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Ponderosa pine responses to elevated CO_2 and nitrogen fertilization

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Abstract. The effects of elevated CO_2 (ambient, +175, and +350 $\mu l\ l^{-1}$) and nitrogen fertilization (0, 100, and 200 kg N $ha^{-1}\ yr^{-1}$ as ammonium sulfate) on C and N accumulations in biomass and soils planted with ponderosa pine (*Pinus ponderosa* Laws) over a 6-year study period are reported. Both nitrogen fertilization and elevated CO_2 caused increases in C and N contents of vegetation over the study period. The pattern of responses varied over time. Responses to CO_2 decreased in the +175 $\mu l\ l^{-1}$ and increased in the +350 $\mu l\ l^{-1}$ after the first year, whereas responses to N decreased after the first year and became non-significant by year six. Foliar N concentrations were lower and tree C:N ratios were higher with elevated CO_2 in the early years, but this was offset by the increases in biomass, resulting in substantial increases in N uptake with elevated CO_2. Nitrogen budget estimates showed that the major source of the N for unfertilized trees, with or without elevated CO_2, was likely the soil organic N pool. There were no effects of elevated CO_2 on soil C, but a significant decrease in soil N and an increase in soil C:N ratio in year six. Nitrogen fertilization had no significant effect on tree C:N ratios, foliar N concentrations, soil C content, soil N content, or soil C:N ratios. There were no significant interactions between CO_2 and N treatments, indicating that N fertilization had no effect on responses to CO_2 and that CO_2 treatments had no effect on responses to N fertilization. These results illustrate the importance of long-term studies involving more than one level of treatment to assess the effects of elevated CO_2.

Introduction

Many field studies to date have found that both tree growth and N uptake are stimulated by elevated CO_2 (e.g., Johnson et al. 1997, 2003, 2004; Oren et al. 2001; Finzi et al. 2002, 2004). The reduced tissue N concentrations that often occur with elevated CO_2 mitigate the increased N demands to some extent, but this is usually outweighed by the increases in biomass, resulting in substantial increases in N uptake even under conditions of N limitation. Two questions then arise: (1) how did the trees manage to obtain this ‘extra’ N in an N-limited environment? and (2) how long can such increases in N uptake be sustained? This has led to speculation that progressive nitrogen limitation (PNL) will ultimately truncate growth responses to elevated CO_2 over the long term

(Luo et al. 2004). These questions are especially pertinent to young developing forests, where N demand is at a very high level because of the necessity of building N-rich tissues such as foliage and fine roots (Switzer and Nelson 1972; Turner 1981).

In this paper, we explore these questions with a data set from an experiment where ponderosa (*Pinus ponderosa* L.) was grown from seed in an open top chamber study under three levels of CO_2 (ambient, +175, and +350 μl l^{-1}) and three levels of nitrogen (unfertilized, 100 and 200 kg ha^{-1} yr^{-1} of N). Previous papers on this study have described the effects of these treatments on roots (Tingey et al. 1995, 1996; Walker et al. 1997), vegetation over the first three years (Johnson et al. 1997) and on soil nutrient concentrations over 6 years (Johnson et al. 2000a, b). Third-year vegetation results showed positive growth responses to elevated CO_2 and N fertilization, and that N uptake increased with elevated CO_2 (Johnson et al. 1997). Soil analyses after 6 years showed that N concentrations had decreased and soil C:N ratio increased with elevated CO_2 in the unfertilized treatments, leading to speculation that elevated CO_2 had facilitated 'mining' of soil N by trees (Johnson et al. 2000a). However, at that time we had no data on the changes in tree N content after 6 years with which to compare to the observed changes in soil N content. In this paper, we present results of the final tree harvest after 6 years of growth, assess the C and N contents of the system as it has changed over the years in response to the treatments, address the two questions posed above, and revisit the soil N mining question.

Site and methods

The site is located at the Institute of Forest Genetics near the town of Placerville, California (840 m elevation; 38°44′ N; 120°45′ W). The soil is Aiken clay loam, a Xeric Haplohumult derived from andesite. During February through April 1991, 24 hexagonal open-top chambers (3.6 m in diameter) were established on the site. The original experimental design consisted of three levels of N representative of common fertilizer application rates (0, 100, and 200 kg ha^{-1} yr^{-1} of N as ammonium sulfate, applied in early spring), and four CO_2 treatments representative of projections for atmospheric levels in the 21st century (ambient, no chamber; ambient, chambered; +175 μl l^{-1} CO_2; and +350 μl l^{-1} CO_2). The 100 kg ha^{-1} yr^{-1} N, +175 μl l^{-1} CO_2 treatment was excluded because of cost limitations. 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.

Ponderosa pine (*Pinus ponderosa* L.) was selected for this study because of its widespread distribution in North America and because it is native to the site. Seedlings were grown from seed (21 planting locations per chamber) and water was delivered to each plot via a timed stand pipe to a looped 2.54 cm diameter manifold and low pressure spray heads.

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. See Walker et al. (1997) for details of the root harvesting methods and results for years 1–3. The final harvest took place in the autumn of 1996. All trees were inventoried for diameter at both 15 cm and diameter at breast height (dbh, 137 cm), and total height just prior to harvest. After that, all trees were cut at the base and weighed for total mass (wet) in the field, later corrected for moisture content obtained from subsamples. Three trees per chamber were cut into components (stem, branched, new and old foliage, candles) which were then dried and weighed separately. After the tops were harvested, fine roots were estimated by coring (10 cores per chamber, 8.6 cm diameter core, at depths of 0–15, 15–30, and 30–45 cm). Roots from these cores were washed out from the cores in the laboratory, sorted by size class (<1 cm, 1–2 cm, >2 cm), dried and weighed. After soil and root samples were taken, taproots were removed by screwing an eyebolt into the top of each stump, lifting with a chain attached to a backhoe blade, and cutting of lateral roots as the taproot was removed.

Biomass estimates for trees were obtained from regressions of the type $\ln(\mathrm{Comp}) = a + b\ln(d^2h)$, where Comp = the tree component in question (foliage, branches, stems, taproots), 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 (Johnson et al. 1997). Aboveground and taproot biomass in 1996 was estimated by linear regression, which was found to be more accurate than log regressions for that data set.

Soils were sampled by horizon (Ap, 0–18 cm and Bw, 18–30 cm) in March 1991 (before treatment), March 1993 (before harvest), and September 1996 (before harvest) by punch auger and analyzed for total C and N on a Perkin-Elmer CHN Analyzer at the Desert Research Institute (DRI) (Johnson et al. 2000a).

Statistical analyses for treatment effects were performed using DataDesk software (Vellman 1997) using ANOVA tests on log-transformed data for the effects of CO_2 and N fertilization at each harvest. Differences among individual treatments in any one harvest were analyzed using Bonferroni post-hoc tests. The log-transformed data were also subjected to repeated measures analysis using DataDesk software.

