

2009

# Primary Productivity and Water Balance of Grassland Vegetation on Three Soils in a Continuous CO<sub>2</sub> Gradient: Initial Results from the Lysimeter CO<sub>2</sub> Gradient Experiment

Philip A. Fay

USDA-ARS Grassland Soil and Water Research Laboratory, [philip.fay@ars.usda.gov](mailto:philip.fay@ars.usda.gov)

Alexia M. Kelley

Duke University

Andrew C. Procter

Duke University

Dafeng Hui

Tennessee State University

Virginia L. Jin

USDA-ARS Grassland Soil and Water Research Laboratory, [virginia.jin@ars.usda.gov](mailto:virginia.jin@ars.usda.gov)

*See next page for additional authors*

Follow this and additional works at: <http://digitalcommons.unl.edu/usdaarsfacpub>

 Part of the [Agricultural Science Commons](#)

---

Fay, Philip A.; Kelley, Alexia M.; Procter, Andrew C.; Hui, Dafeng; Jin, Virginia L.; Jackson, Robert B.; Johnson, Hyrum B.; and Polley, H. Wayne, "Primary Productivity and Water Balance of Grassland Vegetation on Three Soils in a Continuous CO<sub>2</sub> Gradient: Initial Results from the Lysimeter CO<sub>2</sub> Gradient Experiment" (2009). *Publications from USDA-ARS / UNL Faculty*. 425.  
<http://digitalcommons.unl.edu/usdaarsfacpub/425>

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Publications from USDA-ARS / UNL Faculty by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

---

**Authors**

Philip A. Fay, Alexia M. Kelley, Andrew C. Procter, Dafeng Hui, Virginia L. Jin, Robert B. Jackson, Hyrum B. Johnson, and H. Wayne Polley

# Primary Productivity and Water Balance of Grassland Vegetation on Three Soils in a Continuous CO<sub>2</sub> Gradient: Initial Results from the Lysimeter CO<sub>2</sub> Gradient Experiment

Philip A. Fay,<sup>1\*</sup> Alexia M. Kelley,<sup>2</sup> Andrew C. Procter,<sup>2</sup> Dafeng Hui,<sup>3</sup> Virginia L. Jin,<sup>1</sup> Robert B. Jackson,<sup>2</sup> Hyrum B. Johnson,<sup>1</sup> and H. Wayne Polley<sup>1</sup>

<sup>1</sup>USDA-ARS Grassland Soil and Water Research Laboratory, 808 E Blackland Rd, Temple, Texas 76502, USA; <sup>2</sup>Department of Biology and Nicholas School of the Environment and Earth Sciences, Duke University, Durham, North Carolina 27708, USA; <sup>3</sup>Department of Biological Sciences, Tennessee State University, Nashville, Tennessee 37209, USA

## ABSTRACT

Field studies of atmospheric CO<sub>2</sub> effects on ecosystems usually include few levels of CO<sub>2</sub> and a single soil type, making it difficult to ascertain the shape of responses to increasing CO<sub>2</sub> or to generalize across soil types. The Lysimeter CO<sub>2</sub> Gradient (LYCOG) chambers were constructed to maintain a linear gradient of atmospheric CO<sub>2</sub> (~250 to 500 μl l<sup>-1</sup>) on grassland vegetation established on intact soil monoliths from three soil series. The chambers maintained a linear daytime CO<sub>2</sub> gradient from 263 μl l<sup>-1</sup> at the subambient end of the gradient to 502 μl l<sup>-1</sup> at the superambient end, as well as a linear nighttime CO<sub>2</sub> gradient. Temperature variation within the chambers affected aboveground biomass and evapotranspiration, but the effects of temperature were small compared to the expected effects of CO<sub>2</sub>. Aboveground biomass on Austin soils was 40% less than on Bastrop and Houston soils. Biomass differences between soils resulted from variation in biomass of *Sorghastrum*

*nutans*, *Bouteloua curtipendula*, *Schizachyrium scoparium* (C<sub>4</sub> grasses), and *Solidago canadensis* (C<sub>3</sub> forb), suggesting the CO<sub>2</sub> sensitivity of these species may differ among soils. Evapotranspiration did not differ among the soils, but the CO<sub>2</sub> sensitivity of leaf-level photosynthesis and water use efficiency in *S. canadensis* was greater on Houston and Bastrop than on Austin soils, whereas the CO<sub>2</sub> sensitivity of soil CO<sub>2</sub> efflux was greater on Bastrop soils than on Austin or Houston soils. The effects of soil type on CO<sub>2</sub> sensitivity may be smaller for some processes that are tightly coupled to microclimate. LYCOG is useful for discerning the effects of soil type on the CO<sub>2</sub> sensitivity of ecosystem function in grasslands.

**Key words:** carbon dioxide; climate change; grassland; hydrology; net primary productivity; photosynthesis; soil moisture; soil respiration; *Solidago canadensis*.

Received 1 December 2008; accepted 11 March 2009;  
published online 21 May 2009

Author Contributions: PF conceived study, analyzed data, and wrote the paper. AK, AP analyzed data. DH, VJ, RJ, HJ, and WP conceived study, and conducted research.

\*Corresponding author; e-mail: philip.fay@ars.usda.gov

## INTRODUCTION

Atmospheric CO<sub>2</sub> concentration (C<sub>A</sub>) has increased by about 100 μl l<sup>-1</sup> over the last 250 years to approximately 380 μl l<sup>-1</sup>, its highest value since

the pre-industrial era. Further,  $C_A$  has increased more quickly in the last 30 years than in the prior 200 years (Forster and others 2007). Increasing  $C_A$  affects many ecosystem processes. Typically, field studies have examined only two or a few experimental levels of  $C_A$ , making it difficult to detect the presence of non-linear or threshold responses to  $C_A$ . These field experiments also have typically been constrained to one soil type. However,  $CO_2$  change is continuous, and soils differ in hydrologic and biogeochemical properties that can constrain ecosystem responses to increasing  $C_A$ . For example, soil texture mediates the distribution of water in the soil profile (Noy-Meir 1973), its availability to plants (Brady and Weil 2002), the availability of organic substrates to decomposers (Jenkinson 1977; Oades 1988), and the accumulation of organic matter (Hassink 1996). Therefore, the impacts of multiple levels of  $C_A$  on ecosystem function must be understood on different soil types to resolve the shape of ecosystem productivity, carbon cycling, and community structure as a function of  $C_A$ , to understand variation in these and other responses across soils, and ultimately to predict spatial variation in ecosystem structure and function under future climate scenarios (Ainsworth and Long 2005; Rogers and others 2006).

The Lysimeter  $CO_2$  Gradient (LYCOG) facility was constructed to impose a continuous gradient of  $C_A$  representing pre-industrial to mid twenty-first century levels ( $\sim 250$  to  $500 \mu l l^{-1}$ ) on multiple soil types. LYCOG consists of outdoor chambers suited for grassland or other short-statured ( $\leq \sim 1$  m tall) vegetation. The design objectives were to maintain the prescribed gradient in  $C_A$  while also controlling air temperature ( $T_A$ ) and precipitation inputs near ambient values. LYCOG evolved from previous  $C_A$  gradient systems (Mayeux and others 1993; Johnson and others 2000) but incorporates several new features, (1) intact, hydrologically isolated soil monoliths from three soil series initially planted to the same species of native perennial grasses and forbs, (2) weighing lysimeters, and (3) a system to sample drainage water exiting the bottom of the monoliths. These features allow more precise evaluation of soil type effects on ecosystem processes and resolution of the effect of  $CO_2$  on the water and carbon budgets of these soils than would be possible in field plots.

Here our objectives are to (1) review the lineage of  $CO_2$  gradient facilities that led to the LYCOG facility and the major results each provided, (2) document chamber function in terms of control of  $CO_2$  and air temperature, (3) quantify the pre- $CO_2$  treatment plant species assemblages and soil C and

N concentrations, (4) evaluate preliminary responses to  $C_A$  in aboveground net primary productivity, leaf photosynthesis, soil respiration, and the ecosystem water budget.

## PREVIOUS $CO_2$ GRADIENT FACILITIES

LYCOG is the third generation of  $CO_2$  gradient facilities. It was preceded by a prototype gradient experiment that established the viability of the technique, and then by a field facility. All three  $CO_2$  gradient facilities rely on the simple concept that sunlit plants photosynthesizing in an enclosed linear chamber will deplete  $CO_2$  from parcels of air moving directionally through the chamber. This linear chamber approach results in continuously varying  $C_A$ , and a unique capacity to evaluate plants and soils for linear, non-linear, and threshold responses to changing  $C_A$ .

### Prototype Subambient to Ambient Gradient

Mayeux and others (1993) constructed the first  $CO_2$  gradient facility at the USDA laboratory at Temple, Texas, USA. It consisted of a 38 m long  $\times$  45 cm wide serpentine chamber constructed in a greenhouse. The upper portion of the chamber was a clear polyethylene tube enclosing the aerial growth of plants, fixed to a 76 cm deep lower portion filled with soil. The chamber was supplied with ambient air, which during daylight was depleted to approximately  $200 \mu l l^{-1}$ . Air temperature was controlled by a system of chilled water cooling coils and electrical resistance heaters. The system was well suited for leaf-level physiological studies of plant responses to subambient variation in  $CO_2$  concentrations.

Monocultures and simple mixtures of species including *Triticum aestivum*, *Avena sativa*, *Brassica kaber*, and *Schizachyrium scoparium* were successfully grown in this chamber to examine the effects of increases in  $C_A$  from subambient to ambient values on vegetation function. Most species examined showed increases in total biomass, photosynthetic carbon assimilation, water and nitrogen use efficiencies, and decreased stomatal conductance in  $C_4$  species (Polley and others 1992a, 1992b, 1993a, 1993b, 1994, 1995, 1996). These results suggested that sizeable changes in ecosystem function and plant growth may already have occurred in response to rising  $C_A$ , including increased vegetation productivity (Johnson and others 1993; Polley and others 1993a) and increased growth of woody species over dominant grasses (Polley and others

1994) because of reduced water limitation (Polley and others 1995).

### The Prairie CO<sub>2</sub> Gradient (PCG)

The PCG facility was the first field implementation of a self-maintaining CO<sub>2</sub> gradient, on perennial C<sub>4</sub> grassland at Temple, Texas, USA (31°05' N, 97°20' W). Advancements of PCG over the greenhouse prototype included (1) extension of the gradient to superambient concentrations, (2) a nighttime C<sub>A</sub> gradient, created by reversing the direction of air flow, and allowing nighttime plant + soil respiration to progressively increase C<sub>A</sub>. PCG achieved gradients of about 550–200 μl l<sup>-1</sup> during daytime, and approximately 720–370 μl l<sup>-1</sup> at night using two linear chambers enclosing 100 linear m of intact grassland (Johnson and others 2000).

