# Fine and coarse root parameters from mature black spruce displaying genetic $\times$ soil moisture interaction in growth

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**Abstract:** Fine and coarse root biomass, C, and N mass parameters were assessed by root size and soil depths from soil cores in plots of 32-year-old black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) from four full-sib families studied previously for drought tolerance and differential productivity on a dry and wet site. All fine and coarse root size categories had greater root biomass on the dry than on the wet site. Most of the site differences resided in 0-20 cm soil depth. The wet site had greater root N concentration than the dry site, despite the same soil N; thus, virtually no differences were observed in total fine and coarse root N mass between sites. Root N concentration declined with increases in both soil depth and root size. Fine roots (<2 mm) accounted for 73% and 38% of the total fine and coarse N and C mass, respectively. The dry site had lower needle mass and more fine root mass than the wet site, demonstrating an adaptation to moisture stress change through the rebalancing of resource-obtaining organs. Drought-tolerant families had the same quantity of fine roots as drought-intolerant families but were able to support more foliage and aboveground mass per unit fine root mass than intolerant families.

**Résumé :** La biomasse des racines fines et celle des grosses racines, ainsi que les paramètres de masse du C et du N, ont été évalués en fonction de la dimension des racines et de la profondeur dans le sol à partir de carottes de sol prélevées dans des places échantillons où croissent des épinettes noires (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.). Les arbres étaient âgés de 32 ans et représentaient quatre descendances biparentales étudiées précédemment pour leur tolérance à la sécheresse et leurs différences de productivité selon qu'ils croissaient dans une station sèche ou humide. La plupart des différences entre les stations ont été observées dans les premiers 20 cm de sol. La concentration de N dans les racines était plus élevée dans la station humide même si la concentration de N dans le sol était la même dans les deux stations. Par conséquent, pratiquement aucune différence entre les stations n'a été observée dans la masse totale de N de l'ensemble des racines. Les racines fines représentaient respectivement 73% et 38% de la masse totale de N et C dans l'ensemble des racines. La masse d'aiguilles était plus faible et la masse de racines fines plus élevée dans la station sèche que dans la station humide, ce qui implique une adaptation au changement de stress hydrique via le rééquilibrage des organes qui consomment des ressources. Les descendances tolérantes à la sécheresse avaient la même quantité de racines fines que les descendances intolérantes mais elles pouvaient supporter plus de feuillage et de masse aérienne par unité de masse de racines fines que les descendances intolérantes.

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# Introduction

Water availability is a predominant factor in determining the geographic distribution of vegetation, and water stress has long been known to decrease plant growth and gas exchange and change water relations (Kramer 1983). Trees depend on fine roots throughout the soil profile for water and mineral uptake. Fine roots can represent 10%–20% of the total amount of tree root C mass (Jackson et al. 1997; Brunner and Godbold 2007). In studies that examine aboveground growth of mature trees, roots are often ignored or estimated using allometric equations derived from other species. Above- and belowground development in all plants has been linked theoretically with a biophysical model of resource transport (West et al. 1999). Thus, the quantity of fine roots and their distribution in the soil are likely important factors impacting growth and the degree of drought tolerance. Although most moisture stress studies use seedlings, a small number have used mature trees (Comeau and Kimmins 1989; Maier et al. 2004).

To explore the relationship among fine roots, their distribution, and aboveground variation in productivity and drought tolerance, we used one of the oldest existing black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) genetic studies planted on multiple sites (Morgenstern 1974). Spruce

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**Table 1.** Parentage of the four full-sib families (7122, 7125, 7143, and 7146) of black spruce (*Picea mariana*).

Male	Female 59	Female 63		
52	7122	7125		
62	7143	7146		

**Fig. 1.** Height of drought-tolerant and -intolerant black spruce (*Picea mariana*) families (mean and SD) from two sites located at the Petawawa Research Forest, Ontario. Drought-tolerant families are progeny of female 59 (solid symbols) and drought-intolerant families are progeny from female 63 (open symbols). Dry and wet sites refer to sites 2 and 3, respectively.



is the major component in many Canadian forest ecosystems and by far the most important tree genus for the Canadian forest industry, accounting for 33%-40% of Canadian standing inventory (Canadian Council of Forest Ministers 1999). Spruce accounts for 55% of Canadian reforestation activities, with black spruce alone accounting for 35% of the Canadian reforestation activities, most of which use seed from tree improvement programs (Morgenstern and Wang 2001). Genetic studies planted on multiple sites allow biological variation to be more accurately partitioned, and thus, environmental effects as well as genetic and genetic  $\times$  environmental effects can be more accurately ascertained. A standard quantitative genetic analysis of a (F1)  $7 \times 7$  black spruce diallel planted in 1973 on three sites at the Petawawa Research Forest indicated important genetic, environment, and genetic  $\times$  environment interaction effects in aboveground growth characteristics (Boyle 1987; Major and Johnsen 1996). Four families  $(2 \times 2)$ breeding structure) that exhibited this growth variation between two sites were selected for further examination (Table 1). One female parent (59) produced progeny that displayed relatively high productivity on both sites, whereas the other female parent (63) produced progeny that had high growth rates on one site but not on another, less productive site (Fig. 1). Multiple lines of evidence offer strong support that site variation was largely due to differences in soil moisture availability (Johnsen and Major 1995; Major and Johnsen 1996, 2001). On measurement days just after rainfall, physiological parameters, such as predawn xylem water potential, daytime xylem water potential, net photosynthesis ( $P_n$ ), and needle conductance ( $g_{wv}$ ) were similar between sites. Collected on the same dates, under drying conditions, the same physiological parameters were significantly lower on the dry than on the wet site. Soil moisture site differences were confirmed using foliar stable C isotope discrimination (<sup>13</sup>C: <sup>12</sup>C) analysis (Flanagan and Johnsen 1995).