Results

Tree C and N contents are shown in Figure 1 and tree C:N ratios are shown in Figure 2. Statistical analyses using repeated measures analysis (on log-transformed data) are shown in Table 1. ANOVA probabilities for the effects of CO_2 and N treatments in each sampling year are shown in the boxes in Figures 1 and 2. Bonfernni post-hoc tests for significant differences between individual treatments for tree C are indicated by small letters and for tree N are indicated by capital letters. Treatments sharing the same letters are significantly different at the $p < 0.05$ level.

Both CO_2 and N treatments had significant, positive effects on tree C contents over the duration of the study, according to repeated measures analysis (Table 1). The effects of CO_2 and N were only additive, however, as the $CO_2 \times N$ interaction term was not significant in any case. The nature of the responses to CO_2 and N treatments changed over time. CO_2 treatment effects on tree C content were not quite significant ($p = 0.11$) in year 1, and highly significant ($p < 0.01$) in years 3 and 6 (Figure 1). On the other hand, N treatment effects were highly significant in years 1 and 3 but not quite significant ($p = 0.13$) in year 6 (Figure 1). These changes in responses to treatments over time are reflected in the interaction terms: marginally significant for $CO_2 \times$ year ($p = 0.1000$) and highly significant for N × year ($p = 0.0030$) (Table 1).

The previously-noted shift in response to CO_2 from greater growth in the $+175\ \mu l\ l^{-1}$ CO_2 treatment in year 1 to greater growth in the $+350\ \mu l\ l^{-1}$ CO_2 treatment in year 3 (Johnson et al. 1997) held true into year 6. Figure 3 illustrates this shift in growth response by presenting the percentage growth responses to elevated CO_2 using the average of the ambient CO_2 values within each N category as a baseline. By year 6, tree C content in the $+175\ \mu l\ l^{-1}$ CO_2 treatment was either not significantly different from (in the unfertilized treatment) or actually significantly less than (in the 200 kg N ha^{-1} yr^{-1} treatment) that in the ambient CO_2 treatment (Figure 1). There was no major change in the pattern of responses to N but rather an overall diminished response over time.

Tree N contents followed the patterns of tree C contents closely, with only some minor deviations because of slightly higher C:N ratios with elevated CO_2 (Figures 1–3). As was the case with tree C content, CO_2 effects on tree N content were not significant in year 1 but were significant in years 3 and 6, whereas N treatment effects on tree N content were significant in years 1 and 3 but not in year 6 (Figure 1). Repeated measures analysis showed that CO_2 treatment effects on tree N content were not quite significant ($p = 0.1072$) overall but N treatment effects were significant (Table 1). Both the $CO_2 \times$ year and N × year interaction terms were significant for tree N content, indicating significant changes in the responses to these treatments over time, as was the case for C. The $CO_2 \times N$ interaction term was not significant for tree N content in the repeated measures analysis or in years 1 and 3, but was significant ($p = 0.06$) in year 6 (Figure 1).

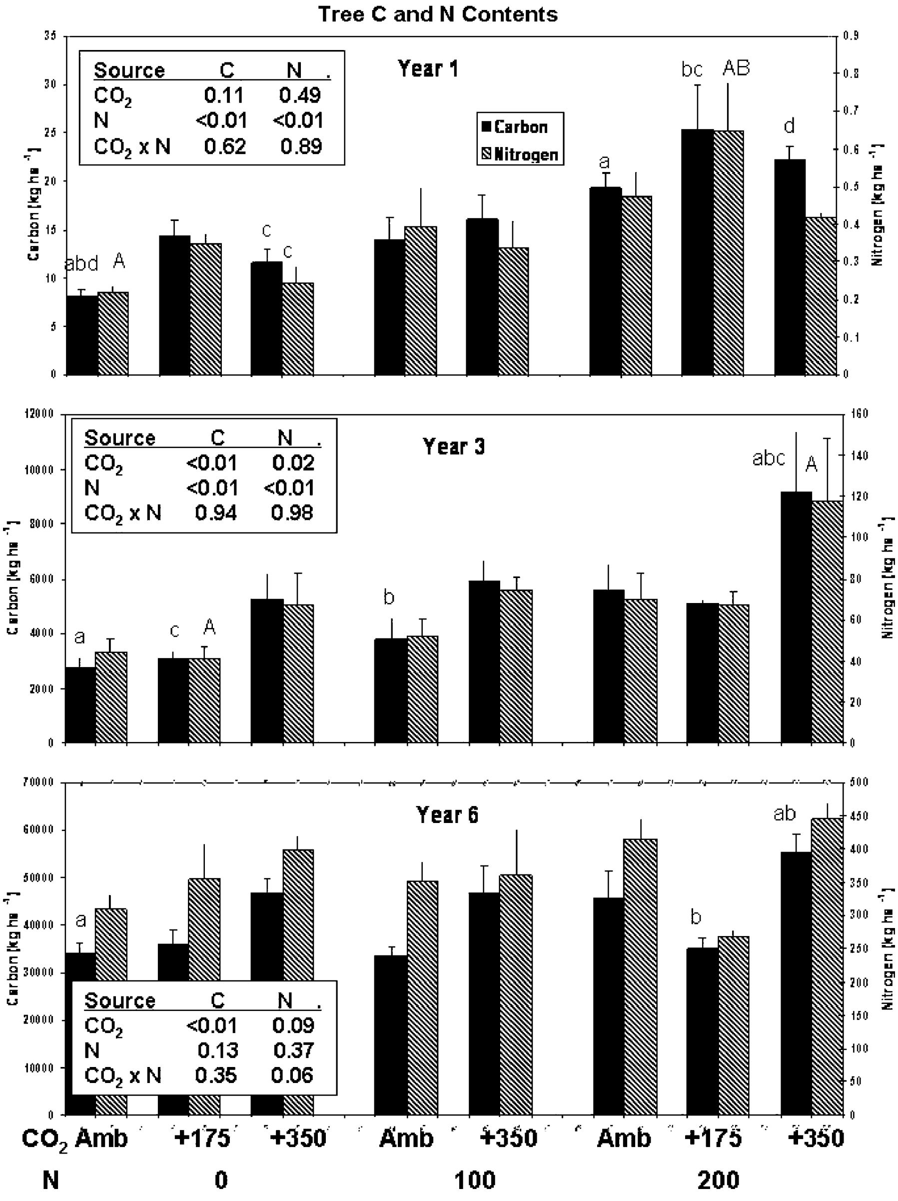

Figure 1. Tree carbon and nitrogen contents in ponderosa pine subjected to three levels of elevated CO_2 (ambient, +175 and +350 $\mu l\ l^{-1}$) and three levels of nitrogen fertilization (0, 100, and 200 kg N ha^{-1} yr^{-1} as ammonium sulfate) in years 1, 3, and 6. ANOVA probabilities for the effects of CO_2 and N treatments in each sampling year are shown in the boxes. Bonfernni post-hoc tests for significant differences between individual treatments for tree C are indicated by small letters and for tree N are indicated by capital letters. Treatments sharing the same letters are significantly different at the $p < 0.05$ level. Standard errors are shown.

