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Cottonwood growth rate and fine root condensed tannin concentration

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Summary We examined the relationship between trunk diameter and diameter relative growth rate (RGR) and fine root condensed tannin concentration in 12 genotypes of eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) planted in three locations across the north central United States. Across genotypes, trunk diameter, diameter RGR and root condensed tannin concentration were negatively correlated at one location (Wisconsin), but showed no significant correlation at the other locations (Iowa and Michigan). The factors responsible for this difference among sites remain unidentified, but may be related to soil fertility.

Keywords: phytochemistry, Populus deltoides, root defense, short-rotation, soil nitrogen, woody biomass.

Introduction

Differences in root composition affect root litter quality and can potentially alter rates of carbon and nutrient cycling in forest ecosystems (Hendricks et al. 2000, Silver and Miya 2001). Root composition may also affect root–herbivore interactions (Karban 1980, 1982, Andersen 1987, Eissenstat et al. 2000, Yanai and Eissenstat 2002, Wells et al. 2002*b*). Trees for short-rotation woody biomass production are selected for rapid growth (Riemenschneider et al. 2001), but it is unknown if these fast-growing genotypes produce root litter low in defensive compounds, and hence with potentially decreased root life span (Yanai and Eissenstat 2002). Studies on growth–leaf composition relationships indicate that slower-growing plant species typically have longer-lived leaves with greater leaf thickness, lower N concentration, higher concentrations of lignin and defensive compounds, and lower photosynthetic assimilation rate per unit leaf mass than faster-growing species (Lambers and Poorter 1992, Reich et al. 1992, Craine et al. 2001). This apparent tradeoff between growth rate and leaf defense (Herms and Mattson 1992) has been observed among genotypes of aspen (*Populus tremuloides* Michx.). Leaves of fast-growing aspen genotypes have lower concentrations of secondary compounds—tannins, lignin and phenolic glycosides—than leaves of slow-growing genotypes (Lindroth and Hwang 1996, Hwang and Lindroth 1997, 1998).

If the tradeoff between growth rate and defense can be generalized from leaves to the whole plant, then slower-growing plants should have better-defended roots, and consequently lower rates of root turnover and root decomposition. In comparisons between fast-growing and slow-growing heathland species, root turnover and root decomposition were consistently slower in slow-growing species (e.g., *Erica tetralix* L.) than in fast-growing species (e.g., *Molinia caerulea* L.) (Berendse et al. 1987, Aerts et al. 1989, Van Vuuren et al. 1993). Slower root turnover in slower-growing plants has also been observed among perennial grasses; both root tissue mass density and root turnover were negatively correlated with plant relative growth rate (Ryser 1996, Wahl and Ryser 2000). Data on root defensive compounds were not presented in these reports.

We hypothesized that there is a negative correlation between tree growth rate and the concentration of root defensive compounds (phenolic glycosides and condensed tannins) (Palo 1984, Bryant et al. 1987) in cottonwood. If true, faster-growing cottonwood genotypes could be more susceptible to damage from root herbivory than slow-growing cottonwood genotypes. To test this hypothesis, we examined relationships among trunk diameter and diameter relative growth rate (RGR), site quality and condensed tannin composition of roots of 12 genotypes of *Populus deltoides* Bartr. ex Marsh., eastern cottonwood, growing at three locations across the north-central USA.

Materials and methods

We studied 12 *P. deltoides* genotypes exhibiting a wide range of trunk diameter growth rates. The genotypes sampled were 180-1, 192-2, 193-5, 51-2, 91.05–06, 91.05–10, D104, D105, D108, D109, D114 and D5 (Riemenschneider et al. 2001). The selected genotypes, which comprise part of a regional trial of 43 *P. deltoides* genotypes (Riemenschneider et al. 2001), were

sampled at three locations across the north-central USA: East Lansing, MI; Arlington, WI; and Ames, IA. All trees were planted in 1995 in a randomized incomplete block design, with 5–10 replicate two-tree plots of each genotype. Each cottonwood genotype was cloned from cuttings originally collected from trees in natural populations throughout the north-central USA. Diameter at breast height (DBH = 1.4 m) had been measured yearly on all trees at all locations. There is a significant genotype × environment interaction on tree growth at the three locations (Riemenschneider et al. 2001, D.I. Dickmann, unpublished data).

