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Physiological and growth responses of switchgrass (*Panicum virgatum* L.) in native stands under passive air temperature manipulation

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Abstract

In the central Great Plains of North America, climate change predictions include increases in mean annual temperature of 1.5–5.5 °C by 2100. Ecosystem responses to increased temperatures are likely to be regulated by dominant plant species, such as the potential biofuel species *Panicum virgatum* (switchgrass) in the tallgrass prairie. To describe the potential physiological and whole-plant responses of this species to future changes in air temperatures, we used louvered open-sided chambers (louvered OSC; 1 × 1 m, adjustable height) to passively alter canopy temperature in native stands of *P. virgatum* growing in tallgrass prairie at varying topographic positions (upland/lowland). The altered temperature treatment decreased daily mean temperatures by 1 °C and maximum temperatures by 4 °C in May and June, lowered daytime stomatal conductance and transpiration, decreased tiller density, increased specific leaf area, and delayed flowering. Among topographic contrasts, aboveground biomass, flowering tiller density, and tiller weight were greater in lowland sites compared to upland sites, with no temperature treatment interactions. Differences in biomass production responded more to topography than the altered temperature treatment, as soil water status varied considerably between topographic positions. These results indicate that while water availability as a function of topography was a strong driver of plant biomass, many leaf-level physiological processes were responsive to the small decreases in daily mean and maximum temperature, irrespective of landscape position. The varying responses of leaf-level gas exchange and whole-plant growth of *P. virgatum* in native stands to altered air temperature or topographic position illustrate that accurately forecasting yields for *P. virgatum* in mixed communities will require greater integration of physiological responses to simulated climate change (increased temperature) and resource availability over natural environmental gradients (soil moisture).

Keywords: aboveground biomass, biofuel, climate change, flowering phenology, gas exchange, grassland, tallgrass prairie

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Introduction

The effects of anthropogenic climate change are creating novel environments for plants, with mean annual air temperatures for the Great Plains projected to increase by 1.5–5.5 °C by 2100 (Houghton, 2001; Parmesan, 2006; Christensen *et al*., 2007; Williams & Jackson, 2007). Although a high degree of variability exists between model predictions, most models agree air temperatures will increase by 2 °C (Christensen *et al*., 2007). Increased mean annual air temperatures are likely to reduce the number of cold days, increase the frequency of hot days, and interact with precipitation changes to shift optimal zones for agricultural and increase the sensitivity of natural ecosystems to pests and invasive species (Karl *et al*., 2009). Physiological adaptations to these changes in climate can drive growth and productivity (Morgan *et al*., 2004), with dominant species influencing the community structure, dynamics, invasibility, and overall ecosystem function (Grime, 1998; Smith & Knapp, 2003; Emery & Gross, 2007). Grassland species responses to altered environments vary based on species-specific responses, including differential sensitivity to changes in temperature or water availability (Sherry *et al*., 2008; Nippert *et al*., 2009). Thus, future productivity of dominant grassland species identified for biofuel cultivation (i.e., *Panicum virgatum*) will reflect leaf-level and whole-plant responses to specific environmental drivers as well as the magnitude of the environmental change (De Valpine & Harte, 2001; Nippert *et al*., 2009; Fay *et al*., 2011).

P. virgatum is a common perennial C_4 grass species adapted to a broad range of environmental conditions (temperature and precipitation) across North America (Parrish & Fike, 2005). In the past, *P. virgatum* was valued

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for use as forage, but in the last 20 years research interest has focused on examining the suitability of the species as a biofuel (McLaughlin & Kszos, 2005; Wright & Turhollow, 2010). *P. virgatum* exhibits large genetic variability and diversity in form (Hartnett, 1993; Casler *et al*., 2004; Das *et al*., 2004). These traits facilitate its suitability for cultivation in a wide range of environmental conditions and geographic ranges across North America (Parrish & Fike, 2005), although this range will likely increase and shift north under future climate change conditions (Barney & DiTomaso, 2010).

Historically, the responses of *P. virgatum* to environmental variability and their agronomic impact have been studied extensively using cultivars developed in breeding programs (Hartman *et al*., 2011) using either pot experiments (Suplick *et al*., 2002; Barney *et al*., 2009) or monocultures (Boe, 2007; Dohleman *et al*., 2009; Hickman *et al*., 2010; Wang *et al*., 2010), with considerably less research on natural populations in a mixed vegetation communities (except see: Knapp, 1985; Vinton & Hartnett, 1992). Because *P. virgatum* is a dominant species in the mesic tallgrass prairie, additional research on native populations will provide greater information on potential population responses and the potential implications of altered climate change conditions for this ecosystem and cultivars grown in mixed assemblages for biofuel production. Although the local responses of *P. virgatum* to resource limitation have been examined for many natural populations (Knapp, 1985; Hartnett, 1993; Hartnett *et al*., 1994; Myster, 2006), few studies have examined the response of native populations to simulated climate changes forecast for the central Great Plains (except see: Hartman *et al*., 2012).

