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ORIGINAL ARTICLE

Intra-organismal variation in the structure of plant vascular transport tissues in poplar trees

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Abstract

Key message **Phloem and xylem conduit structure vary greatly throughout the body of *Populus trichocarpa* trees, particularly between roots and shoots. This has implications for understanding organ and whole plant vascular function.**

Abstract Woody plant vascular transport occurs predominantly within secondary xylem and phloem, which are both produced by the vascular cambium during secondary growth. We examined how vessel and sieve tube structure varied throughout the plant body of *P. trichocarpa* trees and whether xylem and phloem conduit structure was correlated across different positions within the plant. We excavated entire juvenile *P. trichocarpa* trees and measured vessel and sieve tube structural traits of current-year growth in 1 m increments along the main root:shoot axis. Trees were > 4 m tall and had roots that extended 4–5 m at their longest length. We found that both sieve tube and vessel diameters greatly varied throughout the plant body and with organ diameter. Roots had wider diameter conduits than shoots. Sieve tube diameter was strongly correlated with vessel diameter, which may be related to their common developmental origin. Other structural traits, such as pit membrane area and pit density for xylem, and sieve plate area and number of sieve areas per plate for phloem, also varied and were correlated with changes in conduit diameter. The median air-seeding pressure of vessels (P_m) and vessel length did not differ between roots and shoots. Understanding plant vascular function will likely require increased knowledge of whole plant structure and function, since plant performance may be limited by any point along the transport pathway. Considering intra-organismal variation may be a way to evaluate structure–function hypotheses while controlling for confounding sources of variation that may impact inter-specific comparisons.

Keywords Anatomy · Development · Phloem · Pit membrane · Sieve area · Sieve plate · Single-vessel air injection · Transport · Xylem

Introduction

Within woody plants, long-distance transport occurs predominantly within secondary xylem and phloem. Additional resistance to plant transport occurs due to non-vascular components of the whole plant pathway, particularly in fine roots and leaves. Secondary xylem and phloem tissues are produced by the vascular cambium during secondary growth. In angiosperms, the main transport conduits, vessels in the xylem and sieve tubes in the phloem, are both derived from the same type of initial cell within the cambium. The structure of these transport conduits determines plant water use, drought tolerance, growth, and metabolism (Hacke and Sperry 2001; Hacke et al. 2006; Hölttä et al. 2009; Mullendore et al. 2010). Numerous studies have examined the impact of conduit structure on transport function within

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woody plant vascular systems. This includes the relationship between vessel diameter and hydraulic function (reviewed in Hacke and Sperry 2001), the relationship between vessel pit structure and hydraulic function (Wheeler et al. 2005; Hacke et al. 2006; Lens et al. 2011), and the relationship between sieve tube structure and phloem transport (Mullendore et al. 2010; Jensen et al. 2012).

The vast majority of studies on conduit structure and function have been conducted on relatively small-diameter organs (usually around 4–8 mm in diameter) and relatively little is known about how the structure of these conduits changes within the body of a plant. For secondary phloem, variation in sieve tube diameter within shoots has been examined in a few studies (Quilhó et al. 2000; Petit and Crivellaro 2014; Jyske and Hölttä 2015), but phloem structure variation along woody roots has not been examined to date. For secondary xylem, several studies have examined root and shoot conduit structure, but often of only a few selected root or stem size classes. These have included studies on non-vessel-bearing woody plants (Domec and Gartner 2002; Domec et al. 2009; Lintunen and Kalliokoski 2010) and vessel-bearing woody plants (Zimmermann and Potter 1982; Sperry and Saliendra 1994; Ewers et al. 1997; Martínez-Vilalta et al. 2002; McElrone et al. 2004; Pratt et al. 2007; Nygren and Pallardy 2008; Lintunen and Kalliokoski 2010; McCulloh et al. 2010). Within only shoots, there is strong evidence that vessel diameter and length varies greatly with organ diameter for most species (reviewed in Jacobsen et al. 2012), but similar data are not available for roots.

Understanding how conduit structure changes within trees may be particularly important in understanding the function of large woody plants, especially in consideration of their long transport pathways (Tyree and Ewers 1991; Kolb et al. 1996; Givnish et al. 2014). Roots, which appear to differ greatly from stems for many species (Alder et al. 1996; Hacke et al. 2000; Martínez-Vilalta et al. 2002; Pratt et al. 2007), are particularly understudied. This is striking, since roots may be the greatest resistor to hydraulic flow in woody plants (Engelbrecht et al. 2000; Pratt et al. 2010).

We were interested in examining how both sieve tube elements and vessels varied in structure along the main plant root to shoot axis in *Populus trichocarpa* trees. We were particularly interested in examining if phloem and xylem conduit diameters were correlated. We had several non-mutually exclusive predictions regarding the structural relationships between these conduits. Xylem and phloem conduit diameters may be correlated as a result of their common developmental origin or due to physical requirements related to the necessary exchange that occurs between these adjacent tissues. Alternatively, we predicted that xylem and phloem conduit diameters may be inversely correlated due to bulk flow occurring in opposite directions within these tissues. In phloem, bulk flow occurs predominantly from the shoot to root and in xylem occurs predominantly from the root to shoot. This may result in structural changes, such as wider diameter conduits, that reduce resistance at the source versus sink in the transport pathway or that permit a smaller volume of "source tissue" (leaves or roots) to supply a larger volume of "sink tissue". That is, conduits may be larger at their source and smaller at their sink. This pattern could also be linked to different driving forces within these two tissues, cohesion–tension in xylem compared to pressure flow in phloem. For instance, distal branch xylem conduits need to withstand more negative tensions than other portions of the xylem transport pathway and there are known structural differences between the cells that occur in collection, transport, and release phloem. We predicted that samples that were wider in diameter (i.e., a larger diameter roots or wider trunk sections of the shoot) would have conduits that were wider in diameter relative to narrower samples such as small-diameter roots or stems. This would be consistent with allometric scaling models and the transport demands on single trunks and large roots versus many small distal roots or branches (McCulloh et al. 2003). For shoot xylem, this would also be consistent with the globally reported relationship between organ diameter and vessel diameter in Jacobsen et al. (2012) and data from Domec et al. (2009).

