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CONFLICTING HYDRAULIC EFFECTS OF XYLEM PIT STRUCTURE RELATE TO THE
GROWTH-LONGEVITY TRADEOFF IN A CONIFER SPECIES

By

BETH ANN ROSKILLY

B.A., The University of Montana, Missoula, MT, 2010

Thesis

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Approved by:

Scott Whittenburg, Dean of The Graduate School
Graduate School

Anna Sala, Chair
Division of Biological Sciences

Lila Fishman
Division of Biological Sciences

Sharon Hood
W.A. Franke College of Forestry & Conservation

Uwe Hacke
Agricultural, Life & Environmental Sciences

Roskilly, Beth, M.S., Summer 2018 Organismal Biology, Ecology, and Evolution

Conflicting hydraulic effects of xylem pit structure relate to the growth-longevity tradeoff in a conifer species

Chairperson: Anna Sala

Abstract

Consistent with a ubiquitous life history tradeoff, trees exhibit a negative relationship between growth and longevity among and within species. However, the mechanistic basis of this life history tradeoff is not well understood. In addition to tradeoffs among multiple traits based on resource allocation conflicts, life history tradeoffs may arise from tradeoffs based on single traits under opposing selection. While a myriad of factors likely contribute to the growth-longevity tradeoff in trees, we hypothesized that conflicting functional effects of xylem structural traits contribute to the growth-longevity tradeoff. We tested this hypothesis by examining the extent to which xylem morphological traits (i.e. wood density, tracheid diameters and pit structure) relate to growth rates and longevity in two natural populations of the conifer species ponderosa pine. We examined xylem morphological traits and growth rates at the base of the trunk. As hydraulic constraints arise as trees grow larger, xylem anatomical traits are expected to adjust to compensate for these constraints. We therefore disentangled the effects of size through ontogeny and growth rates on xylem traits by sampling each individual tree at multiple trunk diameters. We found that the oldest trees had slower lifetime growth rates compared to younger trees in the populations we studied, indicating a growth-longevity tradeoff. We further provide the first evidence that a single xylem trait, pit structure, with known conflicting effects on xylem function (hydraulic safety vs. efficiency) contributes to the growth-longevity tradeoff in a conifer species.

Introduction

Tradeoffs are central to our understanding of ecology and evolution because they impose limits to the adaptive potential of organisms (Futuyma & Moreno, 1988). A profound ecological consequence of tradeoffs is based on the hypothesis that tradeoffs maintain genetic diversity within species. Diversity among species is also likely maintained by tradeoffs across environments that limit niche breadth and geographic range of species (Agrawal *et al.*, 2010). Therefore, how and why tradeoffs arise has long been of interest to ecologists and evolutionary biologists.

A tradeoff can be defined as any case in which fitness is limited by competing demands on an organism. Two kinds of tradeoffs can be distinguished on the basis of the selection regimes that underlie them. Opposing selection on a single trait by different selective agents (e.g. different environments or different components of fitness) can lead to tradeoffs (Agrawal *et al.*, 2010). For example, a single-trait tradeoff between force and velocity based on beak morphology in Darwin's finches leads to divergence in beak size as an adaptation to different food sources across environments and affects mating song performance (Herrel *et al.*, 2009). On the other hand, when two or more fitness-enhancing traits are favored by selection but compete for a limiting resource, tradeoffs between multiple traits can occur. Examples of multiple-trait tradeoffs include flower size versus number (Worley & Barrett, 2000), offspring size versus number (Messina & Fox, 2001), and growth versus defensive mechanisms in plants (Herms & Mattson, 1992).

Life history tradeoffs are of particular interest to ecologists and evolutionary biologists because they represent adaptive constraints among the most proximal components of fitness. They are typically interpreted as tradeoffs that result from resource allocation conflicts among multiple involved traits (Agrawal *et al.*, 2010). Indeed, life history traits such as growth, reproduction and survival require large investments in available resources. The hierarchical nature of phenotypic traits means that life history traits are the highest order, because they depend on specific functional or morphological traits which require smaller relative investments of available resources (Figure 1). Life history tradeoffs are therefore complex and involve tradeoffs operating on and among lower-order functional and morphological traits. Single-trait tradeoffs also have the potential to contribute to life history tradeoffs. In Darwin's finches, for example, the force-velocity tradeoff based on beak morphology can be related to life history traits by determining which food sources are available to a species, affecting survival under stressful conditions (Boag & Grant, 1981) and mating system divergence (Herrel *et al.*, 2009).

The plant economics spectrum theory is an example that attempts to explain slow-to-fast life history strategies in plants through tradeoffs among traits directly related to resource use (Reich, 2014). This highly-influential theory proposes that tradeoffs among traits lead to universal trait covariation in plants, such as the strong correlation found between leaf mass per area (LMA) and leaf longevity (LL) among the broad range of plant taxa in the leaf economics spectrum (Wright *et al.*, 2004). The strong trait covariation found at broad taxonomic scales is typically explained as tradeoffs due to resource allocation or 'economics'. For example, the positive correlation between LMA and LL is often explained in terms of carbon or nitrogen 'return-on-investment': higher LMA requires more carbon and nitrogen investment in leaf construction, requiring a

longer leaf lifespan to recover the construction cost (Reich, 2014). The same principle is evoked to explain the positive correlation between wood density and woody plant lifespan emerging from the wood economics spectrum (Chave *et al.*, 2009). However, recent studies demonstrate that strong trait correlations like the LMA-LL relationship found at broad taxonomic scales are not consistent at narrower scales, such as within species (Ramírez-Valiente *et al.*, 2017; Anderegg *et al.*, 2018). These exceptions highlight that resource economic principles do not predictably explain trait covariation across taxonomic scales. While tradeoffs based on resource allocation have been the focus of this framework, tradeoffs due to opposing selection on individual traits have received much less attention. Yet understanding their role may enhance our ability to determine which traits predict life history tradeoffs.

A life history tradeoff common across organisms is that between growth and lifespan. Organisms that grow slowly tend to live longer than those with faster growth, indicating that rapid growth is associated with reduced longevity (Arendt, 1997). Consistent with the growth-longevity tradeoff, slow early growth in trees has been associated with longer lifespans in several species (Black *et al.*, 2008; Bigler & Veblen, 2009; Johnson & Abrams, 2009; Di Filippo *et al.*, 2012, 2015; Rötheli *et al.*, 2012; Bigler, 2016). Fluctuating selection dynamics over time in long-lived organisms likely drive this life history pattern. In trees, fast growth rates and large size provide fitness benefits via increased competitive ability, faster time to reproduction, and increased chances of early survival (Lanner, 2002). But selection for fast growth rates early in life may conflict with selection for slow growth at mature stages, as shown in adult ponderosa pine trees under mountain pine beetle outbreak (de la Mata *et al.*, 2017). But while we can identify some of

the selective dynamics influencing these life history patterns, we know little about the tradeoffs on and among the traits that underlie them.