We conducted a field experiment to characterize which physiological and whole-plant traits from native *P. virgatum* populations responded to subtle alterations in air temperature when grown in native prairie. To examine how soil moisture availability impacted responses to air temperature manipulation, plots were established at both upland and lowland locations, as soil moisture in surface soil layers is consistently lower in upland compared to lowland topographic positions (Nippert *et al*., 2011). We hypothesized that warmer canopy temperatures would alter gas exchange responses in *P. virgatum*, with increases in carbon assimilation rates (Zhou *et al*., 2007; Niu *et al*., 2008). In addition, we hypothesized that physiological responses would vary between individuals in upland sites compared to lowland sites, where individuals in lowland areas would be better buffered than upland areas to warmer temperatures. Finally, we hypothesized that the physiological response to warmer temperature would be reflected in whole-plant growth responses.

Materials and methods

Study site and treatments

This research was conducted from May-September, 2010 at the Konza Prairie Biological Station (KPBS). KPBS is native tallgrass prairie site (3487 ha) located in northeastern Kansas, USA (39.1°N, 96.9°W). The region is characterized by a mid-continental climate with cool, dry winters and warm, wet summers. Long-term annual precipitation (1983–2010) at KPBS is 820 mm, with ~75% of the rainfall occurring during the growing season (April–September). January is the coldest month (mean daily air temperature is −1.2 °C), whereas July is the warmest (25.9 °C). Ten locations were chosen across two ungrazed watersheds at KPBS that have been burned annually in the spring for the past 30 years. Watershed K1B included two upland and two lowland locations, whereas watershed 1D contained three upland and three lowland locations. Within each location, paired plots for the warming treatment and control were selected in close proximity (within 2 m), resulting in twenty 1 m^2 plots evenly distributed among upland and lowland locations. Plot locations were selected in areas where switchgrass was the predominant plant canopy (>90%), however, other herbaceous species were present in each plot.

Passive temperature manipulation was conducted using a louvered open-sided chamber (louvered OSC; Fig. 1a) developed by M. Germino, Idaho State University (personal communication). The chamber frame was 1 × 1 m in area and contained 10.15 cm × 1 m wide slats situated at 45° angles.

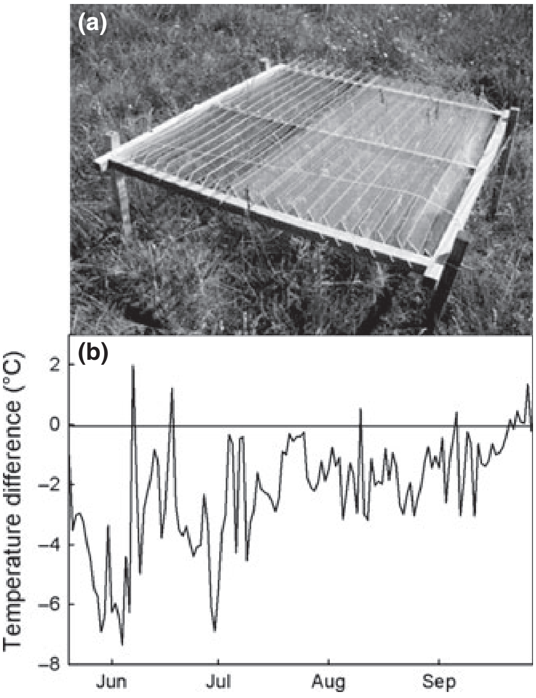

Fig. 1 (a) A louvered open-sided chamber (louvered OSC) as developed by Matt Germino (personal communication) and (b) differences in average daily maximum temperature between the control (line at 0 °C) and altered temperature treatment. The shelter did not reduce/alter precipitation received.

Slats were cut from OPTIX 91.44 × 121.92 × 0.24 cm Acrylic sheets, angled in opposite directions and meeting in the middle. Frame height was adjustable, allowing the frame top to remain ~20 cm above the herbaceous canopy at all times during the growing season. The acrylic slats reduce incoming PAR by ~8% and reduced UV light (250–400 nm) by 80%. Compared to most closed-frame structures, this open-frame design allows for more air movement and mixing. Passive chambers were suitable for temperature manipulation in this experiment because results are optimized in the absence of a tree canopy and under clear sky, and active heating treatments (IR lamps) were not possible given the location of the experiment in native grassland with topographic contrasts.

Sampling procedure & variables measured

Air temperatures above the soil surface were continuously recorded every 10 min from the middle of May 2010, through the middle of October 2010 using DS1922L Thermochron iButtons (Embedded Data Systems, LLC, Lawrenceburg, KY, USA) with a temperature range of −10 °C to 65 °C (±0.5 °C). These temperatures were used for comparison of topographic and treatment differences over the course of the growing season.

