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Growth and C allocation of *Populus tremuloides* genotypes in response to atmospheric CO_2 and soil N availability

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SUMMARY

We grew cuttings of two early (mid Oct.) and two late (early Nov.) leaf-fall *Populus tremuloides* Michx. genotypes (referred to as genotype pairs) for *c.* 150 d in open-top chambers to understand how twice-ambient (elevated) CO_2 and soil N availability would affect growth and C allocation. For the study, we selected genotypes differing in leaf area duration to find out if late-season photosynthesis influenced C allocation to roots. Both elevated CO_2 and high soil N availability significantly increased estimated whole-tree photosynthesis, but they did so in different ways. Elevated CO_2 stimulated leaf-level photosynthesis rates, whereas high soil N availability resulted in greater total plant leaf area. The early leaf-fall genotype pair had significantly higher photosynthesis rates per unit leaf area than the late leaf-fall genotype pair and elevated CO_2 enhanced this difference. The early leaf-fall genotype pair had less leaf area than the late leaf-fall genotype pair, and their rate of leaf area development decreased earlier in the season. Across both genotype pairs, high soil N availability significantly increased fine root length production and mortality by increasing both the amount of root length present, and by decreasing the life span of individual roots. Elevated CO_2 resulted in significantly increased fine root production and mortality in high N but not low N soil and did not affect fine root life span. The early leaf-fall genotype pair had significantly greater fine root length production than the late leaf-fall genotype pair across all CO_2 and N treatments. These differences in belowground C allocations are consistent with the hypothesis that belowground C and N cycling is strongly influenced by soil N availability and will increase under elevated atmospheric CO_2. In addition, this study reinforces the need for better understanding of the variation in tree responses to elevated CO_2, within and among species.

Key words: Atmospheric CO_2, carbon allocation, nitrogen, *Populus tremuloides* Michx. (trembling aspen), fine roots.

INTRODUCTION

A substantial proportion of the C moving into forest soils comes from the turnover of fine tree roots (Pregitzer *et al.*, 1995). Tree allocation of C to fine roots is a function of above- and below-ground resource availability and intrinsic physiological characteristics that are under genetic control (Brouwer, 1983; Bloom, Chapin & Mooney, 1985). For example, root growth increases along with plant size under elevated atmospheric CO_2 (Billès, Rouhier & Bottner, 1993; Larigauderie, Reynolds & Strain, 1994; Rogers, Runion & Krupa, 1994; Pregitzer *et al.*, 1995; Tingey *et al.*, 1996), but elevated CO_2 affects the proportion of C allocated to roots only under certain growth-limiting soil conditions (Stulen & den Hertog, 1993; Curtis & Wang, 1998). By contrast, the rate of fine root turnover is known to increase with soil N availability in some species (Pregitzer, Hendrick & Fogel, 1993; Pregitzer *et al.*, 1995). In turn, organic C in the soil fuels N mineralization (Zak *et al.*, 1993) and soil N availability influences both C assimilation (Field & Mooney, 1986) and allocation (Bloom *et al.*, 1985). Understanding how elevated CO_2 and soil N availability influence C allocation to roots, and to what extent responses might be modified by genetic

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variation, are important aspects of the broader issue of ecosystem C and N cycling.

In northern latitudes, late-season photosynthesis plays an important role in the total C allocated to roots. During active growth of *Populus* trees, the majority of fixed C is allocated to shoots whereas after bud set the majority is allocated to roots (Horwath, Pregitzer & Paul, 1994; Coleman *et al.*, 1995). The ability of deciduous trees to shunt C below ground is functionally related to the longevity and photosynthetic capacity of leaves. Thus, below-ground C allocation in northern latitudes, where growing seasons are relatively short, might be substantially influenced by differences in leaf-area duration among or within species.

This study is one in a series of experiments to examine the interactions between elevated atmospheric CO_2 and soil N availability on tree growth, and to study how trees mediate the cycling of C and N in forest ecosystems (Curtis & Teeri, 1992; Zak *et al.*, 1993; Curtis *et al.*, 1994, 1995; Pregitzer *et al.*, 1995; Kubiske *et al.*, 1997). The purposes of the present study were to measure the effect of elevated atmospheric CO_2 and soil N availability on the production and turnover of fine roots, and to determine the extent to which differences in autumnal leaf-fall phenology of *Populus tremuloides* genotypes might influence late-season C allocation to fine roots under elevated atmospheric CO_2.

MATERIALS AND METHODS

Experimental design

We selected four naturally occurring genotypes of *Populus tremuloides* Michx. at the University of Michigan Biological Station (UMBS), Pellston, MI, USA (45° 34′ N, 84° 40′ W), that were previously studied for phenological characteristics (Barnes, 1959). Consistent with that earlier study, two of the genotypes were observed to have dropped their leaves by mid Oct. 1993 (referred to as the early leaf-fall genotype pair), and two dropped their leaves in early Nov. 1993 (referred to as the late leaf-fall genotype pair). In late Oct. 1993, we collected roots from each genotype and propagated cuttings in Feb. 1994 according to Barry & Sachs (1968).

In spring 1994, we constructed a 4×8 experimental array of open-bottom root boxes ($0{\cdot}5\ m^2 \times 1{\cdot}3$ m deep) at UMBS. The boxes were filled with soils similar to those of our previous experiments: either a mixture of one part A horizon (Kalkaska series, Typic Haplorthod) and four parts native C horizon (Rubicon sand, Entic Haplorthod), referred to as the low N treatment, or Kalkaska A horizon referred to as the high N treatment (initial net N mineralization rates of 89 ± 7 ng N g^{-1} d^{-1}, and 333 ± 16 ng N g^{-1} d^{-1}, respectively, based on laboratory incubations, Mikan & Zak, unpublished). Because of differences in soil colour, each box was topped with *c.* 1 cm of sand to avoid differential soil heating between the two soil treatment. Each box was divided into halves with a plastic-lined plywood divider and each half fitted with a $1{\cdot}5$ m long $\times$ $5{\cdot}2$ cm diameter clear plastic tube (minirhizotron) inserted at an angle of 21°.