Also, under drying conditions, it was found that drought-tolerant families, the progeny of female 59, generated lower osmotic potential, greater turgor, greater  $P_n$ , and lower <sup>13</sup>C discrimination than drought-intolerant families, the progeny of female 63 (Major and Johnsen 1996, 1999, 2001; Johnsen et al. 1999). Furthermore, half the diallel (including selfs) were measured for <sup>13</sup>C:<sup>12</sup>C, which showed drought tolerance to be under strong genetic control, highly heritable (heritability coefficient, 0.54, the highest of all traits measured), and having a strong genetic correlation (-0.97) with growth (Johnsen et al. 1999). Thus, the families being assessed have well-documented differences in aboveground growth, and the mechanisms contributing to this variation are well understood. After an exhaustive literature search, we are not aware of any mature conifer species experiments examining root attributes of pedigreed drought-tolerant and -intolerant families, and consequently, information about the relationship to belowground allocation is lacking. Thus, the role of belowground variation of fine and coarse roots in contributing to the aboveground variation in mature tree family drought tolerance and moisture-driven site productivity differences is unknown. Also, there is some evidence to support a relationship/balance between total foliage and total fine root (<2 mm) mass, as both are the entry points in obtaining resources necessary for growth and both are relatively ephemeral (Helmisaari et al. 2007). Using total needle mass data from Major et al. (2012b), how might the total foliage to total fine root relationship vary with family drought tolerance and moisture-driven site productivity differences?

Our hypothesis was that drought-tolerant families would have greater fine and coarse roots than drought-intolerant families, particularly on the dry site. We also hypothesized that there would be greater root biomass on the dry site, deeper in the dry site soil profile than on the wet site. Another goal was to quantify fine and coarse root component parameters from mature black spruce plantations to contribute to our complete and detailed assessment of the total above- and belowground biomass, C, and N mass pools (Major et al. 2012a, 2012b). Thus, our objectives were to (1) quantify root size components of fine (<1 and 1-2 mm) and coarse (2-5 and >5 mm) root biomass by soil depth, (2) determine root C and N concentrations by root size and soil depth and calculate the C and N mass per hectare, and (3) examine genetic and site variation in fine and coarse root parameters and their relationship to drought tolerance, site productivity, and total needle mass.

# Materials and methods

#### Plant material and location

A complete  $7 \times 7$  diallel cross black spruce experiment was started at the Petawawa Research Forest (46°N, 77°30′W), Ontario, in 1970 (Morgenstern 1974; Boyle 1987). The seven parental trees used for the diallel cross were from a plantation at the Petawawa Research Forest, but the exact origin of the trees is unknown, except that they were grown from seed collected in the Lake Simcoe–Rideau region in Ontario (Morgenstern 1974). The diallel seeds were germinated 21 March 1971, and seedlings were grown for 2 years in a greenhouse before being planted at three field sites at the Petawawa Research Forest in 1973. At each site, trees from a full-sib family were planted in either nine-tree (site 3) or 16-tree (site 2) square (1.83 m  $\times$  1.83 m) spacing. At site 3, there were three replicate blocks, and at site 2, there were four replicate blocks. Family plots were randomized within each block at each site.

As previously reported (Johnsen and Major 1995, 1999; Major and Johnsen 1996, 1999, 2001) and presented in the introduction, the primary difference between sites studied was water availability. Site 2 will be referred to as the "dry" site and site 3 as the "wet" site. The two sites are located within 5 km of each other and are expected to receive similar rainfall; the dry site has a deep sandy substrate and the wet site has a hard pan layer about 30-40 cm below the surface that restricts drainage (S. Brown and R. Ponce-Hernandez, unpublished). A subset of four full-sib families that displayed differences in drought tolerance were used, comprising a two female parent  $\times$  two male parent breeding structure (Table 1). Progeny of female 59 (families 7122 and 7143) are referred as "drought-tolerant" families and progeny of female 63 (families 7125 and 7146) are referred to as "drought-intolerant" families. We note that labels of "wet" and "dry" sites, as well as "tolerant" and "intolerant" families, are relative to this particular study and do not necessarily reflect where these sites, or families, exactly fit along the larger scale environmental and genetic variation spectra within black spruce as a species.

#### Methodology

For the dry site, soil core samples (10.16 cm diameter) were taken from across the four blocks, four families, and three samples from within each plot to a depth of 50 cm by 10 cm depths for a total of 240 subsamples. For the wet site, soil core samples were taken from across the three blocks, four families, and two samples from within each plot to a depth of 50 cm by 10 cm depths for a total of 120 subsamples. Samples were taken from the center of each plot but at least 0.5 m from any tree in late June and early July 2003. Seasonality in fine root development has been documented for northern forest ecosystems, with a tendency for root growth to peak in late spring and early summer followed by a rapid decrease in the fall (Grier et al. 1981; Hendrick and Pregitzer 1993). Thus, the fine roots would be at or near their seasonal maximum. Careful plot root excavations of nontarget families found that >99% of the roots sampled using the coring protocol described above were from their respective family plot trees (data not shown). At the time of sampling, basal area was approximately 36 m<sup>2</sup>·ha<sup>-1</sup>, density was 2900 trees ha-1, live crown length was approximately 50%, and leaf area index was approximately 5.0 (Major et al. 2012b).

The subsamples were sieved for roots and stones. Root samples were categorized first as dead or live, and then, live roots were categorized into size classes: <1, 1–2, 2–5, and >5 mm diameter. The samples were dried at 65 °C for 48 h

and then weighed. Stone mass was also determined and used to correct soil volume. Roots were ground and C and N concentrations were determined for all subsamples and root categories using an elemental analyzer (CNS-2000; LECO Corporation, St. Joseph, Michigan). The within-plot soil core subsamples were averaged together after all measurements were taken for analysis. Concentration and biomass data were used to scale estimates up to the stand level.

