

Fire, hurricane and carbon dioxide: effects on net primary production of a subtropical woodland

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Summary

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Key words: disturbance, elevated CO₂, fire, global environmental change, hurricane, net primary productivity (NPP), nitrogen cycling, oak woodland.

- Disturbance affects most terrestrial ecosystems and has the potential to shape their responses to chronic environmental change.
- Scrub-oak vegetation regenerating from fire disturbance in subtropical Florida was exposed to experimentally elevated carbon dioxide (CO₂) concentration (+350 µl l⁻¹) using open-top chambers for 11 yr, punctuated by hurricane disturbance in year 8. Here, we report the effects of elevated CO₂ on aboveground and belowground net primary productivity (NPP) and nitrogen (N) cycling during this experiment.
- The stimulation of NPP and N uptake by elevated CO₂ peaked within 2 yr after disturbance by fire and hurricane, when soil nutrient availability was high. The stimulation subsequently declined and disappeared, coincident with low soil nutrient availability and with a CO₂-induced reduction in the N concentration of oak stems.
- These findings show that strong growth responses to elevated CO₂ can be transient, are consistent with a progressively limited response to elevated CO₂ interrupted by disturbance, and illustrate the importance of biogeochemical responses to extreme events in modulating ecosystem responses to global environmental change.

Introduction

Rising atmospheric carbon dioxide (CO₂) and nitrogen (N) deposition increase directly the resource availability to plants, and can increase plant growth and net primary productivity (NPP; Norby *et al.*, 2005). Natural disturbance also alters resource availability to plants, creating space for colonization and reducing competition for light after the catastrophic removal of biomass (Sousa, 1984), and mobilizing nutrients bound in organic matter to more available mineral forms (Martin *et al.*, 2012; Block *et al.*, 2013; Michalzik & Martin, 2013). Global environmental change occurs against a background of episodic disturbance and recovery, both altering resource availability and thus ecosystem processes. Despite the importance of the potential interactions between global environmental change and disturbance (Körner, 2006; Leuzinger *et al.*, 2011), very few experiments have evaluated how responses of ecosystems to elevated CO₂ interact with disturbance (Henry *et al.*, 2006; Niboyet *et al.*, 2011; Brown *et al.*, 2012).

The response of NPP to elevated CO₂ varies, at least in part, because the availability of other resources influences the response (Field *et al.*, 1992; de Graaff *et al.*, 2006; Reich *et al.*, 2006a,b;

Wang *et al.*, 2007). Elevated CO₂ can cause a sustained stimulation of NPP (Norby *et al.*, 2005; Drake *et al.*, 2011; Zak *et al.*, 2011), with interannual variation caused by precipitation (Owensby *et al.*, 1999; Smith *et al.*, 2000; Niklaus & Körner, 2004; Seiler *et al.*, 2009) and salinity (Rasse *et al.*, 2005). The stimulation of NPP by elevated CO₂ can be reduced or absent when nutrient availability is chronically low (Schäppi & Körner, 1996; Dukes *et al.*, 2005; Reich *et al.*, 2006a,b; Reich & Hobbie, 2012), or when nutrient availability declines over time (Norby *et al.*, 2010) as a result of progressive nutrient limitation, a theoretically inevitable influence on the response of NPP to elevated CO₂ (or to any growth enhancement), as increased growth and nutrient accumulation in organic matter reduce the nutrient supply to plants (Field, 1999; Luo *et al.*, 2004). N is often the focal element in discussions of progressive nutrient limitation, but the concept applies to any nutrient element limiting to plant growth where the decomposition of plant litter is a major source of the element for plants (e.g. phosphorus, P). Increases in productivity under elevated CO₂ by higher N-use efficiency (NUE; Calfapietra *et al.*, 2007; Finzi *et al.*, 2007; Norby *et al.*, 2010) can mitigate progressive nutrient limitation via flexibility in plant element stoichiometry. Increased nutrient uptake with elevated CO₂ indicates

the mobility and redistribution of nutrients from soils to plants (Johnson *et al.*, 2006; Drake *et al.*, 2011), increased nutrient inputs (Zanetti & Hartwig, 1997) or reduced nutrient losses (Hagedorn *et al.*, 2005), all of which can alleviate progressive nutrient limitation (Luo *et al.*, 2004). In addition, episodic disturbance could reset progressive nutrient limitation by interrupting the plant-mediated accrual of nutrients in organic matter and converting nutrients to available forms. For example, fire disturbance alleviated the suppressive effect of elevated CO₂ on NPP in an annual grassland, possibly by increasing P availability (Henry *et al.*, 2006).

Here, we report the effects of 11 yr of experimental CO₂ enrichment on NPP and N cycling in a subtropical scrub-oak woodland. Our goal in this work was to evaluate the response of NPP in this scrub-oak woodland to elevated CO₂ after recovery from fire disturbance. A hurricane struck the experiment 8 yr after the fire, providing an additional opportunity to assess the role of disturbance in modulating the responses to elevated CO₂.

Materials and Methods

This work was conducted at the Kennedy Space Center, Cape Canaveral, FL, USA (28°38'N, 80°42'W) in a stand of scrub-oak vegetation managed for a fire return cycle approximately every decade (Schmalzer & Hinkle, 1992). In the 20th century, hurricanes made landfall in the state 35 times (Smith, 1999), and thus hurricanes are another frequent agent of disturbance at the site (Li *et al.*, 2007). The climate at the site is subtropical (Mailander, 1990). The 30-yr mean maximum and minimum temperatures are 22.3 and 9.6°C for January and 33.3 and 21.9°C for July. The 100-yr mean precipitation is 1310 mm yr⁻¹, most falling in the wet season from June to October.

The experiment consisted of 16 open-top chambers, built of PVC frames with Mylar windows. The chambers were 2.5 m high with an octagonal surface area of 9.42 m². Blowers distributed air through the chambers, either ambient air or air supplemented with +350 µl CO₂ l⁻¹. The soils at the site are classified as Arenic Haplahumods and Spodic Quartzipsamments, are sandy with a low pH (*c.* pH 4). The vegetation is coastal scrub-oak palmetto, dominated by three oaks (*Quercus myrtifolia* Willd., *Q. geminata* Small and *Q. chapmanii* Sargentii), which accounted for 96% of the aboveground biomass at the site before the experiment began (Dijkstra *et al.*, 2002). Less abundant species include *Serenoa repens* Small, *Vaccinium myrsinites* Lam., *Lyonia ferruginea* Nutt., *Befaria racemosa* Vent. and *Galactia elliottii* Nutt. (Dijkstra *et al.*, 2002; Johnson *et al.*, 2003; Seiler *et al.*, 2009). Many of the species at the site, including the three oaks, resprout from rhizomes after fire disturbance (Webber, 1935; Schmalzer & Hinkle, 1992; Guerin, 1993).