In early June 1994, we planted a cutting from each of the four genotypes into the boxes such that each box half contained either the early or late leaf-fall genotype pair. Genotype pairs were randomly assigned to box halves, and the position of genotypes within box halves was also randomly assigned. Cuttings were $17{\cdot}7 \pm 0{\cdot}5$ cm tall at the time of planting, with no significant differences among genotypes or treatments.

Over the top of each root box, we placed a transparent, open-top chamber ($0{\cdot}64\ m^2 \times 1{\cdot}5$ m tall), as we have used previously. Pure CO_2 was dispensed into the blower system of half of the chambers to elevate the atmospheric CO_2 concentration in the chamber to approx. twice that of ambient. Chamber CO_2 concentrations were monitored continually during the experiment with a Li-Cor® 6252 infrared gas analyser and logged by a microcomputer at *c.* 15-min intervals. The 95% confidence limits for 24 h ambient and elevated CO_2 concentrations over the course of the experiment were 380 ± 88 μmol mol^{-1} and 720 ± 209 μmol mol^{-1}, respectively. On 22 separate occasions, the CO_2 exposure system was turned off (30 min to several h) for various reasons, which contributed to the large confidence limit for elevated CO_2 concentration. We also monitored ambient air chamber temperature (in four chambers) using hooded thermocouples connected to a LI-1000 datalogger. Temperatures were recorded as instantaneous readings taken every 15 min continually throughout the experiment.

Above-ground growth and photosynthesis

Beginning in late June 1994, total leaf area of each tree was non-destructively measured at 1–2-wk intervals. Leaf area was estimated by applying leaf length and width measurements to genotype-specific regression equations. Regression equations were formulated using measured leaf length, width, and area (measured with a Li-Cor 3100 leaf area meter) of senesced leaves following completion of the study.

Late-season net photosynthesis (A) was measured on 8 Sept. and at 2-wk intervals thereafter until leaf senescence. Net photosynthesis was measured on a fully expanded, mid-crown leaf of each tree on relatively cloud-free days under natural sunlight ($\geqslant 1500$ μmol m^2 s^{-1}) to reduce the potential for confounding effects of low light on the CO_2 and N treatments. Two ADC LCA-2 portable photosynthesis systems were used, each one dedicated to either ambient or elevated CO_2 chambers to reduce

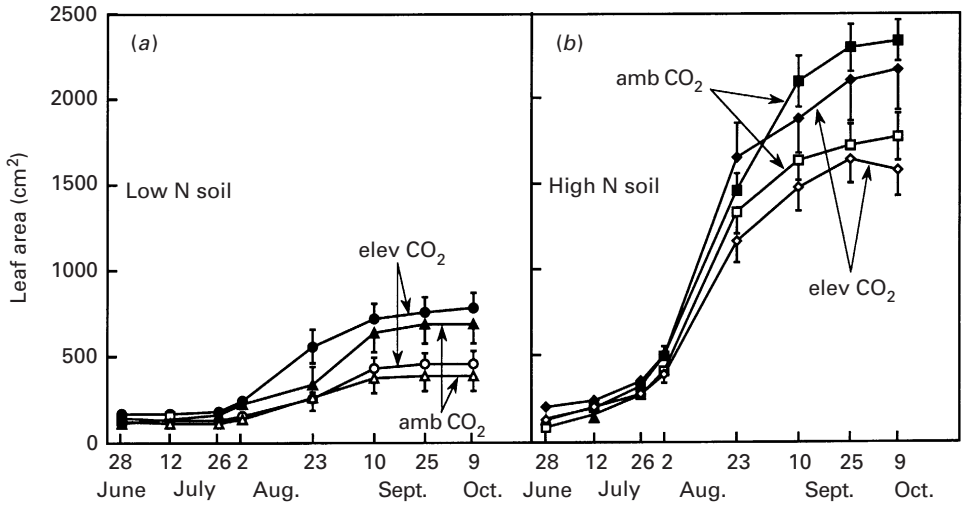

Figure 1. Cumulative plant leaf area for genotypes of *Populus tremuloides* differing in autumnal leaf-fall phenology (open symbols, early leaf-fall; closed symbols, late leaf-fall). There were two genotypes per leaf-fall class and 16 observations per data point. Trees were grown in either ambient (amb) or twice ambient (elev) CO_2 and in soil with low or high N availability. Vertical bars represent 1 SE.

instrument acclimation time to CO_2 concentration and to improve calibration accuracy. The instruments were alternated between CO_2 treatments each measurement date, and we found no systematic instrument bias between CO_2 treatments. Leaf-level photosynthesis calculations followed von Caemmerer & Farquhar (1981).

As a simple estimate of whole-tree photosynthesis, leaf-level *A* was multiplied by total leaf area. Leaf *A* measurement dates were paired with leaf area measurement dates as follows, respectively: 8 and 11 Sept.; 17 and 25 Sept.; 2 Oct. and 25 Sept. Leaf *A* measurements taken on 12 and 22 Oct. and 10 Nov. were multiplied by leaf area data from 9 Oct. Note that all trees had set bud by the end of Sept., and that changes in leaf area after mid Sept. were minimal (see Fig. 1).

Below-ground growth

Concurrent with the leaf area measurements, a microvideo camera system was used to record high-resolution video images of roots growing along the surface of the minirhizotron in each box half. The tubes were etched with a transect of 130, 1·17 cm^2 numbered frames so that the growth, development and mortality of individual roots could be monitored. Root images within the frames were digitized with a microcomputer following the methods of Pregitzer *et al.* (1993, 1995). Individual roots were categorized according to condition (new, live or dead) and the lengths were totalled for the entire transect on each minirhizotron. Roots were considered dead if they appeared black in the video image or if they were missing. The total length of new, live or dead roots was determined for each box half on each sample date.

