

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

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Abstract: Forest canopies are often stratified by species; little is known about the depth distribution of tree roots in mixed stands because they are not readily identified by species. We used diagnostic characteristics of wood anatomy and gross morphology to distinguish roots by species and applied these methods to test for differences in the rooting depth of sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.) in two northern hardwood forests. We also distinguished hobblebush (*Viburnum lantanoides* Michx.) and white ash (*Fraxinus americana* L.) roots. Analysis of plastid DNA fragment lengths confirmed that 90% of the roots were correctly identified. The vertical distribution of fine roots of these species differed by 2–4 cm in the median root depth ($P = 0.03$). There was a significant difference in the distribution of roots by size class, with fine roots (0–2 mm) being more concentrated near the soil surface than coarser roots (2–5 mm; $P = 0.004$). The two sites differed by <2 cm in median rooting depths ($P = 0.02$). The visual identification of roots for the main tree species in the northern hardwood forest allows species-specific questions to be posed for belowground processes.

Résumé : La stratification des essences est fréquente dans le couvert forestier. La distribution verticale des racines des arbres est peu connue dans les peuplements mélangés parce que l'identification des racines n'est pas facile. Nous avons utilisés les caractéristiques diagnostiques de l'anatomie et de la morphologie grossière du bois pour différencier les racines selon l'espèce et nous avons appliqués ces méthodes pour tester les différences de profondeur d'enracinement de l'éclaircie à sucre (*Acer saccharum* Marsh.), du hêtre d'Amérique (*Fagus grandifolia* Ehrh.) et du bouleau jaune (*Betula alleghaniensis* Britt.) dans deux forêts feuillues nordiques. Nous avons aussi distingué les racines de la viorne bois-d'original (*Viburnum lantanoides* Michx.) et du frêne blanc (*Fraxinus americana* L.). L'analyse de la longueur des fragments d'ADN des plastides a confirmé que 90 % des racines ont été correctement identifiées. La distribution verticale des racines fines étaient significativement différente selon l'espèce ($P = 0,03$) et la différence variait de deux à quatre cm en comparant la profondeur médiane des racines. Il y avait une différence significative dans la distribution des racines selon la classe de dimension : les racines fines (0–2 mm) étaient plus concentrées près de la surface du sol que les plus grosses racines (2–5 mm) ($P = 0,004$). La profondeur médiane d'enracinement différait de moins de deux cm entre les deux stations ($P = 0,02$). L'identification visuelle des racines des principales espèces d'arbres dans la forêt feuillue nordique permet de formuler des questions propres à chaque espèce au sujet des processus souterrains.

[Traduit par la Rédaction]

Introduction

Vertical stratification of foliage in the canopy of mixed forests has been associated with interspecific differences in tree growth rates, successional status, and physiological traits, such as shade tolerance (Oliver and Larson 1996). By comparison, we know relatively little about how tree species distribute biomass belowground, although spatial differences in root distributions might be expected to promote species coexistence (Veresoglou and Fitter 1984). Differences in rooting depth have been well studied in conifer plantations, where, for example, shallow-rooted species are more prone to windthrow (Nicoll et al. 2006). However, because of dif-

ferences in distinguishing fine roots of different tree species, few studies have compared depth distributions in mixed stands (Schenk et al. 1999).

Some degree of root depth stratification might be expected in mixed stands because of interspecific differences in age or size structure. In a Scots pine (*Pinus sylvestris* L.) forest in France, understory Norway spruce (*Picea abies* (L.) Karst.) were more superficially rooted (Mikola et al. 1966). Vertical segregation of roots by species might also result from differences in root system architecture or physiological traits in relation to the soil environment. Loblolly pine (*Pinus taeda* L.) was more deeply rooted than red maple (*Acer rubrum* L.) or black locust (*Robinia pseudoacacia* L.)

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in mixed-species stands in the Virginia Piedmont (Frederickson and Zedaker 1995). Western red cedar (*Thuja plicata* Donn ex D. Don), which is flood tolerant, was more deeply rooted than western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) in the very wet climate of British Columbia (Bennett et al. 2002). In northern hardwoods, where species are more similar in their habitat requirements and sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.; hereinafter referred to as beech), and yellow birch (*Betula alleghaniensis* Britt.) are commonly codominant, it is not clear what degree of vertical stratification of fine roots should be expected. Notably, sugar maple is an arbuscular mycorrhizal species and the ectomycorrhizae of yellow birch and beech are quite distinct taxonomically (Newton and Haigh 1998).

