New Phytol. (1995), **129**, 253–263

Interacting effects of soil fertility and atmospheric CO_2 on leaf area growth and carbon gain physiology in *Populus* × *euramericana* (Dode) Guinier

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(*Received 9 June 1994; accepted 7 November 1994*)

SUMMARY

Two important processes which may limit productivity gains in forest ecosystems with rising atmospheric CO_2 are reduction in photosynthetic capacity following prolonged exposure to high CO_2 and diminution of positive growth responses when soil nutrients, particularly N, are limiting. To examine the interacting effects of soil fertility and CO_2 enrichment on photosynthesis and growth in trees we grew hybrid poplar (*Populus x euramericana*) for 158 d in the field at ambient and twice ambient CO_2 and in soil with low or high N availability. We measured the timing and rate of canopy development, the seasonal dynamics of leaf level photosynthetic capacity, respiration, and N and carbohydrate concentration, and final above- and belowground dry weight.

Single leaf net CO_2 assimilation (A) increased at elevated CO_2 over the majority of the growing season in both fertility treatments. At high fertility, the maximum size of individual leaves, total leaf number, and seasonal leaf area duration (LAD) also increased at elevated CO_2, leading to a 49% increase in total dry weight. In contrast, at low fertility leaf area growth was unaffected by CO_2 treatment. Total dry weight nonetheless increased 25% due to CO_2 effects on A. Photosynthetic capacity (A at constant internal $p(CO_2)$, (C_i)) was reduced in high CO_2 plants after 100 d growth at low fertility and 135 d growth at high fertility. Analysis of A responses to changing C_i indicated that this negative adjustment of photosynthesis was due to a reduction in the maximum rate of CO_2 fixation by Rubisco. Maximum rate of electron transport and phosphate regeneration capacity were either unaffected or declined at elevated CO_2. Carbon dioxide effects on leaf respiration were most pronounced at high fertility, with increased respiration mid-season and no change (area basis) or reduced (mass basis) respiration late-season in elevated compared to ambient CO_2 plants. This temporal variation correlated with changes in leaf N concentration and leaf mass per area. Our results demonstrate the importance of considering both structural and physiological pathways of net C gain in predicting tree responses to rising CO_2 under conditions of suboptimal soil fertility.

Key words: Carbon dioxide, nitrogen availability, *Populus x euramericana*, leaf growth, gas exchange.

INTRODUCTION

Rising atmospheric CO_2 has many potential consequences for terrestrial vegetation, ranging from short-term physiological responses to long-term changes in ecosystem structure and function (Strain, 1985). To predict ecosystem level responses better it will be important to know the extent to which dominant plant species exhibit sustained increases in net CO_2 assimilation (A) and growth as CO_2 levels rise (Graham, Turner & Dale, 1990; Zak *et al.*, 1993). The possibility of such sustained increases is open to question on two grounds. First, it is possible that there will be a reduction or elimination of

increases in A following prolonged exposure to elevated CO_2 (Tissue & Oechel, 1987; Sage, Sharkey & Seemann, 1989). Secondly, it is possible that there will be a reduction or elimination of increases in growth at elevated CO_2 when other environmental factors (e.g. nutrients) strongly limit growth (Norby *et al.*, 1992; Diaz *et al.*, 1993). These processes, operating alone or together, could significantly reduce plant responses to elevated CO_2 and hence moderate the direct effects of CO_2 on ecosystems.

The reduction in CO_2 sensitivity of A following long-term (weeks to months) exposure to a CO_2 enriched atmosphere has been viewed as a process of negative adjustment (or 'acclimation') of photosynthetic capacity (Gunderson & Wullschleger, 1994). Specifically, negative adjustment results in lower A at equal internal CO_2 partial pressures (C_i) in high CO_2 relative to ambient CO_2 grown plants. The mechanism(s) causing negative photosynthetic adjustment are not entirely certain but are likely to involve two related processes. Photosynthate production may exceed sink demand to such a degree that non-structural carbohydrates accumulate to high levels in source leaves, leading to feedback inhibition of photosynthetic rate. This inhibition may be a direct result of starch accumulation in chloroplasts (Sasek, DeLucia & Strain, 1985) or due to indirect effects on chloroplast phosphate levels (Sharkey & Vanderveer, 1989). Alternatively, photosynthetic rates may be downregulated by redistribution of N within the plant, away from Rubisco and enzymes involved in RuBP regeneration, and into the formation of additional leaves or other tissues (Stitt, 1991).

It is unclear how prevalent negative photosynthetic adjustment to elevated CO_2 may be under ecologically realistic field conditions. Arp (1991) and Sage (1994) have suggested that restricted rooting volume plays a key role in eliciting this response by limiting belowground sink activity (but see McConnaughay, Berntson & Bazzaz, 1993). Soil fertility also may affect A responses to CO_2. In field based experiments (i.e. full sun and large rooting volumes), plants receiving moderate to high nutrient supply rates exhibited little or no negative photosynthetic adjustment (Idso, Kimball & Allen, 1991; Drake, 1992). In contrast, in cases where nutrients were low, CO_2 sensitivity of A rapidly declined (Tissue & Oechel, 1987; Curtis *et al.*, 1994). Even when negative adjustment occurs, A may remain significantly higher in elevated than in ambient grown plants. This can be considered partial adjustment, and may be a common response in woody species (Gunderson & Wullschleger, 1994).

Seasonal C gain in plants is a function not only of leaf photosynthetic capacity but also of the timing and rate of leaf area growth (Kramer, 1981). Under favourable conditions, CO_2 enrichment generally increases whole plant leaf area (LA) (Sasek & Strain, 1989; Radoglou & Jarvis, 1990), with increases often occurring early in growth and hence contributing to initial growth advantages at high CO_2. As nutrient levels are reduced, this stimulation disappears (El Kohen, Rouhier & Mousseau, 1992) and LA may actually be less at elevated compared to ambient CO_2 (Goudriaan & de Ruiter, 1983). For example, Norby *et al.* (1992) reported that in *Liriodendron tulipifera* growing at elevated CO_2 in unfertilized, unirrigated soil, increased A was balanced by reduced LA, resulting in no net biomass increase compared to ambient CO_2 grown plants. Evaluating the likelihood of sustained CO_2 responses on nutrient poor soils thus requires an assessment of the relative sensitivity of both leaf growth and A to changes in C and N availability.

