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Carbohydrates in individual poplar fine roots: effects of root age and defoliation

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Summary Late-summer starch accumulation in fine roots of poplars (*Populus* × *canadensis* Moench.) defoliated by gypsy moth (*Lymantria dispar* L.) lagged behind that in fine roots of undefoliated trees. If starch concentration declines with age, defoliation-induced changes in root system age structure could be partly responsible for this difference. To test this hypothesis, we measured fine-root starch and soluble sugar concentrations in roots of known age from trees in defoliated and undefoliated plots. There was a significant interaction between the effects of defoliation and root type (white, brown, or dead) on fine root soluble sugar concentration because of the high concentration of soluble sugars in white roots from trees in undefoliated plots. Both root starch and soluble sugar concentrations were variable among individuals of each age class. The population frequency distributions for starch and soluble sugar concentrations were both right-skewed, and fit by exponential functions. These data are most consistent with direct defoliation effects on a labile and dynamic pool of carbohydrates in poplar fine roots, rather than indirect defoliation effects on root system age structure.

Keywords: expandable-wall minirhizotron, herbivory, laparascopic sampling, Populus × canadensis, starch.

Introduction

Root systems are complex and dynamic structures. An individual root system contains roots of many ages, potentially differing widely in composition and metabolic activity. Even in herbaceous annual plants, root systems contain multiple zones under separate developmental control (Zobel 1986). The influence of heterogeneity within a single root system on aggregate root system properties has received attention only recently (Gao et al. 1998, McCully 1999).

Although the transience of woody plant fine roots and the consequent potential for variation in root age are well documented (Hendrick and Pregitzer 1992, Kosola et al. 1994, Eissenstat and Yanai 1997, Ruess et al. 1998, Eissenstat et al. 2000), there is little information available on changes in the composition and function of roots during their life span. This information is critical to understanding the dynamics of root system responses to disturbances such as defoliation. The dynamics of root system response to disturbance or experimental treatments depend on direct effects on existing roots and on shifts in root development and root demography. If a treatment alters root development, roots produced after the treatment will differ from those produced before the treatment. When rates of root production or root mortality change, the mean age of the root system may increase or decrease relative to the control. If root traits vary with age (e.g., Palta and Nobel 1989, Eissenstat and Yanai 1997, Comas et al. 2000, Bouma et al. 2001), shifts in the age structure of a root population can change root system properties in the absence of any other treatment effects.

Bulk samples of roots necessarily combine roots of different ages; determining the mechanisms leading to root system responses to treatments requires sampling individual roots produced before and after the treatment. We applied a new technique for sampling individual roots of known age (Kosola 1999) to investigate the responses of poplar (*Populus* × *canadensis* Moench.) root systems to defoliation by gypsy moth (*Lymantria dispar* L.). This work was part of a larger study of the effects of gypsy moth defoliation on tree physiology, plant–insect interactions and nitrogen cycling (see Kosola et al. 2001 for details). Analysis of bulk root samples from defoliated poplars revealed a transient lag in late-season starch accumulation following defoliation (see Figure 2A). Because defoliation also caused a transient decline in root production (Kosola et al. 2001), this change in starch concentration may have been a result of defoliation effects on root system age structure. We sampled individual roots of known age produced before and after defoliation to test for persistent defoliation-in-

duced shifts in root composition. Methods for analysis of these very small root samples were developed. Because these roots spanned a wide range of ages, we were also able to determine the effects of root age on starch and soluble sugar concentrations in fine roots.

Materials and methods

Fine roots were sampled from defoliated and undefoliated plots in four replicate blocks of Eugenei hybrid poplars on the Kellogg Biological Station Long Term Ecological Research Site. The 400-m^2 plots were in four 1-ha stands of Eugenei poplar, planted as cuttings in May 1989 (Marino and Gross 1998). Weed growth was suppressed from the time of planting with applications of 2% (v/v) glyphosate (Roundup, Monsanto, St. Louis, MO) in May and July of each year. The plots are on a Kalamazoo sandy loam soil (Typic Hapludalf). Trees in the defoliated plots were inoculated with high densities of gypsy moths (*Lymantria dispar*) for the first time in 1996. Trees in the undefoliated plots were protected by sticky barriers (Tanglefoot, Grand Rapids, MI) and manual removal of caterpillars from trees (see Kosola et al. 2001 and Parry 2000 for further details).

