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ROOT ANATOMY, MORPHOLOGY, AND LONGEVITY AMONG ROOT ORDERS IN *VACCINIUM CORYMBOSUM* (ERICACEAE)[1]

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Understanding root processes at the whole-plant or ecosystem scales requires an accounting of the range of functions within a root system. Studying root traits based on their branching order can be a powerful approach to understanding this complex system. The current study examined the highly branched root system of the ericoid plant, *Vaccinium corymbosum* L. (highbush blueberry) by classifying its root orders with a modified version of the morphometric approach similar to that used in hydrology for stream classification. Root anatomy provided valuable insight into variation in root function across orders. The more permanent portion of the root system occurred in 4th- and higher-order roots. Roots in these orders had radial growth; the lowest specific root length, N:C ratios, and mycorrhizal colonization; the highest tissue density and vessel number; and the coarsest root diameter. The ephemeral portion of the root system was mainly in the first three root orders. First- and 2nd-order roots were nearly anatomically identical, with similar mycorrhizal colonization and diameter, and also, despite being extremely fine, median lifespans were not very short (115–120 d; estimated with minirhizotrons). Our research underscores the value of examining root traits by root order and its implications to understanding belowground processes.

Key words: blueberry; Ericaceae; minirhizotrons; mycorrhizas; root architecture; root form; root function; root lifespan; *Vaccinium corymbosum*.

An inadequate understanding of belowground processes is a major limitation to better predictions of crop growth and productivity (Eissenstat et al., 2006) or how climate change may affect terrestrial carbon sequestration (Norby and Jackson, 2000). Unlike the aboveground system, which can be readily divided into absorptive tissues (leaves) and support and transport tissues (stems), roots are much more complex. Traditionally, absorptive, more ephemeral roots had been identified in ecological studies by an arbitrary diameter cut off (e.g., 1 or 2 mm), with little regard to functional considerations (Kummerow et al., 1978; Persson, 1980; Marshall, 1986; Jackson et al., 1997; King et al., 2002). More recently, characterizing roots by their branching order has been reported as a useful approach to identifying functional differences within a root system (Pregitzer, 2002).

Few studies have examined anatomical and functional variation in roots according to their branching order. Anatomically, Eissenstat and Achor (1999) looked at 1st- and 2nd-order roots of citrus and Hishi et al. in two studies (2005a, 2006) examined 1st-, 2nd-, and 3rd-order roots in the conifer *Chamaecyparis obtusa*. The presence or absence of secondary xylem, a strong indicator of the transition from absorptive to transport functions, was commonly observed in 2nd- and higher-order roots in *Chamaecyparis*. An anatomical survey of 23 Chinese temperate tree species indicated that 4th-order roots were nonmycorrhizal and had secondary development, whereas 1st-order roots were mycorrhizal and had primary development (Guo et al., 2008). Depending on the species, 2nd- and 3rd-order roots might be either primarily absorptive or primarily associated with transport and support.

In addition to anatomy, root architecture and morphology has been studied as a function of root order in temperate trees. These include the first three root orders of nine North American temperate trees (Pregitzer et al., 2002), the first five root orders in longleaf pine (Guo et al., 2004), the first five root orders in two Chinese temperate tree species (Wang et al., 2006), and the first five root orders in 23 Chinese temperate tree species (Guo et al., 2008). In these studies, morphology changed systematically with root order with 1st-order roots having the thinnest diameters, highest specific root lengths (SRLs), highest nitrogen concentrations, and largest proportion of the total root length.

When considering variation in root morphology and architecture in plants and possible linkages with function, an extreme example is found in ericoid species, which are noted for their very thin and highly branched roots. For example, *Vaccinium* absorptive roots can have diameters less than 50 μm ("hair roots") (Coville, 1910; Eck and Childers, 1966; Bonfante-Fasolo et al., 1981; Gough, 1994) compared to typically >200 μm in most woody species in other families (Bouma et al., 1997; Wells and Eissenstat, 2001; Pregitzer et al., 2002; Comas and Eissenstat, 2004; Guo et al., 2004; Volder et al., 2005; Withington et al., 2006). Because *Vaccinium* absorptive roots are extremely fine, they might to be expected to be very short-lived compared to other woody plants (Eissenstat, 1992; Eissenstat et al., 2000). In addition, *Vaccinium* species are associated with ericoid mycorrhizal fungi, a distinct group noted for their high extracellular

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enzymatic activities and the ability to use organic forms of N and P (Smith and Read, 1997). Do the form and function of roots of an ericoid plant follow the same patterns as those reported in nonericoid trees?

The objective of this study was to examine anatomical, morphological, and functional traits of *Vaccinium corymbosum* L. roots according to their branching hierarchy following the morphometric approach developed by Fitter (1982). We hypothesized that anatomical traits associated with water transport, such as the diameter of the stele and the diameter and number of xylem vessels, should increase in an exponential fashion as root order increases, to meet the flow demands of water conducted in many conduits converging on fewer conduits. Morphological traits including specific root length and percentage of total root length should increase in an exponential fashion as root order decreases to meet the increased demands for nutrient absorption and the importance of high absorptive surface area for this function. Despite the small diameters and ericoid mycorrhizas in the *Vaccinium* root system, we expected its root function to be similarly organized by branching order as that reported in trees. Thus, we predicted that the absorptive root system would be primarily in the first two orders of roots, that the 4th and higher orders would be primarily associated with transport, and that 3rd-order roots would be primarily transitional.

