

# Linking fine root morphology, hydraulic functioning and shade tolerance of trees

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- **Background and Aims** Understanding root traits and their trade-off with other plant processes is important for understanding plant functioning in natural ecosystems as well as agricultural systems. The aim of the present study was to determine the relationship between root morphology and the hydraulic characteristics of several orders of fine roots (<2 mm) for species differing in shade tolerance (low, moderate and high).
- **Methods** The morphological, anatomical and hydraulic traits across five distal root orders were measured in species with different levels of shade tolerance and life history strategies. The species studied were *Acer negundo*, *Acer rubrum*, *Acer saccharum*, *Betula alleghaniensis*, *Betula lenta*, *Quercus alba*, *Quercus rubra*, *Pinus strobus* and *Pinus virginiana*.
- **Key Results** Compared with shade-tolerant species, shade-intolerant species produced thinner absorptive roots with smaller xylem lumen diameters and underwent secondary development less frequently, suggesting that they had shorter life spans. Shade-tolerant species had greater root specific hydraulic conductance among these roots due to having larger diameter xylems, although these roots had a lower calculated critical tension for conduit collapse. In addition, shade-intolerant species exhibited greater variation in hydraulic conductance across different root growth rings in woody transport roots of the same root order as compared with shade-tolerant species.
- **Conclusions** Plant growth strategies were extended to include root hydraulic properties. It was found that shade intolerance in trees was associated with conservative root hydraulics but greater plasticity in number of xylem conduits and hydraulic conductance. Root traits of shade-intolerant species were consistent with the ability to proliferate roots quickly for rapid water uptake needed to support rapid shoot growth, while minimizing risk in uncertain environments.

**Key words:** Hydraulic conductance, root morphology, shade tolerance, *t/d*, trait plasticity, xylem diameter.

## INTRODUCTION

Canopy gaps and light availability are important factors determining plant community composition and structure (Valladers, 2003; Zavala *et al.*, 2007; Gravel *et al.*, 2010). At the initial stages of stand development, shade-intolerant species often grow in high density and typically allocate a large amount of biomass to fast-growing shoots to compete effectively for light (Casper and Jackson, 1997; Schenk, 2006). A higher level of soil nutrient availability is generally found in early successional forests as compared with late successional forests, which benefits early seral (early successional status), shade-intolerant species typified by fast growth and relatively high above-ground biomass allocation (Walker and del Moral, 2003; Schenk, 2006; Brassard *et al.*, 2009; Freschet *et al.*, 2015). Shade-intolerant species have also been found to be more deeply rooted than shade-tolerant species (Persson *et al.*, 1995; Finér *et al.*, 1997; Chen and Brassard, 2013) with greater ability to absorb soil resources when resource availability is high (Comas *et al.*, 2002; Comas and Eissenstat, 2004; Chen and Brassard, 2013). In contrast, shade-tolerant species may be better able to acquire

nutrients located near the soil surface and also may more effectively suppress the encroachment of neighbours with their relatively shallow, dense root system.

By adapting to more stable environments but longer establishment periods, shade-tolerant species may benefit by having roots with less plasticity, greater structural reinforcement and longer life spans (Chen and Brassard, 2013). This may be reflected in thicker root diameter in more shade-tolerant species (Comas *et al.*, 2002; Comas and Eissenstat, 2004), which is a trait associated with greater root longevity (McCormack *et al.*, 2012), less proliferation in nutrient hot spots and greater dependence on mycorrhizal fungi (Comas *et al.*, 2014; Eissenstat *et al.*, 2015; Liu *et al.*, 2015; Chen *et al.*, 2016; Cheng *et al.*, 2016). While trade-offs in root traits have been considered in terms of nutrient acquisition (e.g. Comas *et al.*, 2002) and interactions with mycorrhizal fungi (e.g. Brundrett, 2002; Comas *et al.*, 2014; Liu *et al.*, 2015), the relationships of root diameter and root order to axial hydraulic conductance and to resistance to loss of hydraulic function have yet to be thoroughly investigated. According to the Hagen–Poiseuille law,

if smaller diameter roots are associated with smaller diameter xylem, then roots of fast-growing, early seral species may also exhibit reduced hydraulic conductance compared with shade-tolerant, late seral species. Larger xylem conduits with greater hydraulic conductance often come at a cost, with increased risk of cavitation (Hacke *et al.*, 2000; Lopez *et al.*, 2005; Gleason *et al.*, 2016). Although high cavitation resistance may be beneficial for roots with a shallow distribution (Sperry and Hacke, 2002; Lopez *et al.*, 2005), shade-tolerant species may experience lower levels of whole-plant water stress, despite being shallow rooted, due to their lower stomatal conductance and slower rates of soil water depletion relative to shade-intolerant species (Hastwell and Facelli, 2003; Poorter *et al.*, 2012).

Considering the biological consequences of the morphological and anatomical properties, there is a need to study root traits and their links to plant adaptations to the environment. Root functioning (absorption and transport) is generally strongly influenced by root position within a branching hierarchy. Absorptive roots, which are typically the first 2–3 root branch orders, function in nutrient and water uptake; while transport roots with a developed vascular cambium and phellem (secondary growth) are found higher in the branching hierarchy and primarily play a structural and transport role with limited or no capacity for water and nutrient absorption (Pregitzer *et al.*, 2002; Guo *et al.*, 2008; Xia *et al.*, 2010). The anatomy of transport roots should theoretically be linked to hydraulic conductance. Few studies, however, have examined hydraulic conductance and cavitation resistance with respect to root position in the branching hierarchy (Long *et al.*, 2013; Kong *et al.*, 2014), where the most distal root orders (absorptive roots) are characterized by the highest rate of water uptake and higher root orders (transport roots), involved in water transport and hydraulic redistribution, play an important role in plant adaptation to water deficits (Rewald *et al.*, 2011). Although there has been increased interest in root traits associated with shade tolerance (Kotowska *et al.*, 2015), limited information exists on xylem traits across different root orders in species that differ in shade tolerance. Moreover, mycorrhizal colonization intensity – related to root position in the branching hierarchy – may have several implications on our understanding of the hydraulic conductance of absorptive roots (Kong *et al.*, 2017). Furthermore,

as temperature and water availability vary among years, examining the characteristics of xylem conduits formed in different years offers an opportunity to examine year to year plasticity in root traits at the anatomical scale.

In the present study, genetically based trait differences were examined among species grown in a common garden in order to standardize environmental variation across species. Several root traits within the same root order were evaluated, including root diameter, development of primary or secondary growth, number of annual growth rings (as a proxy for root age), size and number of xylem conduits (per cross-section), and mycorrhizal colonization. The objective of this study was to determine whether the various root traits were associated with differences in shade tolerance using congeneric contrasts of species in four genera, *Acer*, *Betula*, *Quercus* and *Pinus*. We tested the hypotheses that, compared to shade-tolerant species, shade-intolerant species (1) have greater potential to conduct water; (2) have reduced cell wall structural strength in xylem conducting elements and thus have increased risk of hydraulic failure in roots; (3) have thinner roots with a greater level of branching that is absorptive; and (4) have xylem conducting elements with greater variation in size (diameter) in different years and, thus, exhibit more plasticity in regards to conductance.

