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NITROGEN CYCLING DURING SEVEN YEARS OF ATMOSPHERIC CO_2 ENRICHMENT IN A SCRUB OAK WOODLAND

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Abstract. Experimentally increasing atmospheric CO_2 often stimulates plant growth and ecosystem carbon (C) uptake. Biogeochemical theory predicts that these initial responses will immobilize nitrogen (N) in plant biomass and soil organic matter, causing N availability to plants to decline, and reducing the long-term CO_2-stimulation of C storage in N limited ecosystems. While many experiments have examined changes in N cycling in response to elevated CO_2, empirical tests of this theoretical prediction are scarce. During seven years of postfire recovery in a scrub oak ecosystem, elevated CO_2 initially increased plant N accumulation and plant uptake of tracer ^{15}N, peaking after four years of CO_2 enrichment. Between years four and seven, these responses to CO_2 declined. Elevated CO_2 also increased N and tracer ^{15}N accumulation in the O horizon, and reduced ^{15}N recovery in underlying mineral soil. These responses are consistent with progressive N limitation: the initial CO_2 stimulation of plant growth immobilized N in plant biomass and in the O horizon, progressively reducing N availability to plants. Litterfall production (one measure of aboveground primary productivity) increased initially in response to elevated CO_2, but the CO_2 stimulation declined during years five through seven, concurrent with the accumulation of N in the O horizon and the apparent restriction of plant N availability. Yet, at the level of aboveground plant biomass (estimated by allometry), progressive N limitation was less apparent, initially because of increased N acquisition from soil and later because of reduced N concentration in biomass as N availability declined. Over this seven-year period, elevated CO_2 caused a redistribution of N within the ecosystem, from mineral soils, to plants, to surface organic matter. In N limited ecosystems, such changes in N cycling are likely to reduce the response of plant production to elevated CO_2.

Key words: *^{15}N; nitrogen cycling; progressive nitrogen limitation; rising atmospheric CO_2.*

SPECIAL FEATURE

INTRODUCTION

Will carbon (C) uptake by land ecosystems partly counteract human CO_2 emissions over the next century, and thereby help mitigate climate change? Whether this occurs, and if so by how much, in part depends on how nutrients affect the long-term responses of ecosystems to rising atmospheric CO_2. This is a controversial topic: ''The role of nutrient limitation in modulating the CO_2 responses of NPP [net primary production] and carbon storage is one of the most widely misunderstood and poorly studied issues in ecosystem ecology. This is a topic where proponents of both positions often pass in the dark, with each group basing their analysis on concepts and conditions so far from the mind-set of the other that communication is minimal.'' (Field 1999).

At least some of the confusion arises from the differing perspectives of physiological and biogeochemical disciplines. Clear hypotheses from either will help focus and refine this debate. Luo et al. (2004) offer one, based on biogeochemical theory, the hypothesis of progressive nitrogen (N) limitation. Progressive N limitation postulates that feedbacks through the N cycle strongly shape the potential for C storage in response to elevated CO_2. The accumulation of organic N in plants and soils ultimately reduces N availability to plants and thereby constrains ecosystem C accumulation (Rastetter et al. 1992, Comins and McMurtrie 1993, Field 1999, Kicklighter et al. 1999, Luo et al. (2004).

This hypothesis integrates nutrient modulation of CO_2 responses at the levels of N cycling within plants, between plants and soils, and between ecosystems and

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the atmosphere. For example, the hypothesis accommodates the commonly observed reduction in N concentration in plant tissues in response to elevated CO_2 (Cotrufo et al. 1998). It acknowledges the possibility of CO_2-induced changes in the distribution of N between plants and soils (Zak et al. 2000). It allows for limited effects of elevated CO_2 on whole system N mass, through changes in biological N fixation and N loss (Barnard et al. 2005). Progressive N limitation predicts a sequence of responses along with a number of modulators that, individually, tend to mitigate or exacerbate the condition, but that can all operate at the same time. In short, the hypothesis is complex. In this way, a range of responses might be said to be consistent with its predictions. What then, is not consistent? What experimental results will falsify this hypothesis?

This latter question is obviously an important one to clarify in a paper presuming to offer at least a partial experimental test of this hypothesis. Our answer has three parts. First, we must clarify what constitutes insufficient evidence. Progressive N limitation requires an initial stimulation of NPP with added CO_2, and thus rejects the notion that nutrient limitation precludes any CO_2 response and acknowledges that nutrient cycles are sufficiently flexible to allow this. However, experiments that show no initial NPP response to elevated CO_2 do not falsify PNL. This initial enhancement of NPP is a stipulation, a necessary pre-condition. In ecosystems that are unresponsive to elevated CO_2 (Tissue and Oechel 1987, Oechel et al. 1994), progressive N limitation could still govern their responses to some other environmental forcing (e.g., warming) that causes an initial stimulation of NPP. Second, progressive N limitation unambiguously predicts that the initial stimulation of NPP will decline through time. Third, the causal mechanism for this declining response is a progressive reduction in the availability of N. Nitrogen availability must decline, because there is limited flexibility in the N cycle. The ecosystem is no longer able to dilute N, nor to redistribute internal N, nor to acquire additional exogenous N to support a continued NPP response. Therefore, falsifying PNL requires demonstrating a sustained NPP response to environmental forcing such as elevated CO_2, over a full cycle of plant biomass production and decay, accompanied either by a large reallocation of N (and other nutrients) within ecosystems or by accumulation of N from exogenous sources.

The current generation of ecosystem biogeochemical models represents the fundamental drivers of progressive N limitation, and results from most of these models provide its clearest support (Rastetter et al. 1992, Comins and McMurtrie 1993, VEMAP Members 1995, Kicklighter et al. 1999, Field 1999). Others models illustrate scenarios in which reduced N availability can be mitigated—in the short term, by shifts in N allocation within the system, or, in the long term, by the accumulation of N from exogenous sources (Gifford et al. 1996, Cannell and Thornley 1998). As Field (1999) noted, ''Unless elevated CO_2 is accompanied by increased N inputs, decreased N losses, or increased partitioning of N to high C:N pools within the ecosystem, decreased N availability in response to increased N storage is essentially unavoidable.''

While the models clearly show how progressive N limitation occurs, and what conditions can alleviate it, testing this hypothesis under field conditions is challenging. First, the initial stimulation of NPP by elevated CO_2 must be large enough to elicit the predicted feedbacks such that they are detectable above background variation. In many cases, this may require an NPP enhancement larger than observed in most elevated CO_2 experiments (>25%). Second, these experiments so far have rarely lasted long enough to clearly meet the temporal criterion that a sustained NPP response is maintained over a full cycle of nutrient uptake, senescence in plant tissues, and distribution to various organic N pools with the potential for subsequent re-mineralization.

We have been studying the effects of elevated CO_2 in a Florida scrub oak ecosystem over the last seven years (Hungate et al. 1999, Johnson et al. 2001, Dijkstra et al. 2002) and we believe that this experiment is a good one for testing the concept of progressive N limitation. First, the rapid cycle of fire disturbance and regrowth provides the opportunity to examine changing biogeochemical responses during an entire disturbance cycle (e.g., compared to most forests). Second, during the first four years of the experiment, elevated CO_2 dramatically increased aboveground biomass, by 40% the first year, increasing to 80% the fourth year (Dijkstra et al. 2002). Initial root responses were also dramatic: fine root length increased by 181% two years after fumigation began (Dilustro et al. [2002]; see also Day et al. [1996] for large responses during an earlier CO_2 experiment in this same ecosystem). This large initial growth response was accompanied by nutrient accumulation in plant biomass and surface soils (Johnson et al. 2003), setting the stage for progressive N limitation to occur. Here, we report changes in N cycling, using N budgeting and ^{15}N tracer approaches, during the first seven years of CO_2 enrichment in this scrub oak woodland.

Materials and Methods

Florida scrub oak is a fire-prone woodland occurring on well-drained sandy soils. Soils are acidic (average pH within the experimental plots is 4.3; A. L. Pagel, *unpublished data*) and nutrient poor (Johnson et al. 2003). They are classified as Paola sands (Spodic Quartzpsamment) and Pomello sands (Arenic Haplahumod) (Schmalzer and Hinkle 1987). The stand chosen for this study is dominated by oaks, *Quercus myrtifolia* Willd, *Q. geminata* Small, and *Q. chapmanii* Sargenti. A total of 27 other species have been described in the community (Schmalzer and Hinkle 1987), but these

three oak species accounted for 96% of the preburn aboveground plant biomass at the site chosen for this study (Dijkstra et al. 2002). The stand is located on the Merritt Island National Wildlife Refuge (28°38′ N, 80°42′ W). In 1995, plots of similar biomass and species composition were selected, characterized, and assigned randomly to CO_2 treatments. In the preburn vegetation before the experiment began, densities of oaks in the experimental plots averaged 18 stems/m^2 (Dijkstra et al. 2002). The entire stand was burned in 1996. Densities of oak stems increased markedly in the regrowing vegetation (75 stems/m^2 in January 1997), then declined steadily (51 stems/m^2 by January 2000; Dijkstra et al. 2002).

