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Productivity of well-watered *Panicum virgatum* does not increase with CO_2 enrichment

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Abstract

Aims
Rising atmospheric CO_2 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 CO_2 enrichment for ANPP will vary with soil type and may be reduced if water limitation is low. Little is known about CO_2 effects on ANPP of *Panicum virgatum*, a perennial C_4 tallgrass and potential bioenergy crop. We hypothesized that if water limitation is minimized, (i) CO_2 enrichment would not increase *P. virgatum* ANPP because photosynthetic rates of this C_4 grass would not increase and because decreased transpiration at elevated CO_2 would provide little additional benefit in increased soil moisture and (ii) soil type will have little effect on *P. virgatum* CO_2 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 CO_2 located in central Texas, USA. Plants were watered to replace evapotranspiration, fertilized with NO_3NH_4 and P_2O_5 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 CO_2 except in the planting year. However, biomass removed with clipping strongly increased with CO_2 in years 2 and 3, suggesting that CO_2 enrichment increased the early- to mid-season growth of establishing *P. virgatum* but not later regrowth or that of fully established plants. Furthermore, CO_2 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 CO_2 enrichment. CO_2 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 CO_2 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 CO_2 enrichment when fully established but interacted with clipping to alter allocation patterns during establishment. Soil type effects on ANPP-CO_2 responses will likely become more apparent when water is more limiting.

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

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INTRODUCTION

Atmospheric CO_2 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 CO_2 represents a chronic and cumulative change (Smith *et al.* 2009) in the availability of an essential resource for plant growth and productivity. CO_2 effects on ecosystem primary productivity will depend on the direct effects of CO_2 on plant carbon gain, on the indirect effects of CO_2 on other ecosystem controls such as soil moisture and nitrogen availability and on the extent to which the indirect CO_2 effects either reinforce or offset the direct effects (Polley *et al.* 2011b).

Several mechanisms have been advanced to explain how CO_2 enrichment may increase plant growth and productivity. CO_2 enrichment directly affects photosynthetic processes in several ways, including increased carboxylation efficiency (C_3 species) and reduced stomatal conductance and transpiration (C_3 or C_4 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 CO_2 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 CO_2 enrichment exceeds the capacity of some species to respond, and when other, potentially more productive species are favored by new combinations of CO_2 and resource availability (Smith *et al.* 2009).

However, several factors could limit productivity increases from CO_2 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 CO_2 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 CO_2 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 CO_2 response of perennial grasses in monoculture. Dijkstra et al. (2010) found that the effects of CO_2 enrichment on the productivity of monocultures of perennial C_4 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 C_4 tallgrass, *Panicum virgatum* L. (cv. Alamo), growing at CO_2 ranging from 250 $\mu l\ l^{-1}$ to 500 $\mu l\ l^{-1}$. 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 CO_2 enrichment because the enhancement of physiological efficiencies that may arise from CO_2 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 CO_2 responses would be minimal because of the well-watered conditions.

MATERIALS AND METHODS

Study site

The study was conducted in the Lysimeter CO_2 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 C_4 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 CO_2 concentration of 250 to 500 $\mu l\ l^{-1}$, 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 $\mu l\ l^{-1}$ CO_2. Blowers advect the air, and photosynthesis by the enclosed vegetation progressively depletes the air of CO_2, which exits the chamber at 380 $\mu l\ l^{-1}$. Similarly, on the second linear chamber, the subambient portion of the gradient is created when ambient air is introduced and progressively depleted of CO_2, exiting the chamber at 250 $\mu l\ l^{-1}$.

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. CO_2 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 m^3), 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 CO_2 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 CO_2 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 NH_4NO_3. P was applied as P_2O_5. 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 CO_2, and the selected leaves were recently fully expanded and also of typical vigor. Leaves were measured for net carbon assimilation (A_{CO2}), 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, CO_2 mixer and 85:15 red:blue light source. Leaf chamber illumination was controlled at 1500 $\mu mol\ m^{-2}\ s^{-1}$ photon flux density. Leaf temperature varied between 30 and 35°C, and the leaf cuvette H_2O 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 CO_2 was controlled to values corresponding to the position along the gradient. Instantaneous photosynthetic water use efficiency (WUEt) was computed as $A_{CO2}\ E^{-1}$.

