Identifying roots of northern hardwood species: patterns with diameter and depth

Ruth D. Yanai[1], Melanie C. Fisk[2], Timothy J. Fahey[3], Heather M. Engelman[1], and Byung Bae Park[1,4]

[1] Department of Forest and Natural Resources Management, SUNY College of Environmental Science and Forestry, Syracuse, NY 13210 USA

[2] Department of Zoology, Miami University, Oxford, OH 45056, USA

[3]Department of Natural Resources, Cornell University, Ithaca, NY 14853, USA

[4]Korea Forest Research Institute, Seoul 130-712, South Korea

Abstract

Little is known about the depth distribution of tree roots by species because they have not been readily identifiable. We used diagnostic characteristics of wood anatomy to distinguish between roots of different species, and checked the validity of our separation using molecular genetic techniques. We also learned to recognize roots by their gross morphology, which many researchers thought impossible. We applied these methods to test for differences in the rooting depth of maple (*Acer spp.*), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghenensis* Britton) in two northern hardwood sites.

We studied the distribution of roots with depth by fitting curves of the form $Y = 1 - \beta d$ describing cumulative root fraction (Y) as a function of depth (d). There was no significant difference across species in the decline of root mass with depth (β); similarly, the proportion of species mass was indistinguishable by depth. There was a significant difference in the distribution of roots by size class, with fine roots more concentrated near the soil surface. The two sites differed significantly in rooting depth, with roots at the Hubbard Brook site distributed more deeply than at the Bartlett site.

This method is more time consuming than the already lengthy process of picking roots from soil and sorting them by size class, but it is less expensive than genetic characterization. If the goal were solely to identify the distribution of roots by species, genetic techniques alone could suffice, which might be more efficient than root picking.

Introduction

In some forest types, different tree species occupy distinct vertical positions in the canopy. In northern hardwood forests, species more frequently differentiate by crown class. The position of species in the canopy affects resource capture aboveground.

However, we know relatively little about how tree species distribute biomass belowground. Published studies (Nicoll et al 2006) have focused on windthrown, shallow-rooted species, particularly those grown in monoculture. Identification of roots to species in a mixed-forest presents a much greater challenge that can be approached via genetic techniques (Jackson et al. 1999). As species differ in root morphology and branching patterns (Pregitzer et al. 1999), we attempted to use these characteristics to distinguish roots by species. This method was not thought possible, and thus has not been previously described. We demonstrate the application of these methods to test for differences in the rooting depth of maple (*Acer spp.)* American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghenensis* Britton) in two northern hardwood sites.

Acknowledgements

This project was funded by NSF grants DEB 0235650 and DEB 0423259. It is a contribution to the Hubbard Brook Ecosystem Study, which is maintained by the USDA Forest Service and a site in the NSF-Long-Term Ecological Research program.

Materials and Methods

Study sites

We collected roots from two northern hardwood stands, Bartlett Experiment Forest "Site C9" and Hubbard Brook Experimental Forest "Wedge", in the White Mountains of New Hampshire. We sampled from two 15cmx15cm pits in each of three plots, for a total of six pits from each stand. We excavated in five depth increments; the thickness of individual samples ranged from 2 cm to 7 cm, with greater resolution near the surface.

Root processing

Roots were separated from soil by hand and washed against a 4 mm mesh screen, sorted to species according to wood anatomy and gross morphology (Table 1, Figure 1), and subdivided into size classes: 0-2 mm and 2-5 mm. Dead roots or those > 5 mm in diameter were discarded. Roots were oven-dried and weighed.

Data analysis

We estimated the mass of roots per unit area in 3-cm depth increments for each pit, using the mass of each sampled increment distributed evenly across 1-cm increments and summed in 3-cm increments. We compared the depth distribution of roots of three species by calculating the mass of each 3-cm increment as a fraction of the total across all depths and accumulating these as a function of depth. We studied the distribution of roots with depth by fitting curves of the form $Y = 1 - \beta d$ describing cumulative root fraction (Y) as a function of depth (d). Differences in the fitted curves were evaluated with analysis of variance (ANOVA) by comparing the values of the coefficient β.