We collected two soil cores (6-cm diameter and 10-cm deep) midway between the two trees of each replicate block on April 12 (Wisconsin), April 25 (Iowa) and May 17 (Michigan) 2000, before full leaf-out. The soil cores were stored on ice and transported to the laboratory. The two soil cores were combined before washing. Roots were washed from the soil cores on 2-mm sieves within 3 h of sampling and then stored on ice for up to 8 h before being frozen in liquid nitrogen. Frozen roots were either placed in a –80 °C freezer immediately or stored on dry ice for up to 24 h before being placed in a –80 °C freezer. Roots were lyophilized at –25 °C within 6 months of sampling. The sample collection and processing regime was chosen to reduce potential degradation of phenolic compounds and condensed tannins (Lindroth and Pajutee 1987, Lindroth and Koss 1996). Lyophilized tissue was ground fine enough to pass a 40-mesh screen and stored over desiccant at –20 °C until analyzed.

Root tissues were analyzed for condensed tannin concentration by the acid butanol colorimetric assay (Czochanska et al. 1980). Both intraspecific variation (among dates within a growing season) and interspecific variation in leaf condensed tannin composition have been reported to affect the response of the acid butanol assay (Appel et al. 2001). To control for the possibility that condensed tannin composition might vary among locations, condensed tannin standards were purified from composite samples from each location according to the method described by Hagerman and Butler (1980). The site-specific composite samples comprised equal amounts of lyophilized tissue from each of the12 genotypes.

Three replicate sets of soil samples were taken from each site in October 2002. Each replicate was a composite of twenty 2.5-cm-diameter × 10-cm-deep soil cores sampled from within two adjacent blocks. Nitrogen mineralization potential (ammonium production during anaerobic incubation (Waring and Bremner 1964) and extractable nitrogen (extracted in 2 M KCl, Robertson et al. 1999) were measured for each soil sample. For both assays, nitrogen was measured spectrophotometrically as ammonium by the modified Berthelot reaction; nitrate was converted to ammonium by addition of Devarda's alloy (Rhine et al. 1998).

Analysis of variance (ANOVA) of location and genotype effects on root condensed tannin concentration was carried out with the SAS Proc Mixed program, with blocks as random variables, and locations and genotypes as fixed variables (SAS Institute, Cary, NC). Because distributions of the mean DBH, DBH RGR and root condensed tannin data were significantly right-skewed, we used a rank transformation before ANOVA (Conover and Iman 2001). Because of a violation of the assumption of bivariate normality required for linear regression, and the small sample size, a non-parametric test (Spearman's rank correlation) was used to determine the relationship between trunk diameter growth rate and root condensed tannin concentration.

Results

Standard curves for the acid butanol condensed tannin assay were site-specific (Figure 1). The slope was significantly lower ($P < 0.05$) for tannin purified from the Iowa samples than for tannin purified from the Michigan and Wisconsin samples (Figure 1).

Site quality varied among the three locations. Mean tree DBH across the 12 genotypes differed significantly ($P < 0.0001$) among sites (Figure 2). Trunk diameters were smallest at the Michigan site (DBH = 10.2 ± 0.4 cm), intermediate at the Iowa site (DBH = 11.2 ± 0.5 cm) and highest at the Wisconsin site (DBH = 13.8 ± 0.7 cm). Trunk diameter RGR did not differ significantly among sites, but both trunk diameter and trunk diameter RGR varied significantly ($P < 0.0001$) among genotypes, with the magnitude of the differences depending on site (Figure 3). Nitrogen mineralization was highest in soil samples from Iowa, intermediate in soil samples from Wisconsin, and lowest in soil samples from Michigan (Table 1). Extractable nitrogen was significantly higher ($P < 0.05$) in soils at the Wisconsin site than in soils at the other sites (Table 1), primarily because of high concentrations of extractable NO_3^- (data not shown).

