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Elevated CO_2 and the magnitude and seasonal dynamics of root production and loss in *Betula papyrifera*

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Abstract

The impact of elevated atmospheric CO_2 on belowground plant growth is poorly understood relative to its effects on aboveground growth. We carried out a study of the seasonal dynamics of gross root production and death to determine how elevated CO_2 affected the dynamics of net and gross root production through a full growing season. We quantified gross root production and root loss from sequential, in situ images of fine roots of *Betula papyrifera* in ambient (375 ppm.) and elevated (700 ppm) CO_2 atmospheres from 2 weeks following germination through leaf senescence. We found that elevated CO_2 led to increases in the magnitude of cumulative gross production (ΣP) and cumulative gross loss (ΣL) of roots. However, the effect of elevated CO_2 on these processes was seasonally dependent. Elevated CO_2 led to greater levels of enhancement in ΣP early in the growing season, prior to maximum standing root length (NP). In contrast, elevated CO_2 led to greater levels of enhancement in ΣL in the last half of the growing season, after maximum NP had been reached. This difference in the timing of when elevated CO_2 affects ΣP and ΣL led to a transitory, early enhancement in NP. By the end of the growing season, there was no significant effect of elevated CO_2 on NP, and ΣP was 87% greater than NP for ambient CO_2 and 117% greater in elevated CO_2. We conclude that static assessments of belowground productivity may greatly underestimate gross fine root productivity and turnover and this bias can be exaggerated with elevated CO_2.

Introduction

Since pre-industrial times, atmospheric concentrations of CO_2 have increased by nearly 100 ppm, and they are predicted to double within the next century (Houghton et al., 1996). In addition to feedbacks on climate, elevated CO_2 can lead to significant alterations in plant physiology, plant growth, and plant community dynamics as well as increases in net primary productivity (Bazzaz, 1990; Ceulemans and Mousseau, 1994; Eamus and Jarvis, 1989). To date, most studies of individual plant and plant community responses to elevated CO_2 have focused on leaf gas exchange and or net primary productivity (NPP). However, it is becoming increasingly clear that indirect effects of elevated CO_2 on below ground processes are likely to play a fundamental role in determining the magnitude of carbon storage in terrestrial ecosystems as well as the potential for maintaining long term growth enhancement in elevated CO_2 (Curtis et al., 1994; Rogers et al., 1994).

In temperate forest ecosystems up to 50% or more of NPP is allocated belowground (Fogel, 1983, 1985; McClaugherty et al., 1982; Vogt et al., 1986). Recent developments of non-destructive methods for monitoring in situ root dynamics suggest that these estimates of net belowground production can significantly underestimate gross production (Fahey and Hughes, 1994; Hendrick and Pregitzer, 1992a, 1993a, b). The difference between net and gross belowground productivity represents an input of root derived detritus into the soil. Increased input of detritus into the soil with elevated CO_2 may lead to significant changes in belowground

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food webs and nutrient cycling, possibly leading to alterations in the carbon storage and nutrient availability in the soil (Berntson and Bazzaz, 1997a, b; Curtis et al., 1994; Rogers et al., 1994).

To date, there have been two published reports of the simultaneous effect of elevated CO_2 on gross and net root production for temperate tree species: for *Populus* (Pregitzer et al., 1995) and *Betula papyrifera* and *Acer rubrum* (Berntson and Bazzaz, 1996). The results of these two studies are fairly consistent; when elevated CO_2 leads to significant increases in NPP, the gross production and loss of roots is also increased. An important limitation to both of these studies, however, is that they were carried out over only the first portion of the growing season. Both of these studies found that the effect of elevated CO_2 on root loss was greater toward the end of their experiments.

In temperate forests, net root production is highly seasonal so that standing root length and biomass typically show distinct seasonal patterns (e.g., Burke and Raynal, 1994; McClaugherty et al., 1982). The relative importance of root production is greater earlier in the growing season so the standing quantity of roots tends to increase, and the relative importance of root loss is greater at the end of the growing season and during the winter so the standing quantity of roots tends to decrease.

A key goal of studies of root growth dynamics in response to elevated CO_2 is to better understand the potential for carbon storage belowground (Curtis et al., 1994). A critical component of this understanding is the relationship between net and gross productivity. The seasonal shift from net root production to net root loss implies that short term studies (less than a full growing season) do not include the full dynamics of root loss which take place in a single year (Berntson and Bazzaz, 1996). Thus, studies of root growth dynamics in elevated CO_2 need to encompass at least one full growing season if they are to quantify actual root production and turnover. This paper presents a study which examines the root growth and death dynamics over a full growing season (from germination through leaf senescence) in *Betula papyrifera* seedlings grown in ambient and elevated CO_2 atmospheres.