After burning the site, open-top chambers maintaining ambient or elevated atmospheric CO_2 concentrations were erected over the recovering vegetation (Drake et al. 1989), with eight chambers for each treatment. Chamber frames were octagonal, built from 4″ (~10 cm) PVC pipe, with sides 1.40 m long, in total covering 9.42 m^2. The frames were sealed by eight rectangular panels with 1″ (~2.5 cm) PVC edges and covered with Mylar (Melinex 071, Courtaulds Performance Films, Martinsville, Virginia, USA). Panels also served as doors, locked in place on the chamber frame with PVC clips. To minimize intrusion of outside wind, chambers were topped with a frustum that left an opening of 5.9 m^2, about 60% of the ground area footprint.

On 19 June 1998, 0.18 g $^{15}N/m^2$ (as aqueous 0.1 g N/L $(NH_4)_2SO_4$, 99.9 atom%^{15}N) was added to each experimental plot. This tracer addition was estimated to be sufficient to provide an ecosystem-wide enrichment of 500‰ $\delta^{15}N$, though of course reservoirs of N that vary in size and turnover will differ in $\delta^{15}N$ at any given time. Hand-held sprayers were used to apply the ^{15}N directly to the surface of the O horizon in each study plot.

We measured N and C concentration and stable isotope ratios in oak leaf and stem tissues collected once per year, 1996–2002. Tissue samples were collected between May and July. Each year, leaves of each of the three oak species were haphazardly selected throughout the canopy and composited by chamber. To minimize the more destructive sampling of stem tissues, stem segments were not sampled until 1998. For 1996 and 1997, percentage of N in tissue (%N) and percentage of C in tissue (%C) values from 1998 were used to estimate stem N and C mass. Stems of *Q. chapmanii* were collected only in 1999 and 2002. This is the rarest of the three oak species, constituting less than 10% of oak biomass, and is qualitatively similar to *Q. myrtifolia* in its response to elevated CO_2 (Dijkstra et al. 2002). For years where *Q. chapmanii* stems were not sampled, values of %N, %C, and $\delta^{15}N$ from *Q. myrtifolia* were used to estimate *Q. chapmanii* N, C, and ^{15}N mass. For *Q. myrtifolia* and *Q. geminata*, two to three stem segments (each 5–8 cm long) per chamber were collected for each species in 1998–2002. To minimize destructive sampling, terminal stems were selected. Though this could cause bias toward higher nutrient content compared to whole-stem samples, measured values of %N from stem segments we collected did not differ in %N from entire stem samples reported previously (Johnson et al. 2003). All tissue samples were dried in a forced-air drying oven at 70°C, ground, and analyzed for %N, %C, $\delta^{15}N$, and $\delta^{13}C$ by Dumas combustion (NC 2100; CE Elantech, Lakewood, New Jersey, USA) and continuous flow isotope ratio mass spectrometry (DELTA^{PLUS}-XL; Thermoelectron Corporation, Bremen, Germany) at the Colorado Plateau Stable Isotope Laboratory.

We measured stem basal diameter at ground level on all oak shoots in each plot using electronic calipers once per year (Dijkstra et al. 2002). Measurements were conducted in the winter, between November and January. Allometric relationships were used to estimate stem and leaf biomass (Dijkstra et al. 2002, Johnson et al. 2003; P. Dijkstra, *unpublished data*). Because the allometric equations were developed from biomass harvests conducted in July (Dijkstra et al. 2002), the biomass estimates reflect summer values, near the seasonal maximum of leaf area development (Hymus et al. 2002), coincident with our tissue samples for N and ^{15}N content. The N content of leaves and stems (g N/m^2 ground area) was determined as the product of biomass and N concentration. Tracer ^{15}N mass (mg ^{15}N/m^2 ground area) was determined as the product of N content and the atom percent excess ^{15}N concentration, which is the total minus the natural abundance atom percent ^{15}N.

Litterfall was collected in elongated screened troughs (0.762 long × 0.051 m wide, total area 0.039 m^2), with three troughs per chamber (Johnson et al. 2003). Litter samples were removed monthly. Senesced leaves were sorted by species and category of insect damage, enumerated, and weighed. We thus obtained total litterfall mass as well as the average mass of individual senesced leaves, by species. Monthly samples were combined for quarterly (1997–2001) or annual (2002) analyses of %N, %C, $\delta^{15}N$, and $\delta^{13}C$. Annual litterfall was calculated as the total litter mass collected per year divided by the area (m^2) of the litter collectors. We subdivided total litterfall into oak and non-oak fractions. Annual litterfall N was calculated as the product of litterfall and litter N concentration, and litterfall ^{15}N as the product of litterfall N and atom percent excess ^{15}N concentration. Litter collection began in 1997; litter accumulation during 1996, the first year after fire, was negligible.

We sampled the O horizon in each chamber in April 2001 by collecting all forest floor material within a randomly placed 0.053-m^2 ring (Johnson et al. 2003). Samples were dried, ground, and analyzed for C and N. To avoid additional destructive harvests, we estimated the N and ^{15}N content of the O horizon for April 2002 by summing the April 2001 values with the flux

of N and ^{15}N in litterfall between May 2001 and April 2002.

We calculated leaf and stem N and ^{15}N increment for each year of the study as the difference between N or ^{15}N in leaves and stems over a one-year period. Aboveground N requirement (the amount of N needed to build new aboveground tissues) was calculated as N increment plus N in litterfall. For the two most common oak species in the scrub oak ecosystem (*Q. myrtifolia* and *Q. geminata*), leaf life span is greater than one year (Stiling et al. 2002). To estimate live foliar mass of the senescing cohort for a given year, we multiplied the numbers of leaves collected in litterfall by the average mass of individual live leaves, by species. We enumerated senesced leaves from the litter collections, as described above. The average mass of individual live, green leaves was determined by destructive harvest in 1998 and in 2004. In 1998, average leaf mass was determined by collecting green leaves of *Q. myrtifolia* and *Q. geminata* from within the experimental plots (there were no significant differences in average leaf mass between elevated and ambient CO_2 treatments for either species). In September 2004, 50 leaves were collected from seven plants of each species, *Q. geminata*, *Q. myrtifolia*, and *Q. chapmanii* (total of 350 leaves per species), adjacent to the experimental plots. Leaves were dried and weighed and average leaf mass determined. Because average leaf mass did not differ significantly between the 1998 and 2004 harvests (data not shown), we used the average leaf mass across both harvests for each species for the calculations presented here. Averaging across species and years, oak leaves lost 15.9% of their live mass during senescence, so this amounted to a modest but certainly not negligible correction. We calculated retranslocation of N during leaf senescence as the difference in the mass of N contained in leaves before and after senescence, where the mass of N in leaves before senescence is the product of the mass of the senescing cohort and green leaf N concentration, and the mass of N in leaves after senescence is the flux of N in oak litterfall (litterfall mass times N concentration in litter). This approach estimates retranslocation as a flux of N (g $N \cdot m^{-2} \cdot yr^{-1}$); other methods, such as retranslocation efficiency, are prone to error because they do not account for changes in leaf mass during senescence (Norby et al. 2001). Uptake of N was calculated as the difference between N requirement and N retranslocation.

We measured N and ^{15}N in mineral soils (0–60 cm depth) in May 2002. Five cores (7 cm diameter) were removed from randomly selected sites in each plot. Soil samples were removed in 10-cm depth increments, passed through a 1-mm sieve to remove roots and coarse fragments, and composited into 0–10 cm, 10–30 cm, and 30–60 cm depth increments for soil analyses. Subsamples of 30 g from each depth increment were subjected to sequential density fractionations. Soil samples were suspended in a solution of sodium polytungstate adjusted to a density of 1.5 g/cm^3. Samples were agitated, and then allowed to settle for 24–48 h. The supernatant containing material <1.5 g/cm^3 was then aspirated onto a glass filter, rinsed, oven dried (105 °C), weighed, and analyzed for %N, %C, $\delta^{15}N$, and $\delta^{13}C$ as described above. The procedure was then repeated with the remaining material with a solution of sodium polytungstate adjusted to 1.8 g/cm^3, and then a third time with a solution at 2.2 g/cm^3. We determined N, C, and ^{15}N content in these depth increments by summing over density fractions. For brevity, we report here only the totals for N and ^{15}N. Data separated by individual density fractions as well as data on C and ^{13}C will be presented elsewhere.

