

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Publications from USDA-ARS / UNL Faculty

U.S. Department of Agriculture: Agricultural
Research Service, Lincoln, Nebraska

10-2012

Productivity of well-watered *Panicum virgatum* does not increase with CO₂ enrichment

Philip A. Fay

USDA-ARS, philip.fay@ars.usda.gov

H. Wayne Polley

USDA-ARS, wayne.polley@ars.usda.gov

Virginia L. Jin

USDA-ARS, virginia.jin@ars.usda.gov

Michael J. Aspinwall

University of Texas at Austin, m.aspinwall@unf.edu

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



Part of the [Agricultural Science Commons](#)

Fay, Philip A.; Polley, H. Wayne; Jin, Virginia L.; and Aspinwall, Michael J., "Productivity of well-watered *Panicum virgatum* does not increase with CO₂ enrichment" (2012). *Publications from USDA-ARS / UNL Faculty*. 824.

<https://digitalcommons.unl.edu/usdaarsfacpub/824>

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.

Productivity of well-watered *Panicum virgatum* does not increase with CO₂ enrichment

Philip A. Fay^{1,*}, H. Wayne Polley¹, Virginia L. Jin² and Michael J. Aspinwall³

¹ USDA-ARS Grassland Soil and Water Research Laboratory, 808 E Blackland Rd, Temple, TX 76502, USA

² USDA-ARS Agroecosystem Management Research, 137 Keim Hall, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

³ Section of Integrative Biology, 1 University Station, C0930, University of Texas at Austin, Austin, TX 78712, USA

*Correspondence address. USDA-ARS, 808 E Blackland Road, Temple, TX 76502, USA. Tel: +254-770-6533; Fax: +254-770-6561; E-mail: philip.fay@ars.usda.gov

Abstract

Aims

Rising atmospheric CO₂ has been shown to increase aboveground net primary productivity (ANPP) in water-limited perennial grasslands, in part by reducing stomatal conductance and transpiration, thereby reducing depletion of soil moisture. However, the benefits of CO₂ enrichment for ANPP will vary with soil type and may be reduced if water limitation is low. Little is known about CO₂ effects on ANPP of *Panicum virgatum*, a perennial C₄ tallgrass and potential bioenergy crop. We hypothesized that if water limitation is minimized, (i) CO₂ enrichment would not increase *P. virgatum* ANPP because photosynthetic rates of this C₄ grass would not increase and because decreased transpiration at elevated CO₂ would provide little additional benefit in increased soil moisture and (ii) soil type will have little effect on *P. virgatum* CO₂ responses because of high overall soil moisture.

Methods

Growth and leaf physiology of *P. virgatum* cv. 'Alamo' were studied as plants established for 4 years on silty clay and clay soils along a 250 to 500 µl l⁻¹ gradient in atmospheric CO₂ located in central Texas, USA. Plants were watered to replace evapotranspiration, fertilized with NO₃NH₄ and P₂O₅ and clipped to standard height during mid-season.

Important Findings

ANPP increased through the third year of growth. Soil moisture (0–20 cm), ANPP, tiller numbers and leaf area index were 8–18% higher on

the clay than on the silty clay soil. ANPP did not increase with CO₂ except in the planting year. However, biomass removed with clipping strongly increased with CO₂ in years 2 and 3, suggesting that CO₂ enrichment increased the early- to mid-season growth of establishing *P. virgatum* but not later regrowth or that of fully established plants. Furthermore, CO₂ enrichment differentially affected two components of ANPP in years 2 and 3, increasing tiller mass and reducing tiller numbers. This reallocation of resources in clipped *P. virgatum* suggested increased meristem limitation of productivity with CO₂ enrichment. CO₂ enrichment had little effect on photosynthesis but increasingly reduced stomatal conductance and transpiration as the plants established. As a result, water use efficiency became increasingly coupled to CO₂ as leaf area increased during establishment. These results suggest that for well-watered and clipped *P. virgatum*, ANPP differed between soil types, was not affected by CO₂ enrichment when fully established but interacted with clipping to alter allocation patterns during establishment. Soil type effects on ANPP-CO₂ responses will likely become more apparent when water is more limiting.

Keywords: switchgrass • bioenergy • resource allocation • tillering • meristem limitation

Received: 21 November 2011 Revised: 15 February 2012 Accepted: 15 February 2012

INTRODUCTION

Atmospheric CO₂ concentrations have increased from around 270 µl l⁻¹ in the pre-industrial period to the present level of

390 µl l⁻¹ and are expected to surpass 500 µl l⁻¹ by 2050 (Forster *et al.* 2007). Rising CO₂ represents a chronic and cumulative change (Smith *et al.* 2009) in the availability of an essential resource for plant growth and productivity. CO₂ effects on

ecosystem primary productivity will depend on the direct effects of CO₂ on plant carbon gain, on the indirect effects of CO₂ on other ecosystem controls such as soil moisture and nitrogen availability and on the extent to which the indirect CO₂ effects either reinforce or offset the direct effects (Polley *et al.* 2011b).

Several mechanisms have been advanced to explain how CO₂ enrichment may increase plant growth and productivity. CO₂ enrichment directly affects photosynthetic processes in several ways, including increased carboxylation efficiency (C₃ species) and reduced stomatal conductance and transpiration (C₃ or C₄ species), which can increase photosynthetic water use efficiency (Ainsworth and Long 2005; Anderson *et al.* 2001; Drake *et al.* 1997). Indirect effects of reduced transpiration at elevated CO₂ can include increase soil moisture availability (Lecain *et al.* 2003; Morgan *et al.* 2001) and soil nitrogen mineralization (Austin *et al.* 2004, Dijkstra *et al.* 2008). Furthermore, in multispecies systems, species abundances may begin to change once CO₂ enrichment exceeds the capacity of some species to respond, and when other, potentially more productive species are favored by new combinations of CO₂ and resource availability (Smith *et al.* 2009).

However, several factors could limit productivity increases from CO₂ enrichment. Lack of water limitation, such as in high rainfall years or on soils with high water holding capacity, could limit soil moisture gains accruing from reduced transpiration. In addition, other resources, such as mineral nitrogen (N) availability (Gill *et al.* 2002; Norby *et al.* 2010 but see Dijkstra *et al.* 2008) may limit productivity gains from CO₂ enrichment (Luo *et al.* 2004). Dilution of the N concentration in plant tissues (Ainsworth and Long 2005) may result in lower quality litter inputs. Finally, in monoculture production systems, species reordering is not available as a mechanism by which CO₂ enrichment can increase productivity.