Competing functional demands on the stem xylem may contribute to the growth-longevity tradeoff found in trees. In woody plants, stem xylem performs several physiological functions critical to growth and survival, including efficient water transport, embolism resistance and mechanical safety (Baas *et al.*, 2004; Chave *et al.*, 2009). Trees require water to maintain stomatal conductance, carbon assimilation, and cellular turgor in order to promote tissue growth and maintain function; therefore, water transport efficiency is positively related to growth rate (Tyree *et al.*, 1998; Santiago *et al.*, 2004; Poorter *et al.*, 2010; Smith & Sperry, 2014). Embolism resistance, or the ability to tolerate high tensions in the xylem without catastrophic embolism spread, relates to the ability to maintain water transport and survive during droughts (Brodribb & Cochard, 2009; Kursar *et al.*, 2009). Mechanical safety provides protection against physical damage, such as that caused by wind, pests or pathogens, and cellular implosion under high xylem tension, thus it is also related to survival (Loehle, 1988; Chave *et al.*, 2009). If these multiple xylem functions depend on the same xylem structural traits, tradeoffs can arise with important consequences for tree growth and survival. For example, a tradeoff between hydraulic efficiency and mechanical safety in the gymnosperm xylem is due to the fact that tracheid cells perform both functions (Pittermann *et al.*, 2006b).

While a myriad of factors likely contribute to the growth-longevity tradeoff in trees (Figure 1), here we hypothesized that conflicting functional effects of xylem structural traits contribute to the growth-longevity tradeoff. This hypothesis is based on the expected opposing selection on

these structural traits. We tested this hypothesis by examining the extent to which xylem morphological traits (i.e. wood density, tracheid diameters and pit structure) relate to growth rates and longevity in two natural populations of the conifer species ponderosa pine. We chose a conifer because the limited diversity of cell types in the gymnosperm xylem (compared to that of angiosperms) may lead to more readily-detectable tradeoffs. We focused on a single species to minimize variation in xylem structure in order to explore whether there is support for selection on each xylem trait. We examined xylem morphological traits and growth rates at the base of the trunk. As hydraulic constraints arise as trees grow larger, xylem anatomical traits are expected to adjust to compensate for these constraints (Ryan & Yoder, 1997; Domec *et al.*, 2008). We therefore disentangled the effects of size through ontogeny and growth rates on xylem traits by sampling each individual tree at multiple trunk diameters. We found that the oldest trees had slower lifetime growth rates compared to younger trees in the populations we studied, indicating a growth-longevity tradeoff. We further provide the first evidence that a single xylem trait, pit structure, with known conflicting effects on xylem function (hydraulic safety vs. efficiency) contributes to the growth-longevity tradeoff in a conifer species.

Materials and Methods

Site and tree selection

Two sites were selected for sampling, originally for a study of the long-term effects of fire history on tree growth in 2006 and 2007 (Keeling *et al.*, 2006). The sites were located on ridges above the Salmon River in Idaho; one site near Mackay Bar (MB) and the other site near Bullion Ridge (BR) about 40 km downriver (Figure S1). All sites had a mixture of old growth and younger trees. At each site, relatively open-grown ponderosa pine trees in a range of size classes were sampled from a specified area of similar topography. For smaller size classes, trees

experiencing obvious suppression from neighboring trees were avoided. At each tree, elevation, aspect, slope, GPS coordinates, and tree diameter at breast height (DBH) were recorded. The fire study showed no long-term effects of frequent fire on tree growth in the paired burned and unburned sites at the two locations (Keeling *et al.*, 2011), indicating that occurrence of fire has not had a strong effect on growth rates at these sites. Based on needle carbon isotope ratios and vegetation structure, Mackay Bar was determined to be drier than Bullion Ridge (Keeling *et al.*, 2011).

Growth rates and age estimation

Two cores were collected from each tree and tree diameter at coring height (DCH) at approximately 50 cm from the ground was recorded. Tree heights were measured using a laser range-finder (Impulse 200) from two vantage points located approximately 90 degrees apart. Cores from each tree were visually cross-dated against each other and against a time-series of reconstructed Palmer Drought Severity Index (Cook & Krusic, 2008) for the region. Once cores were crossdated, the year of the innermost ring at coring height established a minimum estimate of tree age. Lifetime annual BAI was calculated as the average of all the annual BAI values for each tree. Trees were first selected for the classes used in this study based on cambial age and lifetime average BAI growth rates: Old trees were above 350 years old cambial age at sampling and young trees were between 85 - 150 years old. We further selected a subset of young trees with fast growth (lifetime BAI > 30 cm²/year) and slow growth (lifetime BAI < 25 cm²/year) for comparison.

Xylem morphological traits

We determined wood density (g/cm³) as the dry mass per saturated volume (after overnight rehydration) and dry mass per air-dried volume (as cores were not freshly collected) following

methods outlined in Williamson & Wiemann, 2010. We found wood densities based on saturated and fresh volume to be strongly correlated when tested in a separate set of freshly-collected ponderosa pine core samples. Heartwood and sapwood were visually distinguished and separated using a razor blade. Wood volume was measured using the water displacement method.

Once wood density was determined, we extracted multiple segments from each mounted core at selected sizes (trunk diameters) by exposing them to steam and cutting them out with a razor blade by hand. Core segments ranged from 3-5 growth rings, depending on how much tissue was needed for a section at least 5 mm in length. Segments were soaked in ethanol for at least an hour, then remounted and covered with a waterproof, gap-filling glue. Transverse sections of ~16 μm were cut using a rotary microtome (Leica RM 2235). Sample surfaces were brushed with a mixture of cornstarch, glycerol and water, which act as a non-newtonian fluid that maintains the cell wall structure of softwoods when cut (Gartner & Schweingruber 2013). Samples were stained using a solution of astrablue and safranin for 10 minutes, rinsed with distilled water, and then gradually dehydrated with 75%, 95% ethanol rinse and finally an anhydrous alcohol solution of 95% ethanol + 2.2 dimethoxypropane. After dehydration, samples were fixed with a drop of Eukitt[®] quick-hardening mounting medium and dried in an oven at 60°C for at least 12 hours. We imaged samples using a light microscope (Amscope T700) connected to a digital camera. For each transversal section, mean tracheid diameters were calculated from at least 100 tracheids throughout the earlywood (Figure S2).

Core segments were then cut tangentially by hand with a razor blade for sampling pit structure. Samples were mounted on aluminum stubs and coated with gold in a vacuum using a sputter

machine (Denton Vacuum Desk V model). Samples were observed and imaged with a scanning electron microscope (Hitachi S-4700 cold field emission, EMtrix electron microscope facility at the University of Montana). Pit aperture diameter (D_a), pit membrane diameter (D_m) and torus diameter (D_t), were measured on 10-20 pits per earlywood segment (Figure 2). Pit measurements were analyzed using ImageJ freeware (<http://rsbweb.nih.gov>). Torus overlap (O) was calculated as in Hacke *et al.*, 2004:

$$O = (D_t - D_a)/(D_m - D_a)$$

Hacke *et al.*'s formula was chosen over alternatives (i.e. Delzon *et al.*, 2010) because it is relative to pit membrane diameter, making the calculation more relevant to water flow resistance, though the two calculations were strongly correlated.

Data analyses

Differences in average lifetime growth rates and wood density between classes were tested using a standard ANOVA. Relationships between xylem traits with tree size through ontogeny between classes of trees sampled at the Mackay Bar site only were tested using a mixed effects model with tree and position within core included as random effects. Linear regressions between xylem traits with lifetime growth rates were tested using a mixed effects model with tree as random effect. Comparison of the relationships between xylem traits and growth rates of trees at both Mackay Bar and Bullion Ridge sites were tested at fixed trunk diameter of 30 cm at coring height for each tree, using a mixed effects model with tree as random effect. All statistical analyses were tested for normality and conducted in R programming software (R Development Core Team, 2013).