The paucity of observations of spatial segregation of fine roots by species reflects the difficulty of distinguishing these plant tissues, whose morphological characteristics are often very similar (Pregitzer et al. 2000). However, in forests with only a few dominant tree species, subtle but significant differences in root anatomy, branching patterns, or gross morphology can be used to sort fine roots by species. An alternative approach is to use genetic techniques (Jackson et al. 1999), but costs would be prohibitive for large sample sizes.

We used a combination of wood anatomy and gross morphology to separate roots of five woody species common in the northern hardwood forest of the northeastern USA. We applied these methods to test for differences in the rooting depth of sugar maple, beech, and yellow birch in two northern hardwood sites in New Hampshire. We also validated the visual identification of roots by species using molecular genetic techniques.

Methods

Study sites

We collected roots by depth in two mature northern hardwood ecosystems in the White Mountains of New Hampshire in June 2005. Overstory vegetation in both forests was dominated by sugar maple, beech, and yellow birch, as characterized by measuring diameters of all trees ≥10 cm in diameter at breast height (Table 1).

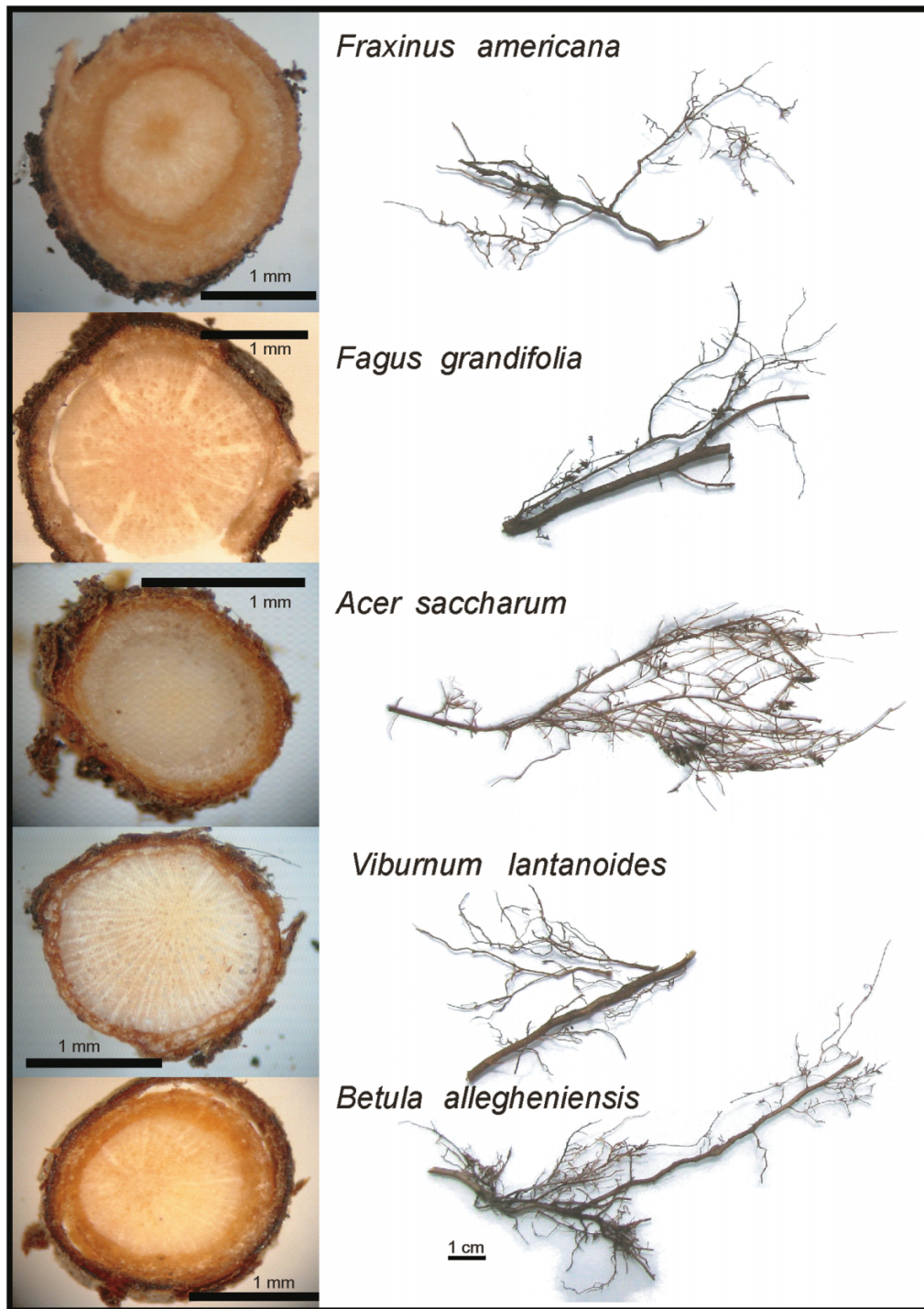
At the Bartlett Experimental Forest, we used a 115-year-old stand (C9) at 440 m a.s.l. in which root biomass with depth had been measured 1 year earlier (Yanai et al. 2006; Park et al. 2007). Soils were coarse-loamy, mixed, frigid, Typic Haplorthods. The stand had three 2500 m² measurement plots, each of which had one soil pit from which roots were previously measured. In June 2005, we collected roots from two small pits (15 cm × 15 cm) in the same 100 m² subplots that had contained the soil pits, for a total of six pits.

