Global Change Biology (1999) **5,** 781–789

Elevated CO_2 increases nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak

BRUCE A. HUNGATE,* PAUL DIJKSTRA,* DALE W. JOHNSON,† C. ROSS HINKLE‡ and BERT G. DRAKE*

Smithsonian Environmental Research Center, Edgewater, MD 21037, †Desert Research Institute, Reno, NV 60220, ‡Dynamac Corporation, Kennedy Space Center, FL 32899, USA

Abstract

We report changes in nitrogen cycling in Florida scrub oak in response to elevated atmospheric CO_2 during the first 14 months of experimental treatment. Elevated CO_2 stimulated above-ground growth, nitrogen mass, and root nodule production of the nitrogen-fixing vine, *Galactia elliottii* Nuttall. During this period, elevated CO_2 reduced rates of gross nitrogen mineralization in soil, and resulted in lower recovery of nitrate on resin lysimeters. Elevated CO_2 did not alter nitrogen in the soil microbial biomass, but increased the specific rate of ammonium immobilization (NH_4^+ immobilized per unit microbial N) measured over a 24-h period. Increased carbon input to soil through greater root growth combined with a decrease in the quality of that carbon in elevated CO_2 best explains these changes.

These results demonstrate that atmospheric CO_2 concentration influences both the internal cycling of nitrogen (mineralization, immobilization, and nitrification) as well as the processes that regulate total ecosystem nitrogen mass (nitrogen fixation and nitrate leaching) in Florida coastal scrub oak. If these changes in nitrogen cycling are sustained, they could cause long-term feedbacks to the growth responses of plants to elevated CO_2. Greater nitrogen fixation and reduced leaching could stimulate nitrogen-limited plant growth by increasing the mass of labile nitrogen in the ecosystem. By contrast, reduced nitrogen mineralization and increased immobilization will restrict the supply rate of plant-available nitrogen, potentially reducing plant growth. Thus, the net feedback to plant growth will depend on the balance of these effects through time.

Keywords: elevated atmospheric CO_2, Florida, *Galactia elliottii*, gross mineralization, nitrogen fixation, scrub oak, soil microbial biomass, soil nitrogen cycling

Received 30 June 1998; resubmitted 19 December 1998 and accepted 8 January 1999

Introduction

Human use of fossil fuels over the last century has increased the concentration of carbon dioxide (CO_2) in the atmosphere by about 90 μL L^{-1} (Keeling 1986), a change in atmospheric composition unprecedented for the past 160 000 years (Barnola *et al.* 1987). Increased CO_2 concentration usually stimulates plant carbon uptake through photosynthesis (Drake *et al.* 1997), and often increases carbon distribution below ground (Canadell *et al.* 1996; Hungate *et al.* 1997c). Increased uptake of carbon by plants and increased distribution to soil in elevated CO_2 could drive changes in the cycling of other elements, such as nitrogen.

Nitrogen fixation – the conversion of dinitrogen gas (N_2) in the atmosphere to ammonium (NH_4^+), a form usable by plants – is the major biological process through which nitrogen enters terrestrial ecosystems (Schlesinger 1997). Initial surveys of experiments conducted on plants in isolated pots found that elevated CO_2 generally caused a larger growth increase in nitrogen-fixing plants than in plants that do not fix nitrogen (Hunt *et al.* 1991; Hunt *et al.* 1993; Poorter 1993). Symbiotic nitrogen fixation is energetically expensive (Benemann & Valentine 1972), but it is usually not directly regulated by the availability of photosynthate (Finn & Brun 1982; Hunt & Layzell

Correspondence: Bruce A. Hungate, Department of Biological Sciences, Box 5640, Northern Arizona University, Flagstaff, AZ 86011, USA, fax +1 / 520 523 7500, e-mail bruce.hungate@nau.edu

1993; Hartwig *et al.* 1994; Weisbach *et al.* 1996). Thus, the relatively large growth enhancements of nitrogen-fixers by elevated CO_2 might be viewed as a realization of the CO_2-limitation of plant growth matched by a ready supply of nitrogen from the atmosphere, rather than a direct CO_2-limitation of N fixation itself (Hartwig *et al.* 1996). Whether elevated CO_2 causes a greater growth enhancement of nitrogen-fixing plants in naturally occurring plant assemblages is more controversial (Mooney *et al.* 1998), with some studies supporting the idea that responses of nitrogen fixers are relatively large compared to nonfixing plants (Soussana & Hartwig 1996; Hebeisen *et al.* 1997; Lüscher *et al.* 1998), and others demonstrating very small or no growth responses of nitrogen-fixing plants in the field (Schäppi & Körner 1996; Leadley & Körner 1996; Niklaus *et al.* 1998).

In addition to altering nitrogen fixation, elevated CO_2 can influence the rates of transfer between organic and inorganic nitrogen pools in the soil. Understanding the overall effects of elevated CO_2 on nitrogen cycling requires investigating all of these processes simultaneously. The hypothesis that elevated CO_2 will increase nitrogen fixation, though unresolved, is relatively straightforward, but hypotheses about CO_2 effects on the transformations of nitrogen in the soil remain highly controversial, and the data inconclusive. Elevated CO_2 could alter these nitrogen transformations by altering the quantity and/or the quality of the organic substrates plants add to soil through litterfall, root turnover, and root exudation (Melillo 1983; Díaz *et al.* 1993; Zak *et al.* 1993), or by altering soil water content (Hungate *et al.* 1997a; Arnone & Bohlen 1998). Increased carbon input to soil is expected to fuel heterotrophic microbial activity, increasing microbial demand for inorganic nitrogen, and thus stimulating nitrogen immobilization (Luxmoore 1981), effects which have been observed in a number of elevated CO_2 studies (Díaz *et al.* 1993; Morgan *et al.* 1994; Rice *et al.* 1994; Rouhier *et al.* 1994). In addition to stimulating microbial nitrogen uptake through increased carbon input to soil, elevated CO_2 could directly decrease the process of mineralization if the quality of root-derived carbon added to the belowground system declines, due to lower nitrogen concentrations or to increased concentration of recalcitrant carbon compounds (Berntson & Bazzaz 1997). By contrast, an initial stimulation of microbial nitrogen uptake could lead to an increase in nitrogen mineralization, if increased carbon input to soil enables microbes to immobilize otherwise inaccessible pools of nitrogen, and if the subsequent expansion of the microbial biomass is accompanied by increased rates of microbial turnover, through predation by protozoa, for example, a process which enhances nitrogen mineralization (Clarholm 1985, 1989). Evidence for this chain reaction also exists in elevated CO_2 studies (Zak *et al.* 1993).

