CO_2 Enhancement of Forest Productivity Constrained by Limited Nitrogen Availability

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Stimulation of terrestrial productivity by rising CO_2 concentration is projected to reduce the airborne fraction of anthropogenic CO_2 emissions; coupled climate-carbon (C) cycle models, including those used in the IPCC Fourth Assessment Report (AR4), are sensitive to this negative feedback on atmospheric CO_2 [1]. The representation of the so-called CO_2 fertilization effect in the 11 models used in AR4 and subsequent models[2,3] was broadly consistent with experimental evidence from four free-air CO_2 enrichment (FACE) experiments, which indicated that net primary productivity (NPP) of forests was increased by 23 ± 2% in response to atmospheric CO_2 enrichment to 550 ppm[4]. Substantial uncertainty remains, however, because of the expectation that feedbacks through the nitrogen (N) cycle will reduce the CO_2 stimulation of NPP[5,6]; these feedbacks were not included in the AR4 models and heretofore have not been confirmed by experiments in forests[7].

Here, we provide new evidence from a FACE experiment in a deciduous *Liquidambar styraciflua* (sweetgum) forest stand in Tennessee, USA, that N limitation has significantly reduced the stimulation of NPP by elevated atmospheric CO_2 concentration (eCO_2). Isotopic evidence and N budget analysis support the premise that N availability in this forest ecosystem has been declining over time, and declining faster in eCO_2. Model analyses and evidence from leaf- and stand-level observations provide mechanistic evidence that declining N availability constrained the tree response to eCO_2. These results provide a strong rationale and process understanding for incorporating N limitation and N feedback effects in ecosystem and global models used in climate change assessments.

Policy decisions to mitigate climate change require dependable predictions of the forcings and feedbacks to the climate provided by the terrestrial biosphere, particularly by forests[8]. Climate models that are coupled to terrestrial and oceanic C cycle models simulate a positive feedback to climate change such that the airborne fraction of anthropogenic CO_2 emissions increases with, and amplifies, climatic warming[1]. However, the uncertainty in these projections is high, largely because of uncertainty in the offsetting negative feedback that occurs if land C storage increases as atmospheric CO_2 concentration increases. Dynamic global vegetation models[9] simulate an increased terrestrial C sink resulting from the physiological responses of plants to eCO_2, and when coupled to climate models, inclusion of the CO_2 fertilization effect slows the increase in atmospheric CO_2 and the trajectory of climatic warming[3]. To reduce the large uncertainties in climate–C cycle projections, C cycle models must be constrained by observational data[1,8]. The AR4 models did not include N dynamics, which are predicted to induce a negative feedback on vegetation NPP response over the longer term[5]. N feedbacks have been observed to reduce the NPP response to eCO_2 in nutrient-poor grasslands[10], and severely N-limited forests show little response to eCO_2 [11], but few forest experiments have continued long enough to determine whether the observed stimulation of NPP would be sustained through time.

The Oak Ridge free-air CO_2 enrichment (FACE) experiment has exposed replicate plots in an established sweetgum (*Liquidambar styraciflua*) plantation forest to an atmosphere with ~550 ppm CO_2 continuously since 1998[12]. Results from the first 6 years of the experiment indicated that NPP was significantly enhanced by eCO_2 (Fig. 1a) and that this was a consistent and sustained response[4]. There was little enhancement of aboveground or wood production, however (Fig. 1b); the bulk of the NPP increase occurred as increased fine-root production, especially in the deeper soil profile[13]; Increased NPP was associated with greater C input to soil[14] and a gain in soil C[15], and it was this 6-year data set that was used in the FACE synthesis product[4]. Now, with 11 years of data, our analysis must be revised. The enhancement of NPP under eCO_2 relative to current ambient CO_2 (aCO_2) declined from 24% in 2001-2003 to 9% in 2008 (Fig. 1a). The diminishing response to eCO_2 since 2004, which coincides with declining NPP in both treatments, is confirmed by a significant CO_2 × year interaction in the NPP data (Fig. 1a). The observed decline in NPP is an expected expression of forest stand development; it can be caused by various environmental or internal factors, and the timing and amount of decline vary with inherent stand productivity[16].