PCG's subambient to superambient gradient allowed for evaluation of the shape (linear or nonlinear) of ecosystem responses under conditions representing a continuum from past to future C<sub>A</sub>. Responses varied among the ecosystem processes that were studied. For example, plant water status, leaf carbon assimilation and water use efficiency increased linearly across the gradient (Anderson and others 2001). However, during the 4 years of the PCG study, aboveground net primary productivity increased linearly with C<sub>A</sub> in some years, but the increase was nonlinear in others, showing less increase at superambient C<sub>A</sub> (Polley and others 2003). Soil organic carbon content also increased from subambient to ambient C<sub>A</sub> but not beyond, and decomposition of older soil C increased at superambient C<sub>A</sub>, whereas N mineralization rates decreased (Gill and others 2002). These results were consistent with N limitation of ANPP at above-ambient C<sub>A</sub>. Together, the studies from the PCG facility suggested that linear increases in plant water status or productivity may not translate into increased soil C if N or other resources limit ecosystem function (Gill and others 2006).

### The Lysimeter CO<sub>2</sub> Gradient (LYCOG)

LYCOG is the second field implementation of a self-maintaining CO<sub>2</sub> gradient. LYCOG built on PCG by incorporating multiple soils and the capacity to completely close ecosystem water budgets. The overarching goal of LYCOG is to test the hypothesis that soil properties will influence, and could even override, the effects of C<sub>A</sub> on NPP, decomposition (Epstein and others 2002; Jenkinson 1977; Oades 1988), C accumulation in soil (Hagedorn and

others 2003), and plant community structure among other processes.

## METHODS

### Study Site

LYCOG is located on the same site as PCG. The original native vegetation was Blackland Prairie, which is the southern extension of the North American tallgrass prairie. Intact Blackland Prairie plant communities are dominated by C<sub>4</sub> grasses accounting for most of the biomass, accompanied by numerous forb species. Less than 5% of this ecosystem remains in Central Texas, but it is an important benchmark for the structure and function of the diverse native grassland ecosystems in this region. The climate is classified as subtropical, and LYCOG is in an area of transition between humid and sub-humid zones. Mean annual precipitation is 914 mm (1971–2000), falling in a bimodal distribution with growing season wet periods in May–June and September–October, and a pronounced July–August dry period. Precipitation patterns are governed by interactions between onshore flows of tropical maritime air from the Gulf of Mexico and colder continental air masses. Temperatures range from a July–August mean maximum of 35°C to a December mean minimum of 2.9°C. The mean frost free period is approximately 250 days, from mid March to late November.

### Chamber Design

LYCOG uses the aboveground chamber, CO<sub>2</sub> enrichment, temperature control, and monitoring systems from PCG to enclose a CO<sub>2</sub> gradient over intact soil monoliths. There are again two linear chambers, arranged in parallel on a north–south axis (Figure 1). Each chamber is 1.2 m wide, 1.5 m tall, and 60 m long, divided into 10–5 m long sections. Each section houses a steel container 5 × 1.2 × 1.6 m<sup>3</sup> deep buried to 1.5 m. Each container accommodates four 1 × 1 × 1.5 m deep intact soil monoliths housed in water-tight steel boxes (Polley and others 2008). Adjacent containers are joined by a 1-m sheet-metal plenum housing a chilled-water cooling coil. The coils are supplied by a 161.4 kW refrigeration unit that circulates coolant at 10°C.

Eighty intact soil monoliths were excavated in 2002 from three soil series: Houston Black clay (32 monoliths), a vertisol (Udic Haplustert) typical of lowlands; Austin (32 monoliths), a high carbonate, silty clay mollisol (Udorthentic Haplustol) typical of

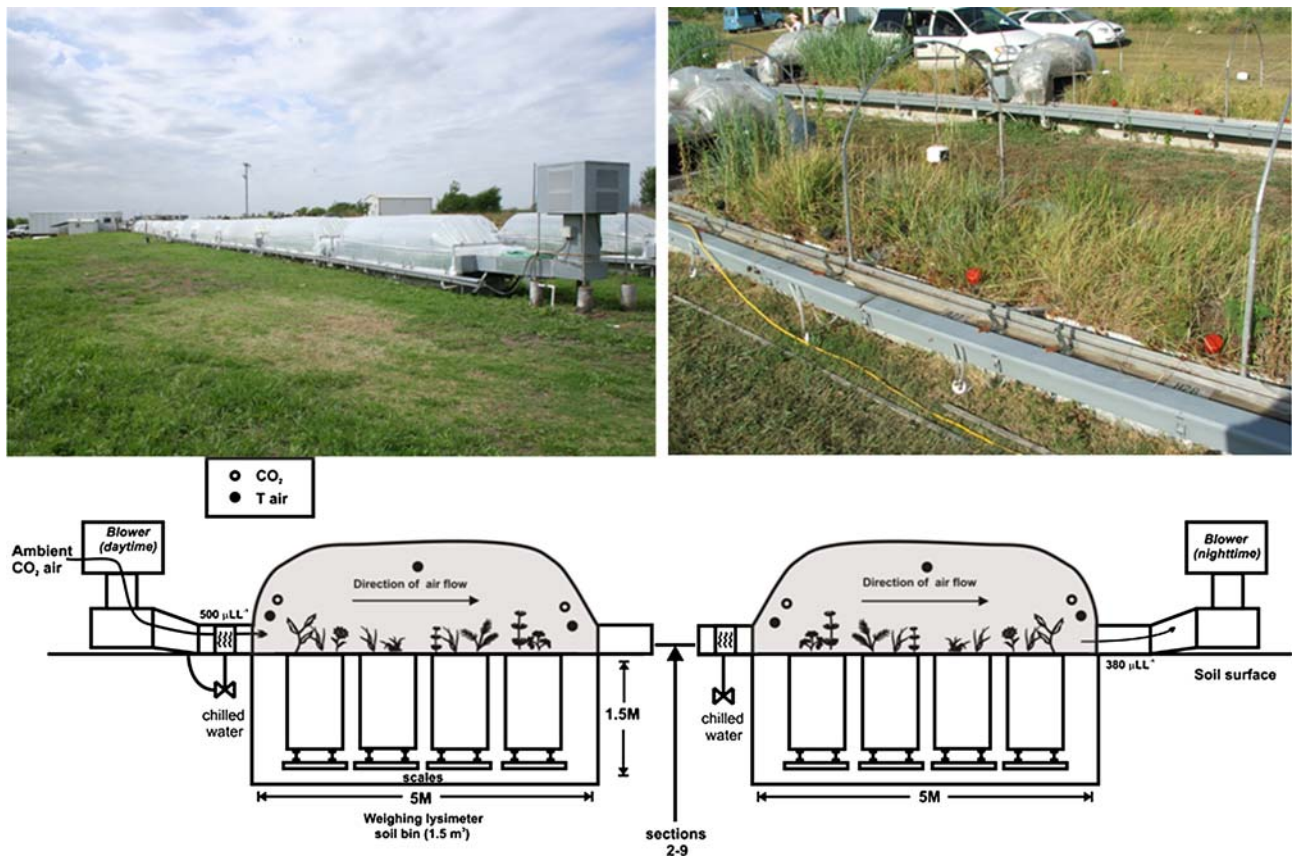


Figure 1. The LYCOG facility for maintaining a continuous gradient of atmospheric  $\text{CO}_2$  concentration on vegetation growing in enclosed soil monoliths. View of the superambient chamber looking from the daytime air exit toward the chamber entrance. The white building at left houses the monitoring, control, and data logging equipment. The subambient chamber runs parallel to the superambient chamber, and is mostly obscured in this view (*Upper left*). One 5-m section of the superambient chamber (*Upper right*). Schematic of the entry (Section 1) and exit (Section 10) sections on the superambient chamber, depicting weighing lysimeters and scales, placement of  $\text{CO}_2$ , temperature, and dew point monitoring locations, and the inputs for controlling the  $\text{CO}_2$  gradient and air temperature (*Lower*).

uplands, and Bastrop (16 monoliths), an alluvial sandy loam alfisol (Udic Paleustalf). These soils were chosen because they represent the broad range of texture, N and C content, and hydrologic properties of grasslands in the southern portion of the U.S. Central Plains. Two monoliths each of two of the soil types were placed randomly in each of the 20 containers. Bastrop soils were included in the pairings in even numbered sections. Sixty of the 80 monoliths (all Bastrop, 22 each of Austin and Houston) are weighed continuously on scales (4500 kg capacity, 450 g precision; Avery Weigh-Tronix, Fairmont, Minnesota, USA). The remaining monoliths serve as non-weighing lysimeters. Water that has drained to the bottom of the monoliths is channeled through a fiberglass wick into a 10 l reservoir attached to the bottom of each steel box. The reservoir is connected to a drainage water measurement system. A vacuum extracts each reservoir's contents for gravimetric

determination of drainage volume and for collection of a water sample.

The vegetation is enclosed with clear polyethylene (.006"/.15 mm; Figure 1). This film transmits more than 90% of incident light with minimal effects on spectral quality, and is similar to polyethylene films used in other global change experiments (Fay and others 2003). The polyethylene is fitted with zippers backed by draft flaps to allow access to the monoliths for sampling. The polyethylene is replaced at the beginning of each growing season to minimize the effects of photodegradation on light transmittance. The polyethylene is removed during winter and the vegetation is exposed to ambient conditions.