Changes in conduit diameter must occur concurrent with changes in other conduit traits for transport resistance not to become limited by other components of the transport pathway, such as pits (Sperry and Hacke 2004), perforation plates (Christman and Sperry 2010), or sieve plates (Mullendore et al. 2010). We measured several structural traits related to transport within xylem and phloem conduits to examine which of these traits varied with changes in conduit diameter. For xylem vessels, these traits included pit membrane area (Sperry et al. 2005), pit membrane density (Jacobsen et al. 2016), maximum vessel length (Jacobsen et al. 2012), and air-seeding pressure of individual vessels, which is determined by the size of the largest pit membrane pore within a vessel (i.e., single-vessel air injection, SVAI; Venturas et al. 2016). For phloem sieve tubes, these traits included the number of sieve areas per sieve plate, the area of the sieve plate, and the area of individual sieve areas. For *P. trichocarpa*, sieve plates are compound and contain three or more sieve areas in the phloem of the stems and roots.

Materials and methods

We sampled juvenile poplar trees (*P. trichocarpa* Hook., Salicaceae) growing on campus in the Environmental Studies Area at California State University, Bakersfield (35°20′N, 119°06′W, 115 m above sea level). Trees were propagated from seed collected near Libby, Montana, USA (Transplant P-1, Lawyer Nursery Inc., personal communication) and

purchased as ~0.5–1-m-tall bare root stock seedlings (Lawyer Nursery Inc., Plains, MT, USA). Trees were grown in a well-watered field plot with trees spaced at 4 m increments along rows that were spaced 4 m apart. The plot contained 288 trees from 4 different species, which were randomized throughout the plot. Poplar trees were 8 years of age during the time of sampling in July 2015 (Table 1). The entire root systems of three trees located within the plot and away from the plot edge were carefully excavated so that the longest root of each tree could be identified for sampling. Each tree took approximately 4–5 days to sample, with five individuals working full time each day. Entire tree root systems were carefully excavated with hand tools so that soil could be cleared without damaging the roots. Shoot samples were collected prior to the complete excavation of the tree to prevent transpiration as root tips were exposed to air and to avoid fall of the tree as roots were excavated and no longer anchoring the tree. Xylem and phloem samples were collected at 1 m increments above and below the root to shoot (*R:S*) junction as well as at the junction itself, following the longest root and the main shoot axis. For each location, a 20-cm-long section was removed from the tree and immediately placed in water. The ends of the sample were then trimmed underwater and two 5-cm-long segments were cut underwater from the center of the sample.

For one set of 5-cm samples from each tree and position, the sample or a section of the sample was preserved for anatomical analyses. For small-diameter samples (< 4 cm diameter), the entire sample was placed in a 10% neutral buffered formalin solution (Fisher Scientific, Protocol, Kalamazoo, MI), while for larger diameter samples, a wedge containing the bark and outer 4 cm was removed underwater from the sample and placed in the same solution. Samples were kept submerged in the formalin solution for > 72 h prior to sectioning. The second 5-cm sample was used for single-vessel air-injection measurements as described below.

Sieve tube and vessel diameters were measured from cross sections. Thin, approximately 40–100-µm-thick cross sections were prepared by hand using a razor blade (GEM single edge stainless steel PTFE-coated blades, Electron Microscopy Sciences, Hatfield, PA, USA) or using a sledge microtome (Model 860 Microtome, American Optical Corp., Buffalo, NY, USA) and mounted on slides in glycerol for anatomical measurements. Photos of the current year xylem and phloem were taken using a transmission light microscope (Leica DM750, Leica Microsystems, Switzerland) with an attached camera (Leica MC120 HD, Leica Microsystems, Switzerland) and were examined using an image analysis program (Leica Application Suite v. 4.6.1, Leica Microsystems, Switzerland). Only the outer most growth ring was analyzed (i.e., current year growth). We did not measure either vessels or sieve tubes that were still developing and expanding near the vascular cambium. *Populus* has previously been identified as having a cambial zone 5–8 cells thick, with all cells potentially dividing (Davis and Evert 1968) and we observed this to be the case in the samples that we examined as well. For each tree and at each sampling location, 77–100 xylem and phloem conduit lumen areas were measured in a wedge that spanned the entire mature portion of current year growth. All of the fully expanded conduits within the sampled sector of tissue were measured by individually outlining their lumen contour. Conduit diameters were calculated from the measured lumen areas based on the assumption that conduits were circular. Conduits tended to be generally circular (Figs. 1, 2) and the use of equivalent circular areas to calculate conduit lumen diameter is consistent with prior studies (Sperry and Hacke 2004; Sperry et al. 2005; McCulloh et al. 2010).

Pit structure and sieve plate structure were measured from longitudinal sections. Thin longitudinal sections were made by hand using razor blades (GEM single-edge stainless steel PTFE-coated blades, Electron Microscopy Sciences, Hatfield, PA, USA) and mounted on slides in glycerol. Prior to mounting, phloem sections were stained using a 1% w/v solution of aniline blue (A967-25, Fisher Chemical, Fair Lawn, NJ, USA). The sieve tube elements of *Populus* contain oblique, compound sieve plates (Davis and Evert 1968) that are extremely inclined. Each sieve plate contains many sieve areas. We measured the sieve plate area, the area of each sieve area within a plate, and counted the number of sieve areas per sieve plate. In *Populus*, phloem differentiation precedes xylem differentiation in the spring, both are formed throughout the summer, and the cessation of cambial activity for both occurs at the same time (Davis and Evert 1968). Our measures occurred in the mid-summer and we were able to identify sections of well-preserved current-year phloem in our samples.

