



## Original article

# Ecophysiological responses of two dominant grasses to altered temperature and precipitation regimes

Jesse B. Nippert<sup>a,\*</sup>, Philip A. Fay<sup>b</sup>, Jonathan D. Carlisle<sup>c</sup>, Alan K. Knapp<sup>d</sup>, Melinda D. Smith<sup>e</sup>

<sup>a</sup>Division of Biology, Kansas State University, Manhattan, KS 66506, USA

<sup>b</sup>USDA-ARS, Grassland Soil and Water Research Laboratory, Temple, TX, USA

<sup>c</sup>Department of Plants, Soils and Climate, Utah State University, Logan, UT, USA

<sup>d</sup>Department of Biology and Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO, USA

<sup>e</sup>Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA

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## ABSTRACT

Ecosystem responses to climate change will largely be driven by responses of the dominant species. However, if co-dominant species have traits that lead them to differential responses, then predicting how ecosystem structure and function will be altered is more challenging. We assessed differences in response to climate change factors for the two dominant C<sub>4</sub> grass species in tallgrass prairie, *Andropogon gerardii* and *Sorghastrum nutans*, by measuring changes in a suite of plant ecophysiological traits in response to experimentally elevated air temperatures and increased precipitation variability over two growing seasons. Maximum photosynthetic rates, stomatal conductance, water-use efficiency, chlorophyll fluorescence, and leaf water potential varied with leaf and canopy temperature as well as with volumetric soil water content (0–15 cm). Both species had similar responses to imposed changes in temperature and water availability, but when differences occurred, responses by *A. gerardii* were more closely linked with changes in air temperature whereas *S. nutans* was more sensitive to changes in water availability. Moreover, *S. nutans* was more responsive overall than *A. gerardii* to climate alterations. These results indicate both grass species are responsive to forecast changes in temperature and precipitation, but their differential sensitivity to temperature and water availability suggest that future population and community structure may vary based on the magnitude and scope of an altered climate.

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## 1. Introduction

A defining characteristic of the climate in the midwestern North American Great Plains is variability (Borchert, 1950). Paleoclimate records indicate that this region has experienced considerable climatic variation at multiple intervals throughout the Holocene (Laird et al., 1996; Laird et al., 2003; Mayewski et al., 2004; Michels et al., 2007). Against this historical backdrop, human-influenced global warming is increasing the probability of rapid climate change, and increased variation and more frequent extreme events for key environmental drivers like precipitation and temperature (Easterling et al., 2000; Houghton et al., 2001; Alley et al., 2003, 2007). Air temperature for the Great Plains has been projected to increase by 4 °C by the year 2100 (range 2–8 °C; Christensen et al., 2007). In contrast, annual precipitation for this region is likely to remain similar to present amounts, but the size distribution of

individual rainfall events and the seasonal distribution of rainfall are likely to change (Gordon et al., 1992; Easterling et al., 2000; Karl and Trenberth, 2003; Meehl et al., 2005; Christensen et al., 2007). As temperatures increase and precipitation becomes more variable, alterations in resource availability are likely to affect the physiological and community responses of the ecosystem (Knapp et al., 2002).

The extant climate and past changes in atmospheric CO<sub>2</sub> concentration, air temperature, and precipitation have been recognized as critical in determining the distribution, persistence, and production of grassland plant communities (Weltzin et al., 2003; Dukes et al., 2005). In the tallgrass prairie ecosystem, there is evidence that historic variability in precipitation and temperature has impacted both species composition and productivity (Wells, 1970; Lauenroth et al., 1999). Indeed, interannual environmental variability can result in a 4-fold range of annual aboveground productivity (200–800 g m<sup>-2</sup>; Knapp et al., 1998). However, plant responses to current levels of climate variability may not adequately predict responses to future climate conditions (Nippert et al., 2006). Experimental manipulations of warming and

\* Corresponding author. Tel.: +1 785 532-0114; fax: +1785 532 6653.

E-mail address: [nippert@ksu.edu](mailto:nippert@ksu.edu) (J.B. Nippert).

precipitation variability have provided some insight into productivity and plant composition responses to climate change (Fay et al., 2002, 2003; Knapp et al., 2002; An et al., 2005) with responses driven primarily by the most abundant or dominant species.

A focus on responses of the dominant species has proven effective for understanding grassland community and ecosystem processes (McNaughton, 1985; Smith and Knapp, 2003; Silletti et al., 2004; Anderson et al., 2006), and the tallgrass prairie is an ideal system for this approach because the response of a few dominant C<sub>4</sub> grasses (i.e., *Andropogon gerardii* Vitman (big bluestem) and *Sorghastrum nutans* (L.) Nash (Indian grass)), strongly influences this ecosystem's response to environmental change (Knapp et al., 1998). These species share many traits and frequently co-occur, but previous studies have suggested that they differ in responses to temperature and water availability (Silletti and Knapp, 2001, 2002; Swemmer et al., 2006). For example, in a retrospective study of long-term plant community composition data, cover of *A. gerardii* was found to be more responsive to variation in summer air temperature, whereas *S. nutans* responded more to changes in rainfall (Silletti and Knapp, 2001). In addition, *S. nutans* generally shows a greater ecophysiological and whole-plant response to alterations in resource availability (Silletti and Knapp, 2002; Silletti et al., 2004; Swemmer et al., 2006).

Our objective was to determine if leaf-level physiological responses to experimental increases in temperature and changes in precipitation regime, as forecast for the Midwest US, differed between these two species and if these responses were consistent with their long-term responses in cover and productivity (Knapp et al., 1998). We hypothesized: (1) ecophysiological responses in *S. nutans* would be more closely related to changes in water availability, while *A. gerardii* would respond more to changes in temperature; and (2) *S. nutans* would exhibit greater responsiveness to variation in key environmental drivers such as increased precipitation variability and warming.

## 2. Materials and methods

This study was performed on plants growing within a long-term climate change field experiment – the rainfall manipulation plots (RaMPs) – in native tallgrass prairie at the Konza Prairie biological station (KPBS). The timing and size of precipitation events in the RaMPs has been manipulated since 1998 (see Fay et al., 2000) and a warming treatment has been included since 2003.