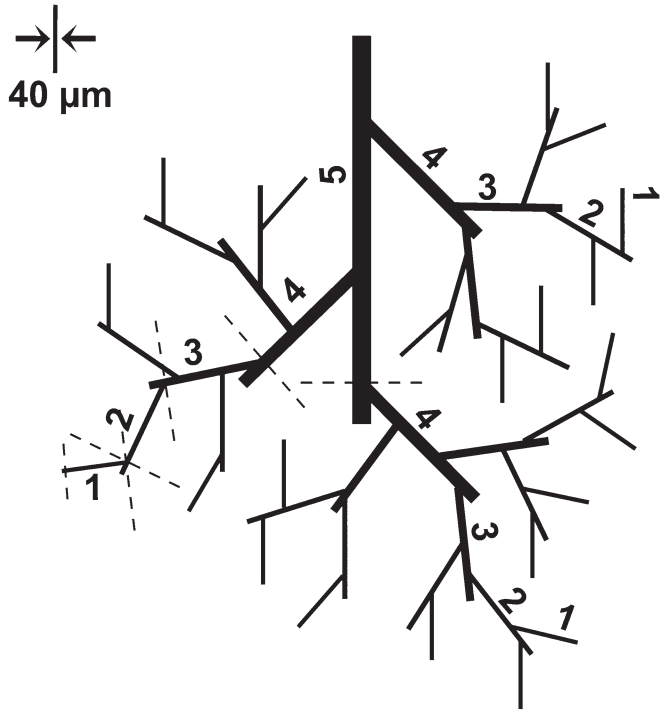

Fig. 1. A schematic diagram of a *Vaccinium corymbosum* root branch network consisting of five root orders classified using a slight modification of the morphometric approach by Fitter (1982). Fitter's approach considered roots with meristematic endings as 1st-order roots. In our approach, meristematic endings that appeared similar to the preceding root section of higher order roots (3rd and higher) without forming a branch junction were not considered 1st-order roots. Dotted lines in the graph indicate the regions of each root order that were considered belonging to that specific root order. Regions between the line and branching point were used for anatomical sectioning. Meristematic endings of 3rd- and higher-order roots (small ending region after the dotted line) were not included. Line thickness is representative of mean diameter for that root order. The length of roots for each branch order represents the proportion of root length accounted for by that root order in the total root length of a branch composed of five orders. *Note:* length is relative to the total length of a 5th-order root branch and not related to the scalar for diameter.

MATERIALS AND METHODS

Study site and plant material—'Blue crop' highbush blueberry (*Vaccinium corymbosum*) plants were planted in 1992 at the Russell E. Larsen Research and Education Center, near State College, Pennsylvania, USA. The soil type was a Hagerstown silt loam. Plants were grown using standard commercial cultural practices, including fertilization every year (73 $kg \cdot ha^{-1}$ applied as urea during the duration of this study) and mulched with rotted sawdust every two to three years, (Demchak et al., 2008). Plants did not receive supplemental irrigation during the study period.

Roots were collected in June and July of 2006 and 2007 at 15–20 cm from the center of the plant and at a soil depth of 10–15 cm. Intact root branches were collected after soil and organic matter were removed from between the roots taking care to minimize breakage. Samples were placed in a plastic bag with moist paper, then kept at 4°C until dissection. For both years, at the time of root collection, plants had a fully developed canopy, and berries were just prior to veraison (changing from green to blue).

Root morphology—Roots were sampled from either three (2006) or six (2007) randomly chosen plants. Root samples in 2007 were pooled into two groups of three plants each to obtain sufficient root material of each order and were sampled twice in June and twice in July.

Before root dissection, root segments were rinsed and cleaned with deionized (DI) water (4°C). Then root segments were transferred to glass petri dishes filled with a 1 mM $CaSO_4$ solution buffered with 5 mM MES adjusted to pH 5.5 with 1 M KOH (4°C) to maintain membrane integrity. Roots were dissected into different branch orders under a 10× stereomicroscope (Olympus SZ-PT; Tokyo, Japan) using a slightly modified version of the morphometric approach developed by Fitter (1982) (Fig. 1). Fitter's approach considered roots with meristematic endings as 1st-order roots; in our approach, meristematic endings that appeared similar (i.e., more pronounced tip, large diameter, long extension) to the preceding root section of higher order roots (3rd and higher) without forming a branch junction were not considered 1st-order roots (see dotted lines in Fig. 1). Root diameter for each order was determined using an Olympus BH-2 compound microscope at 10× to 20× depending on root size in 2006 and a high resolution flat-bed scanner (1200 dpi resolution, 19-µm pixel size , 256-level grayscale, TIFF format; Epson Scanner Perfection 4490, USA) and WinRhizo software (Regent Instruments, Quebec city, Quebec, Canada) in 2007. The root scanning procedure included placing the wet, dissected roots on a glass slide previously misted with DI water (misting the slide helped to spread out the roots) and maintaining them moist during the scanning process. Due to the small size of the finest roots, a high resolution scan was required. No root staining was required. Root length of each order was also determined in 2007 with this system. Once all roots were scanned, they were dried (68°C for 48 h), and their mass was determined to estimate specific root length (SRL).

Root anatomy—In the summer of 2007, roots were sampled from four randomly chosen plants. Roots were prepared for light microscopy differently, depending on order. For higher-order roots, from 4th to 7th order, 12-µm-thick cross sections were cut with a cryostat (Model 77210163GB, Thermo Fisher, Kalamazoo, Michigan, USA) using frozen tissues (O.C.T. embedding medium; Sakura Finetechnical, Tokyo, Japan). For first- to 3rd-order roots, 1-µm cross sections were cut with an ultramicrotome (Model Ultracut E, Leica, Deerfield, Illinois, USA) using a glass knife. Root samples were prepared for ultramicrotomy by first placing them in a fixative solution of 2.8% (v/v) glutaraldehyde in 0.1 M HEPES buffer (pH 7.2) and 0.02% (v/v) Triton X-100 at room temperature for 4 h, then at 4°C overnight. After fixation, roots were placed in 1% OsO_4 in 0.1 M HEPES buffer with 0.02% Triton-100 in a dark container at 4°C for 24 h. They were then dehydrated in an ethanol series and finally embedded in Spurr's resin (Spurr, 1969). For first-order roots, sectioning started at the root tip and continued to at least 500 µm away from the root tip. All root cross sections were then stained with a solution of 0.01% toluidine blue (O'Brien et al., 1964). Using a light microscope and digital camera (Olympus BX51with a digital camera SPOT II RT attachment), images were taken (76 dpi resolution, 1 pixel = 0.16 µm) for each cross section at 4× to 40× depending on root size.

