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Effects of CO_2 and nitrogen fertilization on vegetation and soil nutrient content in juvenile ponderosa pine

D.W. Johnson[1,2], J.T. Ball[1] and R.F. Walker[2]
[1]*Biological Sciences Center, Desert Research Institute, P.O. Box 60220, Reno, NV 89506, USA* and*
[2]*Environmental and Resource Sciences, College of Agriculture, University of Nevada, Reno, NV 98512, USA*

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Abstract

This paper summarizes the data on nutrient uptake and soil responses in opentop chambers planted with ponderosa pine (*Pinus ponderosa* Laws.) treated with both N and CO_2. Based upon the literature, we hypothesized that 1) elevated CO_2 would cause increased growth and yield of biomass per unit uptake of N even if N is limiting, and 2) elevated CO_2 would cause increased biomass yield per unit uptake of other nutrients only by growth dilution and only if they are non-limiting. Hypothesis 1 was supported only in part: there were greater yields of biomass per unit N uptake in the first two years of growth but not in the third year. Hypothesis 2 was supported in many cases: elevated CO_2 caused growth dilution (decreased concentrations but not decreased uptake) of P, S, and Mg. Effects of elevated CO_2 on K, Ca, and B concentrations were smaller and mostly non-significant. There was no evidence that N responded in a unique manner to elevated CO_2, despite its unique role in rubisco. Simple growth dilution seemed to explain nutrient responses in almost all cases.

There were significant declines in soil exchangeable K^+, Ca^{2+}, Mg^{2+} and extractable P over time which were attributed to disturbance effects associated with plowing. The only statistically significant treatment effects on soils were negative effects of elevated CO_2 on mineralizeable N and extractable P, and positive effects of both N fertilization and CO_2 on exchangeable Al^{3+}. Soil exchangeable K^+, Ca^{2+}, and Mg^{2+} pools remained much higher than vegetation pools, but extractable P pools were lower than vegetation pools in the third year of growth. There were also large losses of both native soil N and fertilizer N over time. These soil N losses could account for the observed losses in exchangeable K^+, Ca^{2+}, Mg^{2+} if N was nitrified and leached as NO_3^-.

Introduction

Forest ecosystems throughout the world have experienced and will probably continue to experience a significant increase in both atmospheric carbon dioxide (CO_2) concentrations and nitrogen (N) deposition (Galloway et al., 1995: Kauppi et al., 1992; Strain and Thomas, 1992). The potential growth response to elevated CO_2 is nearly always positive, but there are cases where excessive N deposition has caused deficiencies in other nutrients and growth declines (Schulze, 1989). Growth responses to elevated CO_2 can occur even under N limitation because of reduced foliar N concentrations and consequent increases in the yield of biomass per unit of N uptake (Brown, 1991; Campagna and Margolis, 1989; Norby et al., 1986a, b; Samuelson and Seiler, 1993). One explanation for the often-observed decreases in foliar N concentration with increased CO_2 is that plants may produce lower concentrations of the enzymes of the photosynthetic carbon reduction (PCR) cycle, particularly the carboxylating enzyme Ribulose-1,5-bisphosphatecarboxylase/oxygenase (rubisco) (Tissue et al., 1993). If this is a general response and occurs under most field conditions, it implies that the N deficiencies common to many forest ecosystems will not preclude a growth increase in response to CO_2. No such convenient mechanism exists for increased biomass

* FAX No:+17026737485.E-mail:dwj@maxey.dri.edu

yield per unit uptake of other nutrients, however. Thus, Conroy et al. (1990) found little effect of elevated CO_2 on either growth or tissue P concentrations in P-deficient *Pinus radiata* (D. Don). On the other hand, other investigators have found CO_2 induced reductions in tissue P, K, S, and B in cases where these nutrients were apparently not limiting (Johnson et al., 1994a; Luxmoore et al., 1986; Norby et al., 1986a; O'Neill et al., 1987). These changes were probably due to growth dilution.

The few studies which have addressed the effects of elevated CO_2 on soils have produced conflicting results. Some investigators have found that elevated CO_2 causes a reduction in soil C, presumably because of stimulated decomposition through increased root exudation (Körner and Arnone, 1992; Zak et al., 1993) whereas others have found either no change or a net gain (Johnson et al., 1994b). Some investigators report that elevated CO_2 causes increased soil N availability (Körner and Arnone, 1992; Zak et al., 1993) whereas others report decreased N availability (Diaz et al., 1993) or no effect (Randlett et al., 1996). Norby et al. (1986a) found an increase in soil extractable P with elevated CO_2 whereas Johnson et al. (1994a) found no effect. Norby et al. (1986a) found no effect of elevated CO_2 on exchangeable K^+, but a downward trend which could be attributed to increased uptake, whereas Johnson et al. (1995b) found decreases in exchangeable K^+, Ca^{2+}, and Mg^{2+} with elevated CO_2 in excess of that which could be accounted for by plant uptake.

In this paper, we report the responses of ponderosa pine (*Pinus ponderosa* Laws.) seedlings treated to elevated CO_2 and N fertilization. Based upon the literature cited above, we hypothesized that 1) elevated CO_2 would cause increased growth and yield of biomass per unit uptake of N even if N is limiting, and 2) elevated CO_2 would cause increased biomass yield per unit uptake of other nutrients only by growth dilution and only if they are non-limiting. Vegetation and soil nutrient pools in ponderosa pine subjected to three years of CO_2 and N treatments in a field study were inventoried to test these hypotheses.