We have explored the interaction between CO_2 and N availability using a hybrid poplar, *Populus x euramericana* (Dode) Guinier cv. Eugenei. This hybrid is a cross between *Populus deltoides* Bartr. ex Marsh. and *Populus nigra* L. Here, we tested the general hypotheses that A will increase under elevated CO_2 and that this increase will result in greater plant growth, even under conditions of N limitation (Zak *et al.*, 1993). To examine the mechanisms by which prolonged CO_2 enrichment and/or low N availability might limit the magnitude of the CO_2 response, we have focussed on the seasonal dynamics of C and N allocation at single leaf and whole plant levels.

MATERIALS AND METHODS

Experimental design and plant growth

An array of 20 open bottom root boxes, 0·5 $m^2 \times 1{\cdot}3$ m, were put into the soil in an open field, in early May, 1992, at the University of Michigan Biological Station, in northern Lower Michigan, USA. Each box was lined with plastic and contained two mini-rhizotron tubes mounted at 30° from vertical. Two contrasting soil fertility treatments were established by filling the root boxes with either 100 % locally excavated Kalkaska series topsoil (high fertility treatment) or a homogenized mixture of 20 % topsoil, 80 % native Rubicon sand (low fertility treatment). Net N mineralization in the two treatments was calculated from the production of ammonium-N and nitrate-N over a 9-wk aerobic laboratory incubation at 25 °C (Zak *et al.*, 1993). Nitrogen mineralization was significantly higher ($P < 0{\cdot}0001$) in the high fertility treatment (346 $\mu g\ N\ g^{-1}\ d^{-1}$) than in the low fertility treatment (45 $\mu g\ N\ g^{-1}\ d^{-1}$). Both topsoil and sand were high in inorganic phosphate. Extractable PO_4^{3-}, assayed using an acid extraction procedure, was 110 mg kg^{-1} in the low fertility and 64 mg kg^{-1} in the high fertility treatment.

Five cuttings of *Populus x euramericana* cv. Eugenei (hereafter 'Eugenei') were planted in each

root box on 21 May 1992 (day no. 142). Cuttings were obtained from stock propagated at Michigan State University and were graded for uniformity in diameter and condition. Mean dry weight of cuttings was 5·2 g.

Small open top chambers (0·49 $m^2 \times 1{\cdot}0$ m, Curtis & Teeri, 1992) were used to manipulate atmospheric CO_2 partial pressure around plants growing in the root boxes. Ten chambers received additional CO_2 (elevated CO_2 treatment), and ten chambers received no additional CO_2 (ambient CO_2 treatment). Carbon dioxide treatments were randomized across fertility treatments resulting in a randomized block design with two CO_2 levels (ambient and elevated), two fertility levels (low and high), and five replicate blocks.

Carbon dioxide partial pressure inside elevated CO_2 chambers was increased by dispensing 100 % CO_2 into an input blower; concentrations were controlled with manual flowmeters. The atmosphere inside the chambers was continuously monitored by an infra-red gas analyzer that logged data to a personal computer. Temperatures inside and outside chambers were monitored every 15 min using shaded thermocouples connected to an LI 1000 datalogger (LICOR Inc, Lincoln, NB). Mean seasonal daytime CO_2 partial pressures were $69{\cdot}3 \pm 4{\cdot}5$ Pa (mean $\pm$ SD of 12 h chamber means, $n = 1313$) inside elevated chambers and $34{\cdot}5 \pm 1{\cdot}5$ Pa ($n = 134$) inside ambient chambers. Seasonal nighttime CO_2 partial pressures were $75{\cdot}9 \pm 8{\cdot}6$ Pa and $38{\cdot}6 \pm 2{\cdot}3$ Pa inside elevated and ambient chambers respectively. Daytime temperatures averaged 2·7 °C higher inside chambers than outside across the entire season (range, −0·5 to 4·5 °C temperature difference), but there was no significant temperature difference among CO_2 or fertility treatments. The chamber covering (0·02 cm polyvinyl chloride film with UV inhibitors) transmits *c.* 86 % of total incoming solar radiation (Heagle *et al.*, 1989).

Plants were kept well watered throughout the experiment by addition of 3 l water to each box every third day, except during periods of rainfall. It was our intention to avoid any confounding effects due to water stress. However, while experimental soils were never dry, differing amounts of organic matter in the two fertility treatments could have led to differences in soil water content and hence possible effects on plant water status. To assess this possibility, predawn and midday xylem water potentials were measured on day numbers 239 and 246 respectively, using a Scholander pressure bomb. On these dates, plants had received no water for 3 d (the longest possible dry interval) and those 3 d had been relatively warm and sunny. The summer of 1992 was exceptionally cool in northern Lower Michigan and these sampling dates were chosen to maximize the possibility of detecting differences among treatments in plant water status.

Aboveground growth was monitored non-destructively by censusing stem height, and length (l, cm) and width (w, cm) of leaves from all plants on five dates. Area of each leaf (AL, cm^2) was calculated from the equation $AL = -0{\cdot}377 + 0{\cdot}587\ (l \times w)$, $r^2 = 0{\cdot}99$, obtained from destructive harvests on a separate set of plants. The experiment was terminated after 158 d growth (16 Oct 1992, day no. 290) by complete above- and belowground harvest (= census no. 6). Total tree leaf area was then measured using a LICOR LI-3000 leaf area meter. The entire rooting volume was excavated by hand and coarse and fine roots separated from bulk soil in the field. Final separation of the frozen root system was conducted by hand in the laboratory. Above- and belowground tissues were dried at 70 °C except for subsamples used in carbohydrate analyses.

Seasonal, whole plant net assimilation rate (NAR) was calculated as the ratio of final harvest above- and belowground dry weight (d.wt) to whole plant leaf area duration

$$NAR = \frac{\text{d.wt}}{LAD}, \tag{1}$$

$$\text{where} \quad LAD = \sum_{i=1}^{i=6} \frac{(LA_i - LA_{i-1})(t_i - t_{i-1})}{\ln LA_i - \ln LA_{i-1}} \tag{2}$$

and LA_i = leaf area per plant at the ith census, and t_i = day no. at the ith census (Chiariello, Mooney & Williams, 1989). Belowground biomass was assumed to be equally distributed among plants within a chamber.

