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THE EFFECTS OF ELEVATED CO_2 ON NUTRIENT DISTRIBUTION IN A FIRE-ADAPTED SCRUB OAK FOREST

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Abstract. Elevated carbon dioxide (CO_2) caused greater accumulation of carbon (C) and nutrients in both vegetation and O horizons over a 5-yr sampling period in a scrub oak ecosystem in Florida. Elevated CO_2 had no effect on any measured soil property except extractable phosphorus (P), which was lower with elevated CO_2 after five years. Anion and cation exchange membranes showed lower available nitrogen (N) and zinc (Zn) with elevated CO_2. Soils in both elevated and ambient CO_2 showed decreases in total C, N, sulfur (S), and cation exchange capacity, and increases in base saturation, exchangeable Ca^{2+}, and Mg^{2+} over the 5-yr sampling period. We hypothesize that these soil changes were a delayed response to prescribed fire, which was applied to the site just before the initiation of the experiment. In the ambient CO_2 treatment, the increases in vegetation and O horizon C, N, and S were offset by the losses of soil total C, N, and S, resulting in no statistically significant net changes in ecosystem C, N, or S over time. In the elevated CO_2 treatment, the increases in vegetation and O horizon C content outweighed the losses in soil C, resulting in a statistically significant net increase in ecosystem C content. Nitrogen and S contents showed no statistically significant change over time in the elevated CO_2 treatment, however. Comparisons of vegetation contents and soil pools of potassium (K), calcium (Ca), and magnesium (Mg) suggest that a substantial proportion of these nutrients were taken up from either groundwater or deep soil horizons. This study demonstrates that changes in ecosystem C sequestration due elevated CO_2 or any other factor cannot be accurately assessed in the absence of data on changes in soils.

Key words: *CO_2, elevated; fire; nutrients; O horizon; scrub oak, Florida; soils; uptake.*

INTRODUCTION

The long-term effects of elevated carbon dioxide (CO_2) on growth and carbon (C) sequestration are highly dependent upon the availability and cycling of nutrients, especially nitrogen (N). Elevated CO_2 can mitigate N deficiencies by facilitating increased uptake and/or biomass production per unit uptake (Zak et al. 1993, McGuire et al. 1995, Bernston and Bazzaz 1996, Johnson et al. 1997, Curtis et al. 2000, Medlyn et al. 2000). Some studies have suggested that elevated CO_2 can also facilitate greater soil exploration by increasing root and mycorrhizal biomass (Norby et al. 1987, Rogers et al. 1992, Tingey et al. 1996, Pregitzer et al. 2000), increased N mineralization (e.g., Zak et al. 1993), or ''mining'' of older soil N (Johnson et al. 2000*a*, *b*). On the other hand, elevated CO_2 could also exacerbate nutrient deficiencies by introducing high C:N ratio litter or other organic compounds into the soil, causing N immobilization (Diaz et al. 1993, Rice et al. 1995, Hungate et al. 1999). Elevated CO_2 has been found to cause both increased (Körner and Arnone 1992) and decreased (Torbert et al. 1996) NO_3^- leaching, depending on the degree to which N mineralization and N uptake are affected. Nitrogen status seems to have an effect on how elevated CO_2 affects soil organic matter and N mineralization. Cheng and Johnson (1998) found that elevated CO_2 reduced soil organic matter decomposition by 18% without N fertilization, but increased it by 22% with N fertilization in a greenhouse experiment with wheat.

Studies on the effects of elevated CO_2 on nutrients other than N are fewer in number and have produced conflicting results. Elevated CO_2 often causes reduction in tissue concentrations of nutrients besides N, but there are numerous and inconsistent exceptions that appear to relate to nutrient status and relative growth rates (Luxmoore et al. 1986, Norby et al. 1986, Brown 1991, Johnson et al. 1997). Elevated CO_2 has been found to cause increases (e.g., Norby et al. 1986), decreases (e.g., Johnson et al. 1995, 2000*a*), or no effect (e.g., Johnson et al. 1995, 2000*a*) in soil extractable P and exchangeable Ca^{2+}, K^+, and Mg^{2+} in various greenhouse studies and open-top chamber studies. The often noted increase in soil respiration under elevated CO_2 (Körner and Arnone 1992, Johnson et al. 1994, Vose et al. 1995, Hungate et al. 1997) could cause increased

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carbonic acid production and bicarbonate base cation leaching if soils are not extremely acidic (Andrews and Schlesinger 2001).

The interactions among changes in tissue nutrient concentration, litter quality, root exudation, uptake and recycling of nutrients are especially important in closed-canopy forests; yet, because of logistical and funding constraints, few studies have addressed the effects of elevated CO_2 in closed canopy forests. Exceptions to this include the studies by Oren et al. (2001) who noted a large reduction in growth response to elevated CO_2 after three years of treatment in the prototype Free Air CO_2 Enrichment (FACE) study at Duke. They attributed this decline in CO_2 response to progressive N deficiency, which was supported by increased growth response to CO_2 after the addition of N fertilizer. Allen et al. (2000) did not find significant effects of elevated CO_2 on O horizon C:N ratio during the first year of treatment in the FACE site, and thus the direct causes of the progressive N deficiency noted by Oren et al. (2001) apparently did not include changes in the litter quality–decomposition pathway.

Elevated CO_2 is only one of many factors that could affect C sequestration in terrestrial ecosystems. Among the other factors is the incidence of fire. Fire is an important component of the global carbon cycle: the contributions of fire to global CO_2 emissions have been estimated to rival those of fossil fuel emissions (Olson 1981, Crutzen and Andreae 1990, Mack et al. 1996). Similarly, N emissions from fire appear to make a substantial contribution to the global N budget (Crutzen and Andreae 1990, Galloway et al. 1995). The temperatures at which nutrients are gasified during fire largely determine the effects of fire on nutrient budgets: virtually all N in burned material is gasified, much of the S, much less of the P, and little or none of the Ca, K, and Mg (Raison et al. 1985). Thus, fire causes a net loss of C, N, S, and possibly P (the latter only in hotter fires) and lower concentration of K, Ca, and Mg in ash left on site. Post-fire changes in soils depend largely on the degree of revegetation, especially with regard to nitrogen fixers (Johnson and Curtis 2001). In some cases, invasion of nitrogen fixers can cause a net increase in soil C and N compared to pre-fire conditions (Johnson and Curtis 2001).

We had the opportunity to study the effects of elevated CO_2 on nutrient cycling in a fire-adapted scrub oak system in Florida (Hungate et al. 1999). The Florida scrub oak system offers a unique opportunity to study the combined effects of elevated CO_2 and fire on a model forest ecosystem. Fire is a dominant feature of these ecosystems, with a natural fire return interval of ~5–20 yr (Schmalzer and Hinkle 1996). Because of the small stature of these scrub oak systems and their rapid progression toward canopy closure after fire (Schmalzer and Hinkle 1987, 1996), the feedback effects of elevated CO_2 on stand-level nutrient cycling can be realistically assessed within the lifetime of an experiment. Previous results from this site have shown that elevated CO_2 caused increased biomass for one of the two major species (*Quercus myrtifolia* Willd.), increased fine root biomass (Day et al. 1996), but no effects on root decomposition (Dilustro et al. 2001). Elevated CO_2 was found to have negative effects on soil C, N, and P availability in soils in this study (Hungate et al. 1999, Shortemeyer et al. 2000, Johnson et al. 2001). We hypothesized that the observed reductions in soil N and P availability with elevated CO_2 were due in part to increased uptake of these nutrients by plants under this treatment (Johnson et al. 2001). In this paper, we test this hypothesis and report the results of a reinventory of soil nutrient pools 5 yr after a stand-replacing prescribed fire and after 5 yr of treatment with elevated CO_2.