Minirhizotron data from 1996 and 1997, previously analyzed for root production and mortality (Kosola et al. 2001), were used to determine the mean age of live roots at each observation date. Expandable-wall minirhizotrons (Kosola 1999) were installed in March 1997 in preparation for sampling roots of known age. Five expandable-wall minirhizotrons were installed in the defoliated and undefoliated treatments in each of four replicate blocks (Kosola et al. 2001). Root growth was videotaped with a BTC-100 minirhizotron camera (Bartz Technology, Santa Barbara, CA) every month until November, when roots were sampled with laparascopic forceps (Figure 1) according to the procedures described by Kosola (1999). No significant effects of defoliation on bulk root sample total nonstructural carbohydrate (TNC) concentrations were detected at this date (Figure 2A). A minirhizotron video digitizing program (MSU-ROOTS, Version 8-10-93; W. Enslin et al., Michigan State University, East Lansing, MI) was used to assign a code number to each root as it appeared (Hendrick and Pregitzer 1992; Figure 1). Subsequent observations of each root's size and condition (color and structural integrity) were stored in a database with this code number. Roots typically proceed through white, brown and dead stages. Roots were classified as dead when they lost cortical integrity and were brown in color.

The database was used to record when each sampled root appeared, and its condition at the final observation date (about 2 weeks before sampling). The digitized tracings in each frame from the final observation date were printed as a guide to the position of each root, with the code number recorded on the printout (Figure 1). As each root was laparascopically sampled, it was placed in a 2-ml microcentrifuge tube containing 0.5 ml of ice-cold 80% (v/v) ethanol. Each tube was assigned an accession number. The accession number for each sample was recorded directly on the printed copy of the digitized tracings from the final observation date, and was thus paired with the database code number for that root.

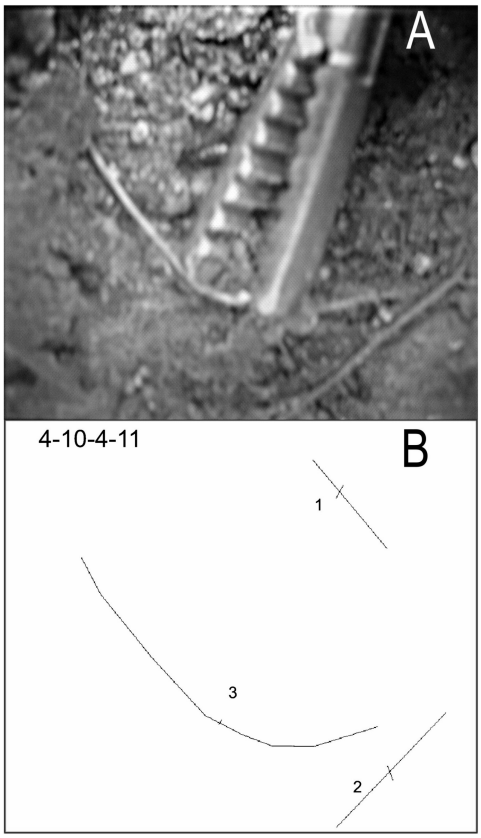

Figure 1. (A) Sampling a root with laparascopic forceps. (B) Digitized tracing of roots from the frame shown in panel A, identified by their database ID numbers. The code (4-10-4-11) at the top of the tracing identifies the plot (4), treatment (10), minirhizotron tube (4) and frame number (11).

Air temperatures ranged between 0 and 4 °C during the entire sampling period. Each sample was stored submersed in 80% ethanol in a microcentrifuge tube on ice. Because starch- and sucrose-degrading enzymes are denatured by ethanol (Hendrix and Peelen 1987), all samples were stored at –20 °C, within 4 h of sampling, until analysis. Soluble sugars (glucose, fructose and sucrose) were extracted from intact roots as described by Hendrix (1993), with the exception that samples were extracted with 0.5 ml of 80% ethanol, and extracts pooled and evaporated to dryness in a 55 °C oven before resuspension in 200 µl of 80% ethanol for analysis.

After extraction of soluble sugars, each root sample was cleansed of any adhering soil by gentle rinsing with 80% ethanol in a watch glass. Clean roots were frozen and lyophilized at –25 °C. The lyophilized roots were weighed and then stored at –20 °C in a desiccator until starch analysis.