Gas exchange

Net CO_2 assimilation was measured using an LCA3 portable photosynthesis system (ADC, Herts UK) equipped with a water-jacketed cuvette. Bottled air enriched with CO_2 was supplied to the LCA3 allowing gas exchange measurements above ambient CO_2. A saturating light intensity (1800 μmol m^{-2} s^{-1}) was supplied by a quartz halogen lamp. Leaf temperature was maintained between 25 °C and 27 °C, and cuvette relative humidity was always greater than 50 %. All gas exchange measurements were conducted on the youngest fully expanded leaf (generally the 11th leaf from the apex with a lamina longer than 1 cm) from one plant per chamber.

Net CO_2 assimilation in ambient and elevated CO_2 plants was routinely measured at two C_i: 27 (A_{27}) and 56 (A_{56}) Pa. These were selected as representative late-morning values from full sun leaves in the ambient and elevated treatments and allowed an evaluation of photosynthetic capacity independent of diurnal or seasonal fluctuations in stomatal conductance. Actual cuvette p(CO_2) varied with stomatal conductance, but was generally within 5 Pa of growth p(CO_2). To determine gas exchange responses to changes in C_i, leaves were first exposed to

their growth $p(CO_2)$ (either 35 or 70 Pa) until steady state gas exchange was observed (7–15 min). Carbon dioxide partial pressure inside the cuvette was then reduced to *c.* 9 Pa and increased in a stepwise manner to *c.* 110 Pa. At each measurement $p(CO_2)$, leaves were allowed to equilibrate for at least 5 min before gas exchange was recorded. Dark respiration was measured at 27 °C on 10 cm^2 leaf discs from the youngest fully expanded leaf using a gas phase Hansatech oxygen electrode and water jacketed cuvette (Hansatech Inst. Ltd., Norfolk, UK). Leaf discs were collected between 10:00 and 16:00 h and immediately placed inside the cuvette at growth $p(CO_2)$. Stable rates of O_2 depletion were achieved within 10 min.

Assimilation *v.* C_i response curves were analyzed according to the methods of Harley *et al.* (1992). A mechanistic model of C_3 photosynthesis (Farquhar *et al.*, 1980; Harley & Sharkey, 1991) was fitted to A/C_i data using nonlinear regression. Model parameters were estimated using a two step curve fitting procedure. At low C_i (< 20 Pa), RuBP levels were assumed to be non-limiting, such that,

$$A = \frac{V_{C,max} C_i}{C_i + K_C(1 + O/K_O)}\left(1 - \frac{0{\cdot}5O}{\tau C_i}\right) - R_d, \quad (3)$$

where $V_{C,max}$ = maximum rate of CO_2 fixation by Rubisco, K_C = Michaelis–Menten constant of Rubisco for CO_2, K_O = Michaelis–Menten constant of Rubisco for O_2, O = oxygen partial pressure (210 kbar), τ = specificity factor of Rubisco for CO_2 *v.* O_2, and R_d = day respiration. K_C, K_O, and τ were assumed to be similar to other C_3 species and assigned values following Harley *et al.* (1992). $V_{C,max}$ and R_d were estimated by nonlinear regression of (3) against A/C_i data at low C_i.

At high C_i (> 20 Pa), the rate of electron transport (J_{max}), or the rate of inorganic phosphate turnover (PiRC) will increasingly limit A, such that,

$$A = \left(1 - \frac{0{\cdot}5O}{\tau C_i}\right)\min\{W_c, W_j, W_p\} - R_d, \quad (4)$$

where

$$W_c = \frac{V_{C,max} C_i}{C_i + K_C(1 + O/K_O)}, \quad (5)$$

$$W_j = \frac{J_{max} C_i}{4(C_i + O/\tau)}, \quad (6)$$

$$W_p = 3\,PiRC + \frac{V_{C,max} O}{C_i \tau}. \quad (7)$$

Using values of $V_{C,max}$ and R_d estimated at low C_i, J_{max} and PiRC were estimated by nonlinear regression of equation 4 against entire A/C_i curves. All parameters were corrected for measured leaf temperature after the methods of Lewis *et al.* (1994). Regressions were conducted on individual curves (9–10 points each), all with goodness of fit (r^2) of 0·94 or higher.

Tissue chemistry

Leaf discs (2·6 cm^2) were excised at various dates from the youngest fully expanded leaf and analyzed for soluble sugar, starch, and total N. Tissue for carbohydrate analysis was collected between 14:00 and 15:00 h, immediately frozen in liquid nitrogen, and stored at −80 °C until lyophilized. Powdered, lyophilized samples were extracted with 80 % ethanol at 80 °C, the supernatant evaporated to dryness and then redisolved in H_2O + polyvinylpolypyrrolidone. Soluble carbohydrates were analyzed enzymatically using a modification of the procedure of Jones, Outlaw & Lowry (1977), with a recovery rate of 95 %. The ethanol extracted tissue pellet was suspended in 0·2 N KOH, boiled for 20 min, and brought to pH 7·0 with 1·0 N acetic acid. Amyloglucosidase was added to the resuspended pellet and incubated for 1 h at 55 °C. Starch concentration was determined as glucose equivalents using the same procedure as for soluble sugars. Starch recovery was 93 %. Total N was determined on a portion of the lyophilized leaf tissue using a Carlo Erba CHN analyzer.

Statistical analysis

All growth, gas exchange, and tissue chemistry data were analyzed by analysis of variance (ANOVA) for randomized block design where the unit of replication was the chamber. Where multiple individuals within a chamber were sampled, results were pooled and expressed on a per plant basis. Sample size therefore was always five ($n = 5$), except for some gas exchange measurements where full replication was not achieved. On day no. 260, $n = 4$ (all measurements) and on day no. 219, $n = 4$ (high fertility), $n = 3$ (low fertility ambient and low fertility elevated, A_{56}), or $n = 2$ (low fertility elevated, A_{27}). Comparisons among means was by least significant difference for *a priori* comparisons (between CO_2 treatments within a fertility treatment), and by minimum significant range for all *a posteriori* comparisons (Sokal & Rohlf, 1981).