#### Statistical analyses

The ANOVA model for testing mass traits by root size class includes the following effects: site (S), soil depth (D), female (F), and male (M), all considered fixed. The ANOVA model used is as follows:

$$\begin{aligned} Y_{ijklm} &= \mu + \mathbf{S}_i + \mathbf{D}_j + \mathbf{F}_k + \mathbf{M}_l + \mathbf{SD}_{ij} + \mathbf{SF}_{ik} \\ &+ \mathbf{SM}_{il} + \mathbf{DF}_{jk} + \mathbf{DM}_{jl} + \mathbf{FM}_{kl} + \mathbf{SDF}_{ijk} \\ &+ \mathbf{SFM}_{ikl} + \mathbf{DFM}_{jkl} + \mathbf{SDFM}_{ijkl} + e_{ijklm} \end{aligned}$$

The ANOVA model for testing overall mass traits, which includes root size class, includes the following effects: site (S), root size class (C), female (F), and male (M), all considered fixed. The ANOVA model used is as follows:

$$Y_{ijklm} = \mu + S_i + C_j + F_k + M_l + SC_{ij} + SF_{ik}$$
  
+ SM<sub>il</sub> + CF<sub>jk</sub> + CM<sub>jl</sub> + FM<sub>kl</sub> + SCF<sub>ijk</sub>  
+ SFM<sub>ikl</sub> + CFM<sub>ikl</sub> + SCFM<sub>ikl</sub> + e<sub>ijklm</sub>

Effects were considered statistically significant at the  $\alpha$  = 0.10 level, although individual *p* values are provided so that readers can make their own interpretations. The general linear model from Systat (Chicago, Illinois) was used for analysis. Full description of the ANOVA model is included in the supplementary section of the online version.<sup>1</sup>

#### Results

Here, we present the significant and biologically meaningful effects in these sometimes complicated analyses, which include genetic (progeny of female and progeny of male), site, root size category, and soil depth effects as well as their interactions. A complete presentation of the four main effects and their interactions of the two ANOVA models and the additional quantification documentation are available in the supplementary section of the online version.

#### Root biomass by root size category

Root biomass <1 mm had significant site (p = 0.009), depth (p < 0.001), female × male (p = 0.076), and site × depth × male interaction (p = 0.003) effects (Table 2). The dry site had more or equal root biomass <1 mm than the wet site at each of the depths (Fig. 2a). Overall, the dry and wet sites had 4.2 and 3.2 Mg·ha<sup>-1</sup>, respectively.

Root biomass 1–2 mm had significant site (p = 0.024), depth (p < 0.001), and male (p = 0.080) effects (Table 2). The dry site had more or equal root biomass 1–2 mm than the wet

<sup>&</sup>lt;sup>1</sup>Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/x2012-144.

**Table 2.** ANOVAs of black spruce (*Picea mariana*) root mass by size category, including source of variation, degrees of freedom (df), mean square values (MS), p values, and coefficient of determination ( $R^2$ ).

	Roots < (Mg·ha	<1 mm <sup>-1</sup> )	Roots (Mg·ha	1–2 mm a <sup>–1</sup> )	Roots 2–5 mm (Mg·ha <sup>-1</sup> )		Roots >5 mm (Mg·ha <sup>-1</sup> )		
Source of variation	df	MS	р	MS	р	MS	р	MS	р
Site (S)	1	1.031	0.009	0.152	0.024	0.450	0.260	3.022	0.428
Depth (D)	4	21.364	< 0.001	0.999	< 0.001	7.074	< 0.001	100.901	< 0.001
Female (F)	1	0.064	0.505	0.074	0.113	0.000	0.995	0.249	0.820
Male (M)	1	0.030	0.646	0.091	0.080	0.001	0.955	0.409	0.770
$S \times D$	4	0.118	0.511	0.034	0.331	0.326	0.450	8.720	0.130
$S \times F$	1	0.018	0.723	0.020	0.403	1.240	0.063	3.819	0.373
$S \times M$	1	0.194	0.248	0.002	0.782	0.003	0.925	1.363	0.594
$D \times F$	4	0.036	0.910	0.038	0.273	0.047	0.969	1.448	0.875
$D \times M$	4	0.142	0.415	0.053	0.131	0.089	0.906	6.492	0.254
$F \times M$	1	1.012	0.009	0.018	0.438	0.112	0.574	2.508	0.470
$S \times D \times F$	4	0.053	0.828	0.027	0.449	0.562	0.180	3.840	0.525
$S \times D \times M$	4	0.627	0.003	0.030	0.400	0.412	0.326	4.146	0.486
$S \times F \times M$	1	0.012	0.771	0.010	0.548	0.337	0.329	1.254	0.610
$D \times F \times M$	4	0.069	0.748	0.007	0.913	0.693	0.104	2.980	0.646
$S \times D \times F \times M$	4	0.007	0.995	0.029	0.419	0.106	0.875	7.129	0.210
Error	100	0.143		0.029		0.350		4.772	
R <sup>2</sup>			0.878		0.679		0.566		0.574

**Note:** *p* values <0.10 are in bold print.

**Fig. 2.** Vertical distribution of black spruce (*Picea mariana*) (*a*) root biomass <1 mm, (*b*) root biomass 1-2 mm, (*c*) root biomass 2-5 mm, and (*d*) root biomass >5 mm (note: *p* values <0.10 are in bold print).



**Table 3.** ANOVAs of black spruce (*Picea mariana*) root size category, including source of variation, degrees of freedom (df), mean square values (MS), p values, and coefficient of determination ( $R^2$ ).

