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Population origin and genome size do not impact *Panicum virgatum* (switchgrass) responses to variable precipitation

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Abstract. Population-level adaptation to broad-scale regional climates or within-population variation in genome size of the genetically and phenotypically diverse C_4 grass, *Panicum virgatum* (switchgrass), may influence the responses of this species to future precipitation variability associated with climate change. Therefore, we investigated *P. virgatum* responses to water variability between natural populations collected across a latitudinal gradient and among individuals spanning a range of genomes sizes within these populations. *P. virgatum* plants from natural populations originating from Kansas, Oklahoma, and Texas, U.S.A, received frequent, small precipitation events ("ambient") or infrequent, large precipitation events ("altered") to simulate contrasting rainfall variability expected for this region. We measured leaf-level physiology, aboveground biomass and genome size for each individual. Gas exchange rates and aboveground biomass varied significantly by population origin but did not differ by genome size. Altered precipitation treatments reduced leaf-level physiological rates; however this result did not vary by population or genome size. Our results suggest that trait variation in *P. virgatum* is primarily attributed to population-level adaptation across a latitudinal gradient, not genome size, and that neither population-level adaptation nor genome size may be important predictors of *P. virgatum* responses to future climatic conditions.

Key words: climate change; genome size; Konza Prairie; *Panicum virgatum*; population-level adaptation; water variability.

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INTRODUCTION

Climate models predict that atmospheric warming will alter global air circulation patterns and result in more variable inter-annual and intra-annual precipitation events over the next century, particularly in the North American Great Plains (Christensen et al. 2007). While the annual net precipitation is forecast to remain similar to the mean historical totals, the timing and magnitude of precipitation events are projected to change (Gordon et al. 1992, Meehl et al. 2005, Christensen et al. 2007). Particularly, rainfall events are expected to decrease in frequency and increase in magnitude, thereby lengthening the interval of dry periods between events (Easterling et al. 2000). In the Great Plains, longer dry periods between precipitation events coupled with elevated atmospheric temperatures will likely reduce soil moisture levels and expose grassland ecosystems to increasingly severe drought conditions throughout the growing

season (Petrie and Brunsell 2012).

Water availability is an important driver of mesic grassland dynamics, having profound impacts on the physiology of individual plants, the structure and productivity of plant populations and communities, as well as ecosystem C fluxes (Fay et al. 2002, Knapp et al. 2002, Nippert et al. 2006, Tucker et al. 2011). Consequently, altered precipitation variability associated with global climate change may have significant consequences for mesic grassland systems within the Great Plains (Knapp et al. 2008). Responses of plant populations to these changes will depend largely on the ability of individuals to respond to abiotic stresses and produce phenotypes that are more fit in these novel environments. Therefore, understanding the mechanisms dictating plastic responses to short-term climatic variability, as well as phenotypic traits that have the potential to influence long-term evolutionary changes, is essential for predicting ecosystem responses to future climatic conditions (Travers et al. 2010). In mesic grasslands, a mechanistic understanding of how dominant C_4 grasses respond to variable precipitation will help forecast future ecosystem dynamics because these species have high abundance and impact ecosystem structure and function more than subdominant C_3 grasses and forbs (McNaughton and Wolf 1970, Callaway et al. 2003, Smith and Knapp 2003, Hughes et al. 2008). C_4 grasses are also characterized by considerable genetic variation that enables a broad range of phenotypic responses to environmental change (Jump and Peñuelas 2005, Liu et al. 2012), which may confer a selective advantage to individuals under future climatic conditions (Avolio et al. 2012). Therefore, it is important to understand how dominant species such as *Panicum virgatum* L. (switchgrass) will respond to future environmental changes.

Panicum virgatum is a co-dominant, warm-season, perennial C_4 grass species that is widely distributed across many grassland ecosystems east of the Rocky Mountains and grows naturally in association with other tallgrasses such as *Andropogon gerardii* (big bluestem), *Sorghastrum nutans* (indiangrass), and *Schizachyrium scoparium* (little bluestem) in the Great Plains region of the United States (Nielson 1944, Parrish and Fike 2005). Ecologically, *P. virgatum* is highly productive over a range of environmental conditions and contributes significantly to the functioning of grassland systems (Sanderson et al. 2006). Due to its high yield, broad adaptability, and ability to grow on marginal land, *P. virgatum* is an important forage feedstock and a candidate for herbaceous bioenergy production (McLaughlin and Kszos 2005, Parrish and Fike 2005, Sanderson et al. 2006, Barney and DiTomaso 2008, Wright and Turhollow 2010). This species is generally more sensitive to water availability than other co-dominant C_4 grasses (Knapp 1985, Stout et al. 1988, Muir et al. 2001) and soil water status is a primary driver of *P. virgatum* physiology, productivity, and distribution (Sanderson 1992, Xu et al. 2006, Hartman et al. 2012). Additionally, *P. virgatum* is sensitive to subtle changes in air temperature (Hartman and Nippert 2012) and is more responsive to enhanced resource availability than other co-dominant grasses (Collins et al. 1998). For example, a recent study by Collins et al. (2012) showed that *P. virgatum* biomass increased in response to supplemental irrigation compared to other co-dominant species in a tallgrass prairie. Therefore, plastic responses at both the leaf-level and whole-plant level to changes in resource availability make it likely this species will have divergent response to future climate changes, such as increased precipitation variability.