Site and Methods

Site

The study site is located on Merritt Island, a barrier island off the east coast of central Florida and part of the Kennedy Space Center (28°38′ N, 80°42′ W). The substrates are well drained Pomello (Arenic Haplahumods) and Poala sands (Spodic Quartzipsamments). Both soils are acidic and low in nutrients, and nutrients tend to be concentrated in the standing biomass and in the O and A horizons (Schmalzer and Hinkle 1996). The experimental site is representative of a fire-maintained, scrub oak palmetto community (Breininger and Schmalzer 1990). Two oak species, *Quercus myrtifolia* Willd and *Q. geminata* Small. constituted up to 87% of aboveground biomass in this system (Schmalzer and Hinkle 1996). Minor species included *Q. chapmannii* Sargent, *Lyonia fruticosa*, *L. lucida*, *Vaccinium myrsinites*, and *Galactia eliotii* (Dijkstra et al. 2002). Also present was the saw palmetto *Serenoa repens* Small, which typically contains considerable biomass in its rhizomes (Schmalzer and Hinkle 1996). The climate is subtropical, warm, and humid, with a 100-yr mean annual precipitation total of 131 cm and high year-to-year variability. One-hundred-year mean maximum and minimum temperatures in July, the hottest month, are 33.3°C and 21.8°C, respectively, and 22.3°C and 9.5°C, respectively, in January, the coldest month (Mailander 1990).

Experimental design

The study site was burned in August 1995 with a few remaining areas burned in January 1996 prior to establishment of 16 open-top chambers (OTCs). Of the 16 OTCs, 8 were maintained at current ambient CO_2 and 8 at ambient plus 350 μL/L CO_2 (elevated). The OTCs were octagonal in design with the largest diameter 3.66 m and with sides 1.4 m long. Each chamber was 3.3 m high with the frustum at the midpoint providing a chamber volume of 18.9 m^3. Eight unchambered control plots of identical surface area were also

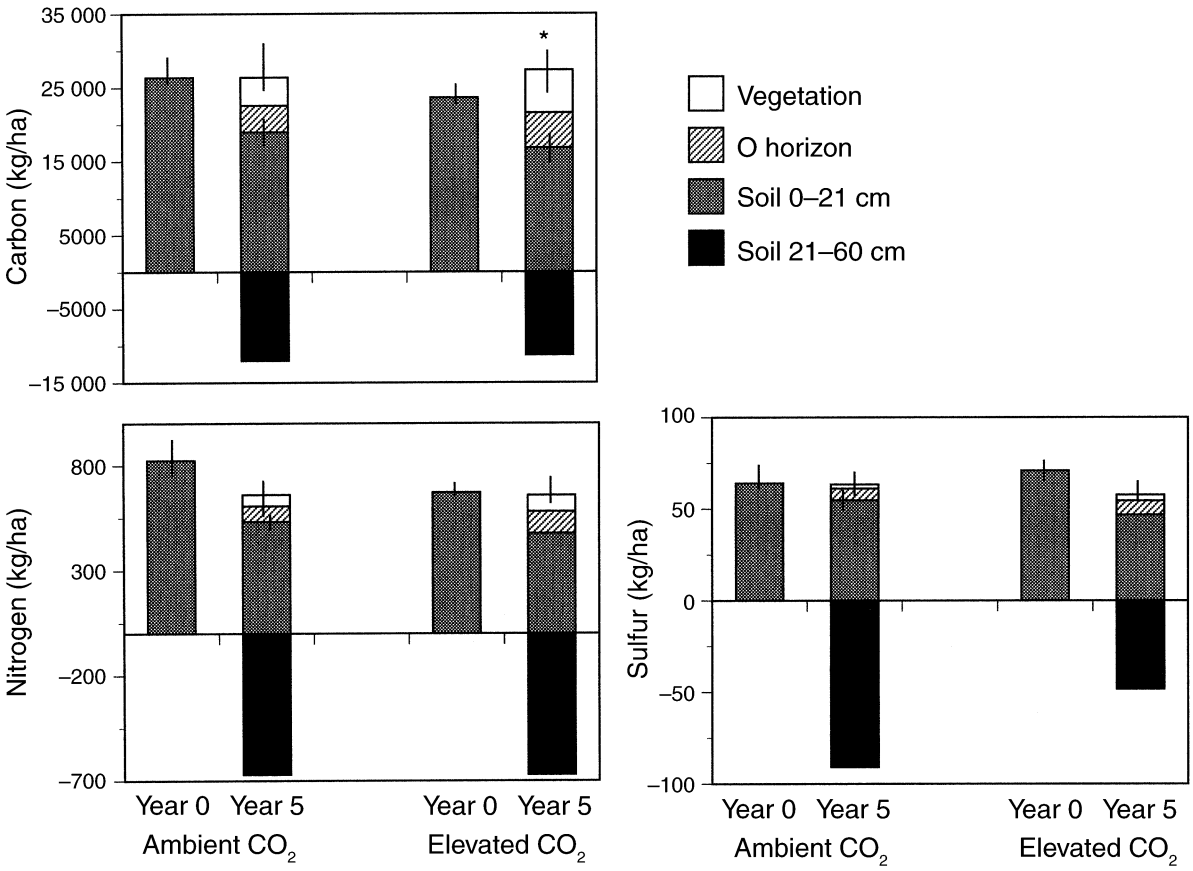

FIG. 1. Carbon, nitrogen, and sulfur contents in soil, O horizon, and vegetation in the study site in 1996 (Year 0) and 2001 (Year 5). Soil contents in the 21–60 cm depth are shown as negative values so that total ecosystem contents using soils at 0–21 cm depths can be compared. (Soils at the 21–60 cm depth were not sampled in 1996.) Standard errors of total ecosystem and soil pools are shown. Statistically significant differences in ecosystem content over time are represented as follows: $*P < 0.05$ (Student's *t* test). See Table 1 for other statistical analyses.

established. The plots were blocked according to preburn aboveground biomass, species composition, and proximity. Each block consisted of one of each of the ambient, elevated, and unchambered plots.

Methods

In April of 1996, after the plots had been chosen but before the chambers had been constructed, we removed soil samples from the A (0–9 cm) and E (9–21 cm) horizons for pretreatment chemical analysis. The cores were taken using a 5-cm diameter corer from two points within 50 cm of the center of each plot.

Five years later, in April of 2001, we sampled soils by depth (0–9, 9–21, and 21–60 cm, corresponding to the A, E, and BC horizons) using a 2.5 cm diameter punch auger at 10 random points in each chamber. The top two depths were used so that analyses between the original sampling in 1996 could be compared to the 2001 sampling; no samples were taken at the 21–60 cm depth in 1996. In order to minimize disturbance to the treatment chambers, soil bulk density pits were dug outside of the chamber area. Five soil pits were dug near the treatment area and sampled at the same depths as the chambers using a coring device. Coarse fragments were negligible throughout the sandy profile. In March of 2001, we collected samples from the two dominant oaks, *Q. myrtifolia* and *Q. geminata*, for nutrient analyses. Two stems of each species were cut at ground level from each plot and divided immediately into leaf and stem subsamples. We also removed one sample of the O horizon from each experimental chamber, collecting all litter material within a randomly placed 0.053 m^2 ring. Plant and O horizon samples were then oven dried at 60°C to constant mass and ground to 40 mesh. Biomass of *Q. myrtifolia* and *Q. geminata* was estimated using regressions between biomass and stem diameter (Dijkstra et al. 2002) and diameter tallies of all oak stems within each chamber. Litterfall was collected over a period of one year in elongated, screened troughs measuring 76.2 × 5.1 cm (387 cm^2), three per chamber. For this analysis, all litterfall was combined by chamber and ground for analysis.