Most studies to date that have examined the effects of elevated CO_2 on soil nitrogen transformations have been conducted in laboratory or outdoor microcosms, with homogenized soil, simplified plant assemblages (many in monoculture), and restricted soil volume, simplifications to the experimental system that are likely to increase statistical power and facilitate interpretation. However, these departures from field conditions restrict the suite of interactions that occurs in the field, and may also introduce experimental artifacts (McConnaughay *et al.* 1993; O'Neill & Norby 1996). Thus, capturing the integrative effects of elevated CO_2 on nitrogen cycling requires studying these processes under natural field conditions.

In this experiment, we investigated the effects of elevated CO_2 on nitrogen transformations and pools in a naturally occurring stand of scrub-oak vegetation in central, coastal Florida, at the Merrit Island National Wildlife Refuge. After several decades of fire suppression, the scrub-oak vegetation in the Merrit Island National Wildlife Refuge is now managed with controlled burns. Resprouting after fire is rapid – the fire return time is ≈7–10 years (Schmalzer & Hinkle 1992a) – and canopy closure occurs as soon as 4 years after fire (Schmalzer, pers. comm.). Establishing our experiment in this ecosystem thus presents the opportunity to examine responses to elevated CO_2 in a woody perennial ecosystem over an entire disturbance cycle. Here, we present results from the first year of the experiment, before substantial input of above-ground litter, and before canopy closure.

Materials and methods

We chose a 10-y-old stand of scrub-oak vegetation, selected sites dominated by *Quercus myrtifolia* Willd., *Q. chapmanii* Sargent, and *Q. geminata* Small, and then burned the above-ground vegetation. The soil at the site is a Histosol, Pomello series (Huckle *et al.* 1974). Sixteen 3.8-m wide × 3.8-m tall octagonal open-top chambers similar in design to Drake *et al.* (1989) were erected following the burn to maintain ambient and ambient + 350 ppmv CO_2 concentrations over the enclosed vegetation, with 8 chambers at each CO_2 level. (For further details of the experimental setup, see Stiling *et al.* 1999.)

Galactia elliottii, a leguminous vine, grows rapidly in scrub oak immediately after fire, but diminishes in relative abundance as the dominant oaks become reestablished (Schmalzer & Hinkle 1992b). *G. elliottii* is perennial, but its above-ground parts senesce nearly

completely during the late autumn (Schmalzer & Hinkle 1992b). We measured above-ground production and nitrogen mass in *G. elliottii* by destructive harvest. In December 1996, all above-ground parts of *G. elliottii* were removed from one-sixth of each plot, separated into stems and leaves, oven dried, weighed, and average mass per shoot calculated. Total emergent shoots were enumerated in the remaining five-sixths of each plot, and total *G. elliottii* mass determined as the product of total shoot number and average mass per shoot. N content in the destructively harvested tissue was measured on a Europa Scientific SL automated CN analyser coupled with a Europa 20–20 isotope ratio mass spectrometer at the UC Berkeley Stable Isotope Laboratory. Total N mass per plot of *G. elliottii* was determined in an analogous manner to total mass.

We measured gross NH_4^+ turnover in soil in August 1996, December 1996, March 1997, and July 1997. Three cm diameter by 15 cm long bags made from fibreglass window screen (1 mm mesh) were filled with C horizon soil from which roots and large pieces of organic matter had been removed by sieving. The C horizon bags were buried vertically in the top 15 cm of soil in May 1996, with 12 bags in each chamber. At each sampling date, one C horizon bag was removed from each chamber for analyses. Additionally, at each sampling date, 3 soil cores (2-cm diameter by 15 cm deep) were removed from each chamber and composited for measurements of N transformations in the O/A horizon soil (hereafter, 'O/A horizon').

In the laboratory, we removed roots and root nodules from the soil samples (though quantitative removal was possible only for the C horizon samples). Roots and nodules were washed in distilled H_2O and placed in a drying oven (65 °C) for 72 h, after which they were weighed. We assume that the root nodules recovered in the C horizon samples were produced by *G. elliottii*, as it is the only legume present in the scrub-oak ecosystem. We removed subsamples of soil from each C and O/A horizon soil sample for gravimetric moisture determination, and placed the remaining soil in a small ziploc plastic bag. To each bag, we added ^{15}N-$(NH_4)_2SO_4$ at a rate of 0.2 μg N in 0.1 mL H_2O per g soil, and mixed the solution with the soil by alternating adding solution and squeezing the soil through the bag. On average, the addition of ^{15}N solution increased the NH_4^+ pools by about 30%, and increased soil water content by about 10%. 15–30 min after adding the ^{15}N solution, ≈20 g of soil was removed from each bag for extraction in 2.0 m KCl. The remaining soil in the ziploc bag was then returned to the field, placed inside the hole in the chamber from which it originated. The following day the samples were retrieved from the chambers and the remaining soil was divided for determination of gravimetric moisture content and for extraction in 2.0 m KCl (20–24 h after the first extraction). Extracts from both days were frozen immediately until analysis, 1–3 months later.

Concentrations of NH_4^+ in the extracts were determined colorimetrically on a Technicon autoanalyser at the Kennedy Space Center Chemistry Environmental Laboratory. NH_4^+ in the remaining extract was concentrated for mass spectrometric analysis by a diffusion procedure (Stark & Hart 1996), as follows: acid traps were constructed by acidifying a 6-mm paper disk (Whatman #3) with 10 μL 2.5 m $KHSO_4$, sandwiching the disk between 2 layers of PTFE tape, and then sealing by pressing the rim of a scintillation vial against the tape. One trap was placed inside each specimen cup containing the extract solution. We then added 0.2 g of MgO to each cup and quickly sealed the cups. We shook the cups daily during the diffusion period. After 7 days, we opened the cups and removed and opened the acid traps. The filter disks were dried for 24 h in a desiccator containing open vials of concentrated H_2SO_4 to remove any atmospheric ammonia. We wrapped each disk in a 6×5 mm tin vessel, and analysed these for ^{15}N composition by isotope-ratio mass spectrometer using a Europa Roboprep/Tracermass system at the UC Berkeley Stable Isotope Laboratory. We calculated gross mineralization and consumption rates using the pool dilution model presented in Wessel & Tietema (1992). Extractable NH_4^+ pools were determined from the t24 sampling time, thereby allowing a 24-h period after ^{15}N addition for the soil NH_4^+ pools to recover.

