Xylem network safety and efficiency are strongly influenced by conduit lengths and, therefore, the frequency and distribution of vessel endings (Fig. 1A). In conduits of woody angiosperms, vessel endings can contribute over 50% of the resistance to the axial flow of water, although this effect may diminish with increasing vessel length (Sperry et al., 2005, 2006). Importantly, however, vessel endings also represent a critical barrier between adjacent vessels that prevent the systemic spread of embolisms that form during drought or freeze–thaw events (Beckman and Keller, 1977; Salleo et al., 1984; Tyree and Zimmermann, 2002; Sperry et al., 2006; Choat et al., 2008) and the spread of disease after wounding (Beckman and Keller, 1977). Despite the importance of vessel endings for xylem safety and efficiency, there have been few detailed anatomical descriptions or measurements of these elusive structures (André, 2005 and references therein).

One reason for the limited research characterizing vessel-ending microstructure is that they are difficult to visualize due to the disproportionate scaling ratio of maximum vessel diameter and length, often measured in micrometers and meters, respectively (Zimmermann and Jeje, 1981; Ewers et al., 1990). Traditional light microscopy most often relies on two-dimensional (2D) sections (e.g., transverse, longitudinal, or radial) through plant xylem networks, thereby making it unlikely to observe a vessel ending within a single section. However, employing serial sectioning techniques can accomplish this task, although the exercise is tedious and requires the analysis of hundreds of sequential cross sections (Tyree and Zimmermann, 2002; Kitin et al., 2004; Huggett and Tomlinson, 2010). Even with serial section analyses (Zimmermann and Tomlinson, 1966, 1968), important characteristics such as the total pitted wall area shared by two overlapping vessels must be approximated by interpolating the data available in sequential cross sections (Zimmermann et al., 1982). Various methods including resin casting (Fujii and Hatano, 2000; André, 2005; Oskolski and Jansen, 2009), macerations (Handley, 1936; Bierhorst and Zamora, 1965; van der Schoot and Bel, 1989), and single vessel dye staining (Choat et al., 2004) have successfully identified vessel endings, but making detailed anatomical measurements using these techniques remains...
challenging due to the limited ability in visualizing their spatial relationship to other vessels within the xylem network.

Besides using serial sections (e.g., Braun, 1959; Zimmermann and Tomlinson, 1966; Burggraaf, 1972; Tyree and Zimmermann, 2002; Kittin et al., 2004), much of our understanding of the spatial distribution of vessel endings comes from proxy measurements where stems are injected with air, paint, fine gauge wires (Kanai et al., 1996), or silicone resin (Zimmermann and Jeje, 1981; Sperry et al., 2005; Scholz et al., 2013). Both the paint and resin fail to pass through the pit membranes in the vessel ending, thereby demarcating the extent of individual vessels. Careful analysis of cross sections at regular intervals along the perfused stem can be used to estimate the vessel ending frequency, although without directly identifying the vessel endings (Zimmermann and Jeje, 1981; Zimmermann and Potter, 1982). As such, our understanding of vessel lengths has improved with various adaptations and statistical techniques applied to these proxy methods (Sperry et al., 2005; Cai et al., 2010; Pan et al., 2015; Oberle et al., 2016), but direct visualization of vessel endings in situ is often lacking, which currently hinders our ability to accurately model xylem–network organization (Loepfe et al., 2007; Brodersen et al., 2010; Lee et al., 2013).

The goal of this study was to determine whether high-resolution x-ray microcomputed tomography (microCT; Brodersen, 2013; Brodersen and Roddy, 2016) could be used to identify and describe the microstructure of vessel endings in situ. If successful, microCT could then serve as a complementary method to existing techniques for improving our ability to understand the role of vessel endings. MicroCT imaging allows for nondestructive, high-resolution 3D visualization and measurement of xylem networks, where the vessel lumen can be “segmented” from other xylem tissue. MicroCT has the distinct advantages of leaving the sample tissue intact while permitting easy exploration and measurement of structures in any plane of view and in three dimension (Brodersen and Roddy, 2016).