Materials and methods

Site

The open-top chamber site for the ponderosa pine research was located at the Institute of Forest Genetics in Placerville, California. The soil is Aiken clay loam, a Xeric Haplohumult derived from andesite. Soils were intensively sampled prior to chamber establishment, and were found to be very uniform. During February - April 1991, 24 hexagonal open-top chambers (3.6 m in diameter) were established on the site. The basic experimental design consisted of three levels of nitrogen (0,10, and 20 g m^{-2} yr^{-1} of N as ammonium sulfate, applied in early spring), and four CO_2 treatments (ambient, no chamber; ambient, chambered; 525 μL L^{-1} CO_2; and 700 μL L^{-1} CO_2). Water was delivered to each plot via a timed stand pipe to a looped one inch diameter manifold, and low pressure spray heads. Each of the chambered treatments was replicated three times, and each of the unchambered treatments was replicated twice. Only the results from the chambered measurements will be reported here. Due to cost limitations, the 10 g m^{-2} yr^{-1} N, 525 μL L^{-1} CO_2 treatment was excluded. Treatments were begun in May, 1991.

Sampling and analysis methods

In May of 1991, Ponderosa pine (*Pinus ponderosa*) was planted in each chamber. Seedlings were grown from seed (21 planting locations per chamber) and seedlings (21 per chamber), the latter being a backup in the event of excessive mortality. Seed-grown seedling survival was very good, and the seedling-grown stock was removed in October 1991.

In October 1991 (year 1), three trees from each chamber were harvested, 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 original plan was for a final harvest in October 1994, but additional funds became available for continuation of the project and this harvest was postponed until October 1996. Root biomass by size class and mycorrhizal infection were analyzed in each case and will be reported in later papers (R F Walker, unpubl. data). Only total root biomass will be reported here. Seedlings were dried, weighed by major component (foliage, branch, stem, roots), and analyzed for N on a Perkin-Elmer 2400 CHN Analyzer and for other nutrients at the Oregon State University (OSU) Soil and Plant Testing Lab. At OSU, plant samples were dry-ashed at 550 °C for 4 hours, dissolved in 5% (v/v) HNO_3, and analyzed by ICP.

Soils were sampled by horizon in March of 1991 and March of 1993 by punch auger. Soils from

Table 1. Regression equations used to estimate seedling biomass ln(Comp) = a + (b)ln(d^2h), where Comp = seedling component, d = diameter at 10 cm, h = height, and k = constant

Component	a	b	r^2
1992 Harvest			
Needles	-2.9797	0.65540	0.692
Stems+Branches	-4.8812	0.86967	0.724
Roots	-4.9412	0.84429	0.615
1993 Harvest			
Needles	-6.2504	1.0395	0.729
Branches	-12.173	1.4685	0.631
Stems	-9.4724	1.3200	0.819
Roots	-6.7686	1.038	0.868

each sampling were analyzed for total C and N on the Perkin-Elmer, NH_4^+, and NO_3^- (2 M NH_4Cl), exchangeable Ca^{2+}, Mg^{2+}, K^+, and Al^{3+} (1 M NH_4Cl); and extractable P (0.5 M HCl plus 1 M NH_4F; Olsen and Sommers, 1982). Soils from the 1991 sampling were also analyzed for SO_4^{2-} (0.016 M NaH_2PO_4; Johnson and Henderson, 1979), but there was insufficient sample for this analysis on the 1993 soils. In 1991, soils were also sampled for bulk density (core method, three replicates per chamber for each horizon). No bulk density samples were taken in 1993 due to the disturbance that would have been created.

Biomass estimates for trees within the chambers in years 2 and 3 were obtained from regressions of the type ln(Comp) = a + (b)ln(d^2h), where Comp = the tree component in question (foliage, branches, stems, roots), d = diameter at 10 cm height, h = height, and a and b are regression constants. This approach to biomass estimation was used because we found that the average diameter and height of the harvested trees differed from that of the total number of trees in the chamber prior to harvest. There were no significant CO_2 or N treatment effects on the regressions but there were significant differences between years, and thus all data were combined into one equation per year (Table 1).

Prior to seedling establishment in March 1991, soils were sampled for bulk density (core method) by horizon (three replicates per chamber) and for nutrient analyses with a punch auger (three replicates per horizon). In March 1993, soils were sampled again by punch auger for nutrient analyses. Bulk density sampling was judged to be too destructive in the 1993 sampling, and thus the values for 1991 were used to calculate changes in soil nutrient content.

Statistical analyses for treatment effects in any given year consisted of analysis of variance for a fractional factorial design using SYSTAT software ($p < 0.10$ was accepted as the level of significance). Analysis for temporal effects consisted of student's t-tests for changes from 1991 to 1993. Means and standard errors are reported in all tables and figures.

Results

Biomass and nutrient uptake

Biomass and nutrient content for the three-year growth period are shown in Table 2. In all three years, there were significant ($p < 0.05$), positive treatment effects of both N and CO_2 on biomass and tree N, P, S, K, Ca, Mg, and B contents. In year 1, there was a trend (non-significant) toward highest biomass and nutrient content in the medium (525 μL L^{-1}) compared to the low and high (350 and 700 μL L^{-1}) CO_2 treatments. This same pattern was found in a previous controlled environment study (Johnson et al., 1994a). However, this effect was transient: in years 2 and 3 the high CO_2 treatment showed the largest biomass and nutrient content.

The fractions of total biomass and N in foliage, woody tissues, and roots during the first three years of growth are shown in Figures 1 and 2. As expected, the fractions of seedling biomass in foliage and roots decreased with time (from $\approx$ 50% foliage and $\approx$ 30% roots in year 1 to $\approx$ 30% foliage and $\approx$ 20% roots in year 3) and the fraction of woody tissues increased with time (from $\approx$ 20% in year 1 to $\approx$ 40-50% in year 3). As noted in a previous paper (Johnson et al., 1996), there was an increase in root/shoot ratio with elevated CO_2 in year 2 (especially in the Low N, Medium CO_2 treatment). As shown in Figure 1, however, this effect was transient: in year 3, the fraction of roots in seedlings treated with elevated CO_2 was either equal to or slightly less than (i.e. high N, high CO_2 treatment) that in seedlings treated with ambient CO_2.