## MATERIALS AND METHODS

### Study site

The study site was located at the Russell E. Larson Agricultural Research Center, near State College, Pennsylvania, USA (40.8°N, 77.9°W). The common garden consisted of 16 species of trees, each planted in eight blocks in 1996 using a randomized complete block design, with the exception for *Betula* trees which were planted in 2004. Plants were obtained as 1-year-old liners from local native-plant nurseries, except for individuals of *Acer negundo*, which were collected in early spring as seedlings from around State College. In each block, each species was planted in a group of six trees in a double row of three trees with a spacing of 3 m between trees within the plot. A 9 m spacing was used between the six-tree plots. Thus, each species was represented by a total of 48 trees. The soil in

TABLE 1. Life history characteristics of the nine North America tree species sampled in this study

| Family      | Species                      | Common name   | Shade tolerance | Successional status | Tree longevity | Growth rate     |
|-------------|------------------------------|---------------|-----------------|---------------------|----------------|-----------------|
| Sapindaceae | <i>Acer negundo</i>          | Boxelder      | Low             | Early               | <100 years     | Fast            |
|             | <i>Acer rubrum</i>           | Red maple     | Moderate        | Midseral            | <150 years     | Intermediate    |
|             | <i>Acer saccharum</i>        | Sugar maple   | High            | Late                | >350 years     | Slow            |
| Betulaceae  | <i>Betula lenta</i>          | Sweet birch   | Low             | Early               | <150 years     | Fast            |
|             | <i>Betula alleghaniensis</i> | Yellow birch  | Moderate        | Midseral            | >300 years     | Moderately fast |
| Fagaceae    | <i>Quercus rubra</i>         | Red oak       | Low-moderate    | Early               | <250 years     | Intermediate    |
|             | <i>Quercus alba</i>          | White oak     | Moderate        | Midseral            | >400 years     | Slow            |
| Pinaceae    | <i>Pinus virginiana</i> *    | Virginia pine | Low             | Midseral            | <120 years     | Fast            |
|             | <i>Pinus strobus</i> †       | White pine    | Moderate        | Midseral            | >400 years     | Moderately fast |

Species contrasted in shade tolerance, successional status, tree longevity and growth rate.

\*Often found on ridgetops and other less fertile but open canopy conditions.

†Common on hill slopes in mid-successional mixed hardwood forests.

the study site was a relatively fertile, well-drained, Hagerstown silt loam with a pH ranging from 6.1 to 6.5. Some areas of the soil were high in calcium. Prior to 1996, the site was used as a grass hayfield and the entire area was fenced to keep out deer. Blocking was used to control for variation in soil characteristics (primarily the depth of the A and B horizons). In the present study, only roots of *Acer negundo*, *Acer rubrum*, *Acer saccharum*, *Betula alleghaniensis*, *Betula lenta*, *Quercus alba*, *Quercus rubra*, *Pinus strobus* and *Pinus virginiana* were examined. These species are known to vary widely in shade tolerance, mainly in seedling and sapling growth stages (Table 1; Burns and Honkala, 1990). This life history strategy is thus linked to their place in succession. The selected species represent genera within the Sapindaceae (*Acer*), Betulaceae (*Betula*), Fagaceae (*Quercus*) and Pinaceae (*Pinus*).

#### Root sampling

Two trees from each of three plots for each species were randomly selected in September 2008. Ten intact root samples were collected from each plot. Samples were collected by carefully digging out plant litter and soil to find the distal root tip, and then gently unearthing subsequent root branches with brushes and spatulas. Roots were confirmed to be from an individual tree by tracing the roots back to the trunks of the selected trees. Roots were sampled up to 1 m from the trunk. Intact samples were cut off from the rest of the root system with scissors and placed on a paper towel moistened with deionized water and then placed into a zippered plastic bag. Soil particles adhering to the root samples were not removed at the time of collection. Root samples were stored in the refrigerator (approx. 5 °C) until further processing. During storage (up to 1 week), the plastic bags were opened and the moistened towels were replaced to prevent any drying and deterioration of the roots.

Root samples were large enough to include at least five levels of branching. The designation of root branch orders followed the topological nomenclature developed in the literature, which identifies root tips as first-order branches, second order as the roots from which first-order roots branch, and so on (Pregitzer et al., 2002). Once removed from plastic bags, root samples were gently washed in a water bath and divided into individual branch orders using a steel scalpel. All roots were kept wet

during the dissection and separation process. Only live roots, as determined by texture and visual appearance, were used for further analysis. Ten roots from each branch order were selected from each of the three plots (30 roots of each branch order; 150 roots from each species).

#### Anatomical analysis

Excised root orders were immediately fixed in FAA (5 mL of formaldehyde + 5 mL of acetic acid + 90 mL of 70 % ethanol, v/v/v) for 48 h. The entire root of lower orders (first, second and third orders) was fixed, while higher root orders were cut into a 10 mm length when the root orders exceeded 15 mm. After 48 h in FAA, each root order was washed twice in 70 % ethyl alcohol, and then dehydrated in an ethyl alcohol series (90, 96 and 100 %) for 1 h at each concentration. Further dehydration was performed in a mixture of ethyl alcohol:butyl alcohol (3:1, 1:1 and 3:1, v/v) for 20 min and subsequently with pure butyl alcohol for 0.5 h. Samples were then left overnight at room temperature in fresh butyl alcohol. Samples were subsequently infiltrated with and embedded in Paraplast Plus using the protocol described by Bagniewska-Zadworna et al. (2012). Cross-sections (10 µm thick) were obtained using a microtome (Leica RM2265) (Leica, Germany) and stained in 0.5 % toluidine blue O in 1 % sodium tetraborate. Observations of cross-sections were made using a Carl Zeiss Axioskop 20 (Carl Zeiss, Germany) light microscope with photographs taken at ×5–40 magnification with an AxioCam (Carl Zeiss) using AxioVision software (Carl Zeiss).

#### Root analysis

Digital images (Fig. 1) were used to measure various root parameters. Root diameter was measured using AxioVision software (Carl Zeiss). Each sample was classified as having primary or secondary development based on the presence of anatomical features such as a vascular cambium and phellem (cork cambium). In temperate climates, growth rings in roots can develop with changes in the seasons and during a period of winter dormancy. The number of growth rings in roots with secondary development can therefore be used to estimate root age

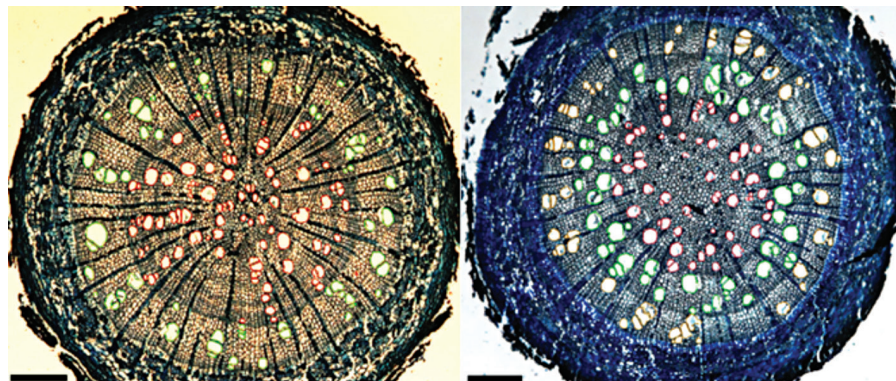


FIG. 1. Transverse section of *A. rubrum* (left panel) and *A. saccharum* (right panel) fifth-order roots showing vessels within two distinct years of xylem growth (annual rings) in *A. rubrum* and three years in *A. saccharum* – first ring (red colour), second ring (green colour) and third ring (yellow colour). Scale bars = 200 µm.