We attribute the observed NPP decline in this ecosystem to a constraint imposed by limited N availability. Additions of N fertilizer to an adjacent part of the same plantation resulted in an immediate increase in aboveground production at the expense of fine roots[17], which was sustained even as tree growth in control plots (and in eCO_2 plots) was declining (Fig. 1b), thereby providing direct evidence that the forest stand was N limited. At the landscape level NPP is linearly related to foliar N concentration[18]. In our sweetgum forest, foliar [N] declined over time (Fig. 2a), so NPP would be expected to decline as well. Our analysis of the relationship between NPP and foliar [N] is guided by the predictions of a simple model of the stand's carbon-nitrogen-water economy[6, 19]. The model predicts an optimal balance with respect to foliar [N], stomatal conductance, and stand leaf area index (LAI) at which NPP is maximized. In aCO_2 and with prescribed water and N availability, the model predicts maximum NPP to occur at a foliar [N] of 16.6 mg g^{-1} (ref. 19). Beginning in 2001 when NPP was at its peak, foliar [N] was less than this optimum, and NPP declined linearly with declining foliar [N] (Fig. 2b); prior to 2001, foliar [N] exceeded this optimum and was not well correlated with NPP.

Nitrogen relationships also can explain the decline in NPP response to eCO_2. Foliar [N] was consistently lower in eCO_2 (Fig. 2a), but this was more than compensated by increased photosynthetic N-use efficiency such that NPP was enhanced over that in aCO_2[20]. As in aCO_2, NPP in eCO_2 declined with foliar [N] beginning in 2001 when NPP was at its peak and foliar [N] corresponded to the modelled optimum of 14.3 mg g^{-1}. However, the slope of the NPP-[N] relationship was steeper in eCO_2 than in aCO_2 (Fig. 2b), which explains the gradual loss of NPP response to CO_2 enrichment. Consistent with empirical observations, the model also shows annual N uptake to aboveground pools in the FACE experiment to decline steadily since 2001. The model predicts reduced CO_2 enhancement of photosynthesis and NPP and reduced foliar [N] over time as N uptake decreases, and it explains the N constraint on CO_2 enhancement of NPP from plant physiological considerations.

These stand-level observations and models have mechanistic support from measurements of leaf-level photosynthesis. Light-saturated photosynthetic rates were significantly greater ($P < 0.05$) at eCO_2 than at aCO_2 in 1999 (means ± s.e.m. were 14.5 ± 1.8 μmol m^{-2} s^{-1} vs. 10.4 ± 1.0 μmol m^{-2} s^{-1}), but photosynthesis was lower in both treatments in 2008 (7.6 ± 0.7 μmol m^{-2} s^{-1} vs. 6.4 ± 0.7 μmol m^{-2} s^{-1}), and there was no longer a significant stimulation by eCO_2. Reductions in leaf photosynthesis through time and with CO_2 treatment reflect differences in foliar [N] and reductions in the parameters of photosynthetic biochemistry, V_{cmax} and J_{max}, but there was no change in the relationships between V_{cmax} or J_{max} and foliar N on a leaf area basis (N_{area}), either with time or with CO_2 enrichment. N_{area} at the top of the canopy was reduced from 1999 to 2008, and was less in eCO_2 than in aCO_2; hence, V_{cmax} and J_{max} were reduced concomitantly. Additionally, leaf mass per unit leaf area (LMA) increased in eCO_2 and with time, which contributed to the decline in N_{mass}. Increased LMA in eCO_2 due to changes in leaf morphology can significantly reduce photosynthesis independently from the effect of LMA on N_{mass}[21].

The long-term relationship among NPP, eCO_2, and N has been predicted by the Progressive Nitrogen Limitation (PNL) hypothesis[22], whereby increased sequestration of N in long-lived biomass or soil pools under eCO_2 causes N availability to decline and induces a negative feedback on further productivity increases in eCO_2. Nitrogen uptake has been consistently greater in eCO_2[7], attributable to increased fine-root production and root proliferation deeper in the soil[14], but after accounting for the N demand for fine-root production, the increased root exploration has not been sufficient to support increased N supply for leaf metabolism and wood production. Annual sequestration of N into perennial tissue (wood) exceeded the input of exogenous N into this ecosystem, implying a drawing down of the soil N capital. N availability should have declined in both treatments. Because the larger N demand in eCO_2 was not compensated for by increased N mineralization in the top 10-15 cm[23,24], N availability should have declined faster in eCO_2. These predictions were confirmed by analysis of the $^{15}N/^{14}N$ ratio in fresh leaf litter (expressed as $\delta^{15}N$). More negative values of $\delta^{15}N$ indicate more N immobilization relative to N mineralization and lower N availability[25]. Leaf litter $\delta^{15}N$ in each plot declined linearly from 1998-2008 ($R^2 = 0.80$ to 0.94), and it declined more steeply in eCO_2 (slopes of -0.32 ± 0.001 ‰ yr^{-1} in eCO_2 compared to -0.17 ± 0.006 ‰ yr^{-1} in aCO_2, $P < 0.001$), indicating a CO_2 effect on N availability as suggested by the PNL hypothesis[22].

