



Effects of [CO₂] and nitrogen fertilization on soils planted with ponderosa pine

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Abstract

The effects of six years treatment with elevated [CO₂] (350, 525, and 700 $\mu\text{l l}^{-1}$) and nitrogen (N) (0, 10, and 20 g N m⁻² yr⁻¹) on soils, soil solution, and CO₂ efflux in an open-top chamber study with ponderosa pine (*Pinus ponderosa* Laws.) are described. The clearest [CO₂] effect was in year 6, when a pattern of lower soil N concentration and higher C/N ratio with elevated [CO₂] emerged. Statistically significant effects of elevated [CO₂] on soil total C, extractable P, exchangeable Mg²⁺, exchangeable Ca²⁺, base saturation, and soil solution HCO₃⁻ and NO₃⁻ were also found in various treatment combinations and at various times; however, these effects were inconsistent among treatments and years, and in many cases (P, Mg²⁺, Ca²⁺, base saturation) reflected pre-treatment differences. The use of homogenized buried soil bags did not improve the power to detect changes in soil C and N or help resolve the inconsistencies in soil C patterns. Nitrogen fertilization had the expected negative effects on exchangeable Ca²⁺, K⁺, and Mg²⁺ in year 6, presumably because of increased NO₃⁻ leaching, but had no consistent effect on soil C, N, or extractable P.

Introduction

The long-term effects of elevated [CO₂] on ecosystem carbon (C) sequestration are highly dependent on the factors affecting C sequestration in mineral soils and the interactions of C with other nutrients. The interactions of C and N under elevated [CO₂] are especially important because N is the nutrient most commonly limiting growth. Studies to date on the effects of elevated [CO₂] on soil N availability have produced conflicting and confusing results, however. Some studies have shown that elevated [CO₂] stimulates N mineralization (Zak et al., 1993; Körner and Arnone, 1992). On the other hand, several studies have shown that the introduction of high C/nutrient ratio litter and/or labile organic compounds into the soil with elevated [CO₂] can cause N immobilization (Bernston and Bazzaz, 1996; Cotrufo et al., 1994; Diaz et al.,

1993; Johnson et al., 1996). Other studies report no effect of elevated [CO₂] on litter quality, decomposition, or N mineralization (O'Neill 1994; Randlett et al., 1996), or a mixture of effects depending upon species (Hungate et al., 1996a).

Studies on the effects of elevated [CO₂] on nutrients other than N are far fewer in number but have also produced conflicting results. Elevated [CO₂] has been found to cause both increases and decreases in soil extractable phosphorus (P). Norby et al. (1986) found an increase in soil extractable P with elevated [CO₂] in a pot study with *Quercus alba* L. and speculated that elevated [CO₂] increased phosphatase activity. On the other hand, Johnson et al. (1995) found reduced soil extractable P levels with elevated [CO₂] which could be explained by increased plant P uptake by ponderosa pine (*Pinus ponderosa* Laws.) in a greenhouse study using a nutrient-poor soil. In another greenhouse study using a richer soil, Johnson et al. (1995) found no effects of elevated [CO₂] on either plant P uptake or soil

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extractable P. Johnson et al. (1995) concluded from these two studies that the effects of elevated $[\text{CO}_2]$ on soil P 'were inconsistent and no general conclusions can be drawn'.

Elevated $[\text{CO}_2]$ could cause reductions in exchangeable base cations by stimulating increased uptake or increased leaching of either bicarbonate or nitrate. The often-noted increase in soil respiration under elevated $[\text{CO}_2]$ (Hungate et al., 1997; Johnson et al., 1994; Körner and Arnone, 1992; Vose et al., 1995) could cause increased carbonic acid production and bicarbonate/base cation leaching if soils are not extremely acidic. McColl and Cole (1968) artificially increased pCO_2 in soil columns, and demonstrated increased carbonic acid leaching. Johnson et al. (1977) found that elevated pCO_2 due to greater biological activity in soils at a tropical site (La Selva, Costa Rica) caused greater carbonic acid leaching rates than in more northern forest soils with lower pCO_2 levels. Increased N mineralization rate in excess of plant demand for N can lead to increased NO_3^- leaching, as found by Körner and Arnone (1992) in their study of an artificial tropical ecosystem. On the other hand, increased N uptake with elevated $[\text{CO}_2]$ can reduce NO_3^- leaching in an N-rich system, as found Torbert et al. (1996) for crop systems.

The direct evidence for $[\text{CO}_2]$ effects on soil exchangeable cations is mixed, as is the case for N and P. Norby et al. (1986) found no statistically significant effect of elevated $[\text{CO}_2]$ on exchangeable K^+ , but a downward trend. Potassium leaching was lower with elevated than with ambient $[\text{CO}_2]$ in the study of Norby et al. (1986), and thus the reduced exchangeable K^+ was attributed to uptake. Johnson et al. (1995) found decreases in exchangeable K^+ , Ca^{2+} , and Mg^{2+} with elevated $[\text{CO}_2]$ in two separate pot studies with ponderosa pine. These decreases were greater than could be explained by plant uptake and thus were attributed to increased leaching.

Studies of the effects of elevated $[\text{CO}_2]$ on soil C sequestration have also produced conflicting results. Isotopic studies usually show increased sequestration of new soil C with elevated $[\text{CO}_2]$ (reviewed by Hungate et al., 1996b; see also Ineson et al., 1996; Torbert et al., 1997), but there are exceptions (the soybean results from Torbert et al., 1997). In a laboratory study using ^{13}C and ^{14}C , Cheng and Johnson (1998) found the elevated $[\text{CO}_2]$ stimulated soil organic matter decomposition when N fertilizer was added but reduced soil organic matter decomposition without N addition. Some studies have shown increased total soil C

with elevated $[\text{CO}_2]$, but the amounts sequestered are often too large to reconcile with measured C inputs (reviewed by Hungate et al., 1996b). Hungate et al. (1996b) concluded from an analysis of results from their own studies and from the literature that, 'either $[\text{CO}_2]$ does not affect soil C content, or that reported $[\text{CO}_2]$ effects on soil C are too large to be a simple consequence of increased plant carbon inputs, suggesting that other mechanisms are involved, or that the differences are due to chance'.

In this paper, we summarize the results of six years of investigation on the effects of $[\text{CO}_2]$ and N fertilization on soils from open-top chamber planted with ponderosa pine. In earlier papers addressing results from year 1 through year 3 of this study, we reported that elevated $[\text{CO}_2]$ caused: 1) elevated soil pCO_2 and soil respiration, 2) increased rates of carbonic acid leaching; 3) reduced soil NO_3^- concentrations; and 4) declines in soil extractable P (Johnson et al., 1996, 1997; Vose et al., 1995). The reductions in soil NO_3^- concentration were attributed to increased inputs of labile C below ground (causing increased N immobilization, denitrification, or both). The only treatment effect at three years was the negative effect of elevated $[\text{CO}_2]$ on extractable P which appeared to be associated with increases in exchangeable Al^{3+} (Johnson et al., 1997).

Site and methods

Site description and experimental design

The open-top chamber site for the ponderosa pine (*Pinus ponderosa* Laws.) research was located at the Institute of Forest Genetics in Placerville, California. The soil is Aiken clay loam, a Xeric Haplohumult derived from andesite. During February – April 1991, 24 hexagonal open-top chambers (3.6 m in dia) were established on the site. The basic experimental design consisted of three levels of nitrogen (0, 10, and 20 $\text{g m}^{-2} \text{yr}^{-1}$ of N as ammonium sulfate, applied in early spring), and four $[\text{CO}_2]$ treatments (ambient, no chamber; ambient, chambered; 525 $\mu\text{l l}^{-1}$ $[\text{CO}_2]$; and 700 $\mu\text{l l}^{-1}$ $[\text{CO}_2]$). Water was delivered to each plot via a timed standpipe to a looped 2.54 cm dia manifold, and low pressure spray heads. Each of the chambered treatments was replicated three times (except for the 10 $\text{g m}^{-2} \text{yr}^{-1}$ N, 525 $\mu\text{l l}^{-1}$ $[\text{CO}_2]$ treatment, which was excluded.) and each of the unchambered treatments was replicated twice. Only the results from

the chambered measurements will be reported in this paper.