Vegetation, litterfall, and O horizon samples were analyzed for nutrient concentrations at A&L Western

TABLE 1. Probability values for repeated-measures ANOVA tests on the effects of treatment on vegetation, litter, and soil nutrient contents and effects of time on soil content.

Sample	C	N	P	K	Ca	Mg	S
Vegetation							
Treatment	0.04	0.08	0.11	0.06	0.05	0.11	0.14
Litter							
Treatment	0.04	0.03	0.35	0.26	0.08	0.39	0.17
Soils, 0–21 cm							
Treatment	0.39	0.30	0.05	0.75	0.60	0.57	0.96
Time	<0.01	<0.01	0.06	0.33	0.13	0.07	0.01
Treatment × time	0.87	0.47	0.74	0.42	0.33	0.08	0.20
Soils, 0–60 cm							
Treatment	0.39	0.41	0.41	0.43	0.61	0.49	0.04
Ecosystem (0–21 cm)							
Treatment	0.80	0.35	0.33	0.09	0.04	0.14	0.97
Time	0.52	0.29	<0.01	<0.01	<0.01	<0.01	0.24
Treatment × time	0.39	0.37	0.40	0.25	0.17	0.67	0.40
Ecosystem (0–60 cm)							
Treatment	0.41	0.48	0.31	0.13	0.04	0.25	0.16

Agricultural Laboratories (Modesto, California, USA). At A&L, nitrogen analyses were performed on a Leco FP428 combustion analyzer (Leco, St. Joseph, Michigan, USA). All other elements were analyzed by inductively coupled plasma emission spectroscopy after microwave digestion (Borkowska-Burnecka et al. 2000) using a nitric acid–hydrogen peroxide digestion mixture. Vegetation nutrient content was calculated by multiplying estimated biomass by mean nutrient concentration by species and component (foliage and stem). For the nutrient content estimates for minor species (which constituted a mean of 13% of total aboveground biomass), total biomass of these species was multiplied by the biomass-weighted mean nutrient concentration of *Q. geminata* and *Q. myrtifolia* for each chamber. Biomass-weighted mean aboveground plant concentration gives an index of overall changes in ''nutrient use efficiency'' with treatment. This value is obtained as

$$\text{WTD} = [(\text{FM})(\text{FC}) + (\text{SM})(\text{SC})]/[\text{FM} + \text{SM}]$$

where WTD = weighted mean concentration, FM = foliage mass (kg/ha), FC = foliage concentration, SM = stem mass (kg/ha), SC = stem concentration. For this calculation, both species were combined.

Soils were analyzed at the Oregon State University Soil Testing Lab (Corvallis, Oregon, USA). At OSU, soil extractable P was analyzed by the bicarbonate method (2 g soil in 50 mL 0.05 mol/L $NaHCO_3$), exchangeable cations and cation exchange capacity by the ammonium acetate method (10 g soil in 50 mL 1 mol/L ammonium acetate, atomic absorption analyses for Ca^{2+}, Mg^{2+}, K^+, and Na^+, followed by soil extraction with 0.1 mol/L HCl to displace the ammonium, with automated colorimetric analysis), and total C, N, and S by PerkinElmer 2400 CHNS analyzer (PerkinElmer, Wellesley, Massachusetts, USA). Analyses of the 1996 soils for exchangeable K^+, Ca^{2+}, and Mg^{2+} were available from the NASA soil analytical laboratory. These were also extracted by 1 mol/L ammonium acetate, but using a different soil : solution ratio (i.e., 1:4, or 5 g soil in 20 mL) than that used in 2001. Perhaps because of the different soil : solution ratios, comparisons of 2001 (''new'') and 1996 (''old'') analyses revealed significant laboratory bias, where the new analyses were substantially greater than the old analyses for Ca^{2+} and Mg^{2+}: old $Ca^{2+} = 0.00 + (0.36 \times \text{new } Ca^{2+})$, $r^2 = 0.36$; old $Mg^{2+} = 0.00 + (0.38 \times \text{new } Mg^{2+})$, $r^2 = 0.70$. In the case of K^+, the 2001 analyses were also substantially greater than the 1996 analyses, and the correlation coefficient was also very poor and not statistically significant ($r^2 = 0.002$). It is not possible to know the true, absolute values for these soils, and thus we avoided the laboratory bias problem by using the 2001 analyses exclusively. No values for C, N, or S were available from the 1996 analysis data set.

In June 2000, soil N and P availability were measured using Plant Root Simulator (PRS) probes (Western Ag Innovations, Saskatoon, Canada). These probes consist of either anion or cation exchange membranes conveniently imbedded in plastic stakes for easy installation and recovery. In this case, the probes were installed for a period of two weeks before recovery. Upon recovery, the probes were sent to Western Ag Innovations for extraction. At Western Ag, each probe was first rinsed of all soil material and then extracted with 17.5 mL of 0.5 mol/L HCl for one hour. The eluent was analyzed for NO_3^- and NH_4^+ by automated colorimetric procedures, and for P, S, Ca, K, Mg, Na, Mn, Zn, Cu, Fe, and B by inductively coupled plasma emission spectroscopy.

Statistical analyses

Statistical analyses were performed using Student's *t* tests for treatment effects in Microsoft Excel for those

TABLE 2. Biomass and nutrient contents of *Quercus myrtifolia* and *Quercus geminata* in ambient and elevated CO_2 treatments. *P* values for Student's *t* tests are given.

	Q. myrtifolia		*Q. geminata*	
	Mean (±1 SE)	*P*	Mean (±1 SE)	*P*
Carbon (kg/ha)				
Ambient	1621 (326)	0.03	1250 (256)	0.39
Elevated	2984 (543)		1129 (350)	
Nitrogen (kg/ha)				
Ambient	25.5 (5.1)	0.11	20.9 (4.1)	0.27
Elevated	35.8 (6.4)		16.8 (5.0)	
Phosphorus (kg/ha)				
Ambient	3.70 (0.67)	0.04	4.73 (0.99)	0.320
Elevated	6.53 (1.30)		3.45 (1.08)	
Sulfur (kg/ha)				
Ambient	0.96 (0.18)	0.22	1.09 (0.24)	0.33
Elevated	1.19 (0.21)		0.92 (0.30)	
Potassium (kg/ha)				
Ambient	11.5 (2.4)	0.05	10.1 (1.8)	0.16
Elevated	21.4 (5.1)		9.6 (2.9)	
Calcium (kg/ha)				
Ambient	24.2 (4.5)	0.03	23.1 (5.0)	0.20
Elevated	46.1 (9.2)		17.0 (5.1)	
Magnesium (kg/ha)				
Ambient	3.93 (0.73)	0.07	3.98 (1.08)	0.19
Elevated	5.98 (1.07)		2.73 (0.86)	
Zinc (g/ha)				
Ambient	128 (21)	0.05	86 (17)	0.45
Elevated	244 (62)		83 (28)	
Manganese (g/ha)				
Ambient	141 (23)	0.02	150 (35)	0.48
Elevated	307 (64)		153 (48)	
Iron (g/ha)				
Ambient	267 (138)	0.33	108 (30)	0.33
Elevated	350 (124)		91 (25)	
Copper (g/ha)				
Ambient	18 (3)	0.02	17 (4)	0.39
Elevated	41 (8)		15 (5)	
Boron (g/ha)				
Ambient	51 (9)	0.06	50 (10)	0.20
Elevated	79 (14)		37 (11)	

ecosystem components that were not measured more than once (e.g., vegetation, O horizons). For soils, General Linear Model (GLM) in DataDesk software (Velleman 1997) was used to detect the effects of treatment and time. Within GLM, analysis of variance was used for overall treatment effects and the repeated measures option was used to detect soil changes.