Controlled burns at the site were conducted in August 1995 and January 1996. Biomass that accumulated after the January burn and was clipped at ground level immediately before initiating the CO₂ treatments. Thus, recovery from the first major disturbance at the site began with treatment initiation on 14 May 1996. In the fall of 2004, the site was struck by a severe hurricane (Frances) on 5 September 2004, with sustained wind speeds of 169 km h⁻¹ (category II). The storm subsided on 9

September 2004. These dates were used as the time-zero points for our analysis of the responses of the ecosystem to elevated CO₂ as a function of disturbance.

Biomass, carbon and nitrogen

Total aboveground biomass was estimated from annual surveys in which the diameter of each individual oak stem was measured in all plots (Dijkstra *et al.*, 2002; Seiler *et al.*, 2009). Total aboveground biomass was calculated using allometric relationships developed previously, relating the stem diameter and total mass for each of the three oak species (Seiler *et al.*, 2009). We developed new relationships to describe the partitioning of aboveground biomass into leaf and stem mass. Oak stems collected before the first burn in 1995 and at the final harvest in 2007 were divided into leaves and stems, the dry mass of each fraction was determined, and the leaf mass fraction was calculated as the mass of leaves divided by the total aboveground biomass. Data were binned into increments of 0.1 log_e (diameter), and the relationship between leaf mass fraction and log_e (diameter) was determined using linear regression on the binned means (Table 1).

Coarse root biomass was measured directly through a combination of coring and ground penetrating radar in 2002, 2005 and for the final harvest in 2007 (Brown *et al.*, 2007; Stover *et al.*, 2007; Day *et al.*, 2013). For years in which direct measurements were not available, coarse root biomass was estimated from aboveground stem biomass, based on the relationship derived from the simultaneously measured values of both variables:

$$\text{Coarse root biomass} = \text{stem biomass} \times 1.9845 + 3132 \text{ g m}^{-2}.$$

Eqn 1

The regression was significant (intercept, $P < 0.001$; slope, $P = 0.025$), but the coefficient of determination was low ($r^2 = 0.16$), and the standard errors were non-negligible: 0.8403 for the slope and 797 for the intercept. With carbon (C) turnover through coarse roots estimated at 0.03 yr⁻¹ (based on the ¹³C tracer described below), it is unlikely that coarse root NPP is extremely dynamic. Even so, these should be considered as rough estimates of coarse root biomass and NPP, and viewed with appropriate caution.

Table 1 Allometric equations^a predicting leaf mass fraction (leaf mass/total aboveground mass) from stem diameter for each of the three oak species

Species	Slope	Intercept	r^2	N (n)
<i>Quercus myrtifolia</i>	-0.4129	-0.2767	0.921	29 (655)
<i>Quercus chapmanii</i>	-0.4345	-0.1660	0.900	30 (301)
<i>Quercus geminata</i>	-0.4715	-0.0269	0.890	15 (82)

^aEquations are of the form: log_e(leaf mass fraction) = slope × log_e(stem diameter) + intercept. Coefficient of determination (r^2) is shown, together with the number of samples included in the regression (binned data, N) and in the total sample (number in parentheses, n).

Fine roots were monitored throughout the experiment using cores and minirhizotron images. Fine root samples were collected from cores in 1997, 1998, 1999, 2000, 2001, 2002 and 2007. Frequent sampling was suspended in 2002 to avoid excessive destructive coring in the plots. Surface cores (0–15 cm) were collected during the first 5 yr. More extensive depth profiles were sampled in 2002 (to 1 m) and 2007 (to 2–3 m, depending on the depth of the Bh horizon in each plot). Roots were hand-picked, washed, oven dried and weighed. These data are presented elsewhere (Langley *et al.*, 2003; Day *et al.*, 2006; Stover *et al.*, 2007; Brown *et al.*, 2009), but are included here as essential components for the calculation of NPP and N cycling. When multiple measurements of fine root biomass were available for a given year, we used the average in our calculations of NPP and N requirement. The average was selected because not all years had sufficient data to identify the annual maximum. The higher frequency of minirhizotron observations gives this technique more influence in the calculations relative to data from the soil cores.

Leaf and stem tissue samples were collected each year for *Q. myrtifolia*, in each year except 2005 for *Q. geminata*, and in each year except 2000, 2001 and 2005 for *Q. chapmanii*. Element concentrations in *Q. chapmanii* and *Q. myrtifolia* were similar, and so the values of the latter were used when measurements of the former were unavailable. For *Q. geminata* in 2005, values were estimated as the average values from 2004 and 2006 for each individual plot. Samples of fine roots for tissue analysis were available from the core samples taken in 1997–2002 and 2007. Coarse roots for tissue analyses were collected from the 1–3-m-deep cores in 2002 and 2007.

Percentage C, %N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of tissue samples were determined at the Colorado Plateau Analytical Laboratory using an elemental analyzer (Costech CE 2100, Valencia, CA, USA) coupled to an isotope ratio mass spectrometer (ThermoFinnigan Delta-PLUS, Delta-PLUS XL or Delta-V, Bremen, Germany). Plant element mass (g C or g N m^{-2}) was calculated as the product of biomass and element concentration for each plant component (leaves, stems, fine roots, coarse roots, litterfall). A ^{15}N tracer was applied directly to the soil surface of all plots in June 1998 at a rate of $0.18 \text{ g }^{15}\text{N m}^{-2}$ as aqueous 0.1 g N l^{-1} ($^{15}\text{NH}_4$) $_2\text{SO}_4$ (99 atom% ^{15}N) using hand-held sprayers. For all organic matter samples collected, tracer ^{15}N mass ($\text{mg }^{15}\text{N m}^{-2}$) was calculated as the atom% excess ^{15}N concentration (measured atom% ^{15}N minus natural abundance ^{15}N) times the mass of N (g N m^{-2}) in the organic matter compartment in question. C, N and ^{15}N in coarse roots were measured directly in 2002 and 2007, and in fine roots in 1998, 1999, 2001, 2002 and 2007; values for the other years were interpolated linearly through time.

Biomass increment and litter production

The annual increments in plant biomass, C and N were calculated for leaves, stems, fine and coarse roots as the difference in average biomass (or C or N) for year $t+1$ minus average biomass in year t .

The annual flux of leaf litter was measured from litterfall collections. Litter traps were placed in each plot and monitored at a

frequency no less than four times per year throughout the experiment. Litter that fell into the traps was sorted by species, dried and weighed (Johnson *et al.*, 2003; Hungate *et al.*, 2006; Stiling *et al.*, 2009). Before 2002, *Q. myrtifolia* and *Q. chapmanii* were not distinguished in the sorting; for this period, we estimated litterfall as a proportion of leaf mass for these species. Stem litter production was assumed to be zero, based on observations of littertrap contents in which stem tissue was never observed. This is an underestimate because standing dead biomass was recovered at the end of the experiment. However, it was a small fraction of the total chamber biomass ($4.5 \pm 1.2\%$), and did not impact appreciably on our estimates of productivity.

