

POTENTIAL NITROGEN CONSTRAINTS ON SOIL CARBON SEQUESTRATION UNDER LOW AND ELEVATED ATMOSPHERIC CO₂

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Abstract. The interaction between nitrogen cycling and carbon sequestration is critical in predicting the consequences of anthropogenic increases in atmospheric CO₂ (hereafter, C_a). The progressive N limitation (PNL) theory predicts that carbon sequestration in plants and soils with rising C_a may be constrained by the availability of nitrogen in many ecosystems. Here we report on the interaction between C and N dynamics during a four-year field experiment in which an intact C₃/C₄ grassland was exposed to a gradient in C_a from 200 to 560 μmol/mol. There were strong species effects on decomposition dynamics, with C loss positively correlated and N mineralization negatively correlated with C_a for litter of the C₃ forb *Solanum dimidiatum*, whereas decomposition of litter from the C₄ grass *Bothriochloa ischaemum* was unresponsive to C_a. Both soil microbial biomass and soil respiration rates exhibited a nonlinear response to C_a, reaching a maximum at ~440 μmol/mol C_a. We found a general movement of N out of soil organic matter and into aboveground plant biomass with increased C_a. Within soils we found evidence of C loss from recalcitrant soil C fractions with narrow C:N ratios to more labile soil fractions with broader C:N ratios, potentially due to decreases in N availability. The observed reallocation of N from soil to plants over the last three years of the experiment supports the PNL theory that reductions in N availability with rising C_a could initially be overcome by a transfer of N from low C:N ratio fractions to those with higher C:N ratios. Although the transfer of N allowed plant production to increase with increasing C_a, there was no net soil C sequestration at elevated C_a, presumably because relatively stable C is being decomposed to meet microbial and plant N requirements. Ultimately, if the C gained by increased plant production is rapidly lost through decomposition, the shift in N from older soil organic matter to rapidly decomposing plant tissue may limit net C sequestration with increased plant production.

Key words: *Bothriochloa ischaemum*; carbon sequestration; decomposition; elevated CO₂; global change; grassland; nitrogen mineralization; nitrogen use efficiency; soil carbon; *Solanum dimidiatum*.

INTRODUCTION

Field experiments and models suggest that terrestrial ecosystems will sequester atmospheric carbon in the coming century, but the magnitude of sequestration depends on the availability of nutrients to sustain productivity. According to the Intergovernmental Panel on Climate Change's Third Assessment Report, land biomes could accumulate the equivalent of 22–57% of anthropogenic CO₂ emissions (IPCC 2001). However, C sequestration by plants and soils may be limited if the accumulation of organic matter results in a progressive nitrogen limitation, PNL (Schlesinger and Lichter 2001, Gill et al. 2002, Hungate et al. 2003). The idea that nutrient feedbacks are important for pre-

dicting plant responses to CO₂ has been acknowledged for decades (Sionit et al. 1981, Patterson and Flint 1982, Zangerl and Bazzaz 1984, Thomas et al. 1991). More recently, Luo et al. (2004) presented a framework to evaluate the potential interactions between changes in atmospheric CO₂ concentrations (hereafter, C_a) and N cycling that is centered on the concept of PNL (Table 1). The PNL concept identifies two feedbacks whereby N could ultimately decrease the production response to rising CO₂. In the first, N is sequestered in plant biomass and litter, reducing its availability in soils and creating a negative feedback to production in an N-limited ecosystem. In the second, increased organic matter inputs lead to the sequestration of N in soil organic matter, again creating a negative feedback to production. The foundation of PNL postulates that if rising C_a increases biomass production, mineral nutrients may be bound in organic material, decreasing their availability in soil (Rastetter et al. 1997, Sterner and

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TABLE 1. Predicted interactions between C and N under elevated CO₂ (Luo et al. 2004).

Components of N limitation and feature measured	Support	Results
Components of progressive N limitation		
Production stimulated		
C ₃ perennial forbs	yes	1998–2000: aboveground biomass production positively correlated with C _a (Polley et al. 2003)
C ₄ grass <i>Bothriochloa ischaemum</i>	no	1998–2000: negative relationship with change in aboveground biomass production (Polley et al. 2003)
Whole-ecosystem	mixed	1997–1998: significant positive relationship between aboveground biomass and C _a 1999–2000: no relationship between aboveground biomass and C _a (Polley et al. 2003); plots exposed to elevated C _a 52% more productive aboveground and 59% more productive belowground (0–30 cm) than subambient plots (Gill et al. 2002)
Increased primary producer N demand	yes	Fig. 1a, b
Decreased labile soil N		
Net N mineralization	yes	threefold decrease in net N-mineralization rates during midsummer (Gill et al. 2002); Fig. 1d
Progressive decrease in production increment	mixed	significance of relationship between ANPP and C _a decreases with time, mostly due to replacement of relatively efficient N user (<i>B. ischaemum</i>) by less efficient C ₃ forbs (Polley et al. 2003)
Mechanisms preventing or alleviating progressive N limitation		
Higher C:N		
Plants	yes	positive relationship between C:N and C _a for roots and crowns of <i>B. ischaemum</i> and aboveground tissue for C ₃ forbs (Gill et al. 2002)
Soil	yes	positive relationship between C:N and C _a for total soil organic matter and POM (Gill et al. 2002)
Movement of N from low to high C:N pools		
Ecosystem	yes	Fig. 1
Soil	yes	Fig. 5
Increased N use efficiency		
Species	yes	positive relationship between photosynthetic NUE for two C ₃ forbs and C ₄ grass (Anderson et al. 2001)
Community	no	negative correlations between N concentration in biomass and C _a ; succession from C ₄ - to a C ₃ -dominated system eliminated or confounded CO ₂ effect on community NUE (Polley et al. 2003)
Interactions		
Decreased C mineralization rates	no	Fig. 3
Decreased N released from litter	yes	Fig. 3
Decreased soil C flux	nonlinear	Fig. 4

Elser 2002). However, the interaction of ecosystem C dynamics and soil N availability with changing C_a remains controversial (Luo et al. 2004).

The formation of organic matter clearly requires N and other nutrients based on biogeochemical stoichiometry. Unless N losses are reduced or N inputs are increased, the availability of labile N eventually should constrain the response of plant productivity to rising C_a (Oren et al. 2001, Gill et al. 2002, Luo et al. 2004). However, it is possible that within-ecosystem redistribution of N from soils (which have low C:N ratios) to plants (with higher ratios) could temporarily ameliorate

the direct effects of PNL. In addition, plants could increase N use efficiency (NUE) to compensate for decreased N availability.

A key assumption of Rastetter et al. (1997) is that the observed change in C:N ratios is sufficient to decrease the availability of mineral N, and that tissue chemistry is the dominant control over litter and soil organic matter decomposition. However, there is substantial evidence that, in many cases, C:N ratios are not significant predictors of decomposition rates (Franck et al. 1997, Gorissen and Cotrufo 2000). In addition, because species differ in C:N ratios, NUE,

and their response to changes in C_a , the need to understand community-level dynamics further complicates our ability to forecast the consequences of rising CO_2 on ecosystem processes (Hungate et al. 1996, Polley et al. 2003, Morgan et al. 2004).

Understanding interactions between vegetation responses to C_a and soil C and N cycling is particularly important in grasslands, where the soil provides the only sustained pool for carbon storage. Globally, the soil C pool in grasslands is $\sim 5.2 \times 10^{17}$ g (Jobbágy and Jackson 2000), $\sim 30\%$ of all terrestrial soil C. Among temperate ecosystems, grasslands have the largest percentage of total ecosystem C stored belowground, and a large portion of soil organic matter cycles slowly or is inert, and thus functions as a long-term sink for CO_2 . An important difference between grasslands and forests, where much of the evidence of PNL has been found, is that plant biomass is the primary sink for anthropogenic CO_2 in forests, whereas soils are the critical sink in grasslands.