While these mechanisms are largely supported by studies in natural mixed-species ecosystems (Knapp *et al.* 1996, Owensby *et al.* 1996) and in annual agricultural crops (Kimball *et al.* 1999; Leakey 2009), an important gap remains regarding the CO₂ response of perennial grasses in monoculture. Dijkstra *et al.* (2010) found that the effects of CO₂ enrichment on the productivity of monocultures of perennial C₄ grasses were not predictable from species responses in mixtures, but there have been few other studies (Oliver *et al.* 2009). The importance of studying perennial monocultures is heightened by the increased interest in their potential for bioenergy production (Perlack *et al.* 2005).

We studied the growth and productivity of monocultures of a mesic C₄ tallgrass, *Panicum virgatum* L. (cv. Alamo), growing at CO₂ ranging from 250 to 500 μl l⁻¹. The study was conducted over 4 years (2007–2010) as *P. virgatum* established on two soils collected from upper (silty clay) and lower (clay) topographic positions in the Blackland Prairie region of central Texas, USA. These soils represent the typical range of texture, N and C contents, and hydrologic properties on which native grasslands (which would have included *P. virgatum*) would be found and on which *P. virgatum* would likely be grown for

bioenergy production in the Blackland Prairie region. The monocultures were well watered and fertilized. With relatively abundant nitrogen and soil moisture, we hypothesized that biomass production would show little response to CO₂ enrichment because the enhancement of physiological efficiencies that may arise from CO₂ enrichment (i.e., increased water use efficiency or nitrogen use efficiency) would accrue little additional soil moisture, and thus provide little additional benefit to plant growth. We also hypothesized that soil type effects on biomass production and CO₂ responses would be minimal because of the well-watered conditions.

MATERIALS AND METHODS

Study site

The study was conducted in the Lysimeter CO₂ Gradient (LYCOG) facility, located at Temple, TX, USA (31°05' N, 97°20' W) in the southern US Central Plains. *Panicum virgatum* is a warm season C₄ grass and native throughout the region. The climate at this site is subtropical, falling in the transition between humid and sub-humid zones. Mean annual precipitation is 914 mm (1971–2000), with growing season wet periods in May–June and September–October and a pronounced July–August dry period. 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 ~ 250°, from mid-March to late November.

The LYCOG facility consists of closed outdoor chambers that maintain a continuous linear gradient in CO₂ concentration of 250 to 500 μl l⁻¹, spanning pre-industrial to anticipated mid-21st century values. The facility is described in detail in Fay *et al.* (2009). LYCOG consists of two linear chambers. Each chamber is 1.2-m wide, 1.5-m tall and consists of 10–5 m long sections. Each section contained four steel-encased 1 × 1 × 1.5 m deep intact soil monoliths enclosed in water-tight steel boxes. One chamber contains the superambient portion of the gradient, created by introducing air enriched to 500 μl l⁻¹ CO₂. Blowers advect the air, and photosynthesis by the enclosed vegetation progressively depletes the air of CO₂, which exits the chamber at 380 μl l⁻¹. Similarly, on the second linear chamber, the sub-ambient portion of the gradient is created when ambient air is introduced and progressively depleted of CO₂, exiting the chamber at 250 μl l⁻¹.

Each section is enclosed with clear polyethylene (0.006"/0.15 mm). This film transmits >90% of incident light with minimal effects on spectral quality and is similar to polyethylene films used in other global change experiments. Zippered openings in the polyethylene backed by draft flaps allow access to the monoliths for sampling. During winter, the polyethylene enclosures are removed and replaced with rain exclusion covers, exposing the dormant vegetation to the ambient atmosphere but continuing to exclude precipitation. The chambers are then reclosed with new polyethylene at the beginning of the next growing season to minimize the effects of photodegradation on light transmittance.

Average air temperature in the chambers is controlled to match outside ambient air temperature by passing the chamber airflow through a chilled water cooling coil separating each 5-m section. CO₂ treatments are maintained for the portion of the growing season when the vegetation has adequate assimilation capacity to maintain the gradient, typically early May to late October.

The chambers contained 80 hydrologically isolated intact soil monoliths (1.5 m³), of which 20 contained *P. virgatum*. The remaining 60 monoliths support tallgrass prairie communities that did not include *P. virgatum* and are considered elsewhere (Polley *et al.* 2008, 2011a).

The 20 *P. virgatum* monoliths came from two soil orders, a silty clay soil typical of uplands ($n = 8$, Austin series, Mollisol, Udorthentic Haplustol) and a moist high organic matter heavy clay found downslope from the silty clay ($n = 12$, Houston Black series, Vertisol, Udic Haplustert). These soils were representative of their respective landscape positions in the Blackland Prairie region where the experiment was conducted. The soils were collected as intact soil monoliths (1 × 1 × 1.5 m deep) in 2002, during construction of LYCOG. The monoliths originally supported mixed native prairie vegetation which was removed by hand weeding and glyphosate application. *Panicum virgatum* was planted in May 2007 at a density of 50 live tillers per monolith. Tillers that died in 2007 were replaced in May 2008. The monoliths were arranged in a stratified random design along the CO₂ gradient, interspersed among the prairie monoliths. Each *P. virgatum* monolith was individually drip irrigated. We irrigated each monolith to field capacity at the start of each growing season and irrigated weekly during each growing season to replace evapotranspiration (ET). We estimated ET from the average change in weight of three monoliths of each soil type exposed to either subambient or elevated CO₂ during 2007 through 2009. Beginning in 2010, all the monoliths were weighed, and ET was calculated for each monolith. Irrigation applications were controlled and logged by a data logger. Each monolith was fertilized with the equivalent of 42 kg N/ha and 22.4 kg P/ha in April 2007 and with 168 kg N/ha and 44.8 kg P/ha in April 2008–2010. N was applied as NH₄NO₃, P was applied as P₂O₅. Fertilizer amounts were chosen to minimize N limitation.

Plants in all monoliths were clipped to 70-cm height two (2009–2010) or three (2008) times per growing season during June–August to prevent plants from outgrowing the chamber volume. The height was chosen to leave ample leaf area for regrowth and not to mimic complete harvests.

Soil and plant measurements

Volumetric soil water content for 0–20 cm (Θ_{20}) of each monolith was measured biweekly with a calibrated neutron attenuation probe (503DR Hydroprobe, CPN International, Concord, CA, USA) at permanently installed access tubes.

Aboveground net primary productivity (ANPP) was determined from the mid-season clipped biomass plus the end of year (November) standing biomass. Tiller numbers were

counted at the final harvest. The end of year biomass was clipped at 10 cm above the soil surface. All biomass was dried to constant mass for 72 h at 60 °C and then weighed. The leaf area index (LAI) in each monolith was measured each July and August using a SunScan canopy analysis system (Delta-T Devices Ltd., Burwell, Cambridge, UK).