Results

Growth rates and ages

The oldest trees at both sites had slower average lifetime growth rates than all younger trees pooled, even early in life ($F_{1,17} = 5.807$, $p = 0.03$; Figure 3). Though not included in this study, differences in lifetime growth rates among age classes were also significant with larger sample sizes of trees from the same sites, even after correcting for climatic differences between the early-life growth periods of old and young trees (Keeling, 2009; see Figure S3 for growth rates by calendar year of selected trees in this study). Some young trees, however, had relatively slow average growth rates compared to other young trees, but no old trees grew as quickly as fast-growing young trees (Figure 3 & 4).

Xylem traits, growth rates and tree size through ontogeny

Torus overlap (the width of pit border covered by the torus) was larger in both old and young slow-growing trees, relative to fast-growing young trees at the single site sampled ($p = 0.03$ for the effect of growth as class; Figure 5a). Torus overlap did not change with tree size through ontogeny ($p > 0.4$ for the effect of trunk size; Figure 5a), though all raw pit dimensions increased with tree size ($p < 0.007$ for the effect of trunk size for all pit dimensional traits; Figure S4).

Tracheid diameters did not differ with growth rates ($p > 0.45$ for effect of growth as class) but increased with tree size through ontogeny ($p < 0.001$ for the effect of trunk size; Figure 5b). The slope of the relationship between tracheid diameter and trunk size was marginally steeper in the young, slow-growing trees ($p = 0.06$ for the interaction effect between size and class; Figure 5b).

We found no differences in heartwood or sapwood density due to growth rates among trees ($p > 0.45$ for each; Figure 6).

Xylem traits and growth rates by site

Torus overlap was negatively related to average lifetime growth rates among trees at both sites when sampled at a given trunk diameter ($p < 0.005$ for the effect of growth as class; Figure 7a). While there was no significant interaction effect between growth rate and site, average torus overlap was larger in trees from the drier site ($p = 0.006$ for site effect; Figure 7a). Tracheid diameters at given trunk size did not differ by site ($p > 0.45$ for the effect of site; Figure 7b).

Discussion

Major findings

This study provides the first evidence that pit structure, a single trait with conflicting consequences on xylem function, contributes to the growth-longevity tradeoff in a conifer species. In the mixed-age ponderosa pine populations we studied, the oldest trees had slower lifetime growth rates compared to younger trees — a pattern found in several other studies (Black *et al.*, 2008; Bigler & Veblen, 2009; Johnson & Abrams, 2009; Di Filippo *et al.*, 2012, 2015; Rötheli *et al.*, 2012; Bigler, 2016) and consistent with a growth-longevity tradeoff. Slow-growing trees from these stands have pits with larger torus overlap compared to fast-growing trees. Torus overlap has been shown to increase embolism resistance in conifers and there is compelling evidence that it also constrains transport efficiency (below). The tradeoff between hydraulic safety and efficiency based on pit structure is consistent with the growth longevity tradeoff: slow-growing trees may reach older ages, in part, because they possess a more drought-tolerant but less efficient xylem. Although the basis of life history tradeoffs are inherently complex and depend on a myriad of traits (Figure 1), this study within a single species provides strong support that a tradeoff based on opposing selection on a single xylem trait, the hydraulic safety-efficiency tradeoff due to pit structure, contributes to growth-longevity tradeoff.

Growth-safety tradeoff due to conflicting hydraulic demands on pit structure

The negative correlation we found between torus overlap and growth rates among trees at both sites (Figure 7a) is most likely due to a xylem safety-efficiency tradeoff based on pit structure. Though we cannot rule out the possibility that the basis of the correlation could be genetic or developmental, there is compelling evidence for conflicting consequences on conifer xylem function based on pit structure. First, it has been shown that larger torus overlap provides greater embolism resistance among conifer species, because a larger torus overlap creates a stronger seal when embolism occurs and prevents its spread to adjacent xylem cells (Delzon *et al.*, 2010; Bouche *et al.*, 2014). Many temperate conifers such as ponderosa pine live in environments that are seasonally dry (Richardson, 2000) and embolism resistance conferred by larger torus overlap likely contributes to their ability to survive droughts (Delzon *et al.*, 2010) and live longer. Moreover, while the slope of the negative correlation between torus overlap and growth rate is consistent across sites, torus overlap was larger on average for a given diameter growth rate in trees from the drier site (Figure 7a). This result is consistent with selection for larger torus overlap and increased embolism resistance in dry environments.

As trees require water to maintain stomatal conductance, carbon assimilation, and cellular turgor in order to promote tissue growth and maintain function, water transport efficiency is positively related to growth rate (Tyree *et al.*, 1998; Santiago *et al.*, 2004; Poorter *et al.*, 2010; Smith & Sperry, 2014). But larger torus overlap may limit water transport efficiency in the xylem by increasing resistance to water flow through pit membranes and thereby constrain growth rates. Indeed, there is compelling support that torus-margo pit structure has strong consequences on hydraulic efficiency and safety. Modeling studies indicate that torus-margo pits can contribute as much as two-thirds of the total resistance in the conifer xylem (Pittermann *et al.*, 2006a, 2010;

Schulte *et al.*, 2015). Further, the larger torus-margo pit structure in conifers compensates for the resistance to flow imposed by the smaller size of tracheids while providing embolism resistance equal to or surpassing that of the angiosperm xylem (Pittermann *et al.*, 2005). Because many factors contribute to total flow (pathway length, conduit size, pit structure and density, etc.), the resistance to water flow contributed by pit structure alone is exceedingly difficult to quantify and is not well resolved at the xylem or whole-plant level (Gleason *et al.*, 2016). However, all else equal, greater torus overlap would create a larger obstruction to water flow through the pit by reducing margo space, creating more resistance. The fact that we focused on a single species likely reduced variability in other xylem traits and contributed to the unmasking of the role of pit structure. Conflicting hydraulic demands based on pit structure are consistent with the negative correlation between torus overlap and growth rates among trees in our study. On the one hand, selection favoring fast growth and increased hydraulic efficiency over embolism resistance could explain why fast-growing trees have smaller torus overlap. On the other hand, selection favoring greater embolism resistance and larger torus overlap over hydraulic efficiency could explain why trees with larger torus overlap grow slowly.