Fine and coarse root litter production were calculated as the product of biomass and turnover rate. Turnover rates of fine and coarse roots were measured using a C tracer approach. The fossil-derived CO_2 added to the elevated CO_2 -treated plots was depleted in ^{13}C relative to atmospheric CO_2 , providing a C isotope tracer: the $\delta^{13}\text{C}$ of plant C fixed after initiation of the CO_2 treatment was *c.* 15‰ lower than that of organic C already present in the ecosystem before the experiment began (Langley *et al.*, 2002). We used the initial rate of incorporation of tracer-derived C into fine and coarse roots to estimate root litter production in $\text{g C m}^{-2} \text{ yr}^{-1}$. Fine roots were collected from ingrowth cores in December 1998 and measured for $\delta^{13}\text{C}$ (Langley *et al.*, 2002); coarse roots were collected in April 2002 and also measured for $\delta^{13}\text{C}$. Coarse root tissue from the ambient CO_2 plots was used as a proxy for the $\delta^{13}\text{C}$ value of 'old' C, and stem tissue from the elevated CO_2 plots was used as an integrative measure of the $\delta^{13}\text{C}$ value of new C input to the ecosystem incorporating the depleted ^{13}C tracer. C turnover (T) through roots was calculated as:

$$T = \frac{(\delta^{13}\text{C}_{\text{Re}} - \delta^{13}\text{C}_{\text{Ra}})}{\delta^{13}\text{C}_{\text{Se}} - \delta^{13}\text{C}_{\text{Sa}}} \cdot \frac{1}{t}, \quad \text{Eqn 2}$$

where $\delta^{13}\text{C}$ is the C isotope signature of roots (R) or stems (S) from the elevated (e) or ambient (a) treatments, and t is the duration of the experiment up to the time of sampling – the time during which the depleted ^{13}C tracer could be incorporated into root tissue. Using this approach, C turnover through fine roots was 0.247 yr^{-1} , and through coarse roots was 0.030 yr^{-1} . These rates are substantially lower than those estimated for fine roots from minirhizotron observations (Stover *et al.*, 2010), a discrepancy often observed (Strand *et al.*, 2008). We favored the approach based on isotopic tracer estimates of C flow, because it is more relevant to ecosystem–atmosphere C exchange (Strand *et al.*, 2008).

Net primary productivity, nitrogen cycling and nitrogen-use efficiency

NPP was calculated as the sum of the net biomass increment and litter production. NPP was expressed in units of biomass ($\text{g biomass m}^{-2} \text{ yr}^{-1}$) and in units of C mass ($\text{g C m}^{-2} \text{ yr}^{-1}$). The N requirement was calculated as the sum of the N increment and N loss in litter production in all measured plant components.

Retranslocation of N during leaf senescence was calculated as the live-equivalent mass of N in litterfall minus the actual N flux of senesced leaves in litterfall (for further details, see Hungate *et al.*, 2006). Retranslocation from fine roots was assumed to be negligible. N uptake was calculated as N requirement minus retranslocation. We estimated NUE as the slope of the relationship between NPP and N uptake, and measured the components of NUE: N productivity was calculated as NPP ($\text{g biomass m}^{-2} \text{yr}^{-1}$) divided by total plant N content (g N m^{-2}), and N residence time (yr) was calculated as total plant N content (g N m^{-2}) divided by total plant N uptake ($\text{g N m}^{-2} \text{yr}^{-1}$).

Soil nutrients

We determined K_2SO_4 extractable N in mineral soil (0–15 cm), the sum of extractable inorganic and organic N, at multiple times during the experiment (July 1997; June, July, September and December 1998; September 1999; and May 2004). Soil subsamples (20–25 g at field moisture content) were extracted in 75 ml 0.5 M K_2SO_4 and filtered through a paper filter, and extracts were dried at 60°C, ground in a mortar and pestle, and the resulting powder was analyzed for %N by Dumas combustion (<http://www.isotope.nau.edu>). Extractable N is expressed as g N m^{-2} ground area using the surface bulk density at the site of 0.83 g cm^{-3} (Hungate *et al.*, 2006). Previously published data on soil P (Johnson *et al.*, 2003) are presented and re-analyzed here as a function of time since disturbance, to assess the role of disturbance on soil nutrient availability (see Data analysis, below).

Data analysis

We compared the importance of increased N uptake and increased NUE in the productivity response to elevated CO_2 . The hypothetical response driven by increased N uptake (ΔNPP_u) was calculated as:

$$\Delta\text{NPP}_u = \Delta U \times E_a, \quad \text{Eqn 3}$$

where ΔU is the effect of elevated CO_2 on N uptake (elevated minus ambient, $E - A$) and E_a is the NUE observed in the ambient CO_2 treatment. Similarly, the hypothetical response driven by increased NUE (ΔNPP_e) was calculated as:

$$\Delta\text{NPP}_e = \Delta E \times U_a, \quad \text{Eqn 4}$$

where ΔE is the effect of elevated CO_2 on NUE ($E - A$) and U_a is the N uptake observed in the ambient CO_2 treatment.

We analyzed NPP, %N concentration in oak stems and soil nutrient availability as a function of time since disturbance. We calculated the time since disturbance as the number of years elapsed between the date of the measurement and the most recent disturbance, whether by fire at the beginning of the experiment or by hurricane in September 2004. We expressed the effect of elevated CO_2 as the absolute difference between elevated and ambient CO_2 plots.

We used a combination of analysis of variance (ANOVA) and resampling to draw inferences about the effects of elevated CO_2 . We used a repeated-measures ANOVA design to analyze time series. We accepted an α value of 0.1. We also used resampling to estimate the magnitude and confidence limits for the observed effects of CO_2 , using resampling with replacement, $n = 8$ and 1000 resamples of the mean, difference between means, relative effect sizes or slope of the relationship between effect sizes and time since disturbance. We estimated 5% and 95% confidence limits as the 5th and 95th percentiles in the resampled population.