Evidence for PNL in a Blackland Prairie ecosystem

A comprehensive analysis of PNL requires an evaluation of long-term responses of intact ecosystems to changes in C_a . Here we present a synthesis of information on C and N dynamics within an ecosystem dominated by C_3 perennial forbs and C_4 grasses exposed to a gradient in C_a from 200 to 560 $\mu\text{mol/mol}$ CO_2 for four years, specifically focusing on canopy N dynamics, soil N availability, and the redistribution of N within the system (see *Methods*, *Site description*, and Table 1).

The first assumption of PNL is that rising C_a will increase net primary productivity (Luo et al. 2004). Between 1997 and 1999, aboveground biomass increased with C_a , while the C_a effect on ANPP was not significant in 2000. However, the ecosystem response to C_a was confounded by community-level dynamics. Polley et al. (2003) found that with increasing C_a there was a shift in community dominance: C_3 forbs increased in abundance and C_4 biomass decreased at elevated C_a . Accompanying this increase in production and shift in community composition was an increased demand for N in aboveground plant tissues (Table 1). Perhaps as a consequence of N accumulation in biomass or changes in litter decomposition dynamics, we observed a net decrease in N mineralization rates as C_a increased (Gill et al. 2002), consistent with the assumptions of PNL (Rastetter et al. 1997, Luo et al. 2004).

Progressive nitrogen limitation also holds that that ecosystems can initially overcome CO_2 -induced N limitations through increased C:N in plants and soils, increased N use efficiency (NUE) for plants, or a transfer of N from organic pools with low C:N ratio to those with higher C:N ratios (Luo et al. 2004). In varying degrees, we found all of these mechanisms in the Blackland Prairie. Ratios of C:N for the two dominant

C_3 forbs and for perennial tissues in the dominant C_4 grasses were positively correlated with growth C_a , the daytime atmospheric CO_2 concentration (Gill et al. 2002), and C:N ratios for total soil organic matter and particulate organic matter increased with increasing C_a . Moreover, photosynthetic NUE was positively correlated with C_a for two C_3 forbs and a C_4 grass (Anderson et al. 2001). However, the response of N concentration in aboveground tissue to CO_2 enrichment varied inter-annually and by plant functional group. As a consequence of this variability and successional dynamics, there was no consistent CO_2 effect on community NUE (Polley et al. 2003). In this paper we present new data indicating that there has been a net transfer of N from SOM, with low C:N ratios, to plants with much larger C:N ratios.

The goal of this paper is to examine the consequences of changes in tissue C:N and within-ecosystem movement of N for N mineralization and carbon sequestration. First, we examine the influence of C_a on canopy and soil N to evaluate the net movement of N from soils to plants. Second, we use laboratory incubations and field measurements of soil respiration to assess the role of growth C_a on C and N mineralization. We hypothesized that increases in C:N with CO_2 enrichment would decrease C and N mineralization rates, potentially explaining the declines in N availability that have been observed. Third, we assess whether altered patterns of litter quality and amount have changed microbial biomass stocks. Finally, we evaluate whether changes in carbon sequestration in soil organic matter due to altered C_a can be explained because of movement of N from older soil organic matter with small C:N ratios to younger, more active pools of SOM with greater C:N ratios.

METHODS

Site description

We analyzed soil C storage and N dynamics at a controlled environment facility in the field at the Grassland, Soil and Water Research Lab (USDA Agricultural Research Service) in Temple, Texas, USA ($31^{\circ}05' N$, $97^{\circ}20' W$; Johnson et al. 2000). This facility controlled atmospheric CO_2 (C_a) along a continuous gradient from 200 to 560 $\mu\text{mol/mol}$ CO_2 . Two parallel, elongated chambers (1 m wide \times 1 m tall \times 60 m long) were constructed in 1996 on an intact grassland dominated by the C_4 perennial grass *Bothriochloa ischaemum* (L.) Keng and the C_3 perennial forbs *Solanum dimidiatum* Raf. and *Ratibida columnaris* (Sims) D. Don. Each chamber was subdivided into 10 sections 5 m long, separated by 1 m long ducts where air was cooled and dehumidified to maintain air temperature and vapor pressure deficit near ambient conditions. The atmospheric concentration of CO_2 was increased by adding pure CO_2 gas to air as it was injected into one chamber to initiate a superambient gradient (560–350 $\mu\text{mol/}$

mol); ambient air was injected into a second tunnel, initiating the subambient CO₂ gradient (350–200 μmol/mol). Gradients were maintained during each growing season (roughly February through November) from 1997 to 2000 by altering the flow rate of air through the chambers. Rubber-coated fabric was used to isolate intact chamber soil from the surrounding soil to a depth of 0.9 m. The soils were highly aggregated, with ~76% of the soil mass in macroaggregates (>250 μm) and an additional 13% in microaggregates (53–250 μm).

Ecosystem N dynamics

The N content of aboveground plant biomass was determined from samples collected yearly in June and October, corresponding to peak biomass for the C₃ or C₄ component of the vegetation, respectively (Polley et al. 2003). Two 0.5 × 0.2 m plots were clipped from each of the 20 sections of the chamber in June and October each year. Harvested plants were sorted by species, dried at 60°C, and weighed. The data reported here are for C₄ grasses and C₃ perennial forbs, which collectively account for >90% of aboveground biomass (Polley et al. 2003). The annual N accumulation for each functional group was calculated by multiplying peak biomass estimates by N concentrations for four years, 1997–2000. To minimize the influence of preexisting variability in vegetation composition, data are reported as the change in N accumulation between 1997 and 2000.

We measured total soil N content annually from 1997 to 2000 to assess whether any losses or gains of N from soil organic matter had occurred. The data reported here are differences in total soil N, calculated by multiplying N concentration in soil by bulk density between the years 2000 and 1997. Soils were collected to 15 cm depth in September of each year and sieved (2-mm mesh), and N concentration was measured using a CE Instruments NC 2100 elemental analyzer (ThermoQuest Italia, Milan, Italy).

Initial decomposition and soil respiration

We performed six-week laboratory incubations to determine rates of potential C and N mineralization from recently senesced plant tissue. The tissue was taken from two of the dominant plant species in the grassland: *Solanum dimidiatum*, the dominant C₃ forb, and *Bothriochloa ischaemum*, the principal C₄ grass. These incubations were conducted to evaluate initial decomposition dynamics for litter collected from plants grown along the field C_a gradient in December 1999 and 2000. Leaves and stems from the whole-plant harvest each year were bulked by species for each 5-m section for this experiment.