In plants where maximum vessel length can be several meters long (Zimmermann and Jeje, 1981; Ewers et al., 1990), the likelihood of observing a vessel ending in a microCT scan, that covers only a few millimeters in length, should be exceedingly low. To increase our chances of observing vessel endings within our microCT scans, we studied roots in the diffuse-porous species *Acer rubrum* L. (Aceraceae). Roots tend to have large diameter vessels (Anfodillo et al., 2013), and although root vessel lengths are rarely studied (Jacobsen et al., 2012; Pratt et al., 2015), the majority of vessels in *A. rubrum* roots are less than 4 cm long (Zimmermann and Potter, 1982). The result was a higher likelihood of clearly observing vessel endings in situ, thereby improving our understanding of xylem vessel endings through quantitative analysis.

Using microCT, we looked for evidence that characteristics of vessel endings differ from nonterminal portions of vessels that might impact hydraulic efficiency or safety from embolism spread. Specifically, we measured the number of intervessel connections (Knipfer et al., 2015), pitfield density (approximation of pitfield fraction visible via microCT; Wheeler et al., 2005), vessel diameter and tapering characteristics (Petit et al., 2008; Anfodillo et al., 2013), vessel element length, and perforation plate angle (Frost, 1930; Ellerby and Ennos, 1998; Christian and Sperry, 2010). Such data collected on a wide range of species and tissue types would improve our understanding of xylem structure and function from both an empirical and theoretical perspective (Loepfe et al., 2007; Brodersen et al., 2011).

**Materials and Methods**

Four woody root segments 1–2 mm in diameter and 2–5 yr old were collected at least 20 cm from the bases of two *A. rubrum* saplings from the understory in Harvard Forest, Petersham, Massachusetts during August 2016. For each tree, two roots that were traced back to the base of the sapling were excavated from the soil, excised with a razor blade, and then oven dried at 60°C for 24 h before transport to the Beamline 8.3.2 at the Lawrence Berkeley National Laboratory Advanced Light Source, Berkeley, California for scanning. Segments were scanned at 23 keV with 1025 2D projection images captured while the segment was rotated over 180°. The 2D projection images were reconstructed into a 3D data set using TomoPy (Gürsoy et al., 2014). To more clearly differentiate between vessel lumen and other xylem tissues, we averaged reconstructions with and without phase contrast image processing. The final 3D data sets had a voxel size of 1.25 μm³. The segments analyzed were 5, 3, 3, and 2 yr old with scanned segment lengths of 2.2, 2.0, 1.9, and 1.9 mm, and segment diameters of 2.1, 1.0, 1.1, and 1.0 mm (average of top and bottom diameters) for each the four segments, respectively.

The reconstructed 3D images were visualized using Avizo 9.3 software (VSG, Burlington, Massachusetts, USA) and a 3D bilateral image filter was applied. Vessel lumens were segmented using the “magic wand” tool and classified as “non-ending” (did not end within the scanned area), “open ended” (vessels that were cut by the top or bottom of the scan but ended within the segment), or “complete” (vessels that both start and end within the scanned area). Vessels were defined by the presence of at least one perforation plate within the scanned area indicating that the vessel contained at least two vessel elements (IAWA Committee on Nomenclature, 1964; Evert et al., 2006).

Using the largest and highest quality microCT scan (Appendix S1, see Supplemental Data with this article), additional detailed measurements were taken on secondary xylem vessels using a combination of 3D renderings and virtual longitudinal and oblique sections. Vessel element length and perforation plate angle (Fig. 1A) were measured in Avizo on 30 elements within non-ending vessels, 20 elements within open-ended vessels, and 10 elements within complete vessels. Differences in vessel element length (distance between center of each perforation plate) and perforation plate angle across vessel types were tested with ANOVA and Tukey’s honest significant difference (HSD). Vessel diameters were calculated for each transverse cross-sectional slice of the segmented image using the “analyze particles” tool in the program FIJI (Schindelin et al., 2012), and differences in vessel diameter among vessel types were tested using ANOVA (“vessel type” interacting with the “slice number”) and Tukey’s HSD.