We have considered other possible explanations for the decline in NPP and the loss in capacity to respond to eCO_2 besides declining N availability. This FACE site had already reached canopy closure before the CO_2 exposure commenced, and maximum annual LAI did not differ significantly between treatments across years. Nevertheless, LAI and its integration over the growing season (leaf area duration, LAD), vary year to year as affected by variations in weather, and variation in LAD explains some of the variation in NPP prior to 2004 (year-to-year coefficient of variation reduced by one-third to one-half). The expression of NPP relative to LAD (Fig. 3) is similar to the Canopy Productivity Index (or growth efficiency[16]) previously proposed[26] as a robust indicator of growth response to eCO_2. The decline in NPP/LAD after 2004 and the loss of response to eCO_2 indicate that factors other than leaf area were responsible for the decline in productivity[16], and this evidence is consistent with a decline in light-use efficiency associated with N limitation. Progressively drier summers from 2004-2007 may be partially responsible for declining NPP, but NPP continued to decline in 2008 despite more mesic conditions. However, carryover effects of the 2007 drought into 2008, and indirect effects of soil moisture on N availability, cannot be dismissed. There has been no effect of eCO_2 on tree height that could have created hydraulic constraints to productivity[16], and sap flow did not decline over time as trees grew taller[27]. Other potential causes of forest growth decline such as reproduction, mortality, or crown abrasion[16] do not apply to this stand. N limitation remains the most likely causative factor.

It is not yet clear whether foliar [N] and CO_2 enhancement of NPP in this experimental forest stand will continue to decline or have reached a new steady state indicative of long-term forest response to eCO_2. Given the importance of the CO_2 fertilization effect in coupled climate-C cycle models that predict future climate change, there is an urgent need for further long-term experiments focusing on interactions between C and N cycles in forests. Failure to characterize these interactions and incorporate suitable algorithms into models will lead to unreliable predictions of the response of the terrestrial biosphere to atmospheric and climatic change. It may be fortuitous, but ultimately misleading, that models, which ignore the N cycle, have matched the previously reported FACE synthesis data, which preceded the onset of N limitation reported here. A longer record of experimental data and more sophisticated modeling are providing more dependable predictions of future responses.

Methods

The experiment was established in Oak Ridge, Tennessee, USA (35° 54' N; 84° 20' W) in 1997 in a fully established 10-year-old plantation forest of the deciduous, broadleaf tree, sweetgum (*Liquidambar styraciflua* L.). The experiment comprises five 25-m diameter plots, each with approximately 90 trees, an initial basal area of 29 m^2 ha^{-1} and peak LAI of 5.7 [12,27]. Beginning in April, 1998, two plots were exposed continuously during daylight hours throughout the growing season to an elevated concentration of CO_2 (~550 ppm) using FACE apparatus[28]. No N fertilizer has been added to the FACE plots; annual N deposition is 12-15 kg ha^{-1}. Urea fertilizer (200 kg N ha^{-1}) was added to replicated plots on an adjacent part of the plantation in early spring, 2004-2008 [17].

NPP was calculated as the sum of wood (bole, branch, and coarse root) increment, leaf litter production, and fine-root production[12,14]. Foliar N concentration was measured in leaves collected from throughout the canopy in August each year using a C-N analyzer[29]. NPP and foliar [N] data were analyzed statistically as repeated measures data using a mixed model with covariance structure chosen to minimize the AIC criterion. $^{15}N/^{14}N$ ratio in archived samples of freshly fallen leaf litter was determined on an Integra-CN, continuous flow, isotope ratio, mass-spectrometer (PDZ-Europa, Cheshire, United Kingdom). Ammonium sulfate ($\delta^{15}N$ = -0.4 ‰), traceable to NIST, was used as an internal standard for the stable isotope measurements.