### 2.1. Study system

The Konza Prairie biological station (KPBS) is a 3487 ha tallgrass prairie preserve located in northeastern Kansas, USA (39°05'N, 96°35'W). This location has a temperate mid-continental climate of cold, dry winters and warm, wet summers. The long-term mean annual precipitation is 835 mm, of which 75% occurs during the growing season (April–September) (Hayden, 1998). The long-term mean daily air temperature is 18 °C in May, peaks at 27 °C in July, and declines to 22 °C in September (Hayden, 1998). While a diverse array of plant species are found on site, the mean annual above-ground net primary productivity (ANPP: 420 g m<sup>-2</sup>; Knapp et al., 1998) reflects the growth of the dominant C<sub>4</sub> grasses, primarily *Andropogon gerardii* and *Sorghastrum nutans* (Smith and Knapp, 2003).

### 2.2. The RaMPs experiment

The RaMPs experiment consists of 12 fixed-location shelters (14 × 9 m) designed to experimentally manipulate precipitation (ambient and altered) and temperature (unwarmed and warmed).

For the ambient precipitation treatment (6 shelters), each rainfall event that occurred was collected and immediately reapplied (mid-Apr to mid-Oct). For the altered treatment (6 shelters), rainfall was collected and accumulated until the interval between precipitation events was increased by 50% and then applied. The altered precipitation pattern thus had: (1) longer inter-rainfall dry periods, (2) greater rainfall event sizes, and (3) fewer rainfall events compared to ambient patterns. The total amount of growing season rainfall is kept equal in both treatments. The warming treatments are nested within the precipitation treatments. Each shelter (ambient and altered treatments) includes four 2 × 2 m subplots. In two of the subplots, infrared heating lamps (Kalglo 240V HS-2420) raise the ambient air temperature year round by ~2 °C. Lamp height is repositioned periodically through the growing season to maintain the lamp 1.2 m above the plant canopy (1.2 m above the soil surface in winter) and provide 20–25 W m<sup>-2</sup> of downward infrared radiation. The control (unwarmed) subplot dedicated to sampling contains a dummy lamp to control for physical shading by the lamps. The soil type in the RaMPs is an Irwin silty clay loam. The permanent wilting point for this soil in the A horizon (0–20 cm) is between 15–17%, while gravimetric water content at field capacity is approximately 31% (NRCS soil series descriptions: <http://www.soils.usda.gov/>). Further details on shelter design, construction, and experimental details have been previously reported and are also available online (Fay et al., 2000, 2002; <http://www.konza.ksu.edu/ramps/>).

### 2.3. Sampling protocol and variables measured

Plant measurements were conducted over two growing seasons with 4 intensive sampling days in 2005 (06/01, 07/17, 07/21, 08/15) and in 2006 (06/01, 06/07, 07/18 and 07/24). For each year, two dates in mid-July targeted a drought-recovery period; the first period (drought) was chosen when soil moisture was limiting and temperatures were >30 °C, and then the second period (recovery) occurred after rainfall was applied to both the altered and ambient treatment plots. Due to the nature of the altered precipitation treatments, this treatment received larger rainfall events than the ambient treatment to initiate the recovery period (50–78 mm vs. 37–38 mm in the ambient treatment).

For each sample period, two morphologically similar tillers (3–5 fully expanded leaves) were identified prior to sampling for each species within the unwarmed control subplot and a randomly selected warmed subplot. Physiological measurements were performed on the newest, yet mature leaves, at the widest portion of the grass blade. Because instantaneous physiological measurements can vary based on time of day, ambient and altered plots were paired and their measurement order was randomized for each sampling period. Sampling was conducted between 09:00–15:00 CST when solar radiation was typically above 70% of full sun levels.

We measured key physiological variables representing responses in photosynthesis, water stress, and water loss. These included the maximum photosynthetic rate at saturating light and ambient CO<sub>2</sub> ( $A_{\text{sat}}$ ), dark-adapted chlorophyll fluorescence ( $F_v/F_m$ ), stomatal conductance to water ( $g_s$ ), instantaneous water-use efficiency (WUE:  $A_{\text{sat}}/E$ ), and midday leaf water potential ( $\psi_{\text{mid}}$ ).  $F_v/F_m$  was measured using a hand-held pulse amplitude modulated fluorometer (OS1-FL, Opti-sciences, Inc., Tyngsboro, MA, USA). Leaves were dark-adapted prior to measurement for 15–30 min immediately preceding diurnal gas exchange measurements. Leaf-level gas exchange was measured on one tiller per species using a LI-6400 gas exchange system with red/blue light source and CO<sub>2</sub>-injector (LicOR, Inc., Lincoln, NE, USA). Flow rate was kept constant at 400  $\mu\text{mol s}^{-1}$ , light intensity inside the cuvette was 1500  $\mu\text{mol}$

$\text{m}^{-2} \text{s}^{-1}$ , the  $\text{CO}_2$  concentration was  $370 \mu\text{mol mol}^{-1}$ . Relative humidity was maintained near ambient by adjusting the desiccant knob, as necessary. Temperature controls were used to maintain the block temperature near the ambient air temperature (measured and adjusted between each sample). We designated stability for our gas exchange measurements when the CV for photosynthesis and conductance rates was  $<1\%$  over a 15-s interval. In general, stability occurred within 5–10 min per sample. One leaf per tiller from each species was measured for leaf water potential ( $\psi_{\text{mid}}$ ) using a Scholander-type pressure bomb (PMS Instruments, Inc., Corvallis, OR, USA).  $F_v/F_m$  and  $\psi_{\text{mid}}$  values were averaged by species/subplot prior to statistical analysis. This measurement protocol was kept constant for all time periods for both years.

To verify that our fluorescence results reflected treatment responses rather than sampling protocol, we analyzed all  $F_v/F_m$  data for each sampling period for a ‘time of day’ effect.  $F_v/F_m$  was measured during the day in the same randomized order used for the gas exchange measurements, and increased daytime light stress and temperatures may decrease  $F_v/F_m$  values (Maxwell and Johnson, 2000). The diurnal sampling order did not significantly influence  $F_v/F_m$  for any of the dates we sampled (all sampling periods had  $p > 0.10$ ).