For the July 1997 harvest, we also measured soil microbial biomass carbon and nitrogen and 24 h $^{15}NH_4^+$ uptake in the O/A horizon samples. We used the chloroform-fumigation direct extraction procedure (Brookes *et al.* 1985), in which microbial biomass is determined as the difference in 0.5 m K_2SO_4 extractable C and N between a subsample of soil exposed to chloroform vapors for 48 h and an unexposed soil sample, divided by a constant for extraction efficiency of 0.54 (Brookes *et al.* 1985). To determine total C, N, and ^{15}N content, the K_2SO_4 extracts were acidified to approximately pH 5 by adding drops of concentrated H_2SO_4, and dehydrated in a forced-air drying oven at 65 °C. The dehydrated extracts were ground in a mortar and pestle, and ≈50 mg placed in a 8×6 mm tin vessel for analysis by combustion/gas chromatography/isotope ratio mass spectroscopy on a Europa SL 20–20 mass spectrometer.

We also measured nutrient accumulation on resin lysimeters during this period. Resin lysimeters were constructed of PVC pipe with a mixed bed (anion and

cation exchange) resin bag set in between two layers of washed sand (modified from Hart & Gunther 1989). The lysimeters were left in place for 1 year and then extracted with 2 m KCl (including two blanks which were left in a refrigerator for 1 year). Being tension-free, the resin lysimeters were used to estimate flux via saturated flow only.

Where appropriate, we used analysis of variance (anova) or *t*-tests to compare ambient and elevated CO_2 treatments. For above-ground biomass and nitrogen mass in *G. elliottii*, the data were severely non-normally distributed, so we used the nonparametric Kruskal–Wallis anova to compare treatments. Because gross nitrogen mineralization and gross ammonium consumption were measured several times throughout the year in each chamber, we used repeated measures analysis of variance to test for treatment differences and seasonal variation.

Results

Elevated CO_2 stimulated the above-ground growth of *G. elliottii* by 128% (Kruskal–Wallis ANOVA $P = 0.027$, Fig. 1), and above-ground nitrogen mass by 96% (Kruskal–Wallis ANOVA $P = 0.046$, Fig. 1). By way of comparison, above-ground nitrogen mass in the oaks was $2.03 \pm 0.37\,g\,m^{-2}$ in ambient CO_2 and $2.21 \pm 0.33\,g\,N\,m^{-2}$ in elevated CO_2 at this time (P Dijkstra, unpubl. data), a nonsignificant difference of about 9% (ANOVA, $P = 0.72$). Additionally, root nodules of *G. elliottii* in the C horizon samples were more numerous in elevated CO_2 for each of the four sampling dates (Fig. 2), with, on average, 7.8 times more nodules occurring in the elevated CO_2 plots (ANOVA, $F_{df} = 5.742_{14}$, $P = 0.031$). Total nodule mass followed the same pattern, increasing in elevated CO_2 at each sampling date (Fig. 2), with an average 5-fold stimulation (ANOVA, $F_{df} = 4.616_{14}$, $P = 0.049$). The relatively larger stimulation of above-ground nitrogen mass of *G. elliottii* (compared to the oaks) together with increased root nodule production, suggest that elevated CO_2 stimulated nitrogen fixation in concert with the increased growth of *G. elliottii*.

On average, rates of gross mineralization were 35% lower in elevated CO_2 compared to ambient CO_2 for both the A/O and C horizon samples (Fig. 3, Repeated Measures ANOVA: A/O, $F_{df} = 14.81_{1,14}$, $P = 0.002$; C, $F_{df} = 4.62_{1,14}$, $P = 0.050$). Elevated CO_2 also reduced rates of gross ammonium consumption in the C horizon ($F_{df} = 14.81_{1,14}$, $P = 0.025$, Fig. 3). The trend was similar for gross ammonium consumption in the A/O horizon samples (Fig. 3), but was not significant ($F_{df} = 2.111_{1,14}$, $P = 0.168$). The effect of elevated CO_2 was particularly apparent for the first three sampling dates, August, December, and March 1996, but was less pronounced in July 1997, where the pattern actually reversed for the C horizon, significantly for gross ammonium consumption (CO_2–time interaction, $F_{df} = 3.427_{1,14}$, $P = 0.026$, Fig. 3).

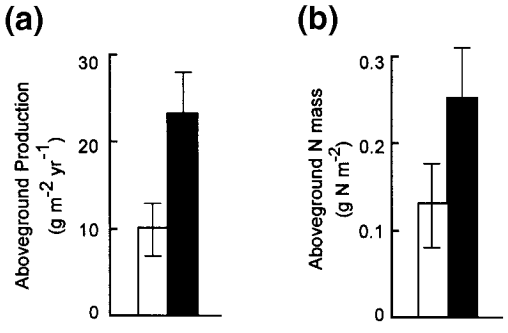

Fig. 1 (a) Above-ground production (g $m^{-2}y^{-1}$) and (b) nitrogen mass (g m^{-2}) for *Galactia elliottii* in ambient (□) and elevated (■) CO_2, after the first growing season of experimental treatment. Values are means ± SE ($n = 8$).