At the Hubbard Brook Experimental Forest we sampled in three permanent plots (500 m²) located in the lower hardwood zone (520–580 m a.s.l.) near the reference watershed, which were approximately 105 years old (Fahey et al. 2005). Soils are Typic Haplorthods developed from unsorted basal tills with a 4–8 cm thick organic horizon. Three 15 cm × 15 cm pits were excavated in each plot.

Table 1. Morphological characteristics used to identify roots to the species or genus level.

Species	Xylem structure (in cross-section)	Fragrance	Root habit	Root epidermis
American beech	Compound rays in xylem form a white star; simple rays and small vessels also present	None	Side branches sparsely branched; may have swollen root tips	Brittle; scrapes off in chunks exposing stark white inner layer
Sugar maple, red maple, and striped maple	Diffuse porous, with uniformly distributed vessels and inconspicuous simple rays	None	Root tips have a club-shaped appearance; pinnately branched with a gradual decrease in size with root order	Scrapes similar to beech, exposing stark white inner layer
Yellow birch	Diffuse porous, with uniformly distributed vessels and inconspicuous simple rays, similar to maple	Wintergreen	Roots are oscillate; root order doesn't correspond to branch diameter; all skinny	Not brittle or easily scrapings; elastic
Hobblebush	Large vessels in xylem; remnants of cortex and epidermis	Malodorous	Similar to maple; few if any clubbed root tips	More fleshy and yellowish; epidermis scrapes off in long soft sections
White ash	Ring porous with alternating large and small vessels; rays are simple	None	Irregularly pinnate branching; little difference in size of 1st, 2nd, and 3rd order roots	Strikingly yellow when fresh

Fig. 1. Gross morphological differences as depicted by xylem structure (right) and root habit (left) of *Fraxinus americana*, *Fagus grandifolia*, *Acer saccharum*, *Viburnum lantanoides*, and *Betula alleghaniensis*.



Root collection and processing

After removing the Oi litter, we collected the soil and roots from each 15 cm × 15 cm pit to a maximum depth of approximately 25 cm. Samples were collected in five depth increments in each pit. It was not practical to sample equal depth increments in all the pits because of variation in the depths of coarse fragments and large woody roots that impeded soil collection. Instead, we measured the actual thickness of each depth increment for each pit. Generally, this

was 2–4 cm for shallow depths and 5–7 cm for deeper samples.