To quantify vessel tapering, we measured vessel diameters of five of the longest open-ended vessels within each slice (in FIJI), and the location of the center of perforation plates and number of vessel–vessel connections for each vessel element were determined in Avizo. Finally, pit density (pitfield fraction was not visible at this resolution; Wheeler et al., 2005) was measured on the terminal vessel elements of the five vessels where they connect to neighboring vessels as well as on five non-ending to non-ending vessel connections using longitudinal slices between intervessel connections and counting individual pits within a subsampled area (~5000 μm²). We then measured the length and diameter of each sample and approximated the total volume by assuming a cylindrical shape. In this way, we were able to calculate the number of vessel endings for a given tissue volume, although with the caveat that the samples were dehydrated, and most of the cortex, phloem, and bark tissue surrounding the xylem had collapsed.
We created a digital model of a vessel group containing multiple vessel endings (as depicted in Fig. 2A) that can be downloaded from the Supplemental Data (Appendices S2 and S3) and printed as a physical model for teaching using a 3D printer. The model was created by cropping the complete root scan to a subvolume immediately surrounding the vessel group such that the vessel lumens are visible as they exist within the xylem tissue when 3D printed with a transparent material. The z-axis was foreshortened four times to make the vessel endings easier to visualize (Appendices S2 and S3).

All graphs were produced and statistics were calculated in R (R Core Team, 2015; Wickham and Chang, 2016).

RESULTS

We observed 214 open-ended, 37 complete, and 385 non-ending vessels within four A. rubrum root segments (Fig. 1B). The four root segments represented a total of 12.3 mm$^3$ of tissue along 8.0 mm of axial root length. Vessel elements were separated by simple perforation plates (Fig. 1C–F) and extensive polygonal alternate pitting to neighboring vessels (Fig. 1E). On average across the four segments, 34 ± 4% (mean ± SD) of the vessels were open-ended to one side but terminate within the segments and 6 ± 2% were complete vessels (i.e., both terminal endings were visible in the scans; Fig. 2A–C). For comparison with injection methods that would only detect vessel endings from one side of the segment and would not capture complete vessels, approximately 22 ± 4% of vessels ended when starting from the top (Fig. 2D) or bottom of each segment (Fig. 2E).

In the largest segment studied, non-ending vessels were 0.9 to 1.5 times larger in diameter than those that ended within the segment (Table 1; $F_{2, 437,052} = 37,641, P < 0.0001$) despite having similar vessel element lengths (Table 1; $F_{2, 57} = 1.01, P = 0.371$). Perforation plate angles were more acute for vessels that ended within the segment than those that did not end (Table 1; $F_{2, 57} = 19.27, P < 0.0001$).

Of the five longest vessels with endings, most vessel diameter tapering occurred in the last one to three vessel elements (Fig. 3A).

**FIGURE 1** Illustration of vessel endings adapted from Tyree and Zimmermann (2002) (A). Vessels are composed of multiple elements separated by perforation plates. Vessels connect to neighboring vessels through bordered intervessel pits (x), with tapered endings. MicroCT imaging (B–F) of Acer rubrum roots reveals the 3D, spatial relationship of vessel endings in situ, and nondestructively. Vessel endings were visible as volume renderings of the vessel lumen segmented from the surrounding tissue (B–D), or as renderings of the xylem network with false coloring (E, F). Connections between a non-ending vessel (blue) and a vessel ending (yellow) are visible from two angles in (B) and (C) and in the surrounding tissue in (D). The same perforation plates are identified with white arrows in (C, E, F). The 3D volume renderings of surrounding xylem tissue (in gray) are shown in (D–F). Black arrows in (E) identify the polygonal alternate intervessel pitting of the penultimate and terminal vessel elements. Panel (E) is a magnified image of the block of xylem in (D), with false coloring to highlight the yellow vessel from panels A–C. Panel F shows an alternate view of the sample in (E) rotated 90°, which bisects the blue and yellow vessels in the same orientation as panel (B), and reveals the simple perforations and intervessel pitting. Bars = 100 μm. This vessel pair is also visible in the lower right corner of Fig. 2A, 2B.
Three of the five vessels (vessels identified as “a”, “b”, and “c”; Fig. 3A, B) had connections with two other vessels along their entire length. Vessel “d” was connected to one other vessel along five of its seven visible elements within the scan (Fig. 3). The fifth vessel (vessel “e” in Fig. 3) drifted laterally over its axial course through the segment and had no vessel–vessel connections for the first 10 elements before having one, three, and one connections, respectively, within the final three vessel elements. Intervessel pit density (black arrows in Fig. 1E) in the five terminal vessel elements (Fig. 3; 0.026 ± 0.005 pits μm⁻²) and pit density between vessels that traverse the entire segment (0.022 ± 0.003 pits μm⁻²) did not differ ($t_{65} = 1.19, P = 0.27$).