### $\text{CO}_2$ Treatment

There were three treatment objectives. (1) Maintain a constant linear daytime gradient in  $C_A$  from

500 to 380  $\mu\text{l l}^{-1}$  in the superambient chamber, and from 380 to 250  $\mu\text{l l}^{-1}$  in the subambient chamber (nighttime:  $\sim 720$ –500 and 500–380  $\mu\text{l l}^{-1}$ , respectively), (2) maintain constant  $T_A$  along the length of the chambers and track the daytime ambient air temperature outside the chambers, and (3) provide precipitation inputs representative of an average year in total amount and seasonal distribution. The CO<sub>2</sub> treatment was first applied in May 2006, and is applied for that portion of each growing season when the photosynthetic capacity of the vegetation is adequate to maintain the  $C_A$  gradient, usually late April to early November. CO<sub>2</sub> treatments were applied with a system consisting of infrared gas analyzers (IRGAs; Li 6262, LiCor Biosciences, Lincoln, Nebraska, USA), filtered air sample lines at the entry and exit of each linear 50 m chamber, a quantum sensor for measuring ambient photosynthetic photon flux (PPFD) densities, and a mass flow controller for injecting precise volumes of CO<sub>2</sub> at the superambient chamber entrance. The gradient endpoints were controlled by measuring chamber entry and exit  $C_A$  at 2-min intervals. At the entrance to the superambient chamber, the mass flow controller adds the appropriate amount of pure CO<sub>2</sub> to enrich the incoming air to 500  $\mu\text{l l}^{-1}$ . The enriched air is advected through the chamber by a blower fan mounted at the entrance to section 1, supplemented by fans in sections 3, 5, and 7. The desired exit  $C_A$  (380  $\mu\text{l l}^{-1}$ ) is controlled by adjusting the blower speed. If the exit  $C_A$  is below the set point, blower speed is increased, resulting in less time for plant uptake and a higher exit  $C_A$ . Likewise, blower speed is decreased if exit  $C_A$  is above the set point. Fan speed is also continuously adjusted for diurnal and cloud-induced variation in PPFD using inputs from the quantum sensor to an algorithm relating the direction and magnitude of PPFD changes to the deviation in  $C_A$  from the set point. Air flow at the chamber entrance at maximum blower speed (800 rpm) is 380  $\text{l s}^{-1}$ , decaying to 160  $\text{l s}^{-1}$  ( $\sim 0.5$ –0.2  $\text{m s}^{-1}$ ) at the chamber exit because of leaks and internal resistance.  $C_A$  and dew point temperature ( $T_{\text{DP}}$ ) at the entry and exit of each section are measured on separate monitoring IRGAs and values stored at 20-min intervals. Nighttime control of  $C_A$  is identical to daytime control except the flow of air is reversed using nighttime blowers (Figure 1), CO<sub>2</sub> is injected into the opposite end of the superambient chamber, and nighttime respiration progressively increases  $C_A$ . The CO<sub>2</sub> treatment requires an external CO<sub>2</sub> source of approximately 3,700 l per month, a small supply requirement compared to other methods of ecosystem-level CO<sub>2</sub>

manipulation, such as free air CO<sub>2</sub> exchange or open top chambers (Kimball 1992; Rogers and others 2006).

$T_A$  is controlled so that the midpoint of each section tracks ambient air temperature. An aspirated thermistor is suspended mid-section at 0.75 m above the monoliths. The thermistor controls the flow of coolant through the cooling coil at the entrance of each section. Separate control of temperature in each section is necessary because evapotranspiration differs among sections, causing differences in energy balance.  $T_A$  at each section entry and exit is measured with shielded fine wire thermocouples.

Water from an onsite well is applied to the monoliths by a metered drip irrigation system in volumes and temporal patterns that replicate an average precipitation year. Applications are controlled and logged by a data logger and are verified by the measurements of monolith mass.

### Pre-Treatment Soil Texture, C, and N

Soil texture was quantified using the rapid method of Kettler and others (2001) on 1 m deep  $\times$  4.2 cm diameter soil cores collected in 2002 during excavation of the monoliths. Soils from 0–10, 10–20, 20–30, 30–50, and 50–100 cm segments were homogenized, and 15 g of soil was suspended in 3% sodium hexametaphosphate. Sand was separated from the suspension by passage through a 0.05 mm sieve. The remaining material was resuspended and allowed to settle for 6 h. The supernatant containing suspended silt was discarded. Clay and sand fractions were weighed after drying to constant mass at 105°C. Silt mass was determined by difference, and all masses expressed as a percentage of the total mass. An additional 0.5–1.0 g of homogenized soil was analyzed at 900°C for total %N and %C and at 600°C for organic %C on a combustion gas chromatograph (Variomax CN, Elementar Instruments, Hanau, Germany).

### Plant Establishment and Pre-Treatment Plant Biomass

To establish experimental plant communities on the three soils, the original vegetation on the monoliths was killed with a non-residual herbicide (glyphosate), and planted in spring 2003 with seedlings of perennial grass and forb species characteristic of Central Texas Blackland prairie. Eight plants of each of five grass species [*Bouteloua curtipendula* (side-oats grama), *Panicum obtusum* (vinemesquite), *Schizachyrium scoparium* (little

bluestem), *Sorghastrum nutans* (Indiangrass), and *Tridens albescens* (white tridens)] and three forb species [*Salvia azurea* (pitcher sage), *Solidago canadensis* (Canada goldenrod), and the legume *Desmanthus illinoensis* (Illinois bundleflower)] were transplanted into each monolith at a total density of 64 plants per m<sup>2</sup> during May 2003. Seedlings were planted in a Latin Square design that was re-randomized for each monolith. Transplants were watered during the initial 2 months to promote establishment, but thereafter received only rainfall until CO<sub>2</sub> treatments began. Other species that emerged in the monoliths were removed. The grass *P. obtusum* proved to be highly aggressive and was removed in 2004 by cutting each plant beneath the crown. In addition, grubs (Coleoptera: Scarabaeidae) infested 20 monoliths of Houston and Austin soils. These were replanted in spring 2007 with well-watered and fertilized monocultures of *Panicum virgatum* (switchgrass) to maintain the assimilation capacity of the gradient, and to examine the response of this species to the C<sub>A</sub> gradient. The *Panicum* monocultures are not considered further here.

Aboveground net primary productivity (ANPP) was measured each November. All current year growth of each species was clipped from the entire 1 m<sup>2</sup> monolith at 10 cm above the soil surface, dried to constant mass, and weighed. ANPP was measured similarly in subsequent years. Vertical profiles of volumetric soil water content (vSWC) in the top meter of each monolith were measured biweekly with a calibrated neutron attenuation probe (503DR Hydroprobe, CPN International, Concord, California, USA) in permanently installed access tubes. Soil temperature at 10 and 30 cm depth was measured with fine wire thermocouples in a total of 18 monoliths, pairs of each soil type at high, intermediate, and low C<sub>A</sub>.

Monolith C and N pools were measured again after the 2005 growing season (early January 2006) prior to the CO<sub>2</sub> treatment and after the third growing season for the experimental plant communities. The monoliths were cored (1 m deep × 2.5 cm) and divided into 0–5, 5–10, 10–20, 20–30, 30–50, and 50–100 cm segments. Roots were separated from soil, dried to constant mass at 60°C, and weighed. Total %N, %C, and organic %C were measured as before.

### Preliminary Responses to CO<sub>2</sub> Treatments

Leaf-level carbon and water exchange were measured on the forb *Solidago canadensis* and grass *Sorghastrum nutans* in 12 of the 20 sections, on one

or two leaves on two plants per species per chamber section. The plants chosen had typical vigor for that soil type and CO<sub>2</sub>, and the chosen leaves were recently fully expanded, and also of typical vigor. Leaves were measured for net carbon assimilation (A<sub>CO<sub>2</sub></sub>) and transpiration rate (E) with an infrared gas analyzer (LI-6400 LiCor Biosciences, Inc., Lincoln, Nebraska, USA) using a 2 × 3 cm leaf cuvette, CO<sub>2</sub> mixer, and 85:15 red:blue light source. Leaf chamber illumination was controlled at 1500 μmol m<sup>-2</sup> s<sup>-1</sup> photon flux density, leaf temperature at 30°C, and leaf cuvette H<sub>2</sub>O mole fraction and [CO<sub>2</sub>] to the conditions maintained at that position along the gradient. The plants were measured on a day when the chamber was opened for monthly sampling (13 June 2007) during the period of rapid plant growth. In preliminary sampling, leaves (both species) measured with the chamber open and the leaf cuvette simulating closed chamber conditions gave readings indistinguishable from leaves measured with the chamber closed ( $P = 0.10\text{--}0.90$ ). Surface (0–10 cm) volumetric soil water content (vSWC) during the gas exchange measurements was measured with a time domain reflectometry (TDR) probe (Fieldscout TDR 200, Spectrum Technologies, Plainfield, Illinois, USA). vSWC was computed from soil-specific calibrations.

Soil CO<sub>2</sub> efflux ( $J_{\text{CO}_2}$ ) was also measured when the chambers were open (13 June 2007) on the 60 monoliths planted to mixtures of prairie species.  $J_{\text{CO}_2}$  was measured with a LiCor LI 6400 IRGA fitted with a soil respiration chamber. Two measurements were taken per monolith by placing the chamber on 10-cm deep PVC collars inserted in the soil to 7.5 cm depth. Each measurement was based on a 20 μl l<sup>-1</sup> change in CO<sub>2</sub> centered on ambient C<sub>A</sub>. This technique provides an estimate of how combined root + microbial respiration differed among soils because of preceding CO<sub>2</sub> treatments. Rates were corrected for soil chamber insertion depth using SoilRecomp Version 1.3 software (LI-COR, Inc., Lincoln, Nebraska).

A water balance was tabulated for the month of May 2008, a period of rapid plant growth. Total irrigation inputs for this month were determined by summing the increases in monolith mass on the days when water was applied. Evapotranspiration (ET) was determined by summing the decreases in monolith mass on non-watering days. The portion of ET that was greater than irrigation inputs was classified as ET from storage, and represented depletion of prior soil moisture stores. Deep drainage was determined by summing the water removed from the reservoirs at the base of the monoliths during this period.



## Data Analysis

$C_A$ ,  $T_A$ , and  $T_{DP}$  from mid April to early November 2007 were examined to characterize daytime (0830–1730) and nighttime (2100–0530) control of these parameters in the chambers. Vapor pressure deficit (VPD) was computed from  $T_A$  and  $T_{DP}$ . ANPP, ET (June 1–July 18 2006), 0–20 cm vSWC (neutron probe), and soil temperature at 10 and 30 cm were averaged by monolith position within the sections to evaluate their association with  $T_A$  variation within sections. The estimated effect of monolith position on ANPP was compared to the effect of CO<sub>2</sub> on ANPP by fitting a second order polynomial to the ANPP data, and then adjusting the resulting predicted ANPP curve for the effect of position within section on biomass.

Pre-treatment differences in aboveground biomass between soil types were evaluated for statistical significance using linear mixed model procedures with the monolith as the experimental unit. Soil type was a fixed effect, and species was considered a spatially repeated measure, because the species biomasses within a monolith are not independent. This enabled computation of a covariance matrix to determine correlations between species in biomass changes during establishment. Differences among soils in ET, leaf physiology, soil respiration, and 0–10 cm vSWC (TDR) were evaluated for statistical significance using linear mixed model procedures, again with the monolith as experimental unit, soil type as fixed effect, and  $C_A$  as a covariate. All analyses were conducted using SAS v9.1.3 (SAS Institute Inc. 2003).