Samples from each pit and depth layer were refrigerated until they could be processed. Roots were separated from soil by hand and washed thoroughly against a 4 mm mesh screen. We then sorted the roots by species according to wood anatomy and gross morphology (Table 1; Fig. 1). Some root material was not classified by species, either because the root fragments were too small to have the diagnos-

tic xylem characteristics or exterior morphological traits, or because the sorters were not confident of the classification. Each species group was subdivided into two size classes, 0–2 mm and 2–5 mm; unidentified roots were not separated by size class. Dead roots and roots >5 mm in diameter were not retained. All other roots were oven-dried and weighed.

Molecular confirmation of root identification

The species of root branches <2 mm in diameter were identified with molecular genetic methods using the procedure described by Brunner et al. (2001). We analyzed 2–5 root branches from the surface layer of each of the six pit samples at Bartlett. In some pits we were unable to amplify DNA from all surface layer root branches, so we used additional root branches from deeper layers for a total of 23–25 root branches per species. We used only the more intact root networks, to ensure adequate sample sizes. Root samples were ground in an amalgamator (Darby Dental, Akron, Ohio). DNA was extracted from approximately 30 mg of each sample using a standard alkaline lysis or chloroform extraction procedure modified with addition of polyvinylpyrrolidone, polyvinylpyrrolidone, betamercaptoethanol, and spermidine, to improve extraction efficiency and inhibitor removal (Brunner et al. 2001). The plastid *trnL* intron was amplified using primers c and d (Taberlet et al. 1991) and PCR products were digested with *taqI*. The *trnL* intron of some samples was also sequenced to verify digest patterns. We extracted DNA from sugar maple, yellow birch, and beech leaf tissues following the same procedures. Roots were identified by comparing fragment sizes with those from leaves of the same species.

We experienced difficulty PCR-amplifying the *trnL* intron of root and leaf tissues despite optimizing salt concentrations and annealing temperatures. We attribute this primarily to the effects of using oven-dried roots and leaves; no such problems were encountered when we PCR-amplified fresh leaf tissue for comparison.

Data analysis

We analyzed differences in rooting depth by species using the soil depth above which 50% of the root mass was found. Because our measurements were not continuous with depth, we first characterized the depth distribution of roots within each site, species, and size class by distributing the observed root mass evenly within each depth increment. We then identified the depth of the median root to the nearest centimetre. The experimental unit was the pit ($n = 15$, with 6 at Bartlett and 9 at Hubbard Brook). Differences in the median root depth were evaluated with analysis of variance (ANOVA), using site, species, and diameter as main effects and including their interactions. We also analyzed the effect of species and diameter within site.

In a second approach to distinguishing rooting depth by species, we fitted an exponential curve to our observations of cumulative root mass as a function of depth. Again, because our measurements were not continuous, we used the mass of roots in 3 cm depth increments derived by evenly distributing the observed root mass over the sampled depths. The cumulative fraction of roots below each 3 cm depth increment (Y) was described as a function of depth (d), using the function $Y = 1 - \beta^d$ for each pit. This approach results in

Table 2. Overstory basal area and mass of roots by species in two northern hardwood stands at Bartlett and Hubbard Brook.

Species	Tree basal area (m ² ·ha ⁻¹)	Root biomass (g·m ⁻²)	
		0–2 mm	2–5 mm
Bartlett			
Sugar maple	17.0 (3.1)	880 (200)	485 (146)
Beech	10.2 (1.5)	216 (82)	168 (46)
Yellow birch	7.8 (1.5)	114 (37)	57 (21)
Hobblebush	na	12 (7)	13 (na)
Other	0.2 (0.1)	313 (80)	
Hubbard Brook			
Sugar maple	16.0 (0.6)	681 (140)	214 (65)
Beech	9.9 (2.0)	235 (59)	386 (71)
Yellow birch	9.0 (1.3)	269 (62)	291 (70)
Hobblebush	na	68 (29)	0
White ash	2.3 (2.3)	67 (31)	30 (na)
Other	0	598 (77)	

Note: Values are means with standard errors in parentheses ($n = 3$ plots). Standard errors were not available (na) for means based on only one observation. Hobblebush is not measured by basal area of trees. Unidentified roots were not divided by size classes, so the mass of “Other” includes 0–2 mm and 2–5 mm size classes.

biased estimates of the coefficient β because we describe the depth of our samples assuming linear, not exponential, distributions (Cook and Kelliher 2006), but this bias applies consistently across all pits and species. The same problem would apply to any nonlinear model; we selected this model because it has only one parameter. We averaged β across pits (two pits per plot at Bartlett and three pits per plot at Hubbard Brook) within plots (three per stand) and used ANOVA to analyze β as a function of species, size class, and site. Duncan’s multiple range test was used to compare values of β as a function of species, size class, and site ($\alpha = 0.05$).