Leaf-level carbon and water exchange were measured in each monolith during June 2008, May and July 2009 and June 2010. Gas exchange was measured on one or two leaves on two tillers per monolith between 0900 and 1400 local time. The chosen tillers had typical vigor for that soil type and CO₂, and the selected leaves were recently fully expanded and also of typical vigor. Leaves were measured for net carbon assimilation (A_{CO_2}), stomatal conductance (g_s), and transpiration (E) with an infrared gas analyzer (LI-6400 LICor Biosciences, Inc., Lincoln, NE, USA) using a 2 × 3 cm leaf cuvette, CO₂ mixer and 85:15 red:blue light source. Leaf chamber illumination was controlled at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density. Leaf temperature varied between 30 and 35 °C, and the leaf cuvette H₂O mole fraction was controlled at 15, 17, and 20 mmol mol⁻¹ in 2008, 2009 and 2010, respectively, corresponding to humidity levels in the gradient during the measurements. Cuvette CO₂ was controlled to values corresponding to the position along the gradient. Instantaneous photosynthetic water use efficiency (WUEt) was computed as $A_{CO_2} E^{-1}$.

Leaves were collected immediately following the gas exchange measurements and measured for leaf water potential (Ψ_l) using a pressure chamber (Model 1000, PMS Instruments, Corvallis, OR, USA). The leaves were then dried, ground to a fine powder and assayed for carbon and nitrogen content in an elemental analyzer (Flash 2000, Thermo Scientific, Waltham, MA, USA).

Data analysis

Statistical analyses of the effects of CO₂ and soil type on soil moisture and plant growth and physiology of *P. virgatum* were conducted in SAS 9.2. Weekly Θ_{20} measurements and the July/August LAI measurements were averaged to yield a single value per monolith for each growing season, to correspond to the ANPP data. We computed the ratio of the sum of mid-season biomass removed to the end of season biomass as an index of clipping intensity. The monolith was the experimental unit in these analyses.

Analyses proceeded in three steps. First, we fit a repeated measures model (Equation 1) to test for soil differences in ANPP, LAI, tiller number and tiller mass using a mixed models procedure (Proc Mixed):

$$y_{ijkl} = \text{intercept} + \text{soil}_i + \text{monolith}_j(\text{soil}_i) + \text{year}_k + \text{soil} \times \text{year}_{ik} + e_{ijkl} \quad (1)$$

Second, to test for CO₂ effects and their interaction with soil type and year, we fit an expanded model to the plant growth and physiology variables (Equation 2):

$$\begin{aligned}
 y_{ijkl} = & \text{intercept} + \text{soil}_i + \text{monolith}_j(\text{soil}_i) + \alpha(\text{CO}_2) \\
 & + \beta_1(\text{CO}_2 \times \text{soil}_i) + \text{year}_k + \text{soil} \times \text{year}_{ik} \\
 & + \gamma_k(\text{CO}_2 \times \text{year}_k) + \delta_{ik}(\text{CO}_2 \times \text{soil}_i \times \text{year}_k) + e_{ijkl}
 \end{aligned}
 \quad (2)$$

In both models, soil was a fixed effect, monoliths nested within soils [monolith(soil)] a random effect, CO₂ a covariate and year a repeated effect. Non-significant terms in this initial model were removed when this improved the fit statistics (Akaike's Information Criteria).

Third, we fit linear regressions of each response variable versus CO₂ for the soils separately and combined and for the years separately and combined (Table 2). When the ANCOVA model (equation 2) returned significant CO₂, soil × CO₂, soil × year or soil × CO₂ × year effects, the corresponding regressions were plotted in Figs 3 and 4.

RESULTS

Soil moisture

Θ₂₀ was higher on the clay (34.4% ± 0.4), compared to the silty clay soil (31.6 ± 0.4%, $P < 0.0001$) and increased weakly with CO₂ for both soils combined ($R^2 = 0.04$, $P = 0.005$, Fig. 1). Θ₂₀ also varied between years (31.5 ± 0.4 in 2009 to 33.8 ± 0.4 in 2010, $P = 0.013$). We found no evidence for interactions among CO₂, soil and year ($0.09 < P < 0.68$).

ANPP and components

Establishment year ANPP of *P. virgatum* averaged 160 g m⁻², increased 8-fold in 2008, followed by a further doubling in 2009, with no further change in ANPP in 2010 (year $P < 0.0001$, Fig. 2a). Tiller numbers and mass both increased during establishment in a similar manner as ANPP ($P < 0.0001$, Fig. 2b and c), whereas LAI increased each year (Fig. 2d). Averaged across all years and CO₂ concentrations, ANPP, tiller

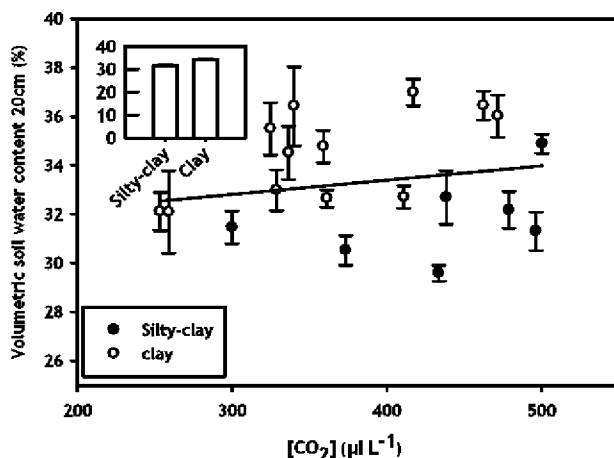


Figure 1: Volumetric soil water content of clay and silty clay soils along the CO₂ gradient, and (inset) mean of all CO₂ concentrations. Each point is the average of 2007–2010 (± standard error). Linear regression is for both soils combined (Table 2).

number and LAI were 12–18% higher on the clay soil than on the silty clay ($P \leq 0.046$, Table 1). Clipping intensity was 38% in 2008, decreasing to 20% in 2010 ($P = 0.0003$, Fig. 3d).

Averaged over both soils, ANPP increased significantly with CO₂ enrichment in the establishment year ($R^2 = 0.21$) but not thereafter (CO₂ × year $P = 0.003$, Fig. 3a, Table 2). The contributions of tiller number vs. tiller mass to ANPP changed as *P. virgatum* established. In the establishment year, ANPP increased with CO₂ enrichment because tiller mass increased with CO₂ ($R^2 = 0.29$, $P = 0.0143$, Fig. 3b). As establishment progressed, tiller numbers decreased with CO₂ enrichment ($0.03 < P < 0.07$, Fig. 3c), whereas tiller masses increased, most strongly on the clay soil in 2008 and 2009 ($R^2 = 0.55$ – 0.79 , $P < 0.006$). However, once *P. virgatum* was fully established in 2010, tiller number and mass no longer varied with CO₂ ($P > 0.19$). Clipping intensity increased with CO₂ enrichment in both 2008 and 2009 ($R^2 = 0.66$ – 0.69 , $P < 0.0001$) because clipped biomass increased with CO₂ enrichment on the clay soil in 2008 and on both soils in 2009 ($R^2 = 0.64$ – 0.72 , $P < 0.008$, Table 2). In contrast, end of season standing biomass

