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THE ALLOMETRY OF ROOT PRODUCTION AND LOSS IN SEEDLINGS OF *ACER RUBRUM* (ACERACEAE) AND *BETULA PAPYRIFERA* (BETULACEAE): IMPLICATIONS FOR ROOT DYNAMICS IN ELEVATED CO_2[1]

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Total root production (ΣP), total root loss (ΣL), net root production (NP), and biomass production were determined for seedlings of *Betula papyrifera* and *Acer rubrum* in ambient and elevated CO_2 environments. ΣP, ΣL, and NP were calculated from sequential, independent observations of root length production through plexiglass windows. Elevated CO_2 increased ΣP, ΣL, and NP in seedlings of *Betula papyrifera* but not *Acer rubrum*. Root production and loss were qualitatively similar to whole-plant growth responses to elevated CO_2. *Betula* showed enhanced ΣP, ΣL, and biomass with elevated CO_2 but *Acer* did not. However, the observed effects of CO_2 on root production and loss did not alter the allometric relationship between root production and root loss for either *Acer* or *Betula*. Thus, in this experiment, elevated CO_2 did not affect the relationship between root production and root loss. The results of this study have important implications for the potential effects of elevated CO_2 on root dynamics. Elevated CO_2 may lead to increases in root production and in root loss (turnover) where the changes in root turnover are largely a function of the magnitude of root production increases.

Key words: *Acer rubrum,* allometry; *Betula papyrifera*; carbon dioxide; root production; root loss.

Like leaves within the canopy of a tree, fine roots are ephemeral organs whose primary function is resource acquisition. Unlike the canopy of most trees, root production and root loss are concurrent and continuous through the year (Hendrick and Pregitzer, 1992, 1993a, b; Berntson, Farnsworth and Bazzaz, 1995). In the last few years several researchers have demonstrated that the fine roots of temperate deciduous trees are extremely dynamic. Within a single year up to 40-50% of fine roots can die and disappear within the soil of many temperate forests (Hendrick and Pregitzer, 1992, 1993a, b; Burke and Raynal, 1994; Fahey and Hughes, 1994). The factors regulating root production and root longevity in forests, and hence root turnover, are not well understood. Both temperature and nitrogen are known to affect root longevity (Hendrick and Pregitzer, 1993a, b; Hendricks, Nadelhoffer, and Aber, 1993; Pregitzer, Hendrick, and Fogel, 1993). Thus, it is likely that edaphic factors are important in regulating patterns of root production and loss in forests.

Atmospheric CO_2 concentrations have increased from pre-industrial levels of 270 μL/L to current levels in excess of 356 μL/L (Boden et al., 1994). If this exponential rise in atmospheric CO_2 continues, atmospheric concentrations will be doubled some time in the 21st century. Increases in atmospheric CO_2 levels can exert a direct influence on both the physical environment and on the biological environment. CO_2 and other greenhouse gases are radiatively active, and thus can cause global warming (e.g., Schneider, 1989). Increasing CO_2 levels can lead to significant alterations in plant physiology, plant growth, and plant community dynamics (reviewed in Bazzaz, 1990). The effects of elevated CO_2 atmospheres on belowground plant growth and associated processes are poorly understood (Rogers, Runion, and Krupa, 1994).

There has been much interest in determining the capacity of terrestrial vegetation for sequestering carbon from the atmosphere as a means of decreasing the future rate of increase in atmospheric CO_2 levels (reviewed in van Veen, Liljeroth, and Lekkerkerk, 1991; Wisniewski and Lugo, 1992; van de Geijn and van Veen, 1993). Many plant species of widely varying life forms and geographic distributions respond to increases in atmospheric CO_2 by increasing overall biomass production (Poorter, 1993). Increases in biomass with increasing CO_2 are typically accompanied by increases in the carbon to nitrogen (C:N) ratios of plant tissues (Bazzaz, 1990). This suggests that the increase in plant growth under elevated CO_2 can be viewed as a dilution of plant nutrients, which are supplied largely by plant roots, and by carbon, which is supplied via photosynthesis. This dilution effect has important implications for several aspects of nutrient cycling. Higher C:N ratios typically lead to slower decomposition rates (Melillo, Aber, and Muratore, 1982; Aber, Melillo, and McClaugherty, 1990), and this has been observed to be the case for plant material produced under elevated CO_2 (Coûteaux et al., 1991; Boerner and Rebbeck, 1995; Cotrufo and Ineson, 1995). Increased atmospheric CO_2 may also result in greater allocation of biomass belowground to maintain a balance of resources given that carbon fixation has increased (e.g., Norby, O'Neill, and Luxmoore, 1986). Recently Rogers, Runion,

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and Krupa (1994) reviewed studies of the growth of 150 different plant species under elevated CO_2 and they found that 87% of the species increased absolute production of roots, and 41% increased root mass relative to shoot mass.

Several studies have also found an increase in the total carbon allocated below ground in the form of roots and root-derived products (e.g., root exudates, sloughed root cells, and senesced roots; Whipps, 1985; Norby et al., 1987; Lekkerkerk, 1990; Billès, Rouhier, and Bottner, 1993; Rouhier et al., 1994). If increases in atmospheric CO_2 levels lead to an increase in the fixation of carbon relative to the acquisition of nutrients, thereby shifting the balance of resource acquisition toward carbon, we might expect that relative belowground carbon investment (including loss to the soil) would be increased (Bloom, Chapin, and Mooney, 1985). However, experimental data available for testing this hypothesis are mixed. Some studies have found that the proportion of organic matter lost from roots relative to root mass is not affected by CO_2 (Whipps, 1985; Norby et al., 1987; Billès, Rouhier, and Bottner, 1993; Rouhier et al., 1994). Others have found that the amount of root-derived carbon (including respiration) relative to net carbon fixation is increased with elevated CO_2 (Lekkerkerk, 1990). These observations suggest that elevated CO_2 may directly influence patterns of root loss (senescence) in relation to root production. Given the magnitude of root turnover in temperate deciduous forests, such an effect would have profound implications for patterns of carbon and nutrient cycling in temperate forest soils.