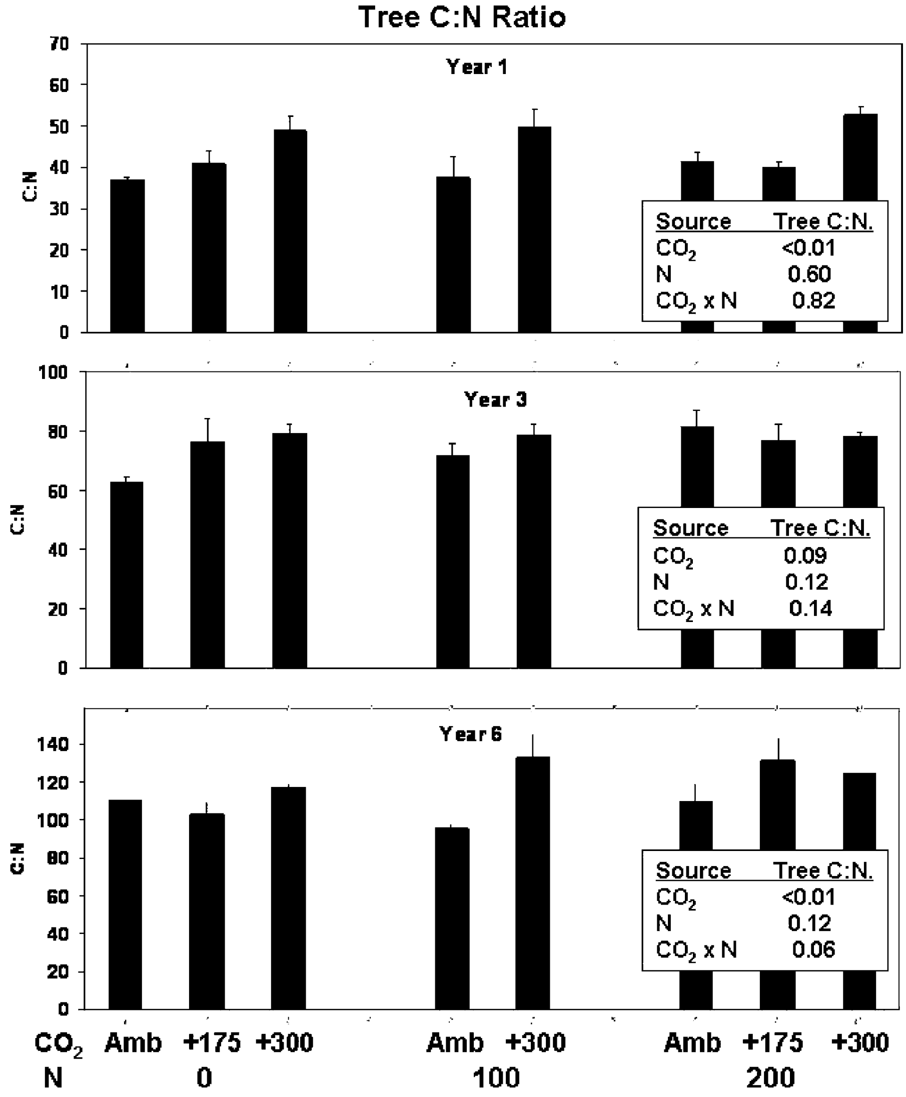

Figure 2. Tree C:N ratios in ponderosa pine subjected to three levels of elevated CO_2 (ambient, +175 and +350 $\mu l\ l^{-1}$) and three levels of nitrogen fertilization (0, 100, and 200 kg N ha^{-1} yr^{-1} as ammonium sulfate) in years 1, 3, and 6. ANOVA probabilities for the effects of CO_2 and N treatments in each sampling year are shown in the boxes. Standard errors are shown.

CO_2 treatment had significant, positive effects on tree C:N ratio by repeated measures analysis (Table 1) and in each sampling year (Figure 2). The $CO_2 \times$year term was significant (Table 1), indicating a change in the pattern of response over time. Nitrogen treatment effects on tree C:N ratio were not quite significant by repeated measures analysis ($p = 0.1087$) nor were they

Table 1. Repeated measures analysis for tree carbon, nitrogen and C:N ratio.

Source	df	SS	MS	*F*-ratio	Prob
Carbon					
Constant	1	715.549	715.549	57993	<0.0001
Chamber	16	0.197417	0.012339	1.6152	0.1210
CO_2	2	0.287704	0.143852	11.659	<0.0001
N	2	0.573756	0.286878	23.251	<0.0001
YR	2	33.309	16.6545	2180.2	<0.0001
CO_2*N	3	0.030668	0.010223	0.8285	0.4970
CO_2*YR	4	0.065031	0.016258	2.1283	0.1000
N*YR	4	0.149018	0.037255	4.877	0.0030
CO_2*N*YR	6	0.006205	0.001034	0.13537	0.9900
Error	32	0.244444	0.007639		
Total	71	152.142			
Nitrogen					
Constant	1	121.094	121.094	9022	<0.0001
Chamber	16	0.214753	0.013422	1.0957	0.3981
CO_2	2	0.069152	0.034576	2.5761	0.1072
N	2	0.402806	0.201403	15.005	0.0002
YR	2	25.2144	12.6072	1029.2	<0.0001
CO_2*N	3	0.030732	0.010244	0.76321	0.5311
CO_2*YR	4	0.152233	0.038058	3.1069	0.0287
N*YR	4	0.174803	0.043701	3.5675	0.0163
CO_2*N*YR	6	0.033695	0.005616	0.45845	0.8336
Error	32	0.391987	0.01225		
Total	71	118.402			
C:N ratio					
Constant	1	247.921	247.921	69280	<0.0001
Chamber	16	0.057256	0.003579	2.0472	0.0414
CO_2	2	0.082889	0.041445	11.581	0.0008
N	2	0.018303	0.009151	2.5573	0.1087
YR	2	0.57558	0.28779	164.64	<0.0001
CO_2*N	3	0.00658	0.002193	0.6129	0.6165
CO_2*YR	4	0.019442	0.004861	2.7806	0.0433
N*YR	4	0.004527	0.001132	0.64745	0.6328
CO_2*N*YR	6	0.02776	0.004627	2.6468	0.0337
Error	32	0.055937	0.001748		
Total	71	2.46561			

significant in any sampling year (Table 1 and Figure 2). The $CO_2 \times N$ term was not significant by repeated measures analysis Table 1) or in years 1 or 3, but was significant in year 6 (Table 1 and Figure 2). Thus, the $CO_2 \times N \times$year term was significant for tree C:N ratio Table 1).