Variation in tracheid diameter and wood density is constrained by other factors

Surprisingly, we did not detect a correlation between tracheid diameters and growth rates among trees in our study (Figure 5b & 7b). We expected that the strong influence of tracheid diameter on water transport efficiency (Hagen-Poiseuille equation; Tyree & Ewers, 1991) would lead to a positive correlation between tracheid diameters and growth rates. Instead, we found that tracheid diameters scaled strongly and positively with tree size (Figure 5b) but not growth rates. Though the slope of the correlation between tracheid diameters and trunk size was marginally steeper in the young slow-growing trees ($p = 0.06$; Figure 5b), which could be because these trees were not

sampled beyond the point at which height growth rate begins to decline (Keeling, 2009). Further, motivated by several studies that report a strong correlation between wood density and tree demographics (Poorter *et al.*, 2008; Martinez-Vilalta *et al.*, 2010; Wright *et al.*, 2010), an emerging hypothesis from the wood economics spectrum posits that denser wood is related with slow growth and higher survival (Chave *et al.*, 2009). However, tracheid size alters the cell wall thickness-to-span ratio and thus determines wood density in the conifer xylem (Pittermann *et al.*, 2006b). Indeed, we did not detect a correlation between wood density and growth rates, which is consistent with the lack of correlation between tracheid diameters and growth rates. We were also surprised to find that tracheid diameters did not vary with site moisture, though we did see an effect of site dryness on the relationship between tree height and diameter — trees at the drier site are shorter for a given trunk diameter (Figure S5). In cold climates, small conduit diameters provide a selective advantage because smaller cells reduce the occurrence of freeze-thaw embolism (Zanne *et al.*, 2013; Hacke *et al.*, 2017). This may be a factor constraining tracheid diameters at a given tree height in these populations that experience regular freeze-thaw events. Therefore, we speculate that both tree height and freezing temperatures impose selection pressures that constrain variation in tracheid diameters and wood density in these populations. These constraints (i.e. lower variability) would help explain the increasing role of pit structure in water flow and growth rates. We speculate that variation in pit structure with diameter growth may be favored due to opposing selection imposed by conflicting hydraulic demands depending on life history and site moisture in these populations.

Xylem safety-efficiency tradeoff depends on which traits are under selection

The tradeoff between hydraulic efficiency and safety in woody plant xylem is a leading hypothesis in the study of plant hydraulics with important implications. Weak evidence among

species (Gleason *et al.*, 2016) demonstrates that the strength of this tradeoff will depend on the selection regimes imposed by the environment on the many traits involved. Some environments may not impose strong selection for traits conferring either greater water transport efficiency or enhanced hydraulic safety. This could produce a pattern exactly like the one found by Gleason *et al.*, in which there is a clear upper constraint line (no species have high efficiency and safety above a certain point) but plenty of variation falls below the constraint line. However, when the environment does impose selection to enhance these functions, the ability to detect a tradeoff will depend on whether or not hydraulic efficiency is achieved through xylem traits that also reduce safety. If different xylem traits contribute to hydraulic efficiency and safety among species a tradeoff between them may not be apparent (Gleason *et al.*, 2016). But this tradeoff may be more readily detected within species (as our results suggest) or in closely-related taxa that possess a similar xylem design, particularly when such a design is based on limited diversity of cell types as in the gymnosperm xylem. Simpler xylem designs enhance the likelihood that increases in hydraulic efficiency will depend on changes in xylem traits that also affect safety (e.g. pit structure). Our results in a single conifer species provide strong support that pit structure is under opposing selection as a result of conflicting consequences on xylem function (safety vs efficiency) providing a mechanistic basis for a growth-longevity tradeoff.

Evolved differences in xylem structure determine which traits relate to life history

Our findings do not support the hypothesis that wood density may be a unifying functional trait that relates to woody plant life history strategies as proposed by the wood economics spectrum (Chave *et al.*, 2009). While we acknowledge that the lack of correlation may be due to small sample size, we offer another reason with important implications for theory development. The associations between wood density and life history patterns may differ between angiosperms and

gymnosperms because of evolved differences in xylem structure. The strong correlations found between wood density and tree demographics tend to be dominated by studies among angiosperm species (Poorter *et al.*, 2008; Martinez-Vilalta *et al.*, 2010; Wright *et al.*, 2010). In studies that include a larger proportion of conifer species, the correlation tends to be weak or absent (Russo *et al.*, 2010; Fan *et al.*, 2012). At the clade level, conifers present an exception to the wood density paradigm, because they tend to grow more slowly and live longer than most angiosperms yet possess lighter wood. The wood of conifers is less dense than that of angiosperms due to the lack of fibers. However, it provides hydraulic and mechanical safety equal to or surpassing that of angiosperm wood due to the reduced size of tracheids (greater cell wall fraction) and the torus-margo pit structure (see above; Sperry *et al.*, 2006). Further, the freeze-induced embolism resistance conferred by the smaller size of tracheids may in part explain the dominance of conifer species over angiosperms in colder climates (Pittermann & Sperry, 2003). Selection for smaller tracheids could constrain variation in wood density and in turn may explain the weak or absent correlation between wood density and growth rates found among conifers. The difficulty of detecting a correlation between wood density and demographics in conifers highlights that the need to account for fundamental evolved differences in xylem structure in order to develop stronger hypotheses for which xylem traits predict life history patterns among woody plant taxa.

Selective basis of tradeoffs predicts which traits relate to life history across taxonomic scales

Recent studies demonstrate that the expected trait co-variation across taxa based on the plant economic spectrum (Reich, 2014; see above) does not necessarily occur within species (Ramírez-Valiente *et al.*, 2017; Anderegg *et al.*, 2018). These results highlight the need to determine which traits truly relate to resource use and life history across taxonomic scales and

environments. An important issue to consider when testing trait covariation across taxonomic scales or environments is how much variation exists in resource acquisition among individuals relative to resource allocation within an organism. Greater variation in resource acquisition can mask a tradeoff between traits by creating a positive correlation between two traits even when there is an inherent resource allocation conflict between them (van Noordwijk & de Jong, 1986). But this issue should not apply to single-trait tradeoffs based on opposing selection. Our study provides strong support that a single-trait tradeoff based on opposing selection, the safety-efficiency tradeoff based on pit structure, may mediate acquisition of a critical resource (water) and thus relate to life history patterns in natural populations of a conifer species. Our findings suggest that determining the selective basis of tradeoffs (i.e. resource allocation or opposing selection) may offer a path forward for predicting which traits relate to life history across taxonomic scales and environments.

Conclusions

We provide the first evidence that a single morphological trait in a conifer species, the torus-margo pit structure, contributes to the growth-longevity tradeoff. Our results support the profound consequences of pit structure for life history tradeoffs and conifer evolution (Pittermann *et al.*, 2005). In light of the evolutionary and ecological relevance of a growth-safety tradeoff based on pit structure in conifers, future research should focus on determining how common it is in natural populations of conifer species. Further research is needed to quantify the contribution of pit structural variation to total xylem hydraulic resistance and efficiency through computational and physical models. Another research priority should be to determine the relevance of pit structural variation to drought survival by measuring pit structure in dead and live trees after drought events. To determine the adaptive potential of pit structure, genetic

differentiation and developmental plasticity should be studied among populations using common garden experiments. However, these research goals will necessitate advances in efficient and reliable methods of measuring pit structure because these measurements are currently prohibitively time consuming at the sample sizes necessary for genetic or ecological studies. Further, if the growth-safety tradeoff that we found in ponderosa pine populations is common broadly among conifers, it predicts declines in the temperate and boreal forest productivity as drought becomes more prevalent with climate change in many parts of the world (Allen *et al.*, 2015). Finally, the findings from this study highlight that determining the underlying nature of tradeoffs in natural populations (the extent to which they arise due to single and multiple trait tradeoffs) provides an important path forward for a unifying plant economic spectrum theory within and across taxa.