		Root biomass (Mg·ha <sup>-1</sup> )		C (%)		N (%)		C:N ratio		C mass (Mg·ha <sup>-1</sup> )		N mass (Mg·ha <sup>-1</sup> )	
Source of variation	df	MS	р	MS	р	MS	р	MS	р	MS	р	MS	р
Site (S)	1	29.494	0.029	0.281	0.839	0.320	< 0.001	4183.246	0.760	8.419	0.030	1.057	0.863
Size class (C)	3	114.686	< 0.001	40.670	0.001	0.935	< 0.001	1.241E+006	< 0.001	32.720	< 0.001	3803.190	< 0.001
Female (F)	1	0.087	0.904	7.044	0.312	0.008	0.439	20207.733	0.503	0.017	0.921	13.129	0.543
Male (M)	1	0.253	0.837	2.009	0.588	0.038	0.097	358868.558	0.006	0.011	0.937	96.913	0.101
$S \times C$	3	4.429	0.530	2.034	0.826	0.011	0.485	40404.433	0.443	1.241	0.545	1.208	0.991
$S \times F$	1	0.017	0.958	0.018	0.960	0.008	0.439	41447.448	0.338	0.035	0.887	44.227	0.265
S  imes M	1	2.615	0.510	0.552	0.776	0.100	0.008	195344.514	0.040	0.464	0.606	158.828	0.036
$F \times C$	3	0.289	0.986	2.077	0.821	0.010	0.538	3618.417	0.970	0.079	0.987	30.059	0.468
$M \times C$	3	0.093	0.997	0.723	0.956	0.013	0.412	344443.039	< 0.001	0.114	0.978	51.693	0.229
F  imes M	1	0.004	0.978	0.015	0.962	0.027	0.161	77396.163	0.192	0.000	0.998	118.471	0.070
$S \times C \times F$	3	5.628	0.424	0.142	0.996	0.034	0.066	37120.483	0.481	1.416	0.488	7.191	0.893
$S \times C \times M$	3	5.799	0.411	1.787	0.852	0.013	0.414	128163.633	0.042	1.733	0.397	54.361	0.209
$S \times F \times M$	1	6.976	0.283	0.918	0.714	0.005	0.525	97998.985	0.142	1.614	0.337	1.124	0.858
$C\times F\times M$	3	3.943	0.579	3.751	0.649	0.010	0.511	34621.987	0.511	1.125	0.585	59.935	0.172
$S \times C \times F \times M$	3	4.365	0.536	1.820	0.849	0.020	0.215	25923.666	0.630	1.141	0.580	0.561	0.997
Error	80	5.972		6.802		0.013		44666.452		1.732		35.135	
<i>R</i> <sup>2</sup>			0.505		0.234		0.770		0.632		0.499		0.819

**Note:** *p* values <0.10 are in bold print.

site at each of the depths (Fig. 2*b*). Overall, the dry and wet sites had 1.1 and 0.8 Mg·ha<sup>-1</sup>, respectively. The significant male effect had progeny of males 52 and 62 with 0.81 and 1.09 Mg·ha<sup>-1</sup>, respectively.

Root biomass 2–5 mm had significant depth (p < 0.001) and female × site (p = 0.063) effects (Table 2). The significant female × site interaction was due to female rank change between sites (Fig. 2c). Progeny of female 59 had lower 2–5 mm root biomass than progeny of female 63 on the dry site; this was reversed on the wet site. Root biomass >5 mm had only a significant depth (p < 0.001) effect (Table 2) (Fig. 2d).

# Root biomass parameters by root size category (summed by depth)

Root biomass had significant site (p = 0.029) and size class (p < 0.001) effects (Table 3). Root biomass on the dry site had significantly greater total fine and coarse root biomass than on the wet site for all size categories (Fig. 3*a*; Table 4). The root mass across sites was, on average, 3.71, 0.95, 1.8, and 5.82 Mg·ha<sup>-1</sup> for the <1, 1–2, 2–5, and >5 mm root size categories, respectively.

Root C concentration only varied significantly for one effect: size category (Table 3). Root C concentration showed an upward and then leveling trend versus greater root size class increase (Fig. 3b) (p < 0.001). Root C concentrations were 50.7%, 52.8%, 53.0%, and 53.4% for the <1, 1–2, 2–5, and >5 mm root size categories, respectively.

Root N concentration had significant site (p < 0.001), root size category (p < 0.001), male (p = 0.097), site × male (p = 0.008), and site × root size × female (p = 0.066) effects (Table 3). Average root N for the dry and wet sites was 0.31% and 0.42%, respectively, and was always greater on the wet site than on the dry site across all root size categories (Fig. 3c).

Mean root N concentration declined with increase in root size category in a near linear decline, with 0.62%, 0.37%, 0.28%, and 0.19% N for the <1, 1–2, 2–5, and >5 mm root size categories, respectively. Progeny of male 52 had lower N concentration than progeny of male 62, with 0.34% and 0.38%, respectively.

Averaged C:N ratio of roots had significant root size class (p < 0.001), male (p = 0.006), site  $\times$  male (p = 0.040), male  $\times$  root size class (p < 0.001), and site  $\times$  root size class  $\times$  male (p = 0.042) effects (Table 3). Root C:N ratio showed a near linear increase with root size class with C:N ratios of 98, 185, 368, and 577 for the <1, 1–2, 2–5, and >5 mm root size categories, respectively (Fig. 3*d*).

Root C mass had significant site (p = 0.030) and size class (p < 0.001) effects (Table 3). Root C mass on the dry site had significantly greater root C mass than on the wet site for all root size categories (Fig. 3*e*). The root C mass across sites was, on average, 1.9, 0.5, 0.9, and 2.9 Mg·ha<sup>-1</sup> for the <1, 1–2, 2–5, and >5 mm root size categories, respectively.

Root N mass had significant size class (p < 0.001) and site × male (p = 0.036) and female × male (p = 0.070) effects (Table 3). Root N mass was 28.2, 3.4, 4.1, and 7.4 kg·ha<sup>-1</sup> the for <1, 1–2, 2–5, and >5 mm root size categories, respectively (Fig. 3*f*).