Standing root biomass, which is what most studies of root growth under elevated CO_2 report, is the difference between root production and root loss. However, it is not possible to infer patterns of gross root production or loss from standing biomass or length alone. To date, there is one study available to assess the effect of elevated CO_2 on root growth dynamics, including root turnover, under elevated CO_2 (Pregitzer et al., 1995). The purpose of this study is to explore these dynamics with other temperate forest species and to develop a new analytical approach for understanding root dynamics in a future high CO_2 world. Specifically, we focus on the following hypotheses: (Hypothesis 1) The influence of elevated CO_2 on whole-plant and belowground growth are qualitatively similar. If the effects of elevated CO_2 on biomass production and root dynamics are qualitatively similar, then we can make qualitative inferences about the patterns of root dynamics in the majority of current studies where whole-plant biomass responses but not root dynamics are available (e.g., Poorter, 1993; Rogers, Runion, and Krupa, 1994). (Hypothesis 2) Total root loss scales directly with both total and net root production. If root loss scales directly with root production, then we can make inferences about the causes and quantity of root loss from an understanding of those factors that alter root production. We decided to use an allometric approach to characterize the scaling relationship between root loss and production because simply examining the ratios of these parameters may lead to erroneous conclusions regarding the scaling of these parameters (e.g., Samson and Werk, 1986). (Hypothesis 3) The relationship between root loss and root production is not modified by an elevated CO_2 atmosphere. If elevated CO_2 does not affect root loss relative to root production, then we can probably deduce a great deal about root length growth under elevated CO_2 from an understanding of root length growth at ambient CO_2 levels.

TABLE 1. Summary of criteria used for selecting species used in this study.

Species	*Acer rubrum* (red maple)	*Betula papyrifera* (white birch)
Mycorrhizal association	Arbuscular	Ectomycorrhizal
Nonwoody roots	small diameter low SRL	very small diameter high SRL
CO_2 responsiveness[a]	1.07[b] 1.27[c] 1.05–1.60[e] 1.17–1.70[f]	1.24[b] 1.31[c] 2.00[d]
Life history	Growth rate variable Shade tolerant g	High growth rate Shade intolerant g

[a] Elevated CO_2 biomass/ambient CO_2 biomass.
[b] Bazzaz et al., 1990.
[c] S. L. Bassow and F. A. Bazzaz, unpublished data.
[d] Rochefort and Bazzaz, 1992.
[e] Bazzaz and Miao, 1993.
[f] Miao, Wayne, and Bazzaz, 1992.
[g] Burns and Honkala, 1990.

To explore these hypotheses we studied whole-seedling growth and root dynamics of two species of temperate deciduous trees common throughout large areas of northwestern North America, white birch (*Betula papyrifera* Marsh.) and red maple (*Acer rubrum* L.). We selected these species because of their contrasting mycorrhizal status, root morphology, responsivess to CO_2, and seedling growth rate and shade tolerance (Table 1).

MATERIALS AND METHODS

Seed collection, germination, and transplanting—Seeds for both species were collected from the Harvard Forest (Petersham, MA). Red maple seeds were collected in spring 1993. White birch seeds were collected in early fall 1992. Seeds were collected from a minimum of three trees per species. Seeds were stored in dry, dark, air-tight containers at 4°C until early spring 1994. On 12 April 1994 seeds of both species were sown into flats (0.75 m × 0.25 m) containing 2:2:1 peat : vermiculite : sand. The flats were kept moist and placed on benches in CO_2 controlled glasshouses. Half the flats were placed in approximately ambient CO_2 (400 μL/L, quartile deviation = 23 μL/L), the other half in elevated CO_2 (700 μL/L, quartile deviation = 20 μL/L). Flats received 70% full sun due to shading by glasshouse structures. On 8 May 1994 seedlings were transplanted from the flats into the growth containers used for the remainder of the study. Prior to transplanting, excess germination mix was gently shaken from the roots. For red maple, all transplanted seedlings had four leaves, two of which were <0.5 cm, and two of which were <2 cm. For white birch, all transplanted seedlings had two leaves that were between 1 and 2 cm in length.

Soil, growth containers, and growth conditions—Plants were grown in containers measuring 22 cm × 36 cm × 2 cm (internal dimensions, 1.6 L volume), constructed of plywood, wood spacers, and clear plexiglass. The wood portion of the pots was lined with polyethylene, which was sealed against the plexiglass with silicon sealant. The plexiglass covered one of the two large sides of each pot to allow nondestructive observations of root growth (e.g., Berntson and Woodward, 1992; Fitter

and Stickland, 1992; Berntson, Farnsworth, and Bazzaz, 1995). Opaque plastic covered the plexiglass to prevent exposing roots to light. Containers were held at 30° angles from vertical with the plexiglass facing down to encourage root growth along the plexiglass surface. The upper surface of each container was painted white to maximize albedo and thereby minimize fluctuations in soil temperature. Soil was a 2:1:1 mix of peat : coarse sand : forest floor material. We used this mix to achieve a soil mix that had intermediate water-holding capacity so that excess water would easily drain and provide mycorrhizal inoculum. The forest floor material (top 10 cm) was collected from a stand adjacent to a stream that was dominated by red maple with a few white birch in Beverly, Massachusetts, USA. No fertilizer was added to the soil.

A total of 48 plants per species were randomly distributed among six separate glasshouse modules. Half of the modules were maintained at approximately ambient CO_2 (400 μL/L, quartile deviation = 23 μL/L), the other half in elevated CO_2 (700 μL/L quartile deviation = 20 μL/L). Glasshouse modules were spatially arranged in pairs to create three blocks of paired CO_2 modules. Plants received 70% full sun due to shading of glasshouse structures. Day/night temperatures were maintained at 26°/19°C with a 13-h day throughout the experiment. Transitions between day/night temperatures were made over a 2-h period. At the beginning of the experiment there were 24 individual plants per species by CO_2 combination. For the first 2 wk of the experiment plants that died were replaced. After this time plant mortality resulted in a decrease in sample sizes. Final sample sizes varied between four and nine for each species × CO_2 × harvest combination.