(Fayle, 1968). Since woody roots frequently form discontinuous rings, only complete rings were counted for consistency, which yielded a conservative estimate of root age.

To assess if shade tolerance was related to root water transport ability of a given root order, transverse (cross-section) images of roots were used to measure various parameters of xylem conduits (vessel or tracheids) with AxioVision 4 software. Data were collected from roots of each branch order (ten roots per branch order) for each species. Conduits from each root and growth ring were counted and measured separately. A hydraulic weighted mean conduit diameter ( $d$ ) was estimated by using each lumen radius ( $r$ ) across all conduits of a cross-section or growth ring, and applying the following equation:  $d = 2(\sum r^5 / \sum r^4)$  (Sperry et al., 1994). The cell wall was not included in the diameter. In the case of coniferous species, *P. strobus* and *P. virginiana*, the fact that tracheid lumens are square was taken into account and following correction  $d = (\text{area})^{1/2}$  was used. The theoretical/calculated root-specific hydraulic conductance for individual roots within each root order was estimated using the Hagen–Poiseuille equation as previously described (Valenzuela-Estrada et al., 2009):

$$Lx = \pi \times n_v \times R_a^4 / 8 \times \eta \times SA$$

where  $Lx$  ( $\text{m}^3 \text{s}^{-1} \text{MPa}^{-1} \text{cm}^{-2}$ ) is the theoretical/calculated root hydraulic conductance,  $n_v$  is the mean number of xylem conduits per root,  $R_a$  (m) is the average weighted conduit radius,  $\eta$  ( $1 \times 10^{-9} \text{MPa s}$  at 20 °C) is the viscosity of water, and  $SA$  ( $\text{cm}^2$ ) is root cross-sectional area.

Five representative conduits were used within each growth ring (if secondary growth was present) or cross-section (if only primary growth was present) to estimate the resistance against implosion, and additional anatomical measurements were taken on these conduits. Conduit cell wall thickness ( $t$ ) was measured on at least five conduits per growth ring or cross-section.

Euler buckling theory suggests that the critical tension resulting in conduit collapse is constant when  $t$  scales proportionately with  $d$  (Hacke et al., 2001; Blackman et al., 2010); as such, the ratio of  $t/d$  (assuming proportional scaling) represents the premium a species places on avoiding conduit collapse. We compared  $(t/d)^2$  among species to evaluate the potential differences in the critical tension that would result in conduit collapse (Hacke et al., 2001), as well as the possible trade-off between  $t/d$  and hydraulic capacity [i.e.  $(t/d)^2 \sim Lx$ ].

#### Mycorrhizal colonization

The fungal colonization intensity of individual roots was assessed with individual root cross-sections classified as one of five categories of intensity based on the proportion of cortical cells colonized by mycorrhizal fungi for arbuscular mycorrhizae or the proportion of cortical cells with Hartig's network for ectomycorrhizae. A transect line was randomly drawn across each cross-section to estimate colonization, and the number of colonized cells along each transect line was counted. Roots were considered to be colonized by ectomycorrhizal fungi based on the presence of a Hartig net and mantle (Peterson et al., 2004), and as being associated with endomycorrhizas by the presence

of arbuscules or vesicles. The following categories of colonization intensity were used: 0, no colonization; I, 1–25 % colonization; II, 25–50 % colonization; III, 50–75 % colonization; and IV, 75–100 % colonization.

#### Statistical analysis

A phylogenetic tree was assembled based upon current evaluations of plant species diversification (Fig. 2; Wikstrom et al., 2001; Magallon and Sanderson, 2005; Gernandt et al., 2008). These evaluations used recent methods to date species divergences, including relaxed-clock analyses and fossil-based calibrations, and estimated branch lengths with maximum likelihood. Branch lengths were unavailable for species divergences within *Betula*, *Quercus* and *Acer*. As a result, these tips were estimated as half-splits from their divergence from their closest relative.

Analyses of phylogenetic trait patterns were conducted on a species average by root order, separately for each root order and by averages of root functional classes, i.e. absorptive vs. transport (McCormack et al., 2015). Root orders were classified as absorptive or transport roots based on anatomical traits. Specifically, first-, second- and almost all third-order roots were considered as absorptive roots. On the other hand, third- (when >75 % showed secondary development), fourth- and fifth-order roots were classified as transport roots.

Correlations between shade tolerance and root traits were assessed using phylogenetically independent contrasts (PICs) (Felsenstein, 1985) to account for phylogenetic sampling of species. PICs have been found to give robust estimates of the correlation between characters with as few as eight terminal taxa (Oakley and Cunningham, 2000). PIC analyses were computed with the PDAP:PDTREE Package version 1.15 (Midford et al., 2010) within the Mesquite system (Maddison and Maddison, 2010) for phylogenetic computing using the fully resolved phylogenetic tree described above.

Overall effects of shade tolerance, family relationship and root order on root traits were analysed with a general linearized model (GLM), and a hierarchical analysis of variance (ANOVA) using mixed effects where shade tolerance (random variable) was nested within taxonomic contrasts. Taxonomic contrast and species within contrast were fixed terms. Root traits were dependent variables. The pooled standard error for individual traits was calculated from the square root of the mean square error in the hierarchical ANOVA. All analyses were conducted using Statistica version 8.0 (StatSoft Tulsa, OK, USA) software.

## RESULTS

#### Hydraulic conductance and likelihood of failure

Contrary to the initial hypotheses, root-specific hydraulic conductance ( $Lx$ ) generally increased with increasing shade tolerance when traits were compared across species of four genera using PIC analyses (second-order, fourth-order and pooled absorptive roots, trend for first order; Table 2). There was no general relationship between  $Lx$  and shade tolerance

TABLE 2. Pearson correlation coefficient ( $r$ ) between pairs of traits among phylogenetically independent contrasts (PICs) analysed separately for each root order (first to fifth order), and by root functional classes, i.e. absorptive (average of first to third order) vs. transport (average of fourth to fifth root order)