To characterize the effects of the precipitation and temperature treatments on environmental conditions, we continuously measured canopy temperature and near-surface soil water content (0–15 cm). Canopy temperature was measured using an infrared thermometer sensor (Apogee) mounted 1.5 m aboveground in one unwarmed and one warmed subplot in four RaMPs. Volumetric soil water content ( $\theta_{0-15}$ ) was measured in the control and warmed subplots in each RaMP using 30-cm time-domain reflectometry (TDR) probes (Campbell Scientific) buried 0.50 m from the edge of each subplot at a  $45^\circ$  angle to sample 0–15 cm soil depth. These sensors were recorded in 30-min intervals using a Campbell CR10X data logger. Air temperature and relative humidity data was available from the nearby (0.3 km) Konza Prairie weather station.

#### 2.4. Statistical analysis

The overall goal of this project was to assess sensitivity of *A. gerardii* and *S. nutans* to variation in soil moisture and temperature generated by the RaMPs treatments over time. To accomplish this goal, we used multiple linear regression (MLR) analysis in which measures of temperature (canopy and leaf) and  $\theta_{0-15}$  were the predictor variables and each of the 5 measured physiological parameters were the response variables. For both years combined, separate MLR were conducted for each physiological variable using the step-wise selection procedure. We chose this approach rather than an ANOVA approach, in order to assess sensitivity (interpreted as the slope of the best-fit MLR) across a range of soil moisture and temperature conditions.

A primary impact of the heating treatments was to elevate canopy temperatures. However, the infrared canopy temperature sensors were present in only a subset of the heated and control subplots. Canopy temperature was correlated to  $T_{\text{leaf}}$  with normally distributed residuals (Fig. 1,  $r^2 = 0.77$ ;  $y = 1.02x + 5.4$ ;  $p < 0.0001$ ; RMSE = 2.7582; K-S normality statistic  $p = 0.3283$ ). The  $5.4^\circ\text{C}$  intercept in Fig. 1 most likely reflects methodological artifacts of temperature measurements using an IRGA versus infrared thermometer. During midday when evapotranspiration is highest, the canopy temperature sensor may slightly underestimate the true leaf temperature as vapor flux passes between the canopy and sensor. The relationship between canopy temperature and  $T_{\text{leaf}}$  holds across a large environmental gradient, but considerable variability in  $T_{\text{leaf}}$  exists at a given canopy temperature (Fig. 1).  $T_{\text{leaf}}$  was used as a predictor variable in the regression analyses. To

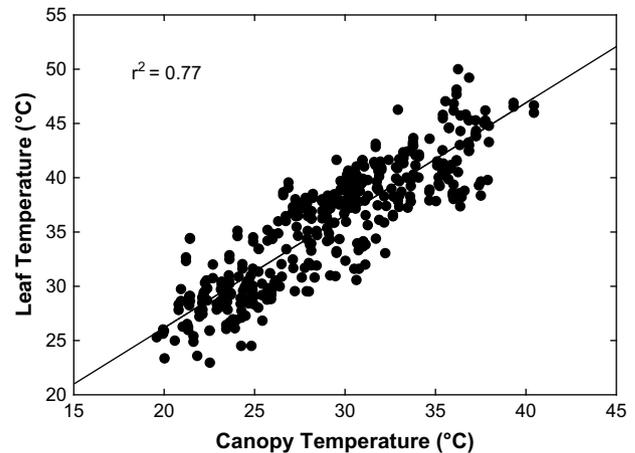


Fig. 1. Simple linear regression relating canopy temperature measured within a RaMPs shelter and leaf temperature measured inside an IRGA during gas exchange measurements ( $y = 1.02x + 5.4$ ; total  $df = 386$ ). Data are from both years and all treatment combinations (precipitation and temperature).

assess sensitivity of response of the two species to the targeted drought-recovery cycles in 2005 and 2006, we compared the differences between the drought and recovery periods using the slope of the response before and after watering for each species for each year separately. All analyses were performed using SAS statistical software (SAS v9.1, Cary, NC, USA).

### 3. Results

The precipitation and warming treatments altered the climate experienced by the plant communities in this experiment (Table 1; Figs. 2 and 3). Responses of surface soil water content ( $\theta_{0-15}$ ) and leaf temperature ( $T_{\text{leaf}}$ ) to the treatments depended on the time periods compared (Table 1; Figs. 2 and 3). Both the precipitation and warming treatments significantly reduced  $\theta_{0-15}$  compared to ambient precipitation patterns and unwarmed subplots, but not at all time periods (Fig. 2). In 2005, the precipitation treatment significantly lowered  $\theta_{0-15}$  during the 06/01 and 07/21 periods (Table 1). In 2006, the precipitation and warming treatments significantly lowered  $\theta_{0-15}$  during 06/07, and the precipitation treatment significantly lowered  $\theta_{0-15}$  during 07/24 (Table 1).  $T_{\text{leaf}}$  was significantly increased by the warming treatment during the first sampling date in 2005, and the first two dates in 2006 (Table 1). Interactions of precipitation and warming treatments significantly increased  $T_{\text{leaf}}$  for the first two sample dates in 2005, but no interaction effects were present in 2006. There were no treatment-induced statistical differences in  $T_{\text{leaf}}$  or  $\theta_{0-15}$  when analyzed by year in 2005 (Table 1), but the warming treatment and precipitation  $\times$  warming significantly reduced  $\theta_{0-15}$  in 2006 ( $p < 0.05$ ; Table 1). Thus, these treatments did not result in a consistent temporal alteration of annual means, but rather the treatments extended and shifted the distribution of environmental variability in ways consistent with predictions of future climate for this location.