RESULTS

Growth

There was a strong interaction between soil fertility and CO_2 on the timing and extent of leaf area growth (Fig. 1, Table 1). At high fertility, leaf area was significantly greater in elevated CO_2 plants by day no. 219. This difference was maintained until final harvest, when elevated CO_2 plants had 35 % greater leaf area than ambient plants. Seasonal leaf area

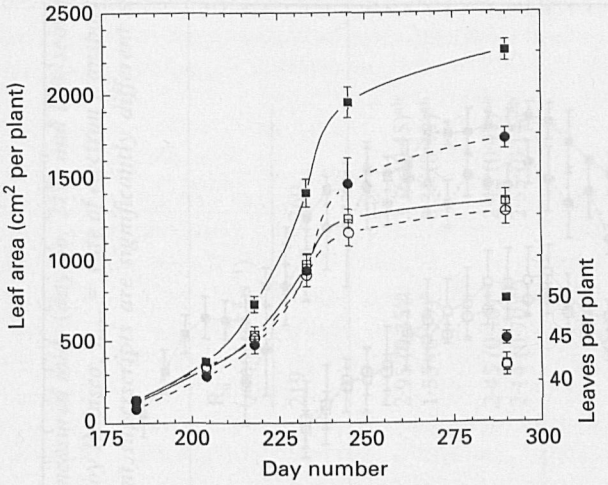

Figure 1. Leaf area per plant and total leaves per plant in Eugenei grown at low or high fertility and at ambient (Amb.) or elevated (Elev.) CO_2. ■, Elev., high; ●, amb., high; □, elev., low; ○, amb., low. Prior to the final harvest (day no. 290), leaf area was estimated from censuses of leaf length and width. Vertical bars indicate one SE (n = 5, individuals pooled within chambers).

duration increased to a similar extent (38%) in elevated compared to ambient CO_2 plants (Table 1). At low fertility, CO_2 had no effect on either final leaf area or LAD. Low soil fertility reduced final leaf area by 24% at ambient CO_2 and by 43% at elevated CO_2 relative to plants at high fertility. Significant soil fertility effects on total leaf area also appeared earlier at elevated than at ambient CO_2.

Treatment effects on final leaf area per plant were due to changes in both the total number of leaves per plant and the size of individual leaves. Total number of leaves per plant increased under elevated CO_2 only at high fertility, and high fertility plants as a group had more leaves than low fertility plants (Fig. 1). The final size of individual leaves displayed a similar pattern of treatment response (Fig. 2). At high fertility, the largest leaves (numbers 31–35) and those produced near the end of the season were significantly larger at elevated than at ambient CO_2. However, leaf numbers 13–18, produced early in the season, were significantly smaller at elevated than at ambient CO_2. Carbon dioxide effects on leaf size were much less at low fertility, although some leaves produced mid-season (numbers 22–26) also were significantly larger at elevated than at ambient CO_2. Plants growing at high fertility had consistently larger leaves than those at low fertility. The largest leaves, and those produced late in the season, were *c.* 40% larger at high than at low fertility. Plants in all treatments had a monopodial growth form, with no lateral branching.

Total plant biomass (roots+shoot) was affected by both CO_2 and fertility treatments (Table 1). Within a fertility treatment, biomass was significantly greater at elevated than at ambient CO_2, but the relative CO_2 stimulation was larger at high compared to low fertility (49% and 25% increases, respectively). Low fertility reduced biomass in both CO_2 treatments. A detailed analysis of whole plant biomass partitioning is reported elsewhere (Pregitzer *et al.*, 1995).

Assimilation

Photosynthetic capacity (i.e. A at constant C_i) varied over time, and with soil fertility and CO_2 treatments (Fig. 3). Seasonally, A_{27} and A_{56} in both CO_2 treatments reached maximum values on day no. 233 at low fertility (Fig. 3*a*) and day no. 260 at high fertility (Fig. 3*b*), declining thereafter with the onset of autumnal senescence. This late seasonal decline was most dramatic in elevated CO_2, high fertility plants, resulting in significantly lower late season A at 27 and 56 Pa C_i relative to ambient CO_2, high fertility plants. At low fertility, elevated CO_2 plants had significantly lower A at 27 and 56 Pa C_i than ambient CO_2 plants by mid-season (day no. 233). Although photosynthetic capacity was negatively affected by growth at elevated CO_2, assimilation at growth C_i was always significantly greater in elevated CO_2 plants than in ambient CO_2 plants except at the very beginning and end of the season at high fertility.

Table 1. *Whole plant dry weight (d.wt) and leaf area (LA) at the final harvest and seasonal leaf area duration (LAD) and net assimilation rate (NAR) of Eugenei grown at high or low fertility and elevated or ambient CO_2. Mean ± (SE), n = 5, with different superscripts indicating significant differences at $P < 0{\cdot}05$*

Fertility CO_2	Final d.wt (g)	Final LA (m^2)	LAD (m^2 d)	NAR (g m^{-2} d^{-1})
High				
Elevated	113 (2)^a	0·23 (0·006)^a	14·3 (0·59)^a	7·9 (0·3)^{ab}
Ambient	76 (3)^b	0·17 (0·006)^b	10·3 (0·47)^b	7·5 (0·5)^{ab}
Low				
Elevated	75 (4)^b	0·13 (0·007)^c	9·3 (0·55)^b	8·0 (0·1)^a
Ambient	60 (4)^c	0·13 (0·008)^c	8·9 (0·58)^b	6·7 (0·2)^b