Year and $CO_2 \times$year were significant for tree C:N ratio because of the increasing C:N ratio of trees over time, as foliar N levels declined and low-N concentration woody tissues became an increasingly important component of total biomass (Figure 2 and Table 1). Indeed, part of the effect of elevated CO_2 on tree C:N ratio was simply the greater size and therefore greater proportion

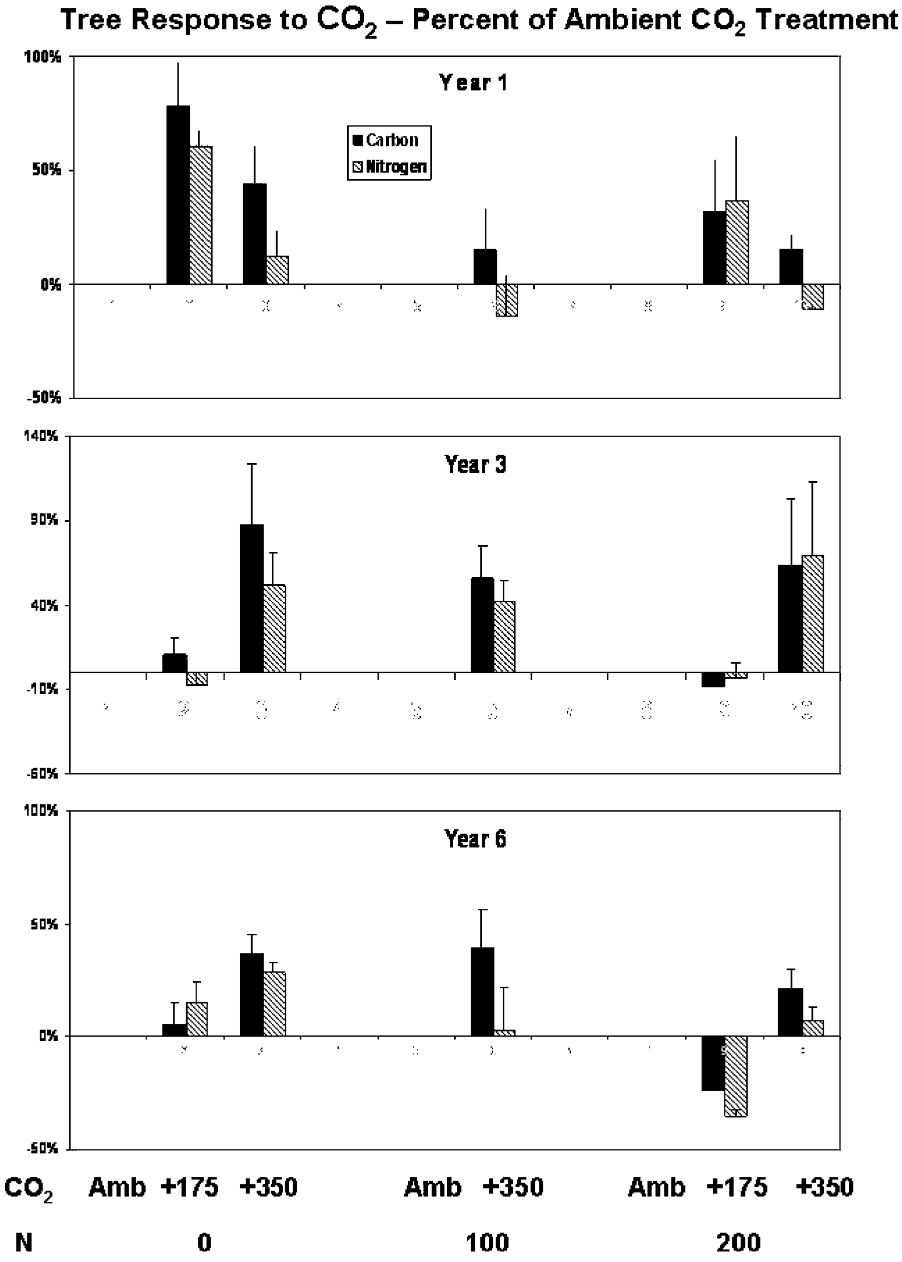

Figure 3. Tree biomass response to CO_2 at +175 and 350 $\mu l\ l^{-1}$ and levels compared to the average of ambient tree biomass at each level of nitrogen fertilization (0, 100, and 200 kg N ha^{-1} yr^{-1} as ammonium sulfate) in years 1, 3, and 6. Standard errors are shown.

of woody tissues in trees subjected to elevated CO_2. However, there was also a significant change in foliar N concentrations with treatment over the 6-year experiment (Table 2). During the first two years, elevated CO_2 caused a significant decrease in foliar N concentrations, as noted previously (Johnson et al. 1997). By year 3, however, foliar N concentrations had declined substantially and the CO_2 effect on foliar N concentration was not significant. The year 6 data now added in this paper shows that the CO_2 effect on foliar N remained non-significant (Table 2). Repeated measures analysis for foliar N concentrations showed that the CO_2 was significant overall ($p = 0.0013$) and that the $CO_2 \times$year term was significant, indicating a change in the response to CO_2 treatment over time (Table 3). Interestingly, there was no significant effect of N fertilization on foliar N concentrations at any time.

Ecosystem C and N contents over the sampling period are shown in Figures 4 and 5, respectively, and associated statistical analyses are given in Tables 4 and 5. Treatment effects on soil C and N concentrations and C:N

Table 2. Nitrogen concentrations (percent) in new foliage (means and standard errors are given).

N treat (kg ha^{-1} yr^{-1})	CO_2 treat (μl l^{-1})	Year 1	Year 2	Year 3
0	Amb	1.72 ± 0.16^{a}	1.21 ± 0.05	1.14 ± 0.06
0	+175	1.56 ± 0.11	1.27 ± 0.06	1.33 ± 0.10
0	+350	1.30 ± 0.11^{b}	1.07 ± 0.15	1.25 ± 0.08
100	Amb	1.74 ± 0.11b^{c}	1.20 ± 0.06	1.64 ± 0.06
100	+350	1.17 ± 0.10^{c}	1.24 ± 0.04	1.18 ± 0.18
200	Amb	1.60 ± 0.13	1.27 ± 0.05	1.33 ± 0.14
200	+175	1.55 ± 0.06	1.43 ± 0.06	1.13 ± 0.15
200	+350	1.19 ± 0.04^{a}	1.19 ± 0.06	1.20 ± 0.10
ANOVA probabilities				
CO_2		< 0.01	0.18	0.18
N		0.69	0.29	0.29
$CO_2 \times N$		0.96	0.57	0.57

Values sharing the same letters are significantly different ($p < 0.05$), Bonferonni post-hoc tests.

Table 3. Repeated measures analysis for foliar N concentrations.