Figures

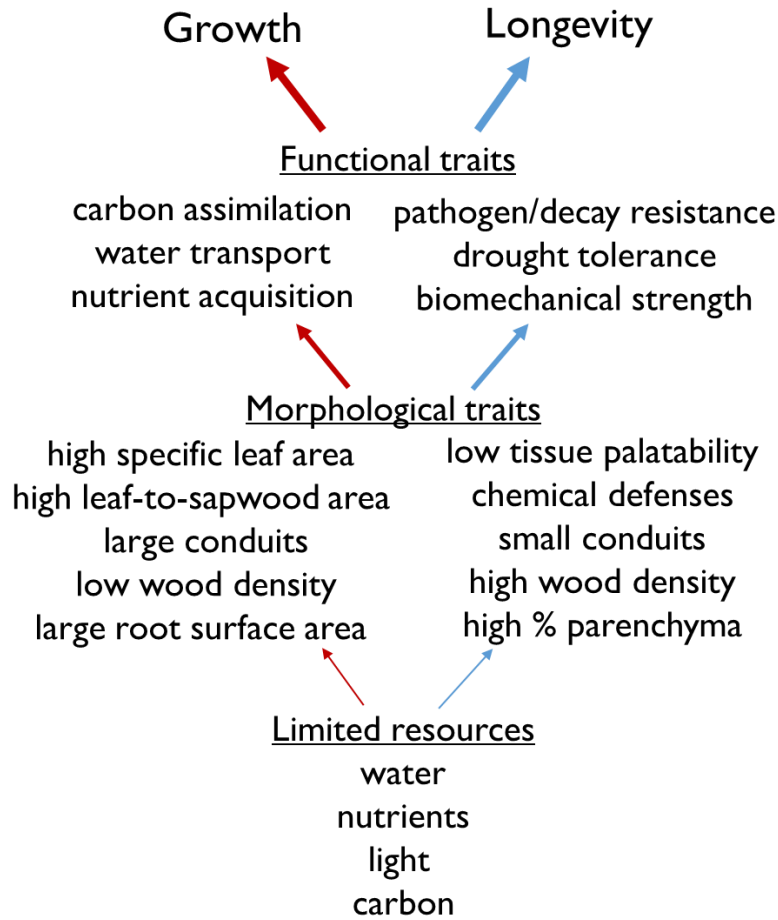


Figure 1. Life history tradeoffs such as that between growth and longevity in trees are the result of tradeoffs among lower-level functional and morphological traits with different rates of resource use. Arrow sizes represent relative amount of resources required at different levels.

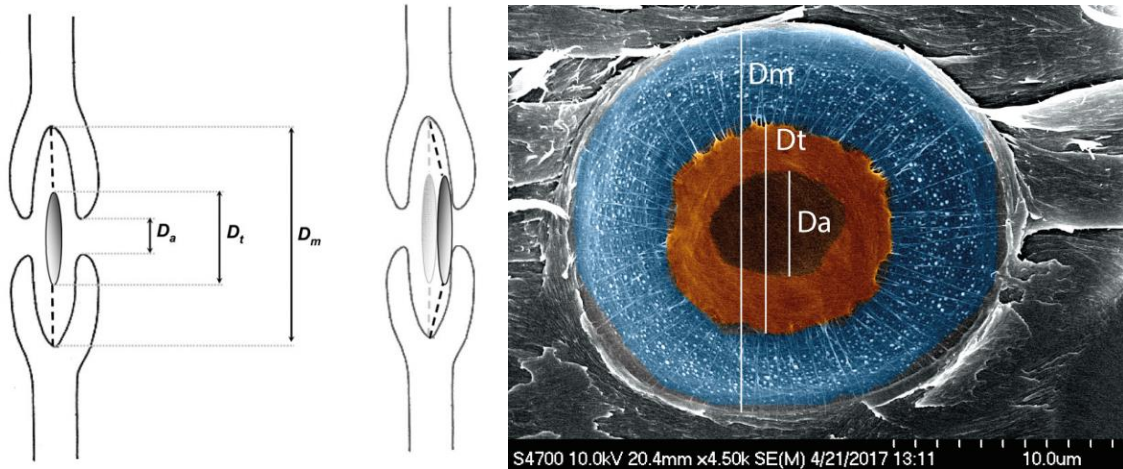


Figure 2. Schematic of an unaspirated pit (left) showing measurements of pit membrane (D_m), torus (D_t), and aperture diameters (D_a) and an aspirated pit (center) from Delzon *et al.*, 2010. Scanning electron microscope image (colorized with Adobe Photoshop[®]) of torus-margo pit surface (right) showing measured pit dimensions.

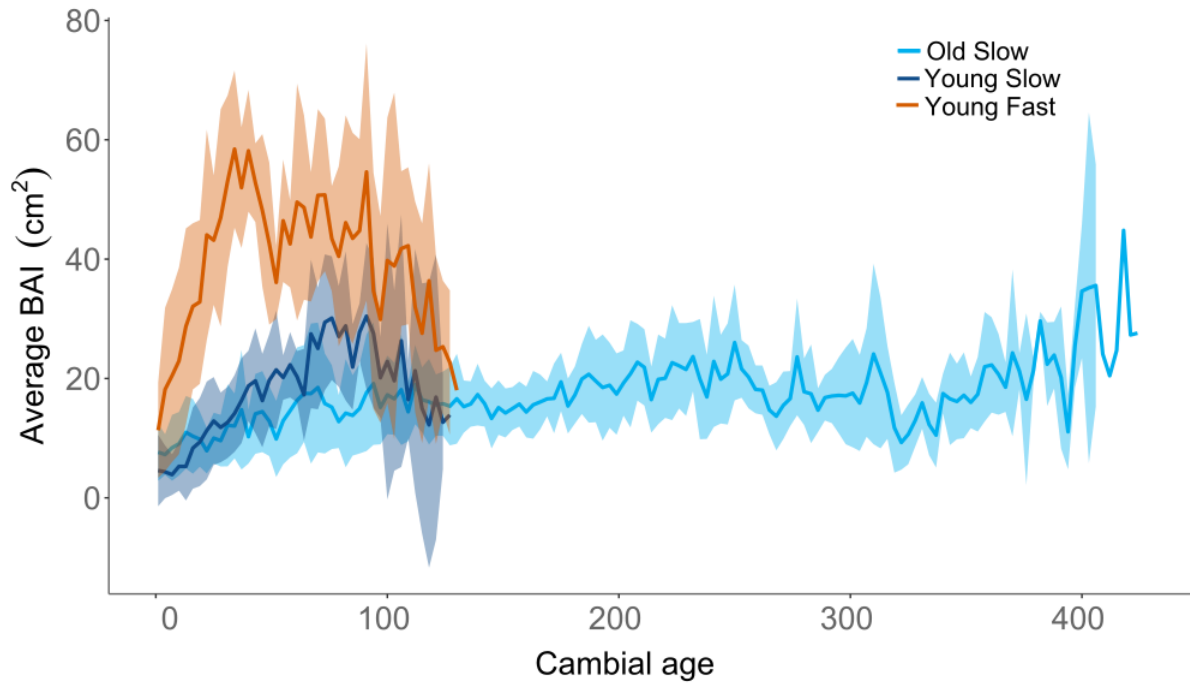


Figure 3. Average annual basal area increment growth rate over cambial age for each class of trees from both sites ($n = 7$ trees per class, 21 total). Shaded bands represent 95% confidence intervals.