In May 1991, ponderosa pine was planted in each chamber. Both seeds and seedlings (21 per chamber), were planted, the latter being a backup in the event of excessive mortality. Seed-grown seedling survival was sufficient such that the seedling-grown stock was removed in October 1991. Three seed-grown trees from each chamber were harvested in October 1991, including complete root systems. In October 1992 (year 2) and October 1993 (year 3), three trees from each chamber were harvested again, but only one complete root system per chamber was obtained because of the increased size of the seedlings and concern for excessive plot disturbance. The final harvest (including roots) took place in October 1996.

Sampling and analysis methods

Measurement and calculation of soil CO₂ efflux

Between March, 1992 and August 1996, soil pCO₂ concentrations were monitored from gas wells established at 15 and 30 cm depths in each chamber. The gas wells consisted of 4 mm tubing inserted to the proper depth in the soil and fitted at the surface with a stoppered, female end of a plastic union. During gas collections, stoppers were removed and 15 ml of soil gas was withdrawn from each well (enough to completely evacuate the tubing and obtain soil gas) using a 50 ml syringe fitted with tygon tubing and the male half of the plastic union. Samples for CO₂ analyses were obtained with Hamilton gas syringes from the section of Tygon tubing between the large syringe and the union. Carbon dioxide analyses were performed on a LiCOR 6250 CO₂ gas analyzer using peak heights compared to a standard gas. Soil temperature and moisture were recorded at each soil gas sampling. Results from the period July 1992 through July 1993 were previously reported by Johnson et al. (1994).

Soil moisture was measured by various methods during the early part of the study. From July 1992 to August 1992 portable tensiometers (Soil Moisture Corp.) were used. The portable tensiometers were replaced by gravimetric analyses between October 1992 and May 1993 because of time considerations. After May 1993, gypsum blocks were calibrated and used for estimations of water content because of concern over the repeated effects of destructive soil sampling.

Cumulative soil respiration over the period March 1992 through August 1996 was estimated using the

profile method (De Jong and Schappert 1972, De Jong et al., 1974; Johnson et al., 1994; Mattson, 1995:

$$q = D \frac{dC}{dz} \quad (1)$$

where q = CO₂ efflux ($g \text{ CO}_2\text{-C m}^{-2} \text{ day}^{-1}$), C = soil [CO₂] concentration ($g \text{ m}^{-3}$), z = depth (m), and D = diffusion coefficient ($\text{m}^2 \text{ day}^{-1}$).

Because CO₂ diffusion in water is much lower than in air, D is strongly affected by soil water content. There are several formulations for D (Collins and Rasmuson, 1988), all of which take soil moisture content into account. In a previous paper, we used the formulation for D given by Collins and Rasmuson (1988), that requires total porosity and soil water content as well as a value for a coefficient that accounts for non-ideal pore shape and dead-end pores. For the Placerville soil, the value of this coefficient was determined from comparisons with measured CO₂ efflux using the IRGA system (Vose et al., 1995). Since the publication of that paper, Moldrup et al. (1996) provided a more general empirical formulation for D which depends upon commonly-measured soil properties:

$$D = (f)(D_a)(E^{-4/3}) \left(\frac{P_{eff}^{(1.5+3/b)}}{E} \right) \quad (2)$$

where f = a factor converting $\text{cm}^2 \text{ sec}^{-1}$ to $\text{m}^2 \text{ day}^{-1}$ (8.64), D_a = diffusion coefficient of CO₂ in air ($\text{cm}^2 \text{ sec}^{-1}$); E = voids ratio, or total soil porosity; P_{eff} = effective porosity = total porosity (E) minus volumetric water content (V_w); $b = [(0.303 - 0.093(\ln BD)) - 0.0565(\ln(CL)) + 0.00003(FS^2)]^{-1}$; BD = bulk density ($g \text{ cm}^{-3}$); CL = percent clay ($<0.002 \text{ mm}$); and FS = percent fine sand (0.02 to 0.20 mm). This formulation for D was found to be an improvement over the previous method and was used to calculate soil CO₂ efflux in this paper. Cumulative CO₂ flux was calculated by trapezoidal integration of respiration values (Cotruffo et al., 1994) for the period September 1992 through August 1996.

Soil sampling and analysis

Soils were sampled by horizon in March 1991, March 1993, and September 1996 by punch auger. Soils from each sampling were analyzed on the Perkin-Elmer CHN Analyzer for total C and N at the Desert Research Institute (DRI). Soils from the 1991 and 1993 samplings were also analyzed at DRI for exchangeable Ca²⁺, Mg²⁺, K⁺, and Al³⁺ and cation exchange

capacity (CEC) (1 M NH₄Cl followed by an ethanol wash to displace interstitial solution and a 1M KCl extract to displace exchangeable NH₄⁺); and extractable P (0.5 M HCl plus 1 M NH₄F; Olsen and Sommers, 1982). Soils from the 1996 sampling were analyzed for exchangeable base cations, CEC, and extractable P at the Soil and Plant Analysis Laboratory, University of Missouri. At the University of Missouri, exchangeable base cations were extracted by ammonium acetate and CEC determined by subsequent displacement of NH₄⁺ with 1M NaCl. Eleven subsamples from the 1991 and 1993 samplings were sent to the University of Missouri for quality assurance checks on laboratory bias. Unfortunately, significant bias was detected (University of Missouri results were lower) in all cases except exchangeable K⁺, precluding analyses of changes in extractable P and exchangeable Ca²⁺, Mg²⁺, and Al³⁺ over time for the 1993-1996 period.

In addition to standard soil sampling, homogenized soil bags (David et al., 1990) were buried in the chambers for the purpose of assessing changes in soil C and N with greater precision. Homogenized samples from the Aiken Ap horizon were placed in 1 mm mesh bags, labelled, and inserted in the Ap horizons of each chamber (three per chamber) in August, 1995. The bags were retrieved in September 1996 and analyzed for C and N on the Perkin-Elmer CHN Analyzer. The homogenized bags were not intended to provide estimates of actual rates of soil change (because the disturbance of homogenizing soils precludes this), but rather as a means of measuring relative treatment effects on soil chemical properties. David et al. (1990) used this technique to detect very small changes in soil chemical properties in response to acidification treatments to a Spodosol in Maine. The buried soil bags were analyzed for C and N as described above and stored for possible future analyses.

Soil solution sampling and analysis

Ceramic cup, falling head lysimeters were installed in each of the unfertilized chambers in November 1993, and collections were made on approximately a bimonthly basis until April 1995. All soil solution samples were analyzed for NH₄⁺ and NO₃⁻ (automated colorimetric analysis), and selected samples were also analyzed for Ca²⁺, K⁺, Mg²⁺, and Na⁺ (atomic absorption), SO₄²⁻, Cl⁻, ortho-phosphate (automated colorimetric analysis), pH, and HCO₃⁻

Table 1. Results of unbalanced ANOVA tests for effects of CO₂ and N treatments on soil chemical parameters

Parameter	Year 0		Year 3		Year 6	
	N	CO ₂	N	CO ₂	N	CO ₂
<i>Ap Horizons</i>						
C	NS	NS	NS	**	NS	NS
N	NS	NS	NS	*	NS	***
C/N	NS	NS	NS	NS	NS	***
P	NS	NS	NS	***	NS	*
Ca ²⁺	NS	*	NS	NS	***	NS
Mg ²⁺	NS	**	**	**	***	NS
K ⁺	NS	NS	NS	NS	***	NS
CEC ¹ NS	NS	NS	NS	NS	NS	
%BS ¹	*	*	*	*	***	NS
<i>Bw Horizons</i>						
C	NS	NS	NS	NS	NS	**
N	NS	NS	NS	NS	NS	***
C/N	NS	NS	NS	NS	NS	***
P	NS	NS	NS	**	NS	**
Ca ²⁺	NS	NS	NS	NS	NS	NS
Mg ²⁺	NS	**	NS	***	***	NS
K ⁺	NS	NS	NS	NS	***	NS
CEC	**	NS	NS	*	NS	NS
%BS	**	**	NS	***	**	NS

¹Cation exchange capacity

¹Base saturation

(by titration to pH 4.5) at the Water Analysis Laboratory at the Desert Research Institute.