Results

Net primary productivity

Elevated CO_2 increased NPP (repeated-measures ANOVA, Tables 2, 3, Fig. 1), but the effect was pronounced in some years and absent in others. The CO_2 effect on total NPP was most apparent in the first (1996), second (1997) and tenth (2005) years of treatment. In these years, the 90% confidence intervals (CIs) for the effect of CO_2 on NPP were positive and did not include zero (Table 3). Excluding these years with unusually strong responses (+142% for 1997 and +135% for 2005), the average effect of elevated CO_2 on NPP was 5%. Elevated CO_2 increased aboveground and belowground NPP (Tables 2, 3,

Table 2 *P* values from repeated-measures analysis of variance of aboveground, belowground and total net primary productivity (NPP; $\text{g C m}^{-2} \text{yr}^{-1}$)

NPP, carbon	CO_2	Year	Year \times CO_2
Aboveground	0.006	0.018	0.879
<i>Quercus myrtifolia</i>	0.078	0.399	0.922
<i>Q. geminata</i>	0.959	0.014	0.516
<i>Q. chapmanii</i>	0.036	0.034	0.623
Leaves	0.014	< 0.001	0.091
<i>Q. myrtifolia</i>	0.125	< 0.001	0.053
<i>Q. geminata</i>	0.964	< 0.001	0.764
<i>Q. chapmanii</i>	0.046	0.003	0.334
Litter production	0.017	< 0.001	0.079
<i>Q. myrtifolia</i>	0.149	< 0.001	0.073
<i>Q. geminata</i>	0.916	< 0.001	0.437
<i>Q. chapmanii</i>	0.048	< 0.001	0.054
Increment	0.012	< 0.001	0.354
<i>Q. myrtifolia</i>	0.091	< 0.001	0.473
<i>Q. geminata</i>	0.905	< 0.001	0.929
<i>Q. chapmanii</i>	0.147	0.002	0.275
Stem increment	0.001	0.001	0.408
<i>Q. myrtifolia</i>	0.033	< 0.001	0.997
<i>Q. geminata</i>	0.813	0.208	0.387
<i>Q. chapmanii</i>	0.055	0.030	0.500
Belowground	0.022	< 0.001	0.600
Coarse root	0.014	0.079	0.999
Litter production	0.056	< 0.001	< 0.001
Increment	0.010	0.122	0.999
Fine roots	0.375	< 0.001	0.614
Litter production	0.216	< 0.001	0.076
Increment	0.625	< 0.001	0.677
Total	0.005	< 0.001	0.461

Bold indicates significant effects ($P < 0.05$).

Table 3 Effect of elevated CO₂ on net primary productivity (NPP): total (NPP), aboveground (ANPP) and belowground (BNPP) (elevated minus ambient, E – A, g C m⁻² yr⁻¹, with 5% and 95% confidence limits^a)

	NPP	ANPP	BNPP
1996	65 (21 to 111)	19 (–20 to 56)	46 (–11 to 99)
1997	662 (350 to 1030)	70 (–8 to 155)	592.1 (319 to 882)
1998	232.4 (–298 to 750)	71.6 (38 to 106)	160.8 (–330 to 637)
1999	–5 (–474 to 479)	94.3 (44 to 151)	–99.6 (–548 to 327)
2000	–132 (–1279 to 986)	59 (–4 to 121)	–191 (–1332 to 986)
2001	–102 (–758 to 506)	44 (–34 to 125)	–145.8 (–750 to 487)
2002	216.9 (–241 to 642)	50 (–24 to 117)	167.3 (–281 to 546)
2003	–58 (–367 to 264)	12.3 (–30 to 60)	–70.8 (–362 to 236)
2004	172 (–28 to 366)	71.3 (11 to 130)	101 (–48 to 273)
2005	810 (218 to 1531)	102 (32 to 178)	708 (168 to 1343)
2006	–298 (–735 to 145)	56 (–36 to 163)	–354.5 (–808 to 75)
2007	–31 (–317 to 267)	120 (11 to 218)	–151 (–413 to 113)

^aBold indicates confidence intervals that do not overlap zero.

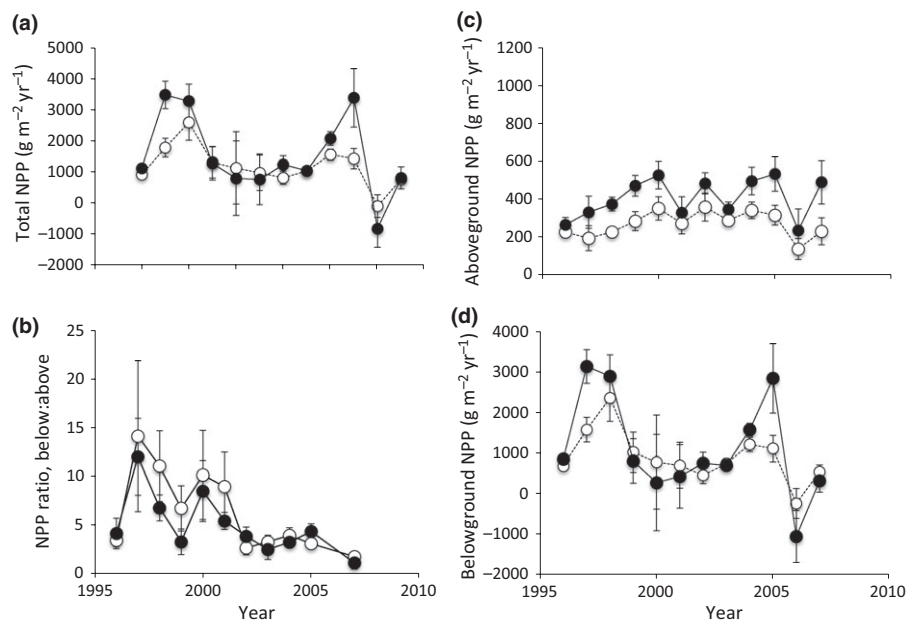


Fig. 1 (a) Total net primary productivity (NPP), (b) ratio of below- to aboveground NPP, (c) NPP aboveground and (d) NPP belowground during 11 yr of CO₂ enrichment of a subtropical oak woodland. Symbols are means ± 2 SE (*n* = 8). Open circles, ambient; closed circles, elevated.

Fig. 1). As with total NPP, positive effects of elevated CO₂ were more pronounced (i.e. 90% confidence limits not overlapping zero) early in the experiment and after the hurricane disturbance (Table 3).

Plants allocated more NPP below ground than above ground (Fig. 1, Supporting Information Table S1). Across all sites and times, the ratio of belowground to aboveground NPP was 5.5 ± 0.6 (mean ± standard error). The relative investment of NPP to structures responsible for resource acquisition was higher below ground than above ground, with 72% of belowground productivity occurring in fine roots compared with 45% of aboveground productivity occurring in leaves. The responses of fine roots to CO₂ varied over time, with increases (e.g. 1997–1998, 2005) followed by sharp declines (1999–2000, 2006, Table S1). Compared with belowground NPP, aboveground NPP was less dynamic (Fig. 1).

The three oak species responded differently to CO₂ exposure. Relative to *Q. geminata*, the aboveground production of *Q. myrtifolia* and *Q. chapmanii* was more responsive to elevated

CO₂, with many (although not all) components of aboveground NPP exhibiting significant positive responses (Tables 2, 3). By contrast, no productivity component of *Q. geminata* responded significantly to elevated CO₂ (Table 2), nor were any interactions between CO₂ and year significant for this species. There was no evidence that the positive growth responses of *Q. myrtifolia* and *Q. chapmanii* reduced the growth of *Q. geminata* through competition: increased growth of the two responsive species was not associated with any reduced growth in *Q. geminata*.