Potential rates of C and N mineralization from plant litter were determined using lab incubations in which 1 g of plant tissue was mixed with a common soil and incubated at field capacity and 22°C for six weeks. These incubations assessed the direct influence of tis-

sue chemistry on decomposition rates. They might differ from field-based decomposition rates because we do not simulate potential C_a effects on the abiotic environment in the lab experiment. To measure N mineralization twice during the six-week incubations, we assembled a filter apparatus contained within a Mason jar. The filter apparatus consisted of a Whatman G/FA filter placed within a Nalgene analytical test filter funnel with a glass wool placed over the filter. We used 25 g of a common soil that was collected in 1999 from the section maintained at ambient C_a. The soil was homogenized and passed through a 2-mm sieve to remove plant litter. Biomass was clipped into ~3 cm lengths and was placed on top of the soil. We used an infrared gas analyzer (EGM-3, PP Systems, Boston, Massachusetts, USA) to measure at 1, 3, 7, 14, 28, and 42 days the amount of CO₂ that accumulated in the headspace of jars after being capped for 1 hour. The CO₂ produced by decomposing plant tissue was determined by subtracting the rate of CO₂ production by soil blanks. At 14 and 42 days, the change in inorganic N within the filter apparatus was determined by adding and then extracting 100 mL of a leaching solution that contained both macro- and micronutrients, but had no inorganic N. The extracts were analyzed for NO₃-N and NH₄-N (QC8000, Lachat Instruments, Loveland, Colorado, USA). The change in inorganic N was determined by subtracting the amount of N in the extracts from the amount that was recovered from the soil blanks.

Rings of PVC pipe (10.5 cm diameter, 86.6 cm² area, 6 cm deep) for soil respiration measurements were installed in the field in each section of the two CO₂ chambers during chamber construction in 1996 (two per section, 40 rings total). Rings were placed in natural vegetation ~20 cm from the outer edge of each chamber and were pushed into the soil to a depth of 2 cm. Rings were clipped free of vegetation immediately after installation. Soil respiration was measured using the LI-6200 infrared gas analyzer with the LI-COR soil respiration chamber (LI-COR, Lincoln, Nebraska, USA). Measurement frequency ranged from three times in 1996 to monthly in 1998. Soil respiration measurements were always taken with the plastic chamber covers removed, to avoid pressure effects on the data (e.g., Nakayama and Kimball 1988, Ham et al. 1995). The PVC rings were clipped free of any small, new standing vegetation at least one hour before each measurement. If the soil within the ring had been disturbed (e.g., by worm activity), the soil was gently smoothed at least three hours before measurement, to minimize effects of soil hummocks on the chamber volume assumed by the LI-6200. At each measurement date, all rings were assessed in one day between 10:00 and 16:00 hours CST. The order in which rings were sampled along the gradient was random to avoid confounding variation due to the diurnal cycle with gradient effects.

Microbial biomass

Active soil microbial biomass (SMB) was determined for soils collected from the field using a substrate-induced respiration technique (Anderson and Domsch 1978). This method measures the response of SMB to a readily available substrate and differs somewhat from the commonly used chloroform fumigation procedure in that it is a measure of active, rather than total, SMB (Bailey et al. 2002). Two soil cores (0–20 cm depth) were collected from beneath *B. ischaemum* individuals along the CO₂ gradient in December 2000, sieved to remove roots and litter, and air dried. We rewetted 50 g of each soil to 50% field capacity and preincubated it in a Mason jar at 22°C for 7 d before adding sucrose as a substrate. We measured the CO₂ accumulated in the headspace during a 30-min interval before adding any substrate as an index of initial respiration rate. We added a sucrose solution to each jar sufficient to provide 4 mg sucrose/g soil and to bring the soils to field capacity. The soils were placed on an orbital shaker for 30 min. We then monitored changes in the rate of respiration at 1, 2, 4, 8, and 24 h. The substrate-induced respiration was calculated as the difference between the initial and maximum respiration rates in microliters of CO₂ per gram of soil per second. Microbial biomass was determined using the equation SMBC (μg/g soil) = (40.04ΔCO₂) + 0.37 (Bailey et al. 2002).

Particulate organic matter fractionation

The distribution of soil organic matter (SOM) within physical and chemical fractions that differ significantly in turnover rates and stoichiometry can provide evidence for how plants and microbes respond to changes in C and N availability (van Groenigen et al. 2002). We measured coarse (>250 μm) and fine (250–53 μm) particulate organic matter (POM) that was found either within soil aggregates or between aggregates from soil samples along the C_a gradient. Several authors have shown that POM has kinetic characteristics similar to the *intermediate* or *slow* pools in many simulation models, with a residence time of between a decade and a century (Cambardella and Elliott 1992, Kelly et al. 1996, Gill et al. 1999). The distribution of particulate organic matter (POM) by size and aggregate class is frequently used to infer temporal C dynamics. Relatively young POM is typically associated with macroaggregates, and older POM is found in microaggregates. In contrast, the youngest POM is thought not to be associated with any aggregates (light or free POM) and the oldest soil organic matter is thought to be mineral associated (<53 μm). The C:N ratios of these fractions suggest that there is a decrease in age from free POM to microaggregates. At our site, C:N ratios for the fractions of soil organic matter at ambient C_a are 32:1 for free POM, 27:1 for macroaggregate POM, and 25:1 for microaggregate POM.

We followed the method of Six et al. (1998) to determine soil POM fractions, using both physical and density fractionation. Briefly, we separated 100-g subsamples of field soils into two aggregate size classes, >250 μm and 250–53 μm, by wet-sieving. Following aggregate separation, free light fraction POM (POM outside of aggregates) was separated by density flotation using 1.85 g/mL sodium polytungstate (Six et al. 1998). After the free light fraction was removed, aggregates were dispersed by shaking for 18 h on a reciprocal shaker in a solution of 5 g/L sodium hexametaphosphate. Dispersed aggregates were then wet-sieved through 250-μm and 53-μm sieves. The organic C content of the material remaining on the sieves was considered intra-aggregate POM. Mineral-associated C was determined by the difference between total C and POM C. We determined POM C for two soil samples from each section ($n = 40$) that were collected in September 1997, 1999, and 2000.

Statistical considerations

We used two approaches to compare and analyze the experimental data. The first was to block responses as coming from either superambient or subambient chambers. This coarse-scale approach was used only to screen initial responses in the system to suggest broad trends in ecosystem responses to past or future C_a. Because of assumptions of independence between replicates, we could not perform ANOVAs for this blocking scheme, but means and standard deviations are reported to provide evidence of potential treatment differences between plots exposed to C_a at either below or above the current concentration. The second, more traditional, approach treated C_a as a continuous variable in regression analyses to determine the influence of CO₂ on N and C dynamics and net changes in soil fractions during the experiment. The relationship between ecosystem response variables and C_a was determined using SAS Proc GLM (SAS Institute 2002) and the Regression Wizard in Sigma Plot 8.0 for Windows (SPSS 2002). We used adjusted r^2 values to determine the best-fit model among linear, quadratic, logarithmic, power, and hyperbolic functions.

RESULTS

Ecosystem N dynamics

We found a general movement of N out of soil organic matter and into aboveground plant biomass with increased C_a (Fig. 1). During the four years of the experiment, there was incrementally more N in aboveground biomass of plants exposed to elevated than subambient C_a (Fig. 1a, b), which corresponded with a general increase in aboveground plant biomass with rising C_a (Polley et al. 2003). Over the same period, there was a significant negative relationship between C_a and the change in bulk soil N stocks ($r^2 = 0.28$, $P < 0.05$); on average, soils in subambient treatments