Physiological sampling was conducted over six dates during the growing season (06/01, 06/18, 06/25, 07/16, 08/13, 09/12). For each sampling date, one *P. virgatum* individual was randomly selected from each plot and gas exchange, dark-adapted chlorophyll fluorescence (F_v/F_m), and mid-day leaf water potentials (Ψ_{mid}) were measured. These physiological measurements were conducted on the newest, fully expanded mature leaf, on the widest portion of the grass blade. For each sampling period, individuals were tagged to ensure that gas exchange and F_v/F_m were conducted on the same individual leaf, and midday water potentials (Ψ_{mid}) were conducted on the same individuals, using different leaf blades from the same individual. This tagging helped to minimize within-population variability. Since physiological measurements vary based on the time of day, measurement order for each plot was randomized for each sampling period.

Gas exchange measurements were conducted using a LI-COR 6400 IRGA with an artificial red/blue LED light source (6400-02B, LI-COR Inc., Lincoln, NE, USA). Measurements were conducted between 9:00–16:00 CST when solar radiation was at least 70% of full-sun levels (~2000 μmol m^{-2} s^{-1}). Plants were placed inside the leaf chamber and allowed to reach steady-state photosynthesis at ambient C_a (400 μmol mol^{-1}) and at a saturating light intensity (2000 μmol m^{-2} s^{-1}). Leaf temperature was allowed to vary with ambient daily air temperature. Relative humidity in the cuvette was adjusted to reflect ambient environmental conditions (generally ranging from 30% to 50%). The variables measured included CO_2 assimilation at ambient C_a (A_{max}), stomatal conductance to water vapor (g_s), leaf-level transpiration (E), and instantaneous water use efficiency (*WUE*: A_{max}/E). Mid-day water potential (Ψ_{mid}) was measured concurrently with gas exchange and was done using a Scholander-type pressure bomb (PMS Instruments, Albany, OR, USA). Dark-adapted maximum photochemical efficiency (F_v/F_m) was conducted using a MINI-PAM photosynthesis yield analyzer (Heinz Walz GmbH, Effeltrich, Germany). F_v/F_m measurements were recorded after individuals were allowed to adapt for a minimum of 1 h following complete darkness. Gas exchange measurements were also conducted during the night on two separate occasions to assess the treatment effect on nighttime respiration (R_d), transpiration (E), stomatal conductance (g_s), and water use efficiency (*WUE*).

Soil moisture measurements were performed concurrently with gas exchange and water potential measurements. Soil moisture (0–10 cm) was measured using a Hydra Probe II Soil Sensor (Stevens Water Monitoring Systems, Inc., Portland, OR, USA) in units of water fraction by volume (wfv). The Hydra Probe II Soil Sensor is a digital probe that comes with soil calibrations for sand, silt, clay, and loam, and the calibration setting was adjusted to reflect the silty clay loams on Konza Prairie. Soil moisture was measured in each plot 10 times over the growing season (05/24, 06/02, 06/18, 06/25, 07/06, 07/19, 07/26, 08/05, 08/13, 09/12).

Specific leaf area (SLA) was measured in late August before senescence, and aboveground biomass was harvested at the conclusion of the growing season (September/October). A 20 × 50 cm clipping frame was used to harvest two replicates randomly located within each plot. Aboveground biomass was dried at 65 °C for at least 48 h and weighed for each replicate. Each plot was monitored for initiation of *P. virgatum* flowering on six dates (07/26, 08/05, 08/10, 08/27, 09/12, 09/26), starting in the middle of the growing season. As a measure of fitness, the number of reproductive (flowering) tillers was counted.

Statistical analyses

Data were analyzed as a split-plot repeated measures design using a mixed effects model ANOVA (Proc Mixed, SAS v9.2, SAS Institute Inc., Cary, NC, USA) blocking by watershed, with topography assigned to the whole plot and treatment to the split plot. Multiple comparison tests between treatments, topographic position, and date were performed using Tukey's HSD.

Temperature differences were analyzed using the same split-plot repeated measures design using the daily minimum, maximum, and average temperatures for each plot. Multiple comparison tests between treatment, topographic position, and month were performed using Tukey's HSD.

Results

Soil moisture was highly variable (0.45–0.13 ± 0.02 wfv) and increased during May-June, declining in July through the rest of the growing season. As expected, soil moisture was significantly different ($P = 0.0116$) between topographic sites, where lowland sites (0.28 ± 0.02 wfv) had ~12% more wfv than upland sites (0.25 ± 0.02 wfv) across the entire summer. No differences in soil moisture ($P = 0.7204$) were detected between the control and altered temperature treatment.