With these data, we constructed growth and mortality functions based on root length for each treatment/genotype pair combination. Fine-root-length mortality was expressed as the cumulative length of dead fine roots at each sampling date. Fine-root-length growth was expressed in two ways: as standing crop and cumulative production. Standing crop was the total length of live roots present in minirhizotron frames at each sampling date, whereas root length production consisted of the total length of live roots plus the cumulative root-length mortality up to that time.

Absolute mortality is a function of both the population size, and the life span of members of the population. Since fine-root mortality was expressed in absolute terms in this study (as cumulative length of dead roots), it was a function of the total length of live roots and life span of individual roots. In order to separate these two effects on fine-root turnover, percentage survival through to the end of the growing season was used as a measure of fine-root life span for cohorts of roots that were first observed in either July or Aug.

Statistical analyses

Our experiment was a split-plot design consisting of four blocks of eight chambers, with two replicates of each soil × CO_2 treatment in each block, for a total of 32 chambers. The main experimental unit was the chamber, with leaf-fall genotype pair as the split-plot factor. Thus, data were analysed by leaf-fall genotype pair and not by individual genotype.

Leaf photosynthesis data were analysed with a split-plot ANOVA model for each sampling date which allowed us to identify specific dates on which photosynthesis differed among genotype pairs and treatments. Leaf area, whole-tree photosynthesis, and root growth and mortality data were pooled by box half (i.e. genotype pair; the sub-unit in the split-plot design) and were analysed using orthogonal

Table 1. *Orthogonal polynomial repeated measure ANOVA for above- and below-ground growth parameters of* Populus tremuloides *genotypes grown at ambient and elevated CO_2 and low and high soil N availability*

		Mean		Linear		Quadratic	
Effect	d.f.	ms ($\times 10^6$)	*P*	ms ($\times 10^6$)	*P*	ms ($\times 10^5$)	*P*
Plant leaf area							
Whole units							
Block	3	1·172		0·748		0·599	
CO_2	1	0·097	0·603	0·094	0·542	0·108	0·305
Error (CO_2)	3	0·288		0·200		0·071	
N	1	63·958	0·007	52·988	0·006	0·473	0·125
Error (N)	3	1·417		1·099		0·106	
$CO_2 \times N$	1	1·474	0·019	0·972	0·107	0·020	0·768
Error ($CO_2 \times N$)	3	0·069		0·041		0·190	
Sub units							
Gen pair	1	2·895	0·008	2·580	0·010	0·645	0·023
Gen pair $\times CO_2$	1	0·037	0·755	0·037	0·749	0·001	0·927
Gen pair $\times$ N	1	0·325	0·355	0·358	0·323	0·273	0·134
Gen pair $\times CO_2 \times$ N	1	0·089	0·627	0·071	0·658	0·018	0·693
Error (Gen pair)	44	0·371		0·358		0·117	
Cumulative root length production							
Whole units							
Block	3	1633		3047		3186	
CO_2	1	22879	0·166	2177	0·230	90	0·010
Error (CO_2)	3	6895		963		3	
N	1	135252	0·030	73649	0·018	1169	0·042
Error (N)	3	8864		3247		100	
$CO_2 \times N$	1	27853	0·105	6020	0·231	0	0·984
Error ($CO_2 \times N$)	3	5277		2678		159	
Sub units							
Gen pair	1	15625	0·156	7781	0·047	158	0·237
Gen pair $\times CO_2$	1	2472	0·568	686	0·547	0	0·948
Gen pair $\times$ N	1	2410	0·573	70	0·847	13	0·734
Gen pair $\times CO_2 \times$ N	1	7890	0·310	591	0·576	39	0·553
Error (Gen pair)	44	7469		1857		110	
Cumulative root mortality							
Whole units							
Block	3	4241		4366		406	
CO_2	1	5939	0·302	3004	0·361	10	0·617
Error (CO_2)	3	3847		2606		34	
N	1	48070	0·014	24433	0·010	169	0·155
Error (N)	3	1795		721		47	
$CO_2 \times N$	1	2656	0·315	483	0·632	343	0·295
Error ($CO_2 \times N$)	3	1835		1706		215	
Sub units							
Gen pair	1	4237	0·218	2171	0·257	29	
Gen pair $\times CO_2$	1	583	0·645	15	0·924	92	0·518
Gen pair $\times$ N	1	667	0·622	469	0·596	57	0·611
Gen pair $\times CO_2 \times$ N	1	2208	0·372	1892	0·289	175	0·374
Error (Gen Pair)	44	2706		1639		216	
Root standing crop							
Whole units							
Block	3	338		298		55	
CO_2	1	9123	0·117	31	0·507	0	0·848
Error (CO_2)	3	1919		68		3	
N	1	23399	0·086	5175	0·014	380	0·022
Error (N)	3	3689		190		20	
$CO_2 \times N$	1	11067	0·081	604	0·204	7	0·500
Error ($CO_2 \times N$)	3	1648		231		15	
Sub units							
Gen pair	1	2605	0·326	195	0·388	1	0·825
Gen pair $\times CO_2$	1	173	0·799	0	0·995	3	0·674
Gen pair $\times$ N	1	1602	0·440	107	0·521	0	0·889
Gen pair $\times CO_2 \times$ N	1	3651	0·246	0	0·980	10	0·460
Error (Gen pair)	44	2629		256		18	

Whole experimental units were the open-top chambers ($n = 32$) and the subunits were half-chambers consisting of early or late leaf-fall genotype pairs (Gen pair). Corresponding data are shown in Figures 1 and 4.

polynomials in repeated measure ANOVA to characterize treatment × time interactions over the duration of the experiment (Meredith & Stehman, 1991). Coefficients of second-order polynomials were calculated according to Robson (1959) for data with unequal sample spacing. This analysis allowed us to compare the mean, linear and quadratic components of shoot and root growth as a function of time among clones and treatments. For example, a significant linear CO_2 effect (i.e., a significant $CO_2 \times$ time interaction) for fine root production indicated that the rate of fine root production was significantly affected by atmospheric CO_2 concentration (cf. Meredith & Stehman, 1991). To analyse treatment effects on fine-root life span, cohort survivorship functions were tested using a Gehen–Wilcoxon test for survivorship data in which not all subjects die before the end of the experiment (Pyke & Thompson, 1986).