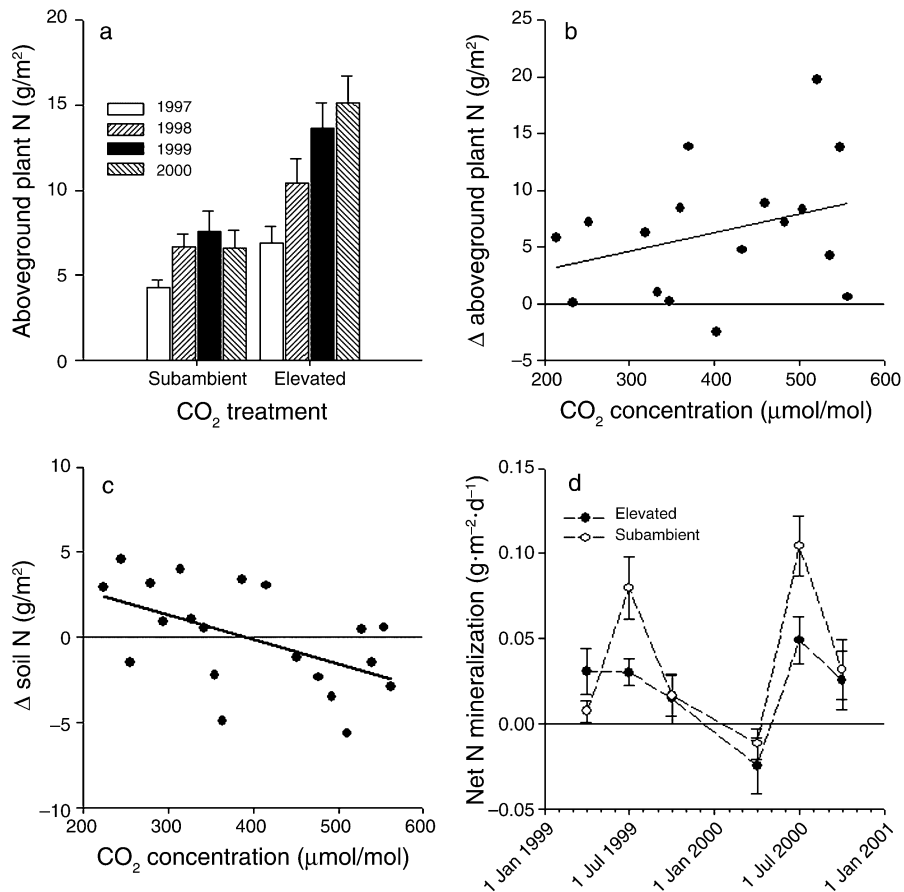


FIG. 1. Plant and soil N dynamics in a grassland exposed to a gradient in atmospheric CO₂: (a) total aboveground N (mean + SE) in C₄ grasses and C₃ forbs blocked by elevated and subambient CO₂ treatments; (b) difference in aboveground N increment in biomass between 2000 and 1997 ($r^2 = 0.10$, $P > 0.10$); (c) difference in total soil N between 2000 and 1997, showing a linear decline with increasing C_a ($r^2 = 0.29$, $P = 0.054$; $\Delta N = -0.14C_a + 55.8$ g/m²); (d) rates of net N mineralization (mean + SE) for six sampling dates during the 1999 and 2000 growing seasons. Solid circles are means for 10 sections exposed to elevated C_a; open circles are means for 10 sections exposed to subambient C_a.

gained 0.84 g N/m² and those at superambient C_a lost 0.96 g N/m² (Fig. 1c). We also found that in situ net N mineralization varied seasonally with C_a (Fig. 1d).

There was a linear increase in C:N for bulk soil organic matter and particulate organic matter (Fig. 2a, b). In addition, there was an increase in photosynthetic nitrogen use efficiency for three of the dominant species (Fig. 2c). Mean rates of N mineralization were low in spring and fall and highest during the warmest portion of the growing season (Fig. 1d). There was a significant, negative exponential relationship between C_a and net N mineralization in midsummer 1999 and 2000 (Gill et al. 2002). Relationships between growth C_a and N mineralization were not significant at other times. We also observed decreasing N concentration in aboveground tissue for both C₄ grasses and C₃ perennial forbs (Polley et al. 2003).

Initial decomposition

There were substantial differences in the impacts of C_a on decomposition dynamics in laboratory incuba-

tions for the two study species (Fig. 3). Carbon mineralized from aboveground tissue of the C₃ forb, *S. dimidiatum*, increased significantly with growth C_a and was greater in 1999 than in 2000 (Fig. 3a; $P < 0.01$ for both years). This positive relationship demonstrated that tissue from *S. dimidiatum* grown at subambient concentrations decomposed more slowly than that grown under elevated C_a. In contrast to the C₃ forb, there was no relationship between C_a and C mineralization for tissue from the C₄ grass, *B. ischaemum*, in either 1999 or 2000 (Fig. 3b; $P > 0.35$).

We also found important species differences in rates of N mineralization (Fig. 3c, d). For *S. dimidiatum*, N mineralization was nearly always positive, whereas *B. ischaemum* tissue consistently immobilized N during the six weeks of initial decomposition. Although C evolution from *S. dimidiatum* was positively correlated with growth C_a in both 1999 and 2000, net N mineralization from *S. dimidiatum* was negatively correlated with growth C_a only in 1999 (Fig. 3c; $r^2 = 0.37$, $P < 0.05$). Patterns of N immobilization from *B. ischaemum*

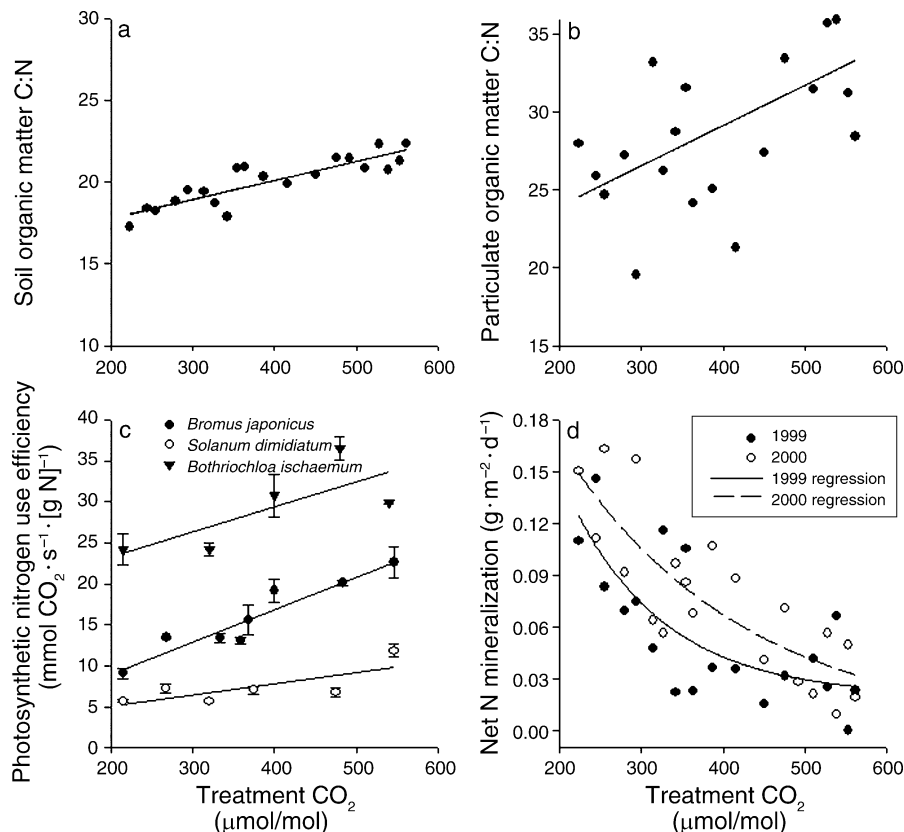


FIG. 2. For soil samples collected in December 2000 (a) whole-soil C:N ($P < 0.01$) and (b) particulate organic matter C:N ($P < 0.05$) increased linearly with C_a (Gill et al. 2002). (c) In 1999, photosynthetic nitrogen use efficiency ($A/\text{leaf N}$ content) was positively correlated with C_a for three of the dominant species. (d) During midsummer in both 1999 and 2000, there was a significant negative exponential relationship between in situ net N mineralization and C_a ($P < 0.001$). Panels (a), (b), and (d) are redrawn from Gill et al. (2002); panel (c) is redrawn from Anderson et al. (2001).

tissue were not correlated with growth CO_2 at all. C:N was not a significant predictor of decomposition rates for either species (data not shown).