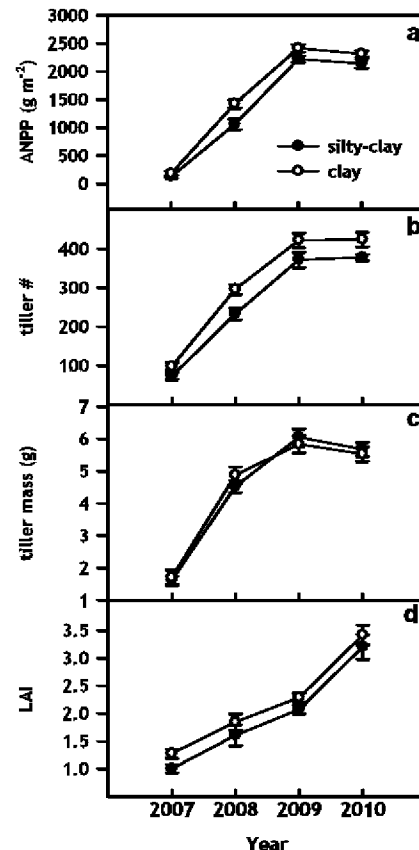


Figure 2: Biomass components on the clay and silty clay soil during the establishment of *Panicum virgatum* in the CO₂ gradient experiment. Each point is the average over all CO₂ concentrations (± standard error). (a) Aboveground net primary productivity (ANPP), (b) number of tillers per monolith, (c) individual tiller mass and (d) Leaf area index (LAI).

Table 1: biomass and physiological variables by soil type, averaged across years and CO₂

	Silty clay (SE)	Clay (SE)	F	P-value
SWC (%vol)	31.59 (0.36)	34.43 (0.36)	28.8	<0.0001
ANPP (g m ⁻²)	1383.03 (157.97)	1580.28 (133.66)	6.9	0.0180
Tiller # (m ⁻²)	263.47 (23.35)	309.77 (20.86)	12.9	0.0006
Tiller mass (g)	4.46 (0.33)	4.48 (0.27)	1.3	0.2724
LAI (m ² m ⁻²)	1.97 (0.16)	2.20 (0.13)	4.6	0.0465
A _{CO2} (μmol m ⁻² s ⁻¹)	22.62 (0.71)	21.38 (0.61)	1.8	0.1834
g _s (mol m ⁻² s ⁻¹)	0.19 (0.02)	0.19 (0.01)	0.1	0.8180
E (mmol m ⁻² s ⁻¹)	4.21 (0.35)	4.23 (0.18)	0.0	0.9986
WUE (A _{CO2} E ⁻¹)	6.21 (0.49)	5.46 (0.31)	1.4	0.2513
Ψ _l (MPa)	-1.19 (0.05)	-1.33 (0.03)	3.3	0.0875
Leaf %C	45.89 (0.12)	45.30 (0.09)	0.7	0.4070
Leaf %N	1.86 (0.09)	1.79 (0.08)	1.0	0.3338
Leaf C:N Ratio	25.64 (1.27)	26.78 (1.25)	0.6	0.4653

in general did not vary with CO₂. No significant CO₂, soil × CO₂ or soil × year interactions were found for LAI.

Leaf carbon and water fluxes

ANPP and tiller responses to CO₂ were accompanied by changes in several measures of photosynthetic carbon uptake and water loss. g_s and E were 14% lower on the clay soil compared to the silty clay, averaged across CO₂ and year ($P < 0.01$, Table 1). However A_{CO2}, WUE_l, Ψ_l and leaf C:N did not differ between the soils ($P > 0.09$). A_{CO2} and g_s increased from 2008 to 2010 while E decreased, leading to a 73% increase in WUE_l, averaged over soils and CO₂ ($P < 0.0001$, Fig. 4e). Leaf %N decreased from $2.2 \pm 0.4\%$ in 2009 to $1.5 \pm 0.4\%$ in 2010, resulting in a 50% increase leaf C:N ($P \leq 0.0001$).

A_{CO2} showed a weak increase with CO₂ enrichment in 2008 ($R^2 = 0.25$, $P = 0.04$, Fig. 4a) but not in 2009–2010. In contrast, g_s and E decreased ~2-fold with CO₂ enrichment, leading to a ~2-fold increase in WUE_l (Fig. 4a–d). Ψ_l also increased ~40% with CO₂ enrichment (Fig. 4e). Furthermore, as *P. virgatum* established, Ψ_l decreased (Fig. 4e), indicating increased plant water stress. g_s and E were unresponsive to CO₂ in 2008 ($P = 0.13$ – 0.25 , Fig. 4b and c), but they declined more steeply and were increasingly tightly correlated with CO₂ from 2008 to 2010 (Fig. 4b and c, Table 2). As a result, WUE_l became increasingly responsive to CO₂ enrichment from 2008 to 2010, with R^2 reaching 0.94 in 2010.

These changes in photosynthetic carbon and water fluxes through time as *P. virgatum* established were accompanied by significant but less dramatic differences between soils in the CO₂ responsiveness of a subset of the parameters. A_{CO2} increased with CO₂ enrichment on the clay soil ($R^2 = 0.18$, $P = 0.01$) but not on the silty clay ($P = 0.69$, Fig. 4a). In contrast, g_s and E decreased with CO₂ enrichment more steeply and were more strongly correlated with CO₂ on the silty clay soil ($R^2 =$

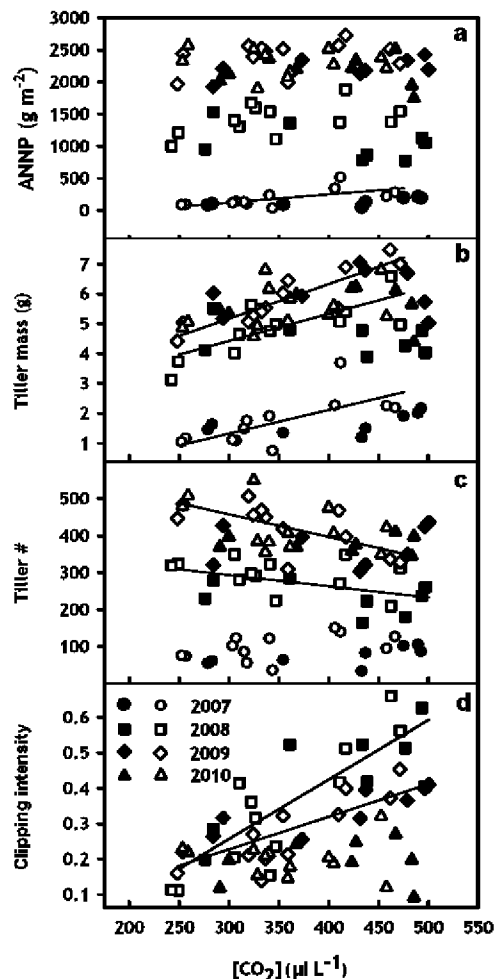


Figure 3: Biomass components of *Panicum virgatum* along the CO₂ gradient on clay (open symbols) and silty clay (closed symbols) soils from the planting year (2007) through full establishment (2010). (a) Aboveground net primary productivity (ANPP), (b) individual tiller mass, (c) number of tillers per monolith, (d) clipping intensity, the ratio of June–August clipped biomass to end of season standing biomass. Regressions are shown for years when significant relationships ($P < 0.05$) were found for both soils combined (Table 2).