|  | $n_v$ | $Lx$ ( $m^3 s^{-1} MPa^{-1} cm^{-2}$ ) | $t$    | $d$     | $(t/d)^2$ | RAD (mm) | Develop  | Rings   | Colonization |
|--|-------|--|--------|---------|-----------|----------|----------|---------|--------------|
| <b>First order</b>                     |       |  |        |         |           |          |          |         |              |
| Shade tolerance                        | -0.45 | 0.56                                   | -0.21  | 0.29    | -0.41     | 0.62*    | —        | —       | 0.16         |
| $n_v$                                  |       | 0.03                                   | 0.58*  | 0.32    | 0.56      | -0.18    | —        | —       | -0.65**      |
| $Lx$ ( $m^3 s^{-1} MPa^{-1} cm^{-2}$ ) |       |  | -0.05  | 0.62*   | -0.34     | 0.37     | —        | —       | -0.26        |
| $t$                                    |       |  |        | 0.57#   | 0.93†     | 0        | —        | —       | -0.60*       |
| $d$                                    |       |  |        |         | 0.24      | 0.46     | —        | —       | -0.20        |
| $(t/d)^2$                              |       |  |        |         |           | -0.22    | —        | —       | -0.63*       |
| RAD (mm)                               |       |  |        |         |           |          | —        | —       | 0.23         |
| Develop                                |       |  |        |         |           |          | —        | —       | —            |
| Rings                                  |       |  |        |         |           |          |          | —       | —            |
| <b>Second order</b>                    |       |  |        |         |           |          |          |         |              |
| Shade tolerance                        | -0.21 | 0.75**                                 | 0.68** | 0.84*** | -0.51     | 0.61*    | 0.18     | —       | 0.6*         |
| $n_v$                                  |       | 0                                      | -0.19  | -0.05   | -0.03     | -0.37    | 0.04     | —       | 0.18         |
| $Lx$ ( $m^3 s^{-1} MPa^{-1} cm^{-2}$ ) |       |  | 0.31   | 0.96†   | -0.85***  | 0.14     | 0.68**   | —       | 0.54*        |
| $t$                                    |       |  |        | 0.42    | 0.13      | 0.73**   | -0.07    | —       | 0.40         |
| $d$                                    |       |  |        |         | -0.84***  | 0.35     | 0.54     | —       | 0.68**       |
| $(t/d)^2$                              |       |  |        |         |           | 0.01     | -0.60*   | —       | -0.49        |
| RAD (mm)                               |       |  |        |         |           |          | -0.40    | —       | 0.24         |
| Develop                                |       |  |        |         |           |          |          | —       | —            |
| Rings                                  |       |  |        |         |           |          |          | —       | —            |
| <b>Third order</b>                     |       |  |        |         |           |          |          |         |              |
| Shade tolerance                        |       | 0.14                                   | 0.15   | 0.54    | -0.59*    | 0.56     | 0.46     | 0.55    | ‡            |
| $n_v$                                  |       | §                                      | §      | §       | §         | §        | §        | §       | §            |
| $Lx$ ( $m^3 s^{-1} MPa^{-1} cm^{-2}$ ) |       |  | 0.29   | 0.84*** | -0.46     | 0.03     | 0.17     | -0.15   | ‡            |
| $t$                                    |       |  |        | 0.48    | 0.45      | 0.57*    | -0.33    | -0.54   | ‡            |
| $d$                                    |       |  |        |         | -0.53     | 0.53     | 0.20     | -0.05   | ‡            |
| $(t/d)^2$                              |       |  |        |         |           | -0.1     | -0.54    | -0.60** | ‡            |
| RAD (mm)                               |       |  |        |         |           |          | -0.12    | -0.12   | ‡            |
| Develop                                |       |  |        |         |           |          |          | 0.66**  | ‡            |
| Rings                                  |       |  |        |         |           |          |          |         | ‡            |
| <b>Fourth order</b>                    |       |  |        |         |           |          |          |         |              |
| Shade tolerance                        | -0.16 | 0.60*                                  | 0.48   | 0.59*   | -0.04     | 0.35     | —        | 0.66**  | —            |
| $n_v$                                  |       | 0.28                                   | 0.09   | -0.57*  | 0.45      | -0.07    | —        | -0.39   | —            |
| $Lx$ ( $m^3 s^{-1} MPa^{-1} cm^{-2}$ ) |       |  | 0.25   | 0.46    | -0.66*    | -0.17    | —        | 0.86*** | —            |
| $t$                                    |       |  |        | 0.40    | 0.56      | 0.81***  | —        | 0.12    | —            |
| $d$                                    |       |  |        |         | -0.42     | 0.69**   | —        | 0.54    | —            |
| $(t/d)^2$                              |       |  |        |         |           | 0.16     | —        | -0.54   | —            |
| RAD (mm)                               |       |  |        |         |           |          | —        | 0.05    | —            |
| Develop                                |       |  |        |         |           |          | —        | 0.06    | —            |
| Rings                                  |       |  |        |         |           |          |          |         | —            |
| <b>Fifth order</b>                     |       |  |        |         |           |          |          |         |              |
| Shade tolerance                        | 0.18  | -0.14                                  | -0.12  | 0.28    | -0.42     | 0.28     | —        | 0.71**  | —            |
| $n_v$                                  |       | -0.42                                  | 0.21   | -0.09   | 0.50      | 0.3      | —        | -0.12   | —            |
| $Lx$ ( $m^3 s^{-1} MPa^{-1} cm^{-2}$ ) |       |  | 0.50   | 0.49    | -0.16     | 0.21     | —        | -0.01   | —            |
| $t$                                    |       |  |        | 0.70**  | 0.33      | 0.75**   | —        | 0.12    | —            |
| $d$                                    |       |  |        |         | -0.33     | 0.91†    | —        | 0.60*   | —            |
| $(t/d)^2$                              |       |  |        |         |           | -0.10    | —        | -0.55   | —            |
| RAD (mm)                               |       |  |        |         |           |          | —        | 0.52    | —            |
| Develop                                |       |  |        |         |           |          | —        |         | —            |
| Rings                                  |       |  |        |         |           |          |          |         | —            |
| <b>Absorptive</b>                      |       |  |        |         |           |          |          |         |              |
| Shade tolerance                        | -0.34 | 0.83†                                  | 0.16   | 0.89*** | -0.53     | 0.62*    | 0.72**   | ¶       | 0.27         |
| $n_v$                                  |       | -0.42                                  | 0.09   | -0.35   | 0.33      | -0.13    | -0.42    | ¶       | -0.58*       |
| $Lx$ ( $m^3 s^{-1} MPa^{-1} cm^{-2}$ ) |       |  | -0.17  | 0.84*** | -0.76**   | 0.30     | 0.94†    | ¶       | 0.64**       |
| $t$                                    |       |  |        | 0.24    | 0.72**    | 0.53     | -0.35    | ¶       | -0.31        |
| $d$                                    |       |  |        |         | -0.48     | 0.71**   | 0.68**   | ¶       | 0.53         |
| $(t/d)^2$                              |       |  |        |         |           | -0.05    | -0.82*** | ¶       | -0.59*       |
| RAD (mm)                               |       |  |        |         |           |          | 0.21     | ¶       | 0.05         |
| Develop                                |       |  |        |         |           |          |          | ¶       | —            |
| Rings                                  |       |  |        |         |           |          |          |         | —            |
| <b>Transport</b>                       |       |  |        |         |           |          |          |         |              |
| Shade tolerance                        | 0.13  | -0.18                                  | 0.06   | 0.37    | -0.28     | 0.32     | -0.02    | 0.74**  | —            |
| $n_v$                                  |       | -0.36                                  | 0.25   | -0.22   | 0.56      | 0.21     | -0.14    | -0.25   | —            |
| $Lx$ ( $m^3 s^{-1} MPa^{-1} cm^{-2}$ ) |       |  | 0.44   | 0.50    | -0.26     | 0.24     | 0.30     | -0.12   | —            |
| $t$                                    |       |  |        | 0.65*   | 0.29      | 0.81***  | 0.17     | 0.06    | —            |
| $d$                                    |       |  |        |         | -0.42     | 0.87***  | 0.39     | 0.58*   | —            |