Changes in the structure of pit membranes were examined using SVAI, which measures the pressure required to seed gas across the largest pit membrane pore within a vessel. These measures were conducted on the second set of 5-cm samples that were harvested from trees as described above. Samples were kept submerged in water until measured and all samples were measured within 3 days of harvest. Prior

Table 1 Plant size characteristics of the sampled *Populus trichocarpa* plants, including the height of the shoot, the length of the longest root, and the diameter of the tree at the root to shoot junction (diameter at *R:S*)

Tree	Shoot height (m)	Root length (m)	Diameter at *R:S* (m)
1	4.45 (4)	4.06 (4)	0.108 (1)
2	4.36 (4)	5.12 (5)	0.126 (1)
3	4.19 (4)	4.86 (4)	0.179 (1)

Numbers in parentheses indicate the number of positions that were sampled within each organ

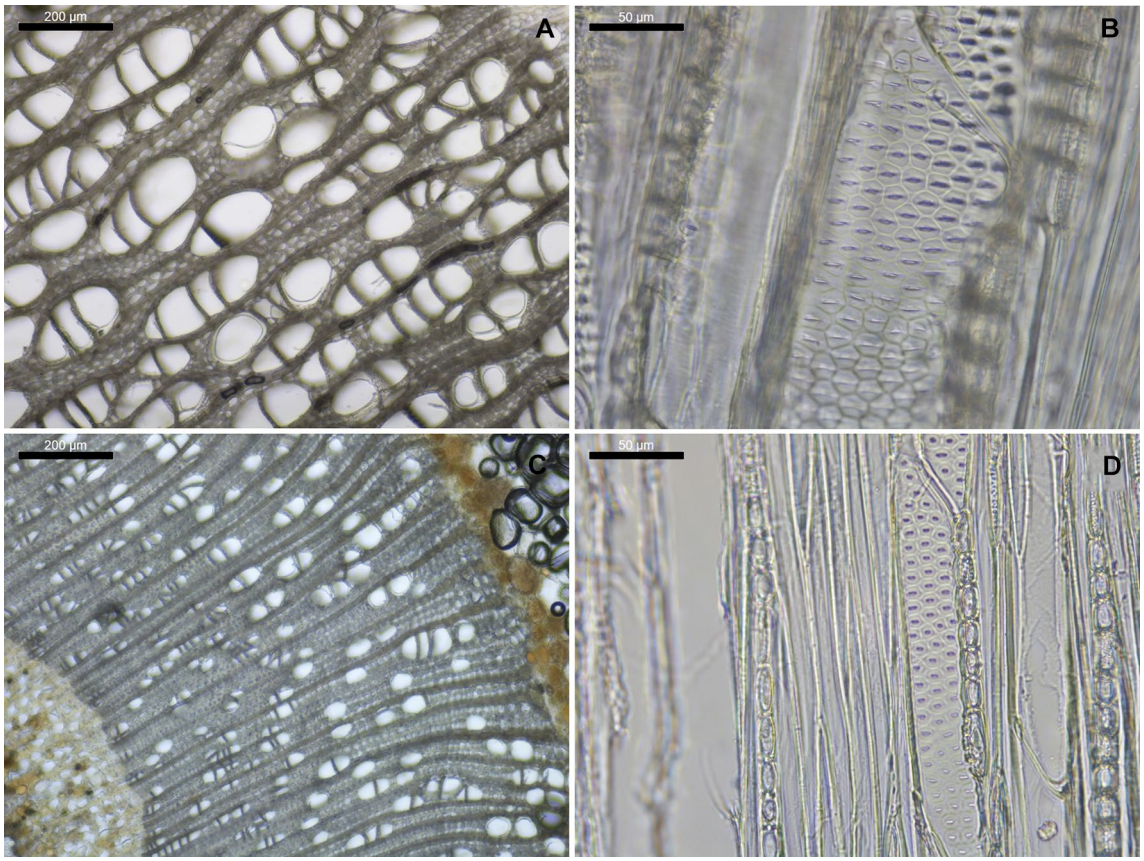

Fig. 1 Xylem tissue micrographs of current year growth from a wide-diameter root (**a, b**) and a narrow shoot (**c, d**) showing the large variability in vessel dimensions found within the plant body. The two micrographs on the left (**a, c**) are both cross sections of the xylem that were taken at the same magnification; scale bars = 200 µm. The two micrographs on the right are both longitudinal sections that were taken at the same magnification; scale bars = 50 µm

to SVAI determination, emboli were removed from samples by submerging them in 20 mM KCl and vacuum infiltrating (−91 kPa) them for 1 h.

Single-vessel air-injection methods follow those described in Venturas et al. (2016). In brief, for each measured vessel, a xylem segment was secured vertically under a stereo microscope (Olympus Corporation, Tokyo, Japan) with a ring-stand clamp, with the distal end facing up and the proximal end submerged in 20 mM KCl degassed solution contained in a 150-ml glass beaker. A glass capillary was inserted into a single vessel and secured with fast-drying glue (Loctite, Super Glue Gel Control, Henkel Corp., Rocky Hill, CT, USA). The other end of the capillary was connected via PEEK (polyetheretherketone) tubing to a pressure chamber that was used to slowly increase the pressure being pushed into the vessel. The proximal sample end was observed until a pressure was reached that resulted in bubbles emerging from a vessel or vessels. This indicated that gas had been seeded through a pit membrane of the injected vessel. The pressure associated with this event indicates that the air-seeding pressure threshold for that vessel had been reached or exceeded (if air had to flow through more than one vessel connection). If bubbles were observed at pressures < 0.05 MPa, it was assumed that the vessel was open through the segment and the measurement was discarded. After each measurement, the capillary and glue were carefully removed to inject another vessel from the same sample.

For each sampling distance and pooled across trees, the air-seeding pressure of eight vessels was determined (2–3 vessels per position per tree). These vessels were selected from around the circumference of the samples, in current-year growth, and not immediately next to the vascular cambium. Air-seeding pressures were not measured on the samples from the root-to-shoot junctions. Because of the time-consuming nature of SVAI measures and the large number of samples collected simultaneously during the sampling of an entire tree, we were not able to sample more extensively on these fresh samples. To allow for analyses of these data, data were pooled within organs and the median air seed pressure (P_m) was determined for roots and shoots using the combined data across all of the trees. SVAI data were plotted as a cumulative distribution function (%) of