Number of epidermal cells and mycorrhizal presence were tallied, and the diameters of the root, stele, and vessels and the number of vessels were determined from the images using Image J software (Abramoff et al., 2004). When some of the epidermal cells were missing or collapsed, measurements were completed between the distal points of the collapsed epidermal cell walls. If all epidermal cells were missing in a root, that root was discarded for the measurements. Total number of roots per root order analyzed varied (1st order, $N = 3$; 2nd, $N = 5$; 3rd, $N = 5$; 4th, $N = 6$; 5th, $N = 4$; 6th, $N = 3$; 7th, $N = 4$).

Root nitrogen concentration and mycorrhizal colonization—Root tissue (3–5 mg dry mass of the same plant material used for root morphological analysis) from each root order was analyzed for total nitrogen concentration by flash combustion chromatography (Fisons CHNS-O elemental analyzer, Model EA-1108, Fisons Instruments, Beverly, Massachusetts, USA). Nitrogen concentration was expressed on the basis of carbon concentration rather than root dry mass because of possible soil particle contamination on very fine roots. Mycorrhizal colonization was assessed on roots collected in July 2006. Roots that were used to assess mycorrhizal colonization were sampled from five randomly chosen plants in July 2006. Mycorrhizal staining was performed by soaking the roots that had been dried (68°C, 48 h) in a solution of KOH (10%) overnight, rinsing with DI water and clearing with an ammonium-hydrogen peroxide mixture solution (10%) for 10 min, and then staining with trypan blue solution (0.01%) (Phillips and Hayman, 1970). For mycorrhizal colonization analysis, we used a light microscope under a 20× magnification (Olympus SZ-PT), with a field of view of uniform length (~50 μm) and scanned across the entire diameter of the root. Each root scan was scored for presence or absence of mycorrhizal coils (Trouvelot et al., 1986). A total of 30 root segments per root order for each of five plants were analyzed.

Root longevity—In May 2003, based on a completely randomized design, 12 clear acrylic minirhizotron tubes were installed by 12 plants in the field (one for each plant) at an angle of 30° from the vertical, 30 cm from the center of the plant either to the left or right side when facing the row of plants. Each tube was 100 cm long with a 3.5 cm outside diameter and etched with a column of 74 numbered, 0.8 × 1.2 cm windows. Beginning in June 2004, a miniature video camera system (BTC-1.125; Bartz Technology, Santa Barbara, California, USA) connected to a computer was used to capture images using specialized software (ICAP v.4.1, Bartz Technology). Root images were collected every two weeks during early spring and late summer and approximately once a month during fall and winter. Root images were analyzed using Win Rhizo Tron MF software (Regent Instruments, Quebec City, Quebec, Canada). The date roots were first observed and disappeared, root diameter, and root order were recorded. No clearly defined root death stage was observed in this species. Thus, our estimate of root lifespan is conservative because it includes decomposition, and it is equivalent to "root persistence" (sensu Peek et al., 2005). We assume that decomposition was relatively rapid in these usually moist soils.

Root lifespan data were analyzed with Cox proportional hazards regression (PROC PHREG; SAS Institute, Cary, North Carolina, USA) (Wells and Eissenstat, 2001). This type of analysis allows for the influence of covariates to be held constant while the "hazard" of an individual covariate is determined (Cox, 1972). The "hazard" of a covariate refers to the possible risk of root mortality at any point in time (Allison, 1995). The covariates used in the model were root diameter, root order, neighboring root number, and soil depth. The Cox's partial likelihood method estimates a parameter coefficient (β) for each tested covariate, and calculates a χ^2 statistic to test the null hypothesis that each β equals zero. The effect of the parameter estimate on each of the covariates can be measure by its negative or positive sign. A negative parameter indicates a decreased hazard of mortality with an increase in the covariate (Wells and Eissenstat, 2001). Root order was used as a covariate in the model. Only the first three orders were well represented in the minirhizotron images. Because points of branching often were not visible in the minirhizotron images, root orders were estimated based on diameter distributions of the different root orders (Fig. 3). Due to overlapping of 1st- and 2nd-order diameter distributions, we combined them as one category for the survival analysis. We used the following limits: 1st- and 2nd-order, <50 μm; 3rd-order, 51–70 μm.

Statistics—Root diameter, root stele diameter, root vessel diameter, vessel count, and SRL were transformed logarithmically to meet assumptions of normality and equal variance across orders. Differences in root diameter, stele diameter, vessel diameter, vessel count, SRL, root tissue density, N:C ratio, and mycorrhizal colonization among root orders were analyzed by the PROC MIXED procedure, using order as a fixed effect (SAS Institute, Cary, North Carolina, USA) and the Bonferroni correction test for multiple comparisons among root branching orders.

RESULTS

Root anatomy—Transverse sections of seven representative root orders indicate minimal cortical layer development in 1st-order ericoid roots (Fig. 2A). We also observed, especially for lowest order roots (1st and 2nd), epidermal cells that were collapsed, squashed, or absent entirely (Fig. 2A). The number of epidermal cells per root from 1st to 3rd order ranged from 7 to 14, 8 to 12, and 10 to 18, respectively ($N = 3$, 5, and 5, respectively). First- through 3rd-order roots had a pentarch xylem pole pattern (Fig. 2). Secondary xylem development occurred in 4th and higher orders.

Mean root diameter varied considerably with root order (Fig. 3). From 1st to 6th order, respectively, mean diameters (in μm) were 40, 48, 75, 120, 177, and 222 ($N = 59$, 52, 54, 55, 53, and 6, respectively), although the range in root diameter for any particular order was as much as ±20 μm for 1st- and 2nd-order roots and much more for higher-order roots.

Vascular tissues also varied substantially among root orders (Fig. 4). Stele diameter varied with root order in a magnitude similar to that of root diameter. Mean stele diameter from 1st- to 7th-order roots was (in μm): 17, 20, 27, 38, 67, 114, and 239, respectively ($N = 3$, 5, 5, 6, 4, 3, and 4 respectively). Mean stele diameters in 1st- and 2nd-order roots were quite similar ($P = 0.39$; Fig. 4A). Unlike root diameter and stele diameter, vessel diameter was quite conserved over root order. Mean vessel diameter from 1st- to 7th-order roots was (in μm) 2.5, 2.9, 3.2, 3.7, 3.7, 3.8, and 4.8, respectively ($N = 3$, 5, 5, 6, 4, 3, and 4, respectively). Maximum vessel diameter from 1st- to 7th-order roots was (in μm) 6.5, 7.2, 9.3, 10.0, 10.0, 25.0, and 31.0, respectively. First- and 2nd-order roots did not differ significantly from each other in mean or maximum vessel diameter; however, they were significantly smaller (40 and 80%, respectively) than those of higher orders (Fig. 4A).