Source	df	SS	MS	*F*-ratio	Prob
Carbon					
Constant	1	1.02139	1.02139	307.64	<0.0001
Chamber	16	0.053121	0.00332	0.99334	0.4868
CO_2	2	0.069059	0.034529	10.4	0.0013
N	2	9.03E−04	4.51E−04	0.13595	0.8739
YR	3	0.009186	0.003062	0.92225	0.4525
CO_2*N	2	0.079426	0.039713	11.882	0.0001
CO_2*YR	4	0.056064	0.014016	4.1935	0.0077
N*YR	4	0.005599	0.0014	0.4188	0.7938
CO_2*N*YR	6	0.030833	0.005139	1.5375	0.1979
Error	32	0.106955	0.003342		
Total	71	0.431395			

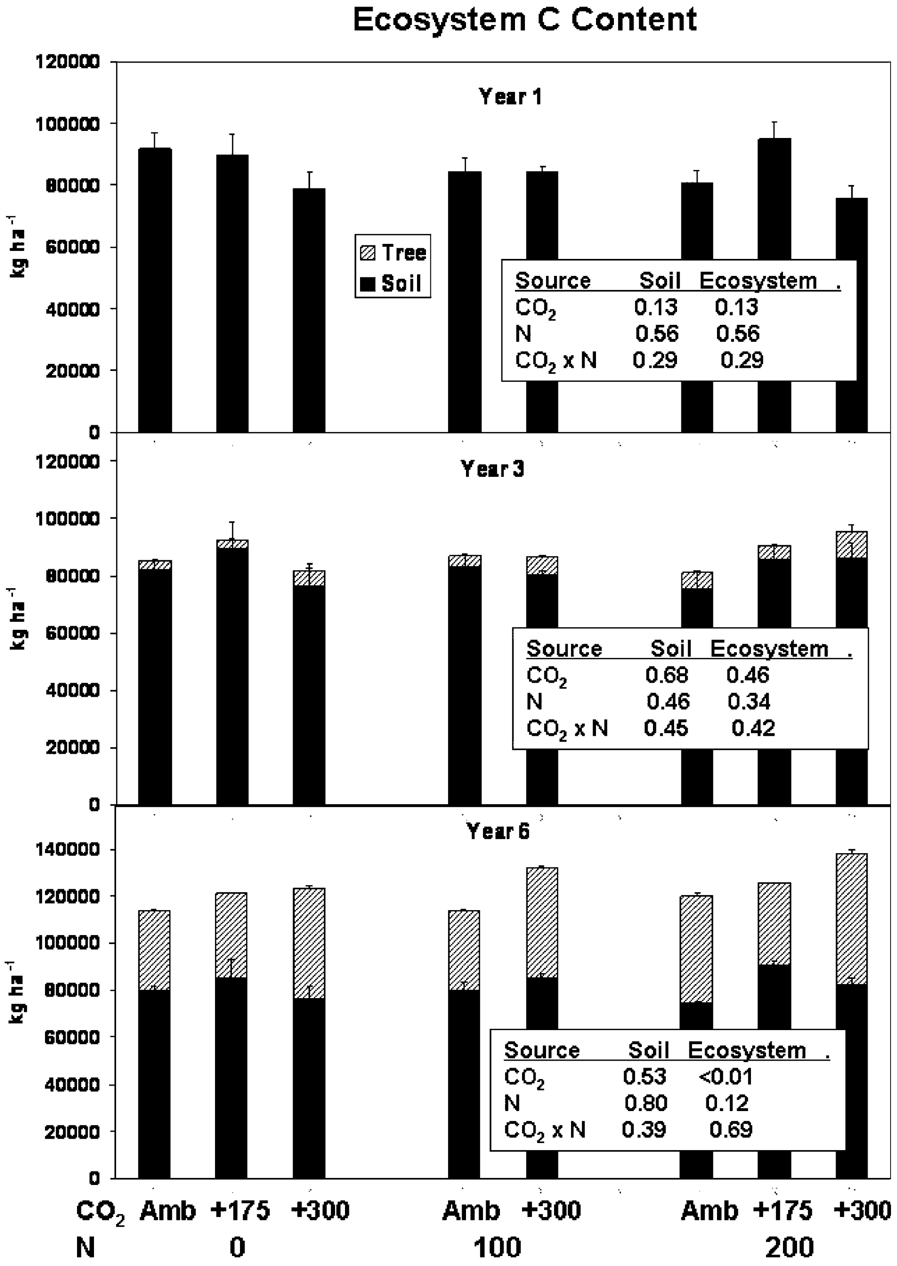

Figure 4. Ecosystem carbon content in ponderosa pine subjected to three levels of elevated CO_2 (ambient, 175 and 350 μl l^{-1}) and three levels of nitrogen fertilization (0, 100, and 200 kg N ha^{-1} yr^{-1} as ammonium sulfate) in years 1, 2, 3, and 6. ANOVA probabilities for the effects of CO_2 and N treatments in each sampling year are shown in the boxes. Standard errors are shown.