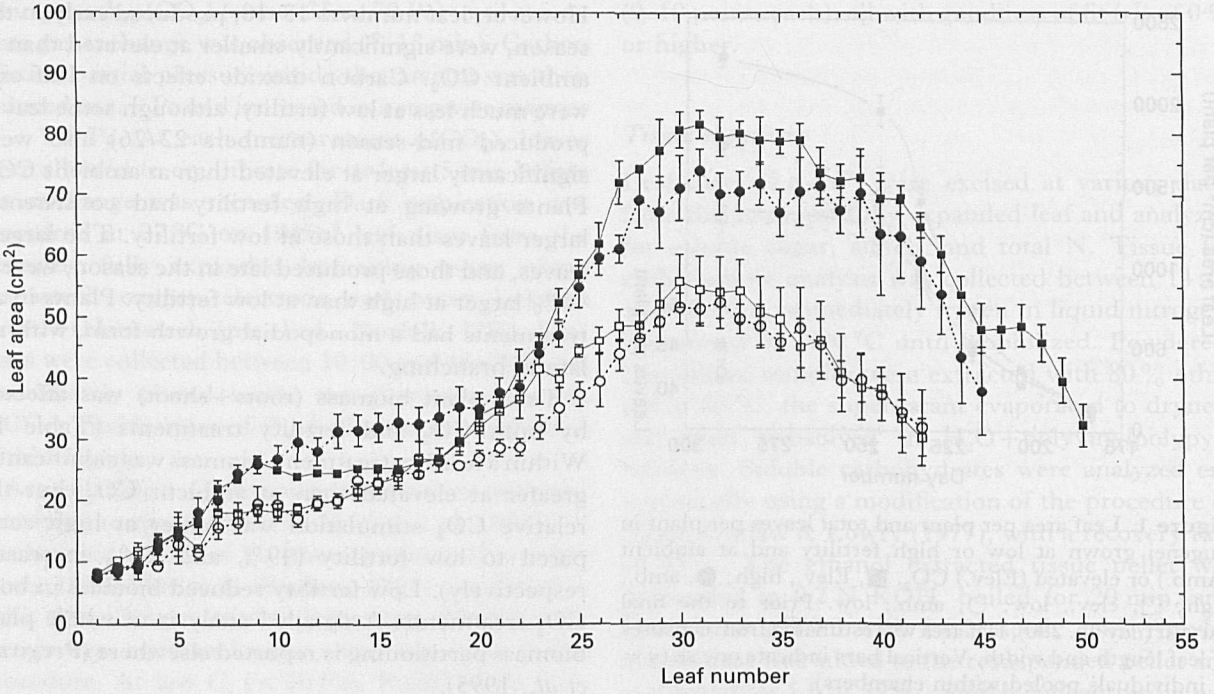

Figure 2. Area of individual leaves of Eugenei after 158 d growth at low or high fertility and at ambient or elevated CO_2. Leaves were numbered starting at the bottom of the stem. Vertical bars indicate one SE ($n = 5$, individuals pooled within chambers). Key as in Fig. 1.

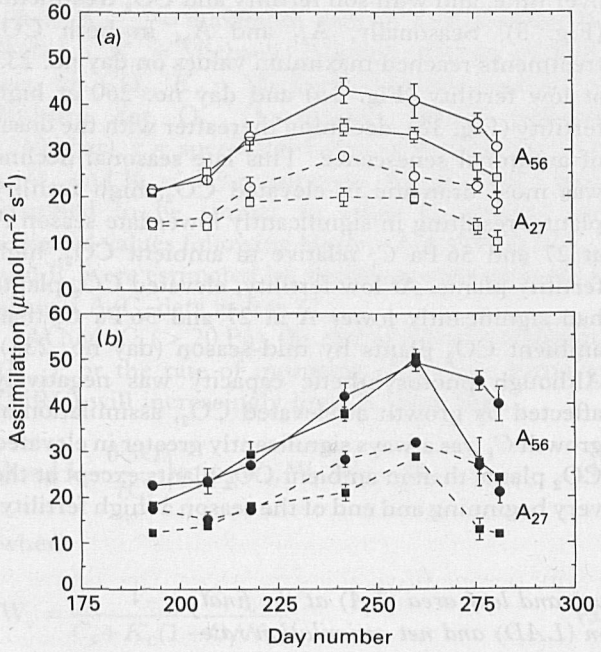

Figure 3. Seasonal change in photosynthetic capacity of Eugenei grown at (*a*) low or (*b*) high fertility and at ambient or elevated CO_2. Key as in Fig. 1. Assimilation was measured at a constant intercellular CO_2 partial pressure (C_i), controlling for diurnal or seasonal differences in stomatal conductance. Plants were measured at $C_i = 56$ Pa (A_{56}) and at $C_i = 27$ Pa (A_{27}). Vertical bars indicate one SE ($n = 2$–5).

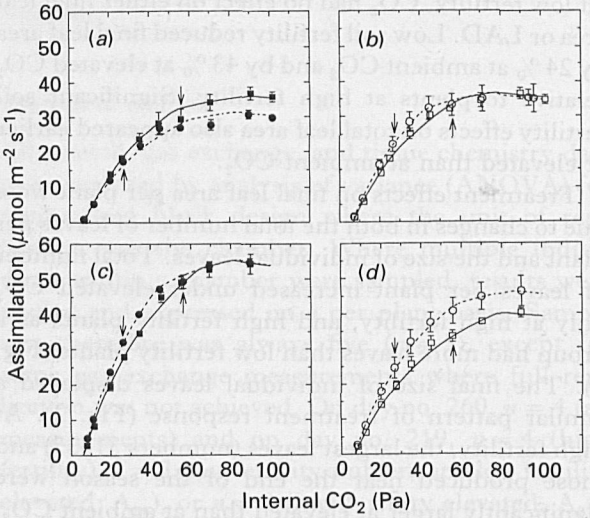

Figure 4. Assimilation as a function of changing C_i in Eugenei grown at low or high fertility and at ambient or elevated CO_2. Key as in Fig. 1. Measurements were made mid- (*a*, *b*; day no. 219) or late-season (*c*, *d*; day no. 260). *Curves were fit by non-linear regression* (see text for details) and arrows indicate growth C_i. Vertical bars indicate one SE ($n = 3$–4).