Roots were ground in a miniature ball mill (Wigg-L-Bug, Crescent Dental Manufacturing, Lyons, IL) before starch digestion, as preliminary assays indicated that starch recovery from intact roots was lower than from ground tissue (data not shown). Roots were ground in microcentrifuge tubes with

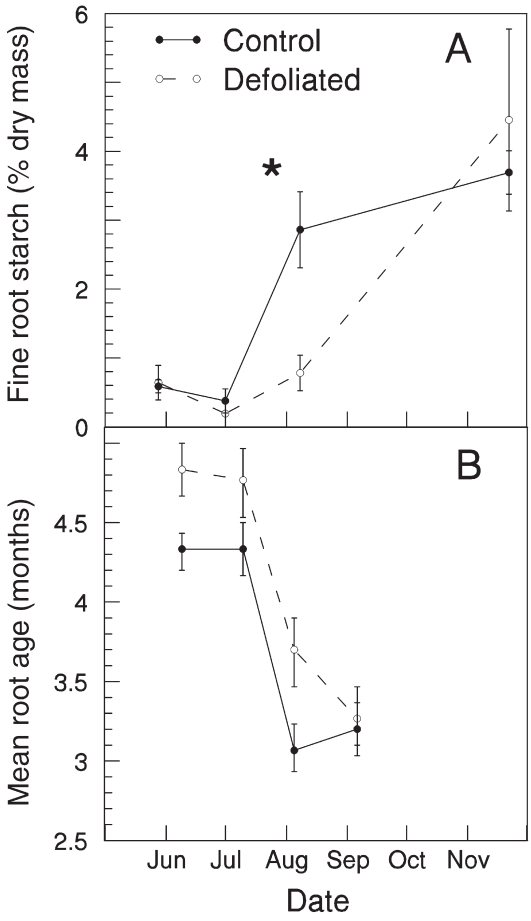

Figure 2. (A) Defoliation effects on bulk-sample fine root starch in 1997. (B) Defoliation effects on mean age (months) of root population observed in minirhizotrons. Values are means ± standard error. Error bars smaller than the symbol are not shown; * = $P < 0.05$ (August sample).

3.5 mm diameter ZrO_2 balls (Glen Mills, Clifton, NJ); the ceramic balls were left in the microcentrifuge tube during all steps of starch gelatinization and digestion. Starch digestion was performed as described by Hendrix (1993), with modifications for small sample sizes described below.

After adding 0.5 ml of H_2O to each sample, starch was gelatinized by heating for 1 h in a water bath set at 100 °C. Two hundred µl of α-amylase (Sigma) was added for the first starch digestion step, and 0.5 ml of amyloglucosidase (Sigma) was used for the second starch digestion step. The digested samples were frozen overnight (–20 °C) and then lyophilized. Lyophilized digests were resuspended in 200 µl of water, mixed, and centrifuged for 5 min to clear the supernatant. Glucose concentration was measured in duplicate 20-µl samples (Hendrix 1993).

Sample ash masses were obtained after starch analysis. Because the starch digests were quite viscous, 200 µl of trypsin (Sigma; 0.5 mg ml^{-1} in 0.2 mol l^{-1} HEPES and 4 mmol l^{-1} $CaCl_2$, pH 7.8) was added to each solution to digest the α-amylase and amyloglucosidase. Digested samples were vacuum filtered onto pre-ashed, pre-weighed 13-mm glass-fiber filters (Gelman Type A/E, No. 61628, Pall Gelman Laboratories, Ann Arbor, MI), each supported on an 11.5 mm Hirsch funnel (Coors No. 60297, VWR Scientific, West Chester, PA). Samples were ashed at 550 °C for at least 4 h.

Ash-free dry masses were used to calculate root TNC concentration. This was necessary to correct for the mass of the clay film that was observed on many roots after washing. Given the small mass of most root samples, inclusion of any soil mass in calculations would lead to substantial errors in estimating TNC concentrations.

Samples less than 0.08 mg dry mass (measured after ethanol extraction) were excluded from the composition analysis. Preliminary examination of the data indicated that soluble sugars in these samples were always below the limit of detection of our colorimetric assay.

Analysis of variance for effects of defoliation treatment, root age and root type on root sugars and starch, and for effects of defoliation on mean root age were carried out with the SAS statistical software package (PROC MIXED, Littell et al. 1996), with defoliation, root age and root type as fixed effects and blocks as a random effect. Logarithmic regression was carried out with CoStat software (Cohort Software, Monterey, CA). Because the starch and soluble sugar data were strongly skewed, we used a rank transformation before analysis (Conover and Iman 1981).