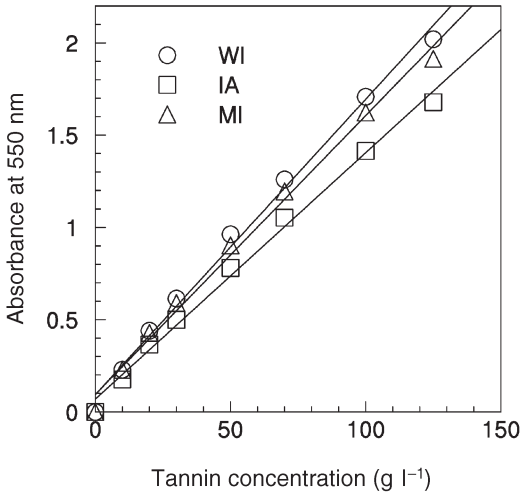

Figure 1. Standard curves derived from the acid butanol assay for condensed tannin. Data are absorbance at 550 nm versus purified fine root condensed tannin concentration (g l^{-1}). For each location, fine root condensed tannin was purified from combined samples containing equal weights of lyophilized, ground root material from each genotype. Error bars are smaller than the symbols. Michigan (MI), $y = 0.09 + 0.015x$, $r^2 = 0.99$; Iowa (IA), $y = 0.07 + 0.013x$, $r^2 = 0.99$; Wisconsin (WI), $y = 0.09 + 0.016x$, $r^2 = 0.99$.