RESULTS

Above-ground growth and photosynthesis

At the end of the experiment (Oct.) trees in high N soil were twice as tall (126 ± 4 and 121 ± 5 cm under ambient and elevated CO_2, respectively) as those in low N soil (52 ± 6 and 62 ± 5 cm under ambient and elevated CO_2, respectively), with no significant CO_2 or genotype pair effects on tree height. Similarly, trees in high N soil had a greater leaf area ($P < 0{\cdot}05$) than those in low N soil across both genotype pairs and at both CO_2 concentrations, but the CO_2 main effect was not significant (Fig. 1, Table 1). The late leaf-fall genotype pair had greater leaf area in all treatments ($P < 0{\cdot}05$) by the end of the growing season. The rate of leaf area increase late in the growing season (after Aug.) was more rapid for the late leaf-fall genotype pair than for the early leaf-fall genotype pair, as indicated by a significant quadratic genotype pair effect (Table 1). However, we did not find phenological differences in the timing of leaf abscission in these chamber-grown trees.

In both soil types, net photosynthesis (A) was significantly higher ($P < 0{\cdot}05$) in elevated than ambient CO_2 on 8 and 17 Sept., but decreased

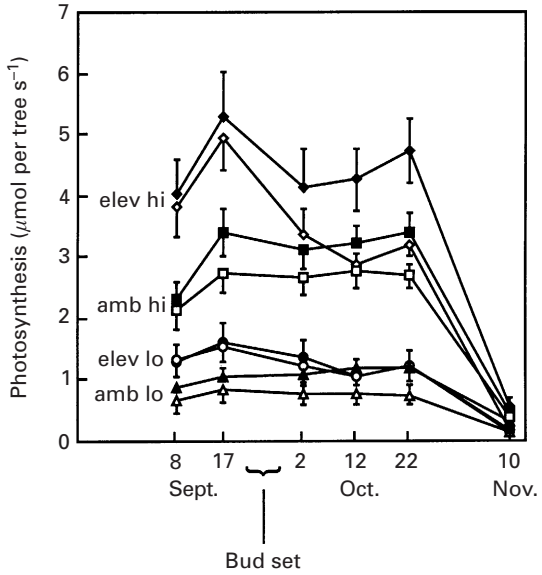

Figure 3. Estimated whole-tree photosynthesis calculated from leaf photosynthesis rate (Fig. 2) times total leaf area of *Populus tremuloides* clones comprising early (open symbols) and late leaf-fall genotypes (closed symbols). trees were grown and measured in either ambient (amb) or twice ambient (elev) CO_2, and in soil with low (lo) or high (hi) N availability.

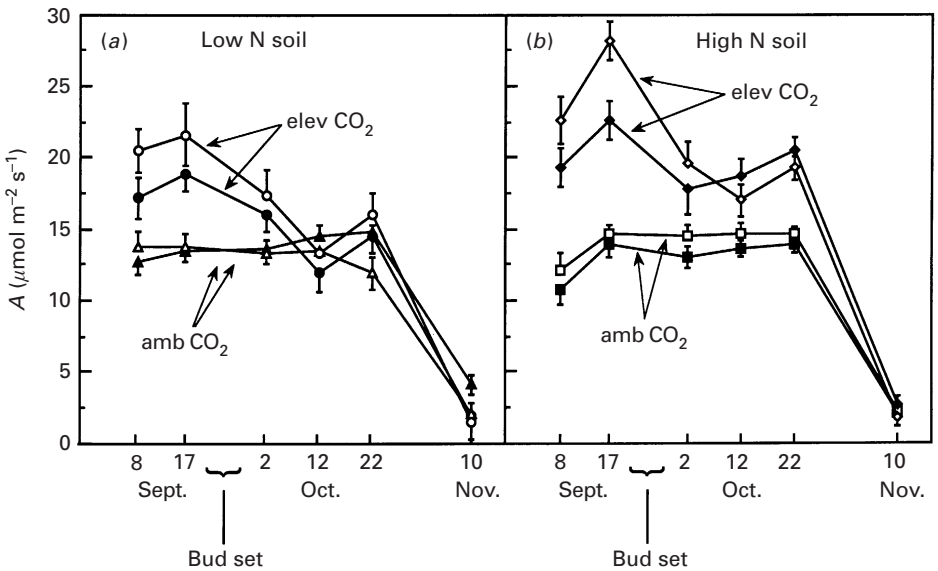

Figure 2. Net photosynthesis (A, photosynthetic photon flux density $\geqslant 1500$ μmol m^{-2} s^{-1}) of mid-crown leaves of *Populus tremuloides* trees differing in autumnal leaf-fall phenology (open symbols, early leaf-fall; closed symbols, late leaf-fall). Trees were grown and measured in either ambient (amb) or twice ambient (elev) CO_2, and in soil with low or high N availability. There were two genotypes per leaf-fall class and 16 observations per data point. Vertical bars are 1 SE.