## RESULTS

### Chamber Function: CO<sub>2</sub>, Temperature, and VPD

Mean growing season daytime  $C_A$  was 502  $\mu\text{l l}^{-1}$  at the entrance to the superambient chamber (target value 500  $\mu\text{l l}^{-1}$ ), declining to 370  $\mu\text{l l}^{-1}$  at the exit (Figure 2A). Mean  $C_A$  of ambient air at the subambient entrance was 372  $\mu\text{l l}^{-1}$ , declining to 263  $\mu\text{l l}^{-1}$  at the subambient exit. The mean growing season daytime  $C_A$  gradient for the two chambers combined was linear ( $R^2 = 0.99$ ,  $P < 0.0001$ ), as was the mean growing season nighttime  $C_A$  gradient ( $R^2 = 0.97$ ,  $P < 0.0001$ ). Nighttime  $C_A$  averaged 143  $\mu\text{l l}^{-1}$  greater than during daytime (Figure 2A), but with a slightly lower slope of  $C_A$  versus distance along chambers (nighttime =  $-1.8 \mu\text{l l}^{-1} \text{ m}^{-1}$ ; daytime =  $-2.1 \mu\text{l l}^{-1} \text{ m}^{-1}$ ).

There was day-to-day variability during the growing season in the mean  $C_A$  in both chambers, but 90% of the daily mean  $C_A$  values fell within 20–50  $\mu\text{l l}^{-1}$  of the growing season mean (Figure 3A). Day-to-day variability in  $C_A$  was greater in the superambient chamber than in the subambient chamber. Variability at the superambient chamber entrance was greater than at the subambient chamber entrance. Variability in  $C_A$  increased with distance downstream in both chambers.

Mean growing season values of daytime  $T_A$  were 23–30°C at the entrance of each 5-m section (Figure 2B), and increased by 3–8°C (mean:  $5.5 \pm 0.4^\circ\text{C}$ ) within each section. The cooling coils at the exit of each 5-m section were generally effective at lowering  $T_A$ . As a result, the mean daytime temperature in each section tracked the mean ambient temperature (26.7°C) except in the last two sections of each chamber, which ran 2–4°C warmer. Mean nighttime  $T_A$  was essentially constant between 19 and 20°C in the superambient chamber (Figure 2B), and declined from 19 to 17°C along the subambient chamber. Daytime and nighttime VPD along the chambers showed the same pattern as their respective  $T_A$  values (Figure 2C).

The increased daytime  $T_A$  within each 5-m section was associated with lower 0–20 cm soil water content (12%) and aboveground biomass (20%), and higher  $T_{\text{SOIL}}$  (1.2–2.3°C) and ET (25%;  $2.8 \leq F \leq 13.3$ ,  $0.05 \geq P > 0.0001$ , Table 1). However, the variation in biomass expected from within-section differences in  $T_A$  was small compared to the variation in biomass associated with soils or the  $C_A$  gradient (Figure 3B).

### Pre-Treatment Soil Texture, C, and N

Houston soils were uniform in texture through the first meter of the profile, with 49–55% clay and 36–39% silt (Table 2). Austin soils were also uniform in texture, with higher silt (46–48%) than the Houston or Bastrop soils, and 40–45% clay. The Bastrop series showed more vertical variation in texture than the Austin or Houston soils, with 60–72% sand and 7–14% clay from 0 to 50 cm, but 48% sand and 26% clay from 50 to 100 cm.

The soils differed in total C, organic C, and total N when monoliths were collected in 2002 (Table 2). The Austin soils contained 8.2–8.7% total C, compared to 4.0–5.0% in the Houston soils and 0.2–0.8% in the Bastrop soils. Organic C was 0.4–2.0% for Austin, 1.0–2.4% for Houston, and 0.2–0.7% for Bastrop. Houston and Austin soils were higher in total N (0.03–0.21%), than the Bastrop soils

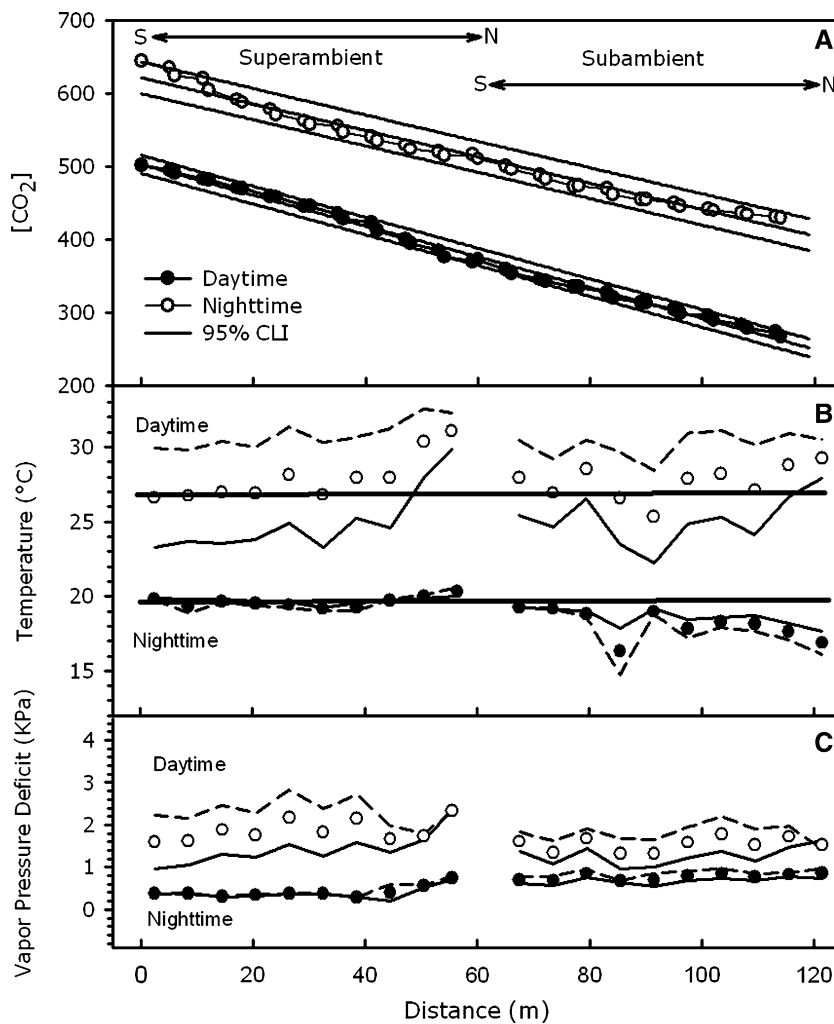


Figure 2. Gradients maintained in LYCOG superambient and subambient chambers during April–November, 2007. Daytime and nighttime, growing-season averages for **A**  $\text{CO}_2$  concentration, **B** air temperature, and **C** vapor pressure deficit along the  $\text{CO}_2$  gradient. In **B**, heavy *horizontal lines* denote ambient mean temperatures. In **B** and **C**, *solid lines* denote air temperature/VPD at the entrance of each 5-m section, and *dashed lines* the values at the exit of each 5-m section.

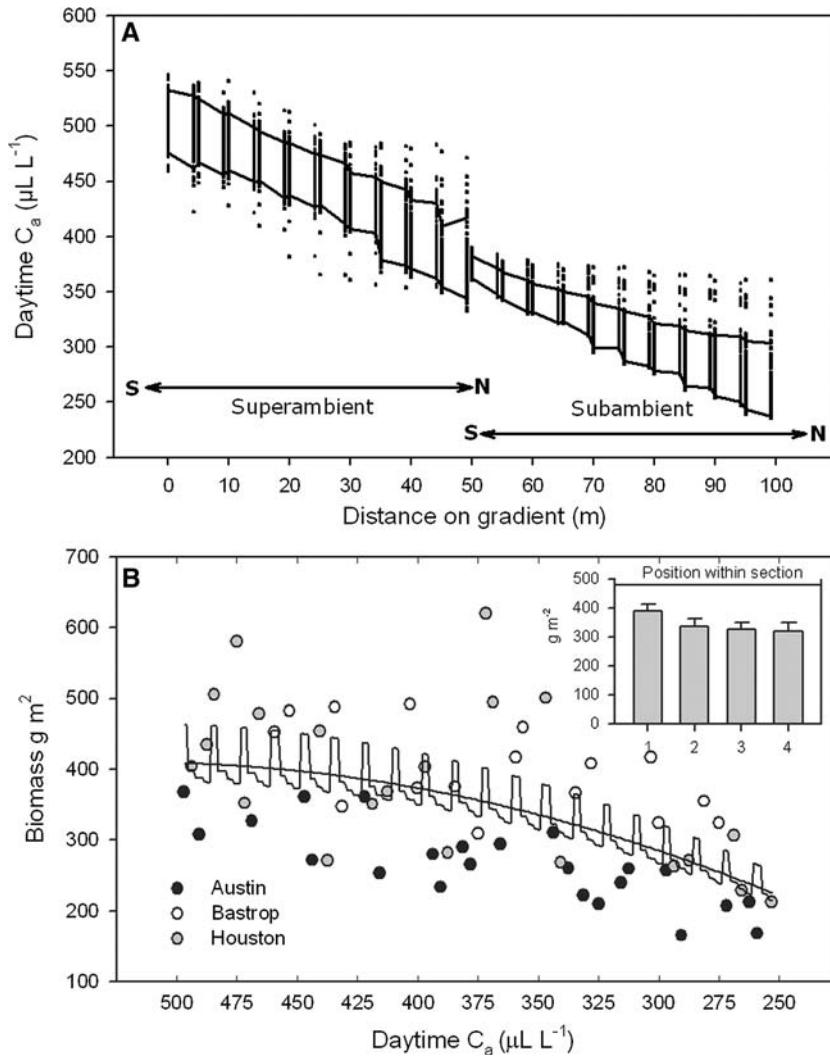
(0.02–0.08%). These C and N concentrations yielded a wide range of total C:N for these soil profiles, from 57 to 289 in the Austin profiles, to 4.8–9.6 in the Bastrop profiles. The C:N was much less variable for organic than total C, ranging from 5 to 10 in the Bastrop profiles, 12.5–14 in Austin profiles, and 11–20 in the Houston profiles.

Total C was essentially unchanged from 2002 to 2005, except for slight changes at 50–100 cm in Bastrop (a decrease) and Houston (an increase) soils ( $.05 < P < .0001$ , Table 2). Total % N declined from 2002 to 2005 by about 10% below 10 cm in Austin soils, but was unchanged in Bastrop and Houston profiles. Organic C below 10 cm depth decreased from 2002 to 2005 in Austin and Bastrop soils. The Austin soils had larger increases in C:N with depth compared to Bastrop and Houston profiles, reflecting greater decreases in total N with depth. There were no systematic changes from 2002 to 2005 in OC:N in the profiles.