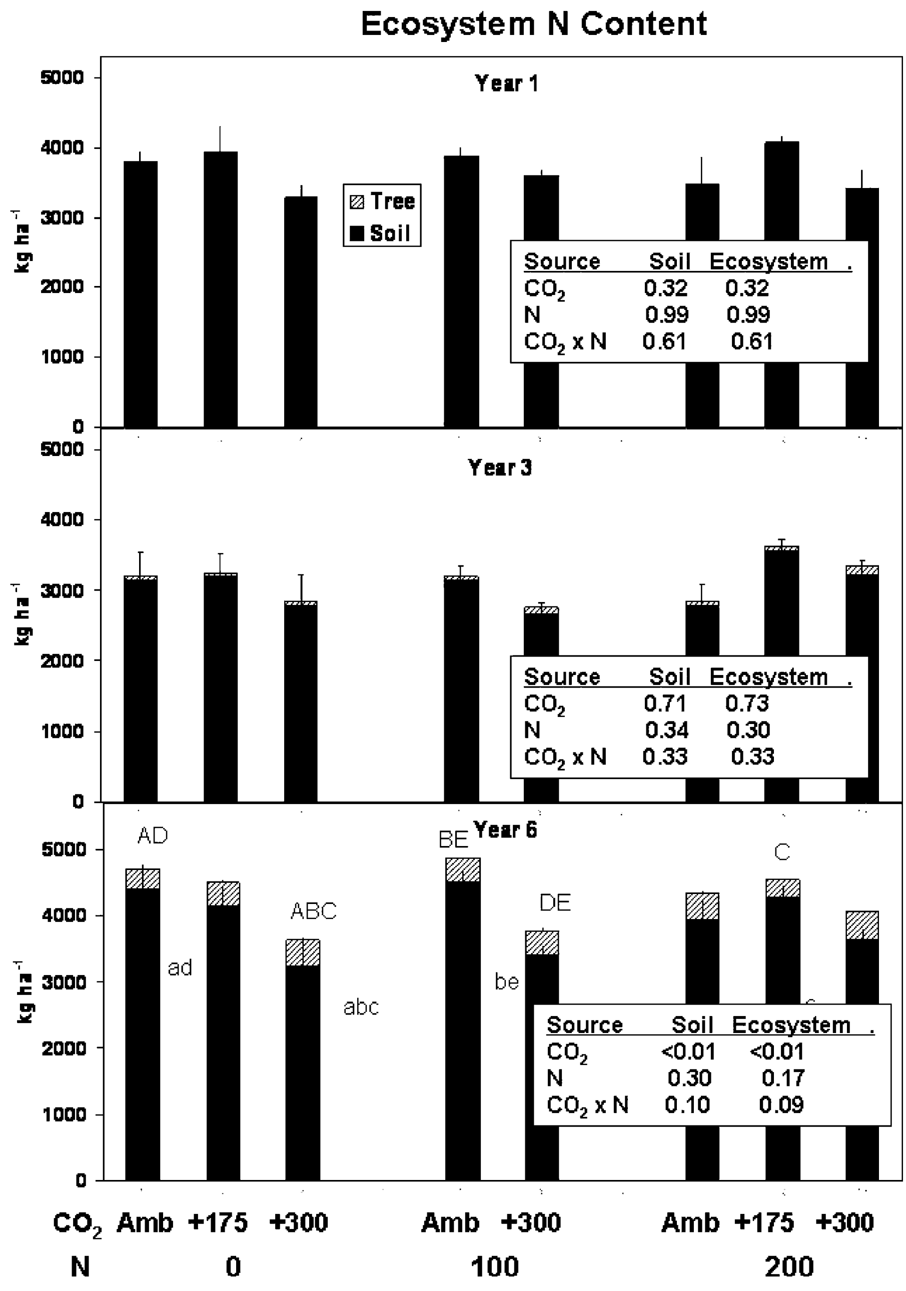

Figure 5. Ecosystem nitrogen content in ponderosa pine subjected to three levels of elevated CO_2 (ambient, +175 and +350 $\mu l\ l^{-1}$) and three levels of nitrogen fertilization (0, 100, and 200 kg N ha^{-1} yr^{-1} as ammonium sulfate) in years 1, 3, and 6. Bonfernni post-hoc tests for significant differences between individual treatments for tree C are indicated by small letters and for tree N are indicated by capital letters. Treatments sharing the same letters are significantly different at the $p < 0.05$ level. Standard errors are shown.

ratios by horizon have been described previously (Johnson et al. 2000a); the data in Figures 4 and 5 show soil contents (kg ha^{-1}) summed over both sampled horizons to a depth of 30 cm. The previous analysis of soil changes indicated that CO_2 treatments caused significant decreases in total N and significant increases in C:N ratio in both Ap and Bw horizons over the 6-year sampling period (Johnson et al. 2000a). CO_2 also caused significant increases in C concentration in the Bw horizons but not in the Ap horizons over this period. When concentrations were converted to aerial amounts (kg ha^{-1}) and horizons were combined to calculate contents for this analysis, the results

Table 4. Repeated measures analysis for soil carbon, nitrogen and C:N ratio.

Source	df	SS	MS	*F*-ratio	Prob
Carbon					
Constant	1	1740.8	1740.8	4.26E + 05	<0.0001
Chamber	16	0.065356	0.004085	4.367	<0.0001
CO_2	2	6.57E−04	3.28E−04	0.080375	0.9230
N	2	3.89E−04	1.95E−04	0.047647	0.9530
YR	2	0.00855	0.004275	4.5706	0.0170
CO_2*N	3	0.011918	0.003973	0.97258	0.4000
CO_2*YR	4	0.011843	0.002961	3.1652	0.0260
N*YR	4	0.007044	0.001761	1.8828	0.1370
CO_2*N*YR	6	0.007633	0.001272	1.36	0.2600
Error	32	0.029932	9.35E−04		
Total	71	0.15294			
Nitrogen					
Constant	1	905.102	905.102	1.50E + 05	<0.0001
Chamber	16	0.096387	0.006024	4.4608	0.0002
CO_2	2	0.034309	0.017155	2.8476	0.0875
N	2	0.008524	0.004262	0.70751	0.5077
YR	2	0.049504	0.024752	18.328	<0.0001
CO_2*N	3	0.026689	0.008896	1.4768	0.2585
CO_2*YR	4	0.017848	0.004462	3.304	0.0225
N*YR	4	0.006626	0.001656	1.2266	0.3191
CO_2*N*YR	6	0.004893	8.16E−04	0.60391	0.7251
Error	32	0.043216	0.00135		
Total	71	0.421318			
C:N ratio					
Constant	1	135.447	135.447	42389	<0.0001
Chamber	16	0.051126	0.003195	2.879	0.0053
CO_2	2	0.031935	0.015968	4.9971	0.0206
N	2	0.005891	0.002946	0.92184	0.4179
YR	2	0.026013	0.013007	11.719	0.0002
CO_2*N	3	0.009672	0.003224	1.009	0.4144
CO_2*YR	4	0.032223	0.008056	7.2582	0.0003
N*YR	4	0.002477	6.19E−04	0.55793	0.6948
CO_2*N*YR	6	0.004782	7.97E−04	0.71814	0.6378
Error	32	0.035517	0.00111		
Total	71	0.326483			

Table 5. Repeated measures analysis for ecosystem carbon, nitrogen and C:N ratio.

Source	df	SS	MS	*F*-ratio	Prob
Carbon					
Constant	1	1789.52	1789.52	4.97E + 05	<0.0001
Chamber	16	0.057581	0.003599	3.9142	<0.0001
CO_2	2	0.002264	0.001132	0.31461	0.7340
N	2	0.002372	0.001186	0.32958	0.7200
YR	2	0.09174	0.04587	49.89	<0.0001
CO_2*N	3	0.007067	0.002356	0.65452	0.5901
CO_2*YR	4	0.023478	0.005869	6.3837	<0.0001
N*YR	4	0.010439	0.00261	2.8384	0.0400
CO_2*N*YR	6	0.009835	0.001639	1.7829	0.1340
Error	32	0.029422	9.19E−04		
Total	71	0.534902			
Nitrogen					
Constant	1	913.417	913.417	1.58E + 05	<0.0001
Chamber	16	0.092785	0.005799	4.4871	0.0002
CO_2	2	0.027369	0.013685	2.3598	0.1264
N	2	0.009672	0.004836	0.8339	0.4524
YR	2	0.064295	0.032148	24.875	<0.0001
CO_2*N	3	0.024054	0.008018	1.3826	0.2841
CO_2*YR	4	0.014707	0.003677	2.845	0.0400
N*YR	4	0.007469	0.001867	1.4449	0.2420
CO_2*N*YR	6	0.005503	9.17E−04	0.70972	0.6442
Error	32	0.041356	0.001292		
Total	71	0.476773			
C:N ratio					
Constant	1	145.936	145.936	57962	<0.0001
Chamber	16	0.040285	0.002518	2.2268	0.0264
CO_2	2	0.044495	0.022247	8.836	0.0026
N	2	0.003125	0.001563	0.62061	0.5501
YR	2	0.027139	0.01357	12.001	0.0001
CO_2*N	3	0.011795	0.003932	1.5616	0.2375
CO_2*YR	4	0.045321	0.01133	10.021	<0.0001
N*YR	4	0.003904	9.76E−04	0.86327	0.4965
CO_2*N*YR	6	0.00517	8.62E−04	0.76208	0.6050
Error	32	0.036182	0.001131		
Total	71	0.325343			

differed somewhat from those reported previously. CO_2 treatment effects on soil C content were not significant by repeated measures analysis (Table 4) or in any sampling year (Figure 4). As in the previous analysis by horizon, N treatment effects on soil C were not significant in any case. CO_2 treatment effects on soil N content were significant by repeated measures analysis (Table 1). The significant $CO_2 \times$year term for soil N content indicated that the effects changed over time: the previously noted pattern of declining N concentration with elevated CO_2 in year 6 was manifested as a decline in soil N content as well (Figure 5 and Table 4). As in the previous analysis by horizon,