Results

Identification of roots by species

Most of the roots were sorted into one of the five taxa using the characteristics described in Table 1 and illustrated in Fig. 1. At Bartlett, 86% of the total root biomass in the top 25 cm of soil was identified by species. At Hubbard Brook, 78% was identified. Sugar maple was the dominant root species at both sites, with beech second, and yellow birch third in total root mass < 5 mm, as expected based on the aboveground inventories (Table 2). The two sites differed somewhat in species composition of the roots, with sugar maple being more dominant at Bartlett than at Hubbard Brook (Table 2; Fig. 2). Hobblebush (*Viburnum lantanoides* Michx.) was an accessory species at the Bartlett site, comprising 4% of the total root biomass of 2180 ± 298 g·m⁻². At the Hubbard Brook site, hobblebush was 2.2% and white ash (*Fraxinus americana* L.) was 1.8% of the root biomass of 2675 ± 265 g·m⁻². Roots of other species were not identified by our methods (Table 1).

Visual identification of roots was verified by molecular genetic identification for 96% of beech, 92% of yellow birch, and 83% of sugar maple roots (Table 3). Of the roots that were visually identified as sugar maple, one was identified as yellow birch by molecular methods, two were shown

Fig. 2. Root biomass distribution with depth of three codominant tree species. Root densities were assigned to uniform depth increments by numerically distributing roots within the measured depth increments, which differed across pits.