Microbial biomass C and soil respiration

Soil microbial biomass (SMB) was relatively constant at C_a concentrations $< 470 \mu\text{mol/mol CO}_2$, but decreased dramatically beyond this threshold in C_a (Fig. 4a; $r^2 = 0.36$, $P < 0.03$). Soil respiration also followed a quadratic function and reached a maximum at $440 \mu\text{mol/mol CO}_2$ (Fig. 4b; $r^2 = 0.48$, $P < 0.003$). Mean respiration rates doubled from 1.9 to $4.0 \mu\text{mol CO}_2\text{-m}^{-2}\text{-s}^{-1}$ between 200 and $400 \mu\text{mol/mol C}_a$, and were variable at $> 400 \mu\text{mol/mol C}_a$. To account for pre-treatment variation in respiration rates, we also calculated the ratio of respiration in year 2000 to respiration in 1996 (Fig. 4c; $r^2 = 0.35$, $P < 0.03$). We found that respiration rates for all plots at $> 220 \mu\text{mol/mol C}_a$ were greater in 2000 than in 1996. In addition, the pre-treatment adjusted measure of soil respiration showed a quadratic relationship with C_a and reached a maximum at $435 \mu\text{mol/mol C}_a$, where respiration rates in 2000 were 3.5 times greater than in 1996. We also observed a general trend of higher respiration rates

during the summer than in the winter, with the difference between the elevated and subambient portions of the experiment increasing with time (Fig. 4d).

Particulate organic matter

The most substantial and consistent change that we observed was a strong, positive relationship between relatively young POM associated with macroaggregates that was equally well fit using either a linear or a power function (Fig. 5b; $r^2 = 0.57$, $P < 0.001$). The magnitude of the gains in POM at elevated C_a was similar to the magnitude of the losses at subambient C_a . We found a weak, positive relationship between C_a and the change in free POM (the youngest fraction of POM) between 1997 and 2000 (Fig. 5a; $r^2 = 0.16$, $P < 0.08$). There was a consistent pattern of an increase in free POM under elevated CO_2 , whereas most soils exposed to subambient C_a exhibited either no change or a decline in free POM (Fig. 5a). Consistent with the distribution of aggregates within the soil, most of the POM was found within macroaggregates. In September 1997, there was $\sim 550 \text{ g POM-C/m}^2$ in macroaggregates, with a range of $300\text{--}1000 \text{ g/m}^2$, whereas in microaggregates there was an average of 72 g POM-C/

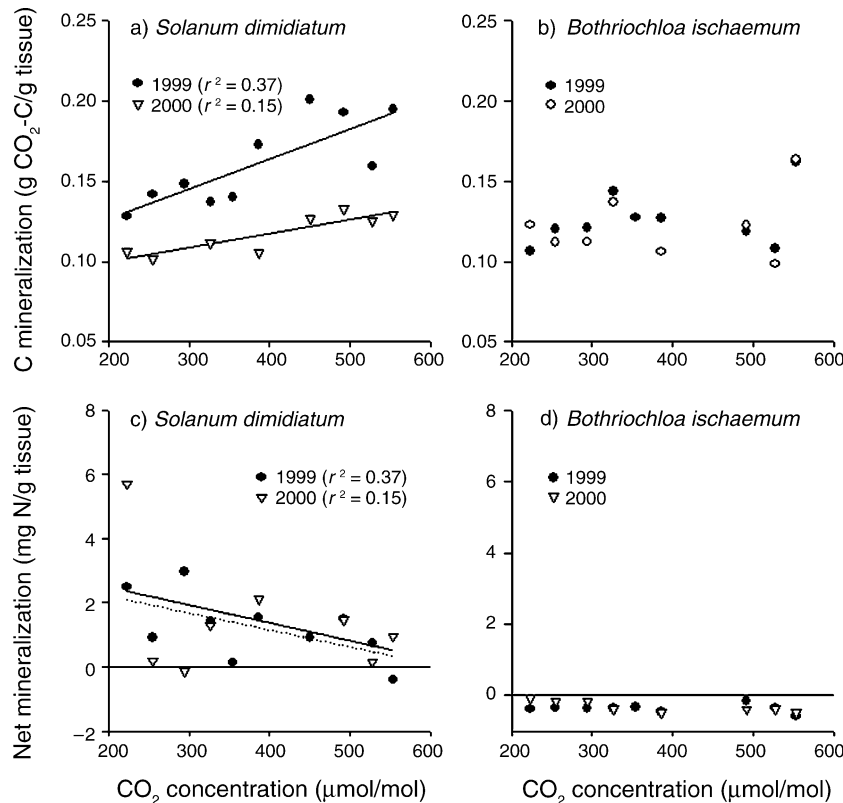


FIG. 3. C and N dynamics during the decomposition of *Bothriochloa ischaemum* (C_4 grass) and *Solanum dimidiatum* (C_3 forb) during six-week laboratory incubations. (a) C mineralization rates were positively correlated with growth C_a for *S. dimidiatum* in both 1999 ($r^2 = 0.64$, $P < 0.01$) and 2000 ($r^2 = 0.78$, $P < 0.01$). (b) C mineralization rates were not correlated with growth C_a for either year for *B. ischaemum*. (c) Net N mineralization was negatively correlated with growth C_a for *S. dimidiatum* tissue collected in 1999 ($r^2 = 0.37$, $P < 0.05$). (d) There was no relationship between growth C_a and net N mineralization rates from *B. ischaemum* tissue, although all samples immobilized N.

m^2 , with a range of 22–250 g/m^2 . The least responsive of the POM fractions was the microaggregate POM, where there appeared to be a weak trend of decreasing POM at elevated C_a with little change at subambient C_a (Fig. 5c; $r^2 = 0.16$, $P < 0.08$). Perhaps the most surprising result was the change in mineral-associated organic matter (MAOM). Although it is considered to be fairly inert, we found that there was a large net decline in MAOM at both elevated and subambient C_a (Fig. 5d). This was surprising both because of the magnitude of the change, with an average decline of 317 $g C/m^2$ under subambient conditions and 86 $g C/m^2$ at elevated C_a , and because there were losses in MAOM at elevated C_a where we anticipated increases in organic matter because of higher litter inputs. It is possible that these declines are due to the loss of dissolved organic carbon during the sieving process or it may point to a poor understanding of the nature of MAOM. The net result of these changes in SOM fractions was a significant nonlinear relationship between C_a and soil C stocks (Gill et al. 2002), with a net loss of soil C at subambient C_a without a concomitant increase at elevated C_a (Fig. 5d).

DISCUSSION

Plant–soil feedbacks

Many of our results were consistent with aspects of progressive N limitation. Even with increases in species-specific N use efficiency (Anderson et al. 2001, Polley et al. 2003), there was more N in aboveground pools because of increased biomass production at elevated C_a . Similar to results from the shortgrass steppe (King et al. 2004), we found that increased NPP with rising CO_2 transferred N from soils to plants. One striking result we found was that the increase in aboveground N stocks was much larger than the observed declines in soil N stocks (Fig. 1b, c). Our measures of soil N were restricted to the upper 20 cm of the soil profile, demonstrating that a significant portion of the N needed to maintain the CO_2 -derived production increment probably came from lower in the soil profile.