Nitrogen

Elevated CO₂ increased total plant N uptake during some years, in concert with the effects of elevated CO₂ on NPP (Tables 4, S2). CO₂ increased N uptake strongly early in the experiment (year 2) and after the 2004 hurricane (Fig. 2). CO₂ increased total aboveground N uptake, especially by the subdominant *Q. chapmanii* (Tables 4, S2). N uptake by *Q. myrtifolia* did not increase significantly in response to elevated CO₂, but tended to

Table 4 *P* values from repeated-measures analysis of variance of nitrogen uptake and translocation

N retranslocation	CO ₂	Year	Year × CO ₂
Aboveground	0.040	< 0.001	0.085
<i>Quercus myrtifolia</i>	0.420	< 0.001	0.180
<i>Q. geminata</i>	0.892	< 0.001	0.646
<i>Q. chapmanii</i>	0.126	< 0.001	0.102
N uptake			
Aboveground	0.019	< 0.001	0.259
<i>Q. myrtifolia</i>	0.110	< 0.001	0.128
<i>Q. geminata</i>	0.810	0.013	0.439
<i>Q. chapmanii</i>	0.035	0.066	0.680
Belowground	0.486	< 0.001	0.520
Coarse root	0.612	0.306	0.137
Litter production increment	0.947	< 0.001	0.303
	0.519	0.415	0.141
Fine roots	0.531	< 0.001	0.587
Litter production increment	0.442	< 0.001	0.023
	0.887	< 0.001	0.668
Total	0.079	< 0.001	0.550

Bold indicates significant effects ($P < 0.05$).

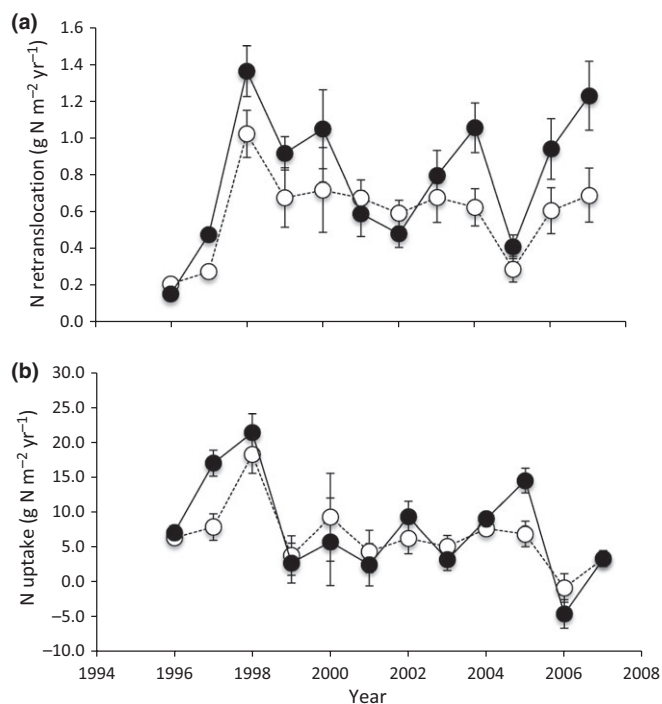


Fig. 2 (a) Nitrogen (N) retranslocation and (b) N uptake during 11 yr of CO₂ enrichment of a subtropical oak woodland. Symbols are means ± 2 SE ($n = 8$). Open circles, ambient; closed circles, elevated.

be higher and, combined with the significant increase shown by *Q. chapmanii*, contributed to the significant increase in total aboveground N uptake by all the oaks combined. Total belowground N uptake was insensitive to elevated CO₂; the only component of belowground N uptake to respond significantly was fine root litter production, for which responses varied over time (Tables 4, S2).

Elevated CO₂ increased total N retranslocation during leaf senescence (Table 4, Fig. 2), but did not consistently increase the

proportion of N retranslocated, which averaged *c.* 30% in both treatments (data not shown). Thus, the effect of elevated CO₂ on N translocation was not a function of increased retranslocation efficiency, but rather reflects the CO₂ stimulation of leaf production and thus a greater pool of leaf N from which reabsorption could occur.

Elevated CO₂ caused a more rapid decline in the ¹⁵N content of plants (Fig. 3), indicating more rapid N turnover. Part of this effect was driven by increased dilution of ¹⁵N in plant tissues ($P < 0.05$), indicating that plants in the elevated CO₂ treatment accessed N not available to plants in the ambient CO₂ treatment.

Elevated CO₂ increased N productivity (Fig. 4); the 90% CI for the difference between treatments (E – A) was positive and did not overlap zero for 1996, 1997, 2004 and 2005. Across all years, N productivity was 12.6 g biomass yr⁻¹ g⁻¹ N higher in the elevated relative to the ambient CO₂ treatment (90% CI, –1.3 to 35.6 g biomass yr⁻¹ g⁻¹ N). Elevated CO₂ had no effect on N residence time in plant tissues (Fig. 4), with a mean difference between the elevated and ambient treatments across all years of –0.4 yr (90% CI, –2.4 to 2.3 yr). Elevated CO₂ increased NUE, the product of N productivity and N residence time (Fig. 4). Averaged across all years, NUE was 164 ± 8 g biomass g⁻¹ N for the ambient CO₂ treatment and 188 ± 11 g biomass g⁻¹ N for the elevated CO₂ treatment ($P = 0.054$).

Increased NUE played a minor role in explaining the N economy underpinning positive NPP responses to elevated CO₂. The effect of elevated CO₂ on N uptake explained nearly all of the observed large stimulation of NPP found in the years 1997 and 2005 (Fig. 5). Thus, acquisition of N from the environment was associated with (and perhaps necessary for) the strong responses of NPP to elevated CO₂. In 2005, the year with the strongest NPP response after application of the ¹⁵N tracer, the dilution of ¹⁵N in plant tissues was less apparent relative to other years (Fig. 3). Thus, the large CO₂ stimulation of NPP and N uptake for this year involved increased uptake of N from the pools of soil N labeled by the tracer, which are probably the rapidly cycling pools of soil organic matter.

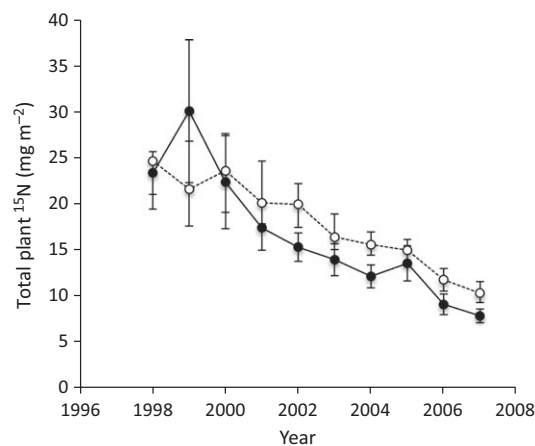


Fig. 3 ¹⁵N content in plant biomass over time (mg ¹⁵N m⁻²). Symbols are means ± 2 SE ($n = 8$). Open circles, ambient; closed circles, elevated.