Light-saturated photosynthetic rate was measured in upper canopy leaves using a LiCor 6400 system from 1998-2000 [20] and again in July 2008. Response curves of assimilation versus internal leaf CO_2 (A-C_i) were used to estimate maximum Rubisco activity, V_{cmax}, and potential electron transport rate, J_{max}, using a consistent set of leaf photosynthesis model equations[30].

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Author Information Data from the FACE experiment are publicly available at: http://public.ornl.gov/face/ORNL/ornl_home.shtml. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to R.J.N. (rjn@ornl.gov).

Fig. 1. Tree growth responses to elevated CO_2 and nitrogen fertilization (a) NPP (g dry matter (DM) per m^2 land area per year) was measured as the sum of stem and coarse root increment, leaf litter production, and fine-root production. Data shown are the means of three aCO_2 plots (open symbols) and two eCO_2 plots (solid symbols) ± s.e.m. The number at each point is the percentage increase

under eCO_2. Repeated measures analysis indicated significant effects of CO_2 ($P = 0.049$), year ($P < 0.001$), and CO_2 × year interaction ($P = 0.041$). (b) Annual increment in stem biomass in the FACE plots (solid lines) and in an adjacent section of the forest stand (dashed lines) where N fertilizer (200 kg N ha^{-1} as urea) was added annually beginning in April, 2004. Elevated CO_2 (solid circles) caused a significant increase in aboveground growth in the first year after treatment initiation (1998), but the response diminished in subsequent years and was not statistically different from FACE controls (open circles). N fertilization (shaded squares) caused an immediate and sustained increase in aboveground growth compared to unfertilized plots (open squares) ($P < 0.001$).

Fig. 2. Nitrogen relationships in FACE plots. (a) N concentration was measured in leaves throughout the canopy of each plot after canopy expansion was complete in July or August of each year. Data shown are the means of three aCO_2 plots (open symbols) and two eCO_2 plots (solid symbols) ± s.e.m. There was no difference prior to treatment initiation (1996-1997); subsequently, the effect of $[CO_2]$ was significant ($P = 0.043$) and there was no CO_2 × year interaction. (b) Beginning in 2001, NPP declined linearly with foliar N concentration. Data are values for each plot-year combination in aCO_2 (open symbols) and eCO_2 (solid symbols) from 2001 to 2008. Regression equations are: $\text{NPP} = 0.209 \times [\text{N}] - 1.67$ ($R^2 = 0.52$) in aCO_2 and $\text{NPP} = 0.371 \times [\text{N}] - 2.17$ ($R^2 = 0.71$) in eCO_2. The slopes are significantly different ($t = -2.54$, $df = 31$, $P = 0.0155$).

Fig. 3. NPP per unit leaf area duration (LAD). LAD was calculated by summing daily values of leaf area index for each growing season. Data shown are the means ± s.e.m. of three plots in aCO_2 (open symbols) and two plots in eCO_2 (solid symbols). CO_2 effect was significant ($P< 0.001$) from 1998-2004, but not significant ($P=0.117$) after 2004.