Measurements using the Thermochron iButtons showed significant increases in daily minimum temperature that correspond with increased ambient air temperature

Table 1 Mixed-effects model ANOVA of altered temperature treatments and topographic position (fixed- treatment, topography, time; random- watershed) effects on physiological responses, soil water content, flowering times, and temperatures. For all physiological variables, flowering, and SWC, time is the specific sampling date. For temperatures, time was month, with daily max, min, and average temperatures averaged for the month. *F*- and *P*-values are provided with significant responses ($P < 0.05$) indicated in bold font, and marginally significant responses ($P < 0.10$) indicated by italics

Response variable	Treatment		Topography		Time		Time × treatment		Time × topography		Treatment × topography		Treatment × topography × time	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
A_{max}	3.03	0.1071	0.65	0.4454	**39.89**	**<0.0001**	**2.32**	**0.0452**	1.66	0.2262	0.04	0.8412	0.87	0.5017
g_s	**5.02**	**0.0445**	2.47	0.1581	**32.08**	**<0.0001**	0.9	0.4827	1	0.4602	0.06	0.8054	0.96	0.4454
E	**10.5**	**0.0071**	*3.93*	*0.0768*	**25.31**	**<0.0001**	0.71	0.6197	1.42	0.2914	0.18	0.6794	0.95	0.4475
WUE	2.21	0.1638	**6.12**	**0.0316**	**6.38**	**0.0053**	**2.85**	**0.0167**	*2.51*	*0.0957*	0.19	0.6749	1.04	0.3965
Ψ_{mid}	0.33	0.5718	0.29	0.6001	**69.55**	**<0.0001**	0.96	0.4455	1.47	0.2744	2.81	0.1144	1.16	0.3274
F_v/F_m	**9.74**	**0.0068**	1.44	0.2798	*4.92*	*0.0646*	**5.37**	**0.006**	0.96	0.4429	**4.81**	**0.0439**	0.6	0.5522
SWC	0.63	0.4399	**6.98**	**0.0166**	**111.65**	**<0.0001**	*1.78*	*0.0791*	0.23	0.9856	0.13	0.7204	1.1	0.3668
Avg T	0.11	0.7414	*3.19*	*0.0937*	**165.9**	**<0.0001**	**2.3**	**0.0426**	0.2	0.956	0.63	0.4412	0.28	0.9221
Max T	**5.03**	**0.0452**	2.09	0.163	**31.32**	**<0.0001**	**8.25**	**<.0001**	0.37	0.8587	0.41	0.535	0.48	0.7914
Min T	0.82	0.375	0.36	0.5569	**920.1**	**<0.0001**	0.43	0.8295	1.08	0.3712	0.1	0.7552	0.38	0.8606
Flowering	*3.13*	*0.0903*	**0.7**	**<0.0001**	**23.26**	**<0.0001**	2.09	0.103	2.12	0.1	1.13	0.2997	1.83	0.1474

over the growing season (Table 1), but no significant differences in daily minimum temperatures between treatments ($P = 0.3750$) or topographic positions ($P = 0.5569$). There was a significant cooling effect from the frames, with an average decrease in the daily maximum temperature of 1.9 ± 0.8 °C (Table 1; Fig. 1b). Daily maximum temperatures ranged from 41 °C in the early summer, to 24 °C in October 2010. The largest differences between treatments occurred in the late spring and early summer (May–June) where mean daily maximum temperatures were on average 4 °C higher in the control plots (Fig. 2a). Significant interactions between the treatments and month were recorded in the mean daily temperatures, with a marginally significant topographic effect where upland plots were warmer by an average of 0.5 °C (Table 1). Mean daily temperatures generally increased from ~23 °C in the beginning of the growing season (May) to ~26 °C in mid-August, and then declined rapidly to ~16 °C at the end of the growing season in October (Fig. 2b).

Altered temperature responses

All physiological variables measured varied significantly over time, with interactions between treatment and time for $A_{\max}$, *WUE*, and F_v/F_m (Table 1). $A_{\max}$ decreased over the course of the growing season, ranging from 30 to 10 μmol CO_2 m^{-2} s^{-1}. Marginally significant differences between the control and altered temperature plots were recorded at the end of the season when carbon assimilation in control plots was twice that observed in the altered temperature plots ($P = 0.0603$; Fig. 3a). Water-use efficiency showed similar trends as carbon assimilation, with significant treatment interactions with date (Table 1). Decreases in *WUE* were recorded over the growing season with an average of 4.71 ± 0.21 μmol $mmol^{-1}$ at the beginning of June, decreasing to 3.16 ± 0.21 μmol $mmol^{-1}$ at the end of September. Control plots showed increased *WUE* compared to the altered temperature plots in September (Fig. 3b), and in general. Changes in dark-adapted chlorophyll fluorescence showed significant interactions between treatment and topography, and treatment responses over time (Table 1). F_v/F_m was significantly higher for altered temperature plots (0.78 ± 0.008) compared to the control plots (0.77 ± 0.008) in the upland sites, but there were no differences between treatments in the lowland sites. F_v/F_m was similar between treatments at the beginning of the growing season, but control plots displayed significantly higher F_v/F_m values at the end of the season compared to the altered temperature treatment (Fig. 3c).