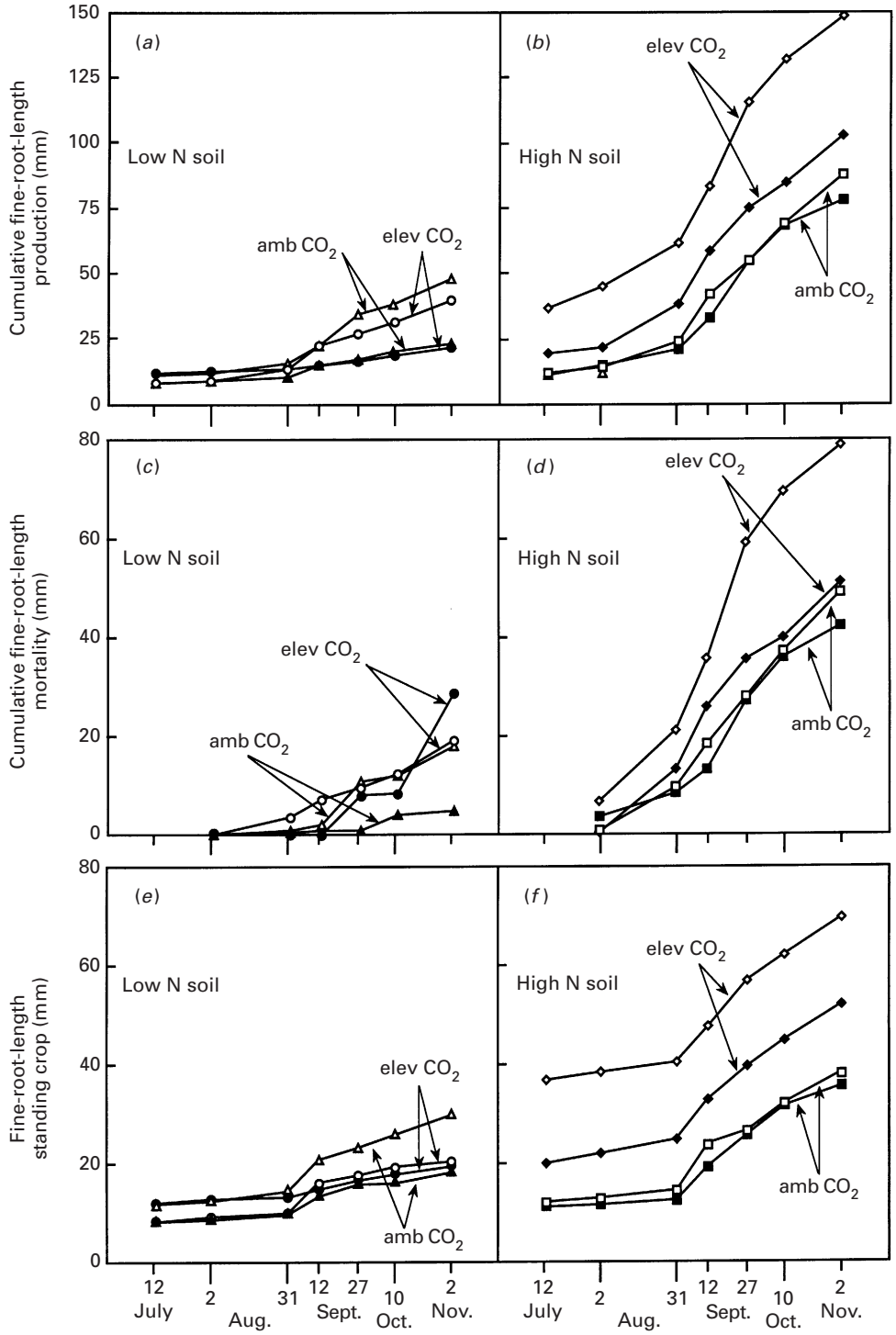

Figure 4. Cumulative fine-root production, cumulative fine-root mortality, and standing crop of fine roots for genotypes of *Populus tremuloides* differing in autumnal leaf-fall phenology (open symbols, early leaf-fall; closed symbols, late leaf-fall). There were two genotypes per phenology class and eight observations per data point. Trees were grown in either ambient or twice ambient (elev) CO_2 and in soil with low or high N availability.

significantly in elevated CO_2 following budset (Fig. 2). Following this decrease, there was no significant CO_2 effect on A for plants grown in low N soil from 2 Oct. through the rest of the study (Fig. 2*a*). In high N soil, trees had higher A under elevated than ambient CO_2 up until leaf senescence in early Nov. (Fig. 2*b*). In contrast to having less leaf area, the early leaf-fall genotype pair had significantly higher A ($P < 0{\cdot}05$) than the late leaf-fall genotype pair under elevated, but not ambient CO_2. These differences were particularly large before budset (on 8 and 17 Sept.).

Whole-tree estimated photosynthesis was significantly affected by elevated CO_2 and soil N availability (Fig. 3). Trees in high N soil or under elevated CO_2 had significantly higher whole tree A

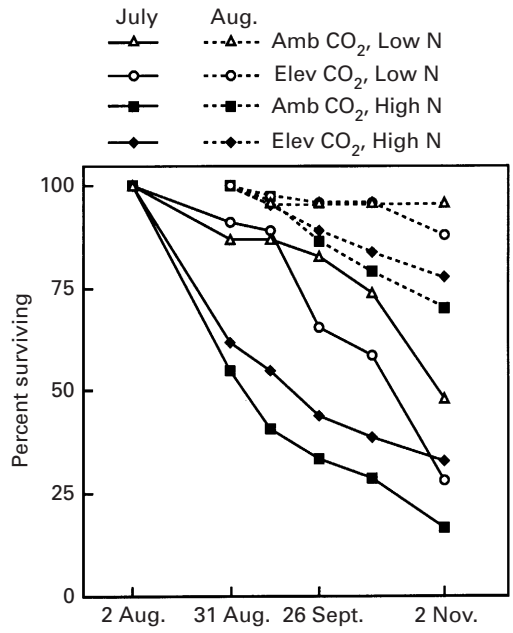

Figure 5. Survivorship curves for cohorts of fine roots produced in July and Aug. for *Populus tremuoloides* clones. Trees were grown in either ambient (amb) or twice ambient (elev) CO_2 and in soil with low or high N availability.

than those in low N soil or under ambient CO_2, respectively. However, whole tree A was influenced by N and CO_2 treatments in different ways: it increased under elevated CO_2 via stimulation of leaf A , and it increased in high N soil due to greater leaf area growth. Lower leaf A in the late leaf-fall genotype pair was more than compensated for by greater leaf area such that whole-tree photosynthesis was consistently greater for the late- compared with the early leaf-fall genotype pair ($P < 0{\cdot}05$). In particular, the late leaf-fall genotype pair in elevated CO_2, high N soil had significantly greater whole-tree photosynthesis late in the season (Oct.).