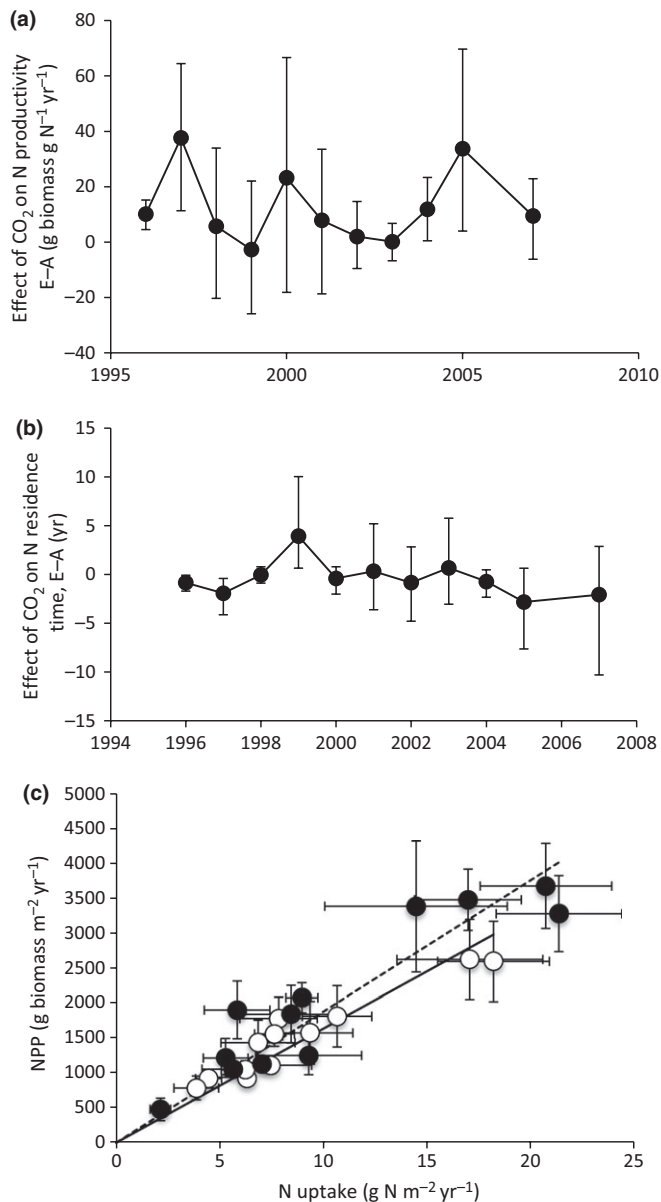


Fig. 4 Effect of the CO₂ treatment on components of nitrogen use efficiency. (a) The absolute effect of elevated CO₂ on nitrogen productivity per unit N in plant tissue and (b) the absolute effect of elevated CO₂ on N residence time, which is plant N content divided by N uptake. Symbols for (a) and (b) show the mean effect size, elevated – ambient, and bars show the 90% confidence intervals (CIs) determined by resampling. (c) Net primary productivity (NPP) as a function of N uptake. The slopes of the relationships are nitrogen use efficiency (g biomass g⁻¹ N) for the ambient (solid) and elevated (dashed) treatments. Open circles, ambient; closed circles, elevated.

Disturbance

The strongest effects of elevated CO₂ on NPP occurred following disturbance, whether by fire at the initiation of the experiment, or by hurricane after 8 yr of exposure to the CO₂ treatment (Fig. 6). Within the time scale of our annual resolution of NPP, responses of NPP to elevated CO₂ exhibited a similar time constant across the two disturbance events, with the maximum response 1–2 yr after the disturbance (Fig. 6). After these peak

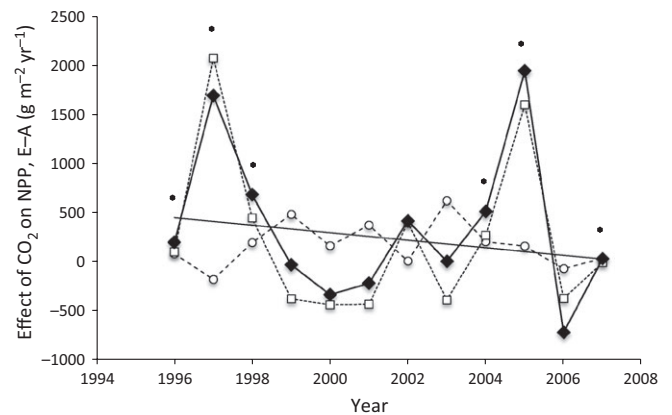


Fig. 5 Observed effect of elevated CO₂ on net primary productivity (NPP; diamonds, observed), and effects explained by altered N uptake (squares, N uptake only) or by altered nitrogen use efficiency (circles, NUE only). Asterisks indicate years in which elevated CO₂ altered significantly aboveground, belowground or total NPP.

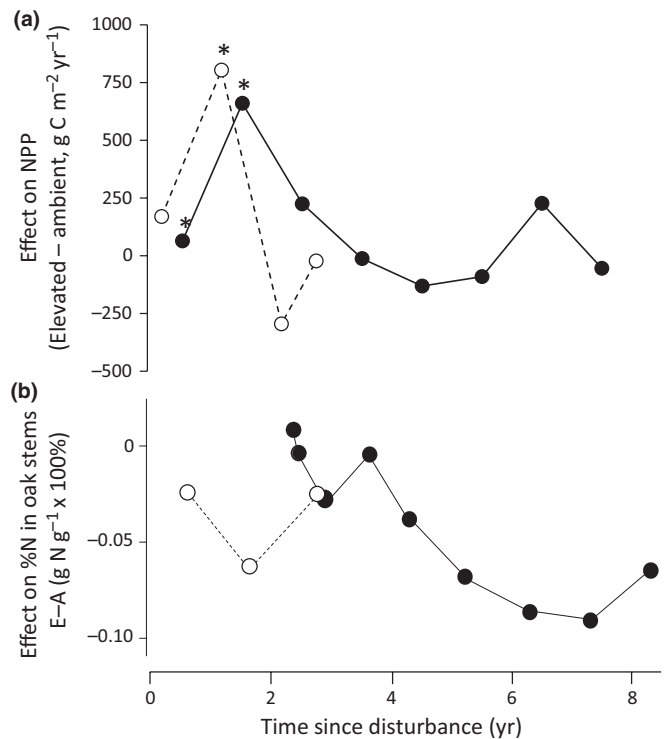


Fig. 6 Temporal pattern of the effect of elevated CO₂ on total net primary productivity (NPP) (a) and %N in stems (b) as a function of time since disturbance, highlighting the roles of the initial fire disturbance before the experiment was established, and disturbance by hurricane in September 2004, 7.4 yr later. Responses are plotted as absolute effect sizes, elevated CO₂ treatment – ambient CO₂ treatment. Asterisks indicate years in which the effect of CO₂ on NPP was statistically significant. Closed circles, fire; open circles, hurricane.