**DISCUSSION**

We found that vessel endings in *A. rubrum* roots were identifiable using high-resolution microCT imaging and confirmed the general notion that vessels end with connections to other vessels, rather than ending blindly (Figs. 1A, 2). Indeed, while some of the non-ending vessels had few intervessel connections, vessels that ended within the scanned segments were always connected to at least one other vessel. Interestingly, we also observed 37 “complete” vessels (often two to four elements in length, tapering at both ends; Fig. 2C) that, to our knowledge, is the first time that a complete vessel has been visualized in this way.

<table>
<thead>
<tr>
<th>Measured trait</th>
<th>Non-ending</th>
<th>Open-ended</th>
<th>Complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of vessels (percentage)</td>
<td>165 (66%)</td>
<td>75 (30%)</td>
<td>10 (4%)</td>
</tr>
<tr>
<td>Vessel diameter (μm)</td>
<td>36.7 ±0.03a</td>
<td>19.8 ±0.06a</td>
<td>14.6 ±0.21b</td>
</tr>
<tr>
<td>Vessel element length (μm)</td>
<td>281 (±15)a</td>
<td>312 (±18)a</td>
<td>307 (±26)a</td>
</tr>
<tr>
<td>Perforation plate angle (°)</td>
<td>43 (±2.4)a</td>
<td>24 (±2.9)b</td>
<td>21 (±4.0)b</td>
</tr>
</tbody>
</table>

*TABLE 1.* Detailed measurements from the largest and highest quality microCT scan of an *Acer rubrum* root (2.2 mm long, 2.1 mm diameter, 5 yr old). Vessels were categorized as non-ending (extend beyond the scanned segment length), open-ended (opened on one side but end within the segment), or complete (entire vessel visible within the segment). Vessel diameters were measured slice by slice. Vessel-element length and perforation-plate angle were measured on 30 non-ending, 20 open-ended, and 10 complete vessels within the segment. Means (±SE) that do not share the same letter within a row indicate the values differed in Tukey’s HSD test ($α = 0.05$).
Vessels traversing the entire segment length tended to be larger in diameter than those that ended within the segment, generally supporting the hypothesis that larger diameter vessels are longer (Zimmermann and Jeje, 1981). However, it is unclear whether vessel diameter tapering over long distances is responsible for this difference (André, 2005) or whether smaller diameter vessels tend to be shorter and therefore more likely to end. Indeed, terminal vessel elements observed in macerations are often, but not always, found to be narrower than non-ending vessel elements (Bierhorst and Zamora, 1965; van der Schoot and Bel, 1989). Our results on intact vessels still suggest, however, that a large portion of vessel diameter tapering occurs within the last few vessel elements.

Vessels that ended within the segment had more acute perforation-plate angles than those that traversed the whole segment, and there was low variability in perforation plate angle. Therefore, changes in perforation plate angle may take place over longer distances than vessel diameter tapering. Similarly, pit density and vessel element length did not vary significantly across vessel types. The trend toward more acute perforation plate angles may permit more lateral movement of vessels near their ends to ensure that they terminate with connections to other vessels. Additionally, the extremely acute perforation plate angles may help reduce resistance to xylem-sap flow near the vessel ending for a given perforation size although this effect may be small in A. rubrum roots with simple perforation plates (Ellerby and Ennos, 1998; Christman and Sperry, 2010). The shift from wide, non-ending vessels with obtuse perforation plate angles toward narrower vessels with more acute perforation plate angles near vessel ends is further supported by studies in tomato using macerations (van der Schoot and Bel, 1989).