Panicum virgatum responses to climate change have been difficult to generalize because natural populations are phenotypically and genetically diverse. Generally, this species is classified into two ecotypes, upland and lowland, according to morphology (Porter 1966, Parrish and Fike 2005). The upland ecotype is smaller, finer-stemmed, and has lower water and nitrogen requirements than the lowland ecotype (Sanderson et al. 2006). Conversely, the lowland ecotype has a large, robust form, and is more sensitive to drought stress than the upland ecotype (Stroup et al. 2003). Regional characteristics, such as latitude and climate, are important drivers of the structure and distribution of *P. virgatum* populations and ecotypes (Casler 2005). The distributions of upland and lowland ecotypes exhibit different geographic ranges, with the upland ecotype found more frequently in cooler, drier climates in northern latitudes and lowland ecotypes frequently found in warmer, wetter habitats of the southern latitudes (Casler et al. 2004),

although both ecotypes overlap in their distribution at mid-latitudes in the central United States (i.e., USDA hardiness zones 5 through 7; Casler et al. 2011). Adaptations to regional characteristics may influence the mechanisms governing *P. virgatum* population responses to abiotic stresses, leading to differential responses to climate change among geographically separated populations.

Responses to climate change may also differ between genetically variable, co-occurring individuals within a single population (Jump and Peñuelas 2005). In *P. virgatum*, genome size varies extensively between ecotypes as well as within and among populations (Porter 1966). *Pancium virgatum* is characterized by a ploidy series, ranging from diploid ($2n = 2x = 18$ chromosomes) to duodecaploid ($2n = 12x = 108$ chromosomes; Nielsen 1944). Generally, lowland ecotypes are tetraploid, while upland ecotypes are hexaploid or octoploid (Porter 1966, Hopkins et al. 1996, Casler 2005). However, upland cultivars have also been reported as tetraploid (Hopkins et al. 1996) and aneuploidy (the gain or loss of a single chromosome from the normal euploid set) is common (Costich et al. 2010). Ploidy levels also differ among individuals within a single population (Hultquist et al. 1996, 1997). Such variation may have evolutionary consequences for future populations if genome size is expressed in the phenotype of an individual and provides a selective advantage under abiotic stresses. Polyploids are thought to have broad ecological tolerances because increased heterozygosity resulting from genome duplication promotes trait variation and phenotypic plasticity within individuals (Otto and Whitton 2000). For example, variation in ploidy level influences anatomical characteristics such as cell size, cell volume, and enzyme concentrations (Otto and Whitton 2000). This variation can influence physiology and, in turn, functional responses to diverse abiotic conditions (Warner et al. 1987, MacGillivray and Grime 1995, Knight et al. 2005, Ohri 2005). Genome size of *P. virgatum* has been associated with variation in photosynthetic rates and chlorophyll concentrations (Warner et al. 1987, Wullschleger et al. 1996). However, it is unknown if these phenotypic traits are a direct consequence of genome size because genome size is closely associated with *P. virgatum* ecotype and geographical distribution. As a result, it is difficult to determine if *P. virgatum* traits are expressed due to genome size or polymorphic adaptation to site-specific conditions. If genome size does influence phenotypic traits, then within-population variation in genome size may be as important or even more important than adaptations to regional climates in driving population responses to climate change.

The current study examined the influence of population origin and genome size on the performance of *P. virgatum* individuals, both within and among natural populations, grown under future climatic conditions. Specifically, we measured physiological traits associated with leaf-level carbon gain, as well as plant productivity and reproductive allocation, to evaluate the influence of population origin and genome size on *P. virgatum* responses to altered timing of watering events. Our objectives were to (1) identify differences in physiology and productivity of *P. virgatum* individuals collected from natural populations across a latitudinal gradient, (2) investigate the response of these *P. virgatum* populations to altered timing of watering events and thus water availability, (3) identify differences in genome size among and within populations, and (4) examine the relationship between genome size and phenotypic traits under different water regimes. We hypothesized that phenotypic responses to extended dry periods between watering events would differ among populations and among individuals representing a range of genome sizes within populations.