Adjustment of the photosynthetic apparatus to growth at elevated CO_2 was analyzed in further detail on two dates by examination of A across a broad range of C_i values (Fig. 4). On day no. 219, the modelled kinetic response parameters obtained from these A/C_i curves were similar among all treatments, indicating no positive or negative adjustment of photosynthetic capacity (Table 2). Later in the season (day no. 260), however, high fertility plants in both CO_2 treatments exhibited increased $V_{C,max}$, J_{max}, and PiRC relative to day no. 219 (Fig. 4*a*, *c*) and also exhibited significantly greater $V_{C,max}$ relative

Table 2. *Photosynthetic response parameters of Eugenei grown at low and high fertility and at ambient and elevated CO_2, measured mid- (day no. 219) and late-season (day no. 260). Parameters were derived from A/C_i data presented in Figure 4, where Vc_{max} = maximum rate of CO_2 fixation by Rubisco, J_{max} = rate of electron transport, PiRC = rate of inorganic phosphate turnover, and R_d = day respiration. Means within a response category with different superscripts are significantly different at $P < 0{\cdot}05$. Mean ± (SE), $n = 3$–5*

	Vc_{max} (μmol m^{-2} s^{-1})		J_{max} (μmol m^{-2} s^{-1})		PiRC (μmol m^{-2} s^{-1})		R_d (μmol m^{-2} s^{-1})	
Day number	219	260	219	260	219	260	219	260
Fertility CO_2								
High								
Elevated	66·2 (2·8)^{a}	93·2 (4·5)^{b}	159·5 (12·5)^{ac}	240·8 (11·2)^{b}	11·2 (0·7)^{ac}	16·4 (1·1)^{b}	2·95 (0·35)^{a}	3·36 (0·45)^{ab}
Ambient	54·4 (2·2)^{a}	106·5 (2·4)^{c}	135·0 (8·0)^{a}	239·3 (14·1)^{b}	9·3 (0·3)^{a}	16·0 (0·5)^{b}	1·55 (0·39)^{b}	2·35 (0·49)^{ab}
Low								
Elevated	65·7 (3·1)^{a}	65·5 (6·5)^{a}	168·5 (8·9)^{ac}	178·6 (10·1)^{ac}	11·1 (0·5)^{ac}	12·3 (0·6)^{ac}	2·45 (0·40)^{ab}	2·42 (0·47)^{ab}
Ambient	73·9 (10·3)^{a}	81·6 (8·5)^{d}	166·4 (17·2)^{ac}	204·0 (14·6)^{bc}	10·4 (1·1)^{a}	14·0 (1·1)^{bc}	2·14 (0·70)^{ab}	2·55 (0·14)^{ab}

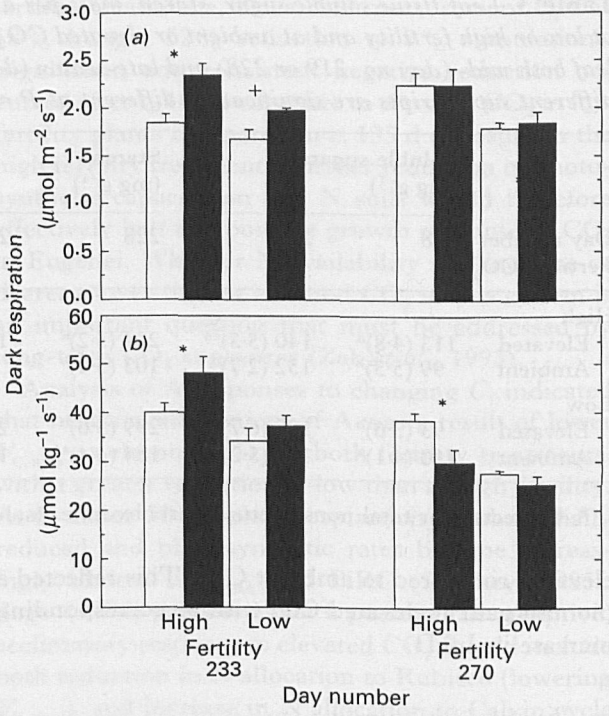

Figure 5. Dark respiration in Eugenei grown at low or high fertility and at ambient or elevated CO_2. □ = Ambient and ■ = elevated CO_2. Measurements were made mid- (day no. 233) and late-season (day no. 270) and are expressed on an area (*a*) or a dry mass less non-structural carbohydrate (*b*) basis. Vertical bars indicate one SE ($n = 4$, day no. 233 or $n = 5$, day no. 270), with * = $P < 0{\cdot}05$, and + = $P < 0{\cdot}10$.

to low fertility plants. At this time there was a small (12%), but significant, decrease in $V_{C,max}$ and consequent decline in A at intermediate C_i in high fertility, elevated relative to ambient CO_2 plants.

At low fertility, A *v.* C_i response characteristics diverged much more strongly between CO_2 treatments on day no. 260 (Fig. 4*d*). In ambient CO_2 plants, $V_{C,max}$ and PiRC increased relative to day no. 219 although not to the extent observed at high fertility (Table 2). This upward shift in capacity was not observed at elevated CO_2, with the result that A was lower than in ambient CO_2 plants across measurement C_i. This reduced photosynthetic capacity (i.e. negative adjustment) was due primarily to lower $V_{C,max}$ in elevated CO_2 plants. There was a consistent trend towards reduction in other estimated response parameters as well, although individual means were not significantly different among CO_2 treatments.

Seasonally averaged, whole plant net assimilation rate (NAR), calculated as the ratio of final above- and belowground biomass to LAD, had a different pattern of treatment response than did single leaf photosynthetic capacity. At high fertility, CO_2 effects on final biomass were matched by increased LAD, resulting in equal NAR among CO_2 treatments (Table 1). We found a significant CO_2 effect on NAR only at low fertility, with an increase of 19% at

Table 3. *Leaf tissue soluble sugar, starch, mass per area (LMA), and nitrogen concentration of Eugenei grown at low or high fertility and at ambient or elevated CO_2. Analyses were performed on the youngest fully expanded leaf both mid- (day no. 219 or 228) and late-season (day no. 266 or 270). Means within a response category with different superscripts are significantly different at $P < 0{\cdot}05$. Mean ± (SE), $n = 5$*