Fig. 1

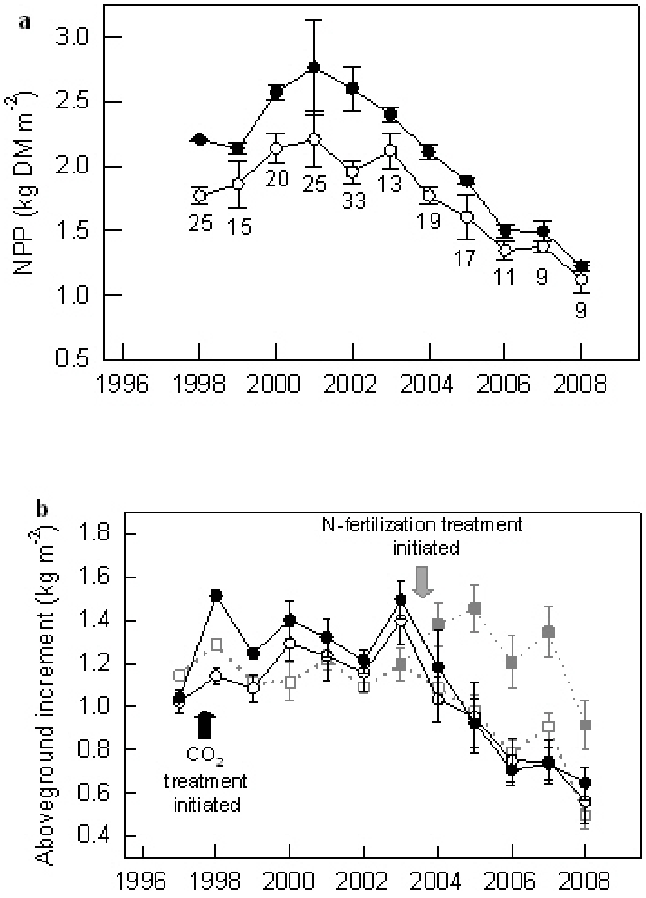

a
NPP (kg DM m^{-2})
3.0
2.5
2.0
1.5
1.0
0.5
25
15
20
25
33
13
19
17
11
9
9
1996
1998
2000
2002
2004
2006
2008
b
Aboveground increment (kg m^{-2})
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
N-fertilization treatment initiated
CO$_2$ treatment initiated
1996
1998
2000
2002
2004
2006
2008

Fig. 2

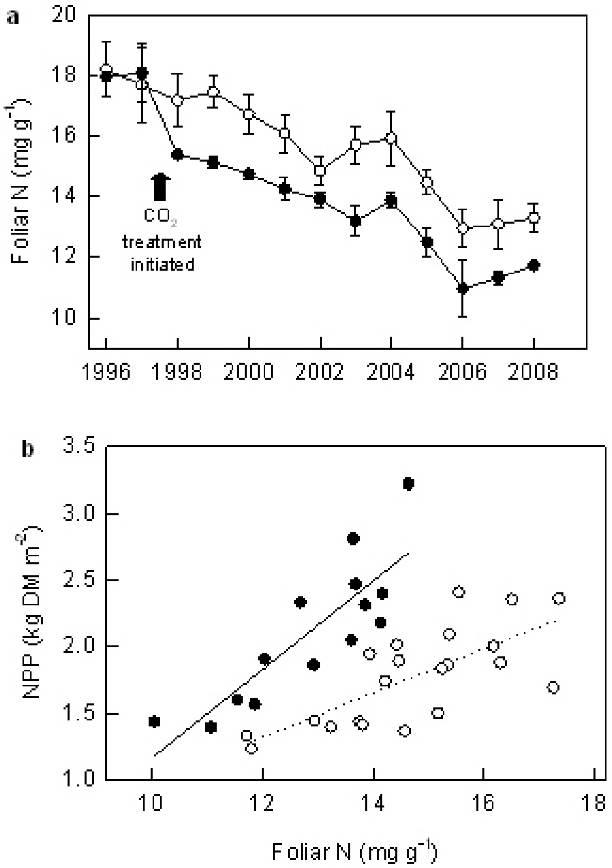
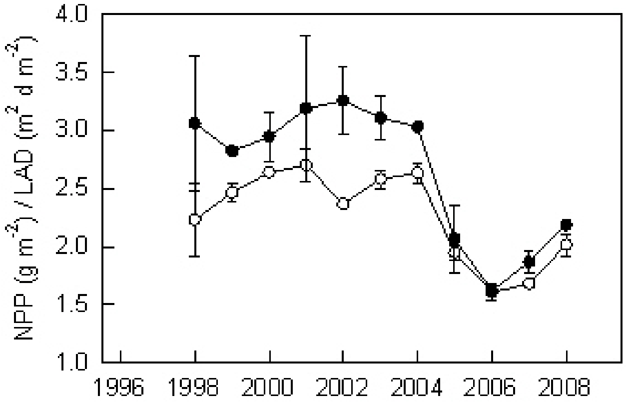

a
20
18
16
14
12
10
Foliar N (mg g^{-1})
CO$_2$
treatment
initiated
1996
1998
2000
2002
2004
2006
2008
b
3.5
3.0
2.5
2.0
1.5
1.0
NPP (kg DM m^{-2})
10
12
14
16
18
Foliar N (mg g^{-1})

Fig. 3



NPP (g m^{-2}) / LAD (m^{2} d m^{-2})
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1996
1998
2000
2002
2004
2006
2008