### Pre-Treatment Plant Biomass

After 3 years of plant establishment and growth, the soils differed in above and belowground biomass. Austin soils supported less aboveground biomass,  $235 \text{ g m}^{-2}$ , compared to  $380\text{--}405 \text{ g m}^{-2}$  on Bastrop and Houston soils (Table 3, Figure 4A). Soils also differed in 0–30 cm root biomass, but differences diminished with depth. vSWC differed among the soils, with highest vSWC in the Houston soil (35–39%) and intermediate vSWC in Austin soils (30%), both with little vertical variation. Bastrop soil had considerably lower vSWC throughout the profile (11–26%), especially in the top 40 cm of the profile (Table 3; Figure 4A).

The biomass of individual species differed among the three soils prior to  $\text{CO}_2$  treatment (Table 3). *Sorghastrum nutans* was strongly dominant on Bastrop and Houston soils (Table 4), whereas *Solidago canadensis* and *S. nutans* were co-dominant on the



**Figure 3. A** Variability in daytime  $C_a$  in LYCOG during 2007. Each point marks 1 day's mean  $C_a$  for each sample point along the chambers. *Lines* represent 5th and 95th percentiles of daily mean  $C_a$ . **B** Aboveground biomass for individual monoliths versus distance along the gradient, a proxy of  $C_a$ . Each point is the mean of 2006 and 2007. The *smooth line* represents the predicted biomass from regression analysis. The *varying line* represents the predicted biomass adjusted for the effect of  $T_A$  on biomass at each monolith position (inset).

**Table 1.** Biomass, Evapotranspiration, Soil Water Content, and Soil Temperature Within Chamber Sections

Position in section	Aboveground biomass ( $\text{g m}^{-2}$ )*	ET ( $\text{mm H}_2\text{O d}^{-1}$ )*	Soil water content (% vol)*	$T_{\text{soil } 10 \text{ cm}}$ ( $^{\circ}\text{C}$ )*	$T_{\text{soil } 30 \text{ cm}}$ ( $^{\circ}\text{C}$ )*
1 (Entrance)	394.4 ± 19.6	3.66 ± 0.13	29.4 ± (0.4)	22.7 ± (0.4)	21.9 ± (0.3)
2	344.8 ± 21.7	4.26 ± 0.14	27.9 ± (0.4)	23.9 ± (0.4)	22.6 ± (0.2)
3	348.8 ± 20.2	4.50 ± 0.13	27.0 ± (0.4)	23.3 ± (0.6)	23.4 ± (0.3)
4 (Exit)	312.1 ± 21.2	4.56 ± 0.14	25.8 ± (0.4)	25.0 ± (0.5)	23.1 ± (0.3)
Effect	$F$ ( $P$ )	$F$ ( $P$ )	$F$ ( $P$ )	$F$ ( $P$ )	$F$ ( $P$ )
Position	2.8 (0.05)	9.5 (<0.0001)	13.3 (<0.0001)	4.2 (0.02)	5.7 (0.009)
Soil	20.2 (<0.0001)	22.1 (<0.0001)	868.4 (<0.0001)	–	–
$P \times S$	1.2 (ns)	1.6 (ns)	2.2 (0.06)	–	–

\*Means ± 1 SE.

less productive Austin soil. The covariance matrix of species biomass suggested there were negative associations among several species. The strongest

negative covariance was between *S. nutans* and *S. canadensis* (Figure 4B), indicating that an increase in *S. nutans* was strongly associated with a

**Table 2.** Depth Profiles of Soil Texture and C and N in 2002 and 2005

2002								
Depth (cm)	% Sand (se)	% Silt (se)	% Clay (se)	Total C, % (se)	Organic C, % (se)	Total N, % (se)	C:N	OC:N
<i>Austin</i>								
0–10	12.2 (0.4)	45.9 (0.7)	41.9 (0.6)	8.55 (0.08)	1.98 (0.04)	0.15 (0.01)	57.0	13.2
10–20	10.6 (0.4)	44.1 (0.7)	45.3 (0.7)	8.18 (0.14)	1.37 (0.03)*	0.11 (0.003) <sup>†</sup>	75.3	12.5
20–30	11.4 (0.6)	45.7 (0.9)	42.9 (0.9)	8.44 (0.17)*	0.99 (0.03)*	0.07 (0.003)**	124.3	14.1
30–50	11.9 (0.8)	46.3 (1.1)	41.8 (1.1)	8.69 (0.11)	0.69 (0.03)**	0.05 (0.002) <sup>‡</sup>	179.6	13.8
50–100	11.7 (0.7)	48.4 (1.3)	39.9 (1.6)	8.66 (0.09)	0.41 (0.03)**	0.03 (0.002)**	289.0	13.7
<i>Bastrop</i>								
0–10	72.8 (0.6)	19.7 (0.4)	7.5 (0.4)	0.77 (0.06)	0.71 (0.06)	0.08 (0.006)	9.6	8.8
10–20	70.2 (1.4)	22.9 (0.8)	6.8 (0.5)	0.35 (0.02)	0.36 (0.01)*	0.04 (0.005)	8.0	9.0
20–30	65.7 (0.2)	25.9 (0.3)	8.3 (0.4)	0.19 (0.01)	0.21 (0.01)	0.02 (0.002)	9.0	10.5
30–50	60.2 (2.8)	25.7 (0.7)	14.0 (3.3)	0.17 (0.01)	0.18 (0.02)*	0.03 (0.007)	5.0	6.0
50–100	48.2 (0.9)	25.7 (1.8)	26.1 (2.3)	0.21 (0.01)**	0.21 (0.02) <sup>†</sup>	0.04 (0.004)	4.8	5.3
<i>Houston</i>								
0–10	11.2 (0.5)	39.5 (1.0)	49.2 (1.2)	5.05 (0.16)	2.38 (0.07)	0.21 (0.009)	26.6	11.3
10–20	10.2 (0.9)	38.8 (1.0)	51.0 (1.7)	4.49 (0.13)	1.77 (0.03)	0.13 (0.005)	35.2	13.6
20–30	9.3 (0.8)	37.0 (1.0)	53.6 (1.1)	4.23 (0.16)	1.52 (0.04)	0.10 (0.005)	44.1	15.2
30–50	9.5 (0.8)	35.5 (0.9)	54.9 (0.9)	4.09 (0.05)	1.32 (0.05)	0.08 (0.004)	51.5	16.5
50–100	8.8 (1.0)	35.7 (0.8)	55.4 (0.7)	3.98 (0.05)**	0.99 (0.08)	0.05 (0.004)	81.4	19.8
2005								
Depth (cm)	Total C, % (se)	Organic C, % (se)	Total N, % (se)	C:N	OC:N	Root mass, mg cm <sup>-3</sup> (se)		
<i>Austin</i>								
0–5	8.60 (0.16)	2.43 (0.11)	0.20 (0.01)	43.0	12.2	41.91 (7.45)		
5–10	8.31 (0.10)	1.72 (0.05)	0.14 (0.006)	59.3	12.3	24.92 (3.21)		
10–20	8.28 (0.10)	1.20 (0.03)	0.09 (0.004)	92.0	13.3	14.70 (1.41)		
20–30	8.70 (0.14)	0.83 (0.04)	0.06 (0.003)	145	13.8	11.58 (1.49)		
30–50	8.99 (0.08)	0.55 (0.03)	0.04 (0.002)	224	13.8	8.58 (0.80)		
50–100	8.67 (0.06)	0.31 (0.05)	0.03 (0.002)	289	10.3	4.70 (0.56)		
<i>Bastrop</i>								
0–5	1.15 (0.22)	1.17 (0.15)	0.11 (0.02)	13.7	10.6	77.61 (11.79)		
5–10	0.49 (0.14)	0.47 (0.07)	0.05 (0.005)	6.1	9.4	30.25 (4.23)		
10–20	0.32 (0.14)	0.31 (0.04)	0.03 (0.003)	10.3	10.3	19.94 (2.43)		
20–30	0.18 (0.19)	0.17 (0.05)	0.02 (0.002)	9.0	8.5	11.84 (1.69)		
30–50	0.15 (0.11)	0.14 (0.04)	0.02 (0.003)	2.5	7.0	8.99 (1.19)		
50–100	0.19 (0.008)	0.18 (0.06)	0.04 (0.002)	4.8	4.5	5.90 (0.88)		
<i>Houston</i>								
0–5	5.92 (0.16)	3.12 (0.11)	0.28 (0.01)	21.1	11.1	99.74 (21.65)		
5–10	4.94 (0.10)	2.10 (0.05)	0.17 (0.008)	29.0	12.4	43.00 (4.82)		
10–20	4.57 (0.10)	1.70 (0.03)	0.12 (0.004)	38.1	14.2	25.58 (2.21)		
20–30	4.41 (0.14)	1.58 (0.04)	0.10 (0.005)	44.3	15.8	15.79 (1.74)		
30–50	4.12 (0.08)	1.32 (0.03)	0.08 (0.003)	51.5	16.5	10.35 (0.86)		
50–100	4.07 (0.06)	1.11 (0.05)	0.05 (0.002)	81.6	22.2	4.57 (0.47)		

\**P* ≤ 0.05, \*\**P* ≤ 0.01, <sup>†</sup>*P* ≤ 0.001, <sup>‡</sup>*P* ≤ 0.0001 from paired *t*-tests of monoliths sampled in both 2002 and 2005.

decrease in *S. canadensis*. This negative association was apparent in the biomass values (Table 4). On Bastrop and Houston soils the biomass of *S. canadensis* was relatively low compared to *S. nutans*, and on Austin soils *S. canadensis* biomass increased

compared to *S. nutans*. Sizeable negative covariances were also found between *Schizachyrium scoparium* and both *S. nutans* and *S. canadensis*, and between *Bouteloua curtipendula* and *S. nutans*. Covariances near zero for the forbs *Tridens albescens*,

**Table 3.** Analysis of Variance Statistics for Aboveground and Root Biomass, Soil Water Content, Leaf Gas Exchange, Soil CO<sub>2</sub> Efflux, and Monolith Water Balance

Response	Effect	df	F	P-value
Aboveground biomass	Soil	2,77	67.1	<0.0001
	Species	6,72	613.3	<0.0001
	Soil × species	12,109	25.5	<0.0001
Root biomass (0–30 cm)	Soil	2,75	7.6	0.001
Soil water content (neutron probe)	Soil	2,77	832.4	<0.0001
	Depth	4,308	250.9	<0.0001
	Soil × depth	8,308	88.9	<0.0001
<i>Solidago canadensis</i>				
$A_{CO_2}$	Soil	2,12	1.30	0.3079
	Soil × $C_A$	3,12	5.58	0.0124
WUE	Soil	2,12	0.80	0.4711
	Soil × $C_A$	3,12	10.20	0.0013
<i>Sorghastrum nutans</i>				
$A_{CO_2}$	Soil	2,15	1.08	0.3658
	Soil × $C_A$	3,15	0.74	0.5437
WUE	Soil	2,15	1.46	0.2636
	Soil × $C_A$	3,15	1.62	0.2260
	Soil × $C_A$	3,15	1.62	0.2260
Soil water content (0–10 cm, TDR)	Soil	2,26	5.93	0.0076
	Soil × $C_A$	3,26	7.65	0.0008
Soil CO <sub>2</sub> efflux	Soil	2,54	3.03	0.0567
	Soil × $C_A$	3,54	3.18	0.0312
Evapotranspiration	Soil	2,37	10.20	0.0003
	Soil × $C_A$	1,37	6.18	0.0175
Storage loss	Soil	2,37	10.08	0.0003
	Soil × $C_A$	1,37	4.32	0.0446

*Salvia azurea*, and *Desmanthus illinoensis* indicated that variation among monoliths in biomass in these species was unrelated to the biomass of the other species.