responses, the effect of elevated CO₂ on NPP declined over time at a rate of 77 g C m⁻² yr⁻¹ (bootstrapped 90% CI, 1–148 g C m⁻² yr⁻¹). Nutrient availability also peaked and then declined following disturbance: total extractable soil N was high after fire disturbance and subsequently declined, and ortho-P captured on resin lysimeters exhibited a similar pattern (Fig. 7;

although this was not the case for inorganic N captured on resin lysimeters; Johnson *et al.*, 2003). Soil extractable micronutrients, including molybdenum, vanadium and iron, were high in 2005, the year following hurricane disturbance, and declined in 2006 and 2007 (B. A. Hungate, unpublished). There was some evidence that the pulse of nutrient availability post-disturbance influenced the response of the oaks to elevated CO₂: the effect of elevated CO₂ on N concentration in oak stems became increasingly negative over time after disturbance (Fig. 6), although this relationship was not as clear for leaves (data not shown). The slope of the effect of elevated CO₂ on stem %N vs time was negative ($-0.010\% \text{N yr}^{-1}$; CI, -0.005 to $-0.014\% \text{N yr}^{-1}$), and the intercept was not significantly different from zero (CI, -0.025 to $0.018\% \text{N}$). Thus, in the first several years after disturbance, elevated CO₂ had little effect on the N concentration of oak stems, but, with increasing time since disturbance, the reduction in N concentration in oak stems became more pronounced.

Discussion

In this experiment, elevated CO₂ ($+350\ \mu\text{l l}^{-1}$) caused an average stimulation of NPP of 24%, comparable with that observed in other experiments on trees (Norby *et al.*, 2005). Yet, this average response was far less striking than its year-to-year variation, where negligible responses to the elevated CO₂ treatment in most years (averaging 5%) were punctuated by a few years with very strong CO₂ enhancements of NPP ($> 100\%$). Such extreme interannual variation in the response of NPP to elevated CO₂ is unusual: most experiments show less variable responses (Dukes *et al.*, 2005; Norby *et al.*, 2005; Reich *et al.*, 2006b). However, episodic responses have been observed previously. For example, a strong CO₂ stimulation of productivity by an invasive annual grass and the dominant shrubs in a desert ecosystem were associated with high rainfall years (Smith *et al.*, 2000; Housman *et al.*, 2006), whereas overall growth responses in this ecosystem were absent (Newingham *et al.*, 2013). In the scrub-oak experiment described here, years with high precipitation also promoted a stronger aboveground growth response to elevated CO₂, particularly in the more responsive species, *Q. myrtifolia* and *Q. chapmanii* (Seiler *et al.*, 2009). Thus, climate partially contributed to the temporal variability in the CO₂ response observed here.

Although climate played a role, the episodic responses of NPP to elevated CO₂ were more strongly associated with disturbance (Fig. 6). Disturbance can cause resource pulses, including pulses of soil nutrients (Matson & Vitousek, 1981), and disturbance is a strong selective agent, often favoring plants with high allocation below ground and plant strategies enabling recovery, such as the ability to resprout (Clarke *et al.*, 2013). The observed pattern of NPP responses to elevated CO₂ following disturbance may be explained by the plant strategies that have evolved in response to episodic disturbance regimes and by the effects of disturbance on nutrient availability.

Resprouting occurred immediately after fire (Dijkstra *et al.*, 2002) and refoliation immediately after hurricane (Li *et al.*, 2007); yet, the maximum response of NPP to elevated CO₂ did not occur immediately, but rather 18–24 months after disturbance (Fig. 6). This was also apparent when analyzed at a finer temporal resolution. For example, root growth responses to elevated CO₂ for the first 7 months after fire were not as large as those observed during the first 24 months (Dilustro *et al.*, 2002). Similarly, the effects of CO₂ on leaf area and net ecosystem C assimilation, which were measured independently from the components of NPP reported here, were present, but not unusually large during the 4 months immediately following the hurricane (Li *et al.*, 2007). The effect of elevated CO₂ was most strongly manifested after the plants had begun to re-establish the capacity for C gain through canopy regrowth, and the effect was most pronounced as increased fine root growth.

Belowground NPP was five times greater than NPP above ground in this subtropical woodland, and therefore belowground NPP was the major driver of the year-to-year variation in total NPP and of the CO₂ response. This may reflect strong relative limitation of productivity by belowground resources, which is probably pronounced in the sandy, low-nutrient soils of this system. High belowground allocation may also occur because of plant strategies favoring resource allocation to organs protected from fire (Clarke *et al.*, 2013). Increased allocation to roots following aboveground disturbance has been observed (Di Iorio *et al.*, 2011) and may be adaptive in scavenging and retaining soil nutrients (Langley *et al.*, 2002), especially if retranslocatable C stored in belowground plant tissues is sufficient to alleviate C limitation after defoliation. Elevated CO₂ enhanced instantaneous net ecosystem C uptake in this ecosystem (Hymus *et al.*,

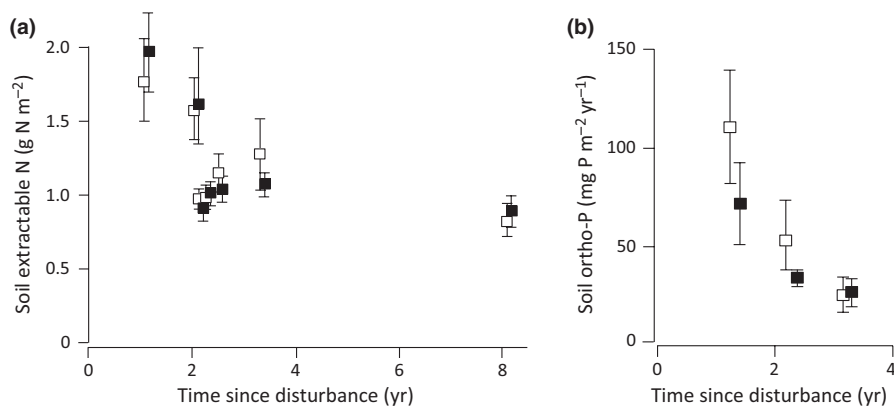


Fig. 7 Soil extractable nitrogen (a) and phosphorus (b) as a function of time since disturbance. Open squares, ambient; closed squares, elevated CO₂. Plots are means \pm 2 SE.