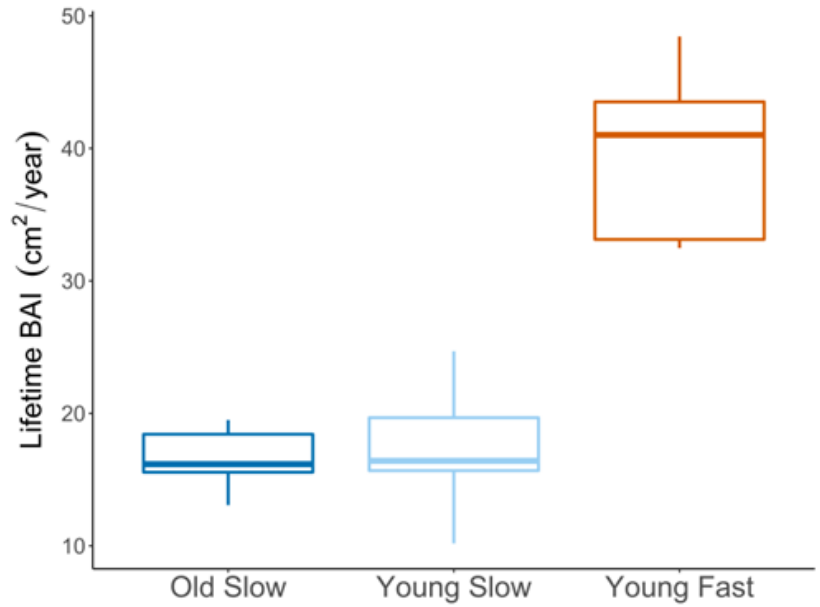


Figure 4. Average lifetime basal area increment growth rate for each class of trees from both sites ($n = 7$ trees per class, 21 total). Cambial ages of old trees at sampling ranged from 375 - 454 years old. Young trees were 88 - 147 years old at sampling. At both sites, old trees had slower average lifetime growth rates than young trees pooled ($p = 0.02$), though we selected some young trees that had slow growth rates (young slow) for comparison with young fast trees.

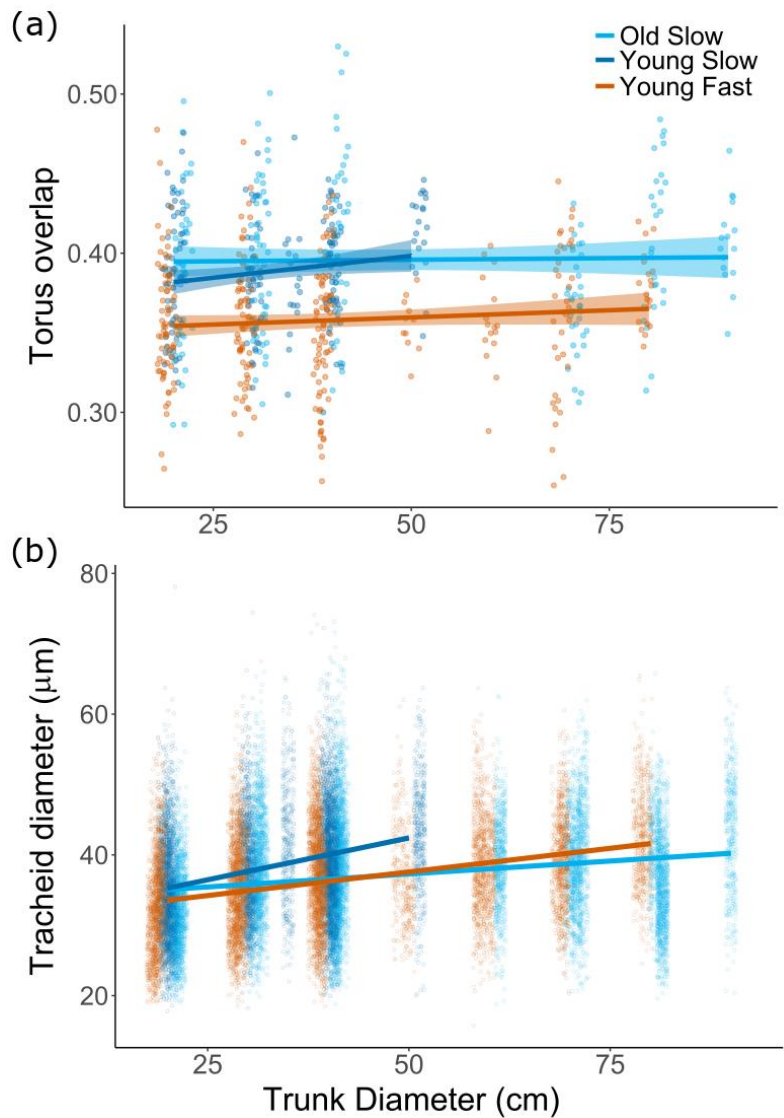


Figure 5. Torus overlap (a) and tracheid diameters (b) by individual trunk diameter through ontogeny for each class at the Mackay Bar site only ($n = 12$ trees total). Shaded bands represent 95% confidence intervals, but too narrow to be visible in (b) due to large tracheid sample size. Points represent individual pit ($n = 708$) and tracheid measurements ($n = 24,989$). Torus overlap was larger in both old and young slow-growing trees, relative to fast-growing young trees ($p = 0.03$). Tracheid diameters increased with tree size through ontogeny ($p < 0.001$) but did not differ by growth rate among trees.

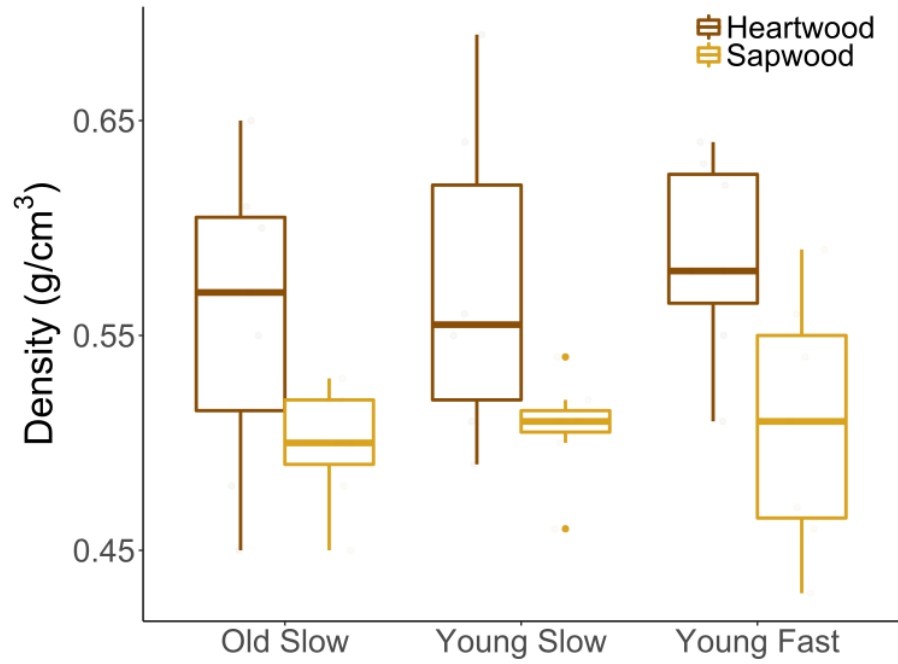


Figure 6. Sapwood and heartwood density (whole core) for each class of trees from both sites ($n = 21$ trees total). No differences in heartwood or sapwood density were found due to growth rate ($p > 0.45$).