### Preliminary Plant and Soil Responses to CO<sub>2</sub>

For *S. canadensis*, leaf  $A_{CO_2}$  and water use efficiency (WUE) increased at higher  $C_A$  (Figure 5A, B). The  $C_A$  sensitivity of  $A_{CO_2}$  and WUE in *S. canadensis* was greater on Houston and Bastrop than on Austin soils (Table 3). However, there was no significant main effect of soil type on  $A_{CO_2}$  or WUE. For *S. nutans*, there were no significant effects of soil type or  $C_A$  on  $A_{CO_2}$  or WUE. vSWC was lower in Bastrop than in Austin or Houston soils (Table 3, Figure 5C) and increased more with  $C_A$  on Austin than on Houston or Bastrop soils. Soil CO<sub>2</sub> efflux was 32% greater on Houston than on Austin or Bastrop soils (Table 3, Figure 5D), however, efflux increased with  $C_A$  more on Bastrop soils.

ET during May 2008 averaged 109 mm on Houston soils, compared to 91 mm on the Austin and Bastrop soils (Table 3, Figure 6). ET was

46 mm greater than irrigation on the Houston soils, and 26 mm greater on Austin and Bastrop soils, indicating that ET depleted previously stored soil water. ET from storage was correlated with changes in monolith soil moisture measured with the neutron probe ( $R^2 = 0.73$ ,  $P < 0.0001$ ). Both total ET and ET from storage increased with higher  $C_A$  (Table 3; Figure 6). The effects of  $C_A$  on ET and storage did not differ between soils ( $P \geq 0.88$ ). Mean drainage from the monoliths was  $1.39 \pm 0.37$  mm (max = 9.20 mm), which was a negligible portion of the water budget. Irrigation inputs to the monoliths averaged about 64 mm during May 2008 (Figure 6).

### DISCUSSION

LYCOG extends the CO<sub>2</sub> gradient approach of Mayeux and others (1993) and Johnson and others (2000) to multiple soil types and allows for closure of monolith water budgets. Preliminary results support the hypothesis that soil properties will influence the effects of  $C_A$  on the productivity, soil CO<sub>2</sub> efflux, hydrology, and plant species composition of these grassland monoliths.

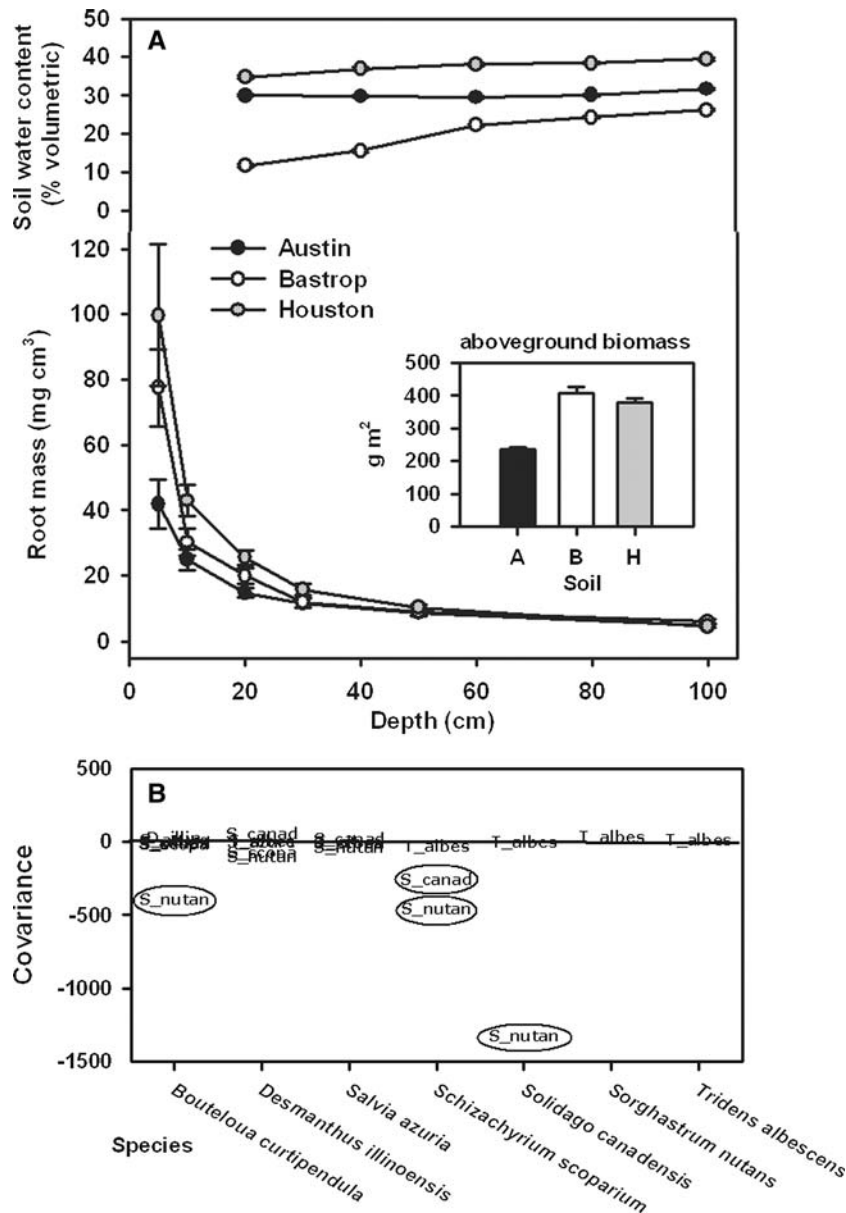


Figure 4. Soil moisture and plant biomass on the three soils in LYCOG following the 2005 growing season, after 3 years of growth in ambient conditions and prior to CO<sub>2</sub> treatment. **A** Mean growing season volumetric soil water content (neutron probe; SE falls within the symbols) and root mass mean  $\pm$  1 SE for the top 1 m of the soil profile; Inset: total aboveground biomass. **B** Covariance for species pairs in monolith-to-monolith variation in biomass.

## Chamber Function

A goal of global change field experiments is to simulate the desired factor while minimizing confounding changes in other factors (Dunne and others 2004). LYCOG achieved the objective of linear daytime and nighttime  $C_A$  gradients. The system performed similarly to the Phase II version of the experiment, which used the same aboveground infrastructure and CO<sub>2</sub> monitoring and control systems (Johnson and others 2000). There were two main artifacts of the system noted in this study. First, day-to-day variability in mean  $C_A$  increased with distance along each chamber. This is due to the directional flow concept used in LYCOG,

where upstream disturbances to  $C_A$  travel downstream, and may be magnified by subsequent downstream disturbances.  $C_A$  variation measured at the subambient chamber entry was caused by day-to-day variability in ambient  $C_A$ . Ambient  $C_A$  varies with soil moisture, temperature, and light because of their effects on photosynthesis. Variation in  $C_A$  entering the superambient chamber results primarily from variation in the function of the mass flow controller that regulates CO<sub>2</sub> injection. Factors contributing additional variability downstream may include patchy light levels due to partial cloud cover, illuminating some part of the 50 m chambers more than other parts, and opening chamber sections for sampling or maintenance. The second

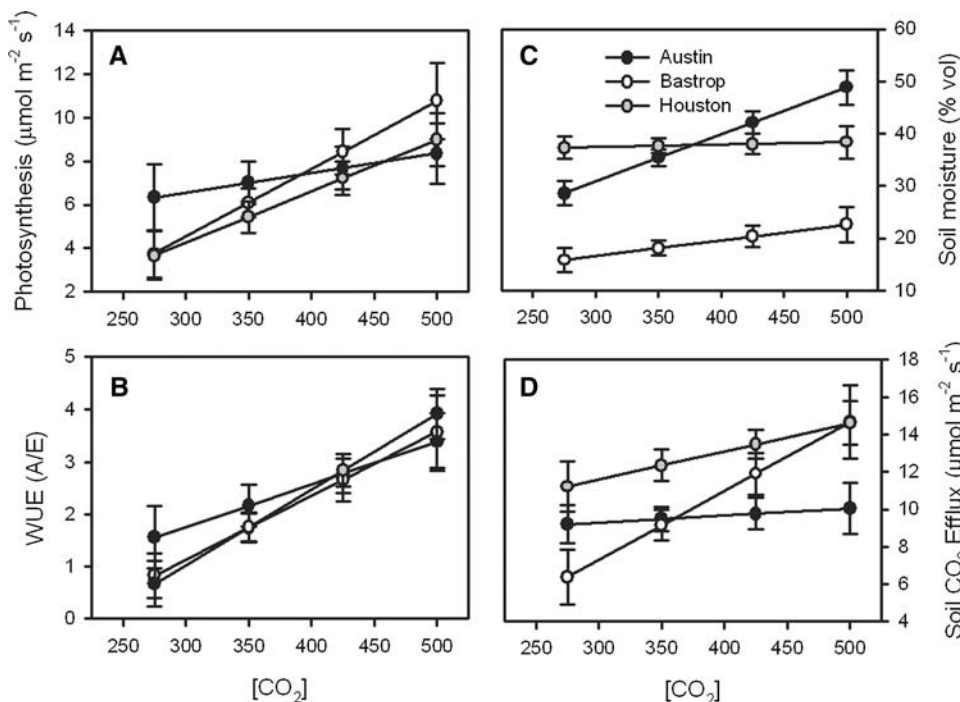
**Table 4.** Mean Aboveground Biomass by Species in LYCOG After the 2005 Growing Season