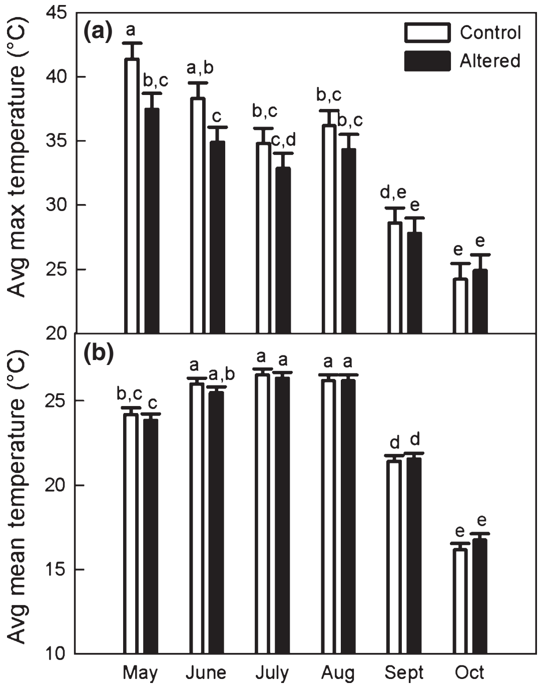

Fig. 2 Average daily (a) maximum and (b) mean temperatures for control and altered temperature treatments for each month. Each bar is the overall mean response (± 1 SE) with letters indicating significant differences ($P < 0.05$) between groups.

E declined over the growing season, similar to other gas exchange responses, with the highest rates of 7.40 ± 0.67 mmol m^{-2} s^{-1} during the beginning of June, and the lowest rates of 2.62 ± 0.67 mmol m^{-2} s^{-1} were in September. Significant differences in E between treatments were observed, with 15% higher E in control plots. Similar to the E responses, g_s declined significantly over the growing season, with significant differences between treatment responses (Table 1). Leaf-level stomatal conductance ranged from 0.1860 ± 0.0092 mol H_20 m^{-2} s^{-1} in June, to 0.06547 ± 0.0092 mol H_2O m^{-2} s^{-1} in September. Individuals in control plots displayed significantly higher g_s values compared to altered temperature plots (Fig. 4d).

Leaf-level water potential decreased significantly over the course of the summer, and there were no overall significant treatment or topographic differences (Table 1). Across all treatments and topography, Ψ_{mid} averaged −0.6 ± 0.1 MPa at the beginning of the growing season (May–June), and ended the season with Ψ_{mid} averaging −2.7 ± 0.1 MPa (September).

Few treatment effects for aboveground biomass responses were present, where only specific leaf area (SLA) and tiller density showed significant differences (Table 2). SLA was 18% higher in altered temperature plots than in control plots (Fig. 4b). There were also significant differences (Table 2) in the number of tillers per square meter between the control and altered temperature plots, with control plots containing on average 100 ± 36 more tillers per square meter compared to plots with the altered temperature treatment.

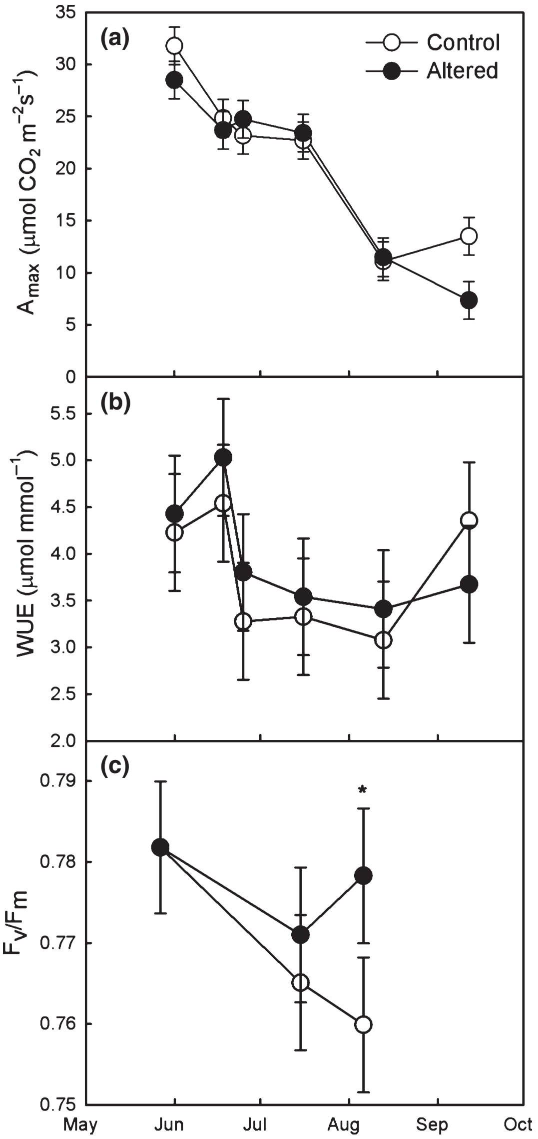

Fig. 3 Physiological responses of (a) carbon assimilation between treatments (b) water use efficiency between treatments, and (c) dark-adapted fluorescence between treatments during the 2010 growing season. Each point is the overall mean response (± 1 SE) for the sample date. Asterisks denote significant ($P < 0.05$) differences between groups within the sampling date.