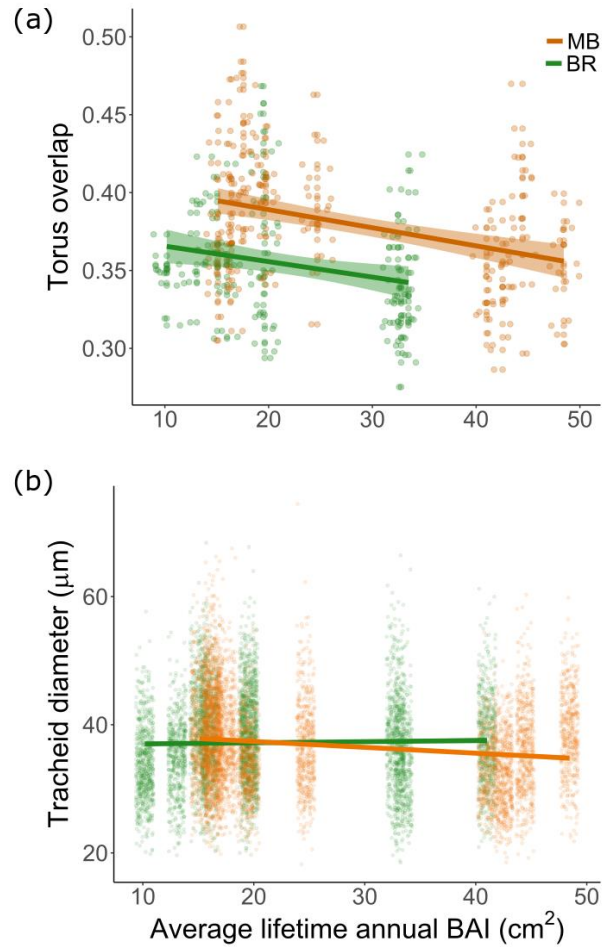


Figure 7. Torus overlap (a) and tracheid diameters (b) by average lifetime annual BAI (cm^2) for each site: Mackay Bar (MB) and Buillon Ridge (BR). Xylem traits were sampled at a fixed trunk diameter of 30 cm for all trees ($n = 21$ total). Shaded bands represent 95% confidence intervals, but too narrow to be visible in (b) due to large tracheid sample size. Points represent individual pits ($n = 322$) and tracheid measurements ($n = 10,470$). Torus overlap was negatively related to average lifetime growth rates among trees at both sites ($p < 0.005$). Average torus overlap was larger in trees from Mackay Bar (MB) ($p = 0.006$). Tracheid diameters at given trunk size did not differ by site ($p > 0.45$).

Supplementary Figures

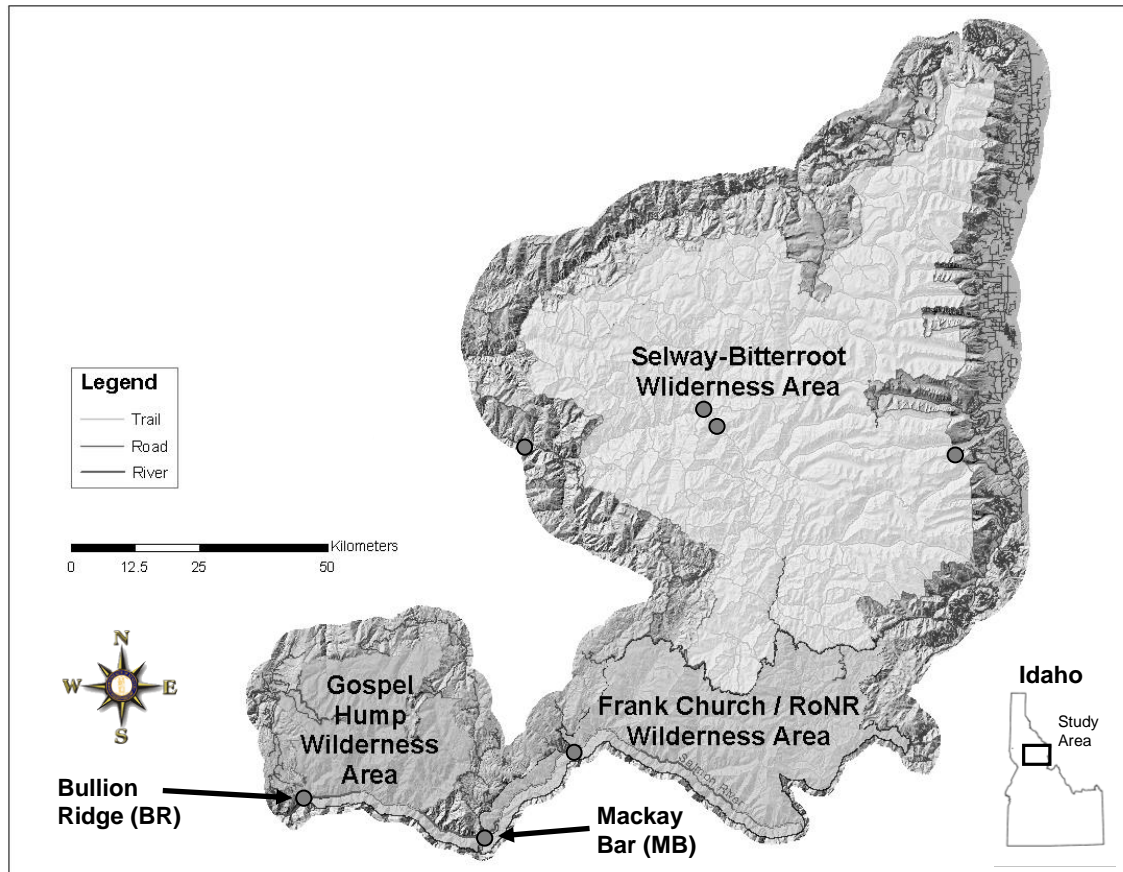


Figure S1. Map of Mackay Bar and Bullion Ridge sites sampled in northern Idaho.

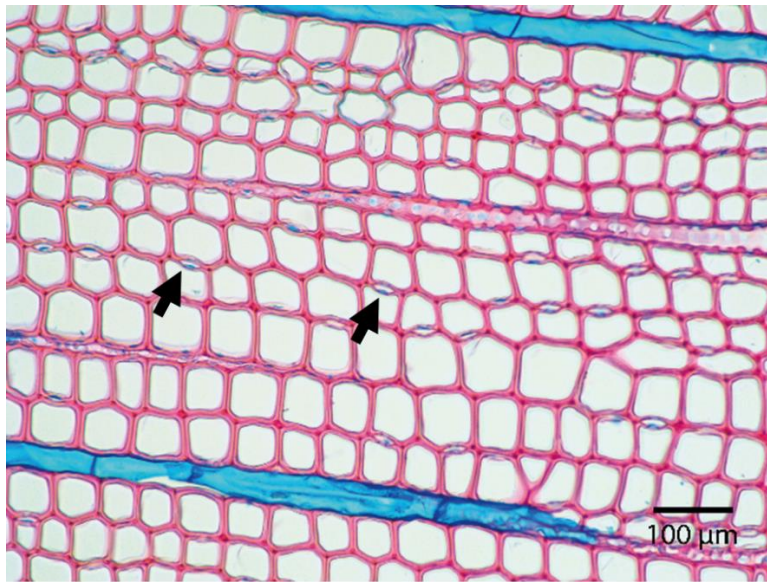


Figure S2. Light microscope image of dyed transverse xylem section used for measuring tracheid diameters with lignified tissues shown in pink and non-lignified in blue (black arrows indicate the torus in the center of pit borders).