As is usually the case in coniferous seedlings, foliage accounted for a disproportionate fraction of seedling N content. Unlike the pattern in biomass, however, there was no clear trend toward decreasing fractions of foliage N with time: foliage contained $\approx$ 60-70% of seedling N in all three years. The lack of change in the foliage N fraction was due to the

Table 2. Biomass and nutrient contents of ponderosa pine at the Placerville site (standard errors are given)

Nitrogen	Unfertilized			10 g m^{-2} yr^{-1}		20 g m^{-2} yr^{-1}		
CO_2 (μL L^{-1})	350	525	700	350	700	350	525	700
Biomass (g tree^{-1})								
Year 1	0.8±0.1	1.5±0.1	1.2±0.2	1.5±0.2	1.7±0.2	1.7±0.4	2.4±0.3	2.2±0.1
Year 2	49±3	62±4	62±6	80±6	83±5	90±4	100±6	114±8
Year 3	689±76	759±94	1244±191	941±121	1407±174	1386±136	1218±85	2363±348
N content (mg tree^{-1})								
Year 1	10.1±1.3	16.1±1.4	12.2±2.1	19.7±4.3	16.4±2.7	18.9±3.8	28.3±4.4	19.4±0.5
Year 2	421±18	455±24	490±32	614±33	561±31	702±26	740±35	816±49
Year 3	4339±341	4794±414	6998±917	5758±508	9082±976	8356±584	8124±493	13491±1289
P content (mg tree^{-1})								
Year 1	1.8±0.2	2.8±0.2	2.0±0.3	3.4±0.8	2.6±0.3	3.2±0.7	3.6±0.5	4.1±0.2
Year 2	61.6±0.5	59.4±0.4	62.7±0.4	74.6±0.6	77.5±0.7	81.9±0.5	94.0±0.7	116.1±2.3
Year 3	792±63	772±63	1193±108	974±79	1317±105	1326±88	1119±63	2257±195
S content (mg tree^{-1})								
Year 1	1.2±0.2	2.2±0.2	1.5±0.3	2.5±0.7	2.1±0.3	2.3±0.5	3.2±0.5	2.8±0.3
Year 2	41.6±0.5	51.4±0.5	40.1±0.3	55.7±0.6	72.3±0.9	73.8±0.5	57.3±0.5	90.5±1.5
Year 3	597±59	607±51	919±86	792±73	1103±91	985±77	826±51	1521±140
K content (mg tree^{-1})								
Year 1	6.3±0.9	9.8±1.6	9.0±1.4	12.4±3.4	11.3±1.5	11.5±2.4	18.1±2.4	15.3±0.3
Year 2	265.4±2.8	242.1±1.8	271.8±2.8	370.6±2.2	382.1±3.5	381.2±1.9	430.6±5.4	480.4±4.3
Year 3	3327±232	4027±323	6113±554	4685±386	7312±557	6325±391	5775±334	11344±1042
Ca content (mg tree^{-1})								
Year 1	1.8±0.2	2.8±0.2	2.5±0.3	3.4±0.8	2.8±0.3	3.2±0.7	5.3±0.8	4.5±0.3
Year 2	102.6±1.1	155.1±1.6	154.6±1.1	192.5±2.2	209.8±2.8	210.9±1.2	241.9±1.7	291.4±4.2
Year 3	1487±115	1534±138	2564±245	1859±152	2754±218	2758±218	2427±153	4379±371
Mg content (mg tree^{-1})								
Year 1	0.8±0.1	1.4±0.1	0.9±0.1	1.6±0.4	1.2±0.1	1.5±0.3	2.3±0.3	1.9±0.1
Year 2	48.8±0.3	59.5±0.5	56.8±0.3	79.1±0.5	72.7±0.5	107.6±2.2	79.6±0.6	114.7±0.8
Year 3	688±48	690±51	1176±123	992±81	1296±104	1379±95	1150±68	2322±206
B content (μg tree^{-1})								
Year 1	0.02±0.00	0.04±0.00	0.04±0.00	0.05±0.01	0.04±0.01	0.05±0.01	0.06±0.01	0.06±0.01
Year 2	0.9±0.2	1.3±0.2	1.2±0.1	1.6±0.2	1.4±0.1	1.8±0.2	2.1±0.2	2.5±0.4
Year 3	10±1	11±2	17±2	16±1	21±2	19±2	19±2	37±4

reductions in N concentrations in woody tissues and roots over time, which offset the effects of increasing biomass fractions in these components. The fraction of N in roots generally decreased over time (from ≈ 15-20% in year 1 to ≈ 6-10% in year 3), and the fraction of N in woody tissues increased with time (from ≈ 10-12% in year 1 to ≈ 22-30% in year 3).