CO_2 treatment had a significant, positive effect on soil C:N ratio (calculated for the entire profile), according to repeated measures analysis (Table 4 and Figure 6). The $CO_2 \times$year interaction term was highly significant, indicating a

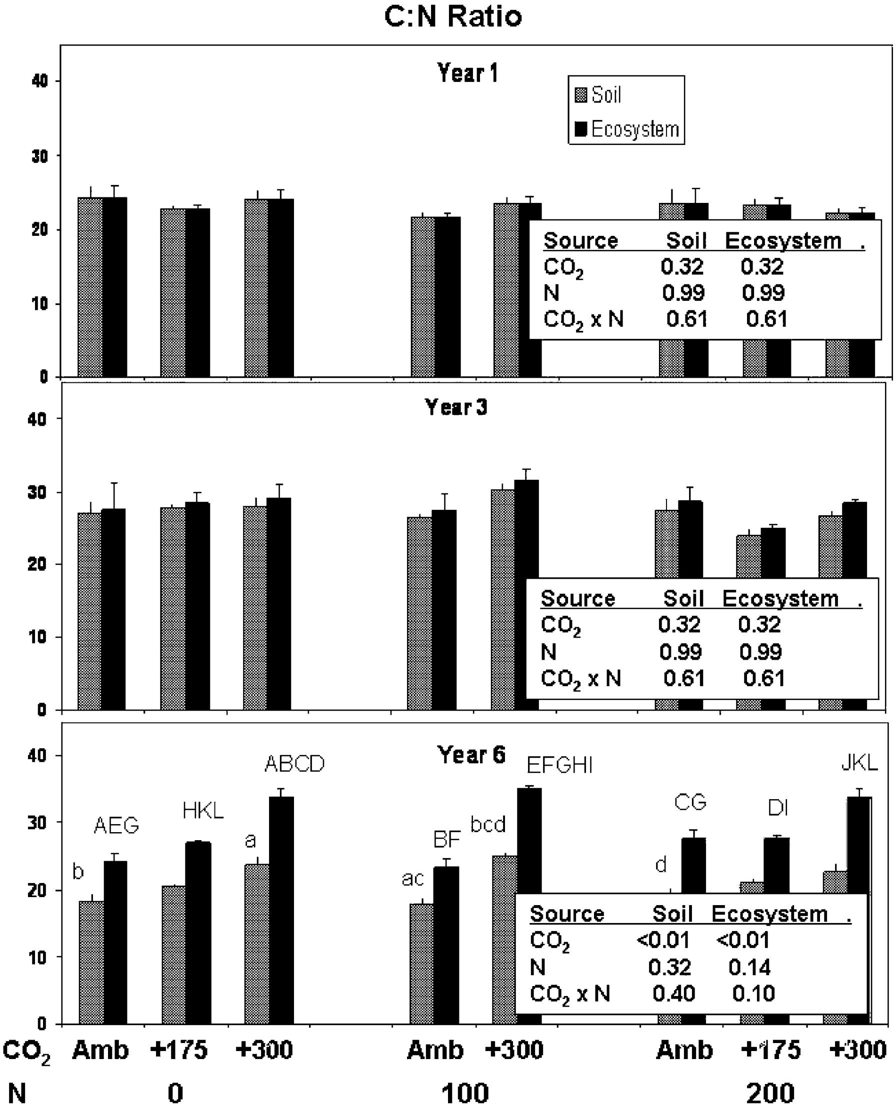

Figure 6. Soil and ecosystem C:N ratios in ponderosa pine subjected to three levels of elevated CO_2 (ambient, +175 and +350 $\mu l\ l^{-1}$) and three levels of nitrogen fertilization (0, 100, and 200 kg N ha^{-1} yr^{-1} as ammonium sulfate) in years 1, 2, 3, and 6. Bonfernni post-hoc tests for significant differences between individual treatments for tree C are indicated by small letters and for tree N are indicated by capital letters. Treatments sharing the same letters are significantly different at the $p < 0.05$ level. Standard errors are shown.

change in CO_2 treatment effects on soil C:N ratio over time. This was due to the fact that CO_2 treatment effects on soil C:N ratio were not significant in years 1 or 3 but highly significant in year 6 (Figure 6), as was the case when N concentrations were analyzed by horizon.

Ecosystem C contents were dominated by soils until year 6: tree C constituted less than 0.05% of ecosystem C in year 1, 3–14% of ecosystem C in year 3, and 24–44% of ecosystem C in year 6 (Figure 4). Thus, there were no significant CO_2 treatment effects on ecosystem C until year 6 when tree C content became a significant component (Figure 4). Repeated measures analysis showed no overall CO_2 treatment effects on ecosystem C content, but a significant $CO_2 \times$year interaction term, indicating a change in CO_2 effects over time Table 5). There were no significant N treatment effects on ecosystem C, according to repeated measures analysis or in any sampling year, but the N × year interaction term was significant (Table 5).

Ecosystem N contents were even more dominated by soils than were ecosystem C contents ($<0.02\%$ in year 1, 2–6% in year 3, and 7–13% in year 6; Figure 5). Thus, CO_2 treatment effects on ecosystem N content paralleled those on soil N content closely: not significant according to repeated measures analysis or in years 1 and 3, but significant and negative in year 6. The $CO_2 \times$year interaction term was significant (Figure 5 and Table 5). There were no significant effects of N treatment on ecosystem N content. CO_2 and N treatment effects on ecosystem C:N ratios paralleled those for soil C:N ratios exactly: an overall significant CO_2 effect and $CO_2 \times$year term according to repeated measures analysis which was driven by an increase in year 6, and no effect of N treatment (Figure 5 and Table 5).

Discussion

This study, like several others (Johnson et al. 1997, 2003, 2004; Finzi et al. 2002, 2004) showed that growth response to elevated CO_2 more than offset declines in tissue N concentrations, necessitating increased N uptake by trees. Thus, we return to the first question posed in the Introduction: how did the trees manage to obtain this ‘extra’ N in an N-limited environment? In the fertilized treatments, the source of the N for uptake is not in question: the N taken up in the elevated CO_2 treatments (270 to 450 kg ha^{-1} over 6 years) was more than supplied by the applied fertilizer (600 and 1200 kg ha^{-1}). In the unfertilized treatments, however, a substantial amount of the N taken up (ranging from 310 kg ha^{-1} in the ambient treatment to 400 kg ha^{-1} in the +350 $\mu l\ l^{-1}$ CO_2 treatment) probably came from the soil. Atmospheric deposition data are not available for the site, but wet deposition values for Davis and Yosemite deposition range from 5 to 6 kg ha^{-1} yr^{-1} for the 1991–1996 period, for a total of 30–36 kg ha^{-1} over 6 years (NADP 2004). Dry deposition might have doubled this amount. No symbiotic N fixer was present in the study plots and the results from Verburg et al. (2004) indicate minimal non-symbiotic N fixation and no stimulation by elevated CO_2 in ponderosa pine.