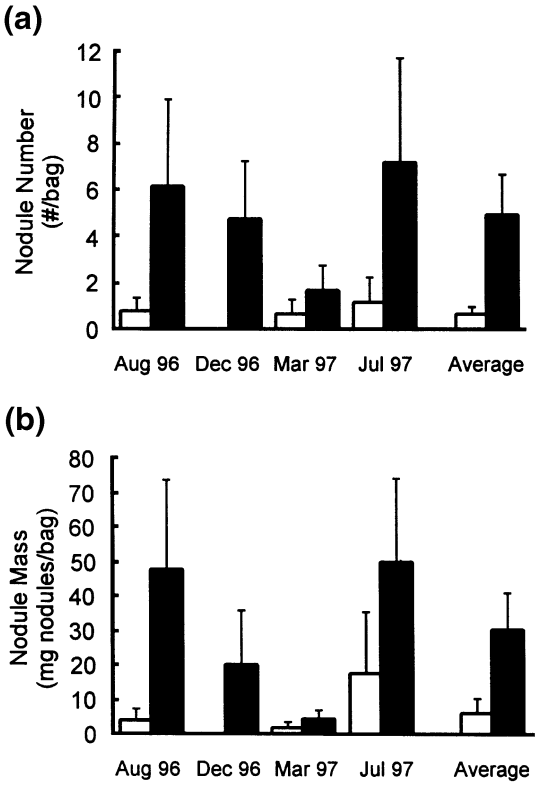

Fig. 2 Legume nodule mass (a) and number (b) for four sampling dates in ambient (□) and elevated (■) CO_2 recovered in the C horizon samples (see methods). Values are means ± SE ($n = 8$) for each sampling date, and for the chamber average across all sampling dates.

Overall, however, elevated CO_2 reduced the flux of nitrogen through the soil ammonium pool. Gross mineralization and consumption varied through time, higher during the first sampling in August, and lower in December, March, and July (Effect of time: A/O_{min} $F_{df} = 4.643_{3,42}$, $P = 0.007$; A/O_{cons} $F_{df} = 5.125_{3,42}$, $P = 0.004$; C_{min} $F_{df} = 18.72_{3,42}$, $P < 0.001$; C_{cons} $F_{df} = 3.427_{3,42}$, $P = 0.026$). At the final sampling in July, soil microbial nitrogen was unchanged in elevated CO_2 (Fig. 4, $t_{df} = 0.633_{1,14}$ $P = 0.537$), but gross immobilization (per gram soil) tended to increase ($P = 0.093$), while the specific rate of immobilization increased significantly ($P = 0.002$). Thus, elevated CO_2 increased microbial demand for nitrogen, but did not result in an expansion of the soil microbial biomass.

Elevated CO_2 reduced NO_3^- recovery on the resin lysimeters (Table 1), indicating a reduction in nitrate leaching from the A horizon. Elevated CO_2 did not significantly affect ammonium or phosphate recovery on the resin lysimeters (Table 1), but extractable NH_4^+-N from the O/A horizon samples was lower in elevated CO_2 ($F_{df} = 4.620_{1,14}$, $P = 0.050$, Table 1). For both treatments, the lysimeters accumulated more mineral P than mineral N.

Discussion

These results show that elevated CO_2 alters nitrogen cycling in the Florida scrub-oak ecosystem. The relatively large increase in above-ground nitrogen mass in *G. elliottii* is most simply explained by increased nitrogen fixation in elevated CO_2: higher rates of photosynthesis in elevated CO_2 stimulate the growth of *G. elliottii*, creating additional nitrogen demand, which is met by increased rates of nitrogen fixation. The positive relationship between atmospheric CO_2 concentration and nitrogen fixation in legumes has long been known (Wilson *et al.* 1933), and has been supported more recently by a number of laboratory and microcosm studies (Masterson & Sherwood 1978; Finn & Brun 1982; Williams *et al.* 1982; Norby 1987; Arnone & Gordon 1990; Ryle *et al.* 1992; Poorter 1993; Ross *et al.* 1995; Stewart & Potvin 1996; Campbell *et al.* 1997; Tissue *et al.* 1997). Results from field studies are less clear, however. In pastures, elevated CO_2 stimulated the biomass of *Trifolium repens* more than that of *Lolium perenne* (Soussana & Hartwig 1996; Lüscher *et al.* 1998), qualitatively similar to the results presented here, where the increase in growth and above-ground nitrogen mass in *G. elliottii* to elevated CO_2 were disproportionately larger than responses of nonfixers.

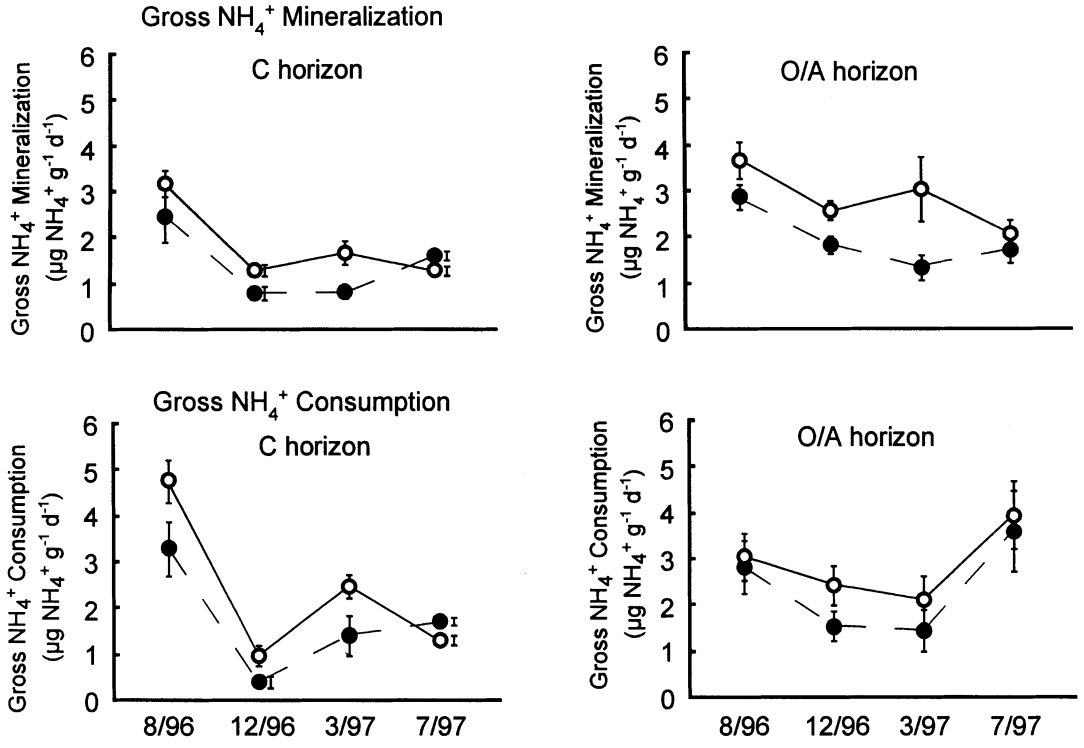

Fig. 3 Gross nitrogen mineralization and consumption for four sampling dates in ambient (□) and elevated (■) CO_2 in the O/A and C horizon samples. Values are means ± SE ($n = 8$).