Although we observed the predicted declines in mineral N availability, these declines were not sufficient to eliminate all of the positive effects of increased C_a on aboveground biomass. C_3 forbs, which have a much lower NUE than C_4 species, often increased at the ex-

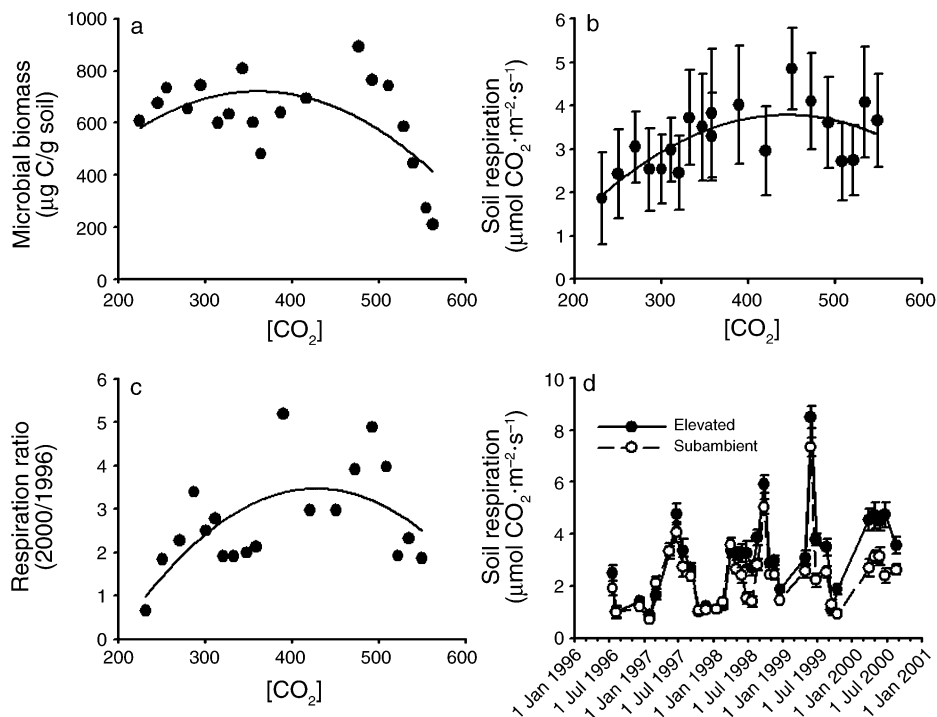


FIG. 4. (a) Relationship between active soil microbial biomass (ASMB) determined using substrate-induced respiration and growth C_a. The best fit was a quadratic relationship ($r^2 = 0.35$, $P < 0.03$). (b) Respiration rates (mean \pm SE, $n = 2$) for the 1999 growing season (April–October) along the CO₂ gradient ($r^2 = 0.48$, $P < 0.003$). (c) Ratio of the mean soil respiration rate in 2000 (five months, March–August 2000) divided by the mean of the growing-season respiration for 1996 (two months, July–August 1996) ($r^2 = 0.35$, $P < 0.03$). (d) Soil respiration, 1996–2000 (mean \pm SE), blocked by CO₂ treatment. The CO₂ treatment was initiated in May 1997. Treatments gradually diverge, on average, as the experiment progresses. In all graphs, the scale for [CO₂] is in units of $\mu\text{mol}/\text{mol}$.

pense of the dominant C₄ grasses with rising C_a, demonstrating that N was not necessarily the only controlling factor on C dynamics during the four-year experiment (Polley et al. 2003).

For decades it has been apparent that the effect of C_a on plant–soil interactions is likely to be mediated by species-specific and community responses (Tolley and Strain 1984, Bazzaz and Garbutt 1988, Bazzaz 1990, Jackson et al. 1994, Hungate et al. 1996, King et al. 2004, Morgan et al. 2004). Finzi and Schlesinger (2002) suggested that direct effects of elevated C_a had little impact on patterns of litter mass loss or nutrient dynamics in a loblolly pine forest, in spite of differences among species in the rate and pattern of litter decomposition. In the shortgrass steppe, a single C₃ grass (*Stipa comata*) was responsible for nearly all of the increased growth with CO₂, and this change in species dominance had substantial influence on forage quality. In our study, we also saw a net decline in C₄ dominance, with an increase in C₃ forb biomass (Polley et al. 2003). This change in species composition probably has a strong influence on soil processes, because there were substantial differences in decomposition dynamics among species. At all growth C_a, the decomposition of the C₃ forb mineralized N, with mineralization declining with increasing C_a. In contrast, the

decomposition of the C₄ grass consistently immobilized N and was unresponsive to C_a. Thus, the shift toward C₃ dominance in this system should increase net N mineralization rates. However, because N mineralization rates from C₃ litter decline with increasing CO₂, there is likely to be a much smaller increase in N availability at elevated CO₂ than we would have predicted based on the succession from C₄ to C₃ vegetation.

Whole-ecosystem N dynamics in this grassland are complicated by large differences in N content and NUE among species. Both C and N dynamics in decomposition differed between the dominant C₃ forb and C₄ grass. Indeed, in spite of linear increases in C:N ratios with C_a, leaf decomposition for the dominant C₃ species was positively correlated with growth C_a. This result is contrary to the predictions of many models suggesting that rising C_a will decrease rates of decomposition (Couteaux et al. 1991, Cotrufo et al. 1998), but is consistent with mounting evidence that the C:N ratio is not always the best predictor of tissue decomposition (Franck et al. 1997, Gorissen and Cotrufo 2000, Norby et al. 2001).

Soil C dynamics

Numerous researchers have traced new C into soil organic matter pools in an attempt to understand the