Molecular Genetic Testing

The effectiveness of visual identification methods was tested by identifying a subsample of roots with molecular genetic methods using the procedure described by Brunner et al. (2001). DNA was extracted from 15-20 roots per species using a standard alkaline lysis/chloroform extraction procedure modified with addition of polyvinylpolypyrrolidone, polyvinylpyrrolidone, and spermidine to improve extraction efficiency and inhibitor removal. The plastid *trn*L intron was amplified using primers c and d (Taberlet et al. 1991) and PCR products were digested with *taq*I. Roots were identified by comparing fragment sizes to those from known leaf tissues (Figure 2, Table 2).

Table 1. Characteristics used to identify roots.

Species	Xylem structure (cross-section)	Fragrance	Root habit	Root epidermis
Fagus grandifolia	Compound rays in xylem form white star	None.	Side branches sparsely branched. May have swollen root.	Brittle, scrapes off in chunks.
Viburnum alnifolium	Large vessels in xylem. Remnants of cortex and epidermis.	Malodorous	Similar to maple, few if any clubbed root tips.	More fleshy, epidermis scrapes off in long soft sections.
Acer sp.	Numerous vessels and inconspicuous rays. Few cortical remnants	None	Root tips have a club-shaped appearance. Pinnately branched with a gradual decrease in size with root order.	Scrapes in patches, white color under bark.
Betula alleghaniensis	Large vessels and inconspicuous rays, similar to maple	Wintergreen	Roots are oscillate, root order doesn't correspond with branch diameter, all skinny.	Not brittle, reddish beneath bark with white underneath reddish layer.

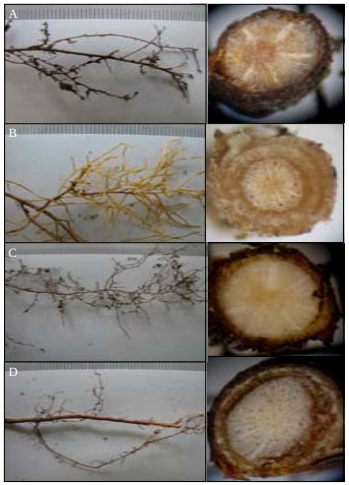

Figure 1. Gross morphology as indicated by Root Habit (Left) and xylem structure (Right) of *Fagus grandifolia* (A), *Viburnum alnifolium* (B), *Acer saccharum* (C), and *Betula alleghaniensis*: (D).

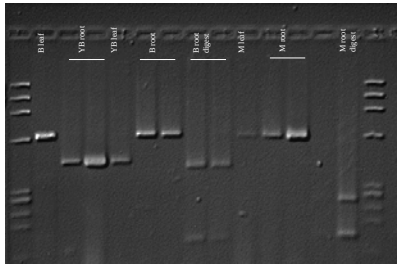

Figure 2. PCR products and *taq*I digests of the plastid *trn*L intron from roots and from leaf tissues of known species. B = American beech, YB = yellow birch, and M = maple.

Table 2. Fragment sizes (base pairs) of *trn* intron PCR product and restriction digests with *taq*I. Digests were not completed for unknown species (ND).

Species	*trn*L intron	*taq*I fragments (>100)
American beech	655	420, 145
Yellow birch	455	455
Sugar maple	635	170,145,110
Red maple	635	255,160
Unknown 1	550	ND
Unknown 2	615	ND

Results

Species Identification

The vast majority (i.e., 86% of the total root biomass at Bartlett; 78% at Hubbard Brook) of the sorted roots were identified to species.

Sugar maple roots were dominant at both sites, with beech second and yellow birch third in total root mass < 5 mm (Table 3); this corresponded to aboveground inventories. The two sites differed somewhat in species composition of the roots, with sugar maple more dominant at Bartlett C9 than at the Hubbard Brook Wedge (Figure 3, Table 3). Viburnum (*Viburnum alnifolium*) was an accessory species at the Bartlett site, comprising 4% of the total root biomass of 2180 ± 298 g/m2. At the Hubbard Brook site, viburnum was 2.2% and ash (*Fraxinus americana*) was 1.8% of the root biomass of 2675 ± 265 g/m^2. Roots of other species were not identified.