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 CO_2 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):

$$\begin{aligned} y_{ijkl} &= \text{intercept} + \text{soil}_i + \text{monolith}_j(\text{soil}_i) \\ &+ \text{year}_k + \text{soil} \times \text{year}_{ik} + e_{ijkl} \end{aligned} \tag{1}$$

Second, to test for CO_2 effects and their interaction with soil type and year, we fit an expanded model to the plant growth and physiology variables (Equation 2):

$$\begin{aligned} y_{ijkl} = {} & \text{intercept} + \text{soil}_i + \text{monolith}_j(\text{soil}_i) + \alpha(CO_2) \\ & + \beta_i(CO_2 \times \text{soil}_i) + \text{year}_k + \text{soil} \times \text{year}_{ik} \\ & + \gamma_K(CO_2 \times \text{year}_k) + \delta_{ik}(CO_2 \times \text{soil}_i \times \text{year}_k) + e_{ijkl} \end{aligned} \qquad (2)$$

In both models, soil was a fixed effect, monoliths nested within soils [monolith(soil)] a random effect, CO_2 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 CO_2 for the soils separately and combined and for the years separately and combined (Table 2). When the ANCOVA model (equation 2) returned significant CO_2, soil × CO_2, soil × year or soil × CO_2 × year effects, the corresponding regressions were plotted in Figs 3 and 4.

RESULTS

Soil moisture

Θ_{20} 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 CO_2 for both soils combined ($R^2 = 0.04$, $P = 0.005$, Fig. 1). Θ_{20} 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 CO_2, 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 CO_2 concentrations, ANPP, tiller number and LAI were 12–18% higher on the clay soil than on the silty clay ($P \leqslant 0.046$, 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 CO_2 enrichment in the establishment year ($R^2 = 0.21$) but not thereafter (CO_2 × 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 CO_2 enrichment because tiller mass increased with CO_2 ($R^2 = 0.29$, $P = 0.0143$, Fig. 3b). As establishment progressed, tiller numbers decreased with CO_2 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 CO_2 ($P > 0.19$). Clipping intensity increased with CO_2 enrichment in both 2008 and 2009 ($R^2 = 0.66$–0.69, $P < 0.0001$) because clipped biomass increased with CO_2 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

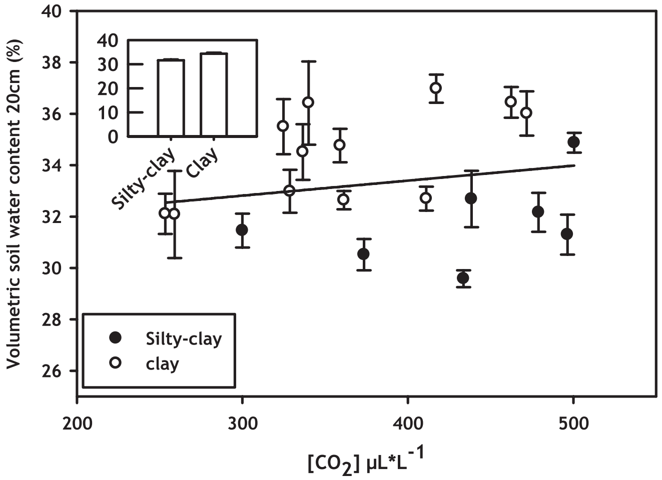

Figure 1: Volumetric soil water content of clay and silty clay soils along the CO_2 gradient, and (inset) mean of all CO_2 concentrations. Each point is the average of 2007–2010 (± standard error). Linear regression is for both soils combined (Table 2).

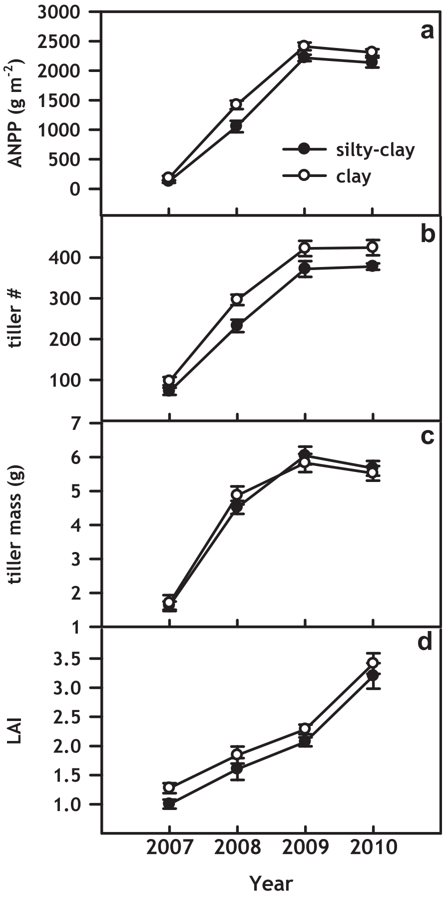

Figure 2: Biomass components on the clay and silty clay soil during the establishment of *Panicum virgatum* in the CO_2 gradient experiment. Each point is the average over all CO_2 concentrations (± standard error). (a) Aboveground net primary productivity (ANPP), (b) number of tillers per monolith, (c) individual tiller mass and (d) Leaf area index (LAI).