We examined some of the possible mechanisms through which progressive N limitation could be alleviated in this ecosystem using a mass balance approach. Progressive N limitation could be avoided, at least in the short term, if plants (or whole ecosystems) are able to acquire more N, or if they are able to use N more efficiently (Luo et al. 2004). To assess the importance of these mechanisms in the scrub oak experiment, we partitioned the C increment caused by elevated CO_2 into that allowed by a reduction in N concentration and that allowed by N acquisition. The increment in C mass caused by elevated CO_2 allowed by reduced N concentration can be quantified as the amount of C accumulated solely due to an increasing C:N ratio in elevated CO_2:

$$(C{:}N_E - C{:}N_A) \times N_A \quad (1)$$

where $C{:}N_E$ and $C{:}N_A$ are the C:N ratios at elevated and ambient CO_2, and N_A is the amount of N (in grams per square meter) in ambient CO_2. The increment in C mass caused by elevated CO_2 and allowed by acquisition of new N can be quantified as the amount of C accumulated solely due to increasing N mass in elevated CO_2:

$$(N_E - N_A) \times C{:}N_A \quad (2)$$

where N_E is the amount of N in elevated CO_2 (g N/m^2). Finally, if elevated CO_2 changes both the C:N ratio and N mass, there will be an interactive component, which can be thought of as the C increment supported by reduced N concentration that was allowed by the increment in acquisition (or vice-versa), and can be expressed as

$$(N_E - N_A) \times (C{:}N_E - C{:}N_A). \quad (3)$$

We applied this analysis at the level of aboveground plant biomass, using N mass and C:N data as described above. We also used the sum of C and N accumulation in mineral soils (0–60 cm depth), the O horizon, and aboveground plant material, to assess the functioning of these mechanisms at the ecosystem level. This analysis should be considered preliminary, because it does not yet take into account roots.

For litterfall, aboveground N and ^{15}N mass and fluxes, we used repeated measures analysis of variance (ANOVA) with CO_2 treatment as the main effect and time as the repeated measure. This statistical test provides an assessment of overall CO_2 responses (and their temporal dependence) during the seven-year period. We analyzed mineral soil and forest floor data using a split-plot ANOVA, with depth as a split-plot effect and CO_2 treatment as the main effect. Yet, for many response variables, we felt that inferring significant results exclusively from significant main effects and interactions from repeated measures or split-plot ANOVAs may be too conservative, causing us to reject what could be biologically significant differences. For this reason, we used two additional inferential approaches. First, even if main effects and interactions in the ANOVAs were not significant, we followed these analyses with *t*-tests comparing responses within individual years, and within individual soil depth increments. We used these results to infer changes in the nature of the CO_2 response from year to year (most changes were clearly driven by mean differences and not by differences in variance from year to year, which were typically small). Second, we used resampling procedures to estimate confidence intervals for the absolute effects of CO_2, and to estimate how these responses changed through time (described next). Resampling provides great flexibility in the types of response metrics that can be compared, and also involves few assumptions (Manly 1997). We report all statistical results, in the spirit of full disclosure, and note which approaches support (or not) particular conclusions.

To test for symptoms of progressive N limitation, we examined the absolute effect of CO_2 (elevated − ambient) through time. The absolute effect of CO_2 is the preferred metric, because in a system recovering from disturbance, biomass accumulation in the ambient CO_2 treatment will cause a declining relative response ((elevated − ambient)/ambient), even with a constant absolute CO_2 stimulation. We used bootstrapping to estimate confidence intervals for the absolute CO_2 effect size and for the response to CO_2 through time, using the program Resampling Stats v 5.0 (Resampling Stats, Arlington, Virginia, USA). Random samples (with replacement) of eight replicates were drawn from the raw data for each treatment, and the absolute difference between means was calculated (elevated − ambient). Eight replicate values were chosen to simulate the actual experiment where $n = 8$ for each treatment. The 95% confidence interval was estimated from 1000 repetitions of this procedure. We also used resampling to estimate the slope of the relationship between the absolute effect of CO_2 and time, focusing on the period between 1999 (the year that absolute CO_2 effects tended to be greatest) to 2002. (For litterfall mass, N, and ^{15}N, we focused on 2000–2002, because of the one-plus-year delay between leaf and litter production). We generated eight estimates of the absolute CO_2 effect (random samples of E–A, with replacement) for each year. With each trial, the absolute effect of elevated CO_2 was regressed against year and the slope recorded; confidence intervals around each slope were then estimated using 1000 trials. Finally, we also used resampling to estimate means and confidence intervals for the partitioning of the aboveground C increment into that supported by N acquisition, reduced N concentration, and their interaction, using Eqs. 1–3. As with the other resampling procedures, eight random samples (with replacement) of N_E, N_A, $C{:}N_E$, and $C{:}N_A$ were drawn, means were calculated, and the means manipulated according to the appropriate equation. The procedure was repeated 1000 times to generate 95% confidence intervals.

Results

Productivity estimated through litterfall

Elevated CO_2 increased oak litterfall (repeated-measures ANOVA, effect of CO_2, $P = 0.038$, Fig. 1), consistent with our previous finding that elevated CO_2 stimulates aboveground growth in this ecosystem (Dijkstra et al. 2002; B. G. Drake, *unpublished data*). Oaks dominated the total litterfall flux, which tended to increase with elevated CO_2 ($P = 0.056$, Fig. 1). Oak leaf mass in a given year estimated by allometry and oak litterfall in the subsequent year were positively correlated (Fig. 2); thus, the CO_2 stimulation of oak litterfall was highest in 2000 ($+82.4\ g{\cdot}m^{-2}{\cdot}yr^{1}$, a 59% stimulation), one year following the largest (nearly 80%) CO_2 enhancement of aboveground biomass estimated by allometry, 1999 (Dijkstra et al. 2002; B. G. Drake, *unpublished data*). These enhancements of biomass and productivity are large compared to those observed in other CO_2 enrichment experiments in forests (Delucia et al. 1999, Norby et al. 2002) and in grasslands (Chiariello and Field 1996, Niklaus et al. 2001, 2002), likely in part because the scrub oak experiment began immediately after fire disturbance, with little competition for light or nutrients (Dijkstra et al. 2002).

After reaching maximum stimulation in 2000, the response of oak and total litterfall to elevated CO_2 declined through time, though statistical support for this conclusion was mixed. In the repeated measures ANOVA for oak litterfall, the interaction term was not significant ($CO_2 \times$ time interaction, $P = 0.570$). The bootstrapping procedure suggests a declining response from 2000 to 2002, at a rate of $-18.1\ g{\cdot}m^{-2}{\cdot}yr^{-1}{\cdot}yr^{-1}$, though the 95% confidence interval slightly overlapped with zero, -35.1 to $0.3\ g{\cdot}m^{-2}{\cdot}yr^{-1}{\cdot}yr^{-1}$. The declining response through time was more apparent for total litterfall. While the $CO_2 \times$ time interaction was not significant ($P = 0.726$), the effect of CO_2 on total litterfall was largest in 2000 ($+80.2\ g{\cdot}m^{-2}{\cdot}yr^{-1}$), was smaller in 2001 ($+65\ g{\cdot}m^{-2}{\cdot}yr^{-1}$), and declined further in 2002 ($+40.2\ g{\cdot}m^{-2}{\cdot}yr^{-1}$). The bootstrapped estimate confirmed the declining CO_2 response: from 2000 to 2002,