	Soluble sugars (mg g^{-1})		Starch (mg g^{-1})		LMA* (g m^{-2})		Nitrogen* (mg g^{-1})	
Day number	228	266	228	266	228	266	219	270
Fertility CO_2								
High								
Elevated	113 (4·8)^{a}	140 (5·3)^{cd}	204 (5·2)^{a}	143 (6·9)^{de}	55 (1·2)^{a}	65 (1·3)^{b}	50 (0·9)^{a}	44 (2·4)^{a}
Ambient	99 (5·5)^{b}	152 (2·7)^{d}	103 (5·4)^{b}	79 (4·9)^{b}	58 (2·1)^{a}	56 (1·1)^{a}	43 (2·8)^{b}	46 (2·1)^{ab}
Low								
Elevated	93 (3·6)^{b}	125 (6·7)^{c}	299 (5·6)^{c}	210 (21·5)^{a}	58 (2·1)^{a}	66 (1·2)^{b}	33 (1·0)^{c}	25 (1·5)^{d}
Ambient	110 (4·1)^{a}	135 (5·2)^{cd}	184 (4·4)^{ad}	133 (16·8)^{e}	54 (1·6)^{a}	59 (1·3)^{a}	37 (5·1)^{cb}	32 (2·2)^{c}

* Corrected for total nonstructural carbohydrates (soluble sugars + starch).

elevated compared to ambient CO_2. This reflected a biomass gain at elevated CO_2 with no corresponding increase in LAD.

Respiration and tissue chemistry

Low fertility reduced leaf dark respiration mid- and late-season and in both CO_2 treatments (Fig. 5). This was true whether respiration was expressed on a leaf area basis (Fig. 5*a*) or on a dry weight basis corrected for TNC (Fig. 5*b*). Carbon dioxide effects, however, depended on fertility treatment, sampling time, and the units of expression. Mid-season respiration was significantly higher in elevated CO_2 plants at both fertility levels when expressed on a leaf area basis, but only at high fertility when expressed on a TNC corrected dry mass basis. Late-season respiration showed no CO_2 effect on an area basis, but a significant decline on a TNC corrected dry weight basis at high fertility.

Day respiration rate (R_d), estimated from A/C_i curves, showed similar CO_2 effects as did leaf area-based dark respiration (Table 2). Mid-season (day no. 219) and at high fertility, R_d was greater in elevated CO_2 plants than in ambient CO_2 plants. This increase was no longer significant by day no. 260. There were no significant CO_2 effects on R_d at low fertility, nor were there significant fertility effects at either CO_2 level.

Leaf starch content showed consistent patterns of variation with CO_2 treatment, soil fertility, and time (Table 3). Mid-season (day no. 228), starch content increased with CO_2 enrichment at both soil fertilities, although this effect was greater at high than at low fertility (98 % *v.* 62 % increase respectively). Within a CO_2 treatment, low fertility also resulted in significantly higher starch content. There were similar results later in the season (day no. 266), with starch content increasing significantly with elevated CO_2 and low fertility. Across all treatments, starch content was lower on day no. 266 than on day no. 228. Soluble sugar content was less variable than starch content and showed no clear pattern of variation with CO_2 or fertility. In contrast to starch content, soluble sugar content increased significantly across treatments from day no. 228 to day no. 266.

Leaf mass per area (LMA), corrected for TNC, did not vary significantly among CO_2 or fertility treatments on day no. 228 (Table 3). By day no. 266, however, LMA at elevated CO_2 was significantly greater than at ambient CO_2. There were no effects of fertility on LMA. Leaf N concentration, corrected for TNC, was reduced at low fertility both early and late in the season but was variable in its response to CO_2 (Table 3). On day no. 219, leaf N increased with elevated CO_2 at high fertility, but was unchanged at low fertility. On day no. 270, there was no difference in leaf N between CO_2 treatments at high fertility, while at low fertility leaf N was significantly lower at elevated compared to ambient CO_2.

The soils used in this experiment varied in organic matter content and water holding capacity, in addition to mineralizable N. Several observations, however, indicated that the dominant effect of the fertility treatment was on plant N nutrition. First, both soil N mineralization rate and leaf N content were reduced significantly in the low fertility treatment. Secondly, the Kalkaska topsoil and Rubicon sand making up the soil mixtures were high in inorganic P, and we observed no indication of nutrient deficiencies in either treatment. Third, both soils were well mixed with very low resistances to root penetration; at harvest roots were found to have explored freely throughout all root boxes. Lastly, all plots were kept well watered, and it was unlikely that differential water stress developed between plants in low and high fertility soils. Mean pre-dawn xylem water potential following 3 d without water was −0·05 and −0·09 MPa in the low and high fertility treatments, respectively. Mean midday xylem water potential, also following 3 d without water, was −1·20 and −1·19 MPa in the low and high fertility

treatments respectively. There were no significant differences in water potential at either time among CO_2 treatments.

DISCUSSION

Leaf initiation and expansion are among the most sensitive responses of plants to environmental stress (Terry, Waldron & Taylor, 1983). Under optimal conditions, CO_2 enrichment generally increases whole-plant leaf area through effects on total leaf number (Pettersson & McDonald, 1992) and/or maximum individual leaf area (Gaudillère & Mousseau, 1989; Sasek & Strain, 1989; Radaglou & Jarvis, 1990; Wong, Kriedemann & Farquhar, 1992). Because of the strong relationship between canopy development, radiation interception, and plant growth, these morphogenetic effects are important in predicting stand productivity at elevated CO_2. In our study, the early stimulation of leaf growth by CO_2 under high fertility conditions led to a 39% increase in seasonal LAD. This stimulation of canopy development was very similar in magnitude to the biomass gain at elevated CO_2. Since hybrid poplars can achieve leaf area indices greater than 10 (Ceulemans, 1990), this early leaf area and biomass advantage at high CO_2 would be expected to compound over several years before canopy closure, significantly reducing stand rotation time in this commercially important species.

Under low fertility conditions, canopy development was unaffected by CO_2 enrichment. Both elevated and ambient CO_2 plants had fewer, smaller leaves at low than at high fertility, although the percentage reduction in these measures was greater at elevated CO_2. Despite equivalent LAD across CO_2 treatments, A, seasonal NAR, and whole plant biomass increased with CO_2 enrichment. Leaf carbohydrate concentration increased at low fertility in both CO_2 treatments, indicating a greater negative effect of N availability on leaf growth than on A (Bouma, 1970). This suggests that stimulation of A by elevated CO_2 will have few mitigating effects on leaf growth reductions due to low N.