Measurements of root production and loss—Starting 9 d after transplanting, the roots visible through the plexiglass were traced onto acetate sheets, and this procedure was repeated every 9 d. Traces were made by hand, using fine-point (0.5 mm) permanent markers. This tracing method was able to resolve roots <1 mm apart (Berntson, Farnsworth, and Bazzaz, 1995). In order to facilitate superimposing sequential images (by hand and within the computer), two position references markers were permanently etched into the top left and top right edges of the plexiglass. These markers were included in all tracings of root length. Tracings were digitized into a computer as 1-bit images using a flatbed scanner at 30 pixels/cm (LaCie Silverscanner™, LaCie, OR). Each digitized image was skeletonized (eroded to a single pixel in width) using the image processing program NIH Image (W. Rasband, NIH, v1.54). Total root length (in centimetres) was determined by summing the number of pixels within the image, correcting for whether a given pixel represented an orthogonal or a diagonal line (e.g., Pan and Bolton, 1991).

Standing root length is equivalent to the net production (NP) of roots. The net production of roots at a given time is the integral of the difference between root production and root loss up to that time.

$$\mathrm{NP}_f = \int_0^f (P - L)\, dt, \tag{1}$$

where f is the time of the measured standing root length, P is root production, and L is root loss. Root production and loss are continuous processes, but measurements of these processes are made over discrete intervals. Thus, it is more convenient to express NP as the difference between the discrete processes of production and loss.

$$\mathrm{NP}_f = \sum P_f - \sum L_f. \tag{2}$$

$\Sigma\ L_f$ and $\Sigma\ P_f$ are calculated by summing the Loss (L_t) and Production (P_t) values, respectively, determined by comparing all of the sequential tracings up to time f.

$$\sum L_f = \sum_{t=1}^{f-1} L_t \tag{3}$$

$$\sum P_f = \sum_{t=1}^{f-1} P_t. \tag{4}$$

L_t and P_t are gross values of root loss and production over the interval t. Interval t corresponds to the *preceding* interval before time f. Thus, L_1 represents the loss of root between t_0 and t_1. There are as many intervals as there are tracings. By definition, $L_1 = 0$ because there are no roots at $t = 0$ ($\mathrm{NP}_0 = 0$).

In this experiment we calculated L_t by directly comparing sequential tracings. Each pair of sequential tracings was placed on a light table and lined up. All of the roots present in the earlier tracing that are not present in the later tracing are traced onto a new sheet of acetate. This "loss composite" tracing was digitized and processed in the same way that the original tracings were to determine length. We decided to create the "loss composite" tracings manually because the individual roots between sequential tracings were sometimes out of alignment with one another by as much as 1–2 mm. Manually creating the loss composites allowed us to correct these misalignments as they were encountered. The use of an automated composite technique (e.g., Heeraman, Crown, and Juma, 1993) would have led to a simultaneous overestimation of both $\Sigma\ L$ and $\Sigma\ P$. Given L_t for all t and NP_f for all tracings, it is possible to solve for both $\Sigma\ P_f$ and P_t.

$$\sum P_f = \mathrm{NP}_f + \sum L_f \tag{5}$$

$$P_t = \mathrm{NP}_{t+1} - \mathrm{NP}_t + L_t. \tag{6}$$

This method of determining root production and loss is similar to the demographic method of monitoring root mortality (Hendrick and Pregitzer, 1992, 1993a; Pregitzer, Hendrick and Fogel, 1993; Pregitzer et al., 1995) and quantitatively identical to a root length dynamics approach (Hendrick and Pregitzer, 1993b). The demographic method of monitoring root mortality involves identifying a cohort of roots at the beginning of the observation period and following the fate of those roots through time. Similarly, the method used here follows individual roots through time. The main differences between the demographic method and the one used here are as follows. First, the demographic method involves following a single cohort through time. The method used here follows all the visible roots through time. Second, the demographic method keeps track of each individual root so that the life-span of individual roots can be determined. The technique used here only considers changes in length. Thus, the technique used here is one that follows the dynamics of root length production and loss, not the demography of roots.

Harvests and correction factors for nondestructive observations—At three times during the experiment one-third of the plants of each species by CO_2 combination were randomly selected and harvested. Harvest dates were 27, 45, and 63 d after transplanting. The following parameters were measured for each plant when it was harvested: fine root length, fine root mass, woody root mass, and shoot mass. Fine and coarse roots were separated for air-dried samples. Coarse roots were classified as those ≥0.5 mm, round (larger diameter fine roots became flattened), stiff, and brittle. Dry mass of roots and shoots was determined after oven-drying at 80°C for 2 d. Fine root length was determined by taking a subsample of fine roots from fresh roots. Each subsample contained ≈5–10% of fresh root mass. This subsample was carefully laid out on a sheet of plexiglass so that roots did not overlap. Roots were then digitized into a computer at 118.1 pixels/cm as an 8-bit gray scale image. Root length for these subsamples were determined using the technique developed by Pan and Bolton (1991). Root subsamples were dried, and masses were obtained after their length was determined. The specific root length (in centimetres per gram) of a given plant's fine root mass was estimated as the product of the total fine root mass and specific root length.