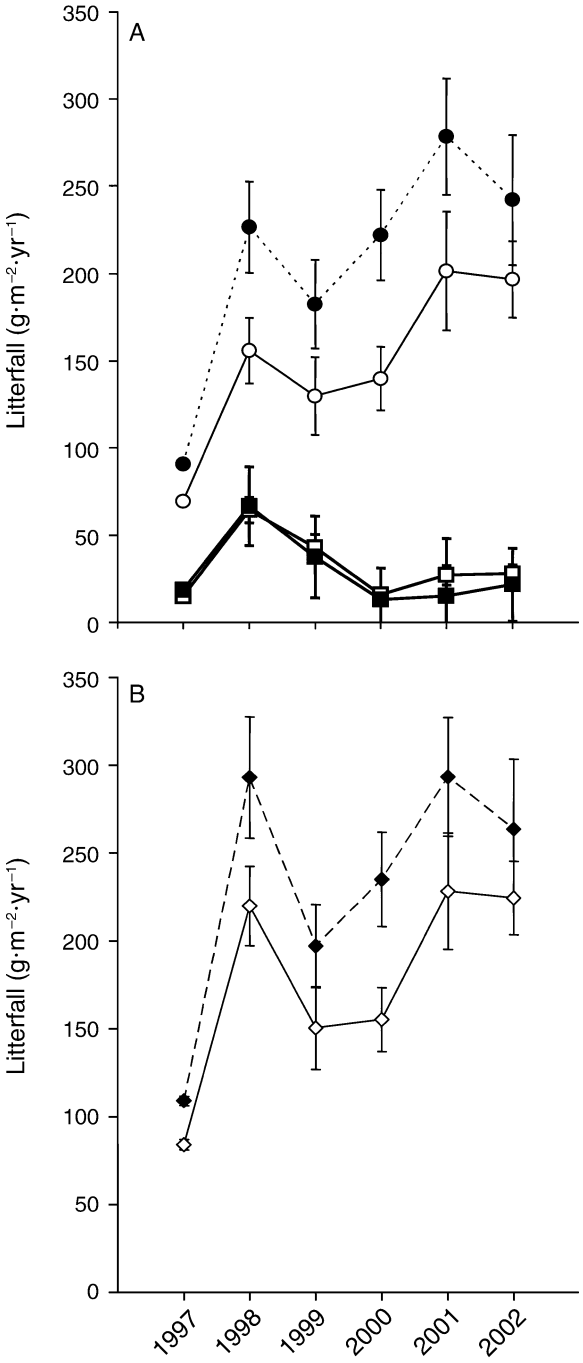

FIG. 1. Litterfall for 1997–2002 for (A) oak (circles) and non-oak (squares) litter and (B) total litterfall (diamonds). Open symbols show the ambient CO_2 treatment; solid symbols show the elevated treatment. Elevated CO_2 increased oak litterfall ($P = 0.038$) and tended to increase total litterfall ($P = 0.056$) but had no effect on litterfall of non-oak species ($P = 0.503$).

the absolute effect of CO_2 on total litter declined at a rate of -20.1 g·m^{-2}·yr^{-1}·yr^{-1} (95% confidence interval, -38.0 to -1.8 g·m^{-2}·yr^{-1}·yr^{-1}). Elevated CO_2 had no effect on litterfall of non-oak species ($P = 0.503$, Fig. 1), 66% of which is litter from the N-fixing species, *Galactia elliottii*, which, after a significantly positive response to elevated CO_2 during the first year, exhibited a negative growth response to elevated CO_2 from 1999 to 2002 (Hungate et al. 2004).

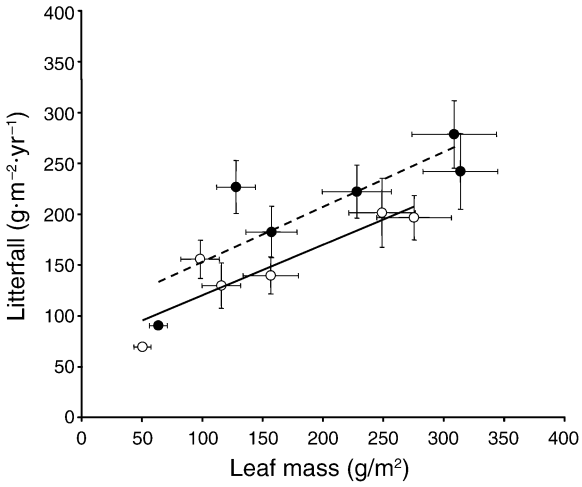

FIG. 2. Relationship between leaf mass in a given year and litterfall in the subsequent year for ambient (open circles) and elevated (solid circles) CO_2 treatments. Values are means ± SE. The solid line shows the least-squares linear relationship for the ambient CO_2 treatment (litterfall = 0.50 × leaf mass + 70.5; $r^2 = 0.81$); the dashed line shows the relationship for the elevated CO_2 treatment (litterfall = 0.54 × leaf mass + 98.8; $r^2 = 0.71$).

The effect of elevated CO_2 on litterfall N was marginally significant for the oak fraction only (Table 1) and overall was less pronounced than the effect on litterfall mass, because elevated CO_2 decreased N concentration in litter (Table 2). For both oak and non-oak litter, the reduction in N concentration was statistically significant in some years but not in others (Table 2). As for litterfall mass, the effect of CO_2 on N in total litterfall declined between 2000 and 2002 at a rate of -0.265 g N·m^{-2}·yr^{-1}·yr^{-1} (95% CI, -0.411 to -0.155). Oak litterfall N also exhibited a mean negative slope, -0.103 g N·m^{-2}·yr^{-1}·yr^{-1}, but the confidence interval overlapped with zero (95% CI, -0.352 to 0.129). CO_2 significantly increased the flux of ^{15}N in litterfall for both oak and total fractions. This effect was most pronounced in 1999 and 2000, decreased in 2001, and had disappeared by 2002 (Table 1). CO_2 had no effect on ^{15}N in litterfall of the non-oak fraction (Table 1).

Aboveground N and ^{15}N

When analyzed over the seven-year period, elevated CO_2 did not cause a statistically significant change in the mass of N in oak leaves (having units of g N/m^2 and hereafter referred to as N content). However, elevated CO_2 increased N content in oak leaves by 50% in 1999, an effect that was marginally significant in a *t* test (Table 3) and exhibited a 95% confidence interval (estimated through bootstrapping) that did not overlap

TABLE 1. Fluxes of N (g $N \cdot m^{-2} \cdot yr^{-1}$) and ^{15}N (mg $^{15}N \cdot m^{-2} \cdot yr^{-1}$) in aboveground litter for each year of the study.

Effect	Oaks				Other	
	Ambient	Elevated	*F*	*P*	Ambient	Elevated
Litter N						
1997	0.710 ± 0.029	0.819 ± 0.026		0.013	0.213 ± 0.016	0.213 ± 0.021
1998	1.068 ± 0.128	1.483 ± 0.151		0.055	0.863 ± 0.103	0.823 ± 0.204
1999	0.925 ± 0.136	1.329 ± 0.189		0.106	0.369 ± 0.061	0.347 ± 0.123
2000	0.982 ± 0.108	1.592 ± 0.175		0.026	0.187 ± 0.033	0.118 ± 0.026
2001	1.690 ± 0.308	2.331 ± 0.305		0.161	0.371 ± 0.076	0.196 ± 0.044
2002	1.777 ± 0.213	1.957 ± 0.307		0.637	0.391 ± 0.068	0.222 ± 0.054
CO_2			3.742	0.074		
Year			20.143	<0.001		
CO_2 × year			1.080	0.379		
Litter ^{15}N						
1999	0.567 ± 0.082	0.991 ± 0.175		0.047	0.160 ± 0.038	0.141 ± 0.022
2000	0.854 ± 0.104	1.312 ± 0.138		0.019	0.016 ± 0.003	0.063 ± 0.025
2001	0.850 ± 0.135	1.099 ± 0.161		0.255	0.016 ± 0.005	0.009 ± 0.002
2002	0.867 ± 0.079	0.857 ± 0.158		0.958	0.021 ± 0.011	0.018 ± 0.007
CO_2			4.547	0.051		
Year			2.961	0.043		
CO_2 × year			1.886	0.147		

Notes: Values are means ± SE, $n = 8$, for the ambient and elevated CO_2 treatments, and for oak litter, non-oak (''other'') litter, and for total litter. *F* and *P* values from repeated-measures ANOVA show effects of CO_2 (df = 1, 14), year (df = 6, 64), and the CO_2 × year interaction (df = 6, 64). *P* values are also shown for the effect of elevated CO_2 during each year, analyzed using *t* tests.

with zero (Fig. 3). After 1999, leaf N content in the ambient and elevated CO_2 treatments converged, and means were nearly identical by 2002. Thus, the effect of CO_2 on leaf N content declined from 1999 to 2002 (Table 4). Elevated CO_2 significantly increased stem N content, and differences were largest in 1999 (Table 3). As with leaf N content, the repeated measures ANOVA did not reveal a significant main effect of CO_2 on aboveground oak N content, nor a CO_2 by time interaction (Table 3). Nevertheless, CO_2 increased aboveground oak N content by more than 2 g N/m^2 (60%) in 1999 (Fig. 3), an effect that was statistically significant in a *t* test (Table 3), and for which the 95% confidence interval did not overlap with zero. Elevated CO_2 also caused marginally significant increases in aboveground oak N content in 1997 and 1998. As with leaf N content, the effects of CO_2 on stem and total aboveground oak N content were largest in 1999, and declined consistently from 1999 to 2002 (Table 4). The effects of elevated CO_2 on ^{15}N content in leaves, stems, and total aboveground oak tissues (mg $^{15}N\ m^{-2}$) were similar to the effects of CO_2 on aboveground N content. CO_2 increased ^{15}N content in oak tissues in 1999, significantly for stems and total aboveground ^{15}N, and marginally so for leaves (Table 3). However, these effects declined between 1999 and 2002 (Table 4, Fig. 4), and for leaves and total aboveground tissues, even reversed, such that, by 2002, the ^{15}N content of aboveground oak tissues was actually lower in the elevated-CO_2 treated plots (Table 3).