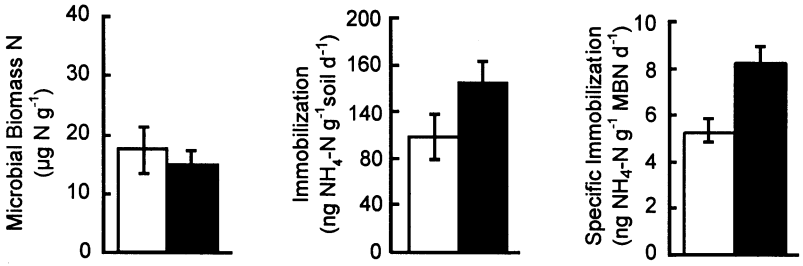

Fig. 4 Soil microbial biomass nitrogen, and ammonium immobilization in the soil microbial biomass for July 1997 in ambient (□) and elevated (■) CO_2. Immobilization is expressed on a per gram soil basis and per unit microbial nitrogen basis. Values are means ± SE ($n = 8$).

Table 1 Nitrogen and phosphorus recovery on resin lysimeters after one year of exposure to ambient or elevated CO_2, and extractable NH_4^+-N pools from the soil O/A horizon from the 4 sampling dates. Values are mg N or P m^{-2}, mean ± SE ($n = 8$). For the resin lysimeters, *P*-values are for *t*-tests comparing the two CO_2 treatments. For the soil cores, the *P*-value is for the main effect of CO_2 in a repeated measures anova for the four sampling dates

Method	Ambient CO_2	Elevated CO_2	*P*-value
Resin Lysimeters			
NO_3^--N	13 ± 3	7 ± 1	0.04
NH_4^+-N	61 ± 12	56 ± 8	0.36
$PO_4^=$-P	110 ± 29	70 ± 22	0.21
Soil extractable NH_4^+-N			
Soil cores	194 ± 14	132 ± 18	0.03

However, elevated CO_2 had no effects on the biomass of nitrogen-fixers in alpine grasslands (Schäppi & Körner 1996) and in calcareous grasslands (Leadley & Körner 1996). Part of the differences among these field studies may be due to variation in phosphorus availability. In the calcareous grassland, low phosphorus availability limits the growth response of nitrogen-fixing legumes to elevated CO_2 (Niklaus *et al.* 1998). By contrast, in the scrub-oak ecosystem studied here soil phosphorus availability is relatively high (Table 1) and thus is unlikely to limit the growth responses of *G. elliottii* to elevated CO_2. However, more field studies in native ecosystems are required to test this idea more broadly.

In addition to stimulating nitrogen fixation, elevated CO_2 depressed gross mineralization and ammonium consumption in soil in this experiment, and caused an increase in the specific rate of ammonium immobilization by soil micro-organisms. Consistent with these changes, elevated CO_2 also reduced nitrate leaching from the A horizon, as indicated by lower recovery of NO_3^- N on the resin lysimeters. Altered carbon partitioning and altered carbon quality in elevated CO_2 are the most likely mechanisms for these changes in soil N transformations. Elevated CO_2 increases photosynthesis in *Q. myrtifolia*, *Q. geminata*, and *Q. chapmanii* in the scrub oak (Vieglais *et al.* 1995; Li *et al.* 1999), and also stimulates root growth (Day *et al.* 1996), thereby causing increased carbon input to soil. The observed increase in the specific rate of NH_4^+ immobilization by soil microorganisms is likely to be a simple consequence of this (Hungate 1999). However, our work demonstrates that elevated CO_2 does not only affect microbial demand for inorganic nitrogen from the soil, but can also affect mineralization, the process through which microorganisms liberate inorganic N from soil organic matter during decomposition. Reduced quality of litter material (higher lignin:nitrogen ratio) in elevated CO_2 may slow mineralization of nitrogen during decomposition (Melillo *et al.* 1983), though support for this hypothesis for leaf litter is thin (Canadell *et al.* 1996; O'Neill & Norby 1996). Whether elevated CO_2 elicits a reduction in the carbon quality of root-derived substrates in our site (higher concentrations of aromatic, aliphatic, and other recalcitrant carbon compounds) is unknown, yet remains a plausible explanation for the decrease in gross N mineralization we observed. In recovering temperate forest microcosms, elevated CO_2 caused changes in soil nitrogen transformations remarkably similar to those observed here: gross mineralization decreased in elevated CO_2 and specific microbial uptake of ammonium increased, together causing reduced nitrogen uptake by plants (Berntson & Bazzaz 1997). In this case, elevated CO_2 increased the C:N ratio of fine roots, a reduction in substrate quality that could account for the changes in microbial N transformations.

Gross ammonium consumption is the sum of all ammonium-consuming processes in soil, including immobilization, but also nitrification, as well as abiotic processes. Even with fairly low soil nitrate pools in the Florida scrub oak ecosystem (Table 1, Schmalzer & Hinkle 1992a), nitrate turnover may still be appreciable (e.g. Stark & Hart 1997). Previous work has shown that decreased gross ammonium consumption in response to elevated CO_2 can reflect decreased nitrification (e.g. Hungate *et al.* 1997b), and the increase in specific NH_4^+ immobilization by soil micro-organisms observed here is consistent with this idea. Indeed, in our experiment, the reduced recovery of NO_3^- on the resin lysimeters in elevated CO_2 indicates reduced nitrification rates in this

treatment. While the resin lysimeters did not differ in NH_4^+-N recovery between the CO_2 treatments, NH_4^+ is extremely immobile in the soil, so the resin lysimeters may not reflect ammonium availability as well as mineralization rates and extractable ammonium pools, which were significantly lower in the elevated CO_2 treatment.