Vessel number varied considerably among orders. Mean number of vessels per root from 1st- to 7th-order was 13, 12, 22, 28, 39, 501, and 812, respectively ($N = 3$, 5, 5, 6, 4, 3, and 4, respectively). Similar to the diameters of the root, stele, and vessel, the number of vessels in 1st- and 2nd-order roots was quite similar (Fig. 4B). Even on a log-scale, there was a considerable increase (1150%) in number of vessels between 5th- and 6th-order roots (Fig. 4B) ($P < 0.0001$), associated with a pronounced radial growth during secondary development (Fig. 2).

Root morphology—Across all seven orders, specific root length ($cm \cdot mg^{-1}$) decreased markedly with root order (Fig. 5A)

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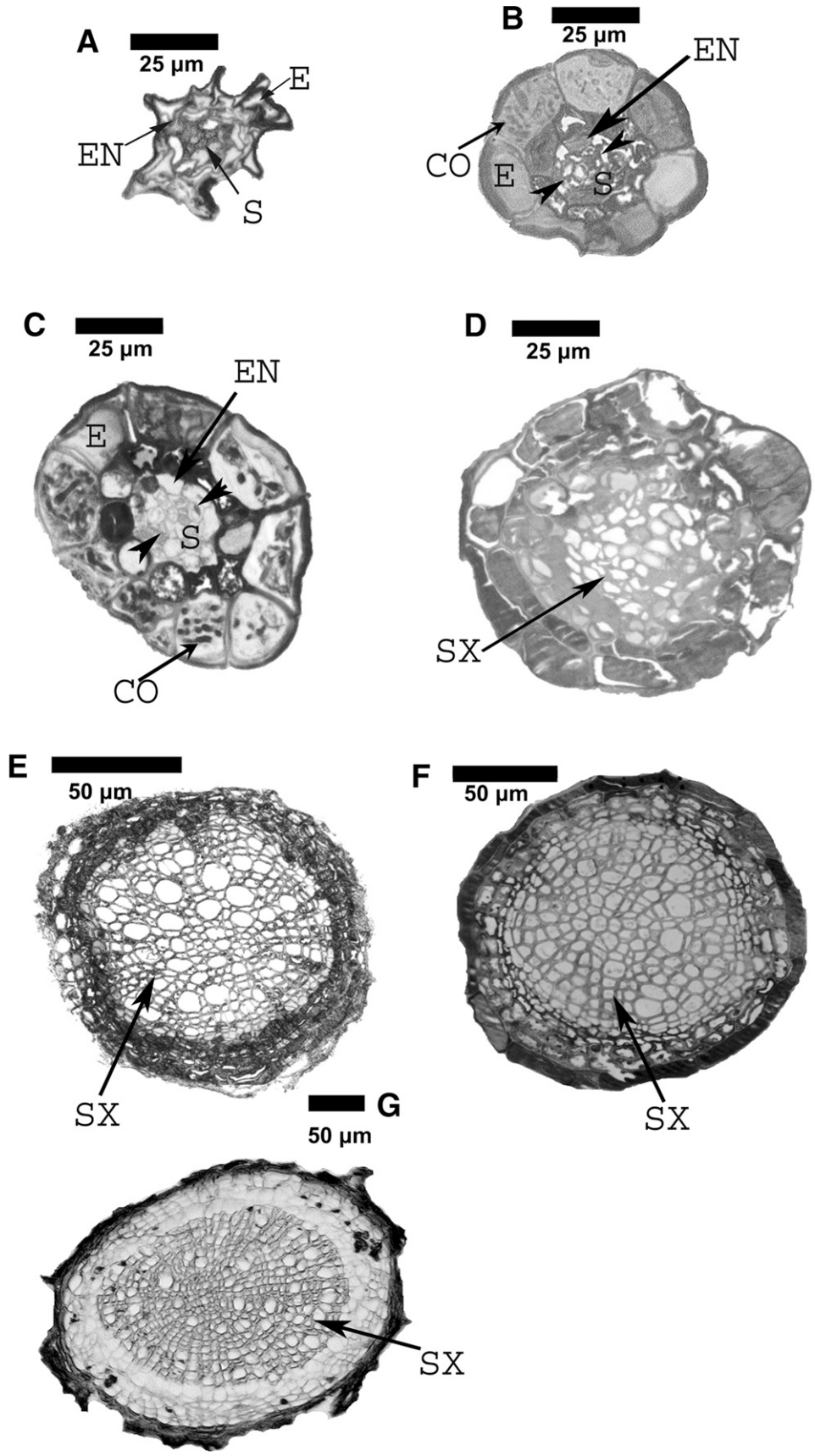
Fig. 2. Cross sections of the first seven root orders of *Vaccinium corymbosum* from roots collected at 15–20 cm soil depth under field conditions. (A) Light micrograph of a 1st-order root about 500 μm from the root tip showing a single cell layer of epidermal cells (E), a single layer of endodermal cells (EN), and stele (S). Cross-sections of (B) 2nd- and (C) 3rd-order roots with no secondary development showing some ericoid fungal coils (CO) in the epidermal cells (E). Also shown is the stele diameter (distance between arrowheads). Cross-sections of (D) 4th-, (E) 5th-, (F) 6th-, and (G) 7th-order roots with secondary xylem (SX). *Note:* scale bar changes in length depending on root order.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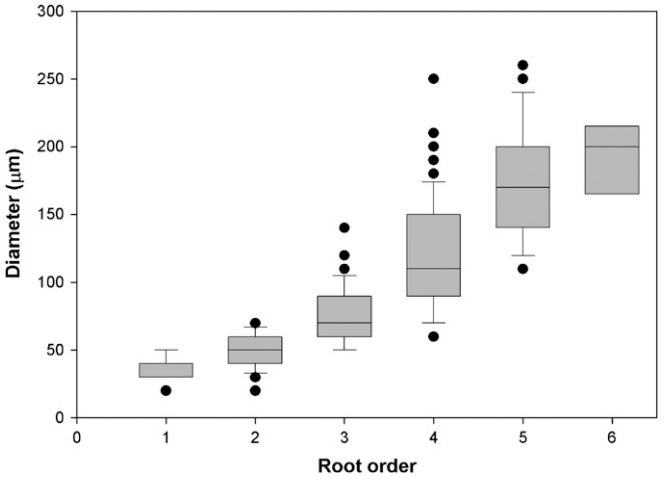