Statistical analyses

Statistical analyses for treatment effects on soils in any given year were performed using PROC GLM in Statistical Analysis System software.

Results

Soil carbon, nitrogen, and C/N ratio

Prior to treatment (year 0), there were no statistically significant differences in total C, total N or C/N ratio among the treatments (Table 1). In year 3, there was a significant effect of [CO₂] treatment on Ap horizon C which was due largely to greater soil C in the low N (0 g N m⁻² yr⁻¹, or unfertilized), 525 μl l⁻¹ [CO₂] treatment (Figure 1; Table 1). Similarly, there was a significant effect of [CO₂] treatment on Ap horizon N due largely to greater soil N in the high N (20 g N m⁻² yr⁻¹), 525 μl l⁻¹ [CO₂] treatment (Figure 2 and

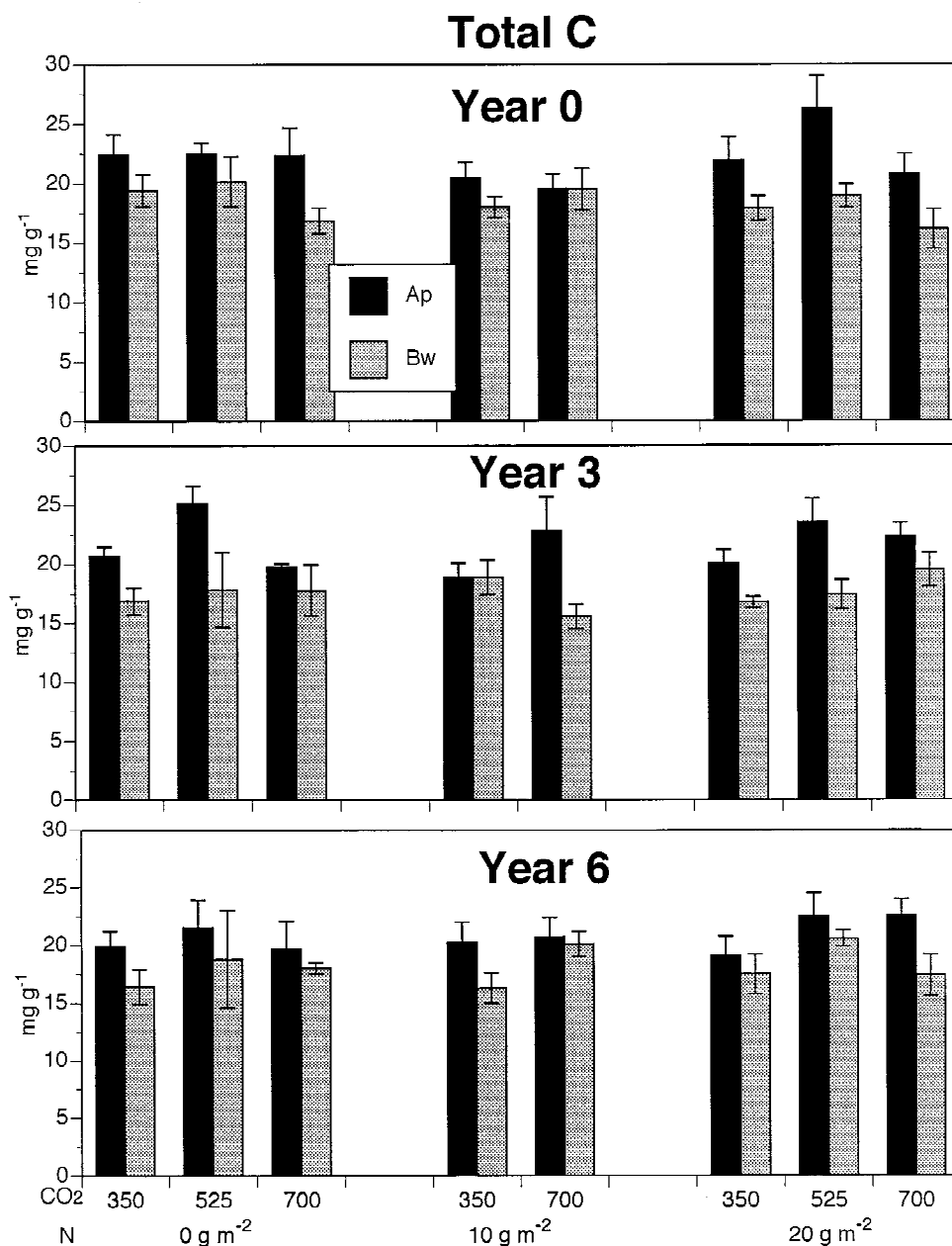


Figure 1. Soil total carbon in 1991 (year 1), 1993 (year 3), and 1996 (year 6) of the study in chambers treated with elevated [CO₂] and N. [CO₂] treatments: 350, 525, and 700 $\mu\text{l l}^{-1}$. N treatments: 0, 10, and 20 $\text{g N m}^{-2} \text{yr}^{-1}$ as ammonium sulfate.

Table 1). In year 6, there were no significant [CO₂] treatment effects on soil C in the Ap horizon, but there was a significant effect in the Bw horizon due largely to greater soil C in the high N, 525 $\mu\text{l l}^{-1}$ and medium N, 525 $\mu\text{l l}^{-1}$ [CO₂] treatments (Figure 1 and Table 1). There was a significant effect of [CO₂] treat-

ment on soil N in both horizons in 1996 due to reduced soil N with elevated [CO₂] in the low and medium N treatments (Figure 2 and Table 1). This in turn caused significantly higher C/N ratio with elevated [CO₂] in the low and medium N treatments in 1996 (Figure 3; Table 1).