The altered precipitation and warming treatments resulted in a wide range in  $T_{\text{leaf}}$  (and canopy temperature) and  $\theta_{0-15}$ , and we focused on comparing the sensitivity of key physiological responses of *A. gerardii* and *S. nutans* to this environmental variation. Our results indicate that  $T_{\text{leaf}}$  and  $\theta_{0-15}$  explained a significant portion of the variation in the five key physiological parameters measured for both species (whole-model  $r^2$  range: 0.24–0.55; Table 2). The total explained variance was similar between species, with the exception of  $A_{\text{sat}}$  responses in which higher  $r^2$  values were present for

**Table 1**

Mixed-effects model ANOVA of treatment effects (fixed, altered precipitation and warming; random, RaMP) on the environmental variables volumetric water content of 0–15 cm soil ( $\theta_{0-15}$ ) and leaf temperature ( $T_{leaf}$ ).

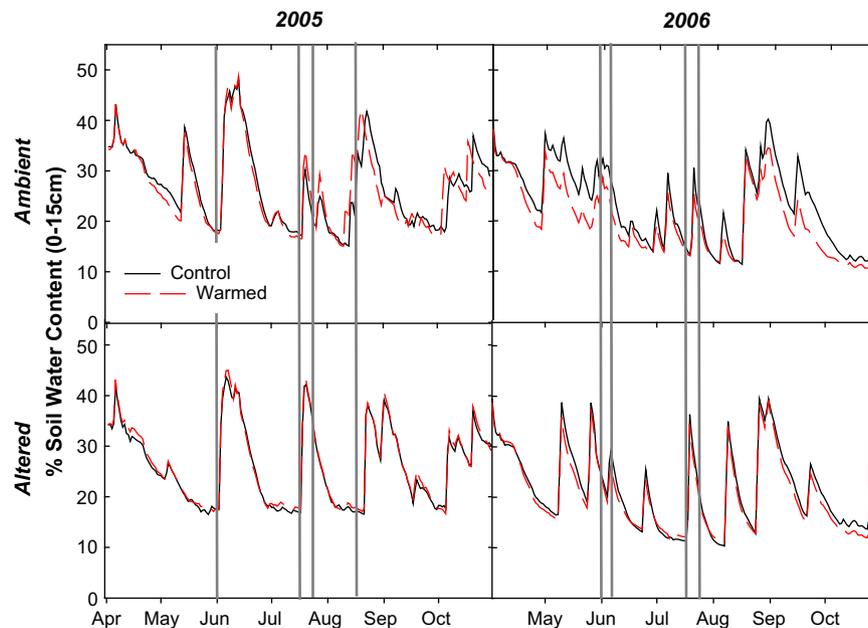
Date		$\theta_{0-15}$			$T_{leaf}$		
		Precipitation	Warming	Precipitation $\times$ warming	Precipitation	Warming	Precipitation $\times$ warming
2005	F	0.20	0.13	1.63	0.09	0.04	0.01
	p	0.662	0.723	0.230	0.759	0.844	0.913
06/01	F	7.37	0.17	2.66	1.64	17.63	4.12
	p	<b>0.022</b>	0.683	0.112	0.209	<b>&lt;0.001</b>	<b>0.050</b>
07/17	F	2.91	1.48	1.70	0.17	2.39	7.27
	p	0.119	0.232	0.201	0.680	0.131	<b>0.011</b>
07/21	F	34.97	2.56	1.36	0.05	1.70	0.57
	p	<b>0.0001</b>	0.119	0.252	0.823	0.201	0.455
08/15	F	3.74	0.37	0.35	0.30	0.43	2.14
	p	0.101	0.549	0.563	0.585	0.519	0.152
2006	F	0.05	20.59	5.22	0.00	0.19	0.00
	p	0.825	<b>0.001</b>	<b>0.046</b>	0.970	0.667	0.988
06/01	F	2.45	3.18	1.84	1.36	20.51	0.28
	p	0.169	0.088	0.189	0.252	<b>&lt;0.001</b>	0.598
06/07	F	5.29	6.63	0.4	0.15	6.97	3.22
	p	<b>0.044</b>	<b>0.015</b>	0.531	0.6992	<b>0.012</b>	0.081
07/18	F	0.04	0.44	1.53	0.00	0.08	0.02
	p	0.855	0.510	0.225	0.9747	0.786	0.899
07/24	F	7.91	1.69	0.06	1.20	2.71	0.37
	p	<b>0.018</b>	0.202	0.803	0.282	0.109	0.550

F- and p-values are provided for both fixed effects and their interaction. Degrees of freedom for individual dates were (1,34) and by year (1,177). Significant responses ( $p < 0.05$ ) are indicated in bold font.

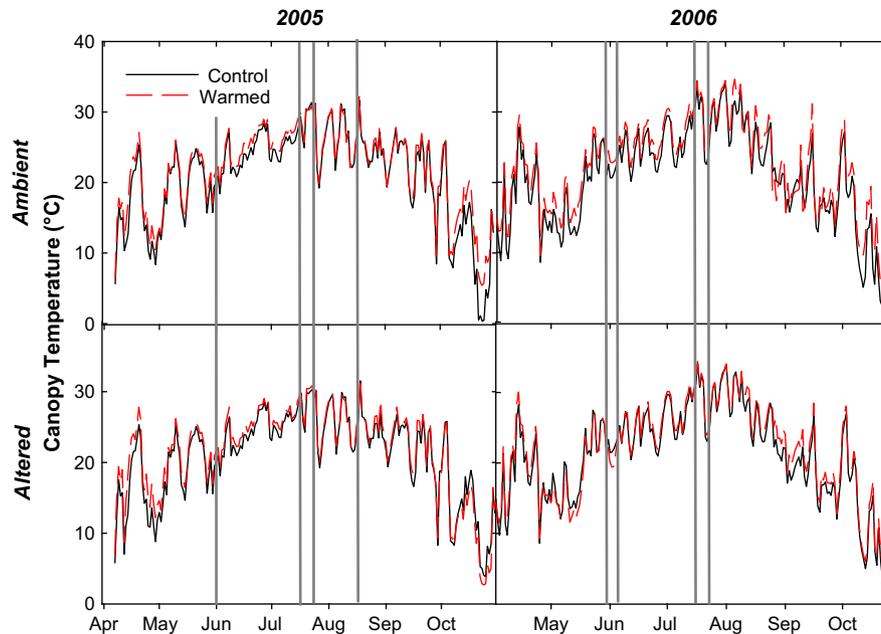
*S. nutans* (Table 2). Additionally, the slope of the relationship between  $\theta_{0-15}$  and  $A_{sat}$  was significantly higher for *S. nutans*, compared to *A. gerardii* (Table 2, final column). For both species,  $T_{leaf}$  had higher partial  $r^2$  values for variation in WUE and  $F_v/F_m$ , whereas  $\theta_{0-15}$  had higher  $r^2$  for  $A_{sat}$  and  $\psi_{mid}$ . Responses of  $g_s$  to environmental variation differed by species. The best predictor for *A. gerardii* was  $T_{leaf}$  (partial  $r^2 = 0.23$ ) while the best predictor of  $g_s$  for *S. nutans* was  $\theta_{0-15}$  (partial  $r^2 = 0.35$ ) (Table 2).