When analyzed over the seven-year period in repeated-measures ANOVAs, elevated CO_2 did not significantly alter the annual N increment in oak leaves, stems, or in total aboveground oak tissues (Table 3). During individual years, however, *t* tests did suggest that elevated CO_2 initially increased annual N increment in oak stems (1997 and 1998, Table 3). Though CO_2 effects on N increments in leaves, stems, and total aboveground tissues were rarely significant, the absolute difference between elevated and ambient treatments declined from 1999 to 2002 for all three parameters (Table 4), reflecting similar patterns found for leaf, stem, and total aboveground oak N content. Responses of ^{15}N increment were similar but even more striking: in 1999, CO_2 increased ^{15}N increment significantly in stem and total aboveground ^{15}N and marginally in leaves (Table 3), but this effect subsequently declined (Table 4). For ^{15}N content and ^{15}N increment, the temporal changes in the magnitude (and even the direction) of the CO_2 effect were large enough to drive significant CO_2 × time interactions in the repeated-measures ANOVA (Table 3). Yet, these responses were qualitatively identical to those observed for N content and N increment: substantial CO_2 enhancements in 1999, followed by declining and even disappearing responses (Fig. 4).

Elevated CO_2 initially increased N requirement, significantly in 1997 and 1999 (Table 5). Increased N requirement was met by both enhanced uptake of soil N and by retranslocation of N during leaf senescence (Table 5, Fig. 3). N requirement in the ambient and elevated CO_2 treatments converged by 2002, and both N uptake and retranslocation of N were not significantly affected by elevated CO_2 after 1999 (Fig. 3). From 1999 to 2002, the effect of elevated CO_2 on all three fluxes declined (Table 4).

Our analysis of N cycling focused on aboveground N mass and fluxes, ignoring roots, because our mea-

TABLE 1. Extended.

Other		Total			
F	*P*	Ambient	Elevated	*F*	*P*
	0.976	0.923 ± 0.040	1.033 ± 0.039		0.066
	0.825	1.935 ± 0.204	2.299 ± 0.287		0.320
	0.874	1.294 ± 0.145	1.675 ± 0.205		0.152
	0.121	1.169 ± 0.105	1.709 ± 0.179		0.021
	0.067	2.060 ± 0.285	2.527 ± 0.316		0.291
	0.448	2.168 ± 0.186	2.179 ± 0.342		0.695
0.687	0.421			2.388	0.145
28.900	<0.001			17.751	<0.001
0.366	0.870			0.538	0.746
	0.674	0.727 ± 0.074	1.133 ± 0.162		0.039
	0.484	0.870 ± 0.102	1.375 ± 0.154		0.020
	0.264	0.865 ± 0.134	1.108 ± 0.160		0.264
	0.792	0.888 ± 0.076	0.875 ± 0.160		0.943
0.217	0.649			4.771	0.046
36.099	<0.001			1.517	0.224
0.190	0.903			1.885	0.147

surements of root production and turnover rely on minirhizotron observations that are not readily calibrated to biomass estimates. These minirhizotron observations show that elevated CO_2 initially increased root biomass (a 181% increase by December 1997), but this increase was transient, declining to a nonsignificant 30% stimulation by mid-1999, and to a 0% to slightly negative relative effect from 2000 through 2004 (Dilustro et al. 2002; F. P. Day, D. B. Stover, A. L. Pagel, B. A. Hungate, J. J. Dilustro, B. T. Herbert, B. G. Drake, and C. R. Hinkle, *unpublished manuscript*). Fine roots exhibited a strong initial response to elevated CO_2 that subsequently declines and disappears—a pattern qualitatively similar but even more pronounced than that described here for aboveground responses. Thus, including root responses is likely to strengthen our conclusion that responses to elevated CO_2 decline through time.

Soil N and ^{15}N

Elevated CO_2 increased tracer ^{15}N and total N in the O horizon by 2002 (Table 6, Johnson et al. 2003). For total N, this increase of 2.27 g N/m^2 in the O horizon approximately reflected the CO_2 treatment difference in cumulative N in litterfall over the seven-year period (9.54 ± 0.80 for ambient and 11.43 ± 1.04 for elevated, a nonsignificant difference of 1.89 g N/m^2, $P = 0.173$). However, the difference in ^{15}N accumulation in the forest floor (6.98 mg ^{15}N/m^2) was larger than the cumulative effect of CO_2 on litterfall ^{15}N flux (1.50 mg ^{15}N/m^2), indicating that some additional mechanisms must be involved, likely microbial immobilization (Hungate et al. 1999) or abiotic ^{15}N fixation due to increased organic matter caused by elevated CO_2 (Johnson et al. 2000, 2003). Because the tracer ^{15}N was applied directly to the forest floor in 1998, it is not surprising that these mechanisms had a stronger influence on ^{15}N than on total N. Elevated CO_2 tended to increase the total accumulation of N in aboveground biomass and in the forest floor, a difference of 3.03 ± 1.65 g N/m^2 ($P = 0.146$). For tracer ^{15}N, the difference was more pronounced, 6.52 ± 1.71 mg ^{15}N/m^2 ($P = 0.006$).

TABLE 2. Litter nitrogen concentrations (mean ± SE) for oak and non-oak fractions; values are g N/g tissue × 100%.

	Oaks				Non-Oaks			
Effect	Ambient	Elevated	*F*	*P*	Ambient	Elevated	*F*	*P*
1997	1.028 ± 0.035	0.906 ± 0.024		0.013	1.446 ± 0.034	1.164 ± 0.017		<0.001
1998	0.687 ± 0.011	0.662 ± 0.010		0.116	1.339 ± 0.037	1.242 ± 0.017		0.033
1999	0.743 ± 0.033	0.726 ± 0.017		0.656	1.593 ± 0.069	1.606 ± 0.171		0.945
2000	0.716 ± 0.026	0.728 ± 0.024		0.738	1.245 ± 0.075	0.952 ± 0.085		0.022
2001	0.832 ± 0.021	0.832 ± 0.025		0.991	1.419 ± 0.097	1.380 ± 0.113		0.798
2002	0.897 ± 0.021	0.803 ± 0.025		0.012	1.477 ± 0.104	1.035 ± 0.087		0.006
CO_2			5.961	0.029			6.934	0.020
Year			44.848	<0.001			9.364	<0.001
CO_2 × year			2.843	0.021			2.169	0.031

Note: F and *P* values from repeated-measures ANOVA show effects of CO_2 (df = 1, 14), year (df = 8, 64), and the CO_2 × year interaction (df = 8, 64).

TABLE 3. Oak N and ^{15}N pools and increments for 1996–2002.