0.64–0.70) than on the clay soil ($R^2 = 0.22$ – 0.25 ; Table 2). There were no differences between soils overall in the correlations of WUE_l and Ψ_l with CO₂.

DISCUSSION

The findings of this study support the hypothesis that ANPP of *P. virgatum* was mostly unaffected by a 250 to 500 μL L⁻¹ gradient of CO₂ concentration. ANPP of established *P. virgatum* in this study was higher than that reported for field-grown plants in this region (Kiniry et al. 1996), suggesting that water and nutrient limitation were relatively low compared to field conditions. However, the findings also show that this range of CO₂ concentrations caused offsetting effects on several components

Table 2: regression parameters and statistical tests for the growth and physiological responses to CO₂ in *Panicum virgatum*

Year	Intercept	Slope	Silty clay			Clay					Both soils				
			R ²	F	P	Intercept	Slope	R ²	F	P	Intercept	Slope	R ²	F	P
ANPP (g m⁻²)															
2007	-67.3	0.469	0.45	4.9	0.0681	-263.6	1.276	0.45	8.3	0.0165	-81.8	0.646	0.21	4.9	0.0396
2008	1670.2	-1.506	0.24	1.9	0.2133	860.3	1.599	0.26	3.1	0.1092	1511.5	-0.634	0.03	0.5	0.4702
2009	1792.4	1.028	0.33	3.0	0.1349	2037.4	1.045	0.11	1.2	0.3006	2143.6	0.499	0.03	0.6	0.4432
2010	2176.6	-0.098	0.00	0.0	0.9394	2481.8	-0.488	0.03	0.3	0.6195	2490.0	-0.666	0.05	0.9	0.3621
All years	1334.1	0.120	0.00	0.0	0.9525	1009.9	1.614	0.01	0.7	0.4167	1364.1	0.366	0.00	0.1	0.7819
# tillers															
2007	2.1	0.172	0.38	3.7	0.1034	12.8	0.241	0.25	3.3	0.1007	39.4	0.128	0.10	2.0	0.1724
2008	307.2	-0.184	0.15	1.0	0.3472	369.0	-0.208	0.12	1.4	0.2600	384.0	-0.303	0.23	5.4	0.0327
2009	332.1	0.097	0.02	0.1	0.7187	639.3	-0.608	0.44	7.8	0.0188	532.8	-0.345	0.18	4.0	0.0607
2010	378.1	-0.001	0.00	0.0	0.9923	585.8	-0.454	0.21	2.6	0.1360	525.2	-0.318	0.17	3.7	0.0701
All years	246.3	0.042	0.00	0.0	0.8874	361.5	-0.146	0.00	0.2	0.6379	347.0	-0.149	0.01	0.5	0.4646
Tiller mass (g)															
2007	0.75	0.002	0.31	2.7	0.1539	-1.06	0.008	0.49	9.6	0.1130	0.07	0.004	0.29	7.4	0.0143
2008	5.49	-0.002	0.16	1.1	0.3310	1.68	0.009	0.55	12.2	0.0059	3.80	0.003	0.70	1.4	0.2602
2009	5.45	0.001	0.03	0.2	0.6924	0.17	0.012	0.79	38.3	0.0001	3.49	0.006	0.37	10.5	0.0045
2010	5.75	0.000	0.00	0.0	0.9544	3.88	0.005	0.16	1.9	0.1940	4.68	0.002	0.06	1.3	0.2789
All years	4.22	0.001	0.00	0.0	0.8838	1.10	0.010	0.13	6.6	0.0133	2.74	0.005	0.04	3.1	0.0807
Clipped mass (g m⁻²)															
2008	214.0	0.024	0.04	0.3	0.6143	-332.4	1.958	0.69	22.2	0.0008	-7.2	0.922	0.27	6.6	0.0195
2009	170.1	0.948	0.72	15.4	0.0078	-158.6	1.873	0.64	17.8	0.0018	11.9	1.368	0.60	26.5	<0.0001
2010	272.4	0.195	0.01	0.1	0.7784	455.5	-0.194	0.02	0.2	0.7005	405.7	-0.089	0.00	0.1	0.8070
All years	198.5	0.512	0.07	1.8	0.1991	-63.9	1.356	0.33	16.4	0.0003	104.0	0.822	0.16	11.4	0.0013
End of year mass (g m⁻²)															
2008	1456.0	-1.745	0.49	5.7	0.0542	1192.7	-0.359	0.03	0.3	0.6165	1518.7	-1.555	0.28	7.0	0.0163
2009	1622.4	0.079	0.00	0.0	0.8880	2196.0	-0.828	0.09	1.1	0.3307	2131.7	-0.869	0.12	2.4	0.1387
2010	1904.2	-0.293	0.03	0.2	0.6639	2026.3	-0.294	0.02	0.2	0.6637	2084.3	-0.578	0.08	1.6	0.2163
All years	1193.4	-0.285	0.00	0.03	0.8579	1089.2	0.504	0.00	0.1	0.7514	1303.6	-0.299	0.00	0.1	0.7763
Clipped: end of year															
2008	-0.05	0.001	0.57	8.0	0.0303	-0.40	0.002	0.77	33.6	0.0002	-0.25	0.002	0.69	40.5	<0.0001
2009	0.11	0.001	0.64	10.7	0.0172	-0.15	0.001	0.71	24.1	0.0006	-0.05	0.001	0.66	35.4	<0.0001
2010	0.15	0.000	0.02	0.1	0.7522	0.22	0.000	0.00	0.0	0.8705	0.19	0.000	0.00	0.0	0.9510
All years	0.04	0.001	0.16	4.31	0.0497	-0.14	0.001	0.36	19.03	0.0001	-0.06	0.001	0.29	23.6	<0.0001
A_{CO2} (μmol m⁻² s⁻¹)															
2008	17.5	0.006	0.03	0.1	0.7292	3.8	0.043	0.47	7.1	0.0283	10.0	0.025	0.25	4.9	0.0425
2009	28.8	-0.011	0.06	0.4	0.5488	19.8	0.008	0.06	0.6	0.4609	22.4	0.003	0.00	0.1	0.7834
2010	28.2	-0.012	0.47	5.4	0.0592	17.6	0.012	0.13	1.5	0.2487	21.0	0.004	0.02	0.4	0.5507
All years	24.1	-0.004	0.01	0.2	0.6901	13.7	0.022	0.18	6.9	0.0132	17.7	0.011	0.06	3.4	0.0691
g_s (mol m⁻² s⁻¹)															
2008	0.42	-0.001	0.67	10.0	0.0251	0.12	0.000	0.05	0.4	0.5245	0.24	0.000	0.09	1.5	0.2479
2009	0.50	-0.001	0.86	37.5	0.0009	0.29	0.000	0.46	8.4	0.0158	0.36	0.000	0.55	22.5	0.0002
2010	0.73	-0.001	0.80	23.9	0.0027	0.77	-0.002	0.79	37.1	0.0001	0.72	-0.001	0.77	61.9	<0.0001
All years	0.54	-0.001	0.70	49.5	<0.0001	0.38	-0.001	0.22	9.0	0.0053	0.43	-0.001	0.39	34.5	<0.0001
E (mmol m⁻² s⁻¹)															
2008	11.0	-0.015	0.68	10.5	0.0230	4.3	0.001	0.01	0.1	0.7703	6.9	-0.005	0.15	2.6	0.1291