To convert the measured root length at the surface of the plexiglass to a value that represented the total length of fine roots for a given plant, we applied simple correction factors. Correction factors were calculated as total plant fine root length divided by trace root length. Root length correction factors were determined for each species × CO_2 ×

harvest combination. All root length parameters estimated directly from tracings (NP_f and L_f) were modified by these correction factors. The correction factors themselves were not significantly different between the CO_2 levels for a given species. Correction factors were applied to information obtained from the tracings by applying a harvest's correction factor to all those tracings made after a previous harvest up to the date of the harvest. The advantage of applying these correction factors is that the values of ΣP, ΣL, and NP reflect whole-root system magnitudes, not just those roots present (or lost) from the observation window.

Statistical analyses—Parameters were analyzed using randomized split-plot analysis of variance with CO_2 as the (fixed) main plot factor using Data Desk v4.2 (Odesta Corp., Northbrook, IL.). Species and harvest were treated as subplot (fixed) factors. Blocks were designated as random effects. Tests of the effects of CO_2 on a given species were performed using Scheffé post hoc tests. All variables were log transformed prior to analysis. Log transformations were selected because they resulted in linear normal probability plots and thus ANOVAs met the assumptions of homoscedasticity and normality of residuals (Velleman, 1994). NP, ΣP and ΣL, were analyzed using these ANOVAs. The NP, ΣP, or ΣL values presented for a given plant are the summation of P_f and L_f up to the time of harvest for that plant.

The allometry between root production and root loss was explored using linear regression analysis. Both root loss and root production measures were log transformed prior to analyses. Measures of root loss and production are made with some error and thus a model II regression technique was used to derive the linear relationships between the variates (Seim and Sæther, 1983). The model II regression technique we selected was the geometric mean regression because approximates to the standard error of the mean can be made, thereby facilitating statistical comparisons of regression slopes (Sokal and Rohlf, 1981). We used linear models because nonlinear regression models did not significantly improve the fit between the variates and are not amenable to model II regression techniques. Tests of differences in regression slopes among main effects were made using minimum significant differences (MSD). Tukey-Kramer, T', and GT2 MSDs were calculated and the method that yielded the smallest MSDs was used (Sokal and Rohlf, 1981).

RESULTS

All measured parameters showed substantial growth through the end of the experiment (Figs. 1, 2). In the analysis of variance the CO_2 main effect was significant in only one of the six measured parameters (Table 2). However, the species × CO_2 interaction was significant or marginally significant for every parameter. In every case, significant species × CO_2 interactions were the result of white birch being more responsive to increasing CO_2 levels than red maple (Table 3, Figs. 1, 2).

Net root production (NP), total production (ΣP) and total root loss (ΣL) did not show an overall response to CO_2 (Table 2). Similar to the other parameters we measured, red maple and white birch showed differential responsiveness in both ΣP and ΣL to increasing CO_2 levels. Increasing CO_2 led to increasing rates of ΣP and ΣL for white birch, but not red maple (Table 3, Fig. 2).

ΣL was highly correlated with both NP and ΣP (Table 4, Fig. 3). The relationships between ΣL and both production terms had correlation coefficients greater than 0.8. Tukey-Kramer minimum significant differences (MSD) were always less that T' and GT2 MSDs and thus were used to compare slopes between treatments. MSDs were greater than the differences between all sets of treatment slopes for both relationships (Table 4). Thus, while

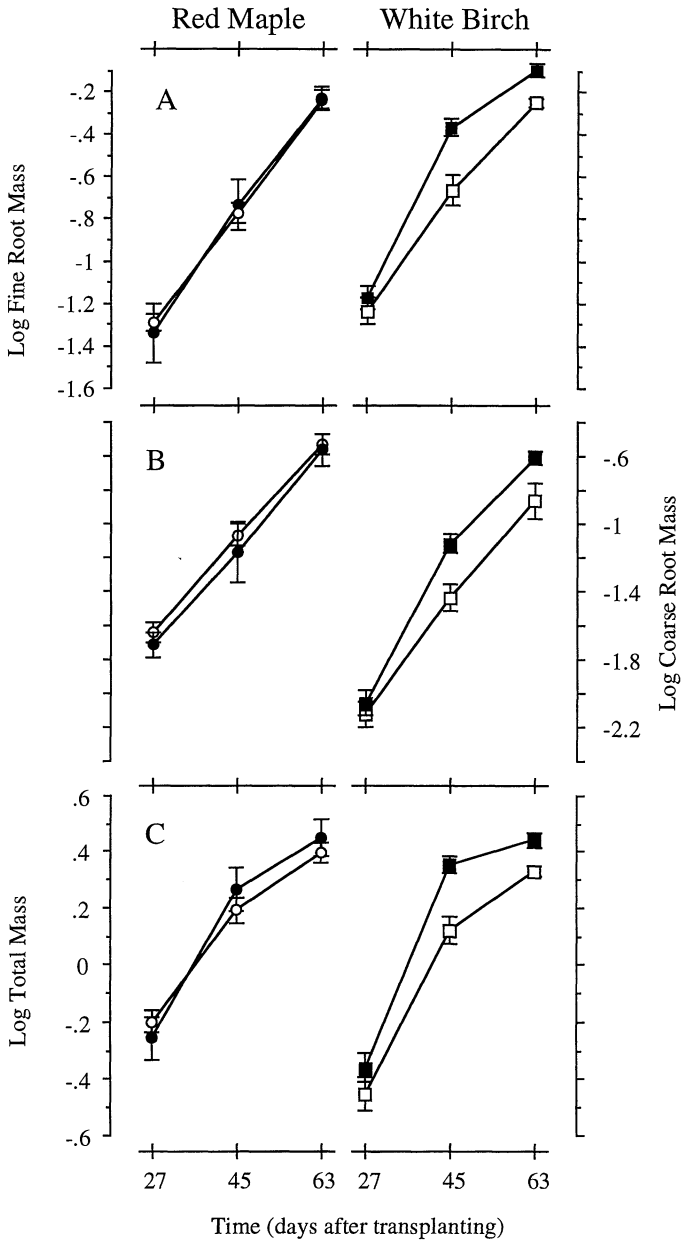

Fig. 1. Patterns of biomass production of (A) fine roots, (B) coarse roots, and (C) whole-plant mass through the experiment. ANOVA least squares means with ±1 SE are presented. Symbol types are as follows: Open square = white birch, ambient CO_2; closed square = white birch, elevated CO_2; open circle = red maple, ambient CO_2; closed circle = red maple, elevated CO_2.