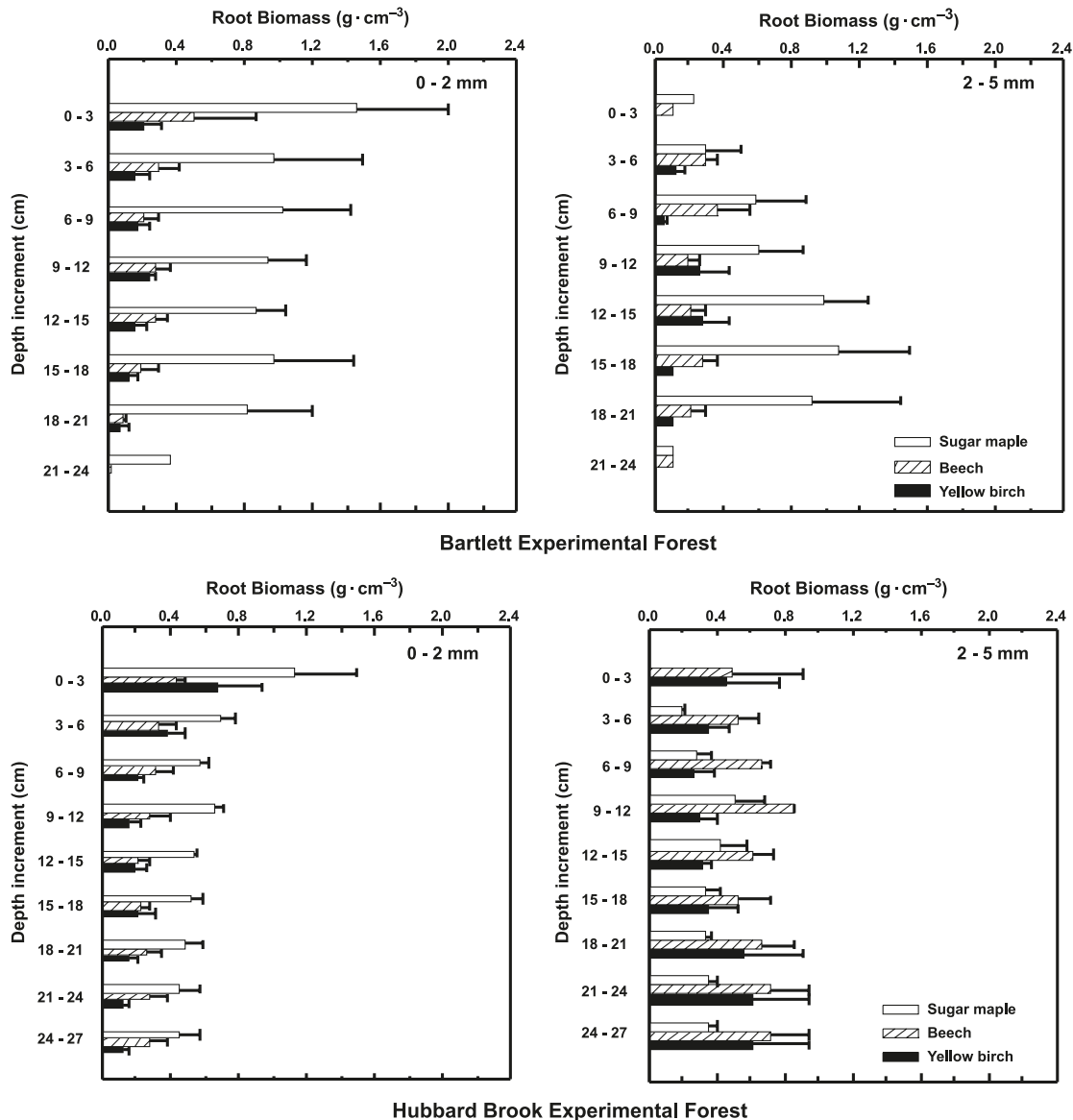


Table 3. Fragment sizes (base pairs) of *trnL* intron PCR product and restriction digests with *taqI* and results of molecular genetic confirmation of visual identifications.

Tree species	<i>trnL</i> intron	<i>taqI</i> fragments (>100)	No. of visually identified samples tested	No. of visual identifications confirmed	Identity of those not confirmed
Beech	582	405, 155	24	23	Sugar maple
Yellow birch	444	405	25	23	Hobblebush
Sugar maple	577	253, 154, 149	23	19	2 beech, 1 yellow birch, 1 hobblebush
Hobblebush	579	240, 219	0		

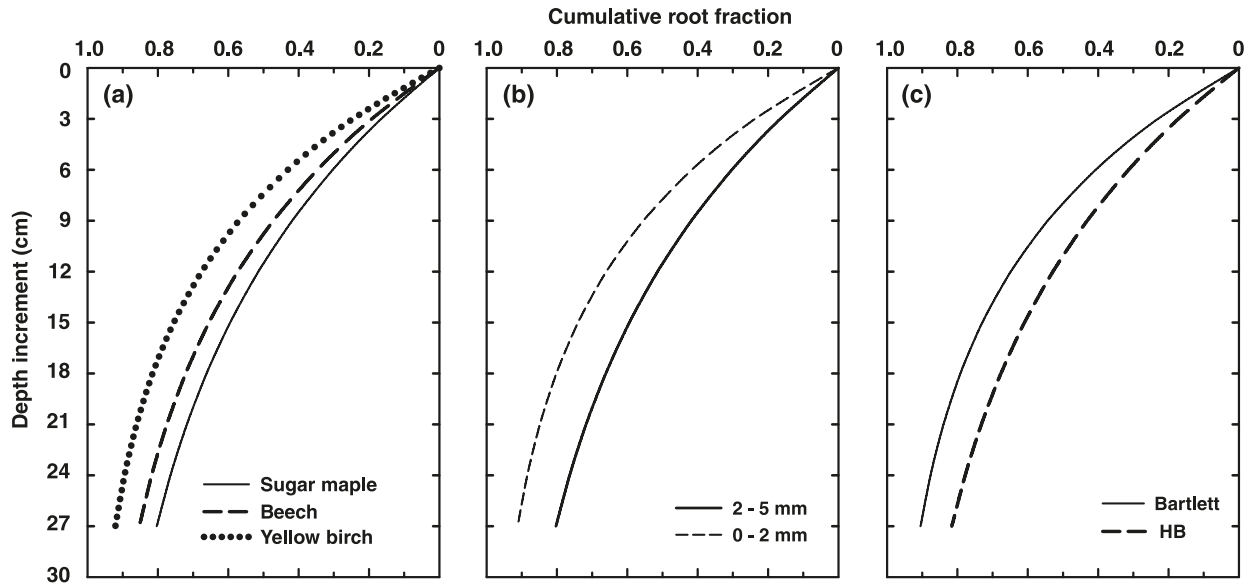
to be beech, and one was shown to be hobblebush. One visually identified beech root was shown to be sugar maple and two visually identified yellow birch roots were shown to be hobblebush. Note that these two hobblebush samples came from the same pit and may represent a single error. Roots were visually identified by species before dividing the roots into size classes; thus, some of the 0–2 mm root branches