Table 2: Continued

Year	Silty clay					Clay					Both soils				
	Intercept	Slope	R ²	F	P	Intercept	Slope	R ²	F	P	Intercept	Slope	R ²	F	P
2009	11.6	-0.018	0.86	36.8	0.0009	7.2	-0.008	0.50	10.1	0.0098	8.6	-0.011	0.58	25.1	<0.0001
2010	10.1	-0.017	0.82	27.5	0.0019	10.3	-0.019	0.88	72.7	<0.0001	9.8	-0.017	0.83	87.3	<0.0001
All years	10.9	-0.017	0.64	36.7	<0.0001	7.0	-0.008	0.25	10.7	0.0026	8.3	-0.011	0.39	35.6	<0.0001
WUEt															
2008	-0.58	0.012	0.93	66.4	0.0005	0.76	0.009	0.53	9.1	0.0167	0.35	0.010	0.68	31.6	<0.0001
2009	-1.79	0.019	0.93	76.7	0.0001	1.00	0.012	0.88	70.1	<0.0001	-0.12	0.015	0.88	127.2	<0.0001
2010	-3.22	0.028	0.93	74.2	0.0001	-5.15	0.033	0.95	200.6	<0.0001	-4.21	0.030	0.94	285.0	<0.0001
All years	-1.84	0.020	0.48	19.5	0.0002	-0.62	0.017	0.43	24.6	<0.0001	-1.06	0.018	0.47	49.4	<0.0001
Ψ_1 (MPa)															
2009	-1.90	0.002	0.74	17.5	0.0058	-1.89	0.002	0.67	20.5	0.0011	-1.96	0.002	0.73	49.8	<0.0001
2010	-1.77	0.001	0.47	5.4	0.0593	-1.66	0.001	0.25	3.4	0.0941	-1.75	0.001	0.42	13.3	0.0018
All years	-1.87	0.002	0.43	10.7	0.0056	-1.79	0.001	0.36	12.6	0.0018	-1.89	0.002	0.46	32.1	<0.0001

that contribute to ANPP and that the physiological coupling of *P. virgatum* to CO₂ concentration increased as establishment proceeded.

CO₂ altered aboveground biomass allocation toward fewer, larger tillers

The finding of a trade-off between tiller mass and numbers suggests a reallocation of resources with CO₂ enrichment, increasingly toward current tiller growth at the expense of tiller production in years 2 and 3. This represents a potential increase in meristem limitation of ANPP for establishing *P. virgatum* at elevated CO₂ (Benson et al. 2004; Dalglish and Hartnett 2009). There are few studies of tiller biomass responses to CO₂ enrichment in *P. virgatum*. The finding contrasts with a meta-analysis of C₃ and C₄ grasses that found increased tiller mass and density with CO₂ enrichment (Wand et al. 1999). CO₂ enrichment would be expected to increase resource availability for tiller production and growth. Previous meta-analyses found that CO₂ enrichment had little effect on plant biomass allocation, even when increasing total plant biomass (Poorter and Nagel 2000; Poorter et al. 2012; Wand et al. 1999).

The clipping applied to the experimental plants is likely the primary explanation for the unexpected pattern in tiller production. Clipping removed 20–40% of total biomass, including some fraction of leaf area, which would lower overall assimilation capacity and contribute to the apparent resource limitation. The biomass removed by clipping increased with CO₂ enrichment during years 2 and 3; however final harvest biomass did not vary with CO₂. This means the early to mid-season growth of *P. virgatum* increased with CO₂ enrichment but not the later-season growth. Plant regrowth following defoliation typically declines through the growing season and increases with light, water and nutrient availability (Whitham et al. 1991). Mechanisms by which tiller regrowth after clipping took increasing precedence with CO₂ enrich-

ment over allocation to additional tillers may include (i) a strong tendency for plants to replacing the removed aboveground biomass, returning to pre-clipping allocation patterns (Poorter et al. 2012), (ii) the activation of new plant sinks for regrowth, which allows for stronger growth responses with CO₂ enrichment (Kirschbaum 2011; Körner 2011), (iii) reduced activation and growth of rhizome buds following leaf removal (Beatty et al. 1978) and (iv) developmental constraints on growth patterns, such as continued stem growth to support later inflorescence production (Körner 2011).

The CO₂ effects on early/midseason growth and tiller masses/numbers were absent in year 4 when the plants were fully established. This suggests that as leaf area continued to increase, increased whole-plant photosynthate supply may have alleviated resource limitations that likely caused the allocation trade-off between tiller numbers and size. In addition, other resources, such as light, soil moisture or N may have become more limiting than in preceding years. The decline in leaf N from 2009 to 2010 may be an early indication of N limitation of photosynthesis and/or biomass production, as suggested by previous studies in C₄ grassland (Schimel et al. 1991; Turner and Knapp 1996). Nutrient effects on allocation are likely stronger than CO₂ effects (Poorter and Nagel 2000).