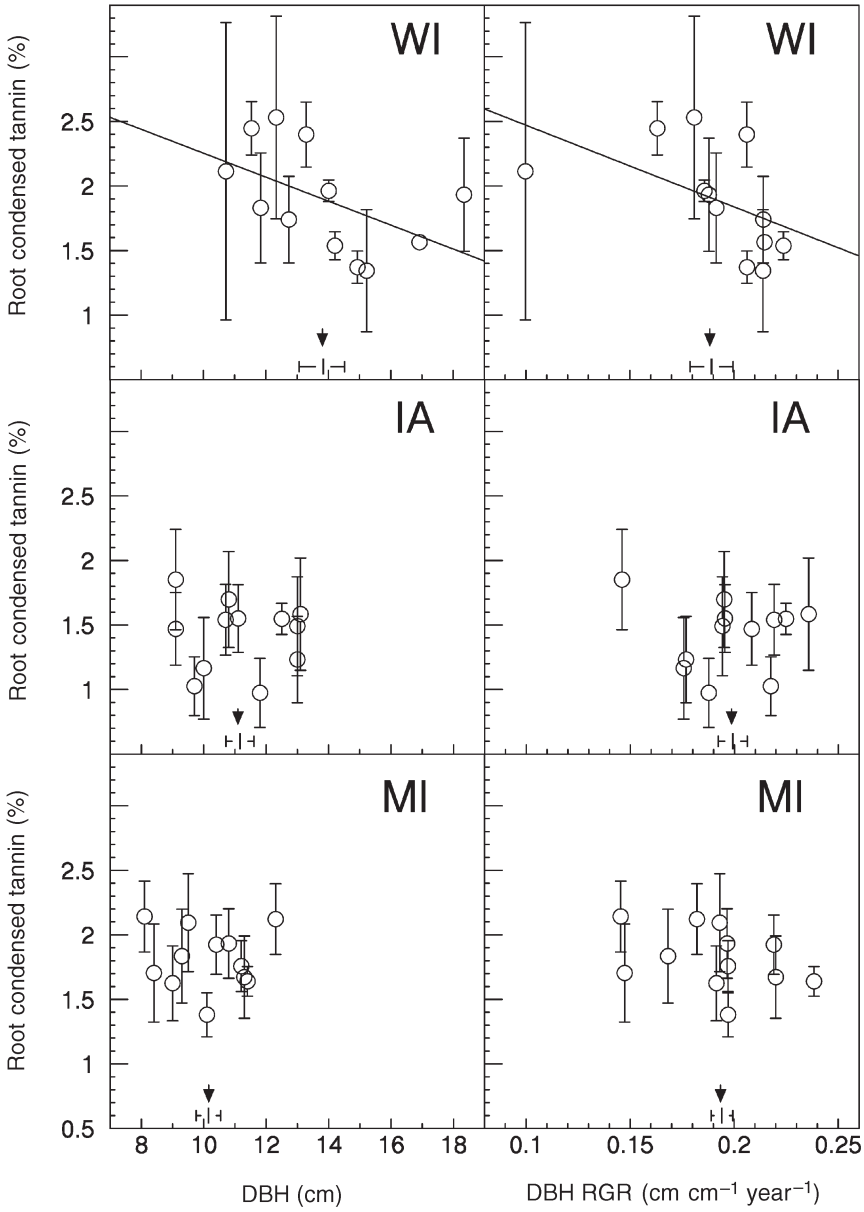

Figure 2. The relationship between fine root condensed tannin concentration and trunk diameter (DBH) and or trunk diameter relative growth rate (DBH RGR) in *Populus deltoides* genotypes at three north-central USA locations. Trees were planted in 1995. Relative growth rates are based on the DBH increase over the 1999 growing season. Samples were collected in East Lansing, Michigan (MI); Ames, Iowa (IA); and Madison, Wisconsin (WI). Each value represents the mean for a single genotype; bars are ± standard error, minimum $n = 3$. The mean DBH or DBH RGR among all cultivars for each location is indicated by a vertical line (arrow) with horizontal error bars, $n = 12$. Both DBH and DBH RGR were significantly negatively correlated with root condensed tannin concentration in Wisconsin (DBH Spearman's $r = -0.62$, $P = 0.03$; DBH RGR Spearman's $r = -0.78$, $P = 0.003$).

Across genotypes, both trunk diameter and trunk diameter RGR were significantly negatively correlated with root condensed tannin concentration at the Wisconsin site (DBH Spearman's $r = -0.62$, $P = 0.03$; DBH RGR Spearman's $r = -0.78$, $P = 0.003$), but not at the Michigan or Iowa sites (Figure 2). Root condensed tannin concentrations did not differ significantly among the 12 genotypes at any site (genotype effects: WI, $F_{11,33} = 1.7$, $P = 0.12$; IA, $F_{11,34} = 0.8$, $P = 0.7$; MI, $F_{11,32} = 0.9$, $P = 0.6$). Mean root tannin concentration differed significantly between sites ($F_{2,102} = 8.23$, $P = 0.0005$). The overall mean tannin concentration was significantly lower in the Iowa samples (1.43 ± 0.08) than in the Michigan (1.82 ± 0.07, $P = 0.006$) and Wisconsin (1.90 ± 0.12, $P = 0.007$) samples. Among genotypes, only D108 had root condensed tannin concentrations that differed significantly among sites ($F_{2,8} = 5.01$, $P = 0.04$), with the highest concentrations in Wisconsin. The genotype × environment (site) interaction was not significant for root condensed tannin concentration ($P = 0.7$).

Despite the absence of a genotype × environment interaction on root condensed tannin concentration, root condensed tannin concentrations showed a tendency to differ among genotypes (Figure 3). One set of genotypes showed a consistent increase in root condensed tannin concentration with increasing soil N, with maximum values at the Wisconsin site (Figure 3). In a second set of genotypes, root condensed tannin concentration reached a maximum value at the intermediate soil N concentration found at the Michigan site (Figure 3).