Foliar nutrient concentrations over the three-year sampling period are shown in Table 3. There were significant negative effects of CO_2 on foliar concentrations of all measured nutrients (N, P, S, K, Ca, Mg, and B) in the low N treatment in year 1 but these effects diminished greatly with time. By year 3, the only significant CO_2 effects on foliar nutrient concentrations

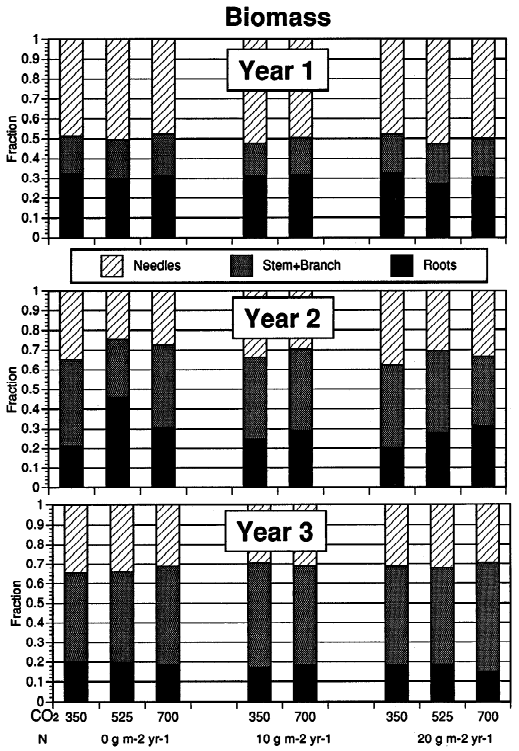

Figure 1. Distribution (fractions of total) of seedling biomass in foliage, stem+branch, and roots in years 1-3.

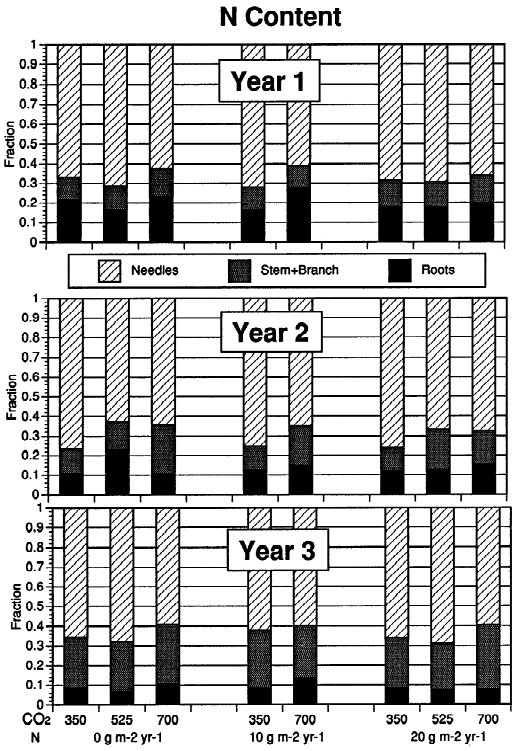

Figure 2. Distribution (fractions of total) of seedling N content in foliage, stem+branch, and roots in years 1-3.

were for P, S, Mn, and Zn in the low N treatment, S, Mg, Fe, and Mn in the medium N treatment, and Fe in the high N treatment. In all cases, these effects could be explained by growth dilution, since there were no reductions in foliar contents with CO_2. N fertilization caused either no change or reductions in foliar concentrations of all nutrients, including N itself.

Foliar concentrations of all nutrients except Cu were above deficiency thresholds for all three years using values from Hüttl et al. (1987). In year 3, however, foliar N, P, Mg, Cu, and Zn concentrations were well below the "interpretative values" ("survey average" values from "5.0 - 7.5 cm tips of young trees") given by Jones et al. (1991) for ponderosa pine. Foliar Cu levels were within the deficiency range for *Pinus radiata* in years 2 and 3 (1.5 - 5.0 μg g^{-1}; Turvey and Grant, 1990).

Soil changes

Analysis of variance for a fractional factorial design revealed no statistically significant overall treatment effects on soil C, N, C/N ratio (Figure 3) or on exchangeable Ca, K, Mg, or cation exchange capacity (CEC) (not shown). The magnitudes of the standard errors suggest that there were a few scattered significant CO_2 treatment effects (i.e. an increase in C in the Low N, Medium CO_2 treatment, an increase in N in the High N, Medium CO_2 treatment, and a reduction in C/N in the High N, Medium CO_2 treatment). There is no consistent pattern with treatment, however, and in one case (the increase in N in the High N, Medium CO_2 treatment) the differences were also present in year 1 (prior to treatment).

Analysis of variance for a fractional factorial design revealed no statistically significant treatment effects on soil mineral N ($NH_4^+ + NO_3^-$), and no significant pre-treatment differences existed (Figure 4). There were significant negative effects of CO_2 on soil extractable P in both Ap and Bw horizons in year 3 and no significant pre-treatment differences existed (although there was a non-significant initial trend toward lower available P in the Ap horizons of the Low N, Medium and High CO_2 treatments). There were trends toward higher Ap horizon exchangeable Al^{3+} with both N and CO_2 which may be relevant to the changes in avail-

Table 3. Foliar nutrient concentration of ponderosa pine at the Placerville site (standard errors are given)