Physiological coupling to CO₂ increased as *P. virgatum* established

The pattern of decreasing CO₂ effects on the components of ANPP were accompanied by increased CO₂ effects on stomatal conductance and transpiration as the plants established and leaf area increased. Overall photosynthetic rates of *P. virgatum*, the absence of increased photosynthesis and lower stomatal conductance and transpiration at elevated CO₂ found in this study agree with previous findings for this and other C₄ tall-grasses (Dohleman et al. 2009; Knapp 1985; Skeel and Gibson 1996). The resulting reduced plant water loss can increase soil moisture compared to that at lower CO₂, providing an indirect

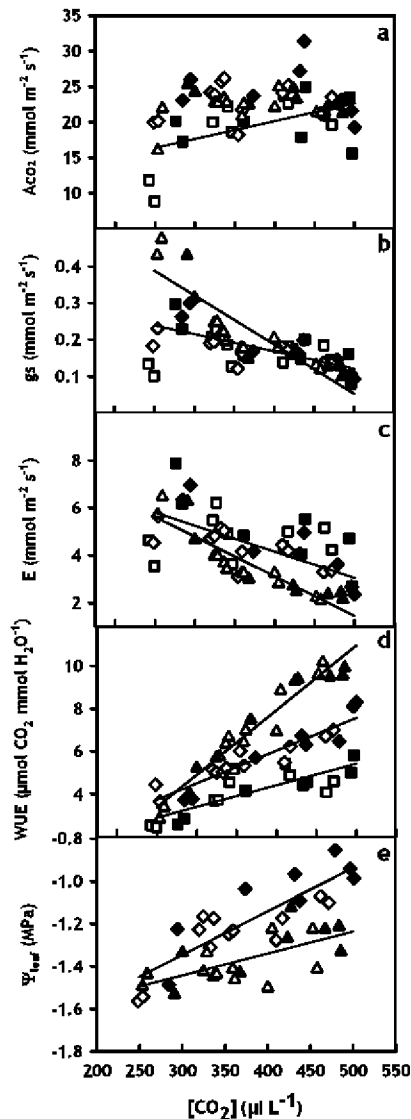


Figure 4: Physiological performance of *Panicum virgatum* along the CO₂ gradient on clay (open symbols) and silty clay (closed symbols) soils from the second year of growth (2008) to full establishment (2010, symbols as in Fig. 3). (a) Leaf net photosynthesis (A_{CO_2}). (b) stomatal conductance (g_s) (c), transpiration (E). (d) photosynthetic WUEt and (e) leaf water potential (Ψ_l). Regressions are shown for years when significant relationships ($P < 0.05$) were found for both soils combined (Table 2).

mechanism by which CO₂ enrichment may stimulate ANPP in water-limited, C₄-dominated vegetation (Morgan et al. 2011). Indeed, we found a modest increase in soil moisture with CO₂ enrichment. A larger increase in soil moisture would likely have occurred if the plants had been less well watered.

The increased soil moisture and decreased stomatal conductance with CO₂ enrichment was accompanied by increased leaf water potential, indicating improved plant water status at elevated CO₂. However, leaf water potential was generally lower

and responded less to CO₂, in year 4 compared to year 3. This suggests that the continued gain in leaf area during establishment increased the transpirational demand for soil moisture. This is the likely mechanism explaining why stomatal conductance and transpiration were progressively more strongly reduced by CO₂ enrichment as plants established, increasing the coupling of WUE to CO₂. We found little evidence to suggest photosynthetic down-regulation with CO₂ enrichment as the plants established. Similar rates of A_{CO_2} were observed each year at high CO₂.

Soil type effects on CO₂ responses were inconsistent

Soil type affected several measures of *P. virgatum* growth and productivity. ANPP, tiller production and LAI averaged 15% higher on the clay than on the silty clay soil. ANPP and root mass of prairie assemblages studied on these same soils were also higher on the clay soil (Fay et al. 2009), suggesting that it was inherently more productive than the silty clay soil. Stout (1992) also reported differences in *P. virgatum* biomass among soil types, in part because of differences in plant water use. However, Sanderson et al. (1999) suggested that soil type had little influence on *P. virgatum* biomass production across several sites in Texas, suggesting that differences in climate can outweigh effects of soil type. Soil texture is a major reason for soil type differences in *P. virgatum* growth (Parrish and Fike 2005). Fine-textured clay soils have higher water holding capacity than more coarse textured soils, resulting in higher soil water contents (Fig. 1; Fay et al. 2009). Soil type effects on ANPP in this study suggest that water limitation was not fully eliminated despite the ET-replacement watering regime.

Differences between soils in the CO₂ responses of some growth and physiology parameters were suggested by the analyses (Table 2). However, these differences were not apparent in the data (Figs 3 and 4), where overall CO₂ effects predominated in years where they occurred. Also, the CO₂ responses were sometimes inconsistent. For example, on the clay soil, stronger tiller mass and clipped mass increases with CO₂ enrichment (compared to the silty clay) were accompanied by weaker stomatal conductance responses (Table 2). Moreover, these soil-specific responses did not culminate in soil-specific ANPP-CO₂ responses. However, at lower soil moisture levels, the lower water holding capacity in the more coarse-textured silty clay soil will likely increase the soil moisture benefits (compared to the clay soil) of reduced transpiration with CO₂ enrichment (Epstein et al. 1997, Morgan et al. 2011).

CONCLUSIONS

The results from this study suggest that ANPP in clipped, well-watered, fertilized *P. virgatum* differed between these soils because of differences in soil moisture. ANPP of established *P. virgatum* did not vary with CO₂ enrichment. CO₂ enrichment affected canopy architecture and tiller growth while plants were establishing, likely because of clipping induced

reallocation of resources that varied with CO₂. Soil type effects on ANPP-CO₂ responses will likely become more apparent when water is more limiting. These findings could have important implications for natural *P. virgatum* populations and for bioenergy production systems based on *P. virgatum*.

FUNDING

United States Department of Agriculture-Agricultural Research Service and the National Science Foundation Plant Genome Research Program (IOS-0922457).

ACKNOWLEDGEMENTS

We thank A. Gibson, K. Jones, C. Kolodziejczyk, A. Naranjo and K. Tiner for technical support, and J. Kiniry for providing the *P. virgatum* rhizomes. USDA is an equal opportunity provider and employer. Mention of trade names or commercial products does not imply recommendation or endorsement by the USDA.