Materials and methods

Seed collection, germination and transplanting

Seeds of *Betula papyrifera* were collected from the Harvard Forest (Petersham, MA). Seeds were collected from three parent trees. Seeds were germinated in flats 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 (375 ppm), the other half in elevated CO_2 (700 ppm). In early May 1995, 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. All transplanted seedlings had two leaves that were between 1 and 2 cm in length.

Soil, growth containers and growth conditions

Plants were transplanted into growth containers (1.6 L volume) which allowed the non-destructive observation of root growth through large plexiglass windows. A detailed description of the design and maintenance of the growth containers is provided in (Berntson and Bazzaz, 1996; Berntson et al., 1995). The soil used to fill the growth containers was a 1:1:1 mix of coarse sand, peat, and field soil. The field soil consisted of forest floor material to a depth of 8 cm, collected from a 40 year-old stand of *Betula papyrifera* in Massachusetts, USA (42° 34′ N, 70° 50′ W). No fertilizer was added to the soil at any time during the course of the experiment.

Thirty plants were randomly distributed among six separate glasshouse modules, for a total sample size of 15 replicate plants per CO_2 level. 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, but received no supplementary artificial lighting. Day/night temperatures were maintained at 26°/19 °C with a 13-h day until the beginning of September. At this time day/night temperatures were ramped down to simulate the onset of autumn. Decreasing temperatures continued until the end of the experiment, when >90% of all leaves had senesced (December 7, 1995).

Measurements of root production and loss

The methods used for calculating root production and loss were based on Berntson and Bazzaz (1996).

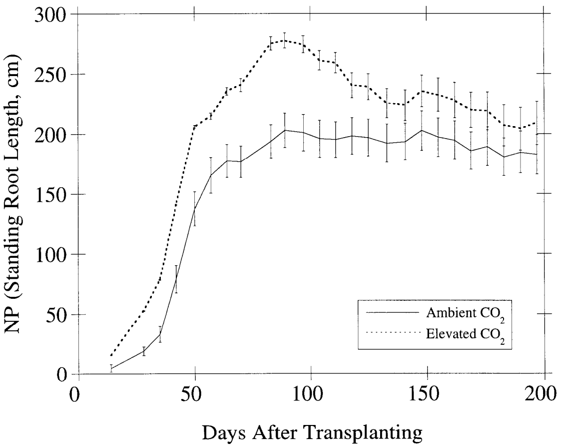

Figure 1. Seasonal dynamics (2 weeks after germination through leaf senescence) of NP (standing root length) for *Betula papyrifera* in ambient (375 ppm) and elevated (700 ppm) atmospheric CO_2. Error bars are a single standard error of the mean (n = 15).

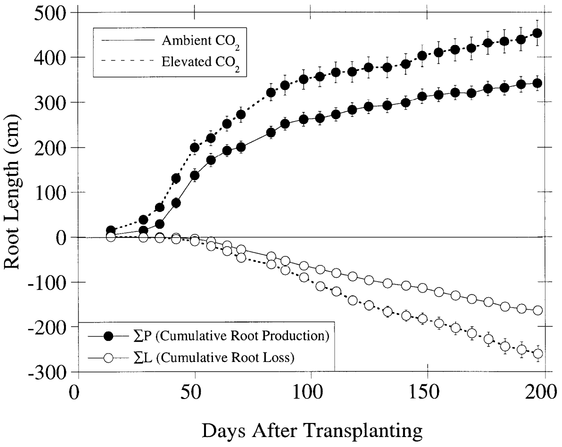

Figure 2. Seasonal dynamics (2 weeks after germination through leaf senescence) of ΣP (cumulative gross root production) and ΣL (cumulative gross root loss) for *Betula papyrifera* in ambient (375 ppm) and elevated (700 ppm) atmospheric CO_2. Error bars are a single standard error of the mean (n = 15). In cases where no error bar is visible, it is smaller than the size of the symbol.