Table 1: biomass and physiological variables by soil type, averaged across years and CO_2

	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 CO_2. No significant CO_2, soil × CO_2 or soil × year interactions were found for LAI.

Leaf carbon and water fluxes

ANPP and tiller responses to CO_2 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 CO_2 and year ($P < 0.01$, Table 1). However A_{CO2}, WUEt, 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 WUEt, averaged over soils and CO_2 ($P < 0.0001$, Fig. 4e). Leaf %N decreased from 2.2 ± 0.4% in 2009 to 1.5 ± 0.4% in 2010, resulting in a 50% increase leaf C:N ($P \leqslant 0.0001$).

A_{CO2} showed a weak increase with CO_2 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 CO_2 enrichment, leading to a ~2-fold increase in WUEt (Fig. 4a–d). Ψ_l also increased ~40% with CO_2 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 CO_2 in 2008 ($P = 0.13$–0.25, Fig. 4b and c), but they declined more steeply and were increasingly tightly correlated with CO_2 from 2008 to 2010 (Fig. 4b and c, Table 2). As a result, WUEt became increasingly responsive to CO_2 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 CO_2 responsiveness of a subset of the parameters. A_{CO2} increased with CO_2 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 CO_2 enrichment more steeply and were more strongly correlated with CO_2 on the silty clay soil ($R^2 = 0.64$–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 WUEt and Ψ_l with CO_2.

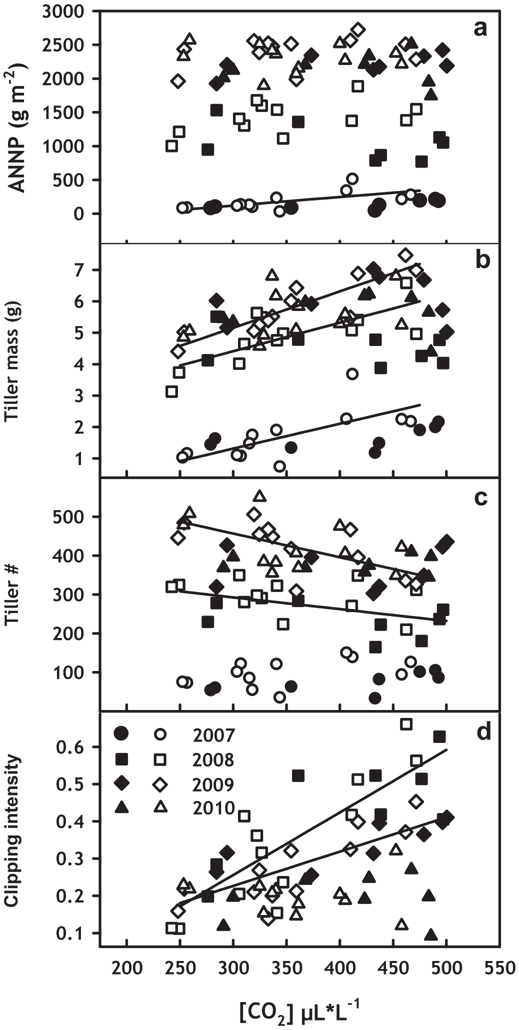

Figure 3: Biomass components of *Panicum virgatum* along the CO_2 gradient on clay (open symbols) and silty clay (closed symbols) soils from the planting year (2007) through full establishment (2010). (a) Aboveground net primary productivity (ANPP), (b) individual tiller mass, (c) number of tillers per 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).

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 CO_2 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 CO_2 concentrations caused offsetting effects on several components

Table 2: regression parameters and statistical tests for the growth and physiological responses to CO_2 in *Panicum virgatum*

	Silty clay					Clay					Both soils				
Year	Intercept	Slope	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

	Silty clay					Clay					Both soils				
Year	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_l (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 CO_2 concentration increased as establishment proceeded.