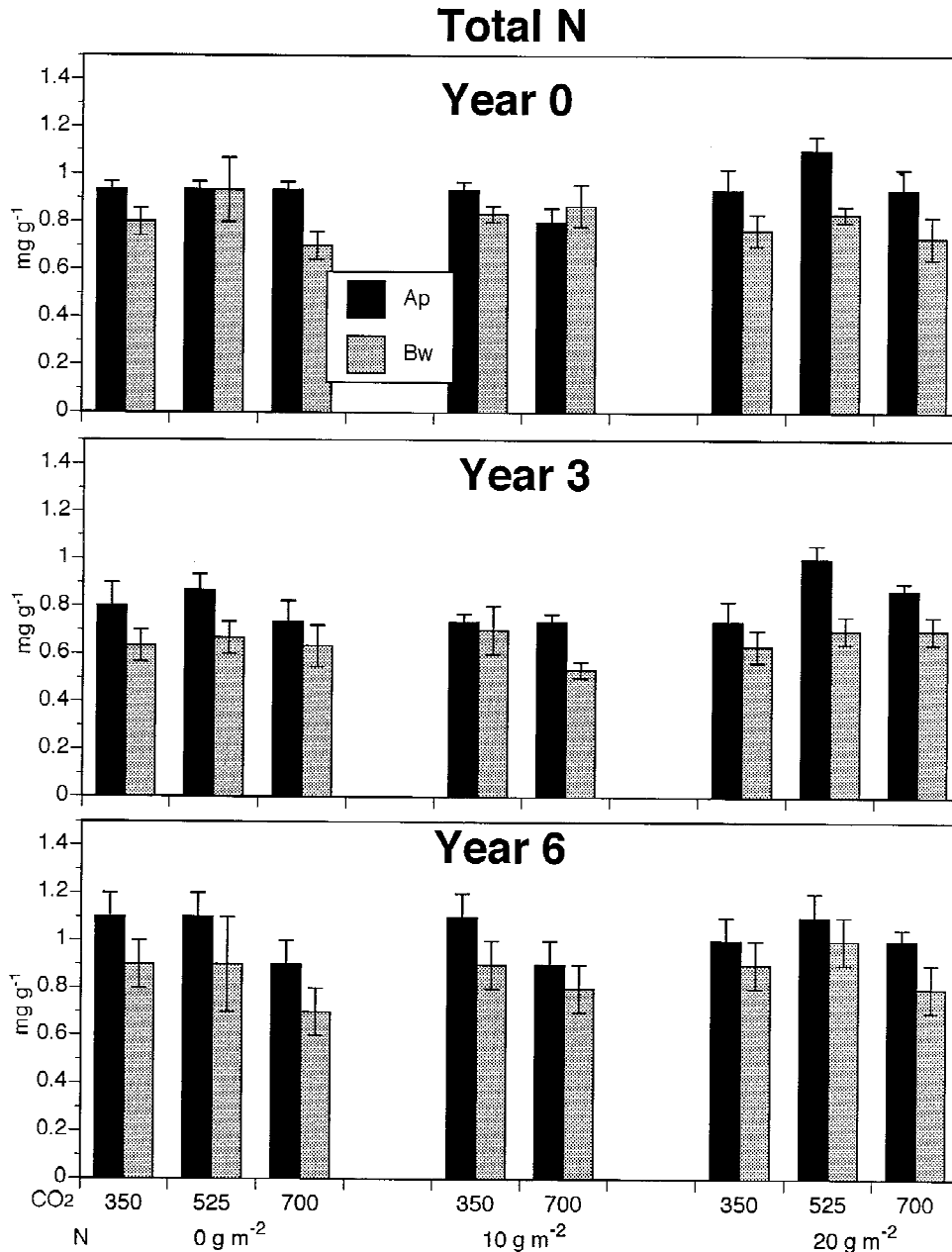


Figure 2. Soil total nitrogen in 1991 (year 1), 1993 (year 3), and 1996 (year 6) of the study in chambers treated with elevated [CO₂] and N. [CO₂] treatments: 350, 525, and 700 $\mu\text{l l}^{-1}$. N treatments: 0, 10, and 20 $\text{g N m}^{-2} \text{yr}^{-1}$ as ammonium sulfate.

The homogenized soil bags showed little change in C concentration after thirteen months and no treatment effects on C (Figure 4). There were net gains in N in soil bags in all treatments, however, and significant ($p < 0.05$) effects of N treatment on soil N and C/N ratio.

Soil pCO₂, CO₂ Efflux, and soil carbon pools

In previous papers, we reported a significant, positive effect of the 525 $\mu\text{l l}^{-1}$ [CO₂] treatment on soil pCO₂ and respiration in the low and high N treatments (0 and 20 $\text{g N m}^{-2} \text{yr}^{-1}$) during the period from July 1992 to

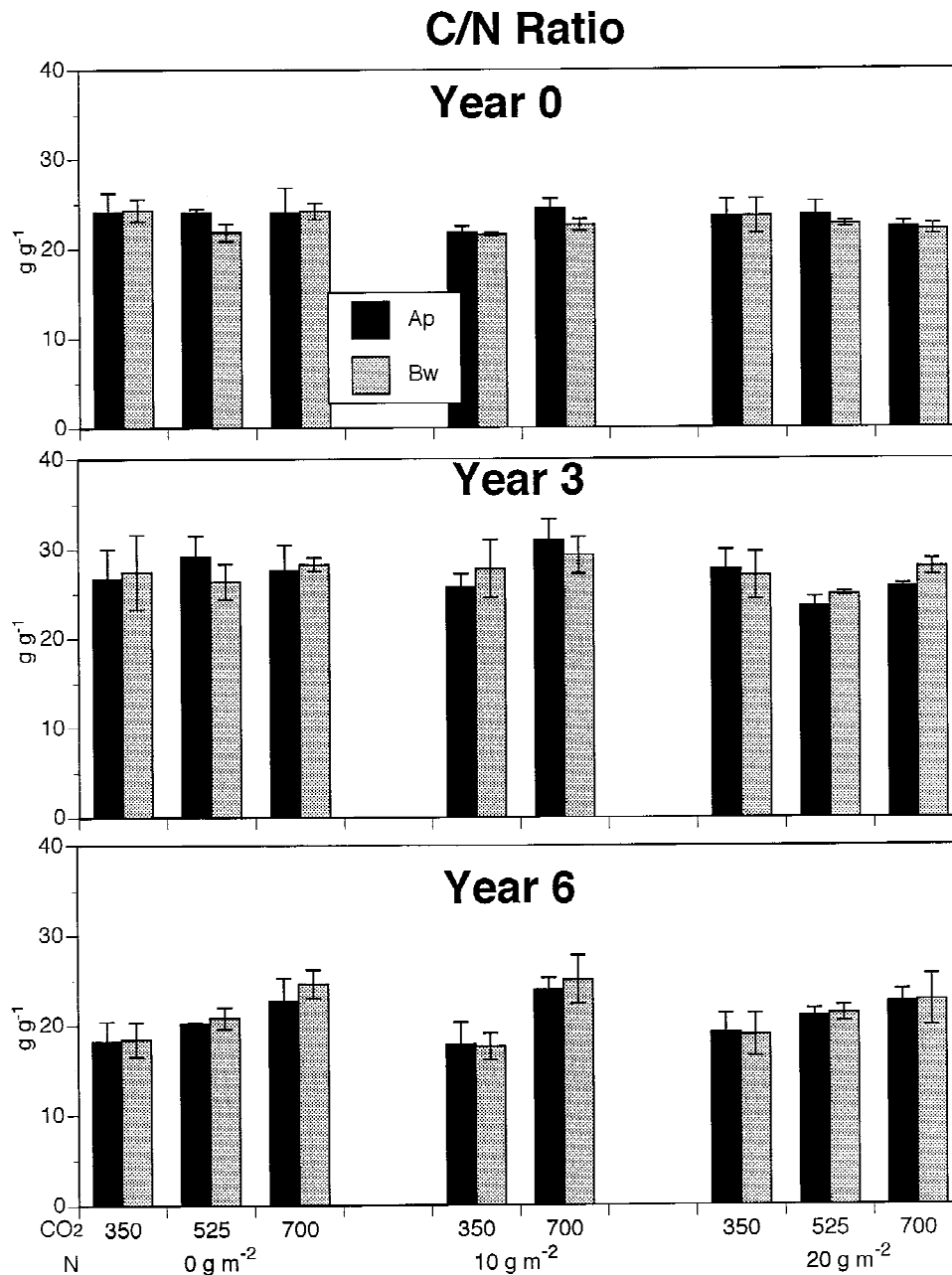


Figure 3. Soil C/N ratio in 1991 (year 1), 1993 (year 3), and 1996 (year 6) of the study in chambers treated with elevated [CO₂] and N. [CO₂] treatments: 350, 525, and 700 $\mu\text{l l}^{-1}$. N treatments: 0, 10, and 20 $\text{g N m}^{-2} \text{yr}^{-1}$ as ammonium sulfate.

July 1993 (Johnson et al., 1994; Vose et al., 1995). This was apparently due to the greater root biomass at the 525 $\mu\text{l l}^{-1}$ [CO₂] level. These effects are evident in Figures 5 and 6, which summarize the pCO₂ data from

March 1992 through August 1996 for the 15 and 30 cm depths, respectively. However, Figures 5 and 6 also reveal that the effects of the 525 $\mu\text{l l}^{-1}$ [CO₂] level treatment faded during the summer of 1993, reappear-