(continued)

TABLE 2. Continued

|           | $n_v$ | $Lx$ ( $m^3 s^{-1} MPa^{-1} cm^{-2}$ ) | $t$ | $d$ | $(t/d)^2$ | RAD (mm) | Develop | Rings | Colonization |
|-----------|-------|--|-----|-----|-----------|----------|---------|-------|--------------|
| $(t/d)^2$ |       |  |     |     |           | -0.07    | -0.68** | -0.54 | -            |
| RAD (mm)  |       |  |     |     |           |          | 0.31    | 0.40  | -            |
| Develop   |       |  |     |     |           |          |         | 0.16  | -            |
| Rings     |       |  |     |     |           |          |         |       | -            |

Traits included the number of conduits ( $n_v$ ), root-specific hydraulic conductance ( $Lx$ ), thickness of individual conduit walls ( $t$ ), hydraulic weighted mean conduit (lumen) diameter ( $d$ ), the critical tension for conduit collapse  $(t/d)^2$ , root average diameter (RAD), proportion of the sample under secondary development (Develop), number of growth rings (Rings) and mycorrhizal colonization (Colonization).

Statistical significance is indicated by \* $P < 0.1$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$  and † $P < 0.001$

– Indicates that analyses were not run. For example, first-order roots do not undergo secondary development, and thus do not form growth rings and periderm. All species had fourth- and fifth-order roots with secondary development, thus this trait was the same among all species so analyses were not run.

‡There was only colonization on third-order roots of *Q. alba* so analyses were not run.

¶Analyses were not run because only two species (*P. virginiana* and *A. saccharum*) formed rings on third-order roots.

§Analyses of  $n_v$  in third-order roots were excluded because these analyses did not pass assumptions for the statistical tests.

in third- and fifth-order roots or in pooled transport root PICs (Table 2). Within comparisons of *Acer*, *Betula* and *Quercus*,  $Lx$  was approx. 140 % higher in moderately shade-tolerant than in shade-intolerant species across all root orders, but in *Pinus*, except for first-order roots, the trend was reversed and species of moderate shade tolerance had 200 % lower  $Lx$  than intolerant species (Fig. 3A).

Also in contrast to our hypotheses, no general pattern was found between root hydraulic mechanical strength ( $t$ ) and shade tolerance assessed by PICs among root orders, or root functional classes, except among second-order roots (Table 2). There was, however, a general pattern of moderately shade-tolerant species in all four families having thicker cell walls ( $t$ ) (Fig. 3B) and a tendency for  $t$  to be thicker with increased  $Lx$  in second- to fifth-order roots and in transport roots, suggesting that roots with greater  $Lx$  were more heavily fortified (Table 2). The critical tension for conduit collapse  $(t/d)^2$

decreased as  $Lx$  increased in second-order, fourth-order and absorptive roots, with tendencies in the others, when assessed with PICs (Table 2).

#### Root morphology, anatomy and mycorrhizal colonization

Root diameter increased with shade tolerance across first- and second-order roots and absorptive roots (Table 2). Across all root classes, diameters of moderate and high shade-tolerant species were thicker than root diameter of shade-intolerant species, with differences increasing in higher root orders (Fig. 4; 50–70 % greater in absorptive and approx. 140 % greater in transport roots). Overall differences in root diameter ( $P < 0.001$ ) were larger in some families than in others, which led to a significant interaction between root diameter and shade tolerance nested

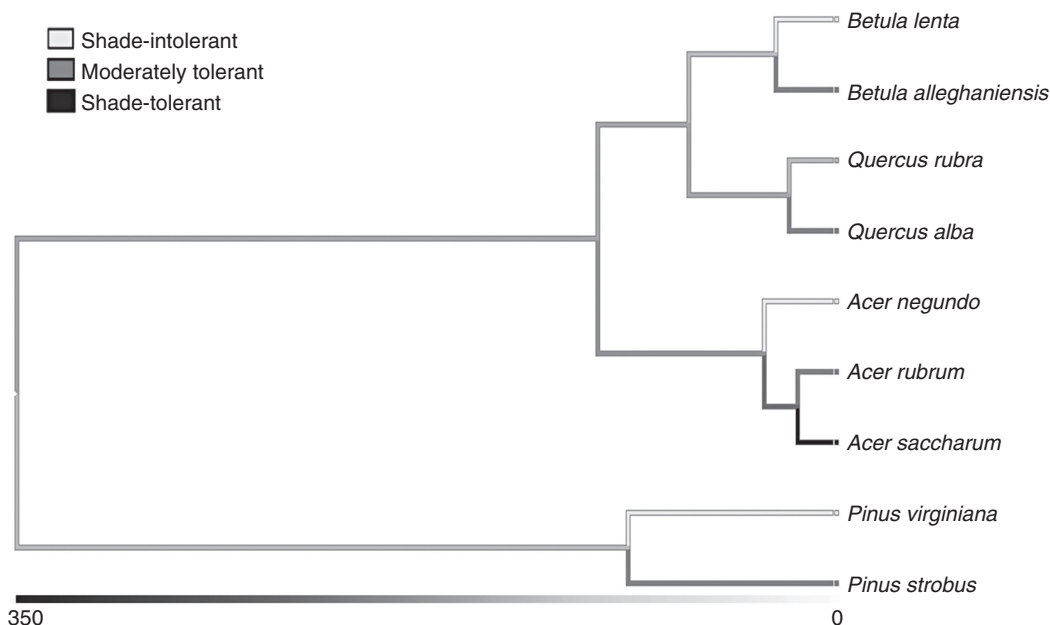


FIG. 2. A phylogenetic tree with shade tolerance mapped was assembled from literature on plant species diversification (Wikstrom *et al.*, 2001; Magallon and Sanderson, 2005; Germant *et al.*, 2008). Branch lengths of the phylogenetic tree indicate time since divergence from a shared ancestor in units of million years.

within a family  $\times$  root order ( $P = 0.019$ ). Differences were smaller among *Acer* species ( $P > 0.53$ ) than among species within the other three genera ( $P < 0.001$ ). Roots with thicker diameter had a thicker xylem lumen diameter ( $d$ ) or a tendency towards it across all root orders and functional classes (Table 2). This relationship was probably the primary driver of the relationship between  $Lx$  and shade tolerance as there was no relationship between the number of conduits and shade tolerance (Table 2; Figs 3A, C, D and 4). The thicker roots in species with greater shade tolerance tended to be more intensely colonized by mycorrhizal fungi than species with moderate or low shade tolerance (approx.

35, 28 and 16 % calculated as a mean of first, second and third orders, respectively) (Fig. 5). This relationship held true in all but one family, the Betulaceae; thus, relationships showed trends but were either only marginally significant ( $P < 0.10$ ) or not statistically significant ( $P > 0.10$ ) when assessed with PICs across root orders (Table 2). There was also increased  $Lx$  associated with mycorrhizal colonization among second-order and absorptive functional class roots when assessed with PICs (Table 2).