A) Pools	Leaf				Stem	
	Ambient	Elevated	*F*	*P*	Ambient	Elevated
N (g/m^2)						
1996	0.669 ± 0.132	0.892 ± 0.175		0.325	0.477 ± 0.083	0.742 ± 0.087
1997	1.081 ± 0.160	1.488 ± 0.210		0.146	0.910 ± 0.163	1.582 ± 0.228
1998	1.701 ± 0.230	2.092 ± 0.296		0.315	1.184 ± 0.198	1.978 ± 0.232
1999	1.706 ± 0.252	2.405 ± 0.248		0.068	1.802 ± 0.293	3.190 ± 0.311
2000	2.507 ± 0.246	2.936 ± 0.324		0.310	1.971 ± 0.253	3.196 ± 0.533
2001	2.905 ± 0.328	2.994 ± 0.318		0.848	2.491 ± 0.381	3.261 ± 0.301
2002	3.566 ± 0.437	3.520 ± 0.386		0.938	3.245 ± 0.417	4.050 ± 0.369
CO_2†			0.839	0.375		
Year‡			80.413	<0.001		
CO_2 × year‡			1.306	0.263		
^{15}N (mg/m^2)						
1999	1.275 ± 0.203	1.662 ± 0.214		0.210	1.014 ± 0.173	1.850 ± 0.160
2000	1.843 ± 0.194	1.891 ± 0.214		0.872	1.153 ± 0.185	1.693 ± 0.286
2001	1.908 ± 0.184	1.477 ± 0.174		0.112	1.415 ± 0.228	1.576 ± 0.162
2002	2.444 ± 0.282	1.690 ± 0.261		0.070	1.761 ± 0.236	2.062 ± 0.244
CO_2†			0.596	0.453		
Year§			5.313	0.003		
CO_2 × year§			5.218	0.004		

B) Fluxes	Leaf increment				Stem increment	
	Ambient	Elevated	*F*	*P*	Ambient	Elevated
N ($g \cdot m^{-2} \cdot yr^{-1}$)						
1996	0.669 ± 0.132	0.892 ± 0.175		0.325	0.477 ± 0.083	0.742 ± 0.087
1997	0.412 ± 0.077	0.595 ± 0.190		0.386	0.433 ± 0.090	0.840 ± 0.183
1998	0.621 ± 0.077	0.604 ± 0.107		0.900	0.274 ± 0.072	0.396 ± 0.134
1999	0.005 ± 0.142	0.314 ± 0.086		0.084	0.618 ± 0.227	1.212 ± 0.224
2000	0.801 ± 0.144	0.531 ± 0.163		0.234	0.169 ± 0.175	0.006 ± 0.353
2001	0.398 ± 0.209	0.058 ± 0.165		0.681	0.521 ± 0.268	0.065 ± 0.269
2002	0.661 ± 0.248	0.526 ± 0.206		0.135	0.754 ± 0.320	0.789 ± 0.169
CO_2†			0.926	0.352		
Year‡			9.273	<0.001		
CO_2 × year‡			0.550	0.768		
^{15}N ($mg \cdot m^{-2} \cdot yr^{-1}$)						
1999	1.275 ± 0.203	1.662 ± 0.214		0.023	1.014 ± 0.173	1.850 ± 0.160
2000	0.569 ± 0.127	0.229 ± 0.184		0.151	0.139 ± 0.104	−0.074 ± 0.224
2001	0.064 ± 0.133	−0.414 ± 0.214		0.079	0.262 ± 0.270	−0.117 ± 0.183
2002	0.536 ± 0.203	0.213 ± 0.206		0.282	0.347 ± 0.236	0.486 ± 0.180
CO_2†			3.854	0.070		
Year§			22.950	<0.001		
CO_2 × year§			1.846	0.153		

Notes: Values are means ± SE, $n = 8$, for the ambient and elevated CO_2 treatments, and for oak leaves, stems, and total aboveground tissues. *F* and *P* values from repeated-measures ANOVA show effects of CO_2, year, and the CO_2 × year interaction.

† df = 1, 14.
‡ df = 6, 84.
§ df = 3, 42.

DISCUSSION

Progressive nitrogen limitation?

The accumulation of organic N and tracer ^{15}N in plants and in the forest floor are consistent with the predictions of progressive N limitation (Luo et al. 2004), but these symptoms are really only the first step. Did this elicit a reduction in N availability to plants and reduce the productivity response to elevated CO_2? Alternatively, were plants able to accumulate N, or to use N more efficiently, alleviating progressive N limitation, at least in the short term? And, at the ecosystem level, did CO_2 decrease N losses or enhance N inputs, possibly mitigating progressive N limitation in the long term?

Reduced N availability?

All of the response variables we examined—litterfall and aboveground N and ^{15}N in various ecosystem compartments—exhibited qualitatively similar responses to elevated CO_2 over this seven-year period. Positive responses to CO_2 tended to reach a maximum four or five

TABLE 3. Extended.

Stem		Total			
F	*P*	Ambient	Elevated	*F*	*P*
	0.045	1.146 ± 0.199	1.634 ± 0.235		0.135
	0.031	1.991 ± 0.317	3.069 ± 0.425		0.061
	0.021	2.885 ± 0.417	4.069 ± 0.502		0.091
	0.006	3.508 ± 0.542	5.595 ± 0.549		0.017
	0.057	4.478 ± 0.464	6.132 ± 0.830		0.104
	0.136	5.396 ± 0.693	6.255 ± 0.605		0.367
	0.171	6.811 ± 0.834	7.570 ± 0.746		0.509
5.864	0.030			2.826	0.115
64.317	<0.001			85.711	<0.001
1.991	0.076			1.617	0.152
	0.003	2.289 ± 0.344	3.512 ± 0.323		0.021
	0.136	2.997 ± 0.358	3.584 ± 0.453		0.326
	0.573	3.322 ± 0.362	3.053 ± 0.306		0.579
	0.390	4.205 ± 0.494	3.753 ± 0.438		0.504
3.772	0.073			0.357	0.560
4.620	0.007			6.474	0.001
1.859	0.151			4.703	0.006
Stem increment		Total increment			
F	*P*	Ambient	Elevated	*F*	*P*
	0.045	1.146 ± 0.199	1.634 ± 0.235		0.135
	0.066	0.845 ± 0.154	1.435 ± 0.315		0.114
	0.435	0.895 ± 0.112	1.000 ± 0.190		0.641
	0.084	0.623 ± 0.348	1.525 ± 0.229		0.048
	0.686	0.970 ± 0.253	0.537 ± 0.434		0.403
	0.250	0.919 ± 0.436	0.123 ± 0.341		0.173
	0.923	1.415 ± 0.529	1.315 ± 0.343		0.877
2.087	0.171			0.460	0.509
3.621	0.003			1.966	0.080
1.316	0.259			1.776	0.114
	0.013	2.289 ± 0.344	3.512 ± 0.323		0.003
	0.403	0.708 ± 0.212	0.155 ± 0.373		0.219
	0.266	0.326 ± 0.363	−0.531 ± 0.224		0.065
	0.645	0.883 ± 0.408	0.700 ± 0.345		0.737
0.787	0.390			0.470	0.504
18.969	<0.001			27.401	<0.001
3.339	0.028			3.418	0.026

TABLE 4. Rates at which the effect of elevated CO_2 declines through time, determined as the slope of the relationship between the absolute effect of CO_2 (elevated − ambient) and time (in years) over the period 1999–2002.

Parameter	Units	Leaf	Stem	Total
^{15}N mass	mg $^{15}N \cdot m^{-2} \cdot yr^{-1}$	−0.385 ± 0.096	−0.202 ± 0.089	−0.575 ± 0.166
^{15}N increment	mg $^{15}N \cdot m^{-2} \cdot yr^{-1} \cdot yr^{-1}$	−0.228 ± 0.083	−0.218 ± 0.084	−0.434 ± 0.144
N mass	g $N \cdot m^{-2} \cdot yr^{-1}$	−0.257 ± 0.141	−0.215 ± 0.137	−0.493 ± 0.271
N increment	g $N \cdot m^{-2} \cdot yr^{-1} \cdot yr^{-1}$	−0.141 ± 0.073	−0.194 ± 0.105	−0.335 ± 0.155
N requirement	g $N \cdot m^{-2} \cdot yr^{-1} \cdot yr^{-1}$			−0.487 ± 0.237
N retranslocation	g $N \cdot m^{-2} \cdot yr^{-1} \cdot yr^{-1}$			−0.078 ± 0.050
N uptake	g $N \cdot m^{-2} \cdot yr^{-1} \cdot yr^{-1}$			−0.409 ± 0.220

Notes: The mean value of the slope and one 95% confidence interval (shown as ± 0.5 CI) are reported for each response variable (confidence intervals were estimated using the bootstrapping procedure described in *Methods*). None of the confidence intervals overlaps with zero; all are negative, describing declining responses through time.

TABLE 5. Oak N requirement, N uptake, and N retranslocation (all in g $N \cdot m^{-2} \cdot yr^{-1}$) for 1996–2002.