Topographic responses

Aboveground biomass responses mainly differed by topographic position, with few treatment differences, and no interactions between the altered temperature treatment and topographic position (Table 2). Significant topographic differences were present in the aboveground biomass, total density of tillers, density of flowering tillers, and tiller weight (Table 2). The lowland plots had a mean of 301 ± 36 tillers m^{-2}; almost double that of upland plots, which had a mean of 179 ± 36 tillers m^{-2} (Fig. 4a). Upland plots had a mean aboveground biomass of 259.38 ± 81.06 g m^{-2}, whereas lowland plots had more than double, with a mean biomass of 558.95 ± 81.06 g m^{-2} (Fig. 4c). Lowland plots also had significantly more flowering tillers (149 ± 35 m^{-2}) than the upland plots (73 ± 35 m^{-2}), and significantly higher weight per tiller (1.93 ± 0.13) compared to the upland plots (1.33 ± 0.13). Specific leaf area was marginally significant between topographic positions (Table 2), where SLA was higher in lowland plots (74.09 ± 2.35 cm^2 g^{-1}) compared to upland plots (68.17 ± 2.35 cm^2 g^{-1}). Upland plots had significantly higher *WUE* (4.13 ± 0.58 μmol $mmol^{-1}$) than lowland plots (3.64 ± 0.58 μmol $mmol^{-1}$).

The percentage of plots with flowering individuals increased significantly over time, and lowland plots flowered more quickly than upland plots (Table 1). The lowland plots flowered earlier than upland plots, with 100% of the control plots in the lowlands flowering before any other treatment × topography combination (Fig. 5). Upland plots showed a delay in flowering compared to the lowlands plots. At the end of the growing season, the upland × altered temperature combination was the only treatment × topography factor that did not have 100% flowering occur across the plots (Fig. 5).

Discussion

In this study, we recorded changes in physiological and whole-plant responses to subtle changes in daily mean and maximum temperatures. The greatest differences in temperature between treatments occurred in May and June, a critical period of maximum biomass production in C_4 grasses (Knapp *et al.*, 1998; Nippert *et al.*, 2011). These *in situ* physiological and whole-plant responses by this potential biofuel species show sensitivity to small temperature manipulations, emphasizing the potential for even larger responses to greater temperature changes forecast for this region by 2100.

Flowering rates were not significantly affected by the decreased mean daily and maximum temperatures, and varied only between topographic positions. Previous studies have shown that increased night time temperature of 2 °C had a significant accelerating effect on the flowering of grasses, but this response was highly variable between years suggesting that the effects of elevated temperatures may be ecosystem-specific (Hovenden *et al.*, 2008). Grassland research from North America has shown that species that flower after peak summer temperatures delay flowering even more in response to warming, and species that flower before peak summer temperatures accelerate flowering in response to warming (Cleland *et al.*, 2006). Sherry *et al.* (2007)

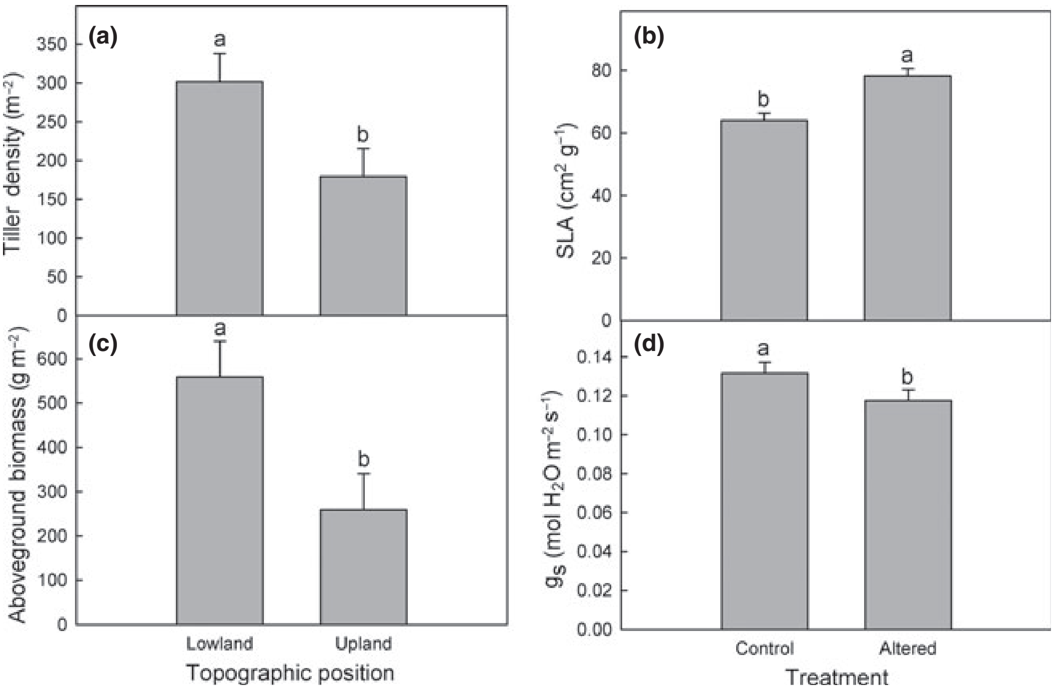

Fig. 4 (a) Tiller density and (c) aboveground biomass for topographic position. (b) specific leaf area, and (d) leaf level stomatal conductance between the control and altered temperature treatment. Each bar is the overall mean response (± 1 SE) with letters indication significant ($P < 0.05$) differences between groups.