Nitrogen	Unfertilized			10 g m^{-2} yr^{-1}		20 g m^{-2} yr^{-1}		
CO_2 (μL L^{-1})	350	525	700	350	700	350	525	700
N (%)								
Year 1	1.72±0.16	1.56±0.11	1.30±0.11	1.74±0.11	1.17±0.10	1.60±0.13	1.55±0.06	1.19±0.04
Year 2	1.76±0.16	1.68±0.11	1.63±0.12	1.71±0.09	1.52±0.12	1.89±0.20	1.57±0.07	1.48±0.10
Year 3	1.21±0.05	1.27±0.06	1.07±0.15	1.20±0.06	1.24±0.04	1.27±0.05	1.43±0.06	1.19±0.06
P (%)								
Year 1	0.26±0.005	0.20±0.002	0.19±0.02	0.29±0.01	0.20±0.01	0.27±0.01	0.19±0.01	0.23±0.01
Year 2	0.20±0.01	0.15±0.01	0.15±0.01	0.17±0.02	0.14±0.01	0.17±0.01	0.14±0.01	0.13±0.01
Year 3	0.18±0.02	0.15±0.01	0.14±0.01	0.14±0.01	0.14±0.003	0.15±0.003	0.14±0.01	0.14±0.01
S (%)								
Year 1	0.20±0.02	0.16±0.00	0.13±0.01	0.21±0.02	0.14±0.02	0.17±0.01	0.15±0.004	0.14±0.02
Year 2	0.12±0.01	0.13±0.02	0.09±0.01	0.11±0.02	0.13±0.02	0.14±0.02	0.08±0.01	0.11±0.03
Year 3	0.14±0.02	0.12±0.01	0.11±0.01	0.14±0.01	0.12±0.003	0.10±0.01	0.09±0.01	0.10±0.01
K (%)								
Year 1	0.99±0.04	0.78±0.02	0.82±0.04	1.04±0.05	0.83±0.02	0.90±0.04	0.91±0.07	0.84±0.04
Year 2	0.81±0.07	0.60±0.04	0.71±0.11	0.82±0.05	0.78±0.02	0.75±0.03	0.68±0.14	0.65±0.03
Year 3	0.65±0.03	0.72±0.05	0.63±0.03	0.67±0.05	0.74±0.04	0.65±0.03	0.66±0.04	0.67±0.05
Ca (%)								
Year 1	0.31±0.01	0.25±0.01	0.28±0.01	0.33±0.01	0.23±0.01	0.28±0.00	0.31±0.02	0.29±0.02
Year 2	0.33±0.06	0.43±0.07	0.36±0.03	0.38±0.06	0.38±0.08	0.39±0.03	0.40±0.04	0.32±0.03
Year 3	0.36±0.02	0.32±0.01	0.33±0.03	0.32±0.02	0.32±0.03	0.32±0.01	0.29±0.02	0.30±0.01
Mg (%)								
Year 1	0.12±0.004	0.10±0.000	0.08±0.01	0.13±0.002	0.08±0.01	0.12±0.004	0.12±0.003	0.10±0.01
Year 2	0.13±0.01	0.14±0.01	0.11±0.003	0.15±0.01	0.10±0.003	0.16±0.01	0.10±0.01	0.13±0.003
Year 3	0.15±0.01	0.13±0.005	0.15±0.02	0.16±0.01	0.13±0.01	0.15±0.01	0.14±0.01	0.15±0.01
B ($\mu g\ g^{-1}$)								
Year 1	41±0	34±0	37±1	42±2	29±3	43±2	29±1	39±3
Year 2	36±9	43±9	33±3	42±4	28±3	44±7	42±2	31±2
Year 3	27±5	26±6	23±3	34±1	26±2	22±3	29±5	30±4
Cu ($\mu g\ g^{-1}$)								
1992	3.7±0.2	3.3±0.2	3.5±0.5	3.9±0.7	4.5±1.1	4.1±0.6	6.0±2.4	3.5±0.5
1993	3.2±0.3	4.1±0.8	3.2±1.0	3.2±0.4	3.8±0.5	2.7±0.3	2.8±0.1	3.2±0.8
Fe ($\mu g\ g^{-1}$)								
1992	325±110	318±60	439±97	411±78	579±107	276±19	470±100	294±60
1993	188±18	200±13	190±4	185±27	149±6	241±24	215±35	163±11
Mn ($\mu\ g^{-1}$)								
1992	338±44	377±49	366±10	325±29	366±47	393±24	349±8	320±39
1993	158±10	115±4	114±5	153±12	127±7	131±7	119±10	133±4
Zn ($\mu g\ g^{-1}$)								
1992	57±10	61±22	49±7	63±8	51±6	67±9	46±5	59±5
1993	63±4	60±7	39±5	48±3	64±4	41±7	40±10	38±4

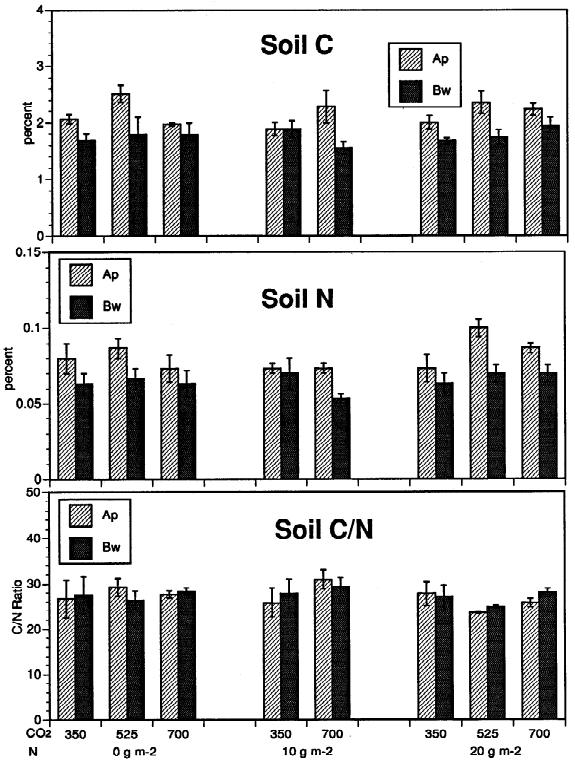

Figure 3. Soil C, N, and C/N ratio in year 3 (standard errors are given).