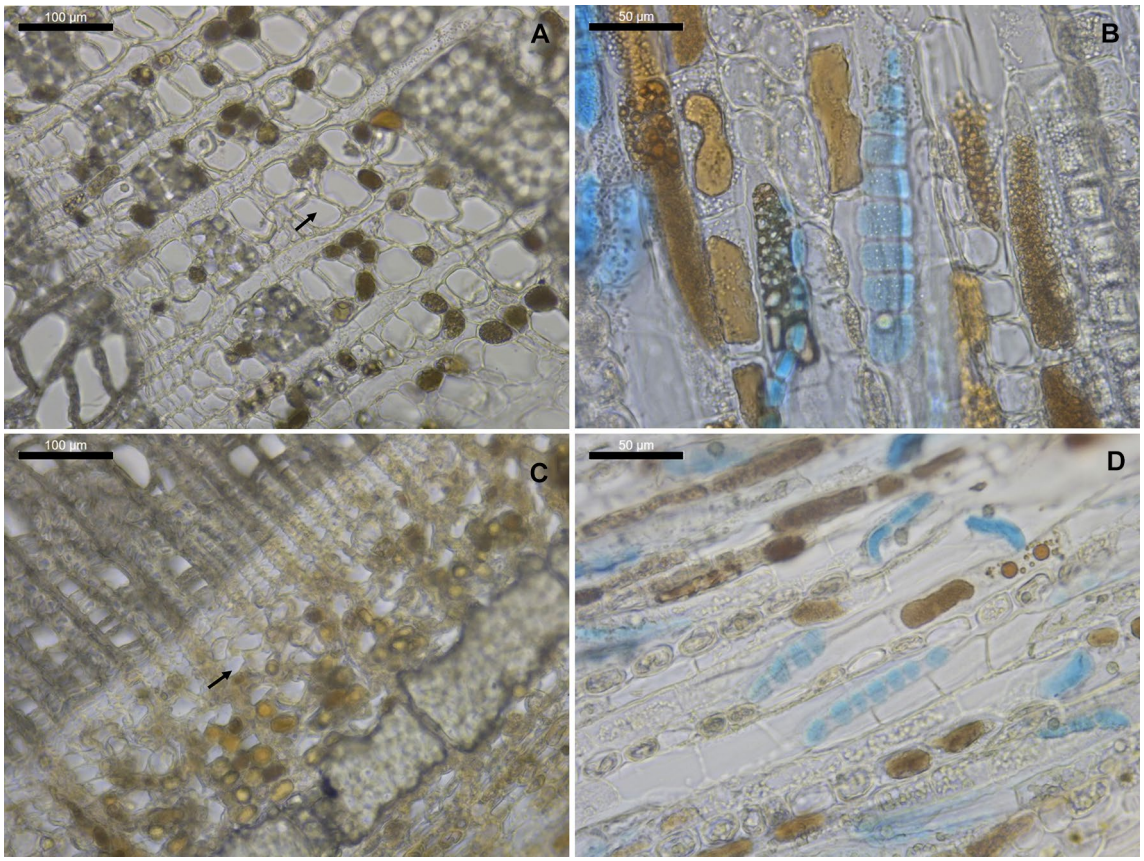

Fig. 2 Phloem tissue micrographs from within a wide-diameter root (**a, b**) and a narrow-diameter shoot (3 m shoot) showing the large variability in sieve tube element dimensions found within the plant body. A black arrow indicates an example sieve tube element in each of the two left panels. The sieve areas within the compound sieve plates visible in panels **b** and **d** have been stained blue using a 1% w/v aniline blue solution. The two micrographs on the left (**a, c**) are both cross sections of the phloem that were taken at the same magnification; scale bars = 100 microns. The two micrographs on the right are both longitudinal sections that were taken at the same magnification; scale bars = 50 µm

the pressure thresholds that seeded air through root or shoot samples. This was then used to determine the P_m which represents the threshold at which 50% of vessels air seeded. The P_m values and 95% confidence intervals were calculated using the methods described in Venturas et al. (2016), with bootstrapping used to generate datasets by randomly resampling the observed datasets.

Maximum vessel length was evaluated using the air-injection method (Greenidge 1952). Large roots and large lateral shoots that were not used for the measures above were collected from the same sampled trees. Different-diameter roots and shoots were injected with gas distally to proximally, so that we could evaluate the relationship between organ diameter and maximum vessel length. Shoot segments were injected with gas at 100 kPa. Initial tests indicated that root vessel length estimates were sensitive to the injection pressure, suggesting that some vessels were air seeding even at these very low pressures. Root segments were injected at 50 kPa, which was the highest pressure that produced stable vessel length estimates. The proximal ends of samples were cut progressively under water removing ca. 5 cm each time when samples were > 1 m in length, ca. 2 cm when samples were < 1 m and > 0.5 m, and ca. 1 cm when samples were < 0.5 m in length. When gas bubbles started to flow from the end of the sample, usually from a single vessel, the remaining sample length was measured and this length plus half of the just previously removed length was used as the estimate for the maximum vessel length.

Mean vessel length was measured for small root (5.4–6.9 mm) and small shoot (5.1–7.7 mm) diameter samples to allow for a more detailed comparison of vessel length between these two organs. These samples came from the same-aged trees in the same research plot and were sampled at the same time, but were not sampled from the same trees as other measures. One shoot and one root sample were injected from each of six trees. Mean vessel length was evaluated using the silicone-injection method (Sperry et al. 2005). Segments 40 cm in length were selected so that

the distal end of the segment corresponded to current year's growth and was approximately 6 mm in diameter. These segments were flushed for 1 h with an ultra-filtered (in-line filter Calyx Capsule Nylon 0.1 µm; GE Water and Process Technologies, Trevose, PA, USA) degassed 20 mM KCl solution at 100 kPa in the direction of bulk hydraulic flow within the plant. Segments were injected with a two-component silicone (RhodorsilRTV-141, Rhodia USA, Cranbury, NJ, USA) containing a UV stain (Uvitex OB, Ciba Specialty Chemicals, Basel, Switzerland) dissolved in chloroform (1% by weight). One drop of the UV stain was added per gram of silicone mixture. Segments were injected into their basal end in the same direction as bulk hydraulic flow in the intact plant at 50 kPa for 24 h. After the injection, the segments were cured for at least 48 h at room temperature. Prior to sectioning, stems were rehydrated to soften tissues by submerging them in water for at least 24 h. Serial thin cross sections (40 µm thick) were obtained with a sledge microtome (Model 860 Sledge Microtome, American Optical Corp., Buffalo, NY, USA) and photographed. Images were captured under fluorescent light with a microscope attached to a digital camera (Zeiss Stereo Discover V.12 with Axiocam HRc digital camera, Carl Zeiss Microscopy, LLC, Thornwood, NY, USA). For each cross section, all silicone-filled vessels were counted and used to evaluate the decline in silicone-filled vessels with distance from the injection point. The mean vessel length was calculated from these measurements using the equations reported by Sperry et al. (2005).