Fig. 3. Box plots of root diameter of *Vaccinium corymbosum* among six root orders collected in the summer of 2006 from three shrubs. The horizontal line in each box plot represents the median, the top and bottom edges of the box represent the 75th and 25th percentiles, respectively; the top and bottom error bars represents the 90th and 10th percentiles, respectively; the black dots represents outliers beyond the 95th and 5th percentiles. Root diameter measurements were completed with a light microscope and a calibrated ocular scale. The number of roots varied per root order as follows: 1st-order roots, $N = 59$; 2nd, $N = 52$; 3rd, $N = 54$; 4th, $N = 55$; 5th, $N = 53$; 6th, $N = 6$.

($P < 0.0001$). First-order roots showed the highest values and therefore the least biomass investment for construction of a centimeter of root. Specific root length decreased 96%, from 83.1 cm·mg^{-1} in the 1st-order to 2.57 cm·mg^{-1} in the 5th-order roots. Compared to the other orders, 1st-order roots had the highest proportion of total root length (46%) in a 5th-order root system with all finer orders attached (Fig. 6A).

Root tissue density (root dry mass/root volume when turgid) tended to increase with root order ranging from 0.25 g·cm^{-3} for the 1st-order to 0.49 g·cm^{-3} for the 5th-order (an increase in 96%; $P = 0.0921$; Fig. 5B). Most of the root biomass in a 5th-order root system containing all lower order roots was in the 3rd, 4th and 5th root orders (24%, 29%, and 26%, respectively; Fig. 6B).

N:C ratio, mycorrhiza, and root lifespan—Because of soil particle contamination on very fine roots, it is often more meaningful to express nitrogen concentration on the basis of carbon concentration rather than root dry mass. Nitrogen to carbon (N:C) ratios decreased markedly with increasing root order (Fig. 5C; $P = 0.004$). First-order roots had the highest N:C ratio among the root orders—91% higher than 5th-order roots.

Mycorrhizal presence significantly decreased as root order increased; there was a decrease in mycorrhizal colonization close to 60% from 1st-order roots to 4th-order roots (Fig. 7). The first three orders had the highest incidence of colonization.

Median root lifespan for 1st- and 2nd-order roots (combined category; Fig. 8) was 120 and 115 d in 2004 and 2005, respectively. Median lifespan of 3rd-order roots was 155 and 136 d in 2004 and 2005, respectively, and significantly longer than that observed in the lower order roots ($P = 0.002$ in 2004 and $P = 0.02$ in 2005).

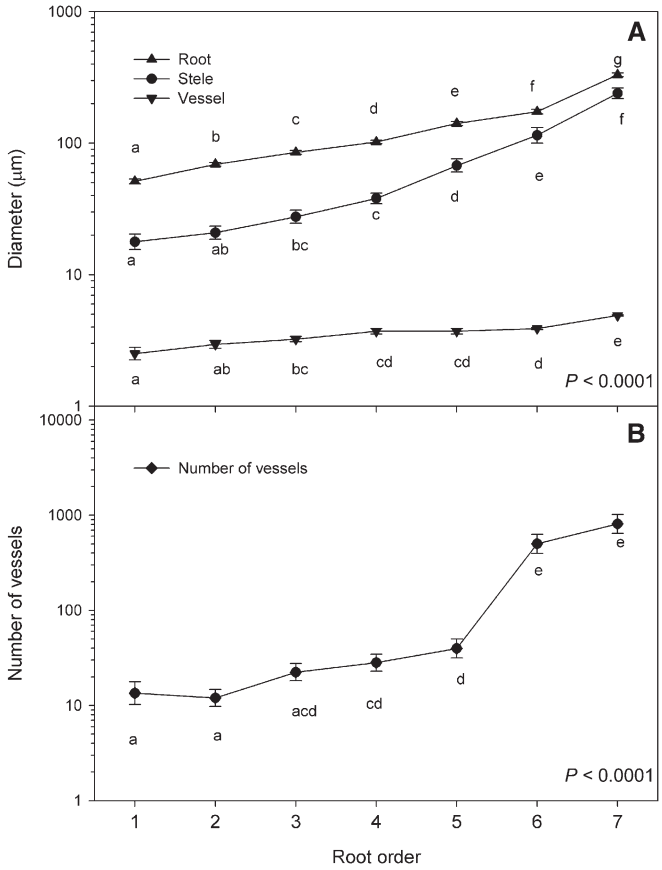

Fig. 4. (A) Mean root (▲), stele (•), vessel (▼) diameter; and (B) mean number of xylem vessels (◆), for the first seven root orders of *Vaccinium corymbosum*. Error bars represent one standard error of the mean of a pooled sample from four shrubs. The number of roots varied per root order, for 1st-order roots, N = 3; 2nd, N = 5; 3rd, N = 5; 4th, N = 6; 5th, N = 4; 6th, N = 3; 7th, N = 4. Lowercase letters that differ between root order indicate a significant difference ($P < 0.05$). A log scale was used for the ordinate.

DISCUSSION

By looking at several root traits and using the root order classification, we were able to identify traits associated with root form and function and also the existence of specific trade-offs in *Vaccinium* roots. We found that the relationship of root order with root function in this ericoid shrub was quite similar to that reported in trees (Pregitzer et al., 2002; Guo et al., 2004, 2008; Wang et al., 2006), despite marked differences in morphology and the mycorrhizal symbiont.