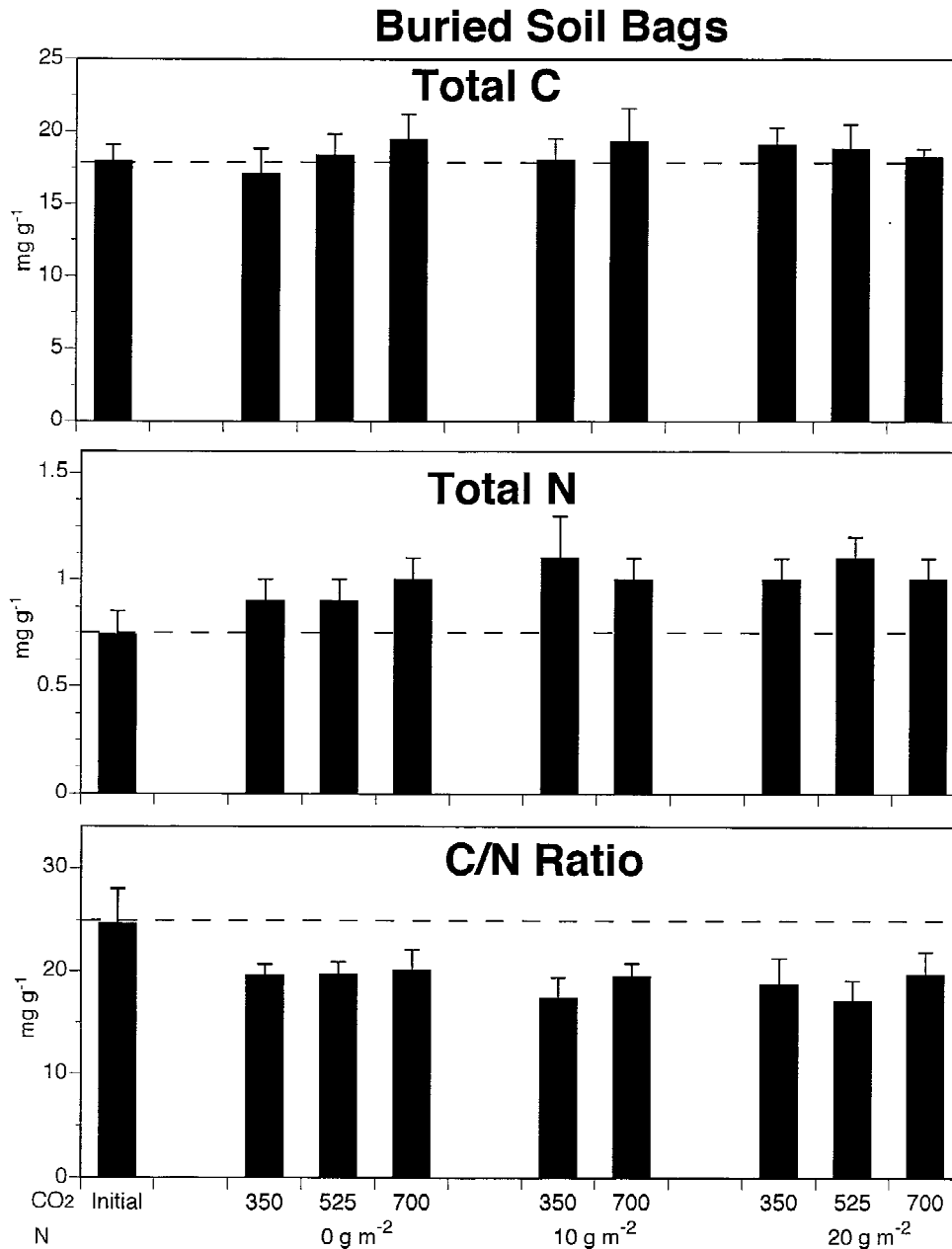


Figure 4. Soil C and N in homogenized, buried bags prior to and 13 months after treatment with elevated [CO₂] and N. [CO₂] treatments: 350, 525, and 700 μl l⁻¹. N treatments: 0, 10, and 20 g m⁻² yr⁻¹ as ammonium sulfate.

ing only sporadically in some N treatments. In the summer of 1994, [CO₂] treatment effects on soil pCO₂ again became significant, but only at the 700 μl l⁻¹, medium and high N treatments. This transition from higher pCO₂ in the 525 μl l⁻¹ to the 700 μl l⁻¹ [CO₂]

matched the patterns observed in tree biomass between 1991 and 1993 as well as in tree diameter and heights between 1993 and 1996: the initially higher values for the 525 μl l⁻¹ [CO₂] treatments faded after year 2, and the 700 μl l⁻¹ [CO₂] treatment became dominant

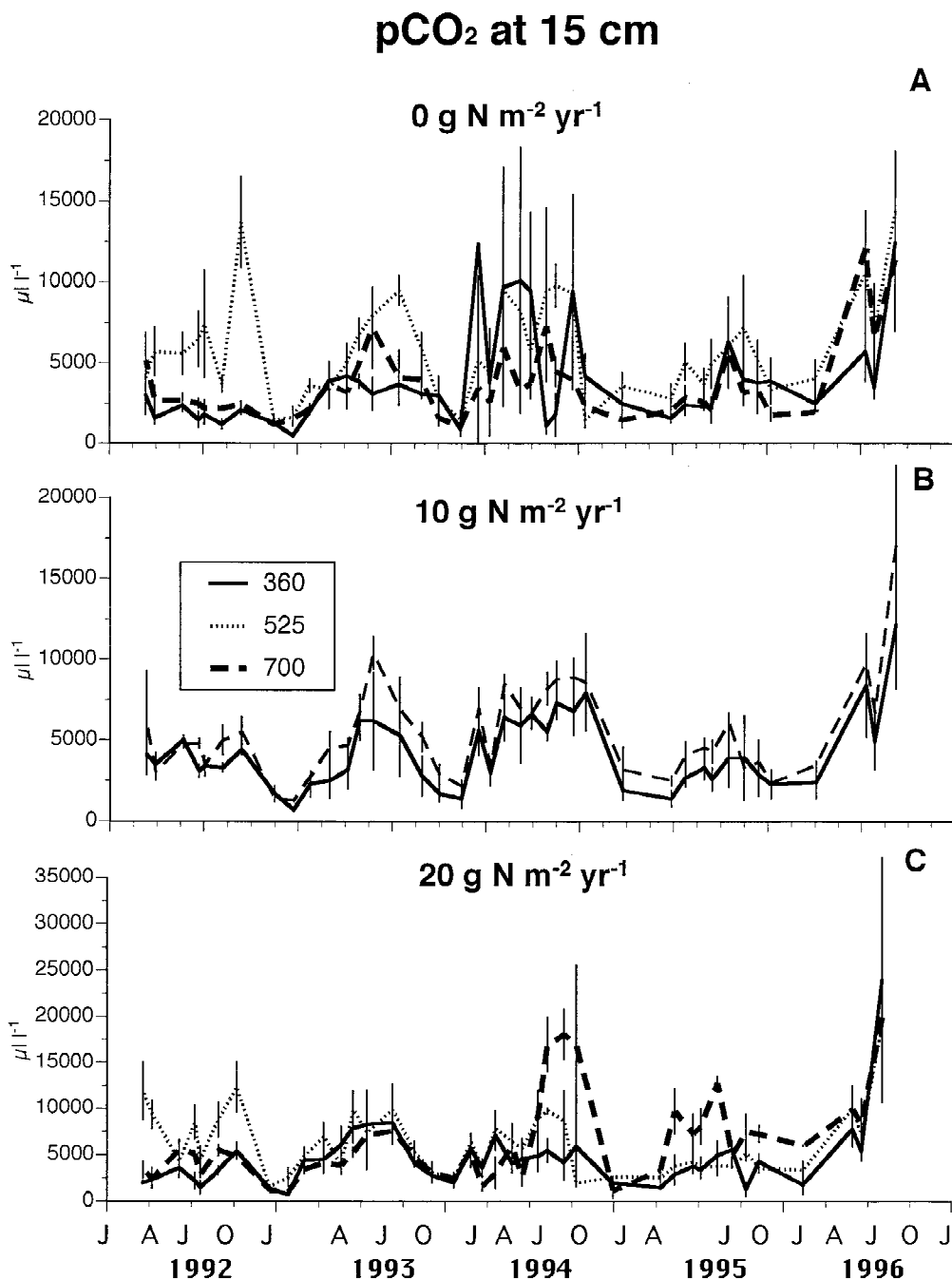


Figure 5. Soil pCO₂ at 15 cm depth in chambers treated with elevated [CO₂] and N. [CO₂] treatments: 350, 525, and 700 µl l⁻¹. N treatments: 0 (panel a), 10 (panel b), and 20 (panel c) g N m⁻² yr⁻¹ as ammonium sulfate.

(Johnson et al., 1997 and J.T. Ball, pers comm.). By the summer of 1996, however, the effects of the 700 µl l⁻¹ [CO₂] had also faded into insignificance, with the

exception of the 700 µl l⁻¹ [CO₂], high N treatment at the 30 cm depth (Figure 6).