Table 2 Mixed-effects model ANOVA of warming treatments and topographic position (fixed- treatment, topography; random- watershed) effects on aboveground biomass responses. *F*- and *P*-values are provided with significant responses ($P < 0.05$) indicated in bold font, and marginally significant responses ($P < 0.10$) indicated by italics

Response variable	Treatment		Topography		Treatment × topography	
	F	*P*	*F*	*P*	*F*	*P*
SLA (g cm^{-2})	**18.29**	**<0.0001**	*3.16*	*0.0808*	0.01	0.9112
Tillers (m^{-2})	**6.39**	**0.0166**	**9.69**	**0.0039**	0.52	0.4742
Flowering tillers (m^{-2})	1.4	0.2441	**7.01**	**0.0121**	0.00	0.9724
Aboveground biomass (g m^{-2})	1.72	0.198	**13.22**	**0.0009**	1.03	0.3169
Tiller weight (g tiller^{-1})	1.07	0.3087	**12.12**	**0.0015**	0.06	0.8132

reported accelerated flowering (17 days) by *P. virgatum* in response to a 4 °C increase in air temperature. In this study, reproductive duration increased for *P. virgatum* individuals in the upland × altered temperature plots, as they were still flowering late in the growing season (57 days after lowland × control plots initiated flowering). The warmer control plots flowered earlier and more quickly than the cooler, altered temperature plots. Our results support the conclusions from Sherry *et al.* (2007), where air temperature can influence the flowering time and duration. Thus, it seems likely a variety of potential phenological responses are possible depending on the magnitude of environmental change and the geographic location studied (Wolkovich *et al.*, 2012).

High air temperatures could also exacerbate plant stress by increasing water deficits (Xu *et al.*, 2009). Sherry *et al.* (2008) showed that even in plots with increased precipitation, increased warming decreased soil water availability enough to produce differences in plant productivity. Furthermore, warming of ~4 °C has been shown to decrease soil moisture throughout the soil profile, even with increased precipitation (Bell *et al.*,

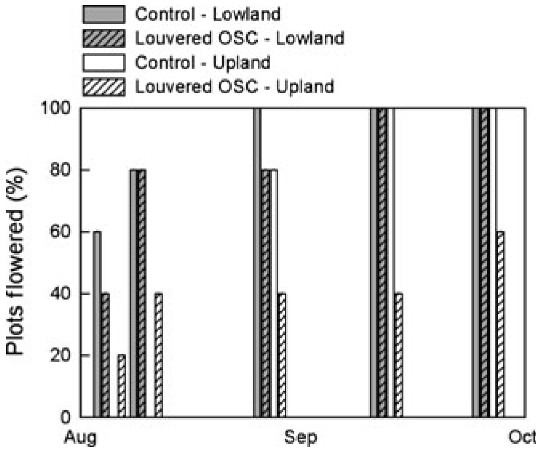

Fig. 5 The percentage of plots flowered for each sampling date during the 2010 growing season. Bars for the Control-Upland plots are absent in the first two sampling periods in August, as there were no plots with flowering individuals.

2010). During 2010, significant differences in water availability existed between the upland and the lowland sites, with upland sites having lower soil moisture (Table 1). Because high air temperatures commonly exacerbate water deficits, individuals in upland sites may be subjected to greater stresses than individuals growing in lowland sites.

Many of the differences seen in this study were between topographic positions. The altered temperature treatment did not alter the biomass production or flowering tiller density, which has been recorded for other grass species under subtle temperature increases of ~1 °C (Zavelata *et al.*, 2003). The genotype of the *P. virgatum* individuals across the topographic sites is not known. However, lowland ecotypes of this species tend to grow in the mesic sites, and the upland types in the more arid sites (Porter, 1966; Parrish & Fike, 2005). Lowland *P. virgatum* types have been shown to produce greater biomass than upland types (Lemus *et al.*, 2002; Alexopoulou *et al.*, 2008; Wullschleger *et al.*, 2010), and more tillers m^{-2} (Madakadze *et al.*, 1998). The lowland sites generally have greater soil water availability, which is an important driver of biomass production, tiller density, and tiller weight (Knapp, 1984; Fay *et al.*, 2003, 2008; Zhou *et al.*, 2009). Results between topographic positions for aboveground biomass production, tiller weight, and flowering tiller density reported here are consistent with other studies performed at this site (Briggs & Knapp, 1995; Nippert *et al.*, 2011) and correspond well with known responses of lowland and upland cultivars (Alexopoulou *et al.*, 2008; Barney *et al.*, 2009).