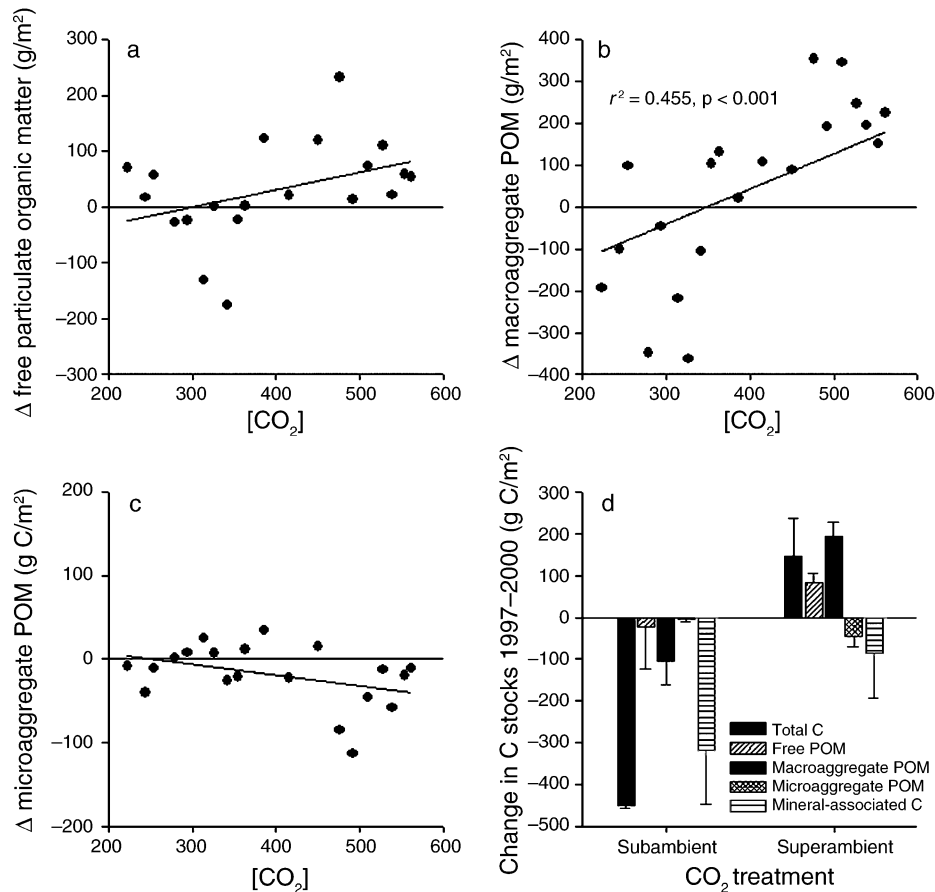


FIG. 5. Changes in particulate organic matter (POM) fractions and total soil C between 2000 and 1997. (a) Change in free POM, thought to be the most labile POM fraction, was not significantly correlated with treatment C_a , although superambient plots tended to show an increase in free POM, while most subambient plots lost free POM. (b) There was a significant linear relationship between macroaggregate POM and treatment C_a ($r^2 = 0.57$, $P < 0.01$). (c) There was a marginal negative polynomial relationship between microaggregate POM, considered to be the least labile POM fraction, and treatment C_a ($r^2 = 0.18$, $P = 0.19$). Although the relationship is poorly correlated, all seven of the plots exposed to >450 $\mu\text{mol/mol}$ CO_2 showed declines in microaggregate POM. In panels (a)–(c), the scale for $[\text{CO}_2]$ is in units of $\mu\text{mol/mol}$. (d) Mean changes in soil C pools at subambient C_a suggest that C is lost from all fractions of SOM. However, there were differences in the patterns of accumulation or loss of C from various SOM fractions at elevated C_a . There was an accumulation of C in the two most labile pools of SOM (free and macroaggregate POM), while there was a net loss from the two most recalcitrant SOM pools (microaggregate and mineral-associated SOM), leading to only modest increases in SOM at elevated C_a .

fate of C at elevated C_a (Van de Geijn and Van Veen 1993, Zak et al. 1993, Cardon et al. 2001, Van Groenigen et al. 2002). In our experiment, aboveground and belowground litter inputs increased with rising C_a (Gill et al. 2002, Polley et al. 2003), leading to an accumulation of organic matter in the two most labile particulate organic matter pools that have the greatest C:N ratios of SOM fractions (Fig. 5a, b). However, at elevated C_a , accompanying this increase in light free POM and macroaggregate-associated POM was a decline in the two most recalcitrant fractions of SOM: microaggregate-associated POM and mineral-associated organic matter (Fig. 5). All fractions of SOM declined at subambient C_a . The net effect of these changes is a decrease in total organic soil C at subambient C_a , and little or no increase in organic C at elevated C_a (see Fig. 5c; Gill et al. 2002).

There are two hypotheses that might explain how rising C_a increased plant production without increasing soil C storage. The first is that litter produced at elevated C_a is preferentially partitioned to labile pools (Hungate et al. 1997). A second hypothesis is that input rates to various organic matter fractions remain unchanged, but that decomposition from older organic matter pools increases because of N limitation. Cardon et al. (2001) found that elevated C_a increased total POM while decreasing the input of new C into mineral-associated organic matter pools. The change in partitioning of C between organic matter pools suggests that elevated C_a might retard the transformation of new litter into recalcitrant organic matter, accumulating organic matter in labile pools. Because new C enters organic matter pools with short residence times, the production increment observed in plants may not scale to carbon

storage in soils (Hungate et al. 1997). Nitrogen released from litter during decomposition of the dominant C₃ forb (Fig. 3c) and net N mineralization (Fig. 2d) both decreased with rising C_a (Gill et al. 2002). As available inorganic N decreases, plant and soil microbial competition for N probably intensifies (Cardon et al. 2001). Hu et al. (2001) suggested that microbial decomposition rates may be retarded as N limitation increases. It is plausible that soil microbes may begin to use the N-rich, but energetically poor, substrate in older organic matter to meet their N stoichiometric requirements.

One of the most consistent patterns that we observed in our data was a maximum in soil CO₂ flux and microbial biomass at between 400 and 500 μmol/mol C_a. This nonlinear response may be the result of the interaction among changes in litter chemistry, plant community composition, and nutrient availability. Based on systematic increase in C₃ forbs with rising C_a and the positive response of *Solanum dimidiatum* decomposition with rising C_a, we could forecast a linear increase in soil respiration. However, it may be that there is a shift in microbial community composition as N availability decreases to some threshold where there might be a decline in bacteria and an increase in fungi that are better adapted to decompose litter in a low-N environment (Zak et al. 2000, Phillips et al. 2002).

By understanding changes in plant community dynamics, net primary production, and soil C dynamics in this C₃/C₄ grassland, we are beginning to understand the mechanisms controlling ecosystem C balance. The potential to partition carbon into organic matter pools with short residence times under rising C_a could have profound implications for the exchange of C from the atmosphere to the terrestrial biosphere. Over the four years of this experiment, production responses to C_a were maintained, in part, because of the redistribution of N within ecosystem N stocks. The long-term ability of this system to be more productive with rising C_a may be constrained by the ability of microbes and plants to access N from older pools of soil organic matter. Ultimately, if the C gained by increased plant production is rapidly lost through decomposition, little benefit in net C sequestration may accrue from the observed shift in N from soil to plants.

