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

Philip A. Fay¹,*, 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. 1996). 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 re-found and on which potential for bioenergy production (Perlack et al. 2005). Monocultures of perennial C₄ grasses were not predictable from CO₂ and resource availability (Smith et al. 2009).

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. Panicles are terminally flattened, with a dark straw-colored spike of flower heads. The fruiting structures are dry, woody seeds, and the mature seed head is removed by the next growing season to minimize the effects of photodegradation on light transmittance.

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 facility 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. The other chamber contains the subambient portion of the gradient, created by introducing air enriched to 250 μl l⁻¹ CO₂. 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 subambient 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 and 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, Udorthent Haplustoll) and a moist high organic matter heavy clay found downslope from the silty clay (n = 12, Houston Black series, Vertisol, Udic Hapludert). These soils were representative of their respective landscape positions in the Black-Land 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 weekly during each growing season to replace evapotranspiration capacity at the start of each growing season and irrigated individually drip irrigated. We irrigated each monolith to field capacity at the start of each growing season and irrigated individually drip irrigated.

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 (Aₜ), stomatal conductance (gₛ), and transpiration (E) with an infrared gas analyzer (LI-6400 LI-Cor 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 μmol m⁻² 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 Aₜ E⁻¹.

Leaves were collected immediately following the gas exchange measurements and measured for leaf water potential (Ψᵝ) 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 Ψᵝ 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):

\[ y_{ijkl} = \text{intercept} + \text{soil}_i + \text{monolith}_j(\text{soil}_i) + \text{year}_k + \text{soil} \times \text{year}_k + e_{ijkl} \]  

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):
\[ y_{ijkl} = \text{intercept} + \text{soil}_i + \text{monolith}_j \text{soil}_i + \delta(CO_2) + \lambda_k(CO_2 \times \text{soil}_i) + \phi_{ijkl} \]  

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 \( \times \) CO2, soil \( \times \) year or soil \( \times \) CO2 \( \times \) year effects, the corresponding regressions were plotted in Figs 3 and 4.

RESULTS

Soil moisture

\( \Theta_{20} \) 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 CO2 for both soils combined (\( R^2 = 0.04, P = 0.005 \), Fig. 1). \( \Theta_{20} \) 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 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 (\( y < 0.0001 \), 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 number and LAI were 12–18% higher on the clay soil than on the silty clay (\( \leq 0.046 \), 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 \( \times \) 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 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).

Figure 2: Biomass components on the clay and silty clay soil during the establishment of \( P. \) 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).
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. gs 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 ACO2, WUEt, Ψ1, and leaf C:N did not differ between the soils (P > 0.09). ACO2 and gs, increased from 2008 to 2010 while E decreased, leading to a 73% increase in WUEt, averaged over soils and CO2 (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 < 0.0001).

ACO2 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 WUEt (Fig. 4a–d). Ψ1 also increased ~40% with CO2 enrichment (Fig. 4c). Furthermore, as *P. virgatum* established, Ψ1 decreased (Fig. 4c), 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, WUEt 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. ACO2 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 = 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 Ψ1 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 1: biomass and physiological variables by soil type, averaged across years and CO2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Silty clay (SE)</th>
<th>Clay (SE)</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWC (%vol)</td>
<td>35.75 (0.36)</td>
<td>35.17 (0.36)</td>
<td>2.8</td>
<td>0.0991</td>
</tr>
<tr>
<td>ANPP (g m⁻²)</td>
<td>1383.03 (157.97)</td>
<td>1380.28 (133.66)</td>
<td>6.3</td>
<td>0.0180</td>
</tr>
<tr>
<td>Tiller # (m⁻²)</td>
<td>263.47 (23.35)</td>
<td>309.77 (20.86)</td>
<td>12.9</td>
<td>0.0006</td>
</tr>
<tr>
<td>Tiller mass (g)</td>
<td>4.46 (0.33)</td>
<td>4.48 (0.27)</td>
<td>1.3</td>
<td>0.2724</td>
</tr>
<tr>
<td>LAI (m² m⁻²)</td>
<td>1.97 (0.16)</td>
<td>2.20 (0.13)</td>
<td>4.6</td>
<td>0.0465</td>
</tr>
<tr>
<td>ACO2 (μmol m⁻² s⁻¹)</td>
<td>22.62 (0.71)</td>
<td>21.38 (0.61)</td>
<td>1.8</td>
<td>0.1834</td>
</tr>
<tr>
<td>g_s (mol m⁻² s⁻¹)</td>
<td>0.19 (0.02)</td>
<td>0.19 (0.01)</td>
<td>0.1</td>
<td>0.8180</td>
</tr>
<tr>
<td>E (mmol m⁻² s⁻¹)</td>
<td>4.21 (0.35)</td>
<td>4.23 (0.18)</td>
<td>0.0</td>
<td>0.9986</td>
</tr>
<tr>
<td>WUE (ACO2 E⁻¹)</td>
<td>6.21 (0.49)</td>
<td>5.46 (0.31)</td>
<td>1.4</td>
<td>0.2513</td>
</tr>
<tr>
<td>Ψ1 (MPa)</td>
<td>-1.19 (0.05)</td>
<td>-1.33 (0.03)</td>
<td>3.3</td>
<td>0.0875</td>
</tr>
<tr>
<td>Leaf %C</td>
<td>45.89 (0.12)</td>
<td>45.30 (0.09)</td>
<td>0.7</td>
<td>0.4070</td>
</tr>
<tr>
<td>Leaf %N</td>
<td>1.86 (0.09)</td>
<td>1.79 (0.08)</td>
<td>1.0</td>
<td>0.3338</td>
</tr>
<tr>
<td>Leaf C:N Ratio</td>
<td>25.64 (1.27)</td>
<td>26.78 (1.25)</td>
<td>0.6</td>
<td>0.4653</td>
</tr>
</tbody>
</table>
### Table 2: regression parameters and statistical tests for the growth and physiological responses to CO2 in *Panicum virgatum*