The following modifications to these techniques were made: (1) Tracings of roots were made every seven days, starting 14 days after transplanting. (2) Tracing was restricted to a rectangular subsection (200 cm^2) which represented 30% of the total viewing area. This subsection of the window reduced the time required to create an accurate tracing, but still captured the spatio-temporal dynamics of root production and loss (Berntson et al., 1995). (3) Calculation of standing root length (cumulative net root production), and gross root production and loss from the tracings were made using an image processing program we developed. The image processing program used the basic quantitative procedures for solving for root production from changes in standing root length and root loss described by Berntson and Bazzaz (1996). For purposes of consistency, all of the terminology and symbols used by Berntson and Bazzaz (1996) will be used without modification in this paper.

Statistical analyses

Root growth dynamics were analyzed using randomized split-plot, nested repeated measures analysis-of-variance with CO_2 as the (fixed) main plot factor, individual plant was nested in CO_2 × Block (Velleman, 1994). Time was treated as subplot (fixed) factor. Blocks were designated as random effects. Tests of the effects of CO_2 at a given time or for a given CO_2 level between two times were performed using Scheffé *post hoc* comparisons with probability of a type I error set to 0.05. Normal probability plots were used to asses the homoscedasticity and normality of residuals (Velleman, 1994). For all of the ANOVAs herein presented, residuals were judged to be normal and homoscedastic by the normal probability plots without any prior transformation of the dependent variables.

Results

The overall effect of elevated CO_2 on standing root length (net production, NP) was not significant, but effect of elevated CO_2 varied significantly through the course of the experiment (see Table 1). Up to day 89, NP increased monotonically for both CO_2 levels, and elevated CO_2 resulted in increasing levels of enhancement in standing root length relative to ambient CO_2 (Figure 1). At day 89 NP reached a maximum level for both ambient (202.8 ± 12.4 cm, mean ± s.e.) and elevated CO_2 (277.7 ± 20.8 cm). After day 89, NP decreased in elevated CO_2 so that by day 118, NP in elevated CO_2 was no longer significantly greater than in ambient CO_2 (p = 0.824). By day 197, when less than 10% of leaves were left on the plants, NP in elevated CO_2 had declined by 25% (p < 0.001), and by only 10% in ambient CO_2 (p = 0.723).

Similar to NP, elevated CO_2 did not have an overall significant effect on cumulative gross root production (ΣP) or loss (ΣL) but the effect of elevated CO_2 varied significantly through the course of the experiment for

Table 1. Results (p-values) of split-plot, nested repeated-measures analysis-of-variance for standing root length (net root production, NP), gross cumulative root production (ΣP), and gross cumulative root loss (ΣL). Total model degrees of freedom = 780. Summaries of average values for each dependent variable are given in Figures 1 and 2, and a summary of CO_2 effects through time are given in Figure 3

Dependent variable	CO_2	Block	$CO_2 \times$ Block	Plant	Time	$CO_2 \times$ Time
NP	0.105	0.932	0.471	< 0.001	< 0.001	< 0.001
ΣP	0.162	0.265	0.066	< 0.001	< 0.001	< 0.001
ΣL	0.143	0.005	0.003	< 0.001	< 0.001	< 0.001

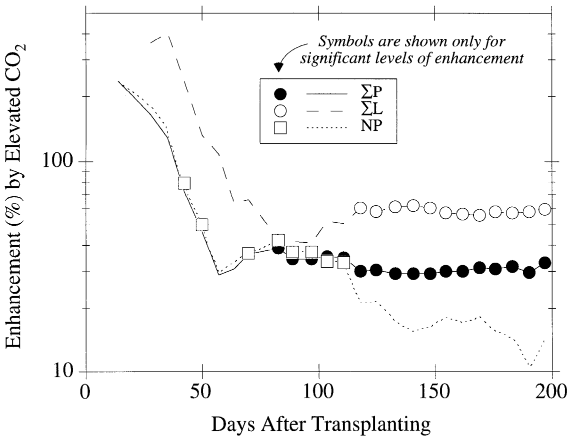

Figure 3. Seasonal dynamics of the effect of elevated CO_2 on NP, ΣP, and ΣL. The effect of elevated CO_2 is expressed as the percent change relative to levels observed in ambient CO_2. Symbols are shown only at times where elevated CO_2 resulted in a significant enhancement ($p < 0.05$) as determined by Sctieffé post hoc tests run after performing the repeated measures ANOVAs described in the 'Materials and methods'.

both of these variables (Table 1). Elevated CO_2 lead to increasing levels of ΣP prior to reaching maximum NP at day 89 (Figure 2). After maximum NP had been reached, the trajectories of ΣP for both CO_2 levels remained parallel to one another. For ΣL, elevated CO_2 had a more pronounced effect after the maximum NP had been reached (Figure 2). In contrast to ΣP, elevated CO_2 lead to increasing magnitudes of ΣL relative to ambient CO_2 only after day 89.