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LITERATURE CITED

- Anderson, J. P. E., and K. H. Domsch. 1978. A physiological method for quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry* **10**:215–221.
- Anderson, L. J., H. Maherali, H. B. Johnson, H. W. Polley, and R. B. Jackson. 2001. Gas exchange and photosynthetic acclimation over subambient to elevated CO₂ in a C-3 C-4 grassland. *Global Change Biology* **7**:693–707.
- Bailey, V. L., A. D. Peacock, J. L. Smith, and H. Bolton, Jr. 2002. Relationships between soil microbial biomass determined by chloroform fumigation-extraction, substrate-induced respiration, and phospholipid fatty acid analysis. *Soil Biology and Biochemistry* **34**:1385–1389.
- Bazzaz, F. A. 1990. The response of natural ecosystems to the rising global CO₂ levels. *Annual Review of Ecology and Systematics* **21**:167–196.
- Bazzaz, F. A., and K. Garbutt. 1988. The response of annuals in competitive neighborhoods: effects of elevated CO₂. *Ecology* **69**:937–946.
- Cambardella, C. A., and E. T. Elliott. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Science Society of America Journal* **56**:777–783.
- Cardon, Z. G., B. A. Hungate, C. A. Cambardella, F. S. Chapin, III, C. B. Field, E. A. Holland, and H. A. Mooney. 2001. Contrasting effects of elevated CO₂ on old and new soil carbon pools. *Soil Biology and Biochemistry* **33**:365–373.
- Cotrufo, M. F., P. Ineson, and A. Scott. 1998. Elevated CO₂ reduces the nitrogen concentration of plant tissues. *Global Change Biology* **4**:43–54.
- Coûteaux, M., M. Mousseau, M.-L. Célérier, and P. Bottner. 1991. Increased atmospheric CO₂ and litter quality: decomposition of sweet chestnut leaf litter with animal food webs of different complexities. *Oikos* **61**:54–64.
- Finzi, A. C., and W. H. Schlesinger. 2002. Species control variation in litter decomposition in a pine forest exposed to elevated CO₂. *Global Change Biology* **8**:1217–1229.
- Franck, V. M., B. A. Hungate, F. S. Chapin, III, and C. B. Field. 1997. Decomposition of litter produced under elevated CO₂: dependence on plant species and nutrient supply. *Biogeochemistry* **36**:223–237.
- Gill, R. A., I. C. Burke, D. G. Milchunas, and W. K. Lauenroth. 1999. Relationship between root biomass and soil organic matter pools in the shortgrass steppe of eastern Colorado: implications for decomposition through a soil profile. *Ecosystems* **2**:226–236.
- Gill, R. A., H. W. Polley, H. B. Johnson, L. J. Anderson, H. Maherali, and R. B. Jackson. 2002. Nonlinear grassland responses to past and future atmospheric CO₂. *Nature* **417**:279–282.
- Gorissen, A., and M. F. Cotrufo. 2000. Decomposition of leaf and root tissue of three perennial grass species grown at two levels of atmospheric CO₂ and N supply. *Plant and Soil* **224**:75–84.
- Ham, J. A., C. E. Owensby, P. I. Coyne, and D. J. Bremer. 1995. Fluxes of CO₂ and water vapor from a prairie ecosystem exposed to ambient and elevated CO₂. *Agricultural and Forest Meteorology* **77**:73–93.
- Hu, S., F. S. Chapin, III, M. K. Firestone, C. B. Field, and N. R. Chiariello. 2001. Nitrogen limitation of microbial decomposition in a grassland under elevated CO₂. *Nature* **409**:188–191.
- Hungate, B. A., J. Canadell, and F. S. Chapin, III. 1996. Plant species mediate changes in soil microbial N in response to elevated CO₂. *Ecology* **77**:2505–2515.
- Hungate, B. A., J. S. Dukes, M. R. Shaw, Y. Luo, and C. B. Field. 2003. Nitrogen and climate change. *Science* **302**:1512–1513.
- Hungate, B. A., E. A. Holland, R. B. Jackson, F. S. Chapin, III, H. A. Mooney, and C. B. Field. 1997. The fate of carbon in grasslands under carbon dioxide enrichment. *Nature* **388**:576–579.
- IPCC (Intergovernmental Panel on Climate Change). 2001. *Climate change 2001: the scientific basis*. Cambridge University Press, New York, New York, USA.

- Jackson, R. B., O. E. Sala, C. B. Field, and H. A. Mooney. 1994. CO₂ alters water use, carbon gain, and yield for the dominant species in a natural grassland. *Oecologia* **98**:257–262.
- Jobbágy, E. G., and R. B. Jackson. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications* **10**:423–436.
- Johnson, H. B., H. W. Polley, and R. P. Whitis. 2000. Elongated chambers for field studies across atmospheric CO₂ gradients. *Functional Ecology* **14**:388–396.
- Kelly, R., I. C. Burke, and W. K. Lauenroth. 1996. Soil organic matter and nutrient availability responses to reduced plant inputs. *Ecology* **77**:2516–2527.
- King, J. Y., A. R. Mosier, J. A. Morgan, D. R. LeCain, D. G. Milchunas, and W. J. Parton. 2004. Plant nitrogen dynamics in shortgrass steppe under elevated atmospheric carbon dioxide. *Ecosystems* **7**:147–160.
- Luo, Y., et al. 2004. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *BioScience* **54**:731–739.
- Morgan, J. A., A. R. Mosier, D. G. Milchunas, D. R. LeCain, J. A. Nelson, and W. J. Parton. 2004. CO₂ enhances productivity, alters species composition, and reduces digestibility of shortgrass steppe vegetation. *Ecological Applications* **14**:208–219.
- Nakayama, F. S., and B. A. Kimball. 1988. Soil carbon dioxide distribution and flux within the open-top chamber. *Agronomy Journal* **80**:394–398.
- Norby, R. J., M. F. Cotrufo, P. Ineson, E. G. O'Neill, and J. G. Canadell. 2001. Elevated CO₂, litter chemistry, and decomposition: a synthesis. *Oecologia* **127**:153–165.
- Oren, R., D. S. Ellsworth, K. H. Johnsen, N. Phillips, B. E. Ewers, C. Maier, K. V. R. Schafer, H. McCarthy, G. Hendrey, S. G. McNulty, and G. G. Katul. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO₂ enriched atmosphere. *Nature* **411**:469–472.
- Patterson, D. T., and E. P. Flint. 1982. Interacting effects of CO₂ and nutrient concentration. *Weed Science* **30**:389–394.
- Phillips, R. L., D. R. Zak, W. E. Holmes, and D. C. White. 2002. Microbial community composition and function beneath temperate tree exposed to elevated atmospheric carbon dioxide and ozone. *Oecologia* **131**:236–244.
- Polley, H. W., H. B. Johnson, and J. D. Derner. 2003. Increasing CO₂ from subambient to superambient concentrations alters species composition and increases above-ground biomass in a C-3/C-4 grassland. *New Phytologist* **160**:319–327.
- Rastetter, E. B., G. I. Ågren, and G. R. Shaver. 1997. Responses of N-limited ecosystems to increased CO₂: a balanced-nutrition, coupled-element-cycles model. *Ecological Applications* **7**:444–460.
- SAS Institute. 2002. SAS version 9.0. SAS Institute, Cary, North Carolina, USA.
- Schlesinger, W. H., and J. Lichter. 2001. Limited carbon storage in soils and litter of experimental forest plots under increased atmospheric CO₂. *Nature* **411**:466–469.
- Sionit, N., D. A. Mortensen, B. R. Strain, and H. Hellmers. 1981. Growth-response of wheat to CO₂ enrichment and different levels of mineral nutrition. *Agronomy Journal* **73**:1023–1027.
- Six, J., E. T. Elliott, K. Paustian, and J. W. Doran. 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. *Soil Science Society of America Journal* **62**:1367–1377.
- SPSS. 2002. SigmaPlot version 8.0. SPSS, Chicago, Illinois, USA.
- Tolley, L. C., and B. R. Strain. 1984. Effects of atmospheric CO₂ enrichment and water stress on growth of *Liquidambar styraciflua* and *Pinus taeda* seedlings. *Canadian Journal of Botany* **62**:2135–2139.
- Van de Geijn, S. C., and J. A. Van Veen. 1993. Implications of increased carbon-dioxide levels for carbon input and turnover in soils. *Vegetatio* **104**:283–292.
- van Groenigen, K.-J., D. Harris, W. R. Horwath, U. A. Hartwig, and C. van Kessel. 2002. Linking sequestration of ¹³C and ¹⁵N in aggregates in a pasture soil following 8 years of elevated atmospheric CO₂. *Global Change Biology* **8**:1094–1108.
- Zak, D. R., K. S. Pregitzer, P. S. Curtis, and W. E. Holmes. 2000. Atmospheric CO₂ and the composition and function of soil microbial communities. *Ecological Applications* **10**:47–59.
- Zak, D. R., K. S. Pregitzer, P. S. Curtis, J. A. Teeri, R. Fogel, and D. L. Randlet. 1993. Elevated atmospheric CO₂ and feedback between carbon and nitrogen cycles. *Plant and Soil* **151**:105–117.
- Zangerl, A. R., and F. A. Bazzaz. 1984. The response of plants to elevated CO₂ II. Competitive interactions among annual plants under varying light and nutrients. *Oecologia* **62**:412–417.