The relationship between organ diameter (i.e., the diameter of the tree root or shoot at the point of sampling) and conduit diameter was examined using an ANCOVA. We were particularly interested in examining if roots and shoots differed in their trait relationships; thus, the model's main factors to explain conduit diameter were "organ" and "tree", and the covariate was "organ diameter". All possible interaction terms were evaluated. Data from *R:S* positions were excluded from these analyses. "Tree" was included in the model because multiple samples were taken from individual trees and thus were not independent; however, tree was not a significant factor in analyses nor did it interact significantly with any other factors. Data were transformed as necessary to meet statistical assumptions. We also evaluated the diameter of xylem and phloem conduits with linear mixed effects models with "tree" as a random effect, "organ" as a fixed effect, and "organ diameter" as a covariate. We included the interaction between "organ" and "organ diameter". The relationship between organ diameter and maximum vessel length was examined using an ANCOVA, with the same factors and covariate as the one analyzing conduit diameter. Relationships between structural traits were examined using Pearson correlations. Differences between mean vessel length in root compared to shoot samples were compared using a *t* test. All analyses were conducted using Minitab 17 (v. 17.2.1, Minitab, Inc., State College, Pennsylvania, USA), except for the linear mixed effects model analyses that were performed with 'lme4' package (Bates et al. 2015) in R version 3.5.0 (R Core Team 2018).

Results

Intra-organismal variation in conduit diameter

The structure of the xylem (Fig. 1) and phloem (Fig. 2) varied greatly throughout the plant body, with mean vessel diameters ranging from 29 to 104 µm and mean sieve tube diameters ranging from 16 to 43 µm diameter. The diameters of conduits within the vascular tissues of poplar trees varied with sample location, with the smallest diameter conduits in terminal shoot samples (31-µm-diameter vessels and 17-µm-diameter sieve tubes when averaged across trees) and the largest diameter conduits within wide-diameter roots (89-µm-diameter vessels and 37-µm-diameter sieve tubes when averaged across trees). The pattern of variation among tree positions was similar for all trees sampled (Fig. 3), with an increase in conduit diameter from the root tip to wide-diameter roots, a sharp decline in diameter at the root to shoot (*R:S*) junction, and a decline in diameter moving up the shoot. Across sampled positions, conduit diameters of vessels and sieve tubes were strongly correlated (Fig. 4; $r = 0.786$, $P < 0.001$).

Conduit diameter differed in roots and shoots

The diameter of the root or shoot from which vascular tissues were sampled (i.e., organ diameter) was strongly associated with the structural traits of the conduits at that position. ANCOVA analyses revealed that organ diameter (logarithmically transformed) was associated with changes in both vessel and sieve tube diameter (Fig. 5), with roots having wider conduit diameters than shoots. In xylem, organ diameter was associated with changes in vessel diameter ($F_{1,24} = 13.95$, $P = 0.002$). Roots and shoots differed in the intercepts for their relationship between these traits ($F_{1,24} = 7.17$, $P = 0.019$), but they did not differ in the slope of the relationship ($F_{1,24} = 0.00$, $P = 0.956$). In phloem, organ diameter was associated with changes in sieve tube diameter ($F_{1,24} = 49.49$, $P = 0.000$). Roots and shoots differed in their intercepts for their relationship between these traits ($F_{1,24} = 7.94$, $P = 0.015$), but they did not differ in the slopes of their relationships between these traits ($F_{1,24} = 0.79$, $P = 0.391$). Different trees were not different nor were there interactions with tree for any other variables ($P > 0.05$ for all comparisons for both xylem and phloem). We obtained similar results when we analyzed xylem and phloem conduit diameters in relation to organ and organ diameter with

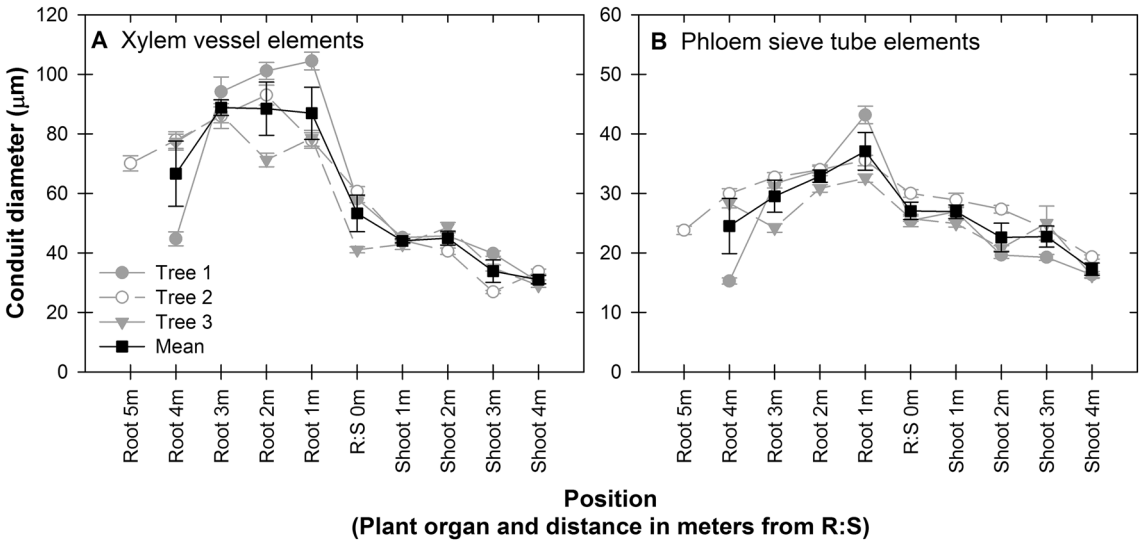

Fig. 3 Three sample trees showed similar patterns of vessel element and sieve tube diameter variation along their main root to shoot axis. Plant position is provided based on distance (m) from the root to shoot junction (*R:S*). Each point represents a mean ± 1 SE for each sampled position