2003), which could enhance the capacity for fine root regrowth following disturbance (Day *et al.*, 2013).

The transient effect of disturbance on nutrient availability also probably contributed to the strong response of NPP to elevated CO₂, as well as the subsequent decline in the response. Hurricanes and fires cause organic matter inputs to soil through leaf fall (hurricane) and ash from combustion of leaves, stems and surface litter. At our site, NMR analysis of the soil in a chronosequence after fire suggests that substantial organic matter inputs from fine root death also occur after fire (Alexis *et al.*, 2012). Rapid mineralization of these organic matter sources probably contributes to the pulse of nutrient availability observed (Fig. 7), and the corresponding NPP response to elevated CO₂. The peak in the CO₂ stimulation of productivity was coincident with the high availability of multiple soil nutrients, including N, P and micronutrients (Fig. 7; B. A. Hungate, unpublished), and the response of NPP to elevated CO₂ declined, together with the availability of these nutrients in soil. The temporal pattern of the CO₂-mediated reduction in stem N concentration is also consistent with increasing N limitation of the CO₂ response (Fig. 6). Together, these results indicate that nutrient limitation can cause a progressive restriction in the response of NPP to elevated CO₂ (Field, 1999; Luo *et al.*, 2004; Reich *et al.*, 2006a,b; Norby *et al.*, 2010), and that interactions between disturbance and nutrient availability can strongly affect productivity responses to elevated CO₂. This interpretation is also complementary to the notion described earlier that fine root regrowth following disturbance is adaptive for capturing the pulse of soil nutrients (Langley *et al.*, 2002), and is consistent with past observations that growth responses to elevated CO₂ are more pronounced when nutrient availability is high (de Graaff *et al.*, 2006; Reich & Hobbie, 2012).

The maximum responses of NPP to elevated CO₂ reported here (>100%) are larger than those observed in most other experiments, even in experiments with fertilizer additions (Reich *et al.*, 2006b). Because of the dominance of belowground production to total NPP, and the importance of fine root production to the belowground total, our conclusions rely on scaling minirhizotron observations to biomass and calculating biomass increments to estimate productivity, a challenge in any ecosystem, and an approach that usually yields productivity estimates higher than those obtained from sequential coring or other approaches (Strand *et al.*, 2008). However, the scaling problem applies equally to both ambient and elevated CO₂ treatments, and so is unlikely to explain the large responses to elevated CO₂ observed here. Remobilization of stored C after disturbance could also skew productivity estimates. We have demonstrated previously that stored C is used to support new root growth in this ecosystem (Langley *et al.*, 2002), creating a multi-year time lag between photosynthesis and root growth. The use of stored C to produce new fine roots will cause an overestimate of belowground productivity if replenishment of stored reserves in belowground storage organs does not occur simultaneously to remobilization, or if the decrement in coarse root C caused by mobilization of stored reserves is difficult to detect. Over multiple years, the influence of this error, if it occurs, will diminish, as what is mistakenly counted as fine root NPP in 1 yr will

be missed in coarse root increment in subsequent years. This phenomenon could dampen the temporal pattern observed after disturbance (Fig. 6), but is unlikely to change it qualitatively. Thus, the large responses of NPP to elevated CO₂ immediately following disturbance probably reflect real properties of the system rather than measurement challenges.

Increased N uptake (Finzi *et al.*, 2007) and increased NUE (Calfapietra *et al.*, 2007; Norby *et al.*, 2010) contribute to the response of NPP to elevated CO₂, consistent with the findings reported here that elevated CO₂ increased both NUE and N uptake (Fig. 4). Our finding that increased N uptake was the more important mechanism, explaining >90% of the response in the years immediately post-disturbance (Fig. 5), underscores the facilitating role of N availability (and of other nutrients) for positive NPP responses to elevated CO₂ (Reich *et al.*, 2006a,b), and is consistent with the finding that nutrient availability increased after disturbance (Fig. 7).

Increased total N uptake and faster turnover of tracer ¹⁵N through plants indicate that elevated CO₂ increased the rates of N cycling through the plant–soil system, enhancing the availability of unlabeled N via priming (Carney *et al.*, 2007; Langley *et al.*, 2009) or access to N deep in the soil profile (McKinley *et al.*, 2009), or both. Both sources could contribute to the increased N uptake and observed ¹⁵N dilution. Yet, the implications of these sources differ. The priming mechanism would involve a net redistribution of N from soil organic matter to plants, from a reservoir with a narrow C to N ratio to one with a wider C to N ratio. This N could become more susceptible to loss from the system through leaching and gaseous pathways, as found for scrub-oak, in which total system ¹⁵N recovery was lower in the high CO₂ treatment (Hungate *et al.*, 2013). Although there is the potential for priming and redistribution of N to enhance C uptake and storage (Drake *et al.*, 2011), this outcome is far from certain. The other mechanism, increased N uptake from the water table, raises issues of experimental design and scale. N inputs from groundwater originate in part from N originally contained in the vertical column defined by the experimental chambers, but also from N flowing laterally from adjacent areas via the water table. In other words, the water table provides hydrological connectivity between the chambers and the surrounding landscape, an avenue for nutrient subsidies to the experimental plots. Such subsidies do not represent viable sources of new nutrients when atmospheric CO₂ concentration is increasing globally, but rather represent an artifact of the spatial scale of the design in which plants can access nutrient subsidies from areas outside the experiment. At this point, we cannot quantify the relative importance of these mechanisms, but we note that there is experimental evidence for both (Carney *et al.*, 2007; Langley *et al.*, 2009; McKinley *et al.*, 2009). To the extent that the latter operates, our results overestimate the CO₂ effect on N uptake and availability.

Conclusions

The subtropical woodland studied here has a short mean fire return interval, and thus the responses shown here may not be apparent in experiments of comparable duration in ecosystems

with longer times between disturbance events, or with plants that lack certain adaptive strategies, such as the ability to resprout using C stored in belowground tissues. Nevertheless, given the widespread occurrence and influence of episodic disturbance on terrestrial ecosystems modulated by changes in resource availability, the phenomena observed here may illustrate general interactions between disturbance and ecosystem responses to the changing atmosphere.

Our work shows that disturbance modulates ecosystem responses to chronic global environmental change. Disturbance can reset the biogeochemical trajectories and trigger plant allocation strategies that shape responses of NPP to elevated CO₂. Transient pulses of nutrient availability, such as those caused by disturbance, may be especially important in explaining the strong NPP responses in some years to elevated CO₂. Our results caution against the interpretation of short-term responses as representative, because they may dissipate over time. Disturbance can influence ecosystem responses to global environmental change, and there is value in incorporating disturbances and other extreme events into the design of global change experiments.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Net primary production 1996–2007 in response to increased atmospheric CO₂ concentrations in a subtropical woodland

Table S2 Nitrogen uptake above- and belowground

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