ΣL was highly correlated with both ΣP and NP, these relationships were not significantly affected by species, atmospheric CO_2 level, or ontogeny.

DISCUSSION

Hypothesis 1) The influence of elevated CO_2 on whole plant and belowground growth are qualitatively similar. Tree seedling biomass responses to elevated CO_2 are variable (e.g., Poorter 1993; Ceulemans and Mousseau, 1994). Several species have shown low levels of biomass

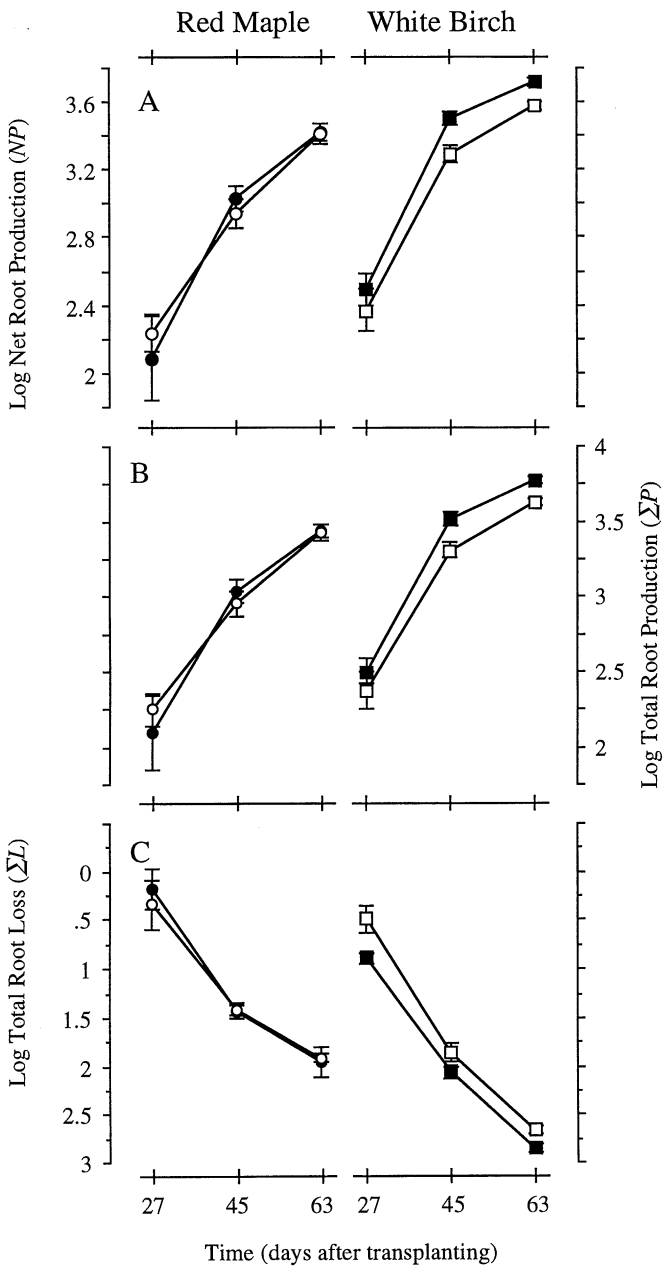

Fig. 2. Patterns of (A) net root production, (B) total root production, and (C) total root loss (measured in centimetres) through the experiment. ANOVA least squares means with ±1 SE are presented. Symbols are the same as in Fig. 1.

enhancement, while others have shown very high levels of enhancement. In this study, the elevated over ambient biomass enhancement ratio for white birch was 1.40, equal to the average biomass enhancement for all the C_3 woody species reviewed by Poorter (1993). In contrast, the biomass enhancement ratio for red maple was 1.06, close to the minimum value for C_3 woody species. These levels of response to CO_2 for white birch and red maple are within the range of those observed previously with these species (Table 1). Thus, the overall biomass enhancements in response to elevated CO_2 for the species used in this study represent variations in magnitudes of response from minimal to average.

The effect of elevated CO_2 on patterns of root production and loss for the two species was qualitatively similar to the effect of CO_2 on biomass (Fig. 1, Tables 2, 3), thus supporting hypothesis 1. Red maple showed no significant changes in biomass production, NP, Σ *L*, or Σ *P*. In contrast, white birch showed significant enhancements in whole-plant biomass, NP, Σ *P*, and Σ *L* with elevated CO_2. If these are general patterns then it is possible to make inferences about patterns of root production and loss with reviews of experimental data such as that provided by Poorter (1993), Rogers, Runion, and Krupa (1994), and Ceulemans and Mousseau (1994). Additional tests of this hypothesis in which a larger number of species with varying magnitudes of response to CO_2 are used is required to provide a more robust test of this hypothesis.

The differences in CO_2 responsiveness observed in this study between red maple and white birch are consistent with predictions based on root morphology and mycorrhizal status. O'Neill and colleagues have found that *Liriodendron tulipifera,* an arbuscular mycorrhizal (AM) species, is less responsive to increasing atmospheric CO_2 than is *Quercus alba,* an ectomycorrhizal (EM) species (O'Neill and Norby, 1988; O'Neill, O'Neill, and Norby, 1991; O'Neill, 1994). They suggested that if this were a general pattern, the increased growth of EM species in an elevated CO_2 atmosphere may lead to shifts in species dominance that have important ecosystem level implications (O'Neill, O'Neill, and Norby, 1991). The results of this study support these data from *Liriodendron* and *Quercus.* White birch, an EM species, was more responsive to elevated CO_2 than was red maple, an AM species. Differences between white birch and red maple in root dynamics are consistent with the observation that root elongation rates (equivalent to production rates in this study) are positively correlated with specific root length (SRL; Eissenstat, 1991, 1992).