#### Total fine and coarse root mass

Total fine and coarse root biomass had significant site (p = 0.091) and soil depth (p < 0.001) effects (Table 4). Overall, the dry site had greater total fine and coarse root biomass than the wet site, with 14.2 and 10.6 Mg·ha<sup>-1</sup>, respectively (Fig. 4*a*). Most of the total fine and coarse root biomass and site differences resided in the top depth (0–10 cm). The dry and wet site fine and coarse roots in the upper depth (0–10 cm) were 10.3 and 7.3 Mg·ha<sup>-1</sup>, respectively. Total fine and coarse

**Fig. 3.** Distribution of black spruce (*Picea mariana*) (*a*) root biomass, (*b*) C concentration, (*c*) N concentration, (*d*) C:N ratio, (*e*) C mass, and (*f*) N mass by root size class (note: p values <0.10 are in bold print).

root C mass only had a significant soil depth (p < 0.001) effect (Table 4). For the record, total fine and coarse root biomass, the dry and wet sites had 7.4 and 5.5 Mg·ha<sup>-1</sup> total fine and coarse root C mass, respectively (p = 0.104).

Total fine and coarse root N mass was significant for soil depth (p < 0.001), male (p = 0.078), site × male (p = 0.024), female × male (family) (p = 0.097), and site × male × depth (p = 0.007) effects (Table 4). Interestingly, sites were almost exactly the same, with 43.4 and 43.3 kg·ha<sup>-1</sup> for the dry and wet sites, respectively (p = 0.967) (Fig. 4b). Progeny of male 52 had lower total root N mass than progeny of male 62, with 39.4 and 47.4 kg·ha<sup>-1</sup>, respectively.

# Summary of root mass allocation

Overall, the top soil depth (0-10 cm) had 8.68 Mg·ha<sup>-1</sup> root biomass, which represented 70.7% of all five depths

(Fig. 5*a*). The next four depths had 15.1%, 4.6%, 2.3%, and 7.2% of the overall root biomass, respectively. Of note, over 50% of the top depth root biomass was >5 mm. Thirty-four percent of the top depth was fine roots (<2 mm) and 66% was >2 mm root size. The 10–20 cm soil depth had approximately 50% in the <2 and >2 mm root size categories. The 40–50 cm soil depth had approximately 80% in the>5 mm root size. Through the 50 cm soil depth, root biomass allocation by root size categories was 30.2%, 7.7%, 14.7%, and 47.4% for root sizes <1, 1–2, 2–5, and >5 mm, respectively (Fig. 5*a*, inset).

Overall fine and coarse root N mass allocation to the top (0-10 cm) soil depth was 31.68 kg·ha<sup>-1</sup>, which is 73.0% of all five depths (Fig. 5*b*). The next four depths had 14.9%, 6.1%, 2.6%, and 3.3% of the root N mass, respectively. Of note,



		Fine root dry mass (<2 mm) (Mg·ha <sup>-1</sup> )		Fine and co dry mass (1	oarse roots Mg·ha −1)	Fine and c C mass (N	coarse roots ⁄Ig·ha <sup>-1</sup> )	Fine and coarse roots N mass (kg·ha <sup>-1</sup> )	
Source of variation	df	MS	р	MS	р	MS	р	MS	р
Site (S)	1	1.976	0.001	10.956	0.091	4.154	0.104	0.042	0.967
Depth (D)	4	31.364	< 0.001	221.552	< 0.001	93.020	< 0.001	4,439.268	< 0.001
Female (F)	1	0.000	0.965	0.363	0.756	0.077	0.824	5.930	0.626
Male (M)	1	0.227	0.268	1.319	0.555	0.226	0.702	78.700	0.078
$S \times D$	4	0.260	0.232	7.151	0.116	2.802	0.131	2.322	0.984
$S \times F$	1	0.000	0.984	0.210	0.814	0.278	0.672	22.888	0.338
$S \times M$	1	0.237	0.257	0.522	0.710	0.039	0.874	129.344	0.024
$F \times D$	4	0.114	0.647	1.353	0.836	0.511	0.855	15.856	0.634
$M \times D$	4	0.294	0.178	3.225	0.492	1.557	0.405	38.096	0.197
$F \times M$	1	1.297	0.009	0.720	0.662	0.135	0.767	69.508	0.097
$S \times F \times D$	4	0.079	0.786	2.537	0.611	0.945	0.653	30.197	0.307
$S \times M \times D$	4	0.916	0.001	0.584	0.960	0.096	0.993	92.674	0.007
$S \times F \times M$	1	0.045	0.619	2.023	0.465	0.458	0.587	0.094	0.951
$F \times M \times D$	4	0.109	0.667	1.271	0.852	0.348	0.923	5.592	0.923
$S \times F \times M \times D$	4	0.036	0.939	6.431	0.154	2.239	0.222	15.784	0.636
Error	100	0.183		3.757		1.538		24.734	
$R^2$			0.894		0.756		0.758		0.895

**Table 4.** ANOVAs of black spruce (*Picea mariana*) total fine and coarse root mass, including source of variation, degrees of freedom (df), mean square values (MS), p values, and coefficient of determination ( $R^2$ ).

Note: p values < 0.10 are in bold print.

68.5% of top depth root N mass was found in roots <1 mm and 75% of root N mass was found in roots <2 mm. Through the 50 cm soil depth, root N mass allocation by root size categories was 65.2%, 7.8%, 9.4%, and 17.6% for root sizes <1, 1–2, 2–5, and >5 mm, respectively (Fig. 5*b*, inset).