Together, these results support the conceptual model that elevated CO_2 increases photosynthesis (Drake *et al.* 1997), carbon input to soil (van Veen *et al.* 1991), stimulating nitrogen demand by heterotrophic microorganisms (Luxmoore 1981; Díaz *et al.* 1993; Zak *et al.* 1993), leading to decreased ammonium availability for nitrifiers and thus reduced nitrification and nitrate accumulation on resin lysimeters. The increase in N fixer growth and N mass are also consistent with elevated CO_2 increasing plant carbon gain, with some of the extra photosynthate powering N fixation to match the increased plant N demand.

However, these results represent opposing effects on nitrogen availability to plants. Reduced mineralization and increased immobilization will decrease ammonium availability, by reducing the flux of N from organic to inorganic form, and by tying up N in microbial biomass or soil organic matter. Though nitrogen in mature Florida scrub oak is concentrated in vegetation, soil organic matter nevertheless contains a substantial amount of nitrogen, around $120\,g\,N\,m^{-2}$ (including the O and A horizons to 30 cm depth), compared to about $45\,g\,N\,m^{-2}$ in live above-ground vegetation two years after fire (Schmalzer & Hinkle 1996). Thus, increased accrual of N in this reservoir, as well as reduced rates of N release from it, could represent a substantial reduction in N availability to plants. By contrast, greater rates of symbiotic nitrogen fixation will increase the mass of labile nitrogen in soil through increased production of above- and below-ground litter of N fixers entering the soil N pool through decomposition, favouring an increase in mineralization rates and delivery of N to plants. Finally, these changes may interact, in that reduced availability of inorganic soil N could increase the reliance of *G. elliottii* on atmospheric N_2, thereby creating a positive feedback to increased N fixation in elevated CO_2 (Soussana & Hartwig 1996; Zanetti & Hartwig 1997). This hypothesis is currently being tested at the site by following a ^{15}N tracer and quantifying N fixation using the ^{15}N dilution method (Warembourg 1993).

Determining the overall effect of these changes in nitrogen cycling is complicated by the short-term nature of this study, and because these nitrogen transformations operate on different timescales. Changes in mineralization and immobilization should influence nitrogen availability to plants fairly quickly, as the soil inorganic nitrogen pools cycle quite rapidly, turning over on a timescale of days to weeks (Jackson *et al.* 1989; Davidson *et al.* 1990). Nitrogen fixation, by contrast, is a fairly minor component of the nitrogen cycle in most ecosystems in the short term, only becoming significant when the small annual fluxes are summed over many years (Comins & McMurtrie 1993; Cannell & Thornley 1998). In the Florida scrub-oak ecosystem, increased N fixation by *G. elliottii* might amount to an important change in the soil N mass over several fire cycles.

Thus, rising atmospheric CO_2 may alter the nitrogen cycle in Florida scrub oak through mechanisms that operate on different timescales, and these effects will tend to cause opposing feedbacks. Modelling studies in a number of systems support the importance of considering different time scales, recognizing that the short-term responses of ecosystems are dominated by the instantaneous increase in production and changes in mineral nitrogen availability, whereas longer-term feedbacks through the nitrogen cycle are dominated by the processes that regulate total ecosystem N mass, such as N fixation, leaching, and gaseous N losses (Comins & McMurtrie 1993; Gifford *et al.* 1996; Rastetter *et al.* 1997; Cannell & Thornley 1998). Since these processes become more important on the timescale of the decades to centuries, the timescale of most interest for predicting the effects of elevated CO_2, the responses of these processes to elevated CO_2 merit greater attention in both empirical and modelling studies.

Acknowledgements

We thank David Johnson, Debra Colavito, Julia Tripp for assistance with the field work, and Teresa Englert for conducting the inorganic nitrogen analyses. Jane Marks provided valuable comments on the manuscript. BAH was supported by a Smithsonian Institution Postdoctoral Fellowship and a Department of Energy Alexander Hollaender Distinguished Postdoctoral Fellowship administered by the Oak Ridge Institute for Science and Education. This research was supported by the Department of Energy's Terrestrial Carbon Program. Dr William Knott, Chief Scientist of the NASA, Kennedy Space Center Biomedical Office, is gratefully acknowledged for his continuing support of this research.

References

Arnone JA III, Bohlen PJ (1998) Stimulated N_2O flux from intact grassland monoliths after two growing seasons under elevated atmospheric CO_2. *Oecologia*, **116,** 331–335.

Arnone JA, Gordon JC (1990). Effect of nodulation, nitrogen fixation and CO_2 enrichment on the physiology, growth, and dry-mass allocation of seedlings of *Alnus rubra* Bong. *New Phytologist*, **116,** 55–66.

Barnola JM, Raynaud D, Korotkevich YS, Lorius C (1987) Vostok ice core provides 160,000-year record of atmospheric CO_2. *Nature*, **329,** 408–414.

Benemann JR, Valentine RC (1972) The pathways of nitrogen fixation. *Advances in Microbial Physiology*, **8,** 59–104.

Berntson GM, Bazzaz FA (1997) Nitrogen cycling in microcosms of Yellow Birch exposed to elevated CO_2: Simultaneous positive and negative feedbacks. *Global Change Biology*, **3,** 247–258.

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, **17,** 837–842.

Campbell BD, Stafford Smith DM, McKeon GM (1997) Elevated CO_2 and water supply interactions in grasslands: a pastures and rangelands management perspective. *Global Change Biology*, **3,** 177–187.

Canadell JG, Pitelka LF, Ingram JSI (1996) The effects of elevated CO_2 on plant-soil carbon belowground: a synthesis. *Plant and Soil*, **187,** 391–400.

Cannell MGR, Thornley JHM (1998) N-poor ecosystems may respond more to elevated [CO_2] than N-rich ones in the long term. A model analysis of grassland. *Global Change Biology*, **4,** 431–442.

Clarholm M (1985) Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. *Soil Biology and Biochemistry*, **17,** 181–187.