To further assess sensitivity in physiological responses between the two  $C_4$  grasses, we focused on two mid-season drought-recovery periods in July, 2005 and 2006. The drought-recovery period was characterized by low water availability and high air temperatures

typical of mid-July for the first sample date, and then alleviation from drought with the application of rainfall to both the ambient and altered treatments for the second sample date. During both years, the rainfall applications significantly increased soil moisture content for the second sampling period (Table 3). However, the two drought-recovery periods differed in that the maximum ambient air temperatures increased between sampling periods in 2005, but decreased in 2006 (Table 3). VPD (air to leaf vapor pressure deficit) increased following the rainfall application in both years, driven largely by decreases in RH (from 60 to 50% in 2005, 50 to 30% in 2006; Table 3). In 2005,  $A_{sat}$ ,  $g_s$  and WUE declined for both species in the recovery period despite the increase in soil water content and  $\psi_{mid}$ .



**Fig. 2.** Treatment effects of altered precipitation pattern and increased air temperature on soil water content from 0–15 cm. Data are 24-h means for the growing seasons of 2005 and 2006. The top row shows the response to ambient precipitation patterns, and the bottom row shows the response to altered precipitation pattern (see Section 2). Line color denotes temperature treatments (black, ambient air temperature; red, warmed). Vertical grey lines indicate dates of physiological sampling.



**Fig. 3.** Treatment effects of altered precipitation pattern and increased air temperature on canopy temperature ( $^{\circ}\text{C}$ ). Data are 24-h means for the growing seasons of 2005 and 2006. The top row shows the response to ambient precipitation patterns, and the bottom row shows the response to altered precipitation pattern (see Section 2). Line color denotes temperature treatments (black, ambient air temperatures; red, warmed). Vertical grey lines indicate dates of physiological sampling.

However,  $A_{\text{sat}}$  and  $g_{\text{m}}$  declined significantly more in *A. gerardii* when compared to *S. nutans* (Fig. 4A,B). In 2006,  $A_{\text{sat}}$ ,  $g_{\text{s}}$ , and WUE increased in the recovery period similarly in both species (Fig. 4A–C) following the rainfall application. Here,  $\psi_{\text{mid}}$  responded significantly more for *S. nutans* in 2006 following water additions, increasing to a higher mean value than *A. gerardii* (Fig. 4E).  $F_{\text{v}}/F_{\text{m}}$  did not vary significantly in response to the increase in water availability by species or between years (Fig. 4D).

Changes in instantaneous WUE were compared by species and year to simultaneous measurements of VPD (Fig. 5). WUE was calculated as a change in the ratio of  $A_{\text{sat}}$  to  $E$  measured on individual leaves, while VPD was calculated using the mean air temperature and relative humidity data from the nearby (0.3 km) Konza Prairie weather station, at the corresponding time periods as gas exchange measurements. Results over both summers and species show a significant negative correlation between VPD and WUE (Fig. 5).

#### 4. Discussion

Previously, community-level comparisons between the two co-dominants *A. gerardii* and *S. nutans* suggested that there was

differential sensitivity to interannual variability in water and temperature in native tallgrass prairie (Silletti and Knapp, 2001, 2002). However, when the physiological responses of *A. gerardii* were related to more subtle intra-annual changes in soil water resulting from the RaMPs treatment protocol, few responses were found (Fay et al., 2002). Our research focused on differences between *A. gerardii* and *S. nutans* within the RaMPs but during years with greater water and temperature stress. In general, these physiological results conducted during drought support previous community-level comparisons of differential sensitivity to water and temperature stress between these co-dominant species (Silletti and Knapp, 2001, 2002; Swemmer et al., 2006).

Previous studies of these two species provide a broad base for describing their ecophysiological traits. *A. gerardii* and *S. nutans* are both NADP-ME subtype  $\text{C}_4$  grasses. Grasses with this  $\text{C}_4$  subtype are characterized by little net  $\text{O}_2$  production or consumption in bundle sheath cells and low rates of  $\text{CO}_2$  leakage out of the bundle sheath (Raven, 1977; Chen et al., 1994). As NADP-ME  $\text{C}_4$  grasses, plant photosynthetic biochemistry varies little among these two species. However, differences in the maximum rates of gas exchange and varying stomatal characteristics do occur (Knapp, 1993). Maximum

**Table 2**  
MLR model (stepwise) results for physiological responses of *A. gerardii* and *S. nutans* to leaf temperature ( $T_{\text{leaf}}$ ) and soil water content ( $\theta_{0-15}$ ).