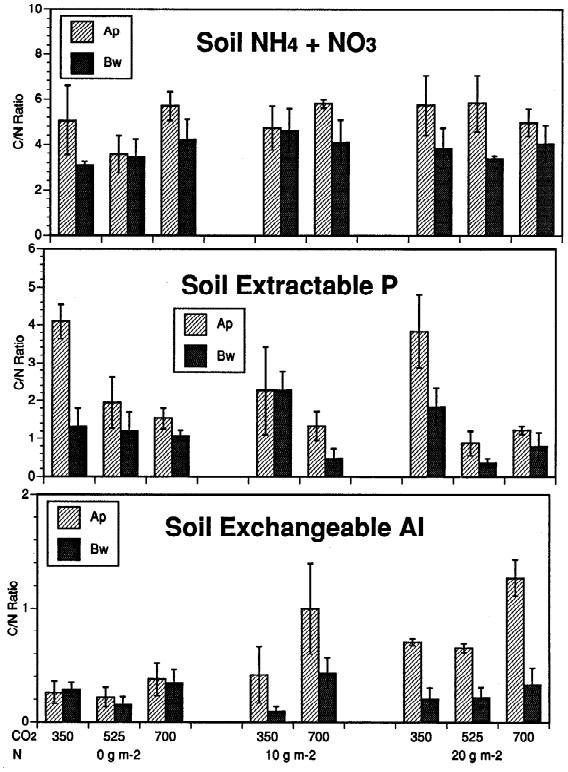

Figure 4. Soil $NH_4^+ + NO_3^-$, extractable P, and exchangeable Al^{3+} in year 3 (standard errors are given).

able P (see Discussion); however, none of the trends in exchangeable Al^{3+} were statistically significant.

There were some significant changes in soil nutrients with time. There was an overall downward trend in total N from year 1 to year 3 in all horizons, resulting in a statistically significant reduction in soil N pools in many cases (Table 4). Thus, although there were no statistically significant changes in soil with time, there was an overall upward trend in C/N ratio (Figure 3). There were general downward trends and several statistically significant reductions in Ca, Mg, and K from year 1 to year 3, but no significant treatment effects at either sampling. There were no consistent patterns of change in CEC (not shown).

By far the largest change in soils with time was in the case of extractable P pools, which decreased by 80-99% between year 1 and year 3. Over this period of time a significant treatment effect of CO_2 also developed: whereas there were no treatment effects on extractable P in year 1 (pre-treatment), there was a statistically significant, overall negative effect of CO_2 extractable P in both the Ap and Bw horizon in year 3 (Figure 4 and Table 4).

Nutrient budgets

The net changes in soil C between year 1 and year 3 ranged from +1048 to -943 g m^{-2} (+12 to -11%), but none was statistically significant (Table 4). Changes in vegetation C (all starting from 0, and therefore positive and statistically significant) ranged from 266 to 876 g m^{-2}, adding +3 to +10% to total ecosystem C capital. If the soil changes are taken at face value (e.g. whether significant or not), the net ecosystem C balance was negative in two cases (Low N, Low CO_2 and High N, Medium CO_2), near zero in one case (High N, Low CO_2), and positive in four cases (Low N, Medium and High CO_2; Medium N, Low CO_2, and High N, High CO_2). There was no relationship between treatment and soil or ecosystem C balance, although there were significant, positive effects of both N and CO_2 on biomass.

Table 4. Ecosystem carbon and nutrient pools in year 3 and changes from year 1 to year 3

N Treat (g m^{-2} y^{-1})	0	0	0	10	10	20	20	20
CO_2 Treat (μL L^{-1})	350	525	700	350	700	350	525	700
	(g m^{-2})							
Carbon content, year 3								
Vegetation	266	293	500	364	565	535	489	876
Soil total	8228	8958	7654	8315	8061	7536	8577	8629
Total	8494	9251	8154	8679	8626	8071	9066	9505
Change, years 1-3								
Soil	-943	5	-234	-121	-374	-545	-925	1048
Total ecosystem	-677	298	266	243	191	-10	-436	1924
Nitrogen content, year 3								
Vegetation	3.7	4.1	6.3	5	8.1	7.2	7.3	11.1
Soil, exchangeable[a]	1.8	1.5	2.0	2.0	2.1	2.0	1.9	1.8
Soil, total	315	321	279	315	268	278	357	323
Total	318.7	325.1	285.3	320	276.1	285.2	364.3	334.1
Change, years 1-3								
Soil, total	-65*	-72	-50	-73*	-80*	0	-50*	-19
Total ecosystem	-61.3	-67.9	-43.7	-68.0	-71.9	7.2	-42.7	-7.9
Phoshorus content, year 3								
Vegetation	0.68	0.66	1.07	0.84	1.18	1.14	1	1.86
Soil, extractable	1.2	0.7	0.5	1	0.4	1.1	0.3	0.1
Total	1.88	1.36	1.57	1.84	1.58	2.24	1.3	1.96
Change, years 1-3								
Soil	-11.4*	-5.9*	-5.7*	11.0*	-8.2*	-8.0*	-22.7*	-7.4*
Total ecosystem	-10.72	-5.24	-4.63	-10.16	-7.02	-6.86	-21.7	-5.54
Potassium content, year 3								
Vegetation	2.9	3.5	5.5	4	6.5	5.4	5.2	9.4
Soil	112	112	117	166	114	110	136	112
Total	114.9	115.5	122.5	170	120.5	115.4	141.2	121.4
Change, years 1-3								
Soil, exchangeable	-42*	-19	-17*	19	-20	-20	-11	-21*
Total ecosystem	-39.1	-15.5	-11.5	23	-13.5	-14.6	-5.8	-11.6
Calcium content, year 3								
Vegetation	1.3	1.3	2.3	1.6	2.5	2.4	2.2	3.6
Soil, exchangeable	382	450	412	531	378	419	424	348
Total	383.3	451.3	414.3	532.6	380.5	421.4	426.2	351.6
Change years 1-3								
Soil	-143*	-37	-33	-86	-57	-46	-56	-65
Total ecosystem	-141.7	-35.7	-30.7	-84.4	-54.5	-43.6	-53.8	-61.4
Magnesium content, year 3								
Vegetation	0.6	0.6	1.1	0.85	1.16	1.18	1.03	1.91
Soil, exchangeable	292	306	278	371	248	299	251	233
Total	292.6	306.6	279.1	371.85	249.16	300.18	252.03	234.91
Change, years 1-3								
Soil	-84	-16	-16	-51	-48	-33	-31	-26
Total ecosystem	-83.4	-15.4	-14.9	-50.15	-46.84	-31.82	-29.97	-24.09

[a] Values obtained one year after fertilization.
*Statistically significant differenca between 1991 and 1993, Student's t-test, p <0.05.