The transitory increase in NP under elevated CO_2 was the result of the different temporal patterns of enhancement in ΣP and ΣL. The seasonal trends in CO_2 enhancement (expressed as % increase relative to ambient CO_2) were different for NP, ΣP, and ΣL (Figure 3). All three variables show similar general trends of high early levels of enhancement, but none of the early observed enhancements are significant due to high relative variation. NP and ΣP are the first to show statistically significant enhancement with elevated CO_2. Starting at day 70 and lasting through the end of the experiment, elevated CO_2 resulted in significantly increased ΣP. At the end of the experiment, elevated CO_2 had resulted in a 33% increase in ΣP. For NP, the significant increase in elevated CO_2 started around day 50 and lasted until day 111. After day 111, NP was not significantly affected by elevated CO_2. Elevated CO_2 did not lead to a significant increase in ΣL until day 118, precisely coinciding with the first incident when NP was no longer significantly increased by elevated. CO_2. ΣL remained significantly enhanced by elevated CO_2 through the end of the experiment. At the end of the experiment, elevated CO_2 had resulted in a 59% enhancement in ΣL.

Discussion

The dynamic nature of root growth and death in temperate trees poses significant methodological challenges. This study provides two important observations regarding the dynamic nature of root production and loss in elevated CO_2 environments.

Observation 1: The seasonal time-courses of enhancement by elevated CO_2 differ for root production and for root loss. Early in the growing season, elevated CO_2 leads to greater enhancement in gross root production. Whereas, later in the growing, season elevated CO_2 leads to greater enhancement in gross root loss.

This finding is consistent with our current understanding of seasonal trends in forest productivity. We know that aboveground and belowground standing biomass peak in the mid- to late summer in most temperate forest ecosystems (Burke and Raynal, 1994;

McClaugherty et al., 1982; Waring and Schlesinger, 1986). Seasonal patterns of net ecosystem exchange of CO_2 in temperate forest ecosystems, measured using eddy-flux correlation techniques, are also consistent with this observation (Wofsy et al., 1993). While these results are not surprising, they have critical implications for how we measure belowground productivity and the potential for carbon storage. Studies of temperate trees which do not follow the full seasonal time course of root dynamics are likely to underestimate, possibly by a large amount, the difference between net and gross root production integrated over a full growing season.

Observation 2. The difference between gross and net root production increases most markedly toward the end of the growing season. Because of enhanced root turnover, this difference will be greater in elevated than in ambient CO_2.

By the end of leaf senescence, net root production underestimated gross root production in ambient CO_2 by 87%, and, in elevated CO_2, by 117%. These discrepancies are so large that any estimate of standing root mass at the end of the growing season needs to be clearly identified as net production, with an understanding that gross production is likely to be much greater. Because the extent of this discrepancy differs between the CO_2 levels, it is problematic to the use end-of-season net root production as an estimate of full season productivity. In this study, standing root length (NP) at the end of the season was not significantly different between the CO_2 levels. However, when we compare ΣP, elevated CO_2 resulted in a significant 33% increase. The greater enhancement in ΣL in elevated CO_2 (59%), which manifested itself later in the season, offset the gains in ΣP leading to the apparent insignificant CO_2 effects.

Interactions with and implications for soil processes

Several others have recently argued that the number, type, and magnitude of belowground effects from rising CO_2 levels is large (Norby, 1994; O'Neill, 1994; Rogers et al., 1994). Nearly every biological and biologically regulated process within the soil can be strongly affected by increasing CO_2 levels due to an increased input of organic carbon. The increased root turnover (elevated root production and loss) observed in this study represents a mechanism whereby the total input of organic material to the soil is enhanced. Soil microflora and -fauna are dependent on plant derived organic material and, in turn, plants are dependent on the soil biota for the cycling of nutrients. The increase in soil labile organic matter derived from plants represents an increase in the substrate which most soil heterotrophic organisms are dependent on for growth (Zak et al., 1994). With this increased food supply, the trophic structure and subsequent cycling and storage of carbon and inorganic nutrients may be significantly altered under elevated CO_2 (Berntson and Bazzaz, 1997a, b; Díaz et al., 1993 Zak et al., 1993).

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