Predictor	Response	<i>A. gerardii</i>			<i>S. nutans</i>			Slope <i>p</i> -value
		$r^2$	SE	Slope	$r^2$	SE	Slope	
$T_{\text{leaf}}$	$A_{\text{sat}}$	0.01	0.08	−0.12	0.04	0.08	−0.26	0.28
	$g_{\text{s}}$	0.23	0.52	−0.93	0.04	0.49	−1.58	0.40
	WUE	0.46	0.02	−0.19	0.43	0.02	−0.22	0.39
	$\psi_{\text{mid}}$	0.07	0.09	0.36	0.07	0.01	0.38	0.81
	$F_{\text{v}}/F_{\text{m}}$	0.40	$4.61 \times 10^{-4}$	$4.61 \times 10^{-3}$	0.35	$5.85 \times 10^{-4}$	$5.25 \times 10^{-3}$	0.55
$\theta_{0-15}$	$A_{\text{sat}}$	0.23	6.85	45.90	0.34	7.20	62.39	0.04
	$g_{\text{s}}$	0.10	45.70	301.89	0.35	42.94	381.88	0.08
	WUE	0.09	1.44	8.26	0.10	1.79	10.53	0.33
	$\psi_{\text{mid}}$	0.25	7.95	65.32	0.25	8.12	68.02	0.95
	$F_{\text{v}}/F_{\text{m}}$	0.04	0.04	0.14	0.06	0.05	0.22	0.78

Each row shows the fit statistics (partial  $r^2$ , standard error, and parameter slope) for predictor variables (leaf temperature ( $T_{\text{leaf}}$ ), and volumetric water content ( $\theta_{0-15}$ )). The *p*-values in the final column indicate differences by species in the slope of predictor and response variables compared. Analyses include data combined across both years.

**Table 3**

Mean environmental conditions during the drought – recovery sampling periods (09:00–15:00 CST) for 2005 and 2006.

	07/17/05	07/21/05	07/18/06	07/24/06
$\theta_{0-15}$	0.14 (0.01)	0.33 (0.01)	0.12 (0.01)	0.19 (0.01)
$T_{\text{air}}$	37.6 (0.5)	39.7 (0.6)	42.6 (0.4)	39.3 (0.3)
VPD	1772.3 (167.2)	2717.9 (109.4)	2246.1 (76.4)	3293.5 (79.5)

Values are whole-site means plus standard error in parentheses. Volumetric soil water content ( $\theta_{0-15}$ ) is expressed as a percent, air temperature ( $T_{\text{air}}$ ) in °C, and leaf to air vapor pressure deficit (VPD) in pascals.

gas exchange rates for both species are similar, but vary based on sampling location, time of year, and by study. In general, the range of values reported across studies is similar (maximum  $A_{\text{sat}}$  mean range 25–35  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and maximum  $g_s$  mean range 250–350  $\text{mmol m}^{-2} \text{s}^{-1}$ ) (Heckathorn and DeLucia, 1991; Knapp, 1993; Silletti and Knapp, 2001; Swemmer et al., 2006). *S. nutans* has a higher stomatal density than *A. gerardii* (mean 324 vs. 168  $\text{mm}^{-2}$ ), but smaller guard cells (mean 24 vs. 38  $\mu\text{m}$  length) (Knapp, 1993). The optimum soil temperature for growth in *A. gerardii* is 25 °C (DeLucia et al., 1992) and the optimum air temperature ranges between 30–34 °C (Knapp, 1985). Both species are highly drought tolerant and maintain gas exchange over a wide range of environmental conditions. During a year with above-average precipitation, the mean  $\psi_{\text{mid}}$  for *A. gerardii* was –1.5 MPa, and the mean  $\psi_{\text{mid}}$  was –2.5 MPa in a dry year (Knapp et al., 1993). However, the minimum water potential at which positive net photosynthesis still occurs is –6.5 MPa for *A. gerardii* and –5.5 MPa for *S. nutans* (Heckathorn and DeLucia, 1991, 1994). Cumulatively, these results show broad similarity by species, but that over a range of environmental conditions, *A. gerardii* exhibits ecophysiological responses characteristic of a higher drought tolerance while *S. nutans* may have a higher tolerance to changes in air temperature. If these species have varying sensitivity to water and temperature, these effects are most likely to be seen during mid-summer when air temperatures remain high but water availability fluctuates with episodic precipitation events and alterations in soil water content.

Despite the high level of variability in this system (McAllister et al., 1998; Nippert et al., 2007), multiple linear regression (MLR) analyses revealed significant relationships between  $T_{\text{leaf}}$  and  $\theta_{0-15}$  and key plant ecophysiological response variables (Table 2). The relative importance of  $T_{\text{leaf}}$  or  $\theta_{0-15}$  as a predictor of the response variables we measured was largely similar for both C<sub>4</sub> grass species. However, the variance explained by  $T_{\text{leaf}}$  and  $\theta_{0-15}$  differed between species for  $g_s$ . For example, more variance in  $g_s$  was explained by  $T_{\text{leaf}}$  in *A. gerardii*, but by  $\theta_{0-15}$  in *S. nutans*. In addition, the physiological responsiveness to changes in temperature and soil moisture (as indicated by slopes of the relationships) was consistently higher (though not always significantly) for *S. nutans* than *A. gerardii* (final column, Table 2).

The results of the mid-season drought – recovery periods when environmental conditions changed over short periods of time provides a unique context for the relationships noted in the MLR analysis (Table 2).  $\theta_{0-15}$  increased during the ‘recovery’ period for both years following rainfall applications, VPD increased concurrently, and  $T_{\text{air}}$  varied between drought and recovery for 2005 and 2006 (Table 3). In 2005, gas exchange variables ( $A_{\text{sat}}$ ,  $g_s$ , WUE) were lower in the ‘recovery’ period despite increases in  $\theta_{0-15}$  (Fig. 4A–C). The most likely explanation for this response is the increase in ambient  $T_{\text{air}}$  and VPD between the drought and recovery sampling periods (Table 3). While rates for both species declined,  $A_{\text{sat}}$  and  $g_s$  in *A. gerardii* decreased more than in *S. nutans* between drought – recovery in 2005, suggesting a greater impact of the higher  $T_{\text{air}}$  on *A. gerardii* during this period (Fig. 4A,B). This response is consistent with our MLR results showing a stronger correlation between  $g_s$