Thus, for the unfertilized treatments, our previous speculation that most N for uptake came from the soil in year 3 (Johnson et al. 1997) continues to be supported by the data for year 6. The additional N taken up and sequestered under elevated CO_2 over the six-year period (89 kg ha^{-1} at the +350 $\mu l\ l^{-1}$ CO_2 level) represents less than a third more than in the ambient CO_2 treatment and is well within the range of error for soil N contents (standard errors ranged from 100 to 350 kg ha^{-1}). Thus, soil N mining for this additional amount of N might well have occurred undetected.

The mechanisms by which trees might mine soil N under elevated CO_2 was not specifically addressed in this study because we did not expect it to occur; indeed, we initially hypothesized that growth response to elevated CO_2 would be facilitated mostly by increased biomass production per unit N uptake. That hypothesis proved to be false. Previous studies at the Placerville site have shown that root growth was substantially increased with elevated CO_2 (Tingey et al. 1996; Walker et al. 1997), perhaps giving an edge to tree N uptake. Some recent developments on the effects of plant roots on soil C and N dynamics suggest that plant roots can effectively compete with soil microbes for N and also cause the decomposition of soil organic matter. In their revised soil N cycling paradigm, Schimel and Bennett (2004) postulate that trees can effectively compete with soil microbes in two basic ways: (1) by taking up organic N released by the depolymerization of N-containing polymers by microbial extracellular enzymes (including those released by mycorrhizae) and (2) by invading N-rich microsites that exist even in relatively N-limited conditions. Using a ^{13}C natural tracer method, Cheng et al. (2003) demonstrated conclusively that the decomposition of native soil organic C was significantly stimulated by the presence of plant roots (*Glycine max* (L.) Merr., and *Triticum aestivum* L.). Thus, the increased root and mycorrhizal biomass observed with elevated CO_2 at this site (Tingey et al. 1996; Walker et al. 1997) may well have facilitated the release of native soil N and greater competition with microbes for soil N in N-rich microsites as per Schimel and Bennett (2004).

The second question posed in the Introduction – how long can such increases in N uptake be sustained? – is more difficult to assess. The results of this study clearly show a decline in the percentage response to elevated CO_2 over the 6-year period, but this decline occurred in both the fertilized and unfertilized treatments, and even appeared to be somewhat more pronounced in the fertilized treatments (Figure 3). In this context, the changing responses to the two CO_2 treatments are interesting and worthy of further comment. The reasons for the apparent acclimation in the +175 $\mu l\ l^{-1}$ CO_2 and the delayed response in the +350 $\mu l\ l^{-1}$ CO_2 treatment are not known; however, these results have implications for the interpretation of results from studies that include only relatively low levels of elevated CO_2. Progressive nitrogen limitation (PNL) cannot be the cause of the declining responses to +175 $\mu l\ l^{-1}$ CO_2 in the 200 kg ha^{-1} yr^{-1} N fertilizer treatment. Even in the unfertilized treatment, the continued positive growth response to the +350 μl l^{-1} CO_2 treatment into year 6 make it improbable that the growth decline at the +175 μl l^{-1} CO_2 level was due to PNL. Thus, the results of this study suggest caution in attributing PNL as a cause of changes in growth and N uptake over time in response to elevated CO_2, and highlight the need for long-term studies at differing CO_2 levels in order to fully understand probable responses and interactions with N uptake.

Simple logic dictates that trees cannot continue to mine soil N indefinitely; however, the long-term studies by Richter and Markewitz (2001) suggest that, while soil mining may not totally preclude the development of N limitations, it can go on for a substantial length of time (at least 40 years). They found that the combined N increment in biomass (366 kg ha^{-1}) and forest floor (740 kg ha^{-1}, for a total of 1106 kg ha^{-1}) was largely compensated by declines in soil N (−823 kg ha^{-1}) over 40 years of stand development in a *Pinus taeda* L. plantation in South Carolina. Thus, the mining of soil N even without elevated CO_2 is not unheard of and must have occurred in the ambient, unfertilized treatment in this study as well. The additional N uptake with elevated CO_2 in the unfertilized treatment (89 kg N ha^{-1}, the difference between tree N content in the +350 μl l^{-1} and ambient CO_2 treatments in year 6) constituted only a 29% increase over N uptake in ambient CO_2, and was actually greater than the additional N taken up in the fertilized treatments (9 and 31 kg ha^{-1} for the 100 and 200 kg ha^{-1} yr^{-1} treatments, respectively). Thus, we see no indication that responses to elevated CO_2 in the unfertilized treatments were being truncated or inhibited by PNL as of year 6 in this study.

The lack of statistical significance in the $CO_2 \times N$ interaction terms is interesting from the point of view of the possible combined effects of increased N deposition and elevated CO_2 in the future. While this study is far too limited in scope to be extrapolated over space and time, it does show that, for ponderosa pine under the conditions imposed in this study, elevated CO_2 has no effect on the responses to N fertilization, and N fertilization has no significant effect on the responses to elevated CO_2. These results contrast to those of Curtis et al. (1994), where severe N limitation curtailed responses to elevated CO_2 in *Populus grandidentata* seedlings, and response to CO_2 in was enhanced by N additions. Similarly, Oren et al. (2001) reversed a reduction in aboveground growth response to elevated CO_2 with N fertilization in the prototype FACE study at Duke.

Summary and conclusions

Both elevated CO_2 and nitrogen fertilization caused increases in growth and tree C and N sequestration in biomass over a 6-year period of exposure in ponderosa pine seedlings/saplings in this open top chamber study. The greatest response to CO_2 was initially at the +175 μl l^{-1} CO_2 level, but later a response only occurred in the +350 μl l^{-1} CO_2 level. The decline in response to the +175 μl l^{-1} CO_2 level cannot be explained by progressive N limitation because it occurred in the fertilized as well as the unfertilized treatment. Elevated CO_2 initially produced the expected decrease in foliar N concentrations and increased tree C:N ratios, but this faded over time and was more than offset by the increased growth, causing greater N sequestration in biomass than in the ambient CO_2 treatments. In the unfertilized treatment, we speculate that increased root and mycorrhizal exploration of soils with elevated CO_2 facilitated additional N uptake from the soil.

Nitrogen fertilization produced positive growth responses and increased tree C and N sequestration until year 6 when N effects were no longer significant. Response was consistently greater at the 200 kg ha^{-1} yr^{-1} level than at the 100 kg ha^{-1} yr^{-1} level. Nitrogen fertilization had no significant effect on tree C:N ratios, foliar N concentrations, soil C content, soil N content, or soil C:N ratios.

There were no significant interactions between CO_2 and N treatments on tree growth, indicating that N fertilization had no effect on responses to CO_2 and that CO_2 treatments had no effect on responses to N fertilization.

In terms of the first questions posed at the beginning of this paper – (1) how did the trees manage to obtain this 'extra' N in an N-limited environment? – we conclude that the additional N needed to respond to elevated CO_2 came from the soil and was facilitated by greater root exploration under elevated CO_2. In terms of the second question – (2) how long can such increases in N uptake be sustained? – we cannot provide an accurate prediction from the results of this study, however, we see no evidence that either growth or additional N uptake at the +350 μl l^{-1} CO_2 level are being inhibited by PNL as of year 6 in this study.

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