.org/10.1093/treephys/tpy065>

Rodriguez-Dominguez, C. M., Forner, A., Martorell, S., Choat, B., Lopez, R., Peters, J. M. R., Pfautsch, S., Mayr, S., Carins-Murphy, M. R., McAdam, S. A. M., Richardson, F., Diaz-

491 Espejo, A., Hernandez-Santana, V., Menezes-Silva, P. E., Torres-Ruiz, J. M., Batz, T. A.,
 492 & Sack, L. (2022). Leaf water potential measurements using the pressure chamber:
 493 Synthetic testing of assumptions towards best practices for precision and accuracy. *Plant,*
 494 *Cell & Environment*, 45(7), 2037–2061. <https://doi.org/10.1111/pce.14330>
 495 Rungwattana, K., & Hietz, P. (2018). Radial variation of wood functional traits reflect size-
 496 related adaptations of tree mechanics and hydraulics. *Functional Ecology*, 32(2), 260–
 497 272. <https://doi.org/10.1111/1365-2435.12970>
 498 Ryan, M. G., & Yoder, B. J. (1997). Hydraulic Limits to Tree Height and Tree Growth.
 499 *BioScience*, 47(4), 235–242. <https://doi.org/10.2307/1313077>
 500 Scholander, P. F., Bradstreet, E. D., Hemmingsen, E. A., & Hammel, H. T. (1965). Sap Pressure
 501 in Vascular Plants. *Science*, 148(3668), 339–346.
 502 <https://doi.org/10.1126/science.148.3668.339>
 503 Soriano, D., Echeverría, A., Anfodillo, T., Rosell, J. A., & Olson, M. E. (2020). Hydraulic traits
 504 vary as the result of tip-to-base conduit widening in vascular plants. *Journal of*
 505 *Experimental Botany*, 71(14), 4232–4242. <https://doi.org/10.1093/jxb/eraa157>
 506 Spatz, H.-C., & Bruechert, F. (2000). Basic biomechanics of self-supporting plants: Wind loads
 507 and gravitational loads on a Norway spruce tree. *Forest Ecology and Management*,
 508 135(1), 33–44. [https://doi.org/10.1016/S0378-1127\(00\)00296-6](https://doi.org/10.1016/S0378-1127(00)00296-6)
 509 Sperry, J. S., Donnelly, J. R., & Tyree, M. T. (1988). Seasonal occurrence of xylem embolism in
 510 sugar maple (*Acer saccharum*). *American Journal of Botany*, 75(8), 1212–1218.
 511 <https://doi.org/10.1002/j.1537-2197.1988.tb08834.x>

491 Sperry, J. S., Meinzer, F. C., & McCulloh, K. A. (2008). Safety and efficiency conflicts in
 492 hydraulic architecture: Scaling from tissues to trees. *Plant, Cell & Environment*, 31(5),
 493 632–645. <https://doi.org/10.1111/j.1365-3040.2007.01765.x>
 494 Sperry, J. S., Nichols, K. L., Sullivan, J. E. M., & Eastlack, S. E. (1994). Xylem Embolism in
 495 Ring-Porous, Diffuse-Porous, and Coniferous Trees of Northern Utah and Interior
 496 Alaska. *Ecology*, 75(6), 1736–1752. <https://doi.org/10.2307/1939633>
 497 Thomas, S. C., & Bazzaz, F. A. (1999). Asymptotic Height as a Predictor of Photosynthetic
 498 Characteristics in Malaysian Rain Forest Trees. *Ecology*, 80(5), 1607–1622.
 499 [https://doi.org/10.1890/0012-9658\(1999\)080%5B1607:AHAAPO%5D2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080%5B1607:AHAAPO%5D2.0.CO;2)
 500 Tyree, M. T., Davis, S. D., & Cochard, H. (1994). *Biophysical Perspectives of Xylem Evolution:*
 501 *Is there a Tradeoff of Hydraulic Efficiency for Vulnerability to Dysfunction?*
 502 <https://doi.org/10.1163/22941932-90001369>
 503 Tyree, M. T., & Ewers, F. W. (1991). The hydraulic architecture of trees and other woody plants.
 504 *New Phytologist*, 119(3), 345–360. <https://doi.org/10.1111/j.1469-8137.1991.tb00035.x>
 505 Tyree, M. T., & Sperry, J. S. (1989). Vulnerability of Xylem to Cavitation and Embolism. *Annual*
 506 *Review of Plant Physiology and Plant Molecular Biology*, 40(1), 19–36.
 507 <https://doi.org/10.1146/annurev.pp.40.060189.000315>
 508 Tyree, M. T., & Zimmermann, M. H. (2002). *Xylem Structure and the Ascent of Sap*. Springer.
 509 <https://doi.org/10.1007/978-3-662-04931-0>
 510 von Arx, G., & Carrer, M. (2014). ROXAS – A new tool to build centuries-long tracheid-lumen
 511 chronologies in conifers. *Dendrochronologia*, 32(3), 290–293.
 512 <https://doi.org/10.1016/j.dendro.2013.12.001>

- Walters, M. B., Kunkle, J. M., Kobe, R. K., & Farinosi, E. J. (2023). Seedling drought responses governed by root traits, site-soil moisture regimes and overstory competition-facilitation. *Forest Ecology and Management*, 544, 121159.
<https://doi.org/10.1016/j.foreco.2023.121159>
- Wheeler, J. K., Sperry, J. S., Hacke, U. G., & Hoang, N. (2005). Inter-vessel pitting and cavitation in woody Rosaceae and other vesselled plants: A basis for a safety versus efficiency trade-off in xylem transport. *Plant, Cell & Environment*, 28(6), 800–812.
<https://doi.org/10.1111/j.1365-3040.2005.01330.x>
- Zanne, A. E., Westoby, M., Falster, D. S., Ackerly, D. D., Loarie, S. R., Arnold, S. E. J., & Coomes, D. A. (2010). Angiosperm wood structure: Global patterns in vessel anatomy and their relation to wood density and potential conductivity. *American Journal of Botany*, 97(2), 207–215. <https://doi.org/10.3732/ajb.0900178>
- Zimmermann, M. H. (1983). *Xylem Structure and the Ascent of Sap*. Springer.
<https://doi.org/10.1007/978-3-662-22627-8>
- Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1(1), 3–14.
<https://doi.org/10.1111/j.2041-210X.2009.00001.x>