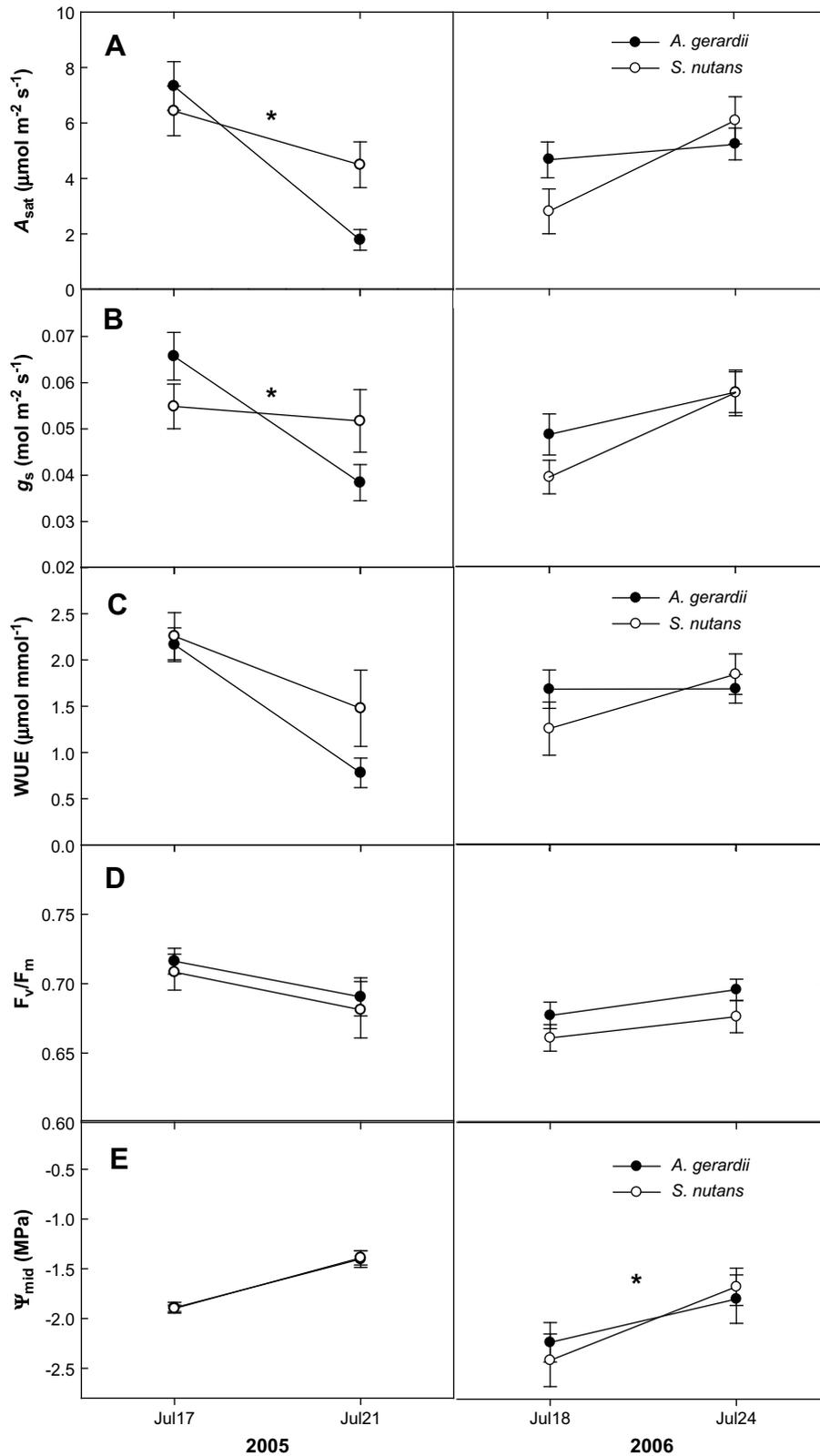
and temperature for *A. gerardii* (Table 2). The environmental conditions in 2006 were similar to 2005, except that  $T_{\text{air}}$  decreased between consecutive sampling periods (Table 3). Both species had higher rates of  $A_{\text{sat}}$  and  $g_s$  following rainfall applications, although in this case, the rate of change was higher for *S. nutans* (Fig. 4A,B). Increased VPD during the recovery period in 2006 did not result in lower WUE for either species (Fig. 4C). WUE nearly doubled for *S. nutans*, likely from increased  $g_s$  and increased water availability. Collectively, the results over both years suggest a greater negative effect of high  $T_{\text{air}}$  on *A. gerardii*, because alleviation of water stress in the recovery period resulted in minimal corresponding increases in  $A_{\text{sat}}$ ,  $g_s$ , and WUE. The positive response of *S. nutans* to increased  $\theta_{0-15}$  only occurred when  $T_{\text{air}}$  also decreased in 2006. For example,  $A_{\text{sat}}$ ,  $g_s$ , WUE and  $\psi_{\text{mid}}$  increased in *S. nutans* when  $\theta_{0-15}$  increased and  $T_{\text{air}}$  decreased in the recovery of 2006 (Fig. 4). The varying species responses during the two drought–recovery cycles provide additional support for both hypotheses. The physiological variables we measured do not show alleviation from stress for *A. gerardii* following water additions because  $T_{\text{air}}$  remained high. The response of *S. nutans* during the ‘recovery’ period in 2006 exhibits the greater responsiveness of this species to improved soil water content compared to *A. gerardii*.

$F_v/F_m$  did not vary significantly by species before or after the imposed drought–recovery cycle for either year (Fig. 4D). Previously, measurements of  $F_v/F_m$  in these C<sub>4</sub> grass species have been relatively insensitive to short-term changes in summer water availability or peak summer temperatures (Nippert et al., 2007). Similarly, Resco et al. (2008) reported minimal changes in  $F_v/F_m$  before and after precipitation pulses in a semi-arid ecosystem except following long periods of drought and considerable loss of photosynthetic activity. These authors suggest  $A_{\text{sat}}$  is likely a better physiological indicator of short-term plant stress responses to changing water availability (Resco et al., 2008).

Changes in instantaneous leaf WUE were negatively related to changes in VPD for all sampling dates during both years (Fig. 5). During periods with high VPD, WUE was the lowest. This relationship was similar by species for both years (but see lower  $r^2$  for *S. nutans* in 2005).