Secondary development was hypothesized to occur to a greater degree in lower root branching orders in shade-tolerant species, presumably because shade-intolerant species produce

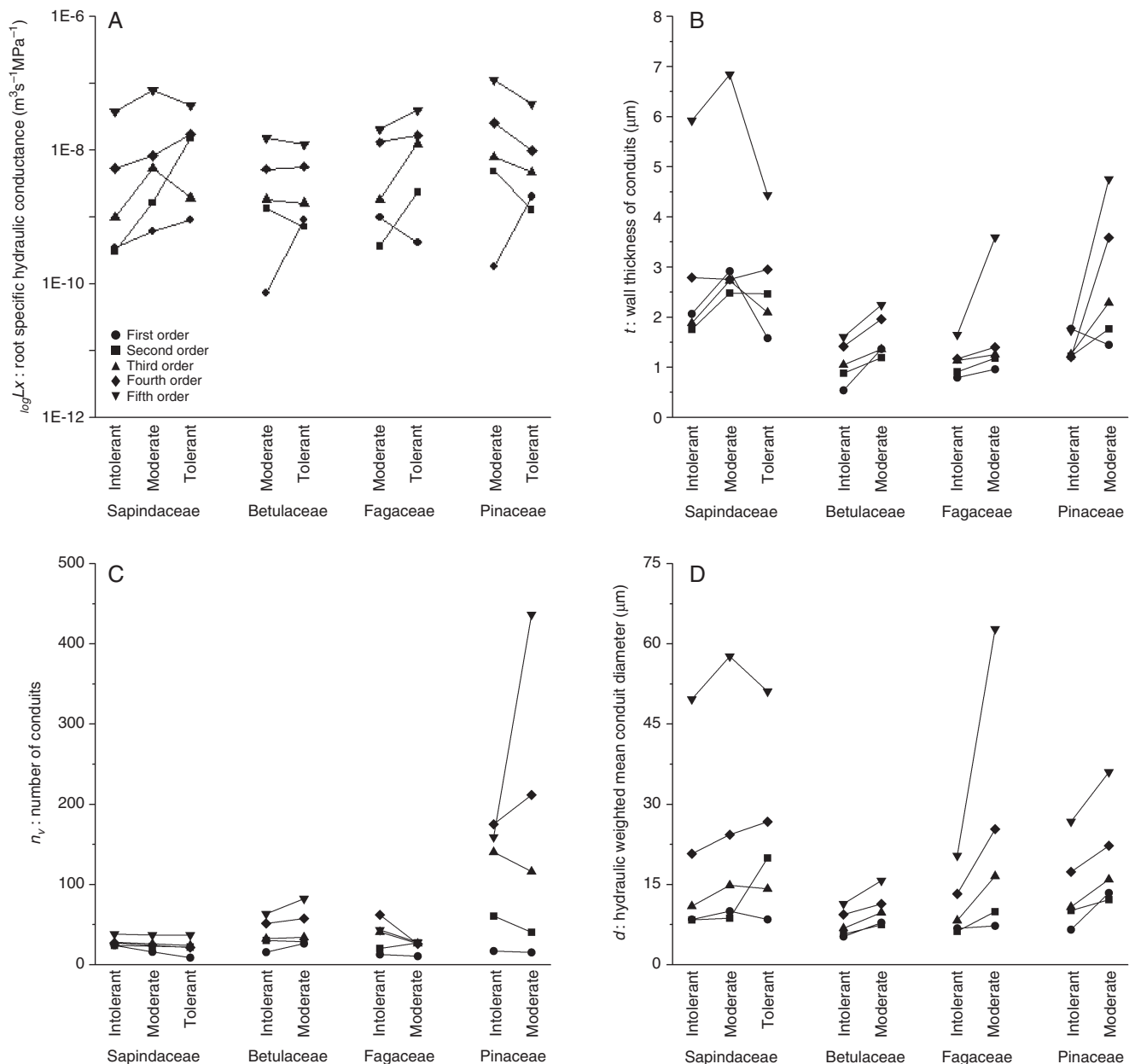


FIG. 3. Root hydraulic traits among species and root orders. Traits include root-specific hydraulic conductance,  $Lx$  (A); wall thickness of conduits,  $t$  (B); number of conduits,  $n_v$  (C); and hydraulic weighted mean conduit (lumen) diameter,  $d$  (D) given separately for first-, second-, third-, fourth- and fifth-order roots among species with low (Intolerant), moderate (Moderate) and high (Tolerant) shade tolerance in Sapindaceae, Betulaceae, Fagaceae and Pinaceae. Species are given in Table 1. Mean square error = 8.E-09 for  $Lx$ ; 0.48 for  $t$ ; 41.78 for  $n_v$ ; and 7.54 for  $d$ .

a more highly branched absorptive root system. First- and second-order roots are generally believed to be ephemeral and rarely showed secondary development among any of the species. Similarly, fourth- and fifth-order roots were mainly woody across the nine species. The main variation occurred in the third-order roots that were primarily absorptive in some species but exhibited more of a role in transport in others. There was no consistent pattern among the four contrasts. Shade-tolerant species had a greater degree of secondary development in third-order roots among *Acer* (Sapindaceae) and *Quercus* (Fagaceae), but an opposite trend was observed in *Betula* species (Betulaceae) and no difference was observed between *Pinus* species (Pinaceae) (Table 3).

Examining the number of growth rings as a proxy for root age, we found that the number of rings generally increased with shade tolerance in higher root orders (fourth and fifth order) and the collective category of transport roots ( $rPIC=0.66-0.74$ ,  $P=0.01-0.05$ ; Table 2), suggesting that higher order roots of moderately and highly shade-tolerant species were generally older (had more growth rings) than roots of shade-intolerant species. This relationship was primarily driven by patterns within *Acer*, *Betula* and *Quercus* (Table 3). The *Pinus* species showed the opposite trend.

#### Year to year hydraulic plasticity

The number of growth rings nested into root order in shade-intolerant species showed a tendency for xylem conduit number to vary among different years ( $P=0.08$ ), suggesting an enhanced level of plasticity, while the number of conduits in growth rings of moderately and highly shade-tolerant species did not vary from year to year, suggesting a low level of plasticity ( $P=0.21$

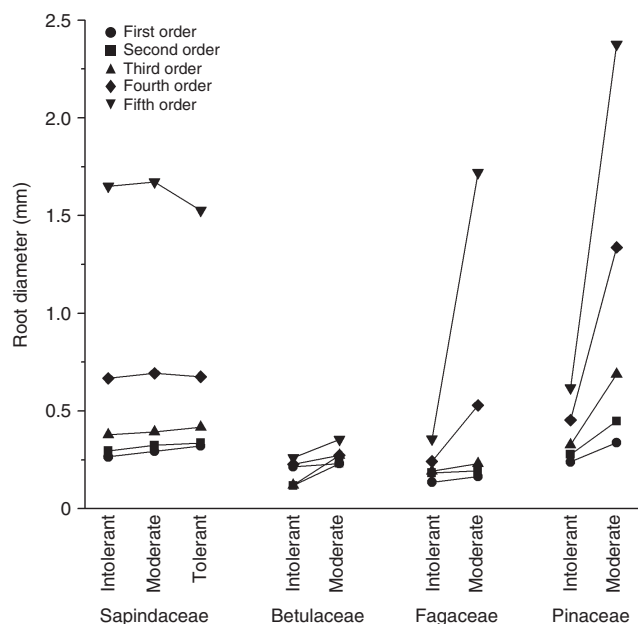


FIG. 4. Root diameter (mm) of first-, second-, third-, fourth- and fifth-order roots among species with low (Intolerant), moderate (Moderate) and high (Tolerant) shade tolerance in Sapindaceae, Betulaceae, Fagaceae and Pinaceae. Species are given in Table 1. Mean square error = 0.11 for root diameter.