Below-ground growth

Total fine root length production (mean effect) and the rate of root length extension (linear effect) were significantly greater in high N than low N soil at both ambient and elevated CO_2 (Fig. 4*a*, *b*, Table 1). Fine root length mortality was also greater in high N compared with low N soil (Fig. 4*c*, *d*, Table 1), yet the length of fine roots present at a given time (i.e, root length standing crop) was also greater in high N than low N soil (Fig. 4*e*, *f*, Table 1).

Trees in high N soil tended to have greater fine root length production, and greater fine root length mortality in elevated than in ambient CO_2, but the differences were not statistically significant due to high variance (Fig. 4*c*, *d*, Table 1). Consequently, atmospheric CO_2 concentration affected root length standing crop differently in each soil type; elevated CO_2 resulted in greater root length standing crop in high N but not low N soil (Fig. 4*e*, *f*; mean $CO_2 \times N$ effect, Table 1).

The two *P. tremuloides* genotype pairs differed significantly in the rate of root length production (Fig. 4*a*, *b*). Whereas total leaf area was less for the early leaf-fall genotype pair than the late leaf-fall genotype pair, the rate of fine root length production was greater for the early than for the late leaf-fall genotype pair (linear genotype pair effect, Table 1). Leaf-fall genotype pair effects on fine root length production did not interact with atmospheric CO_2 or soil N availability.

Cohort survivorship analysis allowed us to separate two influential factors of root mortality: the effect of root life span and the effect of total length production. There were no significant genotype pair effects in the survivorship of either cohort, therefore data were pooled for both genotype pairs. Root cohorts produced in July and Aug. had significantly shorter life spans in high N than low N soil (Fig. 5). Thus soil N availability affected fine root turnover rate both by increasing total root length production and by decreasing the life span of individual roots. There were no significant CO_2 effects on survivorship of fine root cohorts, indicating that elevated CO_2 affected fine root turnover in high N soil by increasing root production, but not by affecting life span.

DISCUSSION

In this study, we found no elevated CO_2-stimulation of above-ground growth in these naturally occurring *P. tremuloides* genotypes. This was unexpected because several studies demonstrated substantial increases in stem height (*c.* 7–90 %), leaf area (*c.* 10–50 %), and above-ground biomass (*c.* 25–47 %) in five *Populus* hybrids in elevated CO_2 (Radoglou & Jarvis, 1990; Bosac *et al.*, 1995; Ceulemans, Jiang & Shao, 1995*a*; Curtis *et al.*, 1995; Pregitzer *et al.*, 1995; Ceulemans *et al.*, 1996). However, *Populus grandidentata*, another naturally occurring species, had no significant height or leaf area response to elevated CO_2 (Curtis *et al.*, 1994). Despite this lack of CO_2 effect on above-ground growth, our estimate for whole-tree photosynthesis was significantly greater in elevated CO_2 (cf. Kubiske *et al.*, 1997). This was due to increased leaf A of 10–15 % during active growth, similar to other studies (Curtis *et al.*, 1994, 1995; Ceulemans, Jiang & Shao, 1995*b*). Elevated CO_2 has a direct effect on photosynthetic efficiency because it increases the diffusion gradient of CO_2 into leaves, and increases leaf N-use efficiency and the efficiency of photosynthetic enzymes (Drake & Gonzàlez-Meler, 1997). This CO_2-stimulation of A decreased following budset, possibly through carbohydrate feedback inhibition of photosynthesis, as seen in other *Populus*

species before and after budset (Bosac *et al.*, 1995; Curtis *et al.*, 1995).

In contrast to above-ground growth, the response of roots to elevated CO_2 might be related to root-system structure and morphology rather than the function of individual roots (Rogers *et al.*, 1992; Stulen & den Hertog, 1993; Larigauderie *et al.*, 1994; Bosac *et al.*, 1995). Authors have reported increased N uptake at elevated CO_2, which they attribute to changes in root architecture that enable plants to explore volumes of soil more thoroughly (Larigauderie *et al.*, 1994; BassiriRad *et al.*, 1996). It has been suggested that the very small terminal roots are most efficient at nutrient uptake, and are also most expensive to maintain (Yanai, 1994; Pregitzer *et al.*, 1997). An increase in C availability might facilitate the production and maintenance of efficient, nutrient absorbing terminal roots, thereby improving the nutrient absorbing capacity of the root system.

Elevated CO_2 significantly increased fine-root growth in high N, but not low N soil. In a previous study, elevated CO_2 increased fine-root production of *Populus* by 7 and 70 % in low N and high N soil, respectively (Pregitzer *et al.*, 1995). If a condition exists where N is limiting for plant growth at ambient CO_2, then it might also be limiting for plant growth at elevated CO_2, despite improvements in leaf N-use efficiency. Plants subjected to sudden N-stress exhibited cessation of root elongation with a concurrent and continuous increase in soluble carbohydrate concentration (Henry & Raper, 1991). *P. grandidentata* had significant stimulation of fine-root growth by elevated CO_2 in low N soil only following N fertilization that resulted in increased root : shoot ratio (Zak *et al.*, 1993; Curtis *et al.*, 1994). Other experiments with *Populus* had no CO_2-induced change in root : shoot ratio (Radoglou & Jarvis, 1990; Bosac *et al.*, 1995; Pregitzer *et al.*, 1995).

Whilst these reports appear to be conflicting, the response of tree roots to atmospheric CO_2 concentration can be modified by soil N availability, species and genotype. For example, root length density (cm root cm^{-3} soil) increased under elevated CO_2 in low N and water, but not high N and water for *Pinus taeda*, whilst it increased with elevated CO_2 irrespective of N or water for *Liquidambar styraciflua* (Jifon, Friend & Berrang, 1995). The comprehensive meta-analysis (over 500 reports) by Curtis & Wang (1998) indicated that the increase in root growth in elevated CO_2 is most commonly a function of increased plant size, and not of altered root : shoot ratio. In terms of below-ground C allocation, however, it is important to note that elevated CO_2 might increase below-ground C flux and rhizodeposition of C, even where no increase in fine-root production or plant C storage is evident (Rouhier *et al.*, 1994).