In theory, WUE should be inherently related to changes in VPD, since WUE is an expression relating changes in  $A_{\text{sat}}/E$ , and the derivation of  $E$  reflects changes in  $g_s$  and VPD. The relationships contrasted in Fig. 5 represent independent data because WUE was derived from gas exchange measurements performed on leaves inside an IRGA, while VPD was estimated concurrently using independent environmental data collected at the plot-scale. These results confirm theoretical expectations that leaf  $E$  would increase with increases in VPD without concurrent changes in  $g_s$  (and thus changes in  $A_{\text{sat}}$ ). If the carboxylation efficiency ( $V_{\text{cmax}}$ ) varied significantly between species, then sensitivity to VPD alone would not explain interspecific differences in WUE. We have previously reported a similar  $V_{\text{cmax}}$  for *A. gerardii* and *S. nutans* across a range of growing conditions at this site (Nippert et al., 2007). Thus, varying sensitivity to VPD is the most likely driver of changes in WUE for both species during 2005 and 2006.

In general, responses of *A. gerardii* and *S. nutans* to changes in water availability and temperature were similar over the entire study period (Table 2), but responses differed following a drought–recovery cycle (Fig. 4). Our results are consistent with how known demographic and biomass allocation strategies vary between the two species. For example, *S. nutans* can reproduce vegetatively throughout the season via new tiller production (McKendrick et al., 1975), and has greater allocation to rhizomes than *A. gerardii* (Swemmer et al., 2006). In contrast, *A. gerardii* reproduces vegetatively only at the beginning of the growing season when soil moisture is highest and temperatures are moderate, and has been

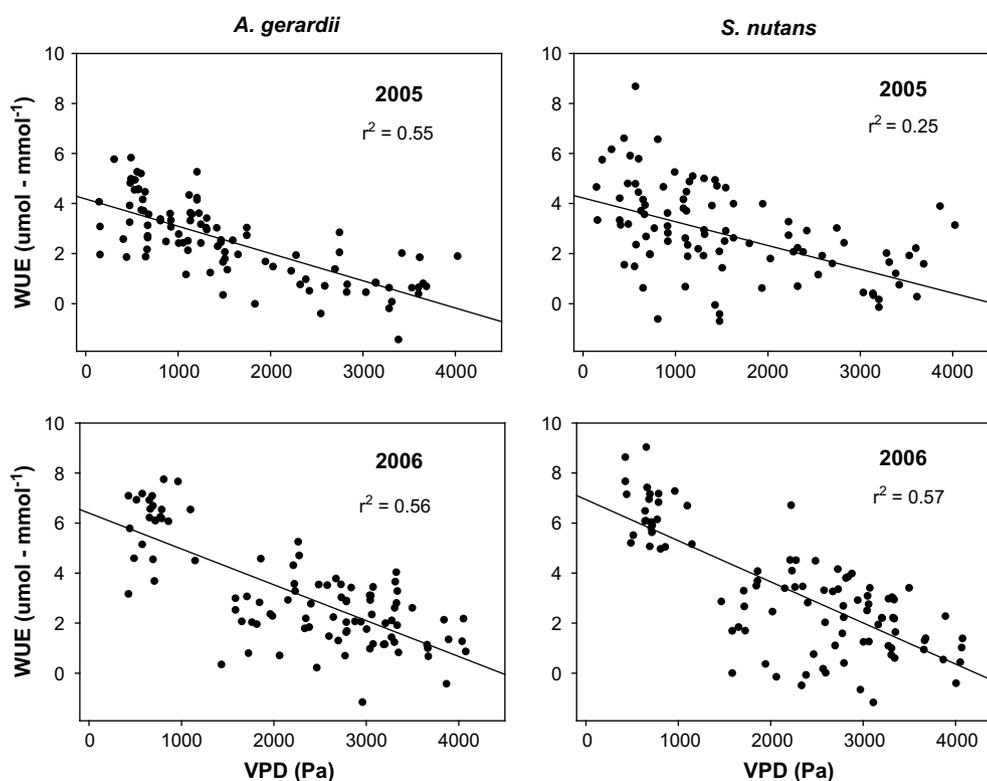


**Fig. 4.** Physiological responses for both species during drought-recovery periods in July 2005 and 2006. Water was applied to all plots (ambient and altered) between drought-recovery during each year. Each point is the overall mean response ( $\pm 1$  SE) by sample date and species. Asterisks indicate a statistically significant difference ( $p < 0.05$ ) between species during the drought and recovery periods.

shown to have greater root production and reduced flowering during drought (Swemmer et al., 2006). This similarity between the physiological and morphological/reproductive responses by species, suggests a consistent whole-plant response to resource

limitation, especially during periods of extreme stress (e.g., drought).

The most likely future environmental change for this region is reduced water availability, as both higher air temperatures and



**Fig. 5.** Changes in instantaneous water-use efficiency (WUE) as a function of the vapor pressure deficit (VPD) for both species and years. WUE was calculated as  $A_{sat}/E$  using gas exchange data, while VPD was calculated using the mean air temperature and relative humidity data from the nearby (0.3 km) Konza Prairie weather station.

more extreme rainfall regimes reduce soil water content. If this change is manifest, our results suggest *S. nutans* may be the most adversely affected, because *A. gerardii* is more stable (and less sensitive) to changes in water availability. Changes in the abundance of either species, particularly *A. gerardii*, have been shown to impact plant diversity and aboveground productivity in the tallgrass prairie (Collins et al., 1998; Knapp et al., 2002; Smith and Knapp, 2003). Thus, if changes in the ecophysiological traits we measured (e.g., reduced photosynthetic rates) translate to changes in growth, abundance, or survivorship for either C<sub>4</sub> grass species, then future changes in precipitation patterns and air temperatures may alter the relative role of each species in the overall dynamics of the tallgrass prairie ecosystem.

## 5. Conclusions

The altered temperature and precipitation treatments reduced soil water availability and increased leaf temperature during portions of the growing season, consistent with predictions of future climate for the study region. Leaf-level responses to these environmental changes indicate that: (1) both species responded similarly for most of the ecophysiological variables we measured; (2) when differences occurred,  $T_{leaf}$  was a better predictor of ecophysiological variability for *A. gerardii*, while  $\theta_{0-15}$  was a better predictor of variation for *S. nutans*; and (3) *S. nutans* was more responsive to alterations in water and temperature stress than *A. gerardii* under the same conditions.

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