and  $P=0.37$ , respectively) (Supplementary Data Fig. S1). Likewise, the root specific hydraulic conductance ( $Lx$ ) differed in shade-intolerant and moderately tolerant species among different years ( $P<0.02$  and  $P<0.01$ , respectively), while no significant relationship was observed in  $Lx$  among growth rings in highly shade-tolerant species ( $P=0.27$ ) (Supplementary Data Fig. S1). However, the hydraulically weighted mean conduit diameter,  $d$ , did not differ in growth rings of shade-intolerant species ( $P=0.1$ ) but differed in those of moderately and highly shade-tolerant species ( $P<0.001$ ), suggesting that plasticity in  $Lx$  of shade-intolerant species was due to variation in xylem conduit number (Supplementary Data Fig. S1).

## DISCUSSION

In the present study, we analysed root traits and strategies for hydraulic conductance associated with shade tolerance among tree species from different taxonomic families. Root samples were collected from species grown in a common garden where environmental conditions, especially light interception, were similar for all individuals. Thus, trait variation should be due to the adaptation of these species to their natural habitat, or reflect phylogenetic inertia. Counter to hypotheses, high  $Lx$  was generally associated with shade tolerance rather than shade intolerance among absorptive roots, potentially fitting with the adaptation of shade-intolerant species to fluctuating and uncertain water status, rather than high conductance associated with fast growth. Increases in  $Lx$  across all roots were generally associated with increases in xylem conduit diameter ( $d$ ), with  $d$  also generally increasing with root average diameter

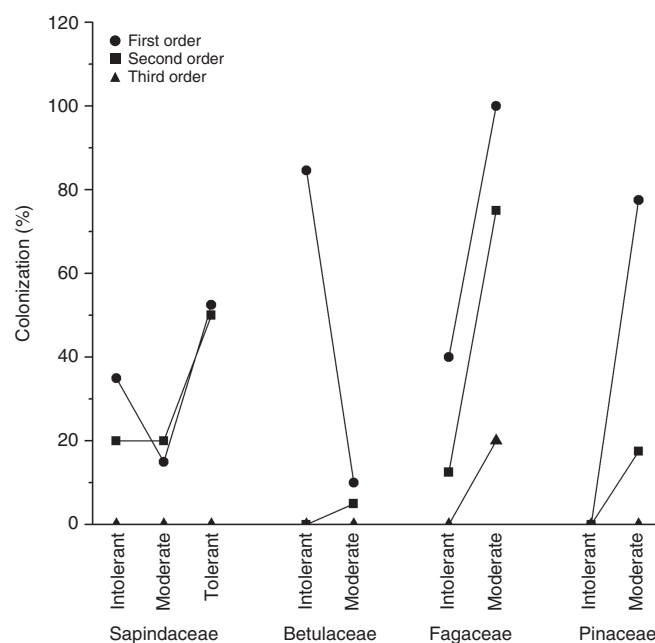


FIG. 5. Mycorrhizal colonization (%) of first-, second- and third-order roots among species with low (Intolerant), moderate (Moderate) and high (Tolerant) shade tolerance in Sapindaceae, Betulaceae, Fagaceae and Pinaceae. Species are given in Table 1. Mycorrhizal colonization was determined from the proportion of cortical cells colonized by mycorrhizal fungi for arbuscular mycorrhizae or the proportion of cortical cells with Hartig's network for ectomycorrhizae. Mean square error = 0.79 for mycorrhiza colonization.



TABLE 3. Percentage of roots with secondary development and number of growth rings for individual root orders (I–V) across nine North American tree species, growing in a common garden contrasting in shade tolerance

| Family      | Species                      | Shade tolerance | I                     |              | II                    |              | III                   |              | IV                    |              | V                     |              |
|-------------|------------------------------|-----------------|-----------------------|--------------|-----------------------|--------------|-----------------------|--------------|-----------------------|--------------|-----------------------|--------------|
|             |                              |                 | Secondary development | No. of rings | Secondary development | No. of rings | Secondary development | No. of rings | Secondary development | No. of rings | Secondary development | No. of rings |
| Sapindaceae | <i>Acer negundo</i>          | Low             | –                     | –            | –                     | –            | –                     | –            | 100                   | 0.6          | 100                   | 2.3          |
|             | <i>Acer rubrum</i>           | Moderate        | –                     | –            | 30                    | –            | –                     | 100          | 100                   | 0.5          | 100                   | 1.9          |
|             | <i>Acer saccharum</i>        | High            | –                     | 20           | 100                   | 0.3          | –                     | 100          | 100                   | 1.5          | 100                   | 3            |
| Betulaceae  | <i>Betula lenta</i>          | Low             | –                     | 29           | –                     | –            | –                     | 100          | 100                   | –            | 100                   | –            |
|             | <i>Betula alleghaniensis</i> | Moderate        | –                     | –            | 10                    | –            | –                     | 90           | 100                   | –            | 100                   | 1.5          |
| Fagaceae    | <i>Quercus rubra</i>         | Low–moderate    | –                     | –            | –                     | –            | 20                    | –            | 100                   | –            | 100                   | 0.8          |
|             | <i>Quercus alba</i>          | Moderate        | –                     | –            | 60                    | –            | –                     | 100          | 100                   | 0.4          | 100                   | 2.2          |
| Pinaceae    | <i>Pinus virginiana</i>      | Low             | –                     | –            | –                     | –            | 100                   | 0.4          | 100                   | 0.7          | 100                   | 1.3          |
|             | <i>Pinus strobus</i>         | Moderate        | –                     | –            | –                     | –            | 100                   | –            | 100                   | –            | 100                   | 1            |

Ten roots from each branch order from plots were selected for observations.

(–) indicates that no roots in the designated root order had secondary development or growth rings. Mean square error was  $\pm 0.16$  for secondary development based on percentile data and  $\pm 0.32$  for no. of rings.

(RAD) across all roots. Interestingly, mycorrhizal colonization also increased with  $Lx$  in absorptive roots, suggesting coupled traits for resource acquisition (nutrient and water). Fitting with our initial hypotheses, thickness of the conduit walls,  $t$ , showed a tendency to increase with  $Lx$  in transport roots across all species, consistent with needs for greater hydraulic structural support with increased  $Lx$ . However,  $d$  generally increased with increased  $Lx$  more quickly than  $t$ , such that the critical tension needed for conduit collapse,  $(t/d)^2$ , generally decreased with increased  $Lx$ . The results suggested that species with greater shade tolerance had root traits associated with longer root life spans, including roots with greater RAD, fewer levels of branching among the absorptive roots (i.e. third-order roots more frequently had secondary development) and transport roots that were older (as evidenced by having fourth- and fifth-order roots with more growth rings). Finally, shade-intolerant species generally had transport roots with more plasticity in conduit numbers and  $Lx$  among growth rings, suggesting further adaptation to fluctuating and uncertain water status.