CO_2 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 CO_2 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 CO_2 (Benson et al. 2004; Dalgleish and Hartnett 2009). There are few studies of tiller biomass responses to CO_2 enrichment in *P. virgatum*. The finding contrasts with a meta-analysis of C_3 and C_4 grasses that found increased tiller mass and density with CO_2 enrichment (Wand et al. 1999). CO_2 enrichment would be expected to increases resource availability for tiller production and growth. Previous meta-analyses found that CO_2 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 CO_2 enrichment during years 2 and 3; however final harvest biomass did not vary with CO_2. This means the early to mid-season growth of *P. virgatum* increased with CO_2 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 CO_2 enrichment 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 CO_2 enrichment (Kirschbaum 2011; Körner 2011), (iii) reduced activation and growth of rhizome buds following leaf removal (Beaty et al. 1978) and (iv) developmental constraints on growth patterns, such as continued stem growth to support later inflorescence production (Körner 2011).

The CO_2 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 C_4 grassland (Schimel et al. 1991; Turner and Knapp 1996). Nutrient effects on allocation are likely stronger than CO_2 effects (Poorter and Nagel 2000).

Physiological coupling to CO_2 increased as *P. virgatum* established

The pattern of decreasing CO_2 effects on the components of ANPP were accompanied by increased CO_2 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 CO_2 found in this study agree with previous findings for this and other C_4 tallgrasses (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 CO_2, providing an indirect

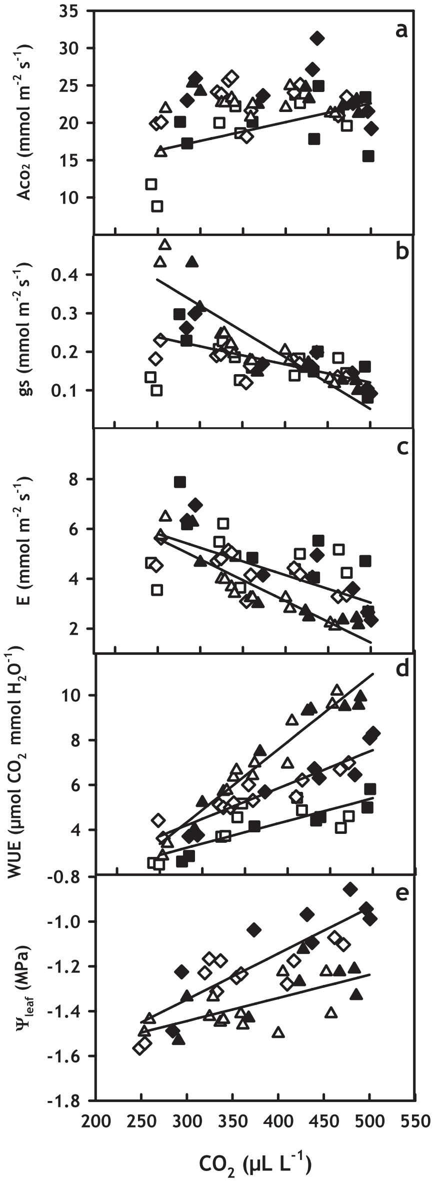

Figure 4: Physiological performance of *Panicum virgatum* along the CO_2 gradient on clay (open symbols) and silty clay (closed symbols) soils from the second year of growth (2008) to full establishment (2010, symbols as in Fig. 3). (a) Leaf net photosynthesis (A_{CO2}), (b) stomatal conductance (g_S) (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 CO_2 enrichment may stimulate ANPP in water-limited, C_4-dominated vegetation (Morgan et al. 2011). Indeed, we found a modest increase in soil moisture with CO_2 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 CO_2 enrichment was accompanied by increased leaf water potential, indicating improved plant water status at elevated CO_2. However, leaf water potential was generally lower and responded less to CO_2, 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 CO_2 enrichment as plants established, increasing the coupling of WUE to CO_2. We found little evidence to suggest photosynthetic down-regulation with CO_2 enrichment as the plants established. Similar rates of A_{CO2} were observed each year at high CO_2.

Soil type effects on CO_2 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 CO_2 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 CO_2 effects predominated in years where they occurred. Also, the CO_2 responses were sometimes inconsistent. For example, on the clay soil, stronger tiller mass and clipped mass increases with CO_2 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-CO_2 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 CO_2 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 CO_2 enrichment. CO_2 enrichment affected canopy architecture and tiller growth while plants were establishing, likely because of clipping induced reallocation of resources that varied with CO_2. Soil type effects on ANPP-CO_2 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*.

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