that we selected for verification may have come from a single larger root.

Distribution of roots by size, depth, and species

Species differed in the distribution of root mass by size class (Table 2). Sugar maple had more root mass in the 0–2 mm than in the 2–5 mm size class at both sites, by a fac-

Fig. 3. Curves fitted to describe variation with depth in root mass by species (a), size class (b), and site (c). Curves are of the form $Y = 1 - \beta^d$, describing cumulative root fraction (Y) by depth (d). Bartlett, Bartlett Experimental Forest site; HB, Hubbard Brook Experimental Forest site.



tor of two or three. Beech and yellow birch tended to have coarser roots; both had slightly more biomass in the larger size class at Hubbard Brook, but less in this size class at Bartlett.

The ANOVA of the median rooting depths did not reveal significant differences among the three dominant tree species in the depth distribution of fine roots (<2 mm) in the top 25 cm of the profile ($P = 0.59$). Across all stands and sites, the median root of yellow birch was at 9.7 cm depth, beech was at 10.0 cm, and sugar maple was at 12.0 cm. Within sites, median fine root depths ranged from 6.3 ± 1.5 cm for beech to 9.2 ± 0.7 cm for yellow birch at Bartlett ($P = 0.15$), and at Hubbard Brook values ranged from 8.5 ± 2.1 cm for yellow birch to 9.8 ± 0.9 cm for sugar maple ($P = 0.86$).

We also analyzed the distribution of roots with depth by fitting curves describing cumulative root fraction (Y) as a function of depth (d). Yellow birch roots declined more steeply with depth than sugar maple ($P = 0.03$); beech was intermediate in depth distribution and not statistically distinguishable from either yellow birch or sugar maple (Fig. 3a). The β function (Fig. 3) imperfectly described our observed root distributions with depth (Fig. 2), which is not surprising, considering it has only one parameter. The median root depths corresponding to the fitted values of β were shallower than those estimated directly. Specifically, $\ln(0.5)/\ln(\beta)$ was 5.9 cm for yellow birch, 8.3 cm for beech, and 10.0 cm for sugar maple.

Differences in rooting depth with diameter were more pronounced than differences with species. The median depth of roots in the 0–2 mm size class was 8.7 ± 1.4 cm, whereas the median depth of roots 2–5 cm in diameter was 13.0 ± 1.9 cm. The exponential model also showed that fine roots were more concentrated near the soil surface (Figs. 2 and 3b) ($P = 0.004$), as previously reported in this forest type (Yanai et al. 2006; Park et al. 2007). This pattern was com-

mon across species; there was not a significant interaction of size class and species on β or on the median root depth.

Finally, the two sites differed significantly in rooting depth based on comparisons of β ($P = 0.01$), with roots at Hubbard Brook distributed more deeply (Fig. 3c). The depth of the median root averaged 11.0 ± 1.9 cm at Hubbard Brook and 9.7 ± 1.4 cm at Bartlett.

Discussion

Morphological traits were sufficient to identify most of the roots we collected in these two northern hardwood sites. At Bartlett, we were unable to identify 14% of the root mass collected. This plot was heavily dominated by sugar maple, beech, and yellow birch (99% of basal area, Table 2), but white birch (*Betula papyrifera* Marsh.) and striped maple (*Acer pensylvanicum* L.) were also present. At Hubbard Brook, 100% of the basal area was in species whose roots we could recognize (including 6% white ash) (Table 2), but we were unable to identify 22% of the root mass. Some of the “unknown” roots were fragments too small to be identified; others simply stumped our root sorters. Both training of the sorters and the method of root collection will influence the success of root separation using our morphological criteria; we collected roots from sizable (15 cm \times 15 cm) pits, so as to produce fewer small root fragments than would be the case for core samples.