SLA responded significantly to the altered temperature treatment. The 8% reduction in PAR from the acrylic sheets and 80% reduction in UV light, coupled with potential shading effects from the frame may explain the significant increase in SLA in the altered temperature plots. Semchenko *et al.* (2012) showed how shading and reduced temperature can facilitate plant growth, including SLA, in temperate grassland species. They found that even in stress tolerant plants that should be adapted to high irradiance, greater growth enhancements were recorded in shaded areas. As a stress-tolerant grassland species, the increased SLA recorded in this study by *P. virgatum* in response to reduced temperatures support the conclusions from Semchenko *et al.* (2012) that increased growth may not actually be an artifact of stress amelioration. The SLA values for both the control and altered temperature plots were an order of magnitude lower than values in other studies using agronomic cultivars (Retta *et al.*, 2000; Dohleman *et al.*, 2009; Trócsányi *et al.*, 2009). This may be a reflection on how cultivars are bred to maximize biomass production, resulting in high SLA values. Long-term warming and heat stress experiments have shown no correlation or decreases in SLA responses (Milbau *et al.*, 2005; De Dato *et al.*, 2008; Wang *et al.*, 2008; Hudson *et al.*, 2011). In this study, we found increases in SLA with decreased mean daily temperatures, but with increases in nighttime warming and subsequently drier surface soils, SLA is likely to decrease, with greater belowground growth in response to reduced water availability (Kalapos *et al.*, 1996).

Temperature manipulation is a critical component in investigating responses of both natural and cultivar populations to climate change, and the need for inexpensive, passive temperature manipulation has been identified previously (Marion & Pidgeon, 1992). The louvered OSC's used in this study effectively altered air temperatures compared to control plots by reducing the daytime average and maximum air temperature. The most likely explanation for this effect is an increase in latent energy between the grass canopy and shelter, serving to increase the relative humidity and decrease temperature. Reduction in UV light can also create a significant decrease in g_s (Knapp, 1993), and this provides for a potential mechanism for the decreased g_s that was recorded. Regardless, the stomatal conductance values measured here were similar (~0.12 mol m^{-2} s^{-1}) to agronomic cultivars (Alamo, Kanlow, Blackwell, Cave-In-Rock) grown in control conditions in a greenhouse (Barney *et al.*, 2009). Unfortunately, these shelters were not instrumented with RH sensors to calculate differences in the VPD between plots. In a previous study in alpine grassland, the louvered OSC's allowed solar energy to accumulate under the frame during the day, and nighttime re-radiation of long-wave IR air increased the air temperature by roughly 1.5 °C (Germino & Demshar, 2008). Thus, while the directional temperature effects of these shelters varied between the ecosystems, the louvered OSC's are shown to effectively alter air temperatures, and are both inexpensive and easy to use for passive temperature manipulations in the field. Drawbacks for this technique include the need of the acrylic slats to remain clean and clear to reduce a shading effect and the potential growth of the vegetation needs to be taken into account. The frame needs to stay above the canopy, and as the height increases, the area the frame covers need to increase to imbue the same warming effect. Other passive warming techniques such as reflection curtains (Beier *et al*., 2004) or open top designs (Marion *et al*., 1997; Godfree *et al*., 2011) also alter air temperatures, but they are either expensive or have additional unintended impacts on the microclimate. In a recent analysis of phenological responses to warming experiments, Wolkovich *et al*. (2012) stated that warming chambers often result in cooling plots or have unintended effects upon daily temperature fluctuations. To improve upon these designs, future studies should incorporate measurements of multiple microclimate variables to better address deficiencies in the design (Wolkovich *et al*., 2012). Ideally, improvement upon the louvered OSC design will produce fewer effects on rainfall interception, soil moisture, wind speed, and relative humidity.

In this study, the responses of native *P. virgatum* within a mixed-community display sensitivity to small air temperature changes, which has the potential to interact with precipitation variability and topographic differences to affect *P. virgatum* physiology, growth, and yield. As a potential biofuel species, monocultures of *P. virgatum* are highly responsive to precipitation variability (Wang *et al*., 2010) and empirical modeling of *P. virgatum* yields have shown strong responses to temperature (Jager *et al*., 2010). Although increases in species diversity have been shown to decrease biofuel yield (Adler *et al*., 2009), large scale cultivation of *P. virgatum* for biofuel will most likely be implemented in mixed communities to produce sustainable production (Gonzalez-Hernandez *et al*., 2009) to reduce potential negative ecological effects (Hartman *et al*., 2011). Thus, for this potential biofuel species, greater integration of physiological responses to simulated climate change (increased temperature) and resource availability (soil moisture) will be required to accurately predict yields across an increasingly larger geographic and environmental range predicted for cultivation of this species.

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