Year	N requirement				N uptake	
	Ambient	Elevated	*F*	*P*	Ambient	Elevated
1996	1.146 ± 0.199	1.634 ± 0.235		0.135	1.146 ± 0.199	1.634 ± 0.235
1997	1.405 ± 0.152	2.348 ± 0.346		0.026	1.300 ± 0.155	2.055 ± 0.332
1998	3.463 ± 0.403	4.286 ± 0.481		0.211	1.962 ± 0.224	2.483 ± 0.288
1999	2.186 ± 0.483	3.687 ± 0.383		0.029	1.547 ± 0.389	2.854 ± 0.326
2000	2.571 ± 0.289	3.009 ± 0.523		0.476	1.952 ± 0.286	2.129 ± 0.453
2001	3.400 ± 0.732	3.211 ± 0.540		0.839	2.608 ± 0.656	2.455 ± 0.457
2002	3.866 ± 0.748	3.973 ± 0.558		0.910	3.191 ± 0.658	3.272 ± 0.476
CO_2			2.251	0.156		
Year			11.005	<0.001		
CO_2 × year			0.943	0.469		

Notes: Values are means ± SE, $n = 8$, for the ambient and elevated CO_2 treatments. *F* and *P* values from repeated-measures ANOVA show effects of CO_2 (df = 1, 14), year (df = 6, 64), and the CO_2 × year interaction (df = 6, 64).

years after the onset of treatment, and subsequently those responses tended to diminish. These changes were not always detected as significant main effects of CO_2 in the repeated measures ANOVAs, and rarely as significant CO_2 × time interactions. However, in at least one year between 1996 and 1999, the bootstrapped 95% confidence intervals and *t* tests suggested significant enhancements caused by elevated CO_2 early in the experiment (Figs. 3 and 4). That these initial enhancements declined later was supported by our bootstrapping estimates of the slope of the relationship between absolute CO_2 effects and time: across the board, these slopes were negative, with 95% confidence limits that did not overlap with zero (Table 4).

We cannot yet rule out the possibility that light limitation contributes to these declining responses. Specifically, the more rapidly developing canopy in the elevated CO_2 treatment is likely to reach closure more quickly than in ambient CO_2 (Hymus et al. 2002), and, when that occurs, the growth response and the demand for soil nutrients are likely to decline (Gielen et al. 2001). Nevertheless, we submit that progressive N limitation is a better explanation for these declining responses. CO_2 caused an accumulation of organic N in aboveground oak tissues and in the O horizon by 2002 of 3.03 g N/m^2 over ambient CO_2. This is more than enough to account for the disappearance, by 2002, of the 1.6 g N/m^2 CO_2 stimulation of N uptake apparent in 1999. Furthermore, the declining response of aboveground litter production from 2000 to 2002 is consistent with the expectation that reduced N availability affected aboveground net primary production. At the same time, the persistent increment in aboveground plant carbon (Fig. 5) suggests that some mechanisms are partially alleviating progressive N limitation.

N acquisition and N concentration: aboveground

Increased plant uptake of N from the soil and reduced plant tissue N concentration (increased C:N ratio) can both alleviate progressive N limitation. Elevated CO_2 can enhance N uptake by stimulating root or mycorrhizal exploration, or by increasing soil N mineralization (Zak et al. 2000). Elevated CO_2 is also well known to increase the C:N ratio of plant tissues, allowing C accumulation without requiring additional N (Cotrufo et al. 1998). To evaluate these mechanisms alleviating progressive N limitation, we used mass balance to partition the aboveground C increment caused by elevated CO_2 into that supported by increased N acquisition, by reduced N concentration, and by the interactive combination of these two mechanisms (Fig. 5). Most of the C increment caused by elevated CO_2 in aboveground biomass was supported initially by increased N acquisition (Fig. 5a). By 1999, elevated CO_2 had caused a nearly 80% enhancement of aboveground biomass (Dijkstra et al. 2002), and this was accompanied by an increase in aboveground plant N mass of >2 g N/m^2 (Table 3). Reduced N concentration oc-

TABLE 6. N and tracer ^{15}N recovered in soils in 2002.

Soil horizon	Total N (g N/m^2)			
	Ambient	Elevated	*F*	*P*
Forest floor	8.42 ± 0.77	10.69 ± 0.64		0.040
0–10 cm (O/A)	70.15 ± 5.37	62.01 ± 8.88		0.446
10–30 cm (E)	26.23 ± 3.13	22.84 ± 3.21		0.462
30–60 cm (C)	13.57 ± 1.65	14.70 ± 1.76		0.647
Total	118.27 ± 8.51	110.24 ± 11.51		0.579
CO_2			0.323	0.579
Depth			95.568	<0.001
CO_2 × depth			0.811	0.495

Note: For CO_2, df = 1, 14; for depth, df = 3, 42; for CO_2 × depth, df = 3, 42.

TABLE 5. Extended.

N uptake		N retranslocation			
F	*P*	Ambient	Elevated	*F*	*P*
	0.135	0	0		
	0.058	0.105 ± 0.043	0.293 ± 0.043		0.008
	0.176	1.501 ± 0.190	1.804 ± 0.207		0.300
	0.022	0.639 ± 0.156	0.833 ± 0.087		0.295
	0.747	0.619 ± 0.117	0.880 ± 0.159		0.207
	0.850	0.792 ± 0.129	0.757 ± 0.147		0.860
	0.923	0.674 ± 0.099	0.701 ± 0.144		0.881
2.167	0.163			1.740	0.208
5.522	<0.001			32.068	<0.001
0.914	0.489			0.639	0.671

curred throughout the experiment, but supported a small proportion of the C increment during the first four years. Between 1999 and 2002, reduced N concentration became increasingly important (Fig. 5b) and by 2002 was the dominant mechanism supporting the CO_2-induced enhancement of aboveground C. At this point, CO_2 had caused substantial N accumulation in the forest floor, and the CO_2 stimulation of N uptake had disappeared. Thus, it appears the ability to acquire additional N had nearly become exhausted by this point (note that the 95% confidence intervals for the C increment caused by N acquisition overlap zero for 2001 and 2002, Fig. 5a). Nevertheless, reduced N concentration was still able to support increased C accumulation in aboveground biomass (Fig. 5b). How long this can be sustained will depend on the limits of N concentration in these oaks, whether N acquisition increases again as the N immobilized in the O horizon is released again in mineral form, or whether plants can acquire N from other sources. Because the fire return time in this system is relatively short, fire disturbance may occur before PNL causes the CO_2 effect on plant C accumulation to disappear entirely.

N acquisition and reduced N concentration: whole system

If elevated CO_2 enhances N accumulation by reducing N losses or increasing N inputs, progressive N limitation could be avoided. In this experiment, total recovery of the ^{15}N tracer provides an integrative measure of N losses, assuming that behavior of the pulse of applied ^{15}N indicates that of total system N. Whereas CO_2 increased ^{15}N recovery in the forest floor, CO_2 significantly reduced recovery of tracer ^{15}N in underlying mineral horizons (Table 6). The reduction in mineral soils (28.42 mg/m^2) exceeded the accumulation in the organic-rich forest floor (6.98 mg/m^2), such that CO_2 reduced total soil ^{15}N recovery by 21.44 mg/m^2. At the same time (2001 and 2002), elevated CO_2 had not caused any accumulation of ^{15}N in plant biomass (Table 3). While changes in total system N mass are not evident in this experiment, reduced recovery of tracer ^{15}N in soil reported here suggests that elevated atmospheric CO_2 increased N losses. We have also recently shown that elevated CO_2 initially increased but later depressed N fixation (Hungate et al. 1999, 2004). Changes in N inputs and losses influence C storage over decades to centuries (Comins and McMurtrie 1993, Cannel and Thornley 1998), time scales not readily accessible in field experiments, so predicting long-term responses based on our observations is problematic. Nevertheless, if the responses we observed persist, the combination of reduced N inputs through fixation and increased N losses are expected to exacerbate—not alleviate—progressive N limitation.

To integrate these responses at the ecosystem level, we partitioned the total C increment in all the stocks of organic matter we have examined so far (aboveground plant parts, O horizon, and mineral soils to a depth of 60 cm) into components allowed by reduced

TABLE 6. Extended.