RESULTS

Carbon, nitrogen, and sulfur

Elevated CO_2 caused a 138% increase in aboveground vegetation C, a 31% increase in O horizon C, and an 82% increase in total aboveground C compared to the ambient treatment (Fig. 1, Table 1). As noted previously (Dijkstra et al. 2002), most of the aboveground vegetation response was due to *Q. myrtifolia*, which comprised approximately half of aboveground vegetation C content and showed a 78% increase with elevated CO_2 (Table 2). The effects of elevated CO_2 on *Q. geminata*, which comprised approximately one third of the vegetation C content, were not statistically significant.

Nitrogen and sulfur contents in vegetation followed the same basic patterns as carbon, with some variation due to differing nutrient concentrations in vegetation components (foliage and wood) and the effects of elevated CO_2 on nutrient concentrations (Table 3). Elevated CO_2 caused reduced tissue nutrient concentrations of N and S, including the biomass weighted mean concentrations, indicating greater ''nutrient use efficiency'' for these nutrients. The differences in N concentration were more than offset by the differences in

TABLE 3. Nutrient concentrations in vegetation and litter.

Treatment	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	S (μg/g)	Zn (μg/g)	Mn (μg/g)	Fe (μg/g)	Cu (μg/g)	B (μg/g)
Foliage											
Q. geminata											
Ambient CO_2	1.13	0.20	0.49	0.15	0.70	679	24	59	48	5.3	31
Elevated CO_2	1.03*	0.17	0.51	0.12**	0.62	621	26	83	40	5.2	23***
Q. myrtifolia											
Ambient CO_2	1.24	0.09	0.46	0.14	0.58	550	34	63	58	5.1	25
Elevated CO_2	1.09**	0.09	0.46	0.12	0.67	418**	38	86*	80	5.5	24
Stems											
Q. geminata											
Ambient CO_2	0.56	0.16	0.32	0.130	1.02	229	42	58	41	6.5	10
Elevated CO_2	0.52	0.14	0.35	0.110*	0.78*	213	37	58	59	7.3	11
Q. myrtifolia											
Ambient CO_2	0.46	0.12	0.26	0.110	0.82	129	41	31	66	5.7	10
Elevated CO_2	0.39	0.11	0.28	0.090	0.77	113	38	35	35	7.1	9*
Weighted mean											
Q. geminata											
Ambient CO_2	0.79	0.18	0.39	0.14	0.85	410	33	57	45	6.3	19
Elevated CO_2	0.72**	0.15*	0.44	0.11**	0.71**	380	33	68*	52	6.4	16
Q. myrtifolia											
Ambient CO_2	0.74	0.11	0.33	0.12	0.73	284	38	48	64	5.5	15
Elevated CO_2	0.59**	0.11	0.33	0.10*	0.74	190***	38	50	49	6.6	13**
Total vegetation											
Ambient CO_2	0.76	0.14	0.36	0.13	0.77	338	35	48	57	5.8	17
Elevated CO_2	0.63**	0.12**	0.36	0.10**	0.74	261**	37	54	51	6.6	14
Litterfall											
Ambient CO_2	1.00	0.09	0.25	0.10	0.66	837	59	66	175	19	54
Elevated CO_2	1.01	0.10	0.27	0.08**	0.71	762*	57	101***	129	7	42***
Standing litter											
Ambient CO_2	0.99	0.05	0.12	0.101	0.93	850	50	73	99	4.3	17
Elevated CO_2	1.03	0.04	0.12	0.085*	1.02	763*	65*	100**	107	7.2	14*

Note: Statistically significant differences between elevated and ambient CO_2 are represented as follows: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

biomass, however, and thus uptake and accumulation of N in vegetation were greater with elevated CO_2 (Fig. 1, Table 1).

Lower foliar N concentrations under elevated CO_2 did not result in lower litterfall and O horizon N concentrations, suggesting that translocation reduced foliar N concentrations to an approximately constant value before senescence (Table 3). In contrast, litterfall and O horizon S concentrations did reflect the reduction with elevated CO_2 seen in foliage. The reduced O horizon S concentrations with elevated CO_2 offset the increases in O horizon biomass, resulting in no statistically significant treatment effect on O horizon S content. The fact that litterfall S concentrations exceeded live foliage concentrations suggests that there was little or no translocation of S (depending on how much mass loss foliage experienced during senescence).

There were no statistically significant CO_2 treatment effects on soil total C, total N, or total S at any depth or time (Fig. 2, Tables 1, 4). The PRS probes showed significantly lower NO_3^-, NH_4^+, and mineral N ($NO_3^- + NH_4^+$) with elevated CO_2 in June 2000, however (Table 5). The negative effect of elevated CO_2 on soil available N was noted previously (Johnson et al. 2001), and was hypothesized to be due to greater vegetation N uptake under elevated CO_2. The current results support this hypothesis.

Statistically significant decreases in C, N, and S concentrations in the A horizon were noted over the 5-yr sampling period (Fig. 2, Tables 1, 4). There were no statistically significant changes in soil C:N ratio, but there was a statistically significant decrease in cation exchange capacity (CEC), probably a result of a decrease in organic exchange sites. The losses of C from soils over the sampling period offset the gains in aboveground biomass and O horizon, so that there was no statistically significant change in ecosystem C contents in the ambient treatment ($P = 0.14$) or when treatments were combined (Fig. 1, Table 1). In the elevated CO_2 treatment, however, the increased aboveground C outweighed the decrease in soil C, resulting in a net increase in ecosystem C ($P = 0.05$) over time. There were no changes in ecosystem N or S content in either treatment because the soil losses offset the gains in