Results

Defoliation transiently increased the mean age of roots observed in the standard minirhizotrons (Figure 2B) from June through August, although the effects were not statistically significant (June, $F_{1,6} = 0.62$, $P = 0.46$; July, $F_{1,3} = 0.24$, $P = 0.66$; August, $F_{1,3} = 0.3$, $P = 0.62$) because of large block × defoliation interactions (June, $\chi^2 = 7.2$, $P < 0.01$; July, $\chi^2 = 27.3$, $P < 0.001$; August, $\chi^2 = 27.1$, $P < 0.001$).

Soluble sugar concentrations in each root type (white, brown or dead) were highly variable (Figure 3). Partly as a consequence of this variability, there was no significant main effect of defoliation ($F_{1,118} = 3.03$, $P = 0.08$) on root soluble sugar concentration (Figure 3). There was a significant effect of root type ($F_{2,116} = 4.39$, $P = 0.01$) on soluble sugar concentration, and a significant interaction between the effects of defoliation and root type on soluble sugar concentration ($F_{2,117} = 4.30$, $P = 0.02$). Both effects were a result of the high concentration of soluble sugars in white roots from undefoliated plots (which significantly exceeded all other treatment combinations; $P < 0.05$, pairwise t-tests). Although most white roots analyzed (29 out of 40) were in the 1-month age class, a few were older (2 months, $n = 5$; 3 months, $n = 4$; 4 months, $n = 1$; 6 months, $n = 1$). Neither defoliation ($F_{1,137} = 0.06$, $P = 0.81$) nor root type ($F_{2,137} = 0.54$, $P = 0.58$) had a significant effect on starch concentration (Figure 3).

Root age effects were analyzed for the population of living roots (white and brown). There were no significant effects of root age on soluble sugar ($F_{6,106} = 1.40$, $P = 0.22$) or starch concentrations ($F_{6,106} = 1.14$, $P = 0.35$) (Figure 4). There were no

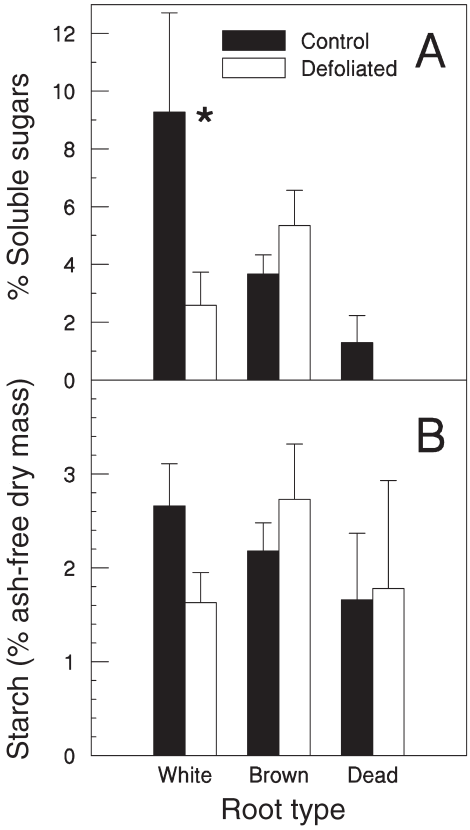

Figure 3. Mean concentrations of (A) soluble sugars (glucose, fructose and sucrose) and (B) starch for roots of each type (brown, dead or white) from trees in the defoliated and undefoliated plots. Values are means + standard error, and include roots of all age classes; * = $P <$ 0.05.