Hypothesis 2) Total root loss scales directly with both total and net root production. Σ *L* scales linearly on a log-log scale with NP and Σ *P,* thereby supporting hypothesis 2 (Fig. 3, Table 4). The slopes of these relationships do not significantly vary between species, CO_2 treatment, or time of harvest. Thus, for the experimental system presented here, Σ *L* scales with NP and Σ *P* independent of the species, CO_2 atmosphere, or ontogeny. It is important to note, however, that the best fit logarithmic regression equations relating Σ *L* to NP and Σ *P* have slopes significantly >1 ($P < 0.0001$ for both regressions). This observation has important implications for how the relationship between patterns of root loss in relation to production are characterized. The slope > 1 in the allometric equation relating root loss to root production implies that larger root systems (greater NP or Σ *P*) have progressively greater relative root loss (Fig. 4A). This result is not surprising as size-dependent shifts in allocation as measured by ratios are common within the biological literature (e.g., Samson and Werk, 1986). The lack of statistically significant differences between allometric equations relating root loss to root production for the different experimental factors examined suggests that these allometries are robust. If this is the case, then any

TABLE 2. Summary of ANOVA for plant biomass, and root production and loss. All variables were log transformed in order to achieve homoscedasticity of residuals. The total degrees of freedom for each ANOVA is 82.

Dependent variable	Species	CO_2	Species × CO_2	Block	CO_2 × Block	Time	Species × Time	CO_2 × Time	Species × CO_2 × Time
Fine root mass	<0.01	0.05	0.01	ns	ns	<0.01	ns	ns	ns
Coarse root mass	ns	ns	<0.01	ns	ns	<0.01	0.04	ns	ns
Whole-plant mass	0.01	ns	0.03	ns	0.08	<0.01	0.01	ns	ns
Fine root length (*NP*)	<0.01	ns	0.08	ns	ns	<0.01	ns	ns	ns
Total root production (ΣP)	<0.01	ns	0.07	ns	ns	<0.01	ns	ns	ns
Total root loss (ΣL)	<0.01	ns	0.03	ns	ns	<0.01	0.02	ns	ns
df	1	1	1	2	2	2	2	2	2

factor that increases the production of roots (total or net) leads to an increase in the relative amount of roots lost. To extrapolate these results to mature trees in forest ecosystems, this hypothesis must be tested with larger trees.

Hypothesis 3) The relationship between root loss and root production is not modified by an elevated CO_2 atmosphere. Several previous studies have observed that elevated CO_2 does not increase the input of carbon to the soil relative to the production of root (Whipps, 1985; Norby et al., 1987; Billès, Rouhier, and Bottner, 1993; Rouhier et al., 1994). In this study we found that the allometric equations relating ΣL to ΣP were not significantly affected by atmospheric CO_2 concentration (Table 4). Thus our results are consistant with previous studies in that we observed that the rate of root loss under elevated CO_2 scales with changes in root production. The allometric characterizations of root loss in relation to root production presented here suggest that larger root systems (greater NP) have a greater relative loss of roots. Thus, we might expect for white birch, the species that showed significant increases in NP and ΣP under elevated CO_2, that we find greater relative loss rates of roots with elevated CO_2. This was not observed. Relative loss ratios were significantly increased only for white birch relative to red maple and then only at the end of the experiment (Fig. 4B). Thus, the general findings of this study support the hypothesis that CO_2 does not affect relative rates of root loss as quantified using allometric relationships or loss/production ratios. The allometric approach taken in this study suggests that the higher relative loss values observed at the end of the experiment for white birch are the product of greater overall levels of root production, not alterations in the relationship between root loss and production per se.

Implications and limitations for inferring root dynamics in elevated CO_2—Scaling up from a controlled-environment study of individual seedlings to the response of forests requires explicit recognition of the impact of seasonal variation, ontogeny, and the difference between steady-state and non-steady-state root dynamics.

Steady-state and non-steady state-dynamics—Fine roots are thought to be in a steady state in many of the temperate deciduous forests where in situ measurements of their production and loss have been made (Hendrick and Pregitzer, 1993a, b; Burke and Raynal, 1994; Fahey and Hughes, 1994). This means that yearly total root loss is approximately equal to yearly total root production; net root production is zero and root density remains approximately constant from year to year. Short-term controlled environment experiments, such as the one presented here, cannot be in a steady state with respect to root production and loss. Controlled-environment studies start with virtually no roots present. Unless the seedlings simply don't grow, total production will greatly exceed total loss. Root dynamics in forests are not in a steady state following disturbance. Following a disturbance in which mature members of the forest system die or are removed root density can decrease significantly (Wilczynski and Pickett, 1993) and subsequently, net root production is much greater then zero (Raich, 1980). Fahey and Hughes (1994) have estimated that the time required for root density to return to predisturbance (steady-state) levels in a temperate hardwood forest may be as little as 5 yr. Applying the results of this study, being a non-steady-state system, we would predict that elevated CO_2 would lead to a decrease in the time for root density to return to predisturbance levels assuming the tree species present and environmental conditions are conducive to a significant growth response with elevated CO_2.

The standing quantity of roots in a forest that is in a steady state is the product of ΣP (on a mass or length basis) and root longevity. The critical factors that need

TABLE 3. Post hoc comparisons (Scheffé) of average species responses to elevated CO_2. Comparisons were made for all variables from Table 2 due to significant ($P < 0.05$) or marginally significant ($P < 0.10$) species × CO_2 interactions for all dependent variables.