A consistent response in our experiments with *Populus* is that of increased root proliferation at high N availability (Zak *et al.*, 1993; Curtis *et al.*, 1994; Pregitzer *et al.*, 1995). In the present study, fine-root production increased in response to high soil N availability at both ambient and elevated CO_2, and more than half of them senesced by the end of the experiment. Across all genotypes, cumulative root length production in low N soil exceeded final standing crop by 46 % and 60 % at ambient and elevated CO_2, respectively. In high N soil, root production exceeded final standing crop by 125 % and 107 % at ambient and elevated CO_2, respectively. For trees grown in high N soil, mortality was a function of increased population size and decreased root life span, whereas elevated CO_2 had no effect on root life span as indicated by cohort analysis (cf. Pregitzer *et al.*, 1995; Berntson & Bazzaz, 1996). Although our results are consistent with reports of higher fine-root turnover in nutrient rich sites compared with nutrient poor sites (Bloom *et al.*, 1985), responses are not consistent among species (e.g. Tingey *et al.*, 1996). In addition, responses of root growth and life span can differ depending upon which soil resources are limiting or abundant (Drew, 1975; Friend, Eide & Hinckley, 1990). Specific mechanisms driving root production and mortality in response to resource availability are currently unknown, but root growth and maintenance are likely to be related to whole plant C and N economies.

We saw no genotypic differences in leaf longevity among our study trees, which we attributed to microclimate modification by the open-top chambers. Air temperatures in the open-top chambers averaged 3·2 °C higher than ambient air during the study (18·7 °C vs. 22·0 °C for ambient and chamber temperatures, respectively). Our study trees retained green foliage whilst the surrounding forest was bare of foliage late in the season, and that foliage remained photosynthetically active until it was killed by frost (positive net photosynthesis rates were measured well into Nov., Fig. 2). These observations suggest that chamber effects modified the normal leaf fall phenology of our study trees, and negated genotypic differences in the timing of leaf senescence. Nonetheless, the late leaf-fall genotype pair had greater rates of leaf area growth than the early leaf-fall genotype pair late in the season. Moreover, the early leaf-fall genotype pair had significantly higher photosynthesis rates, which tend to vary inversely with leaf longevity (Reich, Walters & Ellsworth, 1992). We conclude that our observations, and those of Barnes (1959), on leaf phenology of the field clones represent intrinsic differences in timing of leaf senescence among our study trees that were masked by chamber effects.

Across both genotype pairs, fine-root production continued to increase late in the season, following

cessation of above ground growth, providing an additional sink (other than storage and respiration) for late-season fixed C (cf. Horwath *et al*., 1994; Rouhier *et al*., 1994). In addition, there were clear differences in allocation to roots between early and late leaf-fall genotype pairs that were not related to timing of leaf senescence. The early leaf-fall genotype pair had less leaf area and whole tree A, but greater fine-root production late in the growing season (after Aug.) that coincided with decreasing shoot growth.

These data suggest that, in the late leaf-fall genotype pair, greater leaf area growth increased whole plant A but competed with root growth for fixed C. Perhaps more importantly, the early leaf-fall genotype pair generally had more pronounced responses to elevated CO_2 in terms of leaf-level photosynthesis and root growth. By contrast, the late leaf-fall genotype pair had a large, whole-tree A response to elevated CO_2 in high N soil, but the increased C gain was not reflected in root growth.

In this study, elevated CO_2 resulted in greater C allocation to fine roots for trees grown in high N soil, particularly the early leaf-drop genotype pair. This increased allocation to roots was partly driven by faster root turnover rates in high N soil. Moreover, these genotype pairs of *P. tremuloides* exhibited differential physiological and growth responses to elevated CO_2 and soil N availability. This was true for both shoot and root systems. Late leaf-fall genotype pair had greater rates of shoot growth longer into the growing season which provided an additional sink for C that was not present in the early leaf-fall genotype pair late in the season. Coordination of physiology and growth at the tree level such as this plays an important role in the competitive interactions that structure forest communities. Our experiment clearly demonstrated that growth responses to elevated CO_2 and soil N availability have a genetic foundation (cf. Harper 1982). This implies that we should not expect taxa, at any level, to display identical responses to changes in resource availability. Generalizations about the consequences of global change should be tempered by an understanding of the many different ways individuals respond to the essential resources that limit their growth.

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REFERENCES

Barnes BV. 1959. *Natural variation and clonal development of* Populus tremuloides *and* P. grandidentata *in Northern Lower Michigan*. Ph.D. thesis, University of Michigan, USA, 344.

Barry W, Sachs R. 1968. Vegetative propagation of quaking aspens. *California Agriculture* **22**: 14–16.

BassiriRad H, Griffin KL, Strain BR, Reynolds JF. 1996. Effects of CO_2 enrichment on growth and root $^{15}N_4^+$ uptake rate of loblolly pine and ponderosa pine seedlings. *Tree Physiology* **1**: 957–962.

Berntson GM, Bazzaz FA. 1996. 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. *American Journal of Botany* **83**: 608–616.

Billès G, Rouhier H, Bottner P. 1993. Modifications of the carbon and nitrogen allocations in the plant (*Triticum aestivum* L.) soil system in response to increased atmospheric CO_2 concentration. *Plant and Soil* **157**: 215–225.

Bloom AJ, Chapin FS, Mooney HA. 1985. Resource limitation in plants – an economic analogy. *Annual Review of Ecology and Systematics* **16**: 363–392.

Bosac C, Gardner SDL, Taylor G, Wilkins D. 1995. Elevated CO_2 and hybrid poplar: a detailed investigation of root and shoot growth and physiology of *Populus eruamericana*, 'Primo'. *Forest Ecology and Management* **74**: 103–116.

Brouwer R. 1983. Functional equilibrium: sense or nonsense? *Netherlands Journal of Agricultural Science* **31**: 335–348.

Ceulemans R, Jiang XN, Shao BY. 1995*a*. Effects of elevated atmospheric CO_2 on growth, biomass production and nitrogen allocation of two *Populus* clones. *Journal of Biogeography* **22**: 261–268.