The net changes in soil total N between year 1 and year 3 were negative in all but one case (High N, Low CO_2, where the net change was 0) (Table 4). The losses of soil N ranged from 0 to 80 g m^{-2} (0 to -29% of ecosystem capital) and many were statistically significant. There were significant, positive effects of both N and CO_2 treatments upon vegetation N content in year 3. However, the increases in vegetation N were small compared to the losses in soil N in most cases (ranging from +3.7 to +11.1 g m^{-2}, or 1 to 3% of ecosystem capital). Thus, the ecosystem N balance was negative in all but one case. There were no relationships between either N or CO_2 treatments and soil or ecosystem C although there were significant, positive effects of both N and CO_2 on biomass.

Vegetation N pools were greater than soil mineral N (NH_4^+ + NO_3^-) pools but considerably smaller than soil total N pools, as is usually the case in forest ecosystems (Johnson, 1992). Soil mineral N pools were substantially greater in the fertilized plots after fertilization each spring after the addition of 10 and 20 g m^{-2} in the medium and high N treatments, respectively (data not shown). As is often the case following N fertilization (Johnson, 1992), however, soil mineral N levels in the fertilized chambers dropped to near control levels by the end of the year. Thus, there were no treatment effects on mineral N in March year 3, just prior to refertilization (Table 4).

As noted earlier, there were very large declines in soil extractable P pools between year 1 and year 3. There were positive and significant effects of both N and CO_2 on vegetation P content in year 3; however, the increases in vegetation P were small relative to the declines in extractable P (ranging from 0.68 to 1.86 g m^{-2}, or 4 to 10% of the decline in soil available P). In year 3, the decreases in soil extractable P with elevated CO_2 were approximately the same magnitude as the increases in vegetation P with elevated CO_2, and thus ecosystem P capital (defined here as the sum of vegetation and soil extractable P) was more constant with treatment than either soil or vegetation P content.

There were overall declines in soil exchangeable K^+, Ca^{2+}, and Mg^{2+} pools from year 1 to year 3 (Table 4). These declines ranged from -15 to -27% for K^+, -8 to -27% for Ca^{2+}, and -5 to -22% for Mg^{2+}. The changes were statistically significant in three cases for K^+ (Low N, Low and High CO_2 and High N, High CO_2) and in one case for Ca^{2+} (Low N, Low CO_2). None of the changes were significant for exchangeable Mg^{2+} pools. As was the case for N and P, there were significant and positive effects of both N and CO_2 treatments on vegetation K, Ca, and Mg contents in year 3. These increases were outweighed by the decreases in soil exchangeable pools, however, so that the net ecosystem changes in K, Ca, and Mg were negative in all cases.

Discussion

Growth responses to N and CO_2

Both elevated CO_2 and N fertilization caused growth increases in ponderosa pine in this field study. The growth response to N fertilization indicates that N was a limiting nutrient in this soil, yet N limitation was clearly not severe enough to prevent a growth response to CO_2 in the unfertilized treatments. This growth response to elevated CO_2 was facilitated by increased N uptake more so than an increase in N "use efficiency" (e.g. lower tissue N concentrations). Contrary to the findings of Zak et al. (1993), we have found no evidence that CO_2 treatment caused increased soil N availability: CO_2 had no effect upon soil mineral N (NH_4^+ + NO_3^-) pools at the three year sampling, and ancillary studies have shown that CO_2 has a negative effect upon soil N mineralization rates (Johnson et al., 1996). Details of the effects of CO_2 on soil N availability, litter quality, and decomposition will be reported in another paper. For the purposes of this analysis, it is clear that the growth responses to CO_2 in the absence of fertilization must have been facilitated by increased soil exploration. This increased root/shoot ratio in the Low N, elevated CO_2 treatments during year 2 suggests increased soil exploration at that time (Figure 1; Johnson et al., 1996). Although this root/shoot ratio effect disappeared in year 3, there was still greater root biomass (and total biomass) under the elevated CO_2, which would continue to facilitate greater soil exploration.

The results of this field study contrast sharply with those from previous pot studies with ponderosa pine. In one pot study using an artificial, N-poor soil, ponderosa pine showed no growth response to CO_2 without N fertilization (Johnson et al., 1994b). In other studies, ponderosa pine showed a toxic response to N fertilization which was either mitigated or reversed by CO_2 (Griffin et al., 1995; Johnson et al., 1995a). Collectively, these studies demonstrate that 1) either extreme N deficiency or N toxicity can preclude growth response to CO_2 in ponderosa pine, and 2) N deficiency should be thought of as a continuum rather than as an on/off

situation, with the responses to CO_2 lessening as N supplies become either suboptimal or supra-optimal.

Effects of N and CO_2 on vegetation nutrient status

As of year 3, there were no visual symptoms of nutrient deficiency in the seedlings. By 1996, however, there was some evidence of rosetting on terminal shoots, as is often observed with both B and Cu deficiency (Stone, 1990; Turvey and Grant, 1990). It is interesting that no P deficiency was encountered, even though soil extractable P levels had declined to very low levels by year 3. P uptake may have been facilitated by the greater mycorrhizal colonization with elevated CO_2 noted by Tingey et al. (1995) in this study.