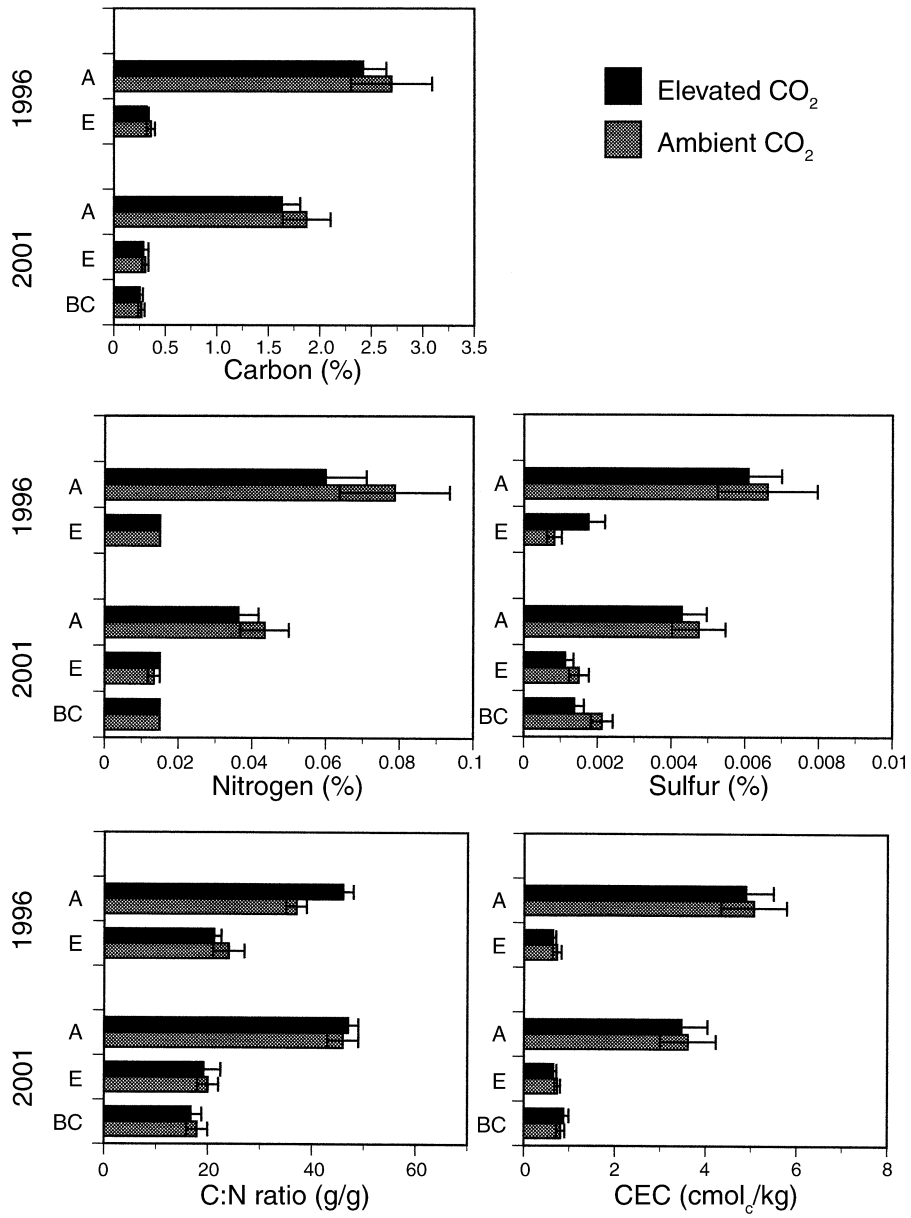

FIG. 2. Total carbon, nitrogen, and sulfur, C:N ratio, and cation exchange capacity (CEC) in soils from the study site in 1996 (before treatment) and in 2001 (five years after treatment). Note that BC horizons were not sampled in 1996. Error bars represent +1 SE.

aboveground and O horizon components (Fig. 1, Table 1).

Phosphorus, potassium, calcium, and magnesium

Elevated CO_2 caused increased vegetation P, K, and Mg contents and increased O horizon Ca content. Elevated CO_2 had no statistically significant effect on vegetation Ca content, O horizon P, K, or Mg contents (Fig. 3, Table 1). Elevated CO_2 caused significantly lower concentrations of P, Ca, and Mg in some cases, but not K. As was the case for S, the reductions in foliar Mg concentrations with elevated CO_2 were also reflected in reduced litterfall and O horizon Mg concentrations. Litterfall concentrations of K and Mg were lower than those in live foliage, indicating positive translocation and foliar leaching (especially in the case of K).

The only statistically significant treatment effect on soils was for extractable P, which was lower with elevated CO_2 in the 2001 sampling but not in the 1996 sampling (Fig. 4, Table 4). This response in soil extractable P may simply reflect a pretreatment difference (even though the initial differences in extractable P were not statistically significant) because the treatment

TABLE 4. Probability values for repeated-measures ANOVA tests on the effects of treatment and time on soil chemical properties.

Soil sample	Total C	Total N	C:N ratio	Cation exchange capacity	Total S	Extractable P	Exchangeable K^+	Exchangeable Ca^{2+}	Exchangeable Mg^{2+}	Percent base saturation
A horizons (0–9 cm)										
Treatment	0.44	0.29	0.26	0.78	0.56	0.06	0.67	0.59	0.7	0.88
Time	<0.01	<0.01	0.06	0.04	0.05	0.62	0.27	<0.01	0.01	<0.01
Treatment × time	0.95	0.49	0.05	0.98	0.98	0.25	0.91	0.11	0.03	0.25
E horizons (9–21 cm)										
Treatment	0.57	0.44	0.57	0.39	0.41	0.19	0.28	0.88	0.94	0.84
Time	0.4	0.74	0.4	0.73	0.98	0.05	0.02	0.11	0.16	0.42
Treatment × time	0.93	0.86	0.93	0.85	0.09	1.00	0.28	0.80	0.81	0.7
BC horizons (21–60 cm)										
Treatment	0.69	1.00	0.69	0.68	0.08	0.55	0.55	0.76	1.00	0.83

× time interaction term was not significant (Table 4). There were no statistically significant CO_2 treatment effects on soil exchangeable K^+, Ca^{2+}, or Mg^{2+} at any depth or time (Fig. 4, Table 4). Similarly, the PRS probes showed no treatment effects on available P, K, Ca, or Mg in June 2000 Table 5).

A horizons showed statistically significant increases in base saturation, exchangeable Ca^{2+} and Mg^2 over the 5-yr sampling period (Figs. 3–4, Table 3). The only change in the E horizons was a statistically significant decrease in exchangeable K^+ over the sampling period. There were no statistically significant changes in soil C:N ratio or extractable P over the sampling period.

There were significant increases in ecosystem P, K, Ca, and Mg contents over the sampling period (where ecosystem content was defined as including only extractable or exchangeable pools to a depth of 21 cm; Fig. 3, Table 1). These gains were due almost exclusively to increased vegetation and O horizon contents. Changes in soil contents of these nutrients were small, and in the cases of K^+ and Ca^{2+} not statistically significant, despite the previously noted increases in A horizon exchangeable Ca^{2+} and Mg^{2+}.

Zinc, manganese, iron, copper, and boron

Elevated CO_2 caused generally lower boron (B) concentrations in live tissues (statistically significant in foliage of *Q. geminata*, stem in *Q. myrtifolia*, weighted mean in *Q. myrtifolia*, and weighted mean overall; Table 3). The lower foliar concentrations apparently resulted in significantly lower B concentrations in litterfall and O horizons. O horizon B concentrations were lower than those in litterfall and foliage, suggesting that B was leached from the O horizon. Elevated CO_2 caused significantly greater concentrations of manganese (statistically significant in foliage of *Q. myrtifolia*), which apparently caused greater concentrations of Mn in both litterfall and O horizons (Table 3). Zinc concentrations in the O horizon were also significantly greater with elevated CO_2, apparently as a result of greater Zn concentrations in foliage (even though these concentrations were not statistically significant). The PRS probes showed significantly lower Zn availability with elevated CO_2, but no treatment effects on any other measured micronutrient (Table 5). Neither tissue analyses nor PRS probe data showed any treatment effects on Fe or Cu.

TABLE 5. Results of plant root simulator probe analysis of soil nutrient availability.

Nutrient	Ambient CO_2 (μ/10 cm^2)	Elevated CO_2 (μg/10 cm^2)	*P*
$NH_4^+ + NO_3^-$	7.05 (1.51)	3.73 (0.37)	0.03
NO_3^-	3.08 (1.26)	1.18 (0.18)	0.08
NH_4^+	4.08 (0.56)	3.20 (0.23)	0.08
Ca^{2+}	160 (12)	145 (7)	0.14
Mg^{2+}	89 (10)	80 (5)	0.21
K^+	131 (9)	141 (34)	0.38
Ortho-P	8.1 (4.1)	4.3 (0.8)	0.20
Fe	3.4 (0.5)	4.0 (0.8)	0.26
Mn	0.5 (0.1)	0.6 (0.1)	0.35
Cu	0.20 (0.00)	0.20 (0.00)	1.00
Zn	2.3 (0.2)	1.9 (0.1)	0.05
B	0.4 (0.1)	0.48 (0.06)	0.57
S	118 (27)	89 (15)	0.95

Note: Data are reported as means, with standard error in parentheses.