Root anatomy and mycorrhizal colonization—One of the most functional aspects related with root anatomy is water transport capacity because it is highly influenced by number and size of the water conductive elements (Esau, 1965; Steudle and Peterson, 1998). Root anatomical studies can provide valuable insight into the mechanical resistance to hydraulic flow within the root system. Another important aspect related with root anatomy is mycorrhizal infection. Ericoid roots require specific anatomical features to be able to host their mycorrhizal fungi, including big, balloon-shaped, almost empty (of cell organelles) epidermal cells (Bonfante-Fasolo et al., 1981; Read, 1983). Our data suggest that functional variation in transport and nutrient

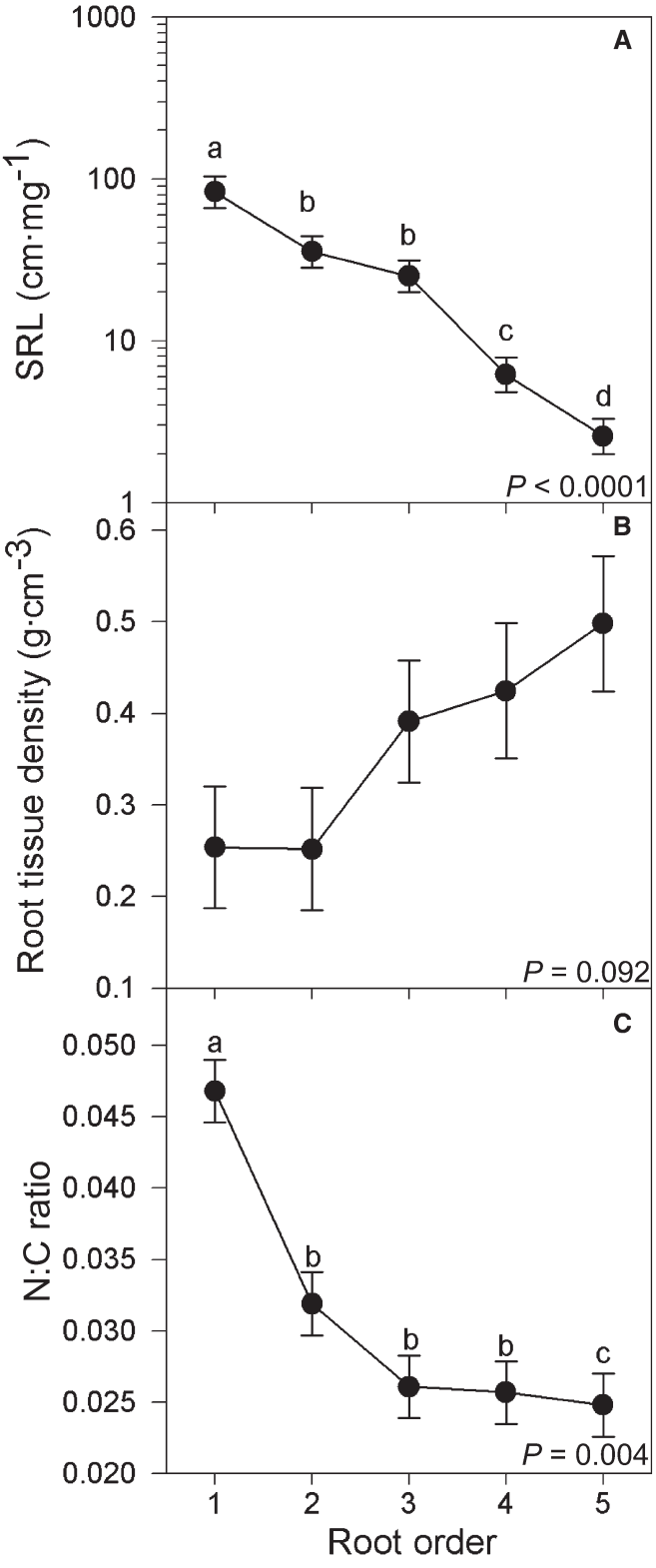

Fig. 5. (A) Specific root length (cm·mg^{-1}), (B) root tissue density (g·cm^{-3}), and (C) nitrogen to carbon ratio (N:C) for the first five root orders of *Vaccinium corymbosum*. Data were pooled into two groups of three plants each and four sampling dates. Error bars represent one standard error of the mean ($N = 2$). Lower case letters that differ between root order indicate a significant ($P < 0.05$) difference. *Note:* for 4A, SRL is plotted on a log scale.

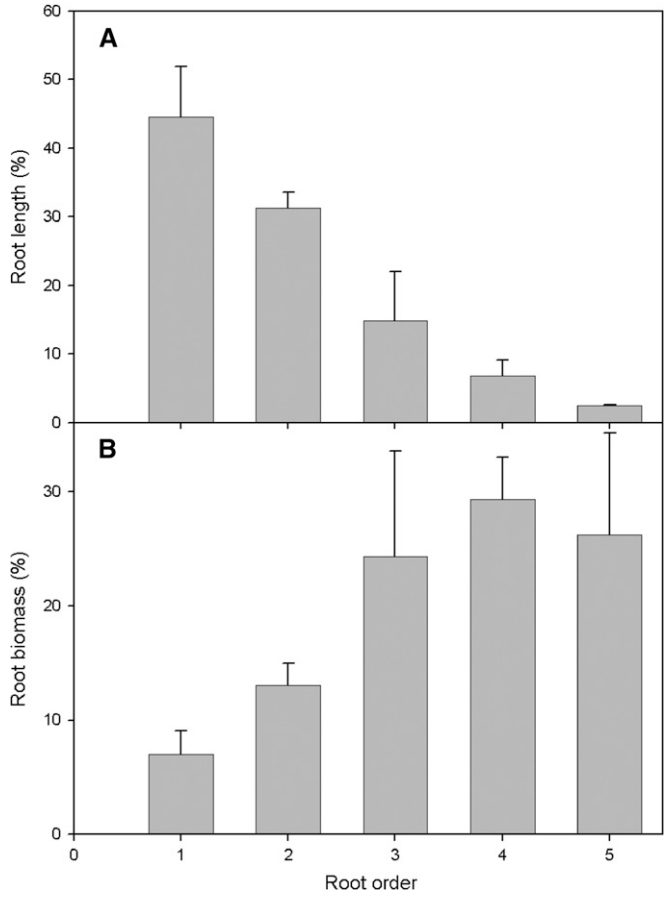

Fig. 6. (A) Percentage root length by root order of the total root length (cm) and (B) percentage root mass by root order of the total root mass (g) measured for *Vaccinium corymbosum*. Data were pooled into two groups of three plants each and four sampling dates. Error bars represent one standard error of the mean ($N = 2$).

uptake associated with mycorrhizal colonization are, at least in part, based on branching order.