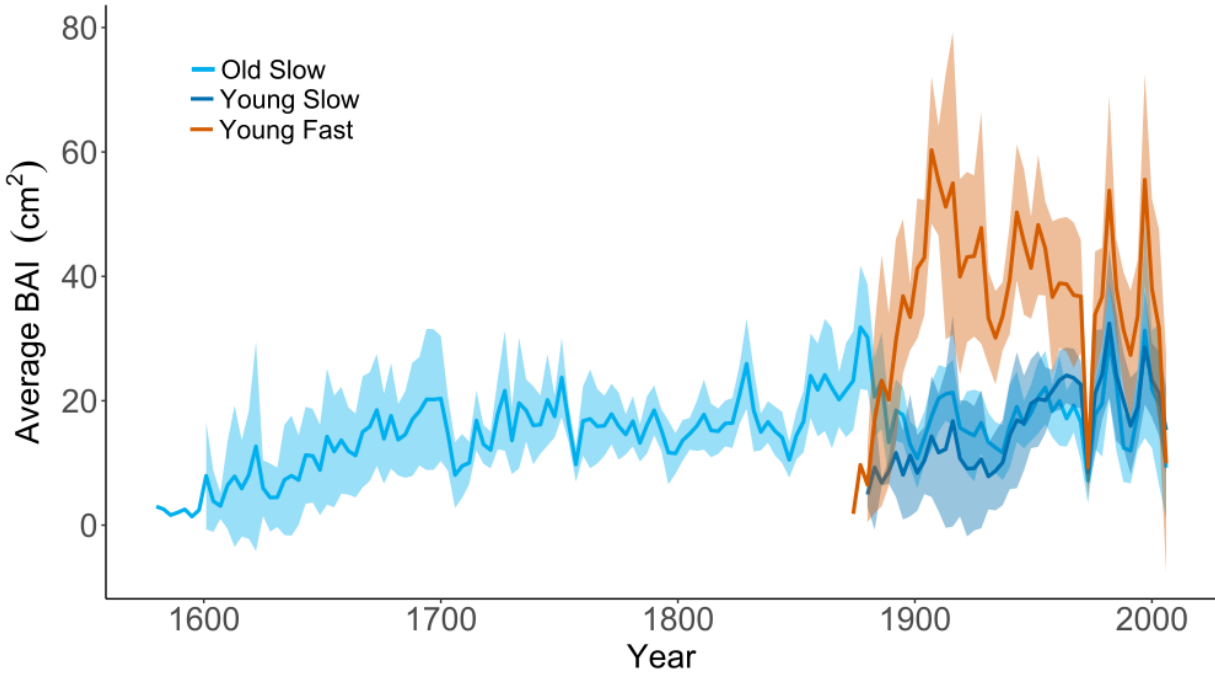


Figure S3. Average annual basal area increment growth rate over calendar year for each class of trees from both sites ($n = 7$ trees per class, 21 total). Shaded bands represent 95% confidence intervals.

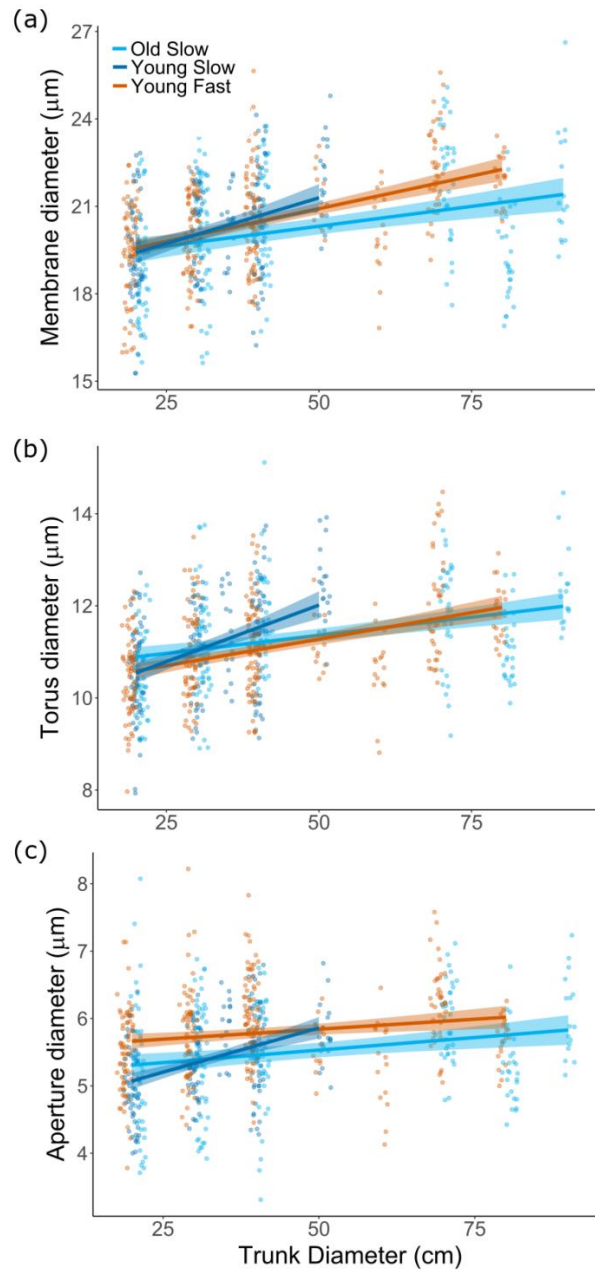


Figure S4. Pit membrane diameter (a), torus diameter (b), and aperture diameter (c) by trunk diameter for each class at Mackay Bar site only ($n = 12$ trees total). Points represent individual pits ($n = 708$). All raw pit dimensions (pit membrane, torus, and aperture diameters) increased with tree size ($p < 0.007$).

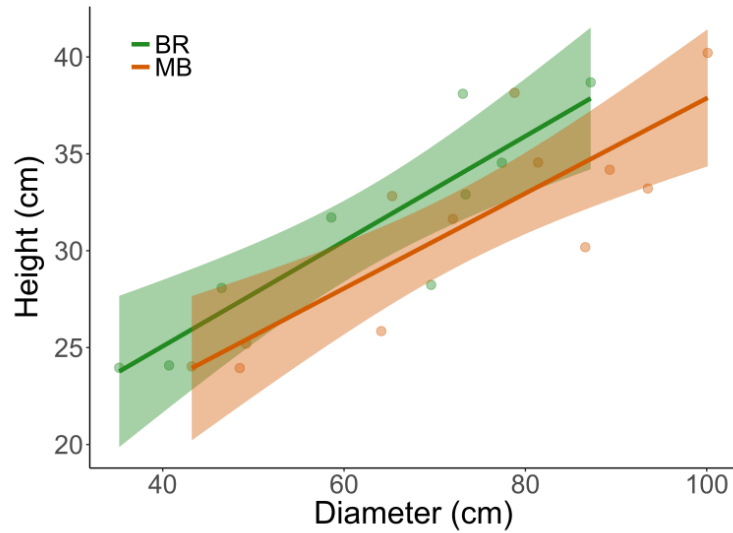


Figure S5. Tree heights by trunk diameters at sampling for each site. Tree heights were shorter for a given trunk diameter at MB, the drier site ($F_{1,19} = 5.105$, $p = 0.04$).

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