#### **Root depth** × root size matrix

Overall, C concentration ranged from 50.7 to 53.4 for root sizes <1 to >5 mm; however, there was an interaction with depth. For roots <1 mm, C concentration declined with depth from 52.9 to 50.6; however, for roots >2 mm, root C was similar across all depths (not shown). Root N concentration declined with increase in both soil depth and root size (Fig. 6*a*). However, there was a strong peak for roots <1 mm in the 0–10 cm soil depth and then a relatively gradual decline downward both ways. Root C:N ratio increased with both root size and root depth (Fig. 6*b*). The magnitude was highly dependent on these two factors. Root C:N ratio ranged from 60 for roots <1 mm at 0–10 cm soil depth to almost 900 for roots >5 mm at 40–50 cm soil depth.

Root biomass had a major peak in the >5 mm root size in the 0–10 cm soil depth with 4.7 Mg·ha<sup>-1</sup> and another in the <1 mm root size in the 0–10 cm soil depth of 2.11 Mg·ha<sup>-1</sup> (Fig. 7*a*). There was only one major peak for root N mass, and that was in the <1 mm root size for 0–10 cm soil depth with 21.7 kg·ha<sup>-1</sup> (Fig. 7*b*).

#### Family drought tolerance $\times$ site interaction

Family drought tolerance × site interaction for fine root (<2 mm) mass was not significant (p = 0.984) (Fig. 8*a*). Both drought-tolerant and -intolerant families had a fine root (<2 mm) mass of 5.3 Mg·ha<sup>-1</sup> on the dry site and 4.0 Mg·ha<sup>-1</sup> on the wet site. Family drought tolerance × site interaction for fine and coarse root mass was not significant (p = 0.814) (Fig. 8*b*). Fine and coarse root masses for drought-tolerant and -intolerant families were, respectively, 14.2 and 14.4 Mg·ha<sup>-1</sup> on the dry site and 10.0 and 11.1 Mg·ha<sup>-1</sup> on the wet site.

#### Discussion

#### Fine roots <1 and 1–2 mm

There is a range of definitions in the literature for fine root size; most are either <1, <2, or <5 mm (Cairns et al. 1997; Helmisaari et al. 2007; King et al. 2007). This, of course, affects direct comparisons of results relating to fine roots among studies. We have provided all three in separate categories for comparisons. However, it appears that <2 mm has become the convention for fine root size definition (Helmisaari et al. 2002, 2007; Ostonen et al. 2005; Finer et al. 2007). Direct comparisons with other reports in the literature are also difficult due to effects of species, age, different depths of organic and mineral soil, and reporting of just root biomass or root C mass. In a study of a much older (123-year-old) black spruce stand in Saskatchewan, the average fine root C mass <2 mm was 3.1 Mg·ha<sup>-1</sup> (Gower et al. 1997), which is comparable, as we had 2.4 Mg  $C \cdot ha^{-1}$  for <2 mm root size categories. In a study of 55-year-old Norway spruce (Picea abies (L.) Karst.) in Maine, USA, biomass of roots <1 mm was 3.6 Mg·ha<sup>-1</sup> (0–40 cm depth; Cronan 2003), similar to our average of 3.6 Mg $\cdot$ ha<sup>-1</sup>. In a chronosequence survey of 10-, 30-, 60-, and 120-year-old Norway spruce in Norway, the <2 mm root biomass (0–60 cm) was 0.49, 3.98, 2.06, and 1.84 Mg·ha<sup>-1</sup>, respectively (Børja et al. 2008). Tree densities per hectare were 9350, 2381, 899, and 431, respectively. The 30-year-old trees had the greatest fine root mass and had a comparable density and fine root mass to our study.

#### Coarse roots 2–5 and >5 mm

The 55-year-old Norway spruce study in Maine had 2.0 and 5.2 Mg·ha<sup>-1</sup> for 1–3 and >3 mm, respectively (Cronan 2003),



**Fig. 4.** Vertical distribution of black spruce (*Picea mariana*) (*a*) total fine and coarse root biomass by site and (*b*) total fine and coarse root N mass by site (note: p values <0.10 are in bold print).

which were similar to our results of 1.8 and 5.5 Mg·ha<sup>-1</sup> for the 2–5 and >5 mm size categories, respectively. Root biomass 2–5 and 5–20 mm for a 40-year-old Norway spruce study in Europe had 1.33 and 4.07 Mg·ha<sup>-1</sup>, respectively (Ostonen et al. 2005). The root biomass distribution among our fine and coarse roots showed that the dominant root categories were very fine (<1 mm) and coarse (>5 mm) with 33% and 47%, respectively. Using the conventional definition of fine roots, it becomes 40% <2 mm fine roots and the remaining 60% is >2 mm coarse roots, similar to Cronan's (2003) findings. However, 40-year-old Norway spruce in Europe had only 22% in fine roots (<2 mm), but the authors noted that the site had good fertility and this can reduce fine root growth (Ostonen et al. 2005).

#### Soil depth effect

The majority, 62% and 54%, of our roots <1 and 1-2 mm were in the top soil depth (0–10 cm), which is consistent with a number of studies of Norway spruce (Cronan 2003; Ostonen et al. 2005; Borken et al. 2007). A larger percentage, 70% and 90%, of our roots 2–5 and >5 mm, respectively, were in the



**Fig. 5.** Vertical distribution of black spruce (*Picea mariana*) (*a*) root biomass by root size class (inset: overall proportion by root size class) and (*b*) root N mass by root size class (inset: overall proportion of root N mass by root size class).

top soil depth. Our root mass decline with depth is relatively more abrupt compared with distribution of fine roots for Norway spruce in Maine and Europe (Cronan 2003; Borken et al. 2007). Thus, with black spruce, the larger the roots, the more likely they would be found in the first 10 cm; however, for Norway spruce, the largest roots were found in the deeper 10–20 cm soil depth (Cronan 2003). Black spruce is known to be a shallow-rooting species, given its typical environment of lowland sites; however, it also grows on upland sites, and it was hypothesized that on the dry site, which is extremely well drained, there would be more coarse roots deeper in the soil. This was not the case, however, and thus, the root distribution pattern appears to be under strong genetic (species) control.