For *Vaccinium* and many other species, many of the anatomical differences are tightly linked with presence or absence of secondary xylem, which defines a key separation in root system function (Esau, 1965; McKenzie and Peterson, 1995; Eissenstat and Achor, 1999; McCully, 1999; Hishi, 2007; Guo et al., 2008). Anatomically, roots have been classified as either absorptive or conductive roots (McCully, 1999), suggesting that roots within the same category are similar in properties and function. Also, root transport capacity has been linked to root aging associated with radial growth, which results in an increase of the number of xylem vessels (Esau, 1965; Steudle and Peterson, 1998; Kumar et al., 2007) and diameter of the largest vessels (Martínez-Vilalta et al., 2002). While this separation is useful, we should not ignore the continuous increase in the capacity to transport water, especially with increases in order in the higher order roots (Figs. 2, 4), nor the continuous shift in nutrient absorptive capacity with a decrease in order among the lower order roots, as seen by the changes in SRL, N concentration, and mycorrhizal colonization (Figs. 5, 7). First- and 2nd-order roots were more similar. Although 1st- and 2nd-order roots had similar diameter, mycorrhizal colonization, and anatomy, 1st-order roots had higher N:C ratio, SRL, and percentage of total length.

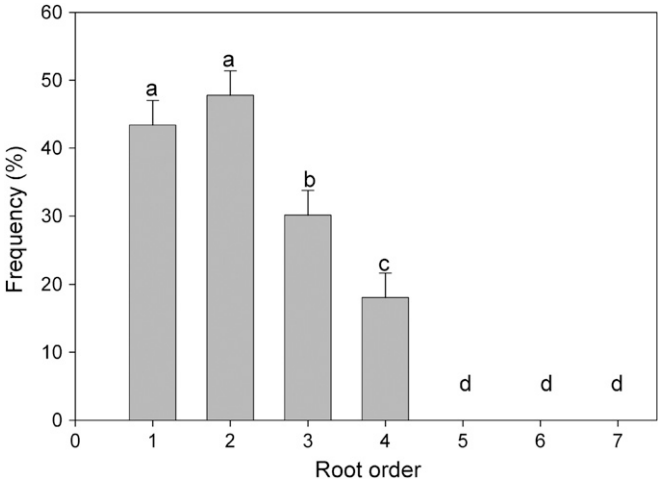

Fig. 7. Frequency of mycorrhizal colonization in the root system of *Vaccinium corymbosum*. Around 30 root segments of 50-μm length per root order were analyzed from five different plants within seven root orders. Error bars represent one standard error of the mean ($N = 5$). Lower case letters that differ between root order indicate a significant ($P < 0.05$) difference.

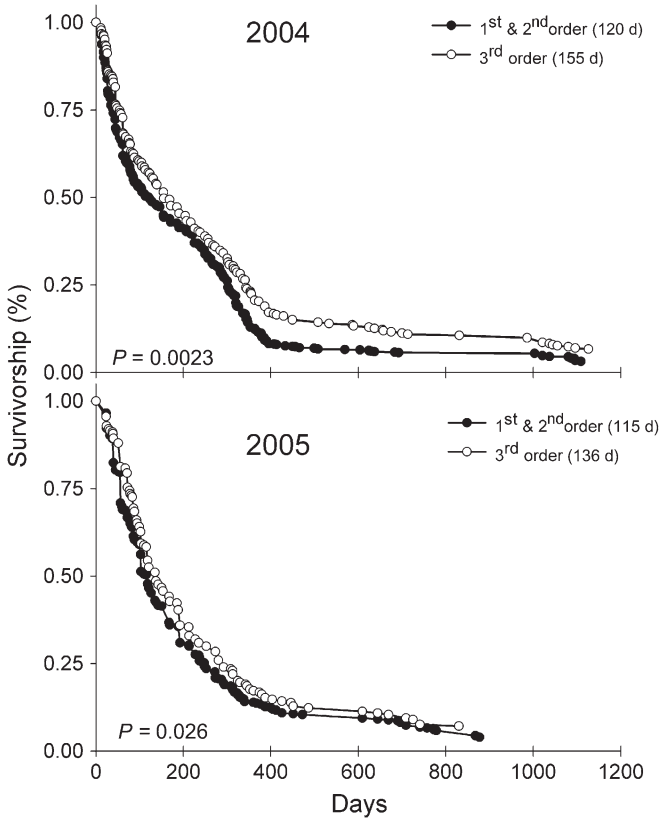

Fig. 8. Root lifespan in a *Vaccinium corymbosum* field near State College, Pennsylvania for 1st- to 3rd-order roots. Median life spans are indicated in parentheses. Data are for all roots observed (1st and 2nd order, 741 individuals for 2004, $N = 430$ for 2005; 3rd order, $N = 293$ for 2004, $N = 215$ for 2005) through minirhizotron windows from a total of 12 plants. First- and 2nd-order roots had shorter root lifespan than 3rd-order roots in both years (2004, $P = 0.002$; 2005, $P = 0.026$).

Hydraulic transport capacity was strongly affected by root order. For example, the mean number of vessels for a 7th-order root was 62-fold greater than that of a 1st-order root (reaching a maximum of 1512 vessels in some roots) suggesting large increases in transport capacity in individual root segments with increased order (Fig. 4B). Interestingly, even though the number of vessels increased greatly across root orders (especially on those with secondary growth), mean vessel diameters did not show the same large changes (Fig. 4A). However, maximum vessel diameter did show large increases with order, suggesting maximum rather than mean diameter was more important for hydraulic transport. Moreover, as indicated by the high number of xylem vessels with a small diameter, *Vaccinium* roots appear to be have a low volume to surface ratio, a property associated with a decreased risk of cavitation and embolisms by drought (Hargrave et al., 1994; Sperry and Saliendra, 1994; Hacke et al., 2000; Martínez-Vilalta et al., 2002), freezing and thawing (Davis et al., 1999), or pathogen infection (Ikeda and Kiyohara, 1995).