Although A increased due to CO_2 enrichment at both fertility levels, we found clear evidence for negative photosynthetic adjustment in high CO_2 plants: when measured at equivalent C_i, A was reduced over time compared to ambient CO_2 plants. Reviewing photosynthetic response data from 39 tree species, Gunderson & Wullschleger, (1994) concluded that some degree of negative adjustment (or 'acclimation') to high CO_2 was typical, with an average reduction in A of 21% when ambient and elevated plants were measured at a common CO_2 partial pressure. They also suggested that nutrient limitation may influence this process. Our data support both observations. The level of negative adjustment we observed in Eugenei, averaged across fertility treatments, dates, and measurement C_i was 18% (range; 52% reduction to 9% stimulation). Significant, and consistent negative reduction occurred after *c.* 100 d exposure to elevated CO_2 in low fertility plants compared to *c.* 135 d exposure in the high fertility treatment. Further reduction in photosynthetic capacity on low N soils would therefore effectively halt any positive growth response to CO_2 in Eugenei. Whether N availability will increase or decrease over time at elevated CO_2 in this system is an important question that must be addressed by long-term CO_2 exposures (Zak *et al.*, 1993).

Analysis of A responses to changing C_i indicated that negative adjustment of A was a result of lower $V_{C,max}$ at elevated CO_2 in both fertility treatments, with a greater reduction at low than at high fertility. At C_i above 50 Pa, photorespiration is substantially reduced and photosynthetic rates become increasingly limited by J_{max} and PiRC (Sharkey, 1985). Sage, (1994) proposed that a positive (i.e. adaptive) acclimatory response to elevated CO_2 should include both reduction in N allocation to Rubisco (lowering $V_{C,max}$), and increase in N allocation to Calvin cycle and electron transport enzymes (raising J_{max}), or sucrose utilization (raising PiRC). Analysis of A/*C*i curves from elevated and ambient CO_2 Eugenei provided no evidence for reallocation of Rubisco N to other photosynthetic enzyme systems. Our results showed either a maintenance of J_{max} and PiRC (high fertility), or a uniform reduction in these parameters (low fertility). This pattern of negative adjustment to elevated CO_2 appears much more common than a positive acclimatory response (Gunderson & Wullschleger, 1994; Sage, 1994), suggesting a lack of plasticity in N reallocation among these biochemical systems in most species. The response parameters we report for Eugenei are high relative to other tree species (Gunderson & Wullschleger, 1994) but comparable to cotton, another highly productive, woody perennial (Harley *et al.*, 1992).

Carbohydrate accumulation in source leaves is typical of plants grown at elevated CO_2 (Arp, 1991; Wullschleger, Norby & Hendrix, 1992) as well as in those grown at low N levels (Bouma, 1970). In either environment, increased TNC results from an increase in source relative to sink strength, and may be a signal through which photosynthetic rate is downregulated in response to this imbalance (Stitt, 1991). Eugenei leaf TNC increased at both elevated CO_2 and low fertility, with the great majority of this accumulation occurring as starch. If downregulation of A had occurred as a result of this accumulation, leaf TNC levels should have been negatively correlated with J_{max}. We observed no such relationship, with equal J_{max} among treatments mid-season but TNC concentrations varying by a factor of two. Low fertility, high CO_2 leaves were 30% starch by weight with no apparent negative consequence of photosynthesis.

Mid-season dark respiration increased under elevated CO_2 and high fertility, coincident with higher leaf N concentration and $V_{C,max}$ compared to ambient CO_2 plants. Carbon dioxide may affect leaf respiration indirectly through changes in tissue composition and growth rate, or directly by as yet unknown mechanisms in which enzyme activities are altered (Amthor, 1991). Where CO_2 effects are rapid and reversible, direct effects may be indicated, but this has been explored in relatively few instances (Bunce, 1990). More commonly, CO_2 induced changes in respiration are linked to changes in tissue composition, particularly N-based compounds (metabolically expensive) or starch (metabolically inexpensive). Poorter *et al.* (1992), in their review of data on over 40 C_3 species, attributed lower mass-based respiration rates of elevated CO_2 grown plants (mean, 15 % reduction) to greater LMA, since on an area basis, respiration generally increased (mean, 14 % increase). Our data support an indirect CO_2 effect based on changes in tissue N concentration and associated maintenance costs. Reduced late-season respiration at elevated CO_2 in Eugenei, expressed on a TNC corrected mass basis, fit the general pattern reported by Poorter *et al.* (1992) and was attributable to increased LMA. Plants growing at low fertility showed similar trends to those at high fertility although in most cases the response was not as strong.

At high fertility, increased biomass accumulation at elevated CO_2 was matched by increased LAD, resulting in equal seasonal NAR among CO_2 treatments. This suggests that CO_2 effects on growth were largely through effects on canopy development rather than on photosynthetic rate, a surprising result given increased A over a majority of the season at high CO_2. However, increased C *loss* via dark respiration, fine root turnover, or other pathways could offset increases in A and hence reduce differences in net 24 h, or seasonal, C gain between CO_2 treatments. Both dark respiration and fine root turnover (Pregitzer *et al.*, 1995) were stimulated in elevated CO_2 grown Eugenei at high fertility, which could help account for the discrepancy between short term measurements of A, and seasonal estimates of NAR. A more complete carbon budget will be necessary to fully account for long-term CO_2 effects on growth, but our data illustrate the importance of considering both structural and physiological pathways of C gain and loss in predicting the response of trees to changing atmospheric CO_2.

ACKNOWLEDGEMENTS

We would like to thank Susan Wadkowski, Brenda Johnson and Diana Randlett for assistance in the field, Tony Sutterley and Richard Spray for crucial logistical and construction support, and Mark Kubiske and Stan Wullschleger for helpful comments on the manuscript. Jim Lewis kindly provided the program for analysis of A/C_i curves. This research was supported by funds from the US Department of Energy (National Institute for Global Environmental Change), USDA Competitive Grant 90-37290-5668, the University of Michigan Project for the Integrated Study of Global Change, and the University of Michigan Biological Station.

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