<table>
<thead>
<tr>
<th>Year</th>
<th>ANPP (g m⁻²)</th>
<th>Intercept</th>
<th>Slope</th>
<th>R²</th>
<th>F</th>
<th>P</th>
<th>Intercept</th>
<th>Slope</th>
<th>R²</th>
<th>F</th>
<th>P</th>
<th>Intercept</th>
<th>Slope</th>
<th>R²</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Silty clay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Clay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Both soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All years</td>
<td>0.54</td>
<td>0.001</td>
<td>0.04</td>
<td>0.03</td>
<td>0.0542</td>
<td>1092.7</td>
<td>0.0303</td>
<td>0.02</td>
<td>0.77</td>
<td>33.6</td>
<td>0.0002</td>
<td>0.002</td>
<td>0.972</td>
<td>0.0283</td>
<td>0.665</td>
<td>1518.7</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.001</td>
<td>0.04</td>
<td>0.03</td>
<td>0.0542</td>
<td>1092.7</td>
<td>0.0303</td>
<td>0.02</td>
<td>0.77</td>
<td>33.6</td>
<td>0.0002</td>
<td>0.002</td>
<td>0.972</td>
<td>0.0283</td>
<td>0.665</td>
<td>1518.7</td>
</tr>
</tbody>
</table>

### Abiotic parameters

#### ACO2 (µmol m⁻² s⁻¹)

<table>
<thead>
<tr>
<th>Year</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>All years</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACO2</td>
<td>17.5</td>
<td>28.8</td>
<td>28.2</td>
<td>25.3</td>
</tr>
</tbody>
</table>

#### gs (mol m⁻² s⁻¹)

<table>
<thead>
<tr>
<th>Year</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>All years</th>
</tr>
</thead>
<tbody>
<tr>
<td>gs</td>
<td>0.42</td>
<td>0.50</td>
<td>0.73</td>
<td>0.54</td>
</tr>
</tbody>
</table>

#### E (mmol m⁻² s⁻¹)

<table>
<thead>
<tr>
<th>Year</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>All years</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
</tr>
</tbody>
</table>
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; Dalgleish 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 tiller 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 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 CO₂ 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 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 photosynthetic 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

<table>
<thead>
<tr>
<th>Year</th>
<th>Intercept</th>
<th>Slope</th>
<th>R²</th>
<th>F</th>
<th>P</th>
<th>Intercept</th>
<th>Slope</th>
<th>R²</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>11.6</td>
<td>−0.018</td>
<td>0.86</td>
<td>36.8</td>
<td>0.0009</td>
<td>7.2</td>
<td>−0.008</td>
<td>0.50</td>
<td>10.1</td>
<td>0.0098</td>
</tr>
<tr>
<td>2010</td>
<td>10.1</td>
<td>−0.017</td>
<td>0.82</td>
<td>27.5</td>
<td>0.0019</td>
<td>10.3</td>
<td>−0.019</td>
<td>0.88</td>
<td>72.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>All years</td>
<td>10.9</td>
<td>−0.017</td>
<td>0.64</td>
<td>36.7</td>
<td>&lt;0.0001</td>
<td>7.0</td>
<td>−0.008</td>
<td>0.25</td>
<td>10.7</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

Table 2: Continued
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 Aₐ 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. Texture is a major reason for soil type differences in _P. virgatum_ biomass (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...
reallocated resources of those 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**