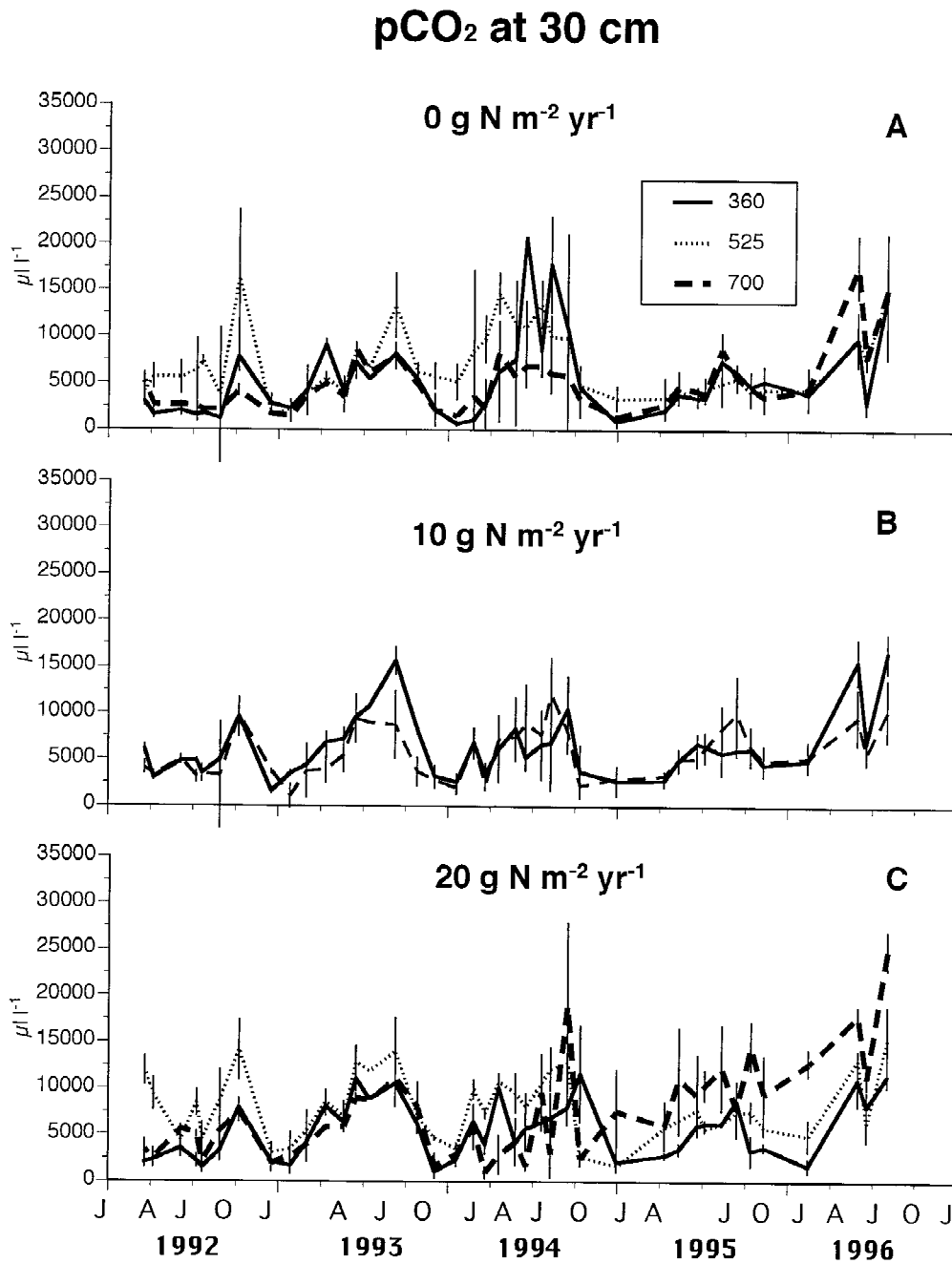


Figure 6. Soil pCO₂ at 30 cm depth in chambers treated with elevated [CO₂] and N. [CO₂] treatments: 350, 525, and 700 μl l⁻¹. N treatments: 0 (panel A), 10 (panel B), and 20 (panel C) g N m⁻² yr⁻¹ as ammonium sulfate.

Estimated cumulative soil CO₂ efflux between September 1992 and August 1996 and soil C contents in year 0, 3, 6 are presented in Table 2. Cumulative soil CO₂ efflux was substantially greater in the high N, 700

μl l⁻¹ [CO₂] treatment than in all other treatments, primarily because of the high pCO₂ concentrations in the summers of 1994 and 1995 in this treatment (Figure 5). The cumulative CO₂ effluxes equaled ap-

Table 2. Estimated cumulative CO₂-C flux from soils at the Placerville field site between March, 1992 and August, 1996

N Treat. (g N m ⁻² yr ⁻¹)	CO ₂ Treat. (μl l ⁻¹)	Cumulative Flux	Soil C Pools		
			Year 0 kg C m ⁻²	Year 3	Year 6
0	350	3.0±0.5	8.4±1.3	7.6±0.8	7.4±0.7
0	525	3.9±1.1	8.6±1.4	8.7±2.1	8.2±1.7
0	700	3.6±1.5	8.0±1.6	7.6±1.3	7.6±0.9
10	350	3.0±1.0	7.8±1.0	7.7±1.2	7.4±0.8
10	700	3.9±0.4	7.9±1.3	7.8±1.8	8.3±0.7
20	350	4.6±0.5	8.1±1.4	7.5±0.8	7.4±0.9
20	525	4.5±1.4	9.2±1.8	8.3±1.4	8.7±0.8
20	700	7.1±1.4	7.5±1.4	8.5±1.1	8.1±0.8

proximately 50 to 100% of the standing soil C pools measured in any year and were many fold greater than any change in soil C which may have occurred.

Soil solution

Soil solution NO₃⁻ and NH₄⁺ concentrations were very low with the exception of the first sampling (May 1993) (Figure 7). The high values in this first sampling may have been due to disturbance associated with lysimeter installation. Soil solution NO₃⁻ was significantly lower with elevated [CO₂] in the first sampling but there were no effects on soil NO₃⁻ or NH₄⁺ thereafter (Figure 7).

As reported previously (Johnson et al., 1996), soil solution HCO₃⁻ concentrations were significantly greater in the 700 μl l⁻¹ [CO₂] treatment (unfertilized N treatments) in one sampling (July 1993 collection) (not shown). Later samplings revealed no significant differences in HCO₃⁻ concentration. There were no other statistically significant differences in the concentration of any other measured ion (Ca²⁺, K⁺, Mg²⁺, Na⁺, SO₄²⁻, Cl⁻, NO₃⁻) in soil solution throughout the sampling period.

Soil extractable P, exchangeable Ca²⁺, Mg²⁺, K⁺, base saturation and cation exchange capacity

Prior to treatment (year 0), there were no statistically significant differences in exchangeable K⁺ or extractable P in any treatment or horizon; but there were significant differences in CEC and base saturation among the N treatments, and exchangeable Ca²⁺, exchangeable Mg²⁺, and base saturation among the [CO₂] treatments (Tables 1 and 3). In year 3, the

differences in exchangeable mg²⁺ and base saturation among [CO₂] treatments remained significant, the differences in exchangeable Ca²⁺ were no longer significant, and the differences in extractable P became significant (Tables 1 and 3). There were also significant (negative) effects of N treatment on exchangeable Mg²⁺ in the Ap horizon in year 3. In year 6, the differences in exchangeable Mg²⁺ and base saturation among [CO₂] treatments were no longer significant, but the differences in extractable P remained significant in both horizons. There were also significant (negative) effects of N treatment on base saturation, exchangeable Ca²⁺, K⁺, and Mg²⁺ in year 6 (Tables 1 and 3).

Discussion

While statistically significant effects of [CO₂] treatment on soil C and nutrient concentrations were detected at various times and treatment combinations, no consistent patterns emerged in most cases, even after 6 years. In the case of soil C, the data suggest that [CO₂] treatment effects first occurred in the Ap horizon in year 3, but in year 6 this effect disappeared and significant [CO₂] treatment effects appeared in the Bw horizon. These effects were rather small, however, and the possibility of Type II errors exist. Unfortunately, the homogenized soil bags did little to clarify the situation: coefficients of variation for C and N concentrations in the bag soils averaged 8 and 11%, respectively whereas those in the 1996 soil samples averaged 9 and 9%, respectively.