First and 2nd-order roots, and to a lesser extent, 3rd- and 4th-order roots, were the root orders primarily used in nutrient absorption. Mycorrhizas are very important for ericoid plants and have been widely studied (Specht, 1979; Cairney and Meharg, 2003; Peterson et al., 2004). However, this root trait had previously never been characterized in relation to root order. By examining mycorrhizal colonization based on the root order classification, we were able to identify the similar colonization in the first two root orders and the decline in colonization in 3rd and 4th root branching orders.

Root morphology, lifespan, and N:C ratios—Root branching order also clearly revealed ecologically relevant information that would be important for an improved understanding of carbon and nitrogen cycling and estimates of belowground net primary productivity. As predicted, specific root length declined in an exponential fashion with an increase in root order (Fig. 5A). Root tissue density was similar in 1st- and 2nd-order roots but then increased in a linear pattern with an increase in order (Fig. 5B). Root N:C ratio declined steeply with an increase in order for the first three orders and then remained relatively stable (Fig. 5C). These results are broadly consistent with other temperate tree species (Pregitzer et al., 1997, 2002; Wells, 1999; Eissenstat et al., 2000; Guo et al., 2004, 2008; Hishi and Takeda, 2005a).While it was challenging to measure just the ephemeral portion in *Vaccinium* (roots 3rd-order and lower), clearly if one wishes to assess the C and N turnover in *Vaccinium*, then including all roots less than an arbitrary diameter (i.e., <1 or 2 mm) would lead to inclusion of a large fraction of relatively permanent roots of relatively low N and very erroneous estimates of below ground net primary productivity and C and N turnover.

Several studies have compared SRL for species from resource-poor and resource-rich environments. The general trend is that species adapted to poor-resource environments have slower growth rates and root traits associated with slower rates of resource acquisition, including relatively low specific root lengths, high root tissue densities, and low absorptive capacities (Eissenstat, 1992; McCrady and Comerford, 1998; Taylor and Peterson, 2000; Comas et al., 2002; Hishi and Takeda, 2005b). For example, in a study comparing 11 temperate tree species of differing potential growth rates (Comas and Eissenstat, 2004), the SRL of 1st- and 2nd-order roots was higher in fast- than slow-growing species within a particular phylogenetic

contrast. In grasses, fast-growing species may have lower root tissue density than that of slow-growing species (Ryser, 1996; Schlapfer and Ryser, 1996; Wahl and Ryser, 2000; Craine et al., 2001). *Vaccinium* and presumably other ericoid species do not fit nicely into this theoretical framework. These species are recognized for their ability to thrive in low-nutrient environments. Yet, when compared to other plant species, ericoid roots would be considered very fine and of high specific root length. Diameters were as little as 20 μm (Fig. 3), or about twice the thickness of typical single-cell root hair (Barber, 1984). The first three root orders had intermediate lifespans and did not follow the hypothesis that small diameter roots should have short lifespans (Eissenstat, 1992). *Vaccinium* roots had median root lifespans of about 115–155 d. In contrast, in a common garden study of 11 temperate trees growing in infertile, sandy soil, median root lifespans ranged from about 200 to 900 d (Withington et al., 2006). Trees with the shortest root lifespans (*Fagus sylvatica,* 200 d; *Pinus sylvestris,* 245 d) had 1st- and 2nd-order roots with diameters about an order of magnitude larger than those of *Vaccinium* (360 and 580 μm vs. 40 μm) but with only slightly longer lifespans. Moreover, other temperate fruit crops, which have approximately 5- to 10-fold coarser 1st-order roots than those of *Vaccinium*, had root lifespans that are somewhat shorter than *Vaccinium* (apple, Wells and Eissenstat, 2001; peach, Wells et al., 2002; grape, Anderson et al., 2003). Thus, in agreement with Withington et al. (2006), we conclude that root diameter is a poor predictor of root lifespan across species of different families.

Our predictions that *Vaccinium* root function would be similarly organized by branching order to that reported in trees was broadly similar, although there were some subtle differences. For example, in *Vaccinium* the first three root orders are dedicated to nutrient and water absorption, unlike the 23 temperate tree species surveyed by Guo et al. (2008) where such function was mainly associated with only the first two root orders. Another difference was the very low biomass construction cost of 1st-order roots in *Vaccinium*, suggested by very high SRL (83 $cm \cdot mg^{-1}$) compared to 3–10 $cm \cdot mg^{-1}$ (8 and 28 times higher) for temperate tree species. In addition *Vaccinium* had similar or slightly lower nitrogen concentrations than common tree species (e.g., 17 $g \cdot kg^{-1}$ in *Vaccinium* and 16.3 $g \cdot kg^{-1}$ in *Pinus palustris* or 32 $g \cdot kg^{-1}$ for *Populus balsamifera*) (Pregitzer et al., 2002; Guo et al., 2004; Wang et al., 2006). Therefore, *Vaccinium* root systems seem to use a slightly different strategy from other tree species for tissue deployment for absorptive surface area that minimizes both C and N allocation, without substantial consequences in terms of tissue longevity.

In summary, we characterized the anatomical, morphological, and functional aspects of the root system in the ericoid plant *Vaccinium corymbosum* with respect to branching order. We found root order to be an effective tool for integrating the changing functions within the hierarchical root system of this species with very fine roots, where even 7th-order roots were less than 1 mm in diameter. The value of examining simple anatomical features was very evident in this study. First- and 2nd-order roots were quite similar and had an almost exclusive absorptive function. Fifth- and higher-order roots had secondary development and were primarily used only for conduction and anchorage. Third- and 4th-order roots were transitional from absorption to conduction. The absence of secondary development suggests that only the first three root orders were ephemeral, with lifespans from 115 to 155d. While ericoid roots have a most unusual root system in regards to its fineness and symbiosis with ericoid fungi, it seems to follow similar basic rules to the relationship of absorptive and transport functions with changing orders as those observed in ecto- and arbuscular-mycorrhizal temperate trees. We hope future work will more directly link absorptive capacity of mineral nutrients, transport of water, and C costs of construction and maintenance to different orders within the root system.

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