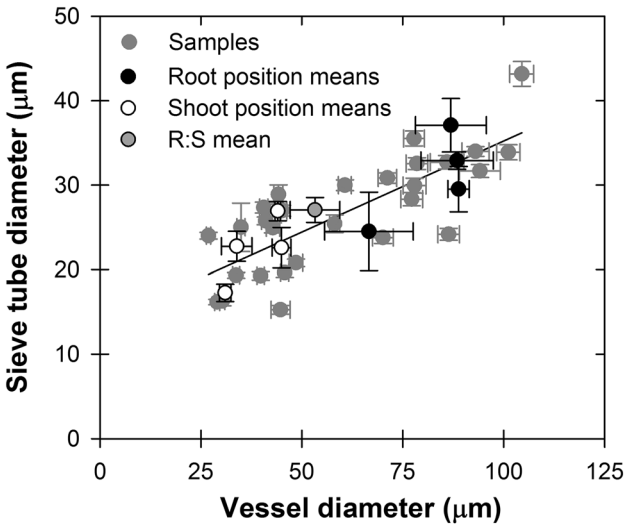

Fig. 4 The diameters of conduits within the xylem and phloem, vessels and sieve tubes, respectively, sampled at different positions within *Populus trichocarpa* trees were correlated ($r = 0.786$, $P < 0.001$). Each point represents a mean ± 1 SE, with values calculated for each individual xylem and phloem sample (gray symbols with gray outlines; $n = 77$–100 vessel diameters or sieve tube diameters per sample) and for each sampled position (i.e., a mean across trees; $n = 3$ trees; symbols with black outlines)

linear mixed effects model (both fixed effects were significant, $P < 0.001$; Supplemental material S1).

Roots and shoots did not differ in their vessel length (Fig. 6). Organ diameter was correlated with maximum vessel length ($F_{1,26} = 15.76$, $P = 0.001$). There was no difference between roots or shoots in either the intercept ($F_{1,26} = 2.22$, $P = 0.150$) or the slope ($F_{1,26} = 1.74$, $P = 0.200$) of this relationship. For small-diameter samples (6 mm diameter), the mean vessel length did not differ between roots and shoots (Fig. 6 inset; $T = -0.37$, $P = 0.721$).

Other conduit structural traits

Other structural traits of the vascular conduits also varied greatly throughout the plant body. Within the xylem, the area of pit membranes within vessel-to-vessel pits varied from 45 to 114 μm^2 and the density of pits within pit fields varied from 0.0065 to 0.0164 pits per μm^2. Within the phloem, sieve plate area varied from a mean of 1039–4954 μm^2, the number of sieve areas per sieve plate varied from an average of 6.1–11.2, and the mean area of each sieve area varied from 94 to 359 μm^2.

Variation in conduit structural traits was strongly correlated with changes in conduit diameter. In the xylem, vessel diameter was correlated to the area of the pit membranes (Fig. 7a, $r = 0.901$, $P < 0.001$) and to the number of pits per wall area (Fig. 7b, pit density; $r = -0.822$, $P < 0.001$). In the phloem, sieve tube diameter was correlated to the area of the sieve plate (Fig. 7c, $r = 0.790$, $P < 0.001$), the number of sieve areas per sieve plate (Fig. 7d, $r = 0.626$, $P < 0.001$) and the area of individual sieve areas within the sieve plate (Fig. 7e, $r = 0.790$, $P < 0.001$).

When pooled by organ, the median pit membrane air-seeding pressure (P_m) was not different between roots and shoots (Fig. 8). The P_m for roots was 0.40 MPa (95% confidence limits of 0.34–0.44 MPa) and for shoots was 0.41 MPa (95% confidence limits of 0.34–0.52 MPa). There was a slight trend for shoots to contain vessels that were more resistant to air seeding as indicated

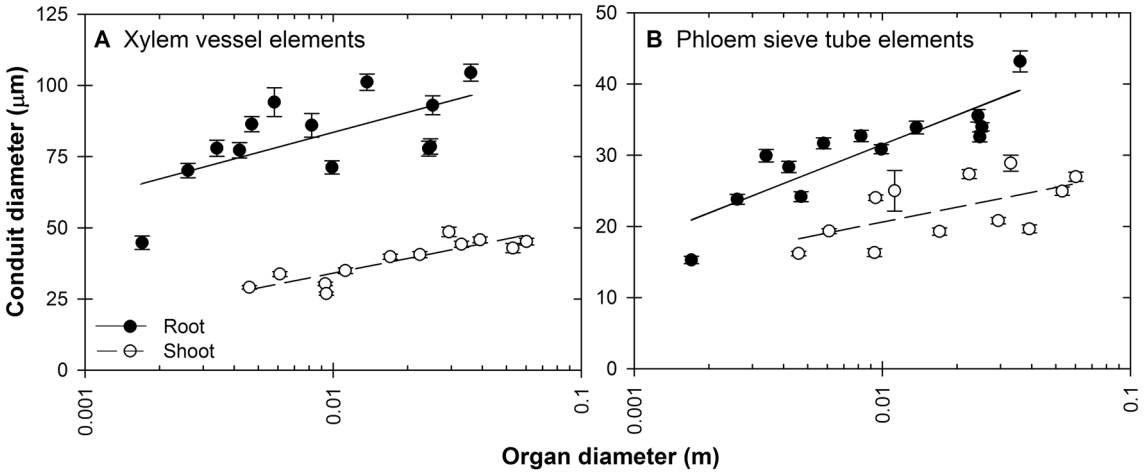

Fig. 5 The diameters of conduits within the xylem (**a**) and phloem (**b**) in both roots (closed symbols) and shoot (open symbols) were correlated with organ diameter. Within both the xylem and phloem, roots and shoots differed in the intercepts, but did not differ in the slope of this relationship. The regression lines included in this figure are included to allow for easier visual interpretation of these relationships and are not based on statistical analysis outputs

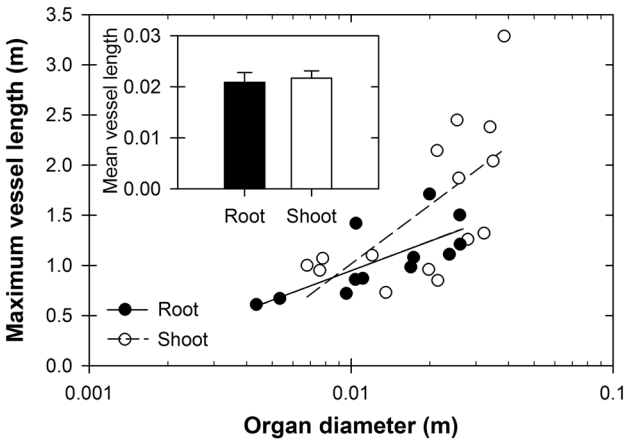

Fig. 6 Maximum vessel length was correlated with organ diameter for both roots and shoots. There was no difference between roots and shoots in this relationship. Each point represents the maximum vessel length of a different large shoot or large root sample and organ diameter represents the diameter at the point of air injection. Mean vessel length (inset) was not different between roots and shoots of similar diameter

by the smaller percentage of vessels seeding at pressures < 0.2 MPa in shoots (3 vs. 20%) and the larger range in P_m across positions, 0.33–0.44 in roots and 0.33–0.54 in shoots (Supplemental material S2). This pattern was apparent across samples from different sample positions, which were all relatively similar, although sample sizes were not great enough for each position to be analyzed separately (Supplemental material S2).