As in several other studies, we were able to detect highly significant effects of elevated [CO₂] on soil

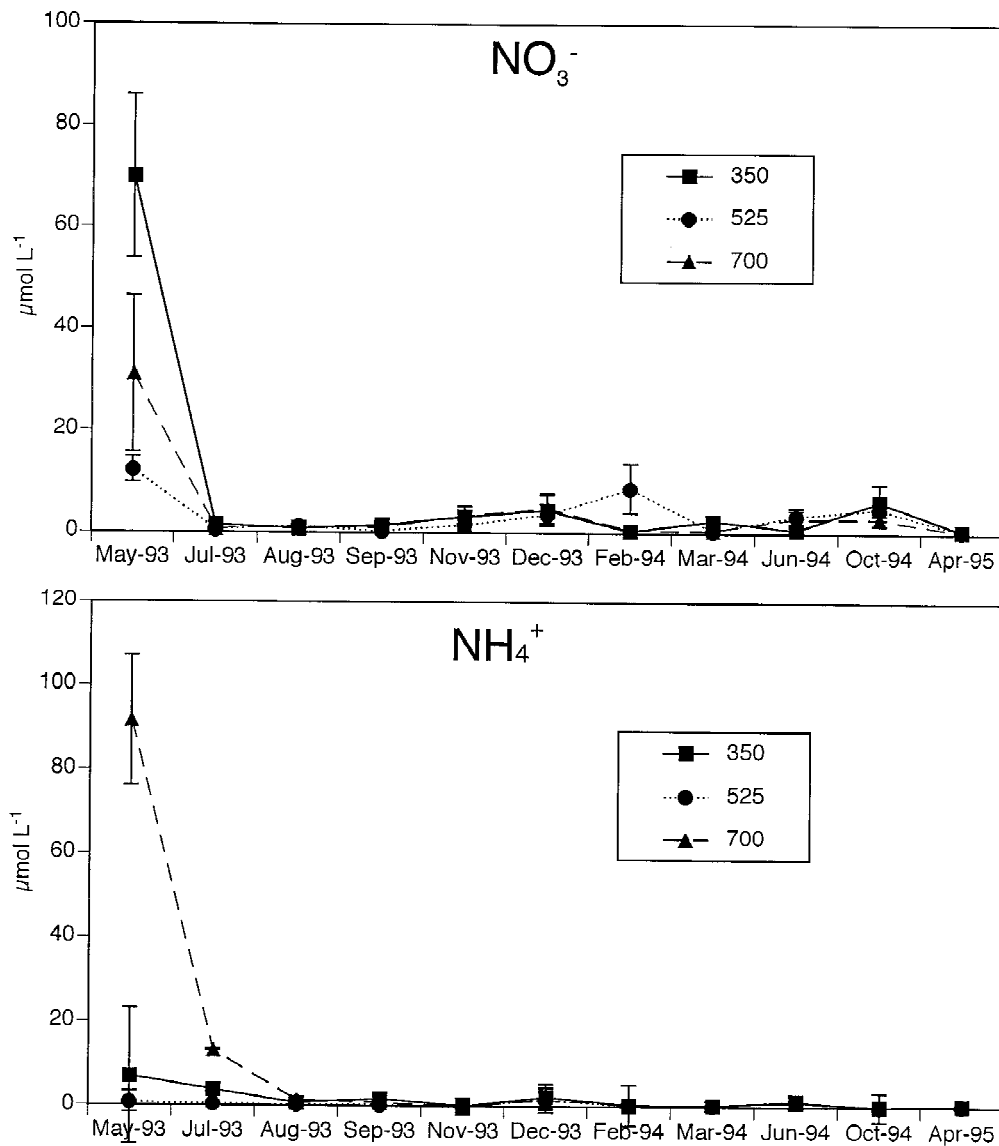


Figure 7. Soil solution NO_3^- and NH_4^+ concentrations in unfertilized chambers treated with elevated $[\text{CO}_2]$ in unfertilized chambers treated with elevated $[\text{CO}_2]$. $[\text{CO}_2]$ treatments: 350, 525, and 700 $\mu\text{l l}^{-1}$. N treatments: 0 (panel A), 10 (panel B), and 20 (panel C) $\text{g N m}^{-2} \text{yr}^{-1}$ as ammonium sulfate.

CO_2 efflux. Also as in many other studies, CO_2 efflux was considerably greater than any change in soil C that may have occurred. The high CO_2 effluxes with elevated $[\text{CO}_2]$ were apparently a result of root respiration (Johnson et al., 1994) and did not coincide with net losses of soil C relative to the low $[\text{CO}_2]$ treatments.

Carbon dioxide efflux is seldom estimated using the profile method, and thus comparisons of the values obtained in this study with those reported in the literature using active or passive chamber methods are

of interest. When expressed on an annualized basis, the values for soil CO_2 efflux in this study (0.8 to 1.8 $\text{kg CO}_2\text{-C m}^{-2} \text{yr}^{-1}$) fall in the low end of the range of values summarized by Raich and Nadelhoffer (1989) from the literature (0.7 to 5.2 $\text{kg CO}_2\text{-C m}^{-2} \text{yr}^{-1}$). This may be in part due to the lack of a well-established litter layer in these open-top chambers. Previous studies at this site suggested that root respiration accounted for more than 90% of total soil CO_2 efflux (Johnson et al., 1994); thus, soil respir-

Table 3. Soil chemical properties before, 3 and 6 years after treatment with CO₂ and nitrogen