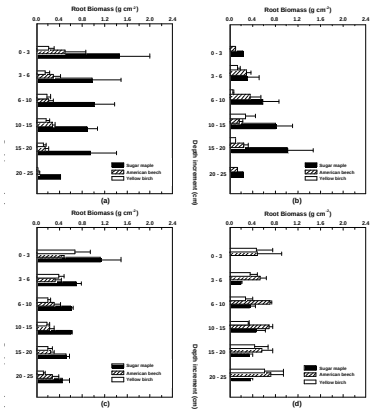

Figure 3. Root biomass distribution with depth of three co-dominant tree species. Root densities were estimated for 3-cm depth increments. Bartlett Experimental Forest (a) 0-2 mm roots, (b) 2-5 mm roots; at Hubbard Brook (c) 0-2 mm roots, (d) 2-5 mm roots.

Table 3. Mass of roots of three dominant species in two northern hardwood stands. Means are followed by standard errors (n = 3 plots). Root diameter distribution in indicated by the root mass in the finer diameter class (0-2 mm) as a percentage of the total root mass < 5 mm, for each species

Site	Species	Root Diameter (mm) 0-2	2-5	Fine Root Proportion (%)
C9	Sugar maple	880(200)	485(146)	67(5)
	American beech	216(82)	168(46)	66(8)
	Yellow birch	114(37)	57(21)	79(7)
HB	Sugar Maple	681(140)	214(65)	78 (3)
	American Beech	235(59)	386(71)	46(8)
	Yellow birch	269(62)	291(70)	64(8)

Molecular Genetic Testing

Visual identification of roots was verified by molecular genetic identification for 89% of maple roots, 87% of beech roots, and 71% of yellow birch roots. One root (of 18 tested) that was visually identified as sugar maple was identified as yellow birch by molecular methods. The *trn*L intron fragment sizes did not match maple, beech, or yellow birch sizes in any of the other roots that were not similarly identified by molecular and visual methods. Hence, we found very little molecular evidence that the visual method was unsuccessful distinguishing among yellow birch, maple, and beech. Sequencing of the *trn*L intron will be used to identify the unknown roots.

Species Distribution

Species differed in the distribution of root mass by diameter class (Table 3). Sugar maple had more root mass in the 0-2 mm class than in the 2-5 mm class at both sites, while at the Hubbard Brook Wedge, both beech and yellow birch had more biomass in the larger size class.

We studied the distribution of roots with depth by fitting curves of the form $Y = 1 - \beta d$ describing cumulative root fraction (Y) as a function of depth (d). Yellow birch roots declined more steeply with depth than those of beech or sugar maple (Figure 3a). There was no significant difference, however, in these curves ($P = 0.55$). There was a significant difference in the distribution of roots by size class, with fine roots more concentrated near the soil surface (Figures 3 and 4b) ($P = 0.05$), as expected. This pattern was common across species; there was not a significant interaction of diameter class and species on β. The two sites also differed significantly in rooting depth, with roots at Hubbard Brook distributed more deeply (Figure 4c).

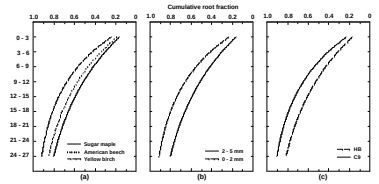

Figure 4. Curves fitted to describe variation with depth in root mass by (a) species, (b) diameter class, and (c) site. The curves are of the form $Y = 1 - \beta d$, describing cumulative root fraction (Y) by depth (d).

Implications

Morphological traits were sufficient to identify most of the collected roots accurately.

The visual sorting process was not without error; genetic validation of species identification is an important step.

The dominant northern hardwood species are not very distinct in their rooting depth, unlike coexisting species in some other ecotypes.

Literature Cited

Brunner, I., Brodbeck, S., Buchler, U., and Sperisen, C. 2001. Molecular identification of fine roots of trees from the Alps: reliable and fast DNA extraction and PCR–RFLP analyses of plastid DNA. Mol.Ecol.10: 2079–2087

Jackson, R.B., L.A. Moore, W.A. Hoffmann, W.T. Pockman, and C.R. Linder. 1999. Ecosystem rooting depth determined with caves and DNA. Proc. Natl. Acad. Sci. U.S.A. . 96: 11387-11392.

Nicoll, B.C., Gardiner, B.A., Rayner, B., and Peace, A.J. 2006. Anchorage of coniferous trees in relation to species, soil type, and rooting depth. Can. J. For. Res. 36: 1871-1883.

Taberlet, P., Gielly, L., Pautou, G., and Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17: 1105–1109.ation to species, soil type, and rooting depth. Can. J. For. Res. 36: 1871-1883.