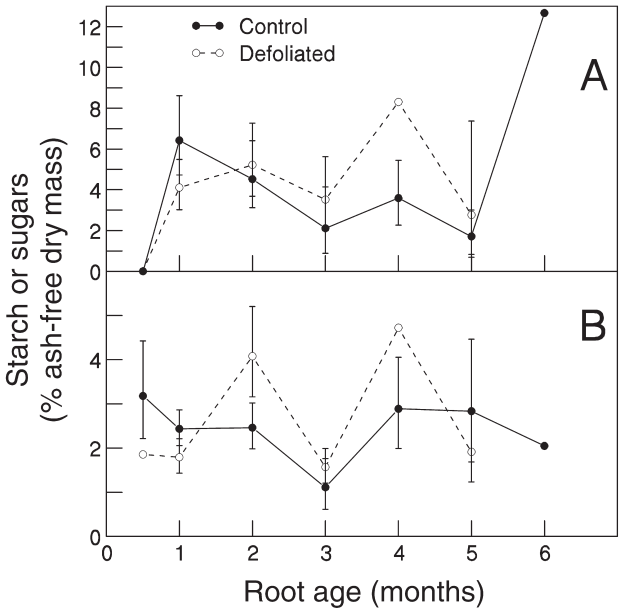

Figure 4. (A) Individual live root soluble sugar (glucose, fructose and sucrose) concentration and (B) starch concentration as a function of root age (months). Values are back-transformed means ± standard error from log-transformed data. Closed symbols (●) and solid lines represent data from trees in control plots; open symbols (○) and dashed lines represent data from trees in defoliated plots.

significant root age × defoliation interactions (sugars, $F_{5,100} =$ 0.98, $P = 0.43$; starch, $F_{5,100} = 0.96$, $P = 0.44$). The median soluble sugar concentration was much lower than the mean for roots of all ages (data not shown). This difference between median and mean soluble sugar concentrations, combined with the high standard error for mean soluble sugar concentrations, suggested that the data were not normally distributed. We combined data from live roots of all ages to investigate the frequency distribution of soluble sugar concentrations. The distribution was highly right-skewed (Figure 5) and could be fit to an exponential function ($F_{1,6} = 23.90$, $P < 0.01$). Although the mean and median root starch concentrations were similar (data not shown), the frequency distribution for root starch concentration in live roots was also right-skewed (Figure 5), and can also be fit to an exponential function ($F_{1,10} = 18.98$, $P < 0.01$).

Neither soluble sugar concentration nor starch concentration of live roots was correlated with sample mass or root diameter (data not shown). There was no correlation between soluble sugar concentration and starch concentration of individual live roots (data not shown). Root diameter growth during the study was negligible; the mean increase in diameter was about 0.05 mm. There was no evidence that older roots were developing into larger-diameter structural roots; root age did not significantly affect root diameter growth ($P > 0.3$, data not shown).

Discussion

Shifts in root system age structure did not play a role in defoliation effects on root starch concentration. Defoliation caused a transient decline in new root production, but had no effect on root mortality (Kosola et al. 2001). Roots from defoliated plots were on average older than roots from control plots early in the season (Figure 2B), although effects varied widely among blocks. The rate of mortality was constant for each age class of fine roots (Kosola et al. 2001). Root soluble sugar and starch concentrations were not significantly correlated with root age (Figure 4) at our sampling date in November. Data from additional sampling dates are needed to determine if this finding is consistent throughout the growing season. It is unlikely that defoliation effects on bulk root starch concentration (Figure 2A) were caused by shifts in root population age structure. The increase in mean root age in response to defoliation (Figure 2B) would cause a decline in root nonstructural carbohydrates (starch and soluble sugars) only if root age and nonstructural carbohydrates were negatively correlated.

Developmental changes in roots produced during defoliation are also unlikely to have caused the transient difference in root starch concentration observed in defoliated and undefoliated trees. Marshall and Waring (1985) hypothesized that the root life span of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings was determined by the initial root