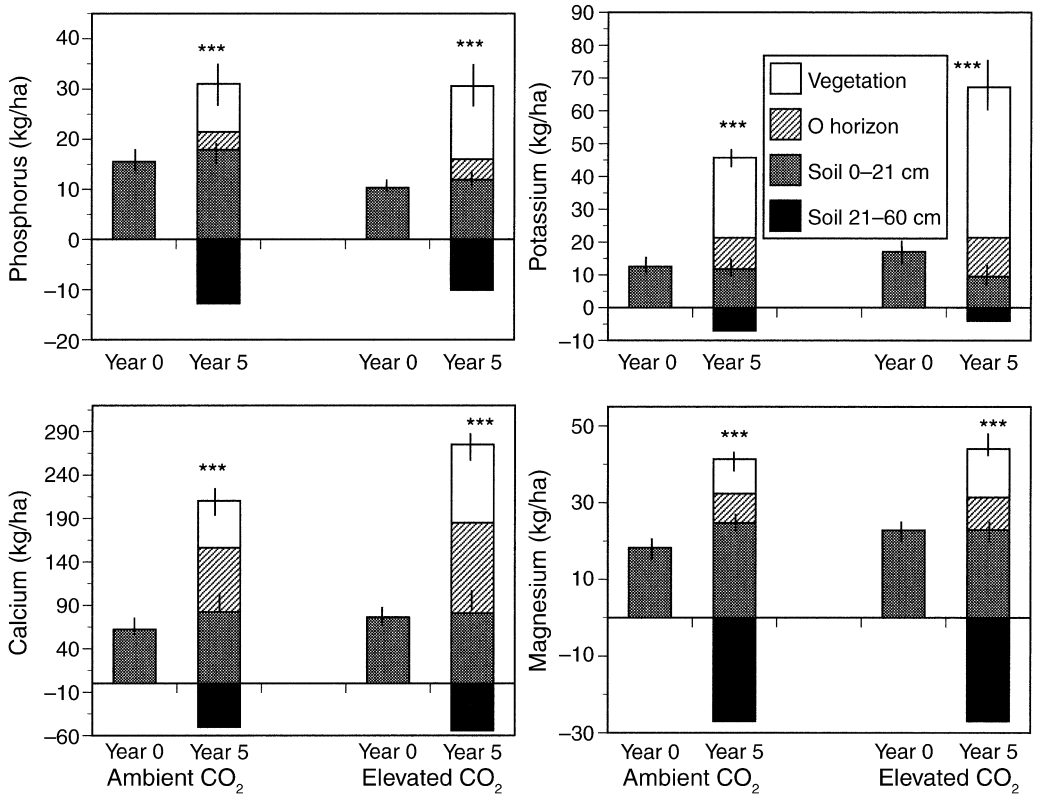

FIG. 3. Phosphorus, potassium, calcium, and magnesium contents in soil (exchangeable for K^+, Ca^{2+}, and Mg^{2+}; extractable for P), O horizon, and vegetation in the study site in 1996 (Year 0) and 2001 (Year 5). Soil contents in the 21–60 cm depth are shown as negative values so that total ecosystem contents using soils at 0–21 cm depths can be compared. (Soils at the 21–60 cm depth were not sampled in 1996). Standard errors of total ecosystem and soil pools are shown. Significant differences in ecosystem content over time are represented as follows: $***P < 0.001$ (Student's t test). See Table 1 for other statistical analyses.

DISCUSSION

These results illustrate the importance of taking soil changes into account when assessing the changes in ecosystem carbon. The prescribed fire prior to the establishment of the experiment eliminated virtually all of the vegetation and O horizon, allowing us to clearly define and measure the reaccumulation of C and nutrients in aboveground components. Ignoring soil changes would result in estimates of increases in ecosystem C capital of 10.5 Mg C/ha with elevated CO_2 and 7.4 Mg C/ha with ambient CO_2, for a difference of 3.1 Mg C/ha. When soil changes are taken into account, however, ecosystem changes were 3.7 Mg C/ha with elevated CO_2 and 0.0 Mg C/ha with ambient CO_2, for a difference of 6.2 Mg C/ha. Thus, while the effects of elevated CO_2 in each case are approximately the same, the net changes in the ecosystem C capital are very different. It is unfortunate that soils were not sampled from greater depths in 1996 so that we could have made a full accounting of soil change.

We hypothesize that the changes observed in the A horizons were a delayed response to fire. Specifically, we hypothesize that (1) soil organic matter continued to oxidize after the fire, before the ground surface was shaded and vegetation regrowth began to add new organic matter to the soil, and (2) Ca^{2+} and Mg^{2+} left in the ash after the fire leached into the A horizon, causing the observed increases there. These results contrast with those of Schmalzer and Hinkle (1987, 1996), who found little effect of fire on soils in their chronosequence studies near this site. Two factors may account for this. First, we found much lower concentrations of exchangeable base cations than Schmalzer and Hinkle did, allowing us to detect changes more readily. Secondly, our studies provide a more accurate assessment of soil change in that they were conducted in real time and not confounded by site differences, as can be the case in chronosequence studies.

The lack of any depletion of soil pools of Ca^{2+} or Mg^{2+} over time demonstrates that the vegetation did not obtain much of its Ca and Mg from the 0–21 cm depth. In the case of K^+, the decreases in exchangeable E horizon concentration were far less than the increases in vegetation and O horizon pools, so the conclusion is similar to that for Ca and Mg (i.e., uptake could not have occurred exclusively from the upper 21 cm of