Discussion

We found that both sieve tube and vessel diameter varied greatly throughout the plant body. Conduit diameter changes were correlated with the diameter of the organ at the position they were sampled. Sieve tube and vessel diameter were strongly correlated. For the xylem, this is consistent with the pattern described in several prior studies, many of which focused on variation within the shoot or at just a few sampled positions (McElrone et al. 2004; Lintunen and Kalliokoski 2010; Schuldt et al. 2013; Petit and Crivellaro 2014; Zhao 2015). This pattern is also consistent with the hydraulic design of woody stems, which, to preserve a constant resistance to water flow, often shows a marked tapering of xylem vessels from the shoot base to the shoot tip (Niklas and Spatz 2004; Anfodillo et al. 2006; Olson et al. 2014). For the phloem, studies have been limited and have examined only patterns within the shoot. Our findings are similar to those of Petit and Crivellaro (2014), but contrast with Quilhó et al. (2000) who found little sieve tube diameter variation within the shoots of *Eucalyptus globulus*.

The correlation between vessel and sieve tube diameters may be related to their common developmental origin from the same initial cells within the vascular cambium. The size of initials within the vascular cambium has been shown to vary with tree position (Butterfield 1973; Ridoutt and Sands 1993). This may be associated with later differences in the cell sizes that are derived from these cambial initials. There may also be similar signals that control cellular expansion, such as auxin concentration (Aloni and Zimmermann 1983; Aloni 2010), that resulted in this pattern of correlated conduit diameters.

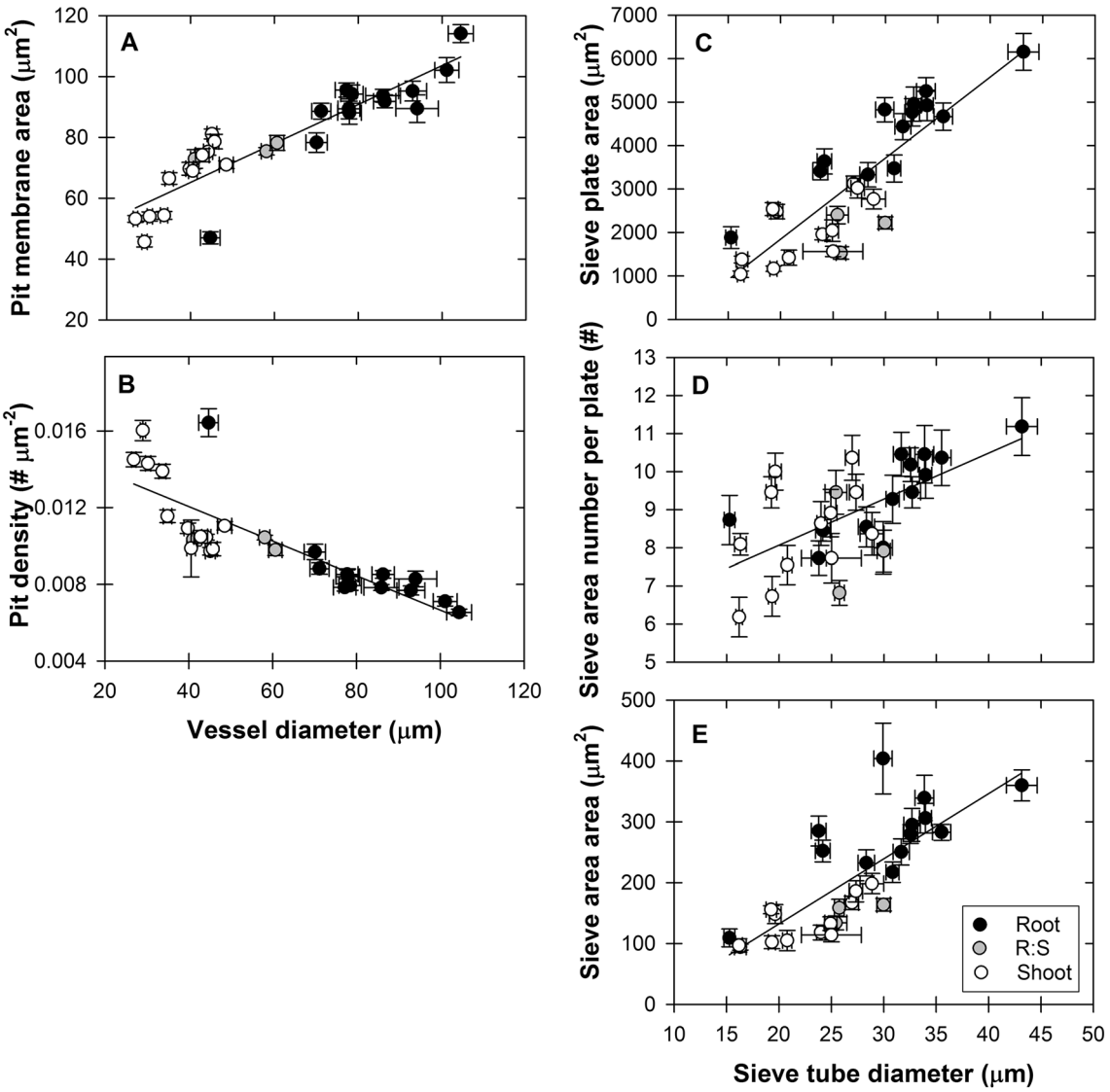

Fig. 7 Variation in conduit structural traits were correlated with conduit diameter in both the xylem (**a, b**) and phloem (**c**–**e**). In the xylem, vessel diameter was correlated to the mean area of individual the pit membranes (**a**) and to the number of pits per wall area within vessel-to-vessel contact areas (**b**). In the phloem, sieve tube diameter was correlated to the area of the sieve plate (**c**), the number of sieve areas per compound sieve plate (**d**), and the mean area of individual sieve areas within the sieve plate (**e**). Each point represents a position mean ± 1 SE, including positions in both roots and shoots. All of the shown relationships were significant ($P < 0.05$)

There were large differences between conduits in roots and shoots for most of the xylem and phloem conduit traits that we examined. We found that roots had larger xylem and phloem conduits than shoots when standardized by organ diameter. Conduits in the roots also had larger pit membranes and sieve plates compared to stems. These differences are consistent with several prior studies that have compared root and shoot xylem structure and function (Hacke and Sauter 1996; Alder et al. 1996; Hacke et al. 2000; Martínez-Vilalta et al. 2002; Pratt et al. 2007) and suggest that roots and shoots are likely functioning differently within trees. The positive correlations between conduit diameter and pit membrane area as well as between sieve tube diameter and sieve area show a coordination between lumen and conduit connection resistances.