Clarholm M (1989) Effects of plant-bacterial–amoebal interactions on plant uptake of nitrogen under field conditions. *Biology and Fertility of Soils*, **8,** 373–378.

Comins NH, McMurtrie RE (1993) Long-term response of nutrient-limited forests to CO_2 enrichments; equilibrium behavior of plant-soil models. *Ecological Applications*, **3,** 666–681.

Davidson EA, Stark JM, Firestone MK (1990) Microbial production and consumption of nitrate in an annual grassland. *Ecology*, **71,** 1968–1075.

Day FP, Weber EP, Hinkle CR, Drake BG (1996) Effects of elevated atmospheric CO_2 on fine root length and distribution in an oak-palmetto scrub ecosystem in central Florida. *Global Change Biology*, **2,** 143–148.

Díaz S, Grime JP, Harris J, McPherson E (1993) Evidence for a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature*, **364,** 616–617.

Drake BG, Leadley PW, Arp WF, Nassiry D, Curtis PS (1989) An open top chamber for field studies of elevated atmospheric CO_2 concentration on saltmarsh vegetation. *Functional Ecology*, **3,** 363–371.

Drake BG, Long SP, Gonzalez-Meler M (1997) Increased plant efficiency: a consequence of elevated atmospheric CO_2? *Annual Review of Plant Physiology*, **48,** 609–639.

Finn GA, Brunn WA (1982) Effects of atmospheric CO_2 enrichment on growth, nonstructural carbohydrate content, and root nodule activity in soybean. *Plant Physiology*, **69,** 327–331.

Gifford RM, Lutze JL, Barrett DJ (1996) Global atmospheric change effects on terrestrial carbon sequestration: Exploration with a global C- and N-cycle model (CQUESTN). *Plant and Soil*, **187,** 369–387.

Hart SC, Gunther AJ (1989) *In situ* estimates of annual net nitrogen mineralization and nitrification in a subarctic watershed. *Oecologia*, **80,** 284–288.

Hartwig UA, Heim I, Lüscher A, Nösberger J (1994) The nitrogen-sink is involved in the regulation of nitrogenase activity in white clover after defoliation. *Physiologia Plantarum*, **92,** 375–382.

Hartwig UA, Zanetti S, Hebeisen T *et al.* (1996) Symbiotic nitrogen fixation: one key to understand the response of temperate grassland-ecosystems to elevated CO_2? In: *Carbon Dioxide, Populations, and Communities* (eds Körner Ch, Bazzaz FA), pp. 253–264. Academic Press, San Diego, CA.

Hebeisen T, Lüscher A, Zanetti S *et al.* (1997) The different responses of *Trifolium repens* L. & *Lolium perenne* L. grassland to free air CO_2 enrichment and management. *Global Change Biology*, **3,** 149–160.

Huckle HF, Dollar HD, Pendleton RF (1974) *Soil Survey of Brevard County, Florida.* USDA Soil Conservation Service, Washington, DC.

Hungate BA (1999) Ecosystem responses to rising CO_2: feedbacks through the nitrogen cycle. In: *Carbon Dioxide and Environmental Stress* (eds Luo Y, Mooney HA). Academic Press, San Diego, CA, in press.

Hungate BA, Chapin FS III, Zhong H, Holland EA, Field CB (1997a) Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. *Oecologia*, **109,** 149–153.

Hungate BA, Holland EA, Jackson RB, Chapin FS III, Field CB, Mooney HA (1997c) The fate of carbon in grasslands under carbon dioxide enrichment. *Nature*, **388,** 576–579.

Hungate BA, Lund CP, Pearson HL, Chapin FS III (1997b) Elevated CO_2 and nutrient addition alter soil N cycling and N trace gas fluxes with early season wet-up in a California annual grassland. *Biogeochemistry*, **37,** 89–109.

Hunt R, Hand DW, Hannah MA, Neal AM (1991) Response to CO_2 enrichment in 27 herbaceous species. *Functional Ecology*, **5,** 410–421.

Hunt R, Hand DW, Hannah MA, Neal AM (1993) Further responses to CO2 enrichment in British herbaceous species. *Functional Ecology*, **7,** 661–668.

Hunt S, Layzell DB (1993) Gas exchange of legume nodules and the regulation of nitrogenase activity. *Annual Review of Plant Physiology*, **44,** 483–511.

Jackson LE, Schimel JP, Firestone MK (1989) Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. *Soil Biology and Biochemistry*, **21,** 409–415.

Keeling CD (1986) *Atmospheric CO_2 Concentrations—Mauna Loa Observatory, Hawaii 1958–86.* NDP-001/R1. Carbon Dioxide Information center, Oak Ridge National Laboratory, Oak Ridge, TN.

Leadley PW, Körner Ch (1996) Effects of elevated CO_2 on plant species dominance in a highly diverse calcareous grassland. In: *Carbon Dioxide, Populations, and Communities* (eds Körner Ch, Bazzaz FA), pp. 159–175. Academic Press, San Diego, CA.

Li J-H, Dijkstra P, Hinkle CR, Wheeler RM, Drake BG (1999). Photosynthetic acclimation to elevated atmospheric CO_2 concentration in the Florida scrub-oak species, *Quercus geminata* and *Q. myrtifolia*, growing in their native environment. *Tree Physiology*, **415,** 229–234.

Lüshscher A, Hendrey GR, Nösberger J (1998) Long-term responsiveness to free air CO_2 enrichment of functional types, species and genotypes of plants from fertile permanent grassland. *Oecologia*, **113,** 37–45.

Luxmoore R (1981) CO_2 and phytomass. *Bioscience*, **31,** 626.

Masterson CL, Sherwood MT (1978) Some effects of increased

atmospheric carbon dioxide on white clover (*Trifolium repens* L.) and pea (*Pisum sativum* L.). *Plant and Soil*, **49,** 421–426.

McConnaughay KDM, Berntson GM, Bazzaz FA (1993) Limitations to CO_2-induced growth enhancement in pot studies. *Oecologia*, **94,** 550–557.

Melillo JM (1983) Will increases in atmospheric CO_2 concentrations affect decay processes? In: *The Ecosystems Center Annual Report*, pp. 10–11. Marine Biological Laboratory, Woods Hole, MA.