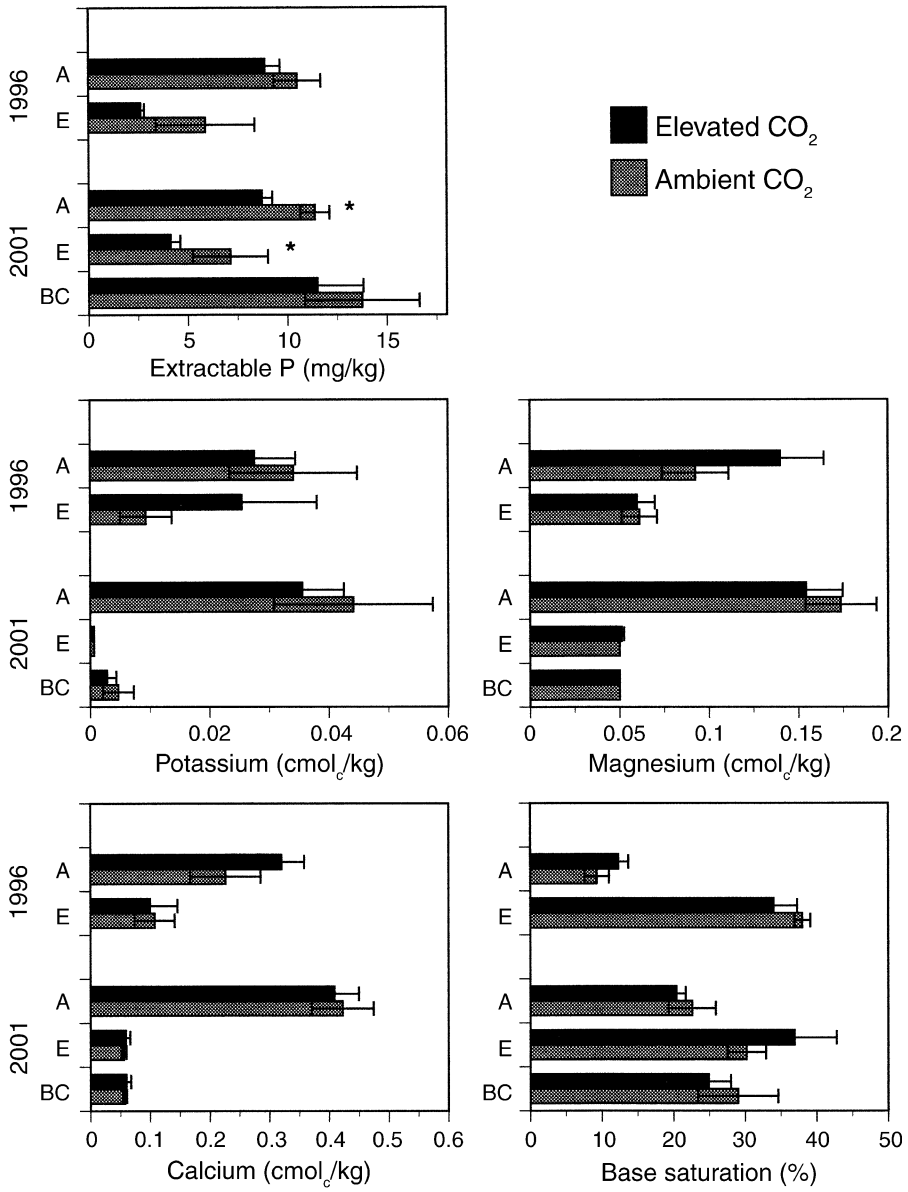

FIG. 4. Extractable P, exchangeable K^+, Ca^{2+}, and Mg^{2+}, and base saturation in soils from the study site in 1996 (before treatment) and in 2001 (five years after treatment). Note that BC horizons were not sampled in 1996. Error bars represent ± 1 SE. Statistically significant difference between elevated and ambient CO_2 are represented as follows: $*P < 0.05$ (Student's *t* test).

soil). It is not possible to determine to what extent (if any) the lower soil horizons (21–60 cm depth) were depleted of base cations over the sampling period because these horizons were not sampled in 1996. These soils are derived from beach sands and are nearly devoid of weatherable minerals, and thus the replenishment of Ca, K, and Mg by soil weathering is not likely. We hypothesize that the uptake of K and Ca, at least (and probably other nutrients as well), originated from either deeper soil horizons or from groundwater.

It is interesting to note that lower foliar N concentrations did not cause lower litterfall and O horizon N concentrations. This suggests that translocation reduced foliar N values to a relatively constant concentration (e.g., Turner 1977) rather than reducing foliar concentrations by a percentage of live foliage concentration (e.g., Nambiar and Fife 1991). Thus, there would seem to be little chance for a CO_2-induced reduction in litter decomposition due to higher C:N ratio in litterfall. These results contrast with those of Norby et al. (2001), who found translocation percentages for ambient and elevated CO_2 to be essentially identical (49% and 48%, respectively) in a meta analysis of the literature.

Summary and Conclusions

1) Elevated CO_2 stimulated growth and nutrient uptake in the scrub oak stand studied here. The increased growth and nutrient uptake were due mainly to one of the two major species, *Q. myrtifolia*, a response noted previously at this site (Dijkstra et al. 2002). Because of the greater primary productivity, there were also greater accumulations of C and nutrients in the O horizon under elevated CO_2.

2) We observed declines in C, N, S, and cation exchange capacity, increases in exchangeable Ca^{2+}, Mg^{2+}, and base saturation in the A horizon soils over the 5-yr sampling period. We hypothesize that these soil changes were a delayed response to prescribed fire applied to the site before the start of the experiment.

3) Over the 5-yr sampling period, the increases in vegetation and O horizon C, N, and S were offset by the losses of soil total C, N, and S, resulting in no statistically significant net changes through time in the ambient CO_2 treatments. In the elevated CO_2 treatments, the increases in vegetation and O horizon C outweighed the losses in soil C, resulting in a net ecosystem gain in C.

4) There were no treatment effects on any measured soil property except extractable P, which was lower with elevated CO_2 after five years. Anion and cation exchange membranes (PRS probes) showed lower available N with elevated CO_2, as in previous studies, and also lower Zn with elevated CO_2.

5) Comparisons of vegetation contents and soil pools of K, Ca, and Mg suggest that a substantial proportion of these nutrients was taken up from either groundwater or deeper soil horizons over the sampling period.

This study demonstrates that the effects of elevated CO_2 or any other perturbation on ecosystem C sequestration cannot be assessed in the absence of data on changes in soils. In this instance, the delayed response of soil C and associated nutrients to fire significantly offset the aboveground responses, and ignoring soil changes could have led to erroneous conclusions about the magnitudes of ecosystem C sequestration and the effects of elevated CO_2 on them.

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Literature Cited

Allen, A. A., J. A. Andrews, A. C. Finzi, R. Matamala, D. D. Richter, and W. A. Schlesinger. 2000. Effects of free-air CO_2 enrichment (FACE) on belowground processes in a *Pinus taeda* forest. Ecological Applications **10**:437–448.

Andrews, J. A., and W. H. Schlesinger. 2001. Soil CO_2 dynamics, acidification, and chemical weathering in a temperate forest with experimental CO_2 enrichment. Global Biogeochemical Cycles **15**:149–162.

Bernston, G. M., and F. A. Bazzaz. 1996. Belowground positive and negative feedbacks on CO_2 and growth enhancement. Plant Soil **187**:119.

Borkowska-Burnecka, J., W. Miazga, and W. Zyrnicki. 2000. Evaluation of digestion procedures for the analysis of vegetables by the ICP emission spectrometry. Analytical Chemistry **45**:429–438.

Breininger, D. R., and P. A. Schmalzer. 1990. Effects of fire and disturbance on plants and birds in a Florida oak/palmetto scrub community. American Midland Naturalist **123**: 64–74.

Brown, K. R. 1991. Carbon dioxide enrichment accelerates the decline in nutrient status and relative growth rate of *Populous tremuloides* Michx seedlings. Tree Physiology **8**: 161–173.

Cheng, W. W., and D. W. Johnson. 1998. Elevated CO_2, rhizosphere processes, and soil organic matter decomposition. Plant and Soil **202**:167–174.

Crutzen, P. J., and M. O. Andreae. 1990. Biomass burning in the tropics: impact on atmospheric chemistry and biogeochemical cycles. Science **250**:1669–1678.

Curtis, P. S., C. S. Vogel, X. Wang, K. S. Pregitzer, D. R. Zak, J. Lussenhop, M. Kubiske, and J. Teeri. 2000. Gas exchange, leaf nitrogen, and growth efficiency of *Populus tremuloides* in a CO_2-enriched atmosphere. Ecological Applications **10**:3–17.

Day, F. P., E. P. Weber, C. R. Hinkle, and B. G. Drake. 1996. Effects of elevated atmospheric CO_2 on fine root length and distribution in an oak–palmetto scrub ecosystem in central Florida. Global Change Biology **2**:143–148.

Diaz, S., J. P. Grime, J. Harris, and E. McPherson. 1993. Evidence for feedback mechanism limiting plant response to elevated carbon dioxide. Nature **365**:616–617.

Dijkstra, P., G. Hymus, D. Colavito, D. A. Vieglais, C. M. Cundari, D. P. Johnson, B. A. Hungate, R. Hinkle, and B. G. Drake. 2002. Elevated atmospheric CO_2 stimulates aboveground biomass in a fire-regenerated scrub-oak ecosystem. Global Change Biology **8**:90–103.

Dilustro, J. J., F. P. Day, and B. G. Drake. 2001. Effects of elevated CO_2 on root decomposition in a scrub oak ecosystem. Global Change Biology **7**:581–589.

Galloway, J. N., W. H. Schlesinger, H. Levy, II, A. Michaels, and J. L. Schnoor. 1995. Nitrogen fixation: anthropogenic enhancement–environmental response. Global Biogeochemical Cycles **9**:235–252.

Hungate, B. A., P. Dijkstra, D. W. Johnson, C. R. Hinkle, and B. G. Drake. 1999. Elevated CO_2 increases nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak. Global Change Biology **5**:1–9.