Dependent variable	Units	Red Maple			White Birch		
		Ambient mean	Elevated mean	*P*	Ambient mean	Elevated mean	*P*
Fine root mass	g	0.173	0.171	0.92	0.187	0.286	0.00
Coarse root mass	g	0.084	0.071	0.33	0.032	0.055	0.00
Whole-plant mass	g	1.339	1.423	0.51	0.998	1.423	0.00
Fine root length (NP)	cm	719.45	707.95	0.92	1 193.99	1 729.82	0.01
Total root production (ΣP)	cm	741.30	726.11	0.91	1 264.74	1 849.27	0.01
Total root loss (ΣL)	cm	16.00	15.63	0.92	46.77	86.87	0.00

TABLE 4. Results of model II linear regression analysis (geometric mean regression) of log total root loss (ΣL) vs. net root production (NP) and total root production (ΣP). The form of the regression equation is: $\log(y) = m \cdot \log(x) + b$. Differences between regression slopes were tested using the Tukey–Kramer minimum significant difference (see Materials and Methods). Significantly different slopes ($P < 0.05$) are indicated by different letters.

Relationship	Factor	Group	m	b	r^2
ΣL (y)	All				
NP (x)		—	1.520	−3.033	0.822
	Species	Red maple	1.333 a	−2.541	0.695
		White birch	1.614 a	−3.301	0.894
	CO_2	Ambient	1.364 a	−2.59	0.548
		Elevated	1.563 a	−3.168	0.894
	Time[a]	27	1.420 a	−2.784	0.283
		45	1.225 a	−2.21	0.704
		63	1.513 a	−2.898	0.487
	CO_2	Red maple, ambient	1.364 a	−2.590	0.548
	×	Red maple, elevated	1.297 a	−2.501	0.907
	Species	White birch, ambient	1.717 a	−3.618	0.931
		White birch, elevated	1.514 a	−2.974	0.855
ΣL (y)	All	—	1.496	−2.995	0.843
ΣP (x)					
	Species	Red maple	1.336 a	−2.57	0.716
		White birch	1.572 a	−3.211	0.911
	CO_2	Ambient	1.542 a	−3.118	0.786
		Elevated	1.459 a	−2.891	0.899
	Time[a]	27	1.432 a	−2.827	0.303
		45	1.224 a	−2.225	0.724
		63	1.507 a	−2.939	0.557
	CO_2	Red maple, ambient	1.376 a	−2.650	0.576
	×	Red maple, elevated	1.289 a	−2.492	0.914
	Species	White birch, ambient	1.668 a	−3.510	0.944
		White birch, elevated	1.478 a	−2.900	0.878

[a] Time, in days after transplanting.

to be understood in order to predict root growth dynamics in a steady-state system are the controls over equilibrium root density. At this time, however, there is no consensus on how changes in standing root biomass are made in response to changes in edaphic factors. For example,

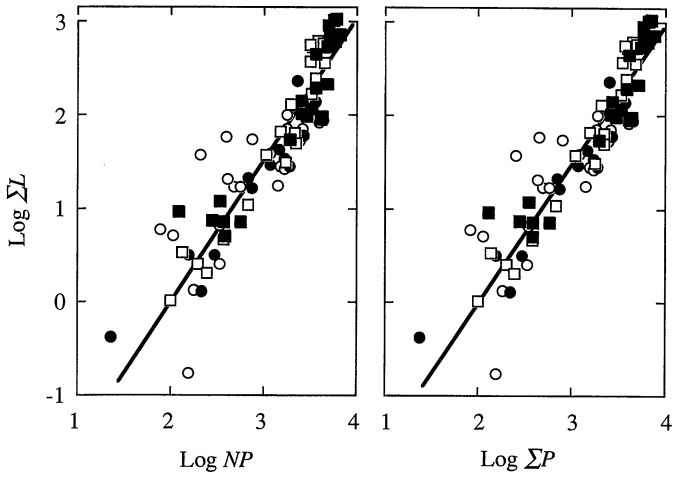

Fig. 3. Scatterplots of total root loss (ΣL), total root production (ΣP), and net root production (NP, measured in centimetres). The bold line in each of the two plots is the model II best fit line. Symbols are the same as in Fig. 1.