Tracer ^{15}N (mg $^{15}N/m^2$)			
Ambient	Elevated	*F*	*P*
9.28 ± 1.21	16.26 ± 1.22		0.001
68.73 ± 5.34	44.56 ± 7.08		0.016
10.47 ± 1.58	6.70 ± 1.23		0.081
4.77 ± 0.82	4.29 ± 0.64		0.651
93.25 ± 7.00	71.81 ± 7.58		0.057
		4.321	0.057
		117.449	<0.001
		8.869	<0.001

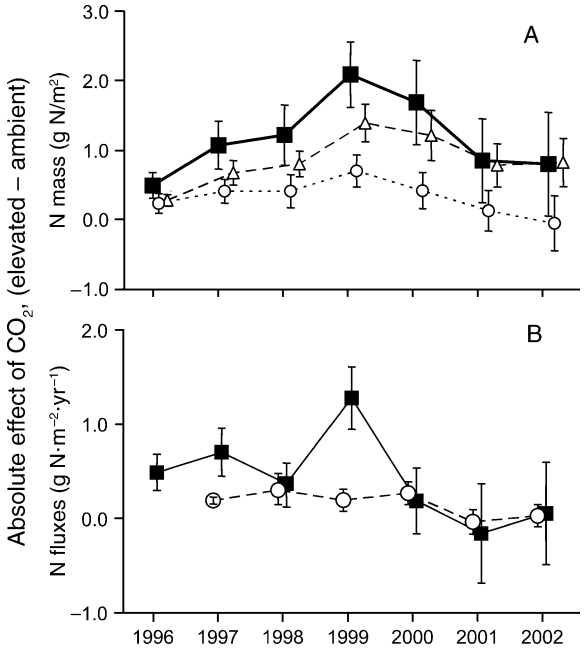

FIG. 3. Absolute effects of elevated CO_2 on (A) aboveground N mass in leaves (open circles), stems (open triangles), and total oak tissues (solid squares), and (B) aboveground N uptake (solid squares) and N retranslocation (open circles). Symbols are mean absolute effect sizes (elevated − ambient), and bars are 95% confidence intervals estimated through resampling.

N concentration, N acquisition (affected by N inputs and losses), and their interaction (Eqs. 1–3). For all components, the 95% confidence intervals overlapped with zero. The C increment supported by N acquisition tended to be negative (−250.4 g C/m²; 95% CI, −1083.5 to 635.2 g C/m²), because the nonsignificant reduction in soil N was larger than any accumulation of N in plant biomass or in the O horizon (Table 6). The C increment supported by reduced N concentration tended to be positive (328.0 g C/m²; 95% CI, −85.1 to 784.1 g C/m²), driven by the observed C increment for aboveground plant biomass (Fig. 5b). The interaction term was small: −16.8 g C/m² (95% CI, −128.3 to 59.3 g C/m²). Whereas both plant N acquisition and reduced N concentration supported a positive C increment in aboveground plant biomass, the tendency towards lower soil N (with no corresponding change in C:N ratio) negated this response at the ecosystem level. The increment in aboveground plant C supported by N acquisition (especially apparent in 1999, Fig. 5a) can be entirely explained by a shift in the allocation of N within the ecosystem, away from mineral soils (Table 6), toward plant biomass by 1999, and subsequently into the O horizon (in 2002).

Conclusions

In this ecosystem, CO_2 elicited changes in N cycling that were broadly consistent with the predictions of progressive N limitation. We found that CO_2 initially supported increased plant N acquisition to match growth responses, but over time, as this N re-entered the litter–soil pool, N availability to plants appeared to decline. Subsequently, plants were still able to maintain an enhancement of cumulative C increment in response to elevated CO_2, but relied strongly on reduced N concentration to do so. So far, the symptoms of progressive N limitation included some evidence for a declining productivity response (litterfall), though aboveground plant C continues to be enhanced by elevated CO_2 (B. G. Drake, *unpublished data*; Fig. 5). Two other studies have found results that more clearly support the idea of progressive N limitation. In an experiment examining grassland response to a gradient of CO_2 concentrations, elevated CO_2 increased plant litter inputs and soil C:N and these changes were associated with decreased soil N mineralization, as predicted by progressive N limitation (Gill et al. 2002). In a plot of *Pinus taeda*, free-air CO_2 enrichment apparently caused progressive N limitation to develop because experi-

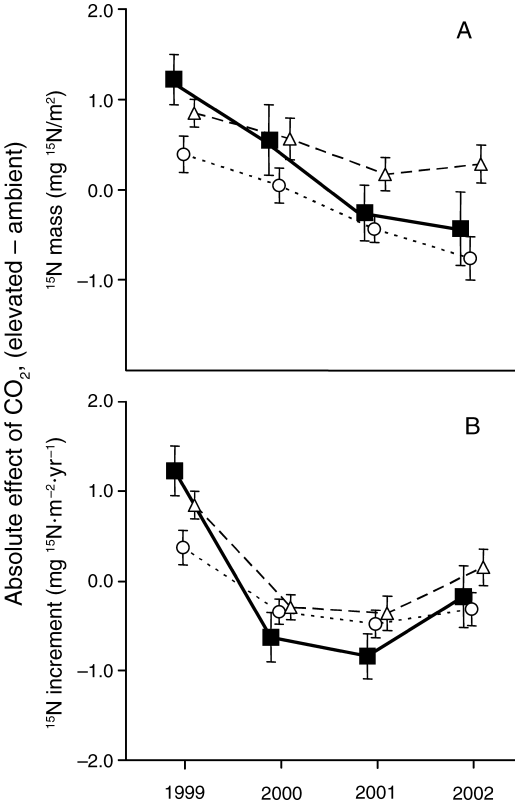

FIG. 4. Absolute effects of elevated CO_2 on (A) aboveground ^{15}N mass in leaves (open circles), stems (open triangles), and total oak tissues (solid squares), and (B) aboveground ^{15}N increments in leaves (open circles), stems (open triangles), and total oak tissues (solid squares). Symbols are mean absolute effect sizes (elevated − ambient), and bars are 95% confidence intervals estimated through resampling. Tracer ^{15}N was added in June 1998, so estimates of annual pools and fluxes begin in 1999.

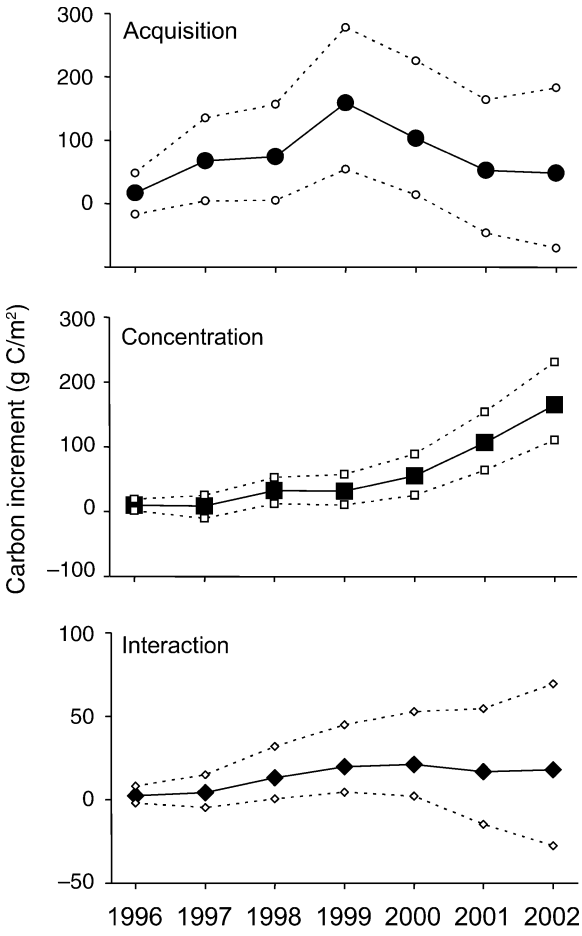

FIG. 5. Mechanisms alleviating progressive N limitation and supporting the CO_2 enhancement of aboveground plant C. (A) N acquisition, the amount of C accumulated because of additional N acquisition by plants (presumably from uptake from soil). (B) N concentration, the increment in C accumulation supported by reduced concentration of N in plant tissues. (C) The interaction between concentration and acquisition, which can be thought of as the carbon increment allowed by reduced N concentration of the increment in acquired N (or vice versa). Values are mean responses (solid symbols, solid lines) and upper and lower 95% CI (open symbols, dashed lines).

mental N addition relieved the growth limitation (Oren et al. 2001). While the theoretical outcome may be clear and inevitable in biogeochemical models, the experimental detection of the phenomenon may in some cases be quite difficult. For example, in some experiments, CO_2 has caused small and variable effects on biomass production, such that progressive N limitation, if it occurs, may be too small to be apparent against background variation (Chiariello and Field 1996, Körner et al. 1997, Leadley et al. 1999, Niklaus et al. 1998, 2001, 2002, Finzi et al. 2002). In this scrub oak ecosystem, the symptoms of progressive N limitation may be more apparent because of the large initial biomass response to elevated CO_2 (Dijkstra et al. 2002). If these symptoms persist, they are likely to elicit more clearly the full suite of predicted responses, including a decline in the CO_2 enhancements of plant growth and C uptake.

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