Discussion

The negative correlation observed in Wisconsin between root

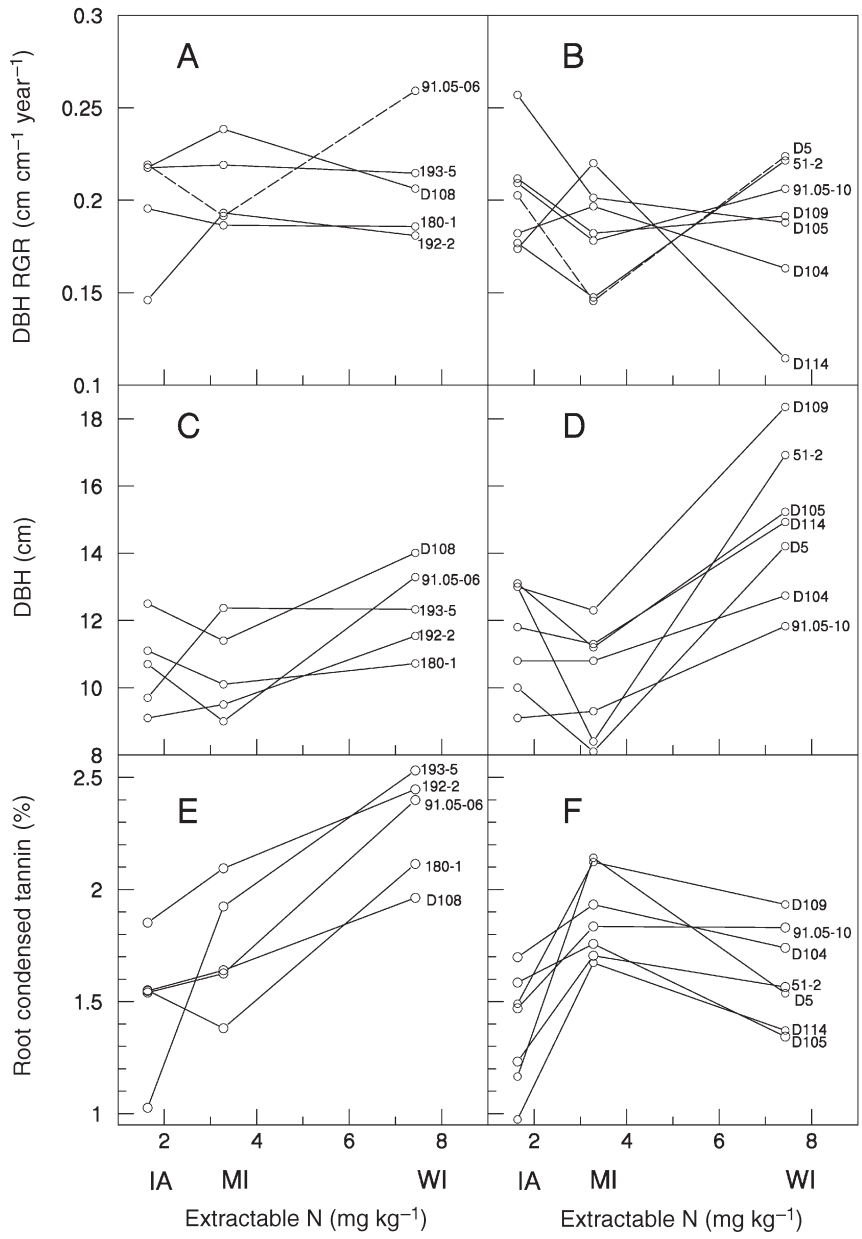

Figure 3. The relationship between extractable soil nitrogen at each site (IA, MI, or WI) and trunk diameter relative growth rate (DBH RGR) (A and B), trunk diameter (DBH) (C and D) and root condensed tannin concentration (E and F) for individual genotypes. Genotypes are separated into two sets based on whether root condensed tannin concentration consistently increases with increasing soil N concentration (panels A, C and E) or plateaus or decreases with increasing soil N concentration (panels B, D and F). Dotted lines in panels A and B are to improve legibility.

condensed tannin concentration and both trunk diameter, which integrates lifetime growth, and trunk diameter RGR, which reflects the past season's growth, is consistent with previous reports of negative correlations between tree growth rate and leaf condensed tannin in aspen grown in moderately fertile soils in pots (Lindroth and Hwang 1996, Hwang and Lindroth 1997, 1998). We observed no genotype × environment (G × E) interaction for root condensed tannin concentration, even though there was a significant G × E effect for trunk diameter and trunk diameter RGR. In contrast, Osier and Lindroth

Table 1. Soil properties at the three sampling locations: Madison, Wisconsin; East Lansing, Michigan; and Ames, Iowa. Soil samples were collected in October 2002.

Soil trait	Wisconsin	Michigan	Iowa
Series and texture	Plano silt loam	Capac loam	Spillville loam
Extractable nitrogen (mg kg^{-1})	7.43 ± 1.46	3.28 ± 0.02	1.64 ± 0.16
N Mineralization potential (mg kg^{-1} day^{-1})	0.50 ± 0.07	0.27 ± 0.08	0.72 ± 0.13

(2001) found a significant fertilizer × genotype interaction for aspen (*Populus tremuloides* Michx.) leaf condensed tannin concentration.

Differences among sites in soil nutrient availability (Table 1) may be partially responsible for variation among sites in the correlation between tree growth and root condensed tannin concentration (Figure 2). Nitrogen availability often has large effects on leaf phenolics in trees, with N stress increasing and N fertilization decreasing leaf phenolic concentrations (Bryant et al. 1983, Lawler et al. 1997). Soil N and K concentrations have also been observed to alter root defensive compounds in several tree species. In loblolly pine, root phenolic concentrations increased with decreasing N availability and increasing C availability (Gebauer et al. 1998). Similarly, concentrations of phenolic compounds in Douglas-fir roots increased with decreasing K availability (Shaw et al. 1998).