The initial hypothesis that shade-intolerant plants evolved greater hydraulic conductance in their root systems than shade-tolerant tree species was proposed based on the fast growth of early seral species and high water requirements anticipated to support this faster growth, as the above-ground plant physiological response should be related to root system development (Norby *et al.*, 2001; Trubat *et al.*, 2006). For example, grasses were found to increase vessel root area with decreasing light supply in order to support sufficient hydraulic conductance (Wahl *et al.*, 2001). As discussed by Hubbard *et al.* (2001) or Lopez *et al.* (2005), in order to provide the amount of water needed to maintain carbon assimilation and evaporative demands, the root system in shade-tolerant species may be designed for high water conductance (Cochard *et al.*, 1997; Maherali *et al.*, 1997) as their leaf dark respiration rate is higher in a standardized light environment (Lusk and Reich, 2000). Shade-tolerant species often have thinner leaves with lower transpiration efficiency (Wyka *et al.*, 2012; Giuliani *et al.*, 2013), and thus may have higher water requirements, congruent with patterns of higher root-specific water conductance ( $Lx$ ) when grown in a common garden. We found general patterns of both thicker root average diameters and greater mycorrhizal colonization associated with shade tolerance, similar to observations in the literature (Comas and Eissenstat, 2004; Chen *et al.*, 2013; Kong *et al.*, 2014). Interestingly, the association of shade tolerance with both increased  $Lx$  and mycorrhizal colonization, and increased  $Lx$  with increased mycorrhizal colonization among absorptive roots functional classes is in accordance with literature results that demonstrated increased hydraulic conductivity with greater mycorrhizal colonization (Muhsin and Zwiazek, 2002). Additionally, shade-tolerant species are suggested to increase their capacity for water absorption through promoting increased external mycelium of long-distance transport types (Lehto and Zwiazek, 2011).

From a mechanical perspective, species with high hydraulic conductance may benefit from mechanisms that decrease the risk of cavitation (Wagner *et al.*, 1998; Lens *et al.*, 2011). Protection of xylem conduits from cavitation can be achieved in two ways: increased cell wall fortification, or reduction in conduit diameter. We showed a tendency of increased  $t$  with  $Lx$  in transport roots, as well as increased  $t$  with increased  $d$

with shade tolerance, supporting the idea that risk of hydraulic failure drives adaptation of xylem anatomy of transport roots to confer protection against embolism (Hacke *et al.*, 2001, 2007; Jacobsen *et al.*, 2007). However,  $d$  increased more rapidly than  $t$  in association with greater  $Lx$ , thus roots with greater  $Lx$  generally had lower  $(t/d)^2$  as often found in the literature (Hacke *et al.*, 2000; Lopez *et al.*, 2005; Gleason *et al.*, 2016). Smaller  $d$  in shade-intolerant species may reflect a strategy of conservative hydraulic regulation by limiting risk of cavitation under water-limiting environment.

Examining relationships between conduit number and  $Lx$  across growth rings, our data suggested greater plasticity in traits among years in shade-intolerant species. Greater plasticity in conduit number and  $Lx$  in shade-intolerant species may be advantageous for growing in early seral habitats, which may be unpredictable and require swift adjustment to prevailing conditions between years for plants to compete for water and nutrients. Moreover, control of conduit number and  $Lx$  may be a means to increase xylem safety in a cost-effective way for handling periodic drought. Inversely, less trait variation in shade-tolerant species may reflect adaptation to growth in an environment with less fluctuation from year to year. Such conservation might, however, be a disadvantage when roots foraging in the soil encounter resource-rich patches. These patterns of root trait plasticity are in agreement with previous observations that species adapted to higher light requirements exhibit greater phenotypic plasticity in morphological and physiological traits than those adapted to shade (Henry and Aarssen, 1997; Sánchez-Gómez *et al.*, 2006a). In contrast, the low plasticity of shade-tolerant species growing under more predictable conditions may reflect a conservative strategy of resource use and greater reliance on a sufficient water supply (Abrams and Mostoller, 1995; Grime and Mackey, 2002; Niinemets and Valladares, 2006; Sánchez-Gómez *et al.*, 2006a,b).

Root traits such as diameter and secondary development impact nutrient uptake. Trait variation in this study aligns with the idea that species with thinner roots are often capable of fast soil foraging, although it was previously considered that thin roots are characterized by high specific hydraulic conductance (Huang and Eissenstat, 2000; Hernández *et al.*, 2010), which was not found here for axial conductance. The association of shade intolerance with smaller root diameter, a more branched absorptive root system and a lower number of growth rings (lower average age) of their transport roots suggests strategies of rapidly proliferating roots with a short life span (*sensu* Eissenstat and Yanai, 1997; Comas *et al.*, 2002). However, nutrient acquisition is, importantly, also influenced by the developmental status of roots, where roots with primary growth (absorptive roots) reflect the ability to take up resources, and roots with secondary development (transport roots) reflect the ability to transport water and resources (Eissenstat and Achor, 1999; Gambetta *et al.*, 2013). Generally, absorptive roots are within the first to third root orders, and roots located higher in the branching hierarchy are transport roots (Pregitzer *et al.*, 2002; Guo *et al.*, 2008; Xia *et al.*, 2010). Roots with secondary development generally occurred more frequently in the lower branching orders in more shade-tolerant species within the Sapindaceae and Fagaceae than in shade-intolerant species, although the opposite trend was observed within the Betulaceae. The patterns observed within the Betulaceae may

be partially accounted for by the growth habitat of the examined species. *Betula alleghaniensis* grows in cooler regions with a shorter growing season than *B. lenta* (Burns and Honkala, 1990). Maintaining absorptive roots without secondary growth is a common attribute in species that grow in cold soils (Chapin, 1974; Zadworny *et al.*, 2016). Additionally, although *B. alleghaniensis* is considered a moderately shade-tolerant species, during the early stages of its life cycle, it also grows rapidly as a pioneer species (Burns and Honkala, 1990). From a root system perspective, secondary development of roots minimizes water loss to dry soils, also referred to as reverse water flow, i.e. from the root to the soil (Gambetta *et al.*, 2013).

In conclusion, results here expand ideas of plant growth strategies to include hydraulic strategies of root systems. This study supports previous ideas of plant growth strategies suggesting that species adapted to high light and resource-rich environments produce thinner roots with shorter life span (Walters and Reich, 1999), and that thinner roots are less reliant on mycorrhizal fungi, in contrast to thicker, extensively colonized roots (Baylis, 1975; Brundrett, 2002; Comas *et al.*, 2012, 2014; Kong *et al.*, 2014; Eissenstat *et al.*, 2015; Liu *et al.*, 2015). We also show that thinner roots of shade-intolerant species also had thinner xylem and lower hydraulic conductance, potentially limiting the risk of cavitation under low water conditions, which may occur with greater frequency under conditions of early succession where competition for water rather than light might be greater. Moreover, our results showed that shade-intolerant species had greater plasticity in number of xylem conduits and hydraulic conductance across different root growth rings of the same root order as compared with shade-tolerant species, also potentially tied to greater fluctuation of water availability in early successional habitats. Expanded species comparisons are needed to improve understanding of how universally these plant life history strategies are linked to hydraulic strategies and interactions with mycorrhizal fungi.

#### SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of Fig. S1: root hydraulic traits by growth ring and shade tolerance.

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