Nitrogen concentration for our roots <1 mm was greatest in the 0–10 cm depth and was 0.95%, almost twice that at the lower depths, which had 0.52% N. This is in agreement with the greater soil N concentration in the top soil depth of 0.45% N, compared with the rest of the depths, which averaged 0.10% soil N (Major et al. 2012*a*). Note that our roots 1–2 mm had 0.4% N in the 0–10 cm depth, much lower than the <1 mm root size. A study examining black spruce found the N concentration in fine roots (<2 mm) was 0.63%–0.74%, and these roots were only from the organic layer (Smith et



**Fig. 7.** Black spruce (*Picea mariana*) (*a*) fine and coarse root biomass by soil depth and root size class and (*b*) fine and coarse root N mass by soil depth and root size class.

al. 2000). The N concentration of fine roots (<5 mm) in a black spruce peat bog (>40 cm) was 0.8% (Bhatti et al. 1998). The greater amount in the peat bog was due to the higher N availability in peat (1%; Smith et al. 2000). The C concentration of our <1 mm roots also declined with soil depth, from 53% in the top to 49.5% in the bottom zone. Borken et al. (2007) also found that the C concentration in fine roots (<2 mm) declined with depth for Norway spruce, with 50.3% in the humus and 48.4% at the10–40 cm depth. The result was a C:N ratio of 57:1 for our roots <1 mm in the top zone and an average of 110:1 in the lower four depths, whereas Borken et al. (2007) found no significant difference in fine root C:N ratio by soil depth, with values of approximately 34:1, driven by the higher site soil N.

#### Root size category effect

We had an almost linear decline in N (average both sites 0.6%-0.2% N) with increased root size (<1 to >5 mm). This has been observed in one other black spruce study, which found approximately 0.45%, 0.25%, and 0.20% N for root sizes <2, 2–5, and 5–20 mm, respectively, from trees of various ages (Ouimet et al. 2008). In a meta-analysis of nutrient concentrations in fine roots in relation to root size, there was a weak but general inverse relationship of fine root nutrient concentrations with root diameters (Gordon and Jackson 2000). They also found that C concentration increased with root size, with 48.0% and 51.5% for roots <2 and 2–5 mm, respectively, consistent with our results. As a result,





**Fig. 8.** Black spruce (*Picea mariana*) (*a*) fine root dry mass (<2 mm) and (*b*) fine and coarse root dry mass of drought-tolerant and -intolerant families (mean and SE) from two sites located at the Petawawa Research Forest, Ontario. Drought-tolerant families are progeny of female 59 (solid symbols) and drought-intolerant families are progeny of female 63 (open symbols). Dry and wet sites refer to sites 2 and 3, respectively.



their C:N ratios for roots <2 and 2–5 mm were, on average, 43:1 and 79:1, respectively (Gordon and Jackson 2000), comparable to our results. Due to the high N in roots <1 mm, 73% of the fine and coarse root N mass was found in the fine roots <2 mm. This is nearly double the <2 mm root C mass, which was 38% total fine and coarse root biomass. With the effect of C and N concentration going in different directions with increased root size, the impact on C:N ratios was large, the greatest differences seen with roots <1 to >5 mm, increasing almost linearly from approximately 100 to 600, respectively.

#### Total fine and coarse root mass

In the study on 55-year-old Norway spruce in Maine, total fine and coarse root biomass (not including the stump coarse roots) was 10.8 Mg·ha<sup>-1</sup> (0–40 cm; Cronan 2003), which is comparable to ours, which averaged 11.8 Mg·ha<sup>-1</sup>. Total fine

and coarse roots for Sakhalin spruce (*Picea glehnii* (F. Schmidt) Mast.) totaled approximately 10 Mg·ha<sup>-1</sup> (Sakai et al. 2007); the allocation to <2 mm fine roots was just under half, whereas ours was just over one-third. Overall, there was 43.4 kg·ha<sup>-1</sup> total fine and coarse root N mass on both our sites. Although the literature is sparse in this area, there is a study by Bhatti et al. (1998) of a 65-year-old black spruce stand in northern Ontario with >40 cm organic matter where the fine roots (<5 mm) had 54 and 20 kg·ha<sup>-1</sup> N mass with alder (*Alnus* sp.) and no alder understory, respectively.

## Soil depth × root size matrix

The only other study documenting soil depth  $\times$  root size matrix for mature trees that we are aware of is that of Pregitzer et al. (1998), which reported root N for sugar maple (*Acer saccharum* Marsh.). They showed a decline in N with root size but no differences with depth. They did not provide a reference to C or C:N ratio. Frequently, investigators assume that root biomass is made up of 50% C for all root sizes and soil depths. However, our results show a significant root size, soil depth, and root size  $\times$  depth interaction for root C concentration, and our numbers range from 49.3 <1 mm to 55.4 >5 mm. Our root C:N ratio showed a very large variation from under 900:1 to 60:1 across the soil depth  $\times$  root size matrix.

#### Site effects

All root size categories had greater biomass on the dry site than on the wet site. Comeau and Kimmins (1989), working with 70- to 80-year-old lodgepole pine (*Pinus contorta* Douglas ex Loudon), found that there were more fine roots (<5 mm) on dry than wet sites in both absolute and percentage of biomass, consistent with what we found. In a review of partitioning with varied belowground resource availability, there was a decrease in belowground C partitioning with increased fertilization and irrigation treatment (Litton et al. 2007) for three genera (*Eucalyptus* spp., radiata pine (*Pinus radiata* D. Don), and loblolly pine (*Pinus taeda* L.)). For loblolly pine, fertilization and irrigation decreased C partitioning belowground by 77% but increased partitioning by 28% to wood and 29% to foliage (Maier et al. 2004).