Mooney HA, Canadell J, Chapin FS III *et al.* (1998) Ecosystem physiology responses to global change. In: *Implications of Global Change for Natural and Managed Ecosystems: a Synthesis of CGTE and Related Research* (eds Walker BH, Steffen WL, Canadell J, Ingram JSI). Cambridge University Press, Cambridge, in press.

Morgan JA, Knight WG, Dudley LM, Hunt HW (1994) Enhanced root system C-sink activity, water relations and aspects of nutrient acquisition in mycotrophic *Bouteloua gracilis* subjected to CO_2 enrichment. *Plant and Soil*, **165,** 139–146.

Niklaus PA, Leadley PW, Stöcklin J, Körner Ch (1998) Nutrient relations in calcareous grassland under elevated CO_2. *Oecologia*, **116,** 67–75.

Norby RJ (1987) Nodulation and nitrogenase activity in nitrogen-fixing woody plants stimulated by CO_2 enrichment of the atmosphere. *Physiologia Plantarum*, **71,** 77–82.

O'Neill EG, Norby RJ (1996) Litter quality and decomposition rates of foliar litter produced under CO_2 enrichment. In: *Carbon Dioxide and Terrestrial Ecosystems* (eds Koch GW, Mooney HA), pp. 87–103. Academic Press, San Diego, CA.

Poorter H (1993) Interspecific variation in the growth responses of plants to an elevated ambient CO_2 concentration. *Vegetatio*, **104/105,** 77–97.

Rastetter EB, Ågren GI, Shaver GR (1997) Responses of N-limited ecosystems to increased CO_2: a balanced-nutrition, coupled-element-cycles model. *Ecological Applications*, **7,** 444–460.

Rice CW, Garcia FO, Hampton CO, Owensby CE (1994) Soil microbial response in tallgrass prairie to elevated CO_2. *Plant and Soil*, **165,** 67–74.

Ross DJ, Tate KR, Newton PCD (1995) Elevated CO_2 and temperature effects on soil carbon and nitrogen cycling in ryegrass/white clover turves of an Endoquaept soil. *Plant and Soil*, **176,** 37–49.

Rouhier H, Billés G, El Kohen A, Mousseau M, Bottner P (1994) Effect of elevated CO_2 on carbon and nitrogen distribution within a tree (*Castanea sativa*) -soil system. *Plant and Soil*, **162,** 281–292.

Ryle GJA, Powell CE, Davidson IA (1992) Growth of white clover, dependent on N_2 fixation, in elevated CO_2 and temperature. *Annals of Botany*, **70,** 221–228.

Schäppi B, Körner Ch (1996) Growth responses of an alpine grassland to elevated CO_2. *Oecologia*, **105,** 43–52.

Schlesinger WH (1997) *Biogeochemistry: an Analysis of Global Change*, 2nd edn. Academic Press, San Diego, CA.

Schmalzer PA, Hinkle CR (1992a) Species composition and structure of oak-saw palmetto scrub vegetation. *Castanea*, **57,** 220–251.

Schmalzer PA, Hinkle CR (1992b) Recovery of oak-saw palmetto scrub after fire. *Castanea*, **57,** 158–173.

Schmalzer PA, Hinkle CR (1996) Biomass and nutrients in aboveground vegetation and soils of Florida oak-saw palmetto scrub. *Castanea*, **61,** 168–193.

Soussana JF, Hartwig UA (1996) The effects of elevated CO_2 on symbiotic N_2 fixation: a link between the carbon and nitrogen cycles in grassland ecosystems. *Plant and Soil*, **187,** 321–332.

Stark JM, Hart SC (1996) Evaluation of a diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. *Soil Science Society of America Journal*, **60,** 1846–1855.

Stark JM, Hart SC (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature*, **385,** 61–64.

Stewart J, Potvin C (1996) Effects of elevated CO_2 on an artificial grassland community: competition, invasion, and neighborhood growth. *Functional Ecology*, **10,** 157–166.

Stiling PS, Rossi AM, Hungate BA *et al.* (1999) Decreased survivorship of leaf-mining insects in elevated CO_2: reduced forage quality and an indirect trophic cascade. *Ecological Applications*, **9,** 240–244.

Tissue DT, Megonigal JP, Thomas RB (1997) Nitrogenase activity and N_2-fixation are stimulated by elevated CO_2 in a tropical N_2-fixing tree. *Oecologia*, **109,** 28–33.

van Veen JA, Liljeroth E, Lekkerkerk LJA, van de Geijn SC (1991) Carbon fluxes in plant-soil systems at elevated atmospheric CO_2 levels. *Ecological Applications*, **1,** 175–181.

Vieglais D, Hinkle CR, Drake BG (1995) The effects of elevated CO_2 on a subtropical scrub oak-palmetto plant community. *IGE Newsletter*, **1995,** 5–6.

Warembourg FR (1993) Nitrogen fixation in soil and plant systems. In: *Nitrogen Isotope Techniques* (eds Knowles R, Blackburn TH), pp. 127–156. Academic Press, San Diego, CA.

Weisbach C, Hartwig UA, Heim I, Nösberger J (1996) Whole nodule carbon metabolites are not involved in the regulation of the oxygen permeability and nitrogenase activity in white clover nodules. *Plant Physiology*, **110,** 539–545.

Wessel WM, Tietema A (1992) Calculating gross N transformation rates of ^{15}N pool dilution experiments with acid forest litter: analytical and numerical approaches. *Soil Biology and Biochemistry*, **24,** 931–942.

Williams LE, Dejong TM, Philips DA (1982) Effects of changes in shoot carbon-exchange rate on soybean nodule activity. *Plant Physiology*, **69,** 432–435.

Wilson PW, Fred EB, Salmon MR (1933) Relation between carbon dioxide and elemental nitrogen assimilation in leguminous plants. *Soil Science*, **35,** 145–165.

Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DA (1993) Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. *Plant and Soil*, **151,** 105–117.

Zanetti S, Hartwig UA (1997) Symbiotic N_2 fixation increases under elevated atmospheric pCO_2 in the field. *Acta Œcologica*, **18,** 285–290.