Both CO_2 and N treatments caused increased uptake of all measured nutrients, even when tissue nutrient concentrations declined. Increased nutrient uptake in combination with reduced tissue nutrient concentrations can be interpreted as growth dilution, and increased uptake with no change in tissue concentration can be interpreted as sufficiency (Timmer and Stone, 1978; Weetman, 1989). Foliar analysis indicated growth dilution responses to CO_2 for N, P, S, K, Ca, Mg, and B in year 1, and to a lesser degree in year 2. CO_2 continued to cause a growth dilution of foliar P, S, and B in year 3, also, but the effects of CO_2 on foliar N, K, and Mg had largely disappeared as concentrations declined.

There is no evidence that N behaved in a unique manner compared to other nutrients in response to elevated CO_2, despite its unique role in rubisco. Although Hypothesis 1 (elevated CO_2 will cause increased growth and yield of biomass per unit uptake of N) was supported in the strictest sense for the first two years of growth, it was not supported in the third year. Hypothesis 2 (elevated CO_2 will cause increased biomass yield per unit uptake of non-limiting nutrients due to growth dilution) was supported: elevated CO_2 caused increased biomass per unit uptake for all major nutrients. In the cases where foliar concentrations were at deficiency thresholds, (e.g. Cu and B in year 3), there were no effects of elevated CO_2, as hypothesized. However, there were also no effects of elevated CO_2 on foliar N in year 3, even though continued growth response to N fertilization indicated continuing N limitation. Thus, once again, there is no evidence that N is behaving in a unique manner compared to other nutrients.

The N responses were somewhat different from the CO_2 responses, as would be expected. First of all, there was no consistent, statistically significant effect of N treatment on foliar N concentration. On a whole-tree basis, N concentration actually decreased with N fertilization (not shown). Thus, although there was a significant growth response to N additions, N itself was diluted by the increased growth that occurred (the "Steenbjerg effect"; Weetman, 1989). The effects of N fertilization on other nutrients was inconsistent. In year 1, N fertilization had no consistent, statistically significant effect on any measured nutrient in foliage. In year 2, N caused growth dilution of foliar P, and in year 3 N caused growth dilution of foliar Cu and Zn.

Effects of N and CO_2 on soil nutrients and nutrient budgets

The declines in soil C and N were probably a result of the plowing that took place prior to planting. Losses of soil C and N with cultivation are well-documented; Mann (1986) found that soil C losses averaged at least 20% over the first 20 years of cultivation. The losses of soil N in this study were surprisingly high, however, as was the increase in C/N ratio. One would normally expect C/N ratios to decline during organic matter mineralization, as C is usually lost prior to N. We speculate that after the initial loss of both C and N due to plowing, there was a re-introduction of C into the soil from root sloughing, exudation, etc. which caused the C/N ratio to increase.

The relatively large losses from the total soil N pool generally cannot be accounted for by vegetation uptake, and therefore must represent either leaching or denitrification. Fertilizer N recovered by trees ranged from $\approx$ 4 to 12% (using unfertilized trees as a base), values which are at the low end but not unusual for fertilizer N recovery by trees (Johnson, 1992). Soil pools are large compared to fertilization inputs and thus fertilizer N recovery by soils is difficult to assess without tracer studies. However, there is no indication that fertilizer accumulated in the soil in these systems: cumulative applied N was of the same order of magnitude as apparent net losses of soil N, and there was no consistent N fertilizer treatment effect on net changes in soil N.

The declines in exchangeable cations were probably caused by increased leaching following plowing. We speculate that the mineralization of C and N after plowing caused increased soil respiration and nitrification, which in turn caused increased rates of cation leaching associated with both bicarbonate and nitrate. A rough reality check of this scenario can be made by

comparing the net losses of N (declines in native soil N plus fertilizer N) with the losses of total base cations in $mol_c\ m^{-2}$, assuming that all N was lost by NO_3^- leaching. This analysis suggests that NO_3^- leaching could account for an average of $\approx$ 90% of the base cation losses from these soils.

Extractable P was the only measured soil nutrient which was affected by treatment, aside from the previously noted negative effect of CO_2 on N mineralization (Johnson et al., 1996). The relatively large declines in soil available P far exceeded vegetation P uptake, and were most likely due to immobilization in the soil (either microbial or chemical). Leaching of P is nearly always minimal due to its high affinity for adsorption sites. It is not possible at this stage to differentiate between microbial P immobilization and adsorption as a cause of reduced soil extractable P with time. The increases in exchangeable Al^{3+} with elevated CO_2 mirrored the decreases in extractable P fairly well and could have caused increased P adsorption. In recent samplings (1996), higher microbial biomass has been found in the soils subjected to elevated CO_2 (W Cheng, pers. comm.).

Conclusions

Elevated CO_2 had a positive effect upon growth and nutrient uptake, with or without N fertilization, in field studies in ponderosa pine. This response occurred despite the fact that N was suboptimal, and was apparently facilitated by increased soil exploration. Despite its unique role in rubisco, there was no evidence that N responded to elevated CO_2 in a unique manner: there were short-term reductions in foliar P, S, B, and Mg as well as N concentrations with elevated CO_2, and all could be explained by simple growth dilution. Elevated CO_2 caused reductions in soil available N and P and increases in exchangeable Al^{3+}, but had no consistent or significant effects upon total C, total N, exchangeable Ca^{2+}, K^+, or Mg^{2+}. The reductions in available P exceed that which could be accounted for by plant uptake and may have been due to increased P adsorption, increased microbial immobilization, or both.

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