Ceulemans R, Jiang XN, Shao BY. 1995*b*. Growth and physiology of one-year old poplar (*Populus*) under elevated atmospheric CO_2 levels. *Annals of Botany* **75**: 609–617.

Ceulemans R, Shao BY, Jiang XN, Kalina J. 1996. First- and second-year aboveground growth and productivity of two *Populus* hybrids grown at ambient and elevated CO_2. *Tree Physiology* **16**: 61–68.

Coleman MD, Dickson RE, Isebrands JG, Karnosky DF. 1995. Carbon allocation and partitioning in aspen clones varying in sensitivity to tropospheric ozone. *Tree Physiology* **15**: 593–604.

Curtis PS, Teeri JA. 1992. Seasonal responses of leaf gas exchange to elevated carbon dioxide in *Populus grandidentata*. *Canadian Journal of Forest Research* **22**: 1320–1325.

Curtis PS, Wang X. 1998. A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia* **113**: 299–313.

Curtis PS, Zak DR, Pregitzer KS, Teeri JA. 1994. Above- and belowground response of *Populus grandidentata* to elevated atmospheric CO_2 and soil N availability. *Plant and Soil* **165**: 45–51.

Curtis PS, Vogel CS, Pregitzer KS, Zak DR, Teeri JA. 1995. Interacting effects of soil fertility and atmospheric CO_2 on leaf area growth and carbon gain physiology in *Populus x euramericana*. *New Phytologist* **129**: 253–263.

Drake BG, Gonzàlez-Meler MA. 1997. More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 609–639.

Drew MC. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and shoot, in barley. *New Phytologist* **75**: 470–490.

Field, C, Mooney HA. 1986. The photosynthesis–nitrogen relationship in wild plants. In Givnish TJ, ed. *On the Economy of Plant Form and Function*. Cambridge, UK: Cambridge University Press, 25–55.

Friend AL, Eide MR, Hinckley TM. 1990. Nitrogen stress alters root proliferation in Douglas-fir seedlings. *Canadian Journal of Forest Research* **20**: 1524–1529.

Harper JL. 1982. After description. In: Newman EI, ed. *The Plant Community as a Working Mechanism*. Oxford, UK: Special Publications Series of the British Ecological Society No. 1. Blackwell Scientific, 11–25.

Henry LT, Raper CD. 1991. Soluble carbohydrate allocation to roots, photosynthetic rate of leaves, and nitrate assimilation as

affected by nitrogen stress and irradiance. *Botanical Gazette* **152**: 23–33.

Horwath WR, Pregitzer KS, Paul EA. 1994. ^{14}C allocation in tree-soil systems. *Tree Physiology* **14**: 1163–1176.

Jifon JL, Friend AL, Berrang PC. 1995. Species mixture and soil resource availability affect the root growth response of tree seedlings to elevated CO_2. *Canadian Journal of Forest Research* **25**: 824–832.

Kubiske ME, Pregitzer KS, Mikan CJ, Zak DR, Maziasz JL, Teeri JA. 1997. *Populus tremuloides* photosynthesis and crown architecture in response to elevated CO_2 and soil N availability. *Oecologia* **110**: 328–336.

Larigauderie A, Reynolds JF, Strain BR. 1994. Root response to CO_2 enrichment and nitrogen in loblolly pine. *Plant and Soil* **165**: 21–32.

Meredith MP, Stehman SV. 1991. Repeated measures experiments in forestry: focus on analysis of response curves. *Canadian Journal of Forest Research* **21**: 957–965.

Pregitzer KS, Hendrick RL, Fogel R. 1993. The demography of fine roots in response to patches of water and nitrogen. *New Phytologist* **125**: 575–580.

Pregitzer KS, Zak DR, Curtis PS, Kubiske ME, Teeri JA, Vogel CS. 1995. Atmospheric CO_2, soil nitrogen and turnover of fine roots. *New Phytologist* **129**: 579–585.

Pregitzer KS, Kubiske ME, Yu CK, Hendrick RL. 1997. Root architecture, carbon and nitrogen in four temperature forest species. *Oecologia*, **111**: 302–308.

Pyke DA, Thompson JN. 1986. Statistical analysis of survival and removal rate experiments. *Ecology* **67**: 240–245.

Radoglou KM, Jarvis PG. 1990. Effects of CO_2 enrichment on four poplar clones. I. Growth and leaf anatomy. *Annals of Botany* **65**: 617–626.

Reich PB, Walters MB, Ellsworth DS. 1992. Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. *Ecological Monographs* **62**: 365–392.

Robson DS. 1959. A simple method for constructing orthogonal polynomials when the independent variable is unequally spaced. *Biometrics* **15**: 187–191.

Rogers HH, Peterson CM, McCrimmon JN, Cure JD. 1992. Response of plant roots to elevated atmospheric carbon dioxide. *Plant, Cell and Environment* **15**: 749–752.

Rogers HH, Runion GB, Krupa SV. 1994. Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environment Pollution* **83**: 155–189.

Rouhier H, Billès G, El Kohen A, Mousseau M, Bottner P. 1994. Effect of elevated CO_2 on carbon and nitrogen distribution within a tree (*Castanea sativa* Mill.) – soil system. *Plant and Soil* **162**: 281–292.

Stulen I, den Hertog J. 1993. Root growth and functioning under atmospheric CO_2 enrichment. *Vegetation* **104/105**: 99–115.

Tingey DT, Johnson MG, Phillip DL, Johnson DW, Ball JT. 1996. Effects of elevated CO_2 and nitrogen on the synchrony of shoot and root growth in ponderosa pine. *Tree Physiology* **16**: 905–914.

von Caemmerer S, Farquhar GD. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**: 376–387.

Yanai RD. 1994. A steady-state model of nutrient uptake improved to account for newly-grown roots. *Soil Science Society of America Journal* **58**: 1562–1571.

Zak DR, Pregitzer KS, Curtis JA, Fogel R, Randlett DL. 1993. Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. *Plant and Soil* **151**: 105–117.