Hungate, B. A., E. A. Holland, R. B. Jackson, F. S. Chapin, III, H. A. Mooney, and C. B. Field. 1997. The fate of carbon in grasslands under carbon dioxide enrichment. Nature **388**:576–579.

Johnson, D. W., J. T. Ball, and R. F. Walker. 1997. Effects of CO_2 and nitrogen fertilization on vegetation and soil nutrient content in juvenile ponderosa pine. Plant Soil **190**: 29–40.

Johnson, D. W., W. Cheng, and J. T. Ball. 2000*a*. Effects of [CO_2] and nitrogen fertilization on soils planted with ponderosa pine. Plant Soil **224**:99–113.

Johnson, D. W., W. Cheng, and J. T. Ball. 2000*b*. Effects of CO_2 and N fertilization on decomposition and N immobilization in ponderosa pine litter. Plant Soil **224**:115–122.

Johnson, D. W., and P. S. Curtis. 2001. Effects of forest management on soil carbon and nitrogen storage: meta analysis. Forest Ecology and Management **140**:227–238.

Johnson, D. W., D. R. Geisinger, R. F. Walker, J. Newman, J. M. Vose, K. J. Elliot, and J. T. Ball. 1994. Soil pCO_2, soil respiration, and root activity in CO_2-fumigated and nitrogen-fertilized ponderosa pine. Plant and Soil **165**:111–121.

Johnson, D. W., B. A. Hungate, P. Dijkstra, G. Hymus, and B. Drake. 2001. Effects of elevated CO_2 on soils in a Florida scrub oak ecosystem. Journal of Environmental Quality **30**:501–507.

Johnson, D. W., R. F. Walker, and J. T. Ball. 1995. Combined effects of nitrogen and elevated CO_2 on forest soils. Water, Air and Soil Pollution **85**:1551–1556.

Körner, C., and J. A. Arnone. 1992. Responses to elevated carbon dioxide in artificial tropical ecosystems. Science **257**:1672–1675.

Luxmoore, R. J., E. G. O'Neill, J. M. Ells, and H. H. Rogers. 1986. Nutrient uptake and growth responses of Virginia pine to elevated atmospheric carbon dioxide. Journal of Environmental Quality **15**:244–250.

Mack, F., J. Hoffstadt, G. Esser, and J. G. Goldammer. 1996. Modeling the influence of vegetation fires on the global carbon cycle. Pages 149–159 *in* J. S. Levine, editor. Biomass burning and global change. MIT Press, Cambridge, Massachusetts, USA.

Mailander, J. L. 1990. Climate of the Kennedy Space Center and vicinity. NASA Technical Memorandum 103498. Kennedy Space Center, Cape Canaveral, Florida.

McGuire, A. D., J. M. Melillo, and L. A. Joyce. 1995. The role of nitrogen in the response of forest net primary production to elevated atmospheric carbon dioxide. Annual Review of Ecology and Systematics **26**:473–503.

Medlyn, B. E., R. E. McMurtrie, R. C. Dewar, and M. P. Jeffreys. 2000. Soil processes dominate the long-term response of forest net primary productivity to increased temperature and atmospheric CO_2 concentration. Canadian Journal of Forest Research **30**:873–888.

Nambiar, E. K. S., and D. N. Fife. 1991. Nutrient translocation in temperate conifers. Tree Physiology **9**:185–20.

Norby, R. J., M. F. Cotrufo, P. Ineson, E. G. O'Neill, and J. P. Canadell. 2001. Elevated CO_2, litter chemistry, and decomposition: a synthesis. Oecologia **127**:153–165.

Norby, R. J., E. G. O'Neill, W. G. Hood, and R. J. Luxmoore. 1987. Carbon allocation, root exudation, and mycorrhizal colonization of *Pinus echinata* seedlings grown under CO_2 enrichment. Tree Physiology **3**:203–210.

Norby, R. J., E. G. O'Neill, and R. J. Luxmoore. 1986. Effects of atmospheric CO_2 enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient-poor soil. Plant Physiology **82**:83–89.

Olson, J. S. 1981. Carbon balance in relation to fire regimes. Pages 327–378 *in* H. A. Mooney, T. M. Bonnicksen, N. L. Christensen, J. E. Lotan, and W. A. Reiners, editors. Fire regimes and ecosystem properties. USDA Forest Service, General Technical Report WO-26. Washington, D.C., USA.

Oren, R., D. S. Ellsworth, K. H. Johnsen, N. Phillips, B. E. Ewers, C. Maier, K. V. R. Schäfer, H. McCarthy, G. E. Hendry, S. G. McNulty, and G. G. Katu. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-rich atmosphere. Nature **411**:469–472.

Pregitzer, K. S., D. R. Zak, J. Maziasaz, J. De Forest, P. S. Curtis, and J. Lussenhop. 2000. Interactive effects of atmospheric CO_2 and soil-N availability on fine roots of *Populus tremuloides*. Ecological Applications **10**:18–33.

Rasion, R. J., P. K. Khanna, and P. V. Woods. 1985. Mechanisms of element transfer to the atmosphere during vegetation fires. Canadian Journal of Forest Research **15**:132–140.

Rice, C. W., F. O. Garcia, C. O. Hampton, and C. E. Owensby. 1995. Soil microbial response in tall grass prairie to elevated CO_2. Plant Soil **165**:67–74.

Rogers, H. H., C. M. Peterson, J. N. McCrimmon, and J. D. Cure. 1992. Response of plant roots to elevated atmospheric carbon dioxide. Plant, Cell, and Environment **15**: 749–752.

Schmaltzer, P. A., and C. R. Hinkle. 1987. Effects of fire on composition, biomass and nutrients in oak scrub vegetation on John F. Kennedy Space Center, Florida. Bionics Corporation, NASA Technical Memorandum 100305. Kennedy Space Center, Cape Canaveral, Florida.

Schmaltzer, P. A., and C. R. Hinkle. 1996. Biomass and nutrients in aboveground vegetation and soils of Florida oak–saw palmetto scrub. Castanea **61**:168–193.

Shortemeyer, M., P. Dijkstra, D. W. Johnson, and B. G. Drake. 2000. Effects of elevated atmospheric CO_2 on C and N pools and rhizosphere processes in a Florida scrub oak community. Global Change Biology **6**:383–391.

Tingey, D. T., M. G. Johnson, D. R. Phillips, D. W. Johnson, and J. T. Ball. 1996. Effects of elevated CO_2 and nitrogen on the synchrony of shoot and root growth in ponderosa pine. Tree Physiology **16**:905–914.

Torbert, H. A., S. A. Prior, H. H. Rogers, W. H. Schlesinger, G. L. Mullins, and G. B. Runion. 1996. Elevated atmospheric carbon dioxide in agroecosystems affects groundwater quality. Journal of Environmental Quality **25**:720–726.

Turner, J. 1977. Effect of nitrogen availability on nitrogen cycling in a Douglas-fir stand. Forest Science **23**:307–316.

Velleman, P. F. 1997. DataDesk, Version 6.0, Statistics guide. Data Descriptions, Ithaca, New York, USA.

Vose, J. M., K. J. Elliot, D. W. Johnson, F. R. Walker, M. G. Johnson, and D. T. Tingey. 1995. Effects of elevated CO_2 and N fertilization on soil respiration from ponderosa pine (*Pinus ponderosa* Doug. ex Laws). Canadian Journal of Forest Research **25**:1243–1251.

Zak, D. R., K. S. Pregitzer, P. S. Curtis, J. A. Teeri, R. Fogel, and D. L. Randlett. 1993. Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant Soil **151**:105–117.