Soil	Species	Aboveground biomass, g m <sup>-2</sup> (SE)
Austin	<i>Solidago canadensis</i>	66.86 (5.87)
	<i>Sorghastrum nutans</i>	60.61 (9.95)
	<i>Bouteloua curtipendula</i>	54.43 (5.16)
	<i>Schizachyrium scoparium</i>	44.01 (5.76)
	<i>Desmanthus illinoensis</i>	7.49 (1.83)
	<i>Salvia azurea</i>	1.99 (0.43)
	<i>Tridens albescens</i>	1.41 (0.59)
	Total	236.79 (6.66)
Bastrop	<i>Sorghastrum nutans</i>	214.28 (23.22)
	<i>Schizachyrium scoparium</i>	104.65 (12.14)
	<i>Solidago canadensis</i>	70.78 (7.75)
	<i>Bouteloua curtipendula</i>	8.31 (1.42)
	<i>Desmanthus illinoensis</i>	7.97 (1.64)
	<i>Salvia azurea</i>	0.76 (0.23)
	<i>Tridens albescens</i>	0.13 (0.13)
	Total	406.88 (19.40)
Houston	<i>Sorghastrum nutans</i>	182.51 (14.54)
	<i>Solidago canadensis</i>	107.32 (5.77)
	<i>Schizachyrium scoparium</i>	39.13 (3.85)
	<i>Desmanthus illinoensis</i>	26.69 (3.20)
	<i>Bouteloua curtipendula</i>	19.30 (2.59)
	<i>Tridens albescens</i>	4.31 (0.92)
	<i>Salvia azurea</i>	0.40 (0.28)
	Total	379.65 (11.21)

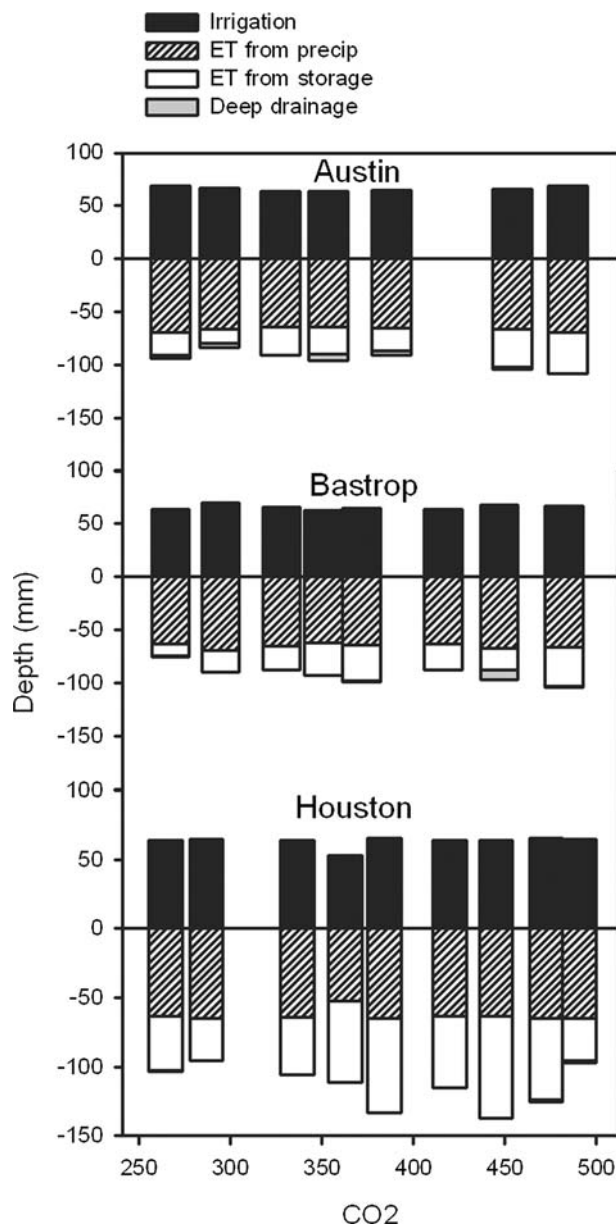
artifact was the presence of within section daytime temperature gradients of about 1°C m<sup>-1</sup>, similar to PCG (Johnson and others 2000). This rate of

warming is greater than reported from other gradient facilities. For example, Rawson and others (1995) reported a temperature gradient of 0.6°C m<sup>-1</sup> during midday full sun in their temperature gradient chamber, and Lee and others (2001) reported 0.25°C m<sup>-1</sup>. Within section warming is greater on days with high radiation loads, and during periods of slow air flow caused by low photosynthetic rates (Johnson and others 2000). Within-section temperature variation was centered on ambient temperature except for the exit sections of the chambers, which trended warmer than the upstream sections.

Within-section warming measurably affected ET and aboveground biomass, but these effects were small compared to those of C<sub>A</sub> and soil type (Figures 3, 4, and 6; Table 1). For example, previous CO<sub>2</sub> gradient studies found that decreasing C<sub>A</sub> from 550 to 250 μl l<sup>-1</sup> increased ET by 35% (Polley and others 2008), decreased aboveground biomass by up to 86% (Polley and others 2003) and decreased J<sub>CO<sub>2</sub></sub> by 300% (Mielnick and others 2001). In contrast, an approximate 2°C increase in T<sub>SOIL</sub> would cause a 15% increase in J<sub>CO<sub>2</sub></sub>, assuming a Q<sub>10</sub> of 2. Although a temperature gradient within sections of the chamber is not ideal, it should pose minimal problem in detecting and interpreting ecosystem effects of the C<sub>A</sub> gradient, given that the temperature gradients are similar among 5-m sections in each chamber. Our primary interest is in comparing the averaged responses of monoliths within each section as a function of C<sub>A</sub> and with



**Figure 5.** Leaf and soil responses to C<sub>A</sub> on the three soil types in LYCOG. **A** Leaf carbon assimilation (A<sub>CO<sub>2</sub></sub>), and **B** photosynthetic water use efficiency for *Solidago canadensis*. **C** 0–10 cm volumetric soil water content (vSWC) and **D** soil CO<sub>2</sub> efflux. Values are least squared means ± 1 SE from linear mixed models analysis.



**Figure 6.** Water balance for the LYCOG soils along the CO<sub>2</sub> gradient for May 2008. Data shown are averages for the 2 monoliths of a given soil type in each 5-m section of the chambers.

determining effects of  $C_A$  and soil type within chambers, rather than comparing the chambers with outside conditions (Rawson and others 1995).

### Pre-Treatment Soils and Plants

Soils differed markedly in texture and C:N pools. Bastrop soils had the lowest total C, organic C, total N, and soil moisture values, typical of sandy soils. Organic C (OC) accounted for nearly all the carbon in these soils, and the low OC:N despite high plant

productivity suggested higher potential decomposition rates versus the other soils. The Houston soils were highest in total C, organic C, total N, and soil moisture. A strong increase in OC:N with depth suggested that decomposition rates are greatest in the top of the soil profile. Austin soils were highest in total C, because of high concentrations of carbonate (V. Jin, unpublished data), and intermediate in soil moisture, organic C and total N. OC:N was higher in Austin than Bastrop soil and comparable to that in the upper layer of Houston soils, implying that decomposition rates are slower in the clay soils compared to the Bastrop soil.

The soils exhibited substantial differences in pre-treatment aboveground and belowground biomass, with Austin soils much less productive than Bastrop and Houston soils. However, productivity did not correlate with either soil water or the pre-treatment C and N pools. Soil texture strongly influences both N and water availability to plants. Other things being equal, decomposition rates and therefore N availability to plants usually are greater in clay than C-poor sandy soils (Paul and others 2001; Johnson and others 2007). In contrast, soil water potential at a given soil water content is greater (less negative) and the permanent wilting point lower in sandy than fine textured soils, resulting in greater plant access to soil moisture (Brady and Weil 2002). This result suggests that productivity differences among soils will depend on soil-specific nutrient  $\times$  water interactions and their variation with  $C_A$ .

The strong negative covariances among *Bouteloua*, *Sorghastrum*, *Solidago*, and *Schizachyrium* imply that CO<sub>2</sub> and soil effects on plant productivity and species composition will be mediated primarily through interactions among these dominant species. We expect to see different trajectories of community change among the soil types in response to the CO<sub>2</sub> gradient because of differential effects of CO<sub>2</sub> on the water budgets of these three soils. For example, we expect CO<sub>2</sub> enrichment to favor the C<sub>3</sub> component of the experimental communities, especially *S. canadensis*, more on the sandy Bastrop soil than on the clay soils. These species are likely to be the most active players in productivity responses to the CO<sub>2</sub> gradient. The differences among LYCOG soils in pre-treatment plant composition are comparable to differences in plant composition both among and within soil types in established tallgrass prairie (Diamond and Smeins 1984; Gibson and Hulbert 1987; Piper 1995). Precipitation may mediate the relationship between soil type and plant community composition. Diamond and Smeins (1984) reported that in



Texas coastal prairies, plant communities located where precipitation was higher showed less association with soil type than communities with lower precipitation.

### Preliminary Responses to $C_A$

Leaf photosynthesis ( $A_{CO_2}$ ) and photosynthetic water use efficiency (WUE) in *Solidago canadensis* increased with  $C_A$  more on the more productive Houston and Bastrop soils in this early summer assessment.  $A_{CO_2}$  and WUE are coupled to soil water and N availability, and to the radiation, temperature, and H<sub>2</sub>O vapor pressure conditions surrounding the leaf. The vSWC differed substantially among soils during the leaf gas exchange measurements (Figure 5C), but the differences in vSWC sensitivity to  $C_A$  among soils did not correspond to the  $C_A$  sensitivity of  $A_{CO_2}$  and WUE in *S. canadensis*. This implies that leaf carbon assimilation and water loss for *S. canadensis* were regulated more strongly by the leaf microenvironment at this time. However, the relative importance of leaf microclimate versus availability of soil moisture or nutrients such as N is not static, and soil resources should become more important for leaf function when soil moisture is more limiting later in the season and at low  $C_A$  (Anderson and others 2001).

$J_{CO_2}$  increased nearly 3-fold with  $C_A$  on Bastrop soils. The magnitude of the Bastrop  $J_{CO_2}$  response was similar to that reported by Mielnick and others (2001) in the PCG experiment. This large response to  $C_A$  may reflect tighter coupling of  $J_{CO_2}$  to vSWC on the Bastrop soils, as the  $C_A$  response of  $J_{CO_2}$  was smaller on the wetter Austin and Houston soils. The greater efflux response to  $C_A$  on the Bastrop than Austin and Houston soils suggests that there was a larger increase in root and microbial activity with higher  $C_A$  on the Bastrop soil. However, control of  $J_{CO_2}$  depends on vSWC, soil temperature, and substrate availability (Luo and Zhou 2006). The response of  $J_{CO_2}$  to  $C_A$  may decrease as the season progresses and photosynthesis decreases and labile soil C and soil moisture are depleted.