REFERENCES

- Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytol* **165**:351–72.
- Anderson LJ, Maherali H, Johnson HB, *et al.* (2001) Gas exchange and photosynthetic acclimation over subambient to elevated CO₂ in a C-3-C-4 grassland. *Glob Change Biol* **7**:693–707.
- Austin AT, Yahdjian L, Stark JM, *et al.* (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia* **141**:221–35.
- Beatty ER, Engel JL, Powell JD (1978) Tiller development and growth in switchgrass. *J Range Manage* **31**:361–65.
- Benson EJ, Hartnett DC, Mann KH (2004) Belowground bud banks and meristem limitation in tallgrass prairie plant populations. *Am J Bot* **91**:416–21.
- Dalglish HJ, Hartnett DC (2009) The effects of fire frequency and grazing on tallgrass prairie productivity and plant composition are mediated through bud bank demography. *Plant Ecol* **201**:411–20.
- Dijkstra FA, Blumenthal D, Morgan JA, *et al.* (2010) Elevated CO₂ effects on semi-arid grassland plants in relation to water availability and competition. *Funct Ecol* **24**:1152–61.
- Dijkstra FA, Pendall E, Mosier AR, *et al.* (2008) Long-term enhancement of N availability and plant growth under elevated CO₂ in a semi-arid grassland. *Funct Ecol* **22**:975–82.
- Dohleman FG, Heaton EA, Leakey ADB, *et al.* (2009) Does greater leaf-level photosynthesis explain the larger solar energy conversion efficiency of *Miscanthus* relative to switchgrass? *Plant Cell Environ* **32**:525–537.
- Drake BG, González-Meler MA, *et al.* (1997) More efficient plants: a consequence of rising atmospheric CO₂? *Ann Rev Plant Physiol Plant Mol Biol* **48**:609–39.
- Epstein HE, Lauenroth WK, Burke IC (1997) Effects of temperature and soil texture on ANPP in the U.S. Great Plains. *Ecology* **78**:2628–631.
- Fay PA, Kelley AM, Procter AC, *et al.* (2009) Primary productivity and water balance of grassland vegetation on three soils in a continuous CO₂ gradient: initial results from the lysimeter CO₂ gradient experiment. *Ecosystems* **12**:699–714.
- Forster P, Ramaswamy V, Artaxo P, *et al.* (2007) Changes in atmospheric constituents and in radiative forcing. In: Solomon S, Qin D, Manning M, *et al.* (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, 129–234.
- Gill RA, Polley HW, Johnson HB, *et al.* (2002) Nonlinear grassland responses to past and future atmospheric CO₂. *Nature* **417**:279–82.
- Kimball BA, LaMorte RL, Pinter PJ Jr, *et al.* (1999) Free-air CO₂ enrichment and soil nitrogen effects on energy balance and evapotranspiration of wheat. *Water Resour Res* **35**:1179–90.
- Kiniry JR, Sanderson MA, Williams JR, *et al.* (1996) Simulating Alamo switchgrass with the ALMANAC model. *Agron J* **88**:602–06.
- Kirschbaum MUF (2011) Does enhanced photosynthesis enhance growth? Lessons learned from CO₂ enrichment studies. *Plant Physiol* **155**:117–24.
- Knapp AK (1985) Effect of fire and drought on the ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a tallgrass prairie. *Ecology* **66**:1309–20.
- Knapp AK, Hamerlync EP, Ham JM, *et al.* (1996) Responses in stomatal conductance to elevated CO₂ in twelve grassland species that differ in growth form. *Vegetatio* **125**:31–41.
- Körner Ch (2011) The grand challenges in functional plant ecology. *Front Plant Sci* **2**:DOI=10.3389/fpls.2011.00001.
- Leakey ADB (2009) Rising atmospheric carbon dioxide concentration and the future of C 4 crops for food and fuel. *P Roy Soc B-Biol Sci* **276**:2333–43.
- Lecain DR, Morgan JA, Mosier AR, *et al.* (2003) Soil and plant water relations determine photosynthetic responses of C3 and C4 grasses in a semi-arid ecosystem under elevated CO₂. *Ann Bot* **92**:41–52.
- Luo Y, Su B, Currie WS, *et al.* (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *BioScience* **54**:731–39.
- Morgan JA, LeCain DR, Mosier AR, *et al.* (2001) Elevated CO₂ enhances water relations and productivity and affects gas exchange in C3 and C4 grasses of the Colorado shortgrass steppe. *Glob Change Biol* **7**:451–466.
- Morgan JA, Lecain DR, Pendall E, *et al.* (2011) C4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature* **476**:202–05.
- Norby RJ, Warren JM, Iversen CM, *et al.* (2010) CO₂ enhancement of forest productivity constrained by limited nitrogen availability. *PNAS* **107**:19368–73.
- Oliver RJ, Finch JW, Taylor G (2009) Second generation bioenergy crops and climate change: a review of the effects of elevated atmospheric CO₂ and drought on water use and the implications for yield. *GCB Bioenergy* **1**:97–114.
- Owensby CE, Ham JM, Knapp A, *et al.* (1996) Ecosystem-level responses of tallgrass prairie to elevated CO₂. In: Koch GW, Mooney HA (eds). *Carbon Dioxide and Terrestrial Ecosystems*. San Diego, CA: Academic Press, 147–62.
- Parrish DJ, Fike JH (2005) The biology and agronomy of switchgrass for biofuels. *Crit Rev Plant Sci* **24**:423–59.
- Perlack RD, Wright LL, Turhollow AF, *et al.* (2005) *Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-Ton Annual Supply*. Oak Ridge, TN: USDA and DOE, Oak Ridge National Laboratory, 1–60.

- Polley HW, Jin VL, Fay PA (2011a) CO₂-caused change in plant species composition rivals the shift in vegetation between mid-grass and tallgrass prairies. *Glob Change Biol* **18**:700–10.
- Polley HW, Johnson HB, Fay PA, et al. (2008) Initial response of evapotranspiration from tallgrass prairie vegetation to CO₂ at subambient to elevated concentrations. *Funct Ecol* **22**:163–71.
- Polley HW, Morgan JA, Fay PA (2011b) Application of a conceptual framework to interpret variability in rangeland responses to atmospheric CO₂ enrichment. *J Agr Sci* **149**:1–14.
- Poorter H, Nagel O (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO₂, nutrients and water: a quantitative review. *Aust J Plant Physiol* **27**:595–607.
- Poorter H, Niklas KJ, Reich PB, et al. (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol* **193**:30–50.
- Sanderson MA, Reed RL, Ocumpaugh WR, et al. (1999) Switchgrass cultivars and germplasm for biomass feedstock production in Texas. *Bioresource Technol* **67**:209–19.
- Schimel DS, Kittel TGF, Knapp AK, et al. (1991) Physiological interactions along resource gradients in a tallgrass prairie. *Ecology* **72**:672–84.
- Skeel VA, Gibson DJ (1996) Physiological performance of *Andropogon gerardii*, *Panicum virgatum*, and *Sorghastrum nutans* on reclaimed mine spoil. *Restor Ecol* **4**:355–67.
- Smith MD, Knapp AK, Collins SL (2009) A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. *Ecology* **90**:3279–89.
- Stout WL (1992) Water-use efficiency of grasses as affected by soil, nitrogen, and temperature. *Soil Sci Soc Am J* **56**:897–902.
- Turner CL, Knapp AK (1996) Responses of a C-4 grass and three C-3 forbs to variation in nitrogen and light in tallgrass prairie. *Ecology* **77**:1738–49.
- Ward SJE, Midgley G, Jones MH, et al. (1999) Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO₂ concentration: a meta-analytic test of current theories and perceptions. *Glob Change Biol* **5**:723–41.
- Whitham TG, Maschinski J, Larson KC, et al. (1991) Plant responses to herbivory: the continuum from negative to positive and underlying physiological mechanisms. In: Price FW, Lewinsohn TM, Fernandes GW, et al. (eds). *Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions*. New York: Wiley, 227–56.