Treatments		Extr. P ($\mu\text{g g}^{-1}$)	Exch. Ca ²⁺	Exch Mg ²⁺ mol _c kg ⁻¹	Exch K ⁺	CEC	Base Sat. %
CO ₂ ¹	N ²						
Year 0							
Ap Horizons							
350	0	9.2 ± 4.1	57.2 ± 3.4	7.5±0.6	9.8±0.2	110.3 ± 4.7	68 ± 3
525	0	7.2 ± 3.0	54.2 ± 3.1	6.8±2.3	8.0±1.9	105.0 ± 3.9	66 ± 5
700	0	5.2 ± 0.2	54.4 ± 9.9	6.1±0.9	8.8±0.3	120.7 ± 22.9	60 ± 18
350	10	6.4 ± 1.9	70.2 ± 23.4	8.8±2.4	8.9±0.7	123.1 ± 18.3	71 ± 11
700	10	5.2 ± 0.2	48.2 ± 9.7	5.7±1.6	8.5±0.6	99.9±9.1	63±14
350	20	7.8 ± 5.2	52.9 ± 6.6	6.9±1.2	8.6±0.4	107.1 ± 3.3	64 ± 5
525	20	5.1 ± 0.1	51.2 ± 2.0	5.4±0.5	9.2±0.6	113.7 ± 3.0	58 ± 3
700	20	5.4 ± 0.2	42.8 ± 9.1	4.8±1.4	8.3±0.6	124.8 ± 12.2	46 ± 11
Bw Horizons							
350	0	47.6 ± 6.4	61.8 ± 5.9	7.7±0.8	8.1±0.5	117.6 ± 9.5	66 ± 2
525	0	23.2 ± 2.5	61.1 ± 10.5	6.9±0.8	8.0±0.4	120.4 ± 16.7	64 ± 7
700	0	24.0 ± 10.7	55.2 ± 5.9	6.8±1.3	8.0±0.3	120.4± 10.1	59 ± 11
350	10	46.3 ± 11.1	70.4 ± 24.9	8.4±1.6	8.2±0.7	101.3 ± 28.4	88 ± 19
700	10	33.2 ± 14.4	52.5 ± 7.0	6.5±0.7	7.5±0.3	109.2 ± 14.3	62 ± 12
350	20	36.0 ± 11.6	60.7 ± 7.8	7.6±0.7	7.7±0.7	122.3 ± 7.4	63 ± 10
525	20	99.1 ± 110.2	62.2 ± 5.6	6.5±1.1	8.6±1.8	125.8 ± 3.7	62 ± 4
700	20	30.1 ± 6.7	56.3 ± 5.4	6.3±1.0	8.2±0.4	137.5 ± 13.7	52 ± 3
Year 3							
Ap Horizons							
350	0	4.1 ± 0.8	55.3 ± 6.7	7.2±0.4	8.0±0.3	101.6 ± 18.2	73 ± 11
525	0	2.0 ± 1.2	49.9 ± 2.6	6.4±1.8	7.0±0.3	108.4 ± 2.2	60 ± 6
700	0	1.5 ± 0.5	51.1 ± 14.6	6.1±1.8	7.6±0.8	132.1 ± 33.1	51 ± 6
350	10	2.3 ± 2.0	60.9 ± 27.1	7.3±2.3	11.2±7.0	110.1 ± 15.4	73 ± 27
700	10	1.3 ± 0.7	38.5 ± 16.7	4.6±1.9	6.5±0.7	105.3 ± 4.1	49 ± 21
350	20	3.8 ± 1.7	44.2 ± 2.6	5.5±0.2	6.5±0.6	116.3 ± 8.8	50 ± 3
525	20	0.9 ± 0.6	44.8 ± 2.4	4.5±0.3	8.0±0.4	120.3 ± 28.7	50 ± 9
700	20	1.2 ± 0.2	33.1 ± 6.4	3.8±0.7	6.3±0.9	100.4 ± 14.0	45 ± 12
Bw Horizons							
350	0	1.3 ± 0.9	50.2 ± 2.8	7.3±0.2	7.9±1.1	93.8±7.1	72±2
525	0	1.2 ± 0.9	56.8 ± 9.1	6.6±1.3	7.2±0.7	119.5 ± 5.6	61 ± 9
700	0	1.1 ± 0.2	50.6 ± 8.0	6.0±0.9	7.1±0.5	106.8 ± 5.1	61 ± 9
350	10	2.3 ± 0.9	60.8 ± 12.4	7.8±0.5	7.8±0.2	105.8 ± 20.7	76 ± 17
700	10	0.5 ± 0.4	48.8 ± 9.5	5.6±1.4	7.0±0.3	104.8 ± 6.5	60 ± 9
350	20	1.9 ± 0.9	58.5 ± 11.3	7.5±1.4	7.3±0.6	102.7 ± 28.4	75 ± 8
525	20	0.4 ± 0.2	55.3 ± 10.9	6.0±0.5	8.4±1.5	119.3 ± 7.3	60 ± 7
700	20	0.8 ± 0.6	50.1 ± 6.0	6.1±0.9	7.6±0.5	108.8 ± 1.9	60 ± 7
Year 6							
Ap Horizons							
350	0	7.8 ± 1.7	58.0 ± 5.6	8.0±0.0	4.7±0.6	190.7 ± 8.1	38 ± 3
525	0	6.2 ± 1.6	55.0 ± 4.4	6.7±2.3	4.3±0.6	188.3 ± 11.7	36 ± 6
700	0	8.4 ± 2.2	54.7 ± 7.1	6.7±2.3	4.7±0.6	190.0 ± 9.5	36 ± 7
350	10	7.7 ± 1.0	64.0 ± 23.4	6.7±2.3	4.0±0.0	187.0 ± 5.3	41 ± 14
700	10	6.9 ± 3.3	46.0 ± 9.8	5.3±2.3	4.0±0.0	177.0 ± 21.8	32 ± 4
350	20	8.9 ± 2.1	44.7 ± 3.5	4.0±0.0	3.7±0.6	191.0 ± 4.6	28 ± 3
525	20	5.7 ± 0.9	39.0 ± 1.7	4.0±0.0	3.0±0.0	191.3 ± 5.5	25 ± 2
700	20	8.5 ± 3.2	39.0 ± 6.6	4.0±0.0	3.3±0.6	189.3 ± 7.0	25 ± 4

Table 3. continued

Bw Horizons							
350	0	6.7 ± 1.1	54.0 ± 4.4	8.0±0.0	4.3±0.6	180.3 ± 8.1	38 ± 3
525	0	3.7 ± 1.3	53.3 ± 2.9	5.3±2.3	4.3±0.6	182.3 ± 17.6	36 ± 4
700	0	4.9 ± 1.2	52.3 ± 10.0	6.7±2.3	4.3±0.6	171.3 ± 15.9	38 ± 9
350	10	5.6 ± 0.6	62.3 ± 19.9	6.7±2.3	4.0±0.0	179.3 ± 7.8	42 ± 14
700	10	5.6 ± 2.4	48.7 ± 8.3	5.3±2.3	3.7±0.6	189.0 ± 2.6	32 ± 6
350	20	6.4 ± 0.4	48.7 ± 4.0	4.0±0.0	3.7±0.6	184.3 ± 8.4	31 ± 1
525	20	4.0 ± 0.9	44.3 ± 7.4	4.0±0.0	3.0±0.0	183.0 ± 9.8	29 ± 3
700	20	4.0 ± 1.7	45.3 ± 4.7	4.0±0.0	3.7±0.6	184.7 ± 10.8	29 ± 4

¹ μl CO₂.²0, 10 and 20 g N m⁻² yr⁻¹ as ammonium sulfate.

ation is not necessarily related to any change in soil C pools. Given that root respiration is the dominant source of soil CO₂ efflux, one would expect a trend of increased soil pCO₂ and soil CO₂ efflux over time as root biomass increased (Johnson et al., 1997). In this study, we identified weak ($r^2 < 0.30$) upward trends in pCO₂ (Figures 5 and 6) and calculated soil CO₂ efflux (not shown), except in the low N, 525 μl l⁻¹ [CO₂] treatment.

In contrast to previous greenhouse studies (including one study on the same soil; Johnson et al., 1996), there were no consistent [CO₂] treatment effects on soil exchangeable base cation concentrations or base saturation over the six-year duration of this field study. Despite the problems in laboratory bias between the 1991–3 and 1996 analyses (see methods), the statistical analyses of treatment effects within each year indicate that elevated [CO₂] had no consistent effect on exchangeable base cations or base saturation. The statistically significant differences in exchangeable Mg²⁺ and base saturation in year 3 were clearly artifacts of pretreatment biases, and all effects of [CO₂] on base cations and base saturation disappeared in year 6. The increase in base cation uptake with elevated [CO₂] (Johnson et al., 1997) was apparently insufficient to cause any detectable depletion of soil exchangeable base cation pools. Elevated [CO₂] caused temporary increases in soil solution NO₃⁻ and HCO₃⁻, but these were transient and quantitatively unimportant for either N or base cation leaching. The significant reductions in exchangeable Ca²⁺, K⁺, and mg²⁺ with N fertilization were undoubtedly due to increased nitrate leaching in the fertilized treatments (which was not measured due to funding constraints).

The effects of [CO₂] treatment on extractable P, while statistically significant, were inconsistent and reflective of pretreatment patterns. There was cer-

tainly no pattern of significant soil P depletion or immobilization with elevated [CO₂]. Extractable P, like exchangeable NH₄⁺ and NO₃⁻, is an ephemeral quantity which is known to vary on a seasonal basis (e.g., Johnson et al., 1988).

Perhaps the clearest [CO₂] treatment effects on soils were those on soil N and C/N ratio in year 6 (Figures 2 and 3; Table 1). The apparent negative effect of [CO₂] treatment on soil N raises interesting questions regarding the source of N for tree uptake under elevated [CO₂]. As noted in a previous paper, N uptake rates were significantly greater with elevated [CO₂], including the unfertilized treatments (Johnson et al., 1997). This leads to the question of whether the additional N for uptake with elevated [CO₂] came from: 1) increased mineralization, 2) increased soil exploration, or 3) non-symbiotic N fixation (cf., Bormann et al., 1993). Nitrogen mineralization rates during the course of this study were initially reduced (during year 1) and later unaffected (during year 4) by elevated [CO₂] (Johnson et al., 1996). Thus, our conclusion at the end of year 3 was that the source of additional N uptake was either increased soil exploration or non-symbiotic N fixation. These results suggest that trees subjected to elevated [CO₂] were able to ‘mine’ soil N in the unfertilized N treatments, even though this was not detected by laboratory N mineralization tests or in the buried bag studies. This possibility will be explored in a future paper using ¹⁵N analyses of plant tissues and soils.

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