In contrast to these observations, we did not find a consistent negative correlation between root condensed tannin concentration and extractable soil nitrogen. Variation in mean condensed tannin concentrations among sites was associated with complex across-site variations in root condensed tannin concentrations among genotypes (Figure 3). Genotype diameter relative growth rates were not closely linked to root condensed tannin concentrations across sites (Figure 3), and so do not conform to the theoretical predictions (Bryant et al. 1983, Herms and Mattson 1992). The poor correlation between tree diameter relative growth rate and root condensed tannin concentration (Figure 3) suggests that growth rate and defense responses are not tightly linked in these trees, and may be responding independently to different environmental signals. The genotypes can be separated into two classes (Figure 3), that appear to differ in the threshold value of soil N above which root condensed tannin concentration plateaus or decreases.

Climate varied among sites. Although it is likely that climate variation influenced tree diameter growth, it is unclear if it also affected root condensed tannin concentration. The Michigan and Iowa sites were both slightly warmer than the Wisconsin site (mean annual air temperature in 1992–2002 for Madison, WI = 8.2 °C, for Ames, IA = 8.9 °C, and for Lansing, MI = 8.4 °C; Midwest Regional Climate Center, mcc.sws.uiuc.edu/html/prodserv.htm). Total yearly precipitation was similar among the study sites during 1995–2000 (mean annual precipitation for WI = 85.2 cm, for IA = 80.1 cm, for MI = 74.7 cm; Midwest Regional Climate Center, mcc.sws.uiuc.edu/html/prodserv.htm). The sites vary significantly in local topography. The Wisconsin site is in a low spot among rolling hills, and may capture local precipitation runoff; the Iowa site is on a riparian floodplain; and the Michigan site is relatively higher in the local topography than the other two sites. These topographical differences may lead to differences in water availability among the sites.

Differences between sites in tree size reflect differences in site quality. Trunk diameter RGR for genotypes combined did not vary among sites, although the range of variation was greatest in Wisconsin. The range of variation in root condensed tannin concentrations was also greatest in Wisconsin. The lack of correlation between trunk diameter growth rate and root condensed tannin concentration at the Michigan and Iowa sites may be partially associated with the smaller range of trunk diameters and root condensed tannin concentrations at these sites compared with the Wisconsin site. This possibility could be tested by sampling more genotypes with a wider range of growth rates at the lower-quality sites.

Because the extinction coefficient for condensed tannin isolated from the Iowa samples was slightly different from that for the condensed tannins isolated from the Wisconsin and Michigan samples (Figure 1), we used separate standards purified from samples at each site. Had we, instead, used a bulked standard containing equal quantities of roots from each genotype at each site the results would likely have been similar, given the small differences in extinction coefficients among sites. Individual standards for each genotype at each site and sampling date may be advisable to reduce artifacts in future studies (Appel et al. 2001), at least until qualitative variation in root condensed tannin is better characterized for that study.

The small block size is likely to have contributed to variation in the root samples, because our study trees had overlapping root systems. Sampling midway between the two trees minimized the potential biomass contribution from adjoining trees. Replicate sampling from the randomized planting design also reduced the potential for systematic errors introduced by neighboring genotypes. Genotype or site differences in root system age structure (Kosola et al. 2001) or size class structure (Wells and Eissenstat 2001, Wells et al. 2002*a*) may also contribute to the variation in bulk samples of roots, because new roots are likely to contain lower amounts of defensive compounds than older roots.

For leaves, where there is a negative correlation between plant growth rate and leaf defense, faster-growing plants are more susceptible to herbivory, but possess a competitive advantage over slower-growing plants in the absence of herbivory (Herms and Mattson 1992, but see Siemens et al. 2002). If this holds true for roots, we hypothesize that roots from genotypes with low root tannin concentrations in Wisconsin will be more susceptible to herbivory than roots from genotypes with high root tannin concentrations. The quantitative importance of root herbivory in forest carbon and nitrogen cycling is largely unknown, although recent studies indicate that forest root losses to herbivory may be substantial (Stevens et al. 2002). Clarifying the links between plant growth rate, root defense, nutrient availability, root life span and root decomposition rates is crucial for understanding the belowground components of forest ecosystem carbon and nutrient cycling.

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