The water budgets showed that ET and the portion of ET supplied by pre-existing stores of soil moisture increased at higher  $C_A$  on all three soils. Stomatal closure at higher  $C_A$  typically reduces transpiration per unit leaf area (for example, Ward and others 1999; Anderson and others 2001). In this preliminary study, transpiration was not significantly affected by  $C_A$  for either species on any of the soils ( $P = 0.15\text{--}0.27$ ). Increased aboveground biomass at higher  $C_A$  may be responsible for the

higher ET. As with leaf carbon assimilation and  $J_{CO_2}$ , the effects of soil and  $C_A$  on ET are likely to change through the season as soil moisture becomes depleted.

### CONCLUSIONS

The CO<sub>2</sub> gradient approach has been used successfully to study plant and soil responses to subambient-to-ambient (Mayeux and others 1993) and subambient-to-superambient (Johnson and others 2000) ranges of  $C_A$ . LYCOG maintained linear gradients of daytime and nighttime  $C_A$ , with good overall control of  $T_{air}$ . The facility advances our capability to discern effects of pre-industrial to mid-twenty-first century levels of  $C_A$  on ecosystem structure and function on soils that differ in water and nutrient availability, primary productivity, and plant species composition. The initial results of this study indicate that aboveground net primary productivity, ET, leaf C assimilation in an abundant C<sub>3</sub> forb, soil C efflux, and soil moisture depletion all increased with enriched  $C_A$ , particularly on the sandy soil. No counteracting effect of  $C_A$  on stomatal conductance was detected. These results suggest that increased  $C_A$  may accelerate rates of carbon cycling on these soils. Over the longer term, plant and soil responses to  $C_A$  may be mediated by differences in hydrologic properties among the soils and in particular by differences in both soil water content and the availability of soil water to plants and microbes. The larger implication is that variation in soil texture and other properties has the potential to cause considerable variation across the landscape in grassland responses to continuing CO<sub>2</sub> enrichment.

### ACKNOWLEDGMENTS

We thank A. Gibson, A. Griffith, K. Jones, C. Kolodziejczyk, A. Naranjo, K. Tiner, and scientists and staff involved in the previous CO<sub>2</sub> gradients for their contributions to this experimental approach, and to the development of LYCOG and to this manuscript. R.B.J. acknowledges financial support from the Department of Energy's Program for Ecosystem Research (#ER64242).

### REFERENCES

- Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO<sub>2</sub> enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO<sub>2</sub>. *New Phytol* 165:351–72.
- Anderson LJ, Maherali H, Johnson HB, Polley HW, Jackson RB. 2001. Gas exchange and photosynthetic acclimation over

- subambient to elevated CO<sub>2</sub> in a C-3-C-4 grassland. *Glob Chang Biol* 7:693–707.
- Brady NC, Weil RR. 2002. The nature and properties of soils. Upper Saddle River, NJ: Prentice Hall, p 960.
- Diamond DD, Smeins FE. 1984. Remnant grassland vegetation and ecological affinities of the upper costal prairie of Texas. *Southwest Nat* 29:321–34.
- Dunne JA, Saleska SR, Fischer ML, Harte J. 2004. Integrating experimental and gradient methods in ecological climate change research. *Ecology* 85:904–16.
- Epstein HE, Burke IC, Lauenroth WK. 2002. Regional patterns of decomposition and primary production rates in the US Great Plains. *Ecology* 83:320–27.
- Fay PA, Carlisle JD, Knapp AK, Blair JM, Collins SL. 2003. Productivity responses to altered rainfall patterns in a C4-dominated grassland. *Oecologia* 137:245–51.
- Forster P, Ramaswamy V, Artaxo P, Bernsten T, Betts R, Fahey DW, Haywood J, Lean J, Lowe DC, Myhre G, Nganga J, Prinn R, Raga G, Schulz M, Van Dorland R. 2007. Changes in atmospheric constituents and in radiative forcing. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Avery KB, Tignor M, Miller HL, Eds. *Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge, United Kingdom and New York, NY: Cambridge University Press. p 129–234.
- Gibson DJ, Hulbert LC. 1987. Effects of fire, topography and year-to-year climatic variation on species composition in tallgrass prairie. *Vegetatio* 72:175–86.
- Gill RA, Anderson LJ, Polley HW, Johnson HB, Jackson RB. 2006. Potential nitrogen constraints on soil carbon sequestration under low and elevated atmospheric CO<sub>2</sub>. *Ecology* 87:41–52.
- Gill RA, Polley HW, Johnson HB, Anderson LJ, Maherali H, Jackson RB. 2002. Nonlinear grassland responses to past and future atmospheric CO<sub>2</sub>. *Nature* 417:279–82.
- Hagedorn F, Spinnler D, Bundt M, Blaser P, Siegwolf R. 2003. The input and fate of new C in two forest soils under elevated CO<sub>2</sub>. *Glob Chang Biol* 9:862–71.
- Hassink J. 1996. Preservation of plant residues in soils differing in unsaturated protective capacity. *Soil Sci Soc Am J* 60:487–91.
- Jenkinson DA. 1977. Studies on the decomposition of plant material in soil. *J Soil Sci* 28:424–34.
- Johnson JMF, Barbour NW, Weyers SL. 2007. Chemical composition of crop biomass impacts its decomposition. *Soil Sci Soc Am J* 71:155–62.
- Johnson HB, Polley HW, Mayeux HS. 1993. Increasing CO<sub>2</sub> and plant-plant interactions: effects on natural vegetation. *Vegetatio* 104–105:157–70.
- Johnson HB, Polley HW, Whitis RP. 2000. Elongated chambers for field studies across atmospheric CO<sub>2</sub> gradients. *Funct Ecol* 14:388–96.
- Kettler TA, Doran JW, Gilbert TL. 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses. *Soil Sci Soc Am J* 65:849–52.
- Kimball BA. 1992. Cost comparisons among free-air CO<sub>2</sub> enrichment, open-top chamber, and sunlit controlled-environment chamber methods of CO<sub>2</sub> exposure. *Crit Rev Plant Sci* 11:265–70.
- Lee JS, Usami T, Oikawa T. 2001. High performance of CO<sub>2</sub>-temperature gradient chamber newly built for studying the global warming effect on a plant population. *Ecol Res* 16:347–58.
- Luo Y, Zhou X. 2006. *Soil respiration and the environment*. Burlington: Academic Press.
- Mayeux HS, Johnson HB, Polley HW, Dumesnil MJ, Spanel GA. 1993. A controlled environment chamber for growing plants across a subambient CO<sub>2</sub> gradient. *Funct Ecol* 7:125–33.
- Mielnick PC, Dugas WA, Johnson HB, Polley HW, Sanabria J. 2001. Net grassland carbon flux over a subambient to superambient CO<sub>2</sub> gradient. *Glob Chang Biol* 7:747–54.
- Noy-Meir I. 1973. Desert ecosystems: environment and producers. *Annu Rev Ecol Syst* 4:25–51.
- Oades JM. 1988. The retention of organic matter in soils. *Biogeochemistry* 5:35–70.
- Paul EA, Morris SJ, Bohm S. 2001. The determination of soil C pool sizes and turnover rates: biophysical fractionation and tracers. In: Lal R, Kimble JM, Follett RF, Stewart BA, Eds. *Assessment methods for soil carbon*. Boca Raton, FL: CRC Press. p 193–206.
- Piper JK. 1995. Composition of prairie plant communities on productive versus unproductive sites in wet and dry years. *Can J Bot* 73:1635–44.
- Polley HW, Johnson HB, Derner JD. 2003. Increasing CO<sub>2</sub> from subambient to superambient concentrations alters species composition and increases above-ground biomass in a C-3/C-4 grassland. *New Phytol* 160:319–27.
- Polley HW, Johnson HB, Fay PA, Sanabria J. 2008. Initial response of evapotranspiration from tallgrass prairie vegetation to CO<sub>2</sub> at subambient to elevated concentrations. *Funct Ecol* 22:163–71.
- Polley HW, Johnson HB, Mayeux HS. 1992a. Carbon dioxide and water fluxes of C3 annuals and C4 perennials at subambient CO<sub>2</sub> concentrations. *Funct Ecol* 6:693–703.
- Polley HW, Johnson HB, Mayeux HS. 1992b. Growth and gas exchange of oats (*Avena sativa*) and wild mustard (*Brassica kaber*) at subambient CO<sub>2</sub> concentrations. *Int J Plant Sci* 153:453–61.
- Polley HW, Johnson HB, Marino BD, Mayeux HS. 1993a. Increase in C3 plant water-use efficiency and biomass over glacial to present CO<sub>2</sub> concentrations. *Nature* 361:61–4.
- Polley HW, Johnson HB, Mayeux HS, Malone SR. 1993b. Physiology and growth of wheat across a subambient carbon dioxide gradient. *Ann Bot* 71:347–56.
- Polley HW, Johnson HB, Mayeux HS. 1994. Increasing CO<sub>2</sub>: comparative responses of the C4 grass *Schizachyrium* and grassland invader *Prosopis*. *Ecology* 75:976–88.
- Polley HW, Johnson HB, Mayeux HS. 1995. Nitrogen and water requirements of C3 plants grown at glacial to present carbon dioxide concentrations. *Funct Ecol* 9:86–96.
- Polley HW, Johnson HB, Mayeux HS, Brown DA, White JWC. 1996. Leaf and plant water use efficiency of C4 species grown at glacial to elevated CO<sub>2</sub> concentrations. *Int J Plant Sci* 157:164–70.
- Rawson HM, Gifford RM, Condon BN. 1995. Temperature gradient chambers for research on global environment change. I. Portable chambers for research on short-stature vegetation. *Plant Cell Environ* 18:1048–54.
- Rogers A, Ainsworth EA, Kammann C. 2006. FACE value: perspectives on the future of free-air CO<sub>2</sub> enrichment studies. In: Nosberger J, Long SP, Norby RJ, Stitt M, Eds. *Managed ecosystems and CO<sub>2</sub>: case studies, processes, and perspectives*. Berlin: Springer. p 431–49.
- SAS Institute Inc. 2003. *The SAS system for windows*. [9.1.3]. Cary, NC: SAS Institute.
- Ward JK, Tissue DT, Thomas RB, Strain BR. 1999. Comparative responses of model C3 and C4 plants to drought in low and elevated CO<sub>2</sub>. *Glob Chang Biol* 5:857–67.