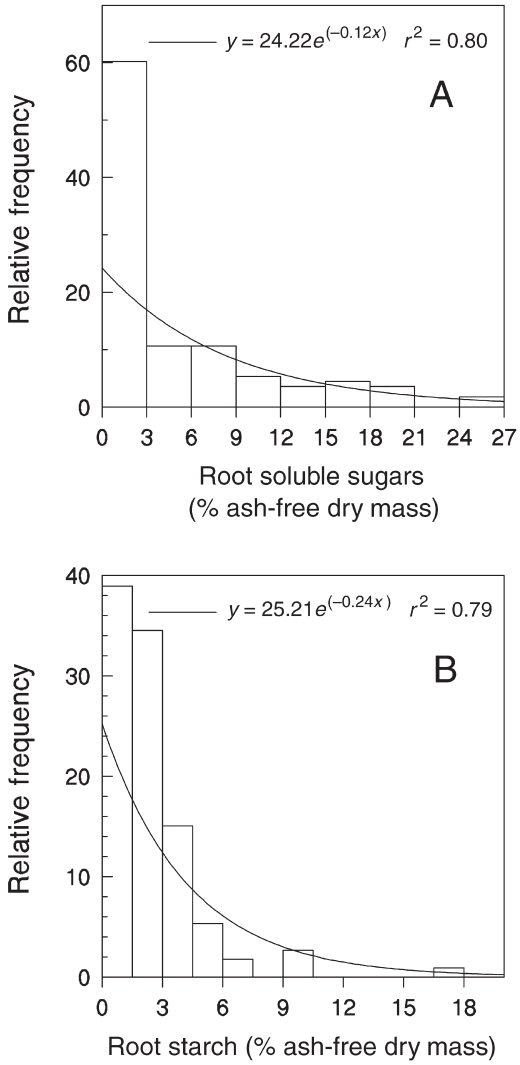

Figure 5. Frequency distribution for (A) root soluble sugar (glucose, fructose and sucrose) concentration and (B) root starch concentration. Data are for live roots of all age classes and defoliation treatments combined; $n = 113$. Class sizes are 3% for soluble sugars and 1.5% for starch.

starch concentration established during root formation, and that roots died when the initial pool of starch was depleted. If this hypothesis applies to poplar roots, we would expect root starch concentration to decline with root age. Furthermore, transient declines in root starch concentration caused by defoliation (Figure 2A) would lead to long-term differences in root starch concentration. Roots produced during a defoliation event would always have less starch than roots of the same age produced either before defoliation or after recovery from defoliation. Our data did not support the hypothesis that starch concentration is determined at root formation. We did not observe a significant decrease in starch concentration with increasing root age, nor was there a long-term effect of defoliation on fine root starch concentration. Our data are most consistent with a labile and dynamic pool of starch in fine roots of Eugenei poplar, as previously suggested by Nguyen et al. (1990), based on their observations of large seasonal changes in root starch concentration.

Defoliation had no effect on soluble sugars in bulk samples of roots, even in samples collected at peak defoliation (Kosola et al. 2001). In contrast, there was a significant interaction between defoliation and root type on soluble sugar concentration in individual roots sampled in November. This was a result of the high concentration of soluble sugars in white roots from trees in undefoliated plots (Figure 2). Separate analysis of white roots from a bulk sample may provide a sensitive indicator of defoliation effects on carbon allocation to roots.

The high variation in soluble sugar and starch concentrations, and the logarithmic frequency distribution of root soluble sugar and starch concentrations are both striking. The negative exponential frequency distribution of soluble sugar and starch concentrations may reflect developmental or structural variation among roots. Fine-root branch order is known to have a strong effect on fine root C/N ratio (Pregitzer et al. 1997), and it may affect soluble sugar or starch concentrations as well. The probability of sampling a root from any particular branching order increases exponentially from the most proximal (least frequent) to the most distal and most frequently sampled roots; the rate of increase depends entirely on the pattern of branching. We speculate that roots with the lowest soluble sugar concentration are the highest order roots, because they are most distal from the source of photosynthate. Models incorporating carbon transport resistance (Thornley 1972) can generate longitudinal patterns in carbon partitioning along a kiwifruit cane that match field observations (Greaves et al. 1999); similar processes are likely to play a role in root systems. It is also possible that we were sampling roots from different trees, which may have differed in carbon status. Both excavation on our site and published reports (Friend et al. 1991) indicate that the lateral roots of hybrid poplars in high-density plantings can spread at least 5 m from the trunk. Tracing of roots back to their origin would be required to test the relative influence of either root branch order or tree of origin on root TNC status.

By including rhizosphere soil with some roots, we have introduced root exudates in the ethanol extract. There was no significant correlation between rhizosphere soil mass and soluble sugar concentrations measured in roots (data not shown), so there was no systematic bias introduced by root exudates in rhizosphere soil. Although inclusion of some rhizosphere soil introduced additional variation in soluble sugar measurements, we believed that washing roots would also have introduced error by leaching soluble sugars from the roots. It must also be considered that the pattern of variation we saw in root soluble sugar and starch concentrations was an artifact caused by systematic variation in extraction efficiency. This is a particular concern for extraction of soluble sugars from intact root segments, particularly large roots, because of diffusion limitations. The lack of correlation between sample mass (data not shown) or root diameter (data not shown) and soluble sugar concentration indicates that soluble sugar extraction was not significantly affected by these technical difficulties.

Tree root systems may contain a population of roots with a

wide range of ages and conditions. As a result, attempts to create detailed mechanistic models of short-term forest ecosystem responses to disturbance will require information on the role of individual roots in the aggregate response of the entire root system. Root age effects on composition and metabolism, the rate of root turnover, and the degree of individual root plasticity may all influence the rate of change in aggregate root system properties following a disturbance.

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