Molecular genetic analysis confirmed the visual identification of beech and yellow birch roots in most cases; visual identification of sugar maple was most problematic. We expected high success in beech identification because of the distinctive xylem anatomy relative to the other species (Table 1). We were also successful in distinguishing between sugar maple and yellow birch (in 49 of 50 roots), despite the similarity of gross morphological traits between these two species. Confusion with hobblebush occurred for both yellow birch and sugar maple. Given the relatively low

abundance of hobblebush roots (Table 2), we would expect these errors to be infrequent but perhaps patchily distributed. Since hobblebush is more superficially rooted than the tree species (data not shown), we would also expect this error rate to decrease with soil depth.

The differences we found in fine root depth distributions among the three dominant northern hardwood species in these two mature forests were modest (2–4 cm in median root depth) compared with the differences reported for more disparate species mixtures. Using the root densities and soil layer thicknesses reported by other researchers and applying the approach we adopted of distributing root densities evenly within each layer, we estimated the median rooting depth of western red cedar to be approximately 20 cm deeper than that of western hemlock in British Columbia (Bennett et al. 2002). Loblolly pine was 10 cm more deeply rooted than red maple but only 3 cm more deeply rooted than black locust in the Virginia Piedmont (Frederickson and Zedaker 1995). In a mixed spruce and pine forest in France, the median depth of pine roots was only 3 cm greater than spruce (Mikola et al. 1966). Data from mixed beech and pine in Finland (McQueen 1968) show approximately 2 cm greater median depth for pine, although the author, seeing fewer pine roots at depth, concluded that pine was more superficially rooted.

Although none of these studies reported the median root depth, some of them reported the percentage of roots of each species above a common depth. In British Columbia, hemlock and cedar had 93% and 96% of the fine root biomass located in the forest floor (Bennett et al. 2002). In an oak–beech forest in Germany, 35% of oak and 51% of beech fine root biomass was located in the uppermost organic horizon of the profile (Büttner and Leuschner 1994). The proportion of mycorrhizal root tips in the humus layer was 84% for spruce and 41% for pine in mixture in France (Mikola et al. 1966). For our site at Bartlett, where the forest floor averages 7.7 cm (Park et al. 2007), we can calculate that an average of 53% of sugar maple, 60% of beech, and 67% of yellow birch fine roots should occur in the forest floor, using the fitted β by species for that site. At the Hubbard Brook site, where the forest floor averages 4.5 cm (Fahey and Hughes 1994), we estimate that 35% of sugar maple, 32% of beech, and 46% of yellow birch fine roots occur above that depth. Collection of roots by horizon showed 43% to be in the forest floor (Fahey and Hughes 1994). Both forest floor depth and rooting depth are spatially quite variable in these ecosystems. Some of the variation we observed in depth distributions of roots might be accounted for by collecting roots from genetic soil horizons.

The calculation of median root depth or the proportion of roots above a given depth depends on the depth to which roots are sampled. Our study collected roots to 25 cm; some studies have sampled to approximately 60 cm (Büttner and Leuschner 1994; Bennett et al. 2002), but others have been similar to ours (McQueen 1968; Frederickson and Zedaker 1995), and Mikola et al. (1966) sampled to only 8 cm. It would be difficult to estimate the proportion of roots not sampled, except that we had excavated quantitative soil pits into the C horizon in our plots at Bartlett as part of an earlier study (Park et al. 2007); 70% of fine root biomass (<2 mm) was in the upper 25 cm of the profile. Species dif-

ferences in the deployment of roots below this depth could potentially have important implications for water and nutrient uptake.

It is also important to note that roots may serve different functions at different depths. For example, the proportion of nutrients taken up from the forest floor may not be the same as the proportion of roots occurring there (Yanai 1992), owing to differences in nutrient availability with depth. Different species may also derive somewhat different benefits from roots in different soil layers. The distribution of absorbing mycorrhizal hyphae may be more important than, and imperfectly related to, the distribution of fine roots. Identifying roots by species is an important step towards better understanding the nature and intensity of interspecific competition belowground.

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