Roots had wider diameter vessels than shoots, but they did not differ from shoots in either their maximum vessel length, when standardized by organ diameter, or their mean vessel length in samples that were matched in diameter. This is consistent with the findings of Pratt et al. (2015) who found that root vessel lengths were generally similar to shoot vessel lengths for seven species, with some species having slightly shorter root vessel lengths and some slightly longer when compared to stems. Additionally, a global stem dataset

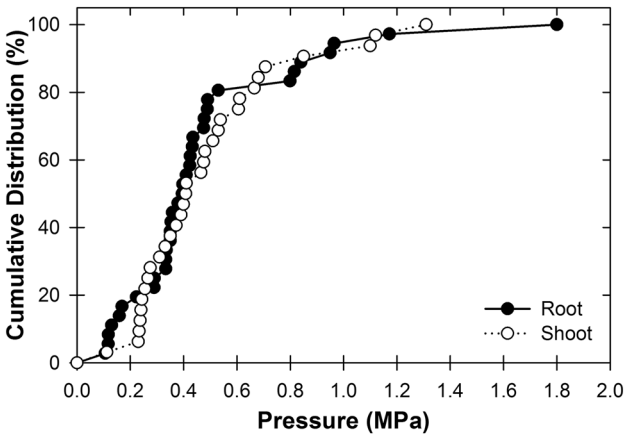

Fig. 8 The cumulative distribution of air-seeding pressures using the single-vessel air-injection (SVAI) method for vessels within root (closed symbols) and shoots (open symbols) for 5-cm-long segments. The median air-seeding pressure (P_m) for roots was 0.40 MPa and for shoots was 0.41 MPa

showed that vessel diameter and length were not correlated across species (Jacobsen et al. 2012), suggesting that these parameters may vary independently.

Roots are more buffered from cold temperature extremes and rapid temperature changes than shoots, because they are insulated by soil. Roots are also likely to be buffered from frequent freeze–thaw cycles under negative pressure. Soil and root freezing and thawing are most likely to occur during winter when *Populus* species have shed their leaves and xylem pressures are high (close to zero), which reduces the chances for cavitation induction because air bubbles can dissolve in sap before low xylem pressures are sustained due to transpiration and soil drying. Thus, it may be that roots do not experience the same strength of selection for reduced diameter associated with risk of freezing-induced embolism, whereas shoot vessels would be selected to have narrower diameters to tolerate more frequent freeze–thaw cycles while experiencing negative pressures (Langan et al. 1997; Davis et al. 1999). Freezing embolism risk is linked to conduit diameter, but not length, so vessel length would not experience freezing-related selection (Pittermann and Sperry 2003).

When examined within an organ, wider diameter organ positions contained both longer and wider vessels compared to narrow-diameter terminal positions for both roots and shoots. This is consistent with several studies that have found that vessel diameter and length are correlated within an individual (Zimmermann and Jeje 1981; Zimmermann and Potter 1982; Ewers and Fisher 1989; Ewers et al. 1990; Liu et al. 2018). However, these studies have examined only patterns within the shoot. We also found evidence for this relationship, but only within an organ and not when applied across organs.

Median air-seeding pressures (P_m) did not differ between roots and shoots, which was surprising given the large changes between roots and shoots in vessel diameter and pit membrane area. The air-seeding threshold of a vessel is determined by the size of the largest pore within the pit membranes of a vessel. The lack of difference between roots and stems suggests that pit membrane structure was relatively stable throughout the plant even though other traits were changing. Although other species have been shown to have large differences in cavitation resistance between their roots and shoots (Hacke et al. 2000; Martínez-Vilalta et al. 2002; Pratt et al. 2007), it appears that in some *Populus* spp. roots and stems may not differ greatly in their root and stem resistance to cavitation (Hacke and Sauter 1996; Hukin et al. 2005). Indeed, we found that there was little variation in root and stem cavitation resistance in *P. trichocarpa* within our study plot (−0.19 and −0.50 MPa P_{50} for roots and shoots, respectively; Jacobsen, unpublished data). Similarities in vulnerability to cavitation, which occurs via the air seeding of vessels, support our P_m measures that suggest that our *P. trichocarpa* roots and shoots were similar in their pit membrane porosity. *P. trichocarpa* is fairly vulnerable to cavitation relative to other woody species (Maherali et al. 2004). It is possible that there may be stronger selection for changes in pit membrane characteristics in more water-stress-tolerant species that experience a greater pressure gradient from root to shoot during transpiration.

Conclusions

Our results suggest that different organs within a plant are structurally different and that conduit structure varies with cambium age, diameter, and organ. However, some conduit traits may be less plastic than others within the plant body. For instance, in the xylem, neither vessel length nor median air-seeding pressures (P_m) differed between roots and shoots. Because conduit structure determines conduit function, intra-plant variation in structural traits has important implications for understanding how plants transport water and sugars. Understanding plant vascular structure and function will likely require increased knowledge of whole plant structure, since plant performance may be limited by any point along the transport pathway. Surprisingly, very few studies have examined root structure or changes in vascular structure throughout the plant body, especially for phloem. Our data suggest that roots, in particular, may be important in determining whole plant function because wider/older roots have the largest vessel and sieve tube diameters, which may make them particularly susceptible to drought-induced failure.

Author contribution statement ALJ conceived of and designed the study and wrote the initial manuscript. MDV

contributed to the experimental design and contributed to the writing of the manuscript. All authors collected data and contributed to analyses. All authors reviewed the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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