There was significantly greater fine root N concentration on the wet site than on the dry site, despite having no significant soil N concentration site or site  $\times$  depth effects (Major et al. 2012*a*). The greater fine root N concentration may be due to the same N resource availability but a smaller amount of fine root mass on the wet site. Helmisaari et al. (2007), working with Scots pine (*Pinus sylvestris* L.) and Norway spruce over a number of sites, found that the greater the C:N ratio of the organic matter (or less site N), the greater the fine root mass. The dry site had greater C:N ratio for fine roots, and the only other related study was a metadata review that found a positive relationship between fine root biomass and organic soil layer C:N ratio (Finer et al. 2007).

Differential resource allocation to foliage and fine roots has been observed in response to site fertility (Vanninen and Makela 1999; Helmisaari et al. 2007). The overall foliage to fine root (<2 mm) ratio by mass for our study was 2.3 and 4.0 for the dry and wet site, respectively (foliage data from Major et al. 2012*b*). Helmisaari et al. (2007) examined the foliage to fine root ratio of 16 stands of Norway spruce throughout Finland and found that it ranged from 2.1 to 6.4. This ratio decreased from south to north as well as from fertile to more infertile site types. This trend implies that more fine roots are needed to maintain a certain amount of foliage when belowground resources are scarce. In congruence, the dry site had lower needle mass and more fine root mass than the wet site, demonstrating an adaptation to a change in water availability through the rebalancing of resource-obtaining organs (needles and fine roots) in response to a limiting environmental resource, water.

#### Genetic effects

We hypothesized that there would be greater fine and coarse root mass for drought-tolerant families, particularly on the dry site. This was not found; drought-tolerant and -intolerant families had, respectively, 14.2 and 14.4 Mg·ha<sup>-1</sup> total fine and coarse root biomass on the dry site and 10.0 and 11.1 Mg·ha<sup>-1</sup> total fine and coarse root biomass on the wet site. In one of the few genetic quantifications of above- and belowground biomass variation of field-grown trees, Oleksyn et al. (1999), using a 12-year-old Scots pine provenance test, found regional differences among northern, central, and southern populations in a common-garden plot in central Poland. All three regions allocated similar amounts to fine roots (<2 mm), approximately 2.3 Mg·ha<sup>-1</sup>. However, allocation to larger roots (>2 mm) was, respectively, 5.4, 9.1, and 2.9 Mg·ha<sup>-1</sup>, similar to aboveground biomass, which was, respectively, 25, 43, and 12 Mg·ha<sup>-1</sup> for northern, central, and southern populations. Thus, similar to our study, there was no difference in fine roots among genetic entries despite aboveground growth differences. Although a seedling study, Johnsen and Seiler (1996) found that northern provenances of black spruce allocated more biomass to roots than southern sources.

Do more productive families allocate more or less to foliage in relation to fine roots? In drought-tolerant and -intolerant families, the foliage to fine root mass ratios were 3.8 and 2.6, respectively (foliage data from Major et al. 2012b). Although they have the same fine root mass, drought-tolerant families are able to allocate and support more foliage mass in relation to fine root mass than the drought-intolerant families. Furthermore, examining N partitioning for drought-tolerant and -intolerant families, the foliage N mass to fine root N mass ratios were 8.4 and 6.1, respectively. Thus, more N and C mass is allocated to foliage than to fine roots for wet compared with dry sites and for drought-tolerant compared with droughtintolerant families. Although drought-tolerant and -intolerant families had the same fine and coarse root mass, droughttolerant families were able to support more foliage and aboveground mass per unit fine root mass than droughtintolerant families, demonstrating variation and adaption to moisture stress. This rebalance is a reflection of heritable drought tolerance, which is the maintenance of greater growth and photosynthesis per unit fine root biomass under limiting water availability.

Where might these four families fit within the context of black spruce genetic variation? The area that the parent trees (seed) came from is large and as long as the trees selected are at least 50–100 m from each other to avoid issues such as inbreeding. This would have been evident when they had been grown for breeding but would have been carefully avoided by a geneticist such as Morgenstern (1974) who wrote on this subject and supervised the breeding. In fact, as part of the

complete  $7 \times 7$  diallel, he produced inbred trees that were half the size of the outcrossed trees, which we have reported on previously for growth and gas exchange (Johnsen et al. 2003). There is substantial genetic variation within populations, and in most cases, there is much more genetic variation within a population than among populations (Morgenstern 1996). From this diallel, the genetic variation was very large, and these four families fall within the upper fifth percentile for productivity and drought tolerance (Johnsen et al. 1999). Although traditional forest genetics research has clearly shown that tree genotypes can vary greatly in aboveground volume growth, it is not at all certain that these aboveground growth increases will result in overall increases in C sequestration. This is because trade-offs can exist among above- and belowground C sinks (Maier et al. 2004; Litton et al. 2007). Thus, a genetically superior aboveground volume-producing genotype may well divert less C belowground than a slower aboveground volume-producing genotype. Among other questions, this will be explored in the companion paper examining total belowground C and N partitioning of mature black spruce displaying genetic  $\times$  soil moisture interaction in aboveground growth (Major et al. 2012a).

#### Conclusions

Empirical information on fine and coarse root biomass and C and N mass parameters by root size and soil depth as well as their contribution to aboveground variation is scarce for mature trees and particularly for mature pedigreed trees. All fine and coarse root size categories had greater root biomass on the dry site than on the wet site. Most of the site differences resided in 0-20 cm soil depths. This difference occurred despite less aboveground biomass on the dry site and appears to be a direct response to limiting water availability. The dry site had lower needle mass and more fine root mass than the wet site, demonstrating an adaptation to change through the rebalancing of resource-obtaining organs (needles and fine roots) in response to a limiting environmental resource, water. Drought-tolerant families were able to support more foliage mass per unit fine root mass than drought-intolerant families, demonstrating variation and adaption to moisture stress. This rebalance is a reflection of heritable drought tolerance, which allows the maintenance of greater growth and photosynthesis per unit fine root biomass under limiting water availability.

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