The proportion of each vessel type (i.e., non-ending, open-ended, and complete) identified within a segment was relatively consistent between our four segments and may be representative of A. rubrum sapling roots of this size. Zimmermann and Potter (1982) reported that approximately 60% of vessels should be shorter than 4 cm in roots of canopy-height A. rubrum trees growing in the same forest as our saplings (Harvard Forest, Petersham, Massachusetts). There are at least two possible interpretations of how those data compare to the apparently much shorter vessel lengths in this study. One possibility is that our sapling root segments had very short vessels compared to larger trees (Zimmermann and Potter, 1982), which may be indicative of the different hydraulic demands and water supply available to trees of these two size classes associated with rooting depth and path length (Petit et al., 2008; Anfodillo et al., 2013). A second possibility is that, regardless of tree age, a subset of vessels are very short and likely to end within a ~2 mm segment, whereas the remaining vessels are much longer and less likely to end within the root segment—effectively shifting the peak of a typical vessel-length distribution even closer to zero. Indeed, there is limited data on root vessel lengths, challenging the common assumption that root vessel lengths are longer than in other tissues (Jacobsen et al., 2012; Pratt et al., 2015) despite having larger diameters (Anfodillo et al., 2013). Measuring vessel length distributions using small intervals in conjunction with a series of microCT scans of the same tissue will help resolve these two possibilities and potentially identify others.

During this investigation, we encountered two important factors that are recommended for continued research on xylem vessel anatomy. First, the presence of long vessel-element “tails” extending beyond the perforation plate (Evert et al., 2006), visible
above the white arrows in Fig. 1C, may appear to be separate vessels when observed in cross section (i.e., a transverse section taken at the dotted horizontal line in Fig. 1C). If counted as separate vessels, these tails could influence estimates of Hagen–Poiseuille flow depending on the vessel diameters. Second, we encountered several apparent vessel elements of large diameter, shaped like other elements within the vessel group, and with extensive pitting to neighboring vessels; however, they had no perforation plates on either end would therefore be classified as vascular tracheids (IAWA Committee on Nomenclature, 1964); the apparent single vessel element gap visible in Fig. 2B (black arrow) is an example. Further analysis of plant tissues via microCT will likely improve our understanding of gradations between different xylem cell types and their spatial organization (IAWA Committee on Nomenclature, 1964; Wheeler et al., 1989; Carlquist, 2013).

Overall, we found that vessel endings were surprisingly common in A. rubrum roots and that microCT imaging of xylem vessel networks can provide a more complete understanding of the spatial distribution of vessel endings within the xylem network. Perforation plate angles were dimorphic, with more acute plate angles and narrower diameters in vessels that ended within the scanned area, compared with vessels that traversed the entire segment, suggesting different developmental signals during xylogenesis (Frost, 1930). Interestingly, vessel element lengths and pit density were consistent across vessel types within our samples, suggesting that some xylem vessel characteristics may be less variable over the length of a vessel than others. If such patterns hold for a greater number of samples, tissue types, and species, this information could simplify xylem network model parameterization and facilitate future modeling efforts to better understand the relationship between xylem microstructure and function. Finally, this new approach to visualizing vessel endings with microCT imaging shows potential as a complementary tool to be used with existing methods for determining vessel length distributions (Cai and Tyree, 2014).

ACKNOWLEDGEMENTS

The authors thank D. Parkinson of Beamline 8.3.2 for technical assistance and three anonymous reviewers for helpful suggestions. This research used resources of the Advanced Light Source, which is a DOE Office of Science User Facility under contract no. DE-AC02-05CH11231. This research was funded by National Science Foundation Grant #1557917.

DATA ACCESSIBILITY STATEMENT

Complete microCT image data sets for the four A. rubrum root segments are available upon request.

LITERATURE CITED


IAWA Committee on Nomenclature. 1964. Multilingual glossary of terms used in wood anatomy. Konkordia, Winterthur, Switzerland.