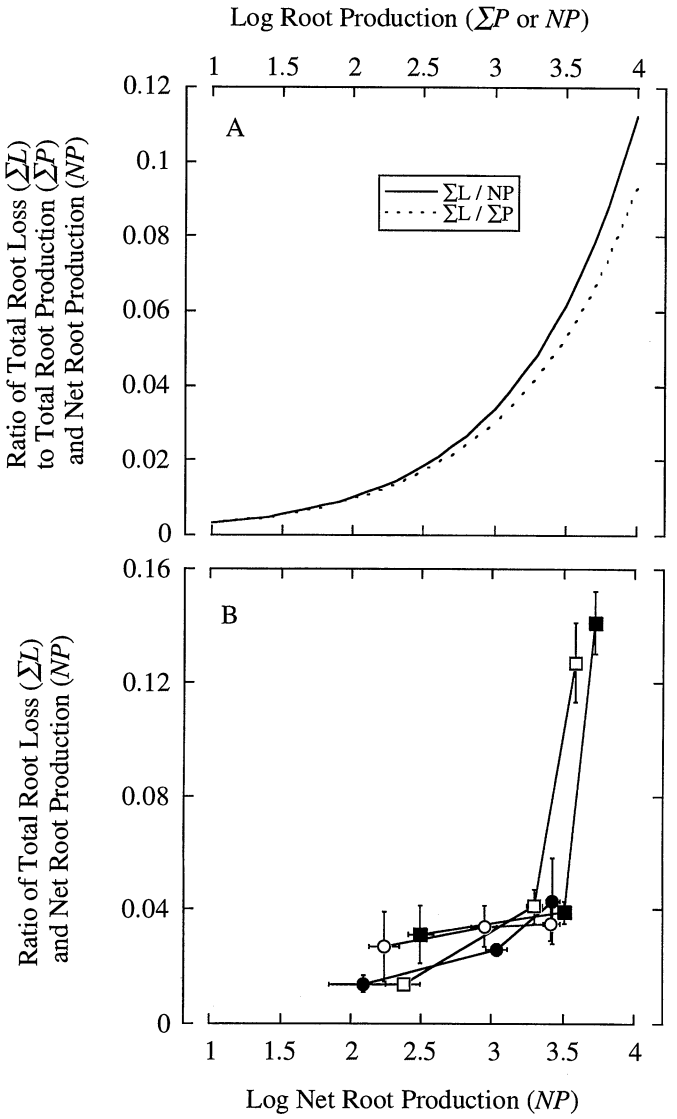

Fig. 4. (A) Ratio of root loss to root production, measured in centimetres (net and total, left axis) and ratio of total root production to net root production (right axis) as a function of root production (denominator of ratio). The relationships presented in this figure were derived using the model II regression equations presented in Table 4 and Fig. 3. (B) Ratio of ΣL to NP as a function of NP for actual experimental data. Each symbol is the average of a given species by CO_2 combination at a single harvest. Error bars are +1 SE of the mean. Symbols are the same as in Fig. 1.

from a review of the literature Hendricks, Nadelhoffer, and Aber (1993) concluded that increasing soil nitrogen availability typically results in a decrease in standing root biomass. They suggested that the decrease in root biomass was the result of increased root turnover rates. In direct contrast to this conclusion, Pregitzer, Hendrick, and Fogel (1993) observed that root longevity was increased in experimental high-nitrogen patches within the soil. If Pregitzer et al.'s observations are general, then we would expect that decreases in the standing root biomass of temperate forests in response to increasing nitrogen availability would result from decreases in ΣP. In this study ΣP was increased under elevated CO_2 for one species

and not for another. If root longevity were decreased by increasing CO_2, we would expect that the amount of loss for a given amount of production would increase, thereby altering the bivariate scaling relationship between root loss and root production. This did not occur. Thus, in this study it appears that root longevity was not affected by elevated CO_2. If elevated CO_2 leads to increased gross production and unchanged root longevity for larger trees as well, the standing quantity of roots in steady-state forests may increase.

Seasonal patterns of production and loss—In temperate forests, net root production is highly seasonal. Even though the production and loss of roots occurs throughout the growing season (Hendrick and Pregitzer, 1992, 1993a, b; Berntson, Farnsworth, and Bazzaz 1995), the total standing quantity of roots typically shows distinct seasonal patterns (e.g., McClaugherty, Aber, and Melillo, 1982; Burke and Raynal, 1994). The relative importance of root production is greater earlier in the growing season as the standing quantity of roots tends to increase. Conversely, the relative importance of root loss is greater at the end of the growing season and during the winter as the standing quantity of roots tends to decrease. The present study, because it focused on the earlier half of the growing season in which standing quantity of roots monotonically increased, provides only a partial picture of root production and loss dynamics. In this study white birch showed a significant increase in gross and net root production under elevated CO_2. But would it also show an increase in total root loss over the winter under elevated CO_2 and would this root loss result in an equalization of net root production between the different CO_2 levels? This question is of central importance to identifying the potential longer term implications of elevated CO_2 for patterns of root production and loss for long-lived perenials in seasonal environments.

Ontogeny—One of the greatest challenges in predicting the response of forests to rising atmospheric CO_2 levels is the role ontogeny plays in mediating tree responses to increasing atmospheric CO_2 levels (Ceulemans and Mousseau, 1994). To date, all studies of tree responses to elevated CO_2 have either focused on growth responses of seedlings and saplings (see Poorter, 1993; Ceulemans and Mousseau, 1994) or the responses of individual branches of trees exposed to elevated CO_2 inside bags (e.g., Barton and Jarvis, 1993). Studies of multiyear growth responses of a variety of tree species to elevated atmospheric CO_2 have demonstrated that some species show marked declines in growth enhancements within 2–3 yr, while others maintain earlier levels of growth enhancement (e.g., Bazzaz, Miao, and Wayne, 1993; Norby et al., 1992; Norby, 1994).

Because the allometric relationships we observed between root loss and root production had slopes >1, it is almost certain that this relationship would eventually break down for larger trees. If loss is greater than production, then net root growth is negative and roots are being lost. More likely, the allometry between root loss and production over longer time frames (e.g., multiple years) is nonlinear (e.g., Weiner and Thomas, 1992; Weiner and Fishman, 1994). If the allometry of root loss and root production over a longer time is nonlinear, it would be misleading to blindly use seedling responses to make predictions about the root dynamics of mature trees or even saplings in a future elevated-CO_2 world. The results from this study suggest that root loss scales as a simple function of root production independent of the CO_2 environment. However, we would do well to direct future longer term experiments with elevated CO_2 and trees to include observations of fine root dynamics to improve our understanding of the impact of elevated CO_2 on this critical component of the carbon cycling in temperate forest communities and other terrestrial ecosystems.

Conclusion—In this study we have shown that an elevated CO_2 atmosphere can increase total root production (ΣP), total root loss (ΣL), and net root production (NP) in seedlings of white birch but not red maple. These observations are qualitatively similar to whole-plant growth responses to elevated CO_2 where white birch showed significantly greater biomass, but red maple did not. However, the observed effects of CO_2 on root production and loss did not alter the bivariate scaling relationship between root production and root loss for either red maple or white birch. Thus, it appears that elevated CO_2 does not affect the relationship between root production and root loss (e.g., root longevity). The results of this study have important implications for the potential effects of elevated CO_2 on root dynamics in temperate forest ecosystems. However, because this study was conducted in controlled environments over a relatively short period of time the results of this study need to be taken as a potential indicator of what may happen in forests in the future. To better understand the potential effects of elevated CO_2, and other aspects of global change, on root dynamics in temperate forests, future studies should be of a long enough duration to gain an understanding of seasonal and ontogenetic trends and should focus on a range of tree species of contrasting life history, root morphology, mycorrhizal symbioses, and CO_2 responsiveness.

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