Methods

Site description

Field work was conducted in the Rainfall Mesocosm Facility at the Konza Prairie Biological Station (KPBS), a Long Term Ecological Research (LTER) site. KPBS is a 3487 ha tallgrass prairie located in the Flint Hills region of northeastern Kansas, USA (39.1° N, 96.9° W). The site is characterized by a temperate mid-continental climate of cool, dry winters (−3°C average) and warm, wet summers (27°C average). For the 2011 growing season (May 1–September 30), the mean (±1 SEM) maximum air temperature was 30.83 ± 0.54°C and the mean minimum air temperature was 16.39 ± 0.54°C. Long-term mean annual

precipitation at KPBS is 860 mm (1891–2006), approximately 75% of which occurs during the growing season (April–September). The mesocosm facility was built in 2003 and contained 64 individual (1.44 $m^2 \times 1.8$ m) mesocosm "cells" arranged in two 2×16 arrays under an 11×25 m rainout shelter (Rainbow Plus, Stuppy Greenhouse Manufacturing, North Kansas City, MO, USA). Each cell was assembled using plastic-lined plywood and contained 30 cm of well-mixed A-horizon topsoil overlying B-horizon subsoil collected from native soil at KPBS (Nippert et al. 2007). The rainout shelter had open walls and ends that maximized air movement and heat dissipation, 2.4 m high eaves, and a roof constructed from clear corrugated polycarbonate (DynaGlas Plus, SPS International, San Jose, CA, USA) that allowed >90% light transmission. This facility was previously used for a precipitation manipulation experiment until 2007 (Fay et al. 2008), at which point all above and belowground biomass was removed by hand. The top 30 cm of soil was then homogenized and all subsequent volunteer plant growth was weeded by hand during the 2008–2011 growing seasons.

Plant material

P. virgatum rhizomes were collected from three geographically separated populations across a latitudinal gradient: KPBS, the Nature Conservancy's Tallgrass Prairie Preserve in northeast Oklahoma (36.8° N, 96.5° W), and the United States Department of Agriculture–Agriculture Research Service landholdings in east-central Texas (31.1° N, 97.3° W). Hereafter, these locations are referred to as Kansas, Oklahoma, and Texas populations, respectively. Each site along this latitudinal transect is native tallgrass prairie characterized by a similar environmental history and similar mean annual precipitation (see: Hartman et al. 2012). Approximately 50 rhizomes were collected from each population in 2008, as described by Hartman et al. (2012). Rhizomes were then randomly assigned to each mesocosm cell so that each cell contained individuals of a single geographic location. Individuals were spaced 40 cm apart, a density that promotes interplant competition but still facilitates high tiller numbers per plant (Sanderson and Reed 2000). Additional rhizomes from Kansas and Oklahoma were planted in 2009 to augment the plant density in cells which had a lower number of surviving individuals remaining from the previous year. During the 2011 growing season, 21 cells contained individuals from Kansas, 16 from Oklahoma, and 15 from Texas. For the research presented here, one plant was randomly selected per mesocosm cell for use in all subsequent analyses to ensure that all physiological measurements were collected on the same day within each sampling period.

Water treatments

Because *P. virgatum* is slow to establish, all mesocosm cells were watered equally, every 3–5 days, to promote rhizome establishment prior to the start of the experiment. For the current experiment, precipitation treatments were applied in the 2011 growing season (May 1–September 30) that altered the timing of watering events ("ambient" or "altered") without varying total growing season precipitation. For the ambient treatment, plants received 21 mm water every 6 days, the long-term mean ambient precipitation interval at KPBS. Altered treatment plants received 42 mm water every 12 days. Therefore, all plants received 626.25 mm water over the growing season, (long-term mean growing season precipitation), but in either frequent, small events or infrequent, large events. The ambient and altered treatments were designated so that a single water treatment was assigned to each mesocosm cell. Of the populations, 10 Kansas, 8 Oklahoma, and 8 Texas mesocosm cells received the ambient treatment, and 11 Kansas, 8 Oklahoma, and 7 Texas cells received the altered treatment. Watering events were scheduled so only ambient cells received water every sixth day and both ambient and altered cells received water every twelfth day.

Water from an onsite well was applied directly to the soil with a metered hand sprayer (model TM075; Great Plains Industries, Wichita, KS, USA) to prevent water loss to canopy interception or runoff. Relative water content (RWC) within the top 10 cm of soil immediately adjacent to plants used in analyses was measured using a Hydra Probe II Soil Sensor (Stevens Water Monitoring Systems, Portland, OR, USA) in units of water fraction by volume (wfv). Soil water content was measured immediately before and

one day after watering events when both ambient and altered treatments received water, beginning in June and ending in mid-September.

Physiological measurements

Leaf-level physiological responses to water treatments were evaluated throughout the 2011 growing season. Steady-state gas exchange, midday leaf water potential, and chlorophyll fluorescence were measured halfway through the growing season (July 18) and at the end of the growing season (September 18). Gas exchange and leaf water potential were measured 1 day before watering events in which ambient and altered cells were both watered. All gas exchange measurements were made on clear days between 1000 hours and 1600 hours (CST) when solar radiation was greater than 70% of full sun levels. Gas exchange was measured on the youngest, fully expanded leaf of each replicate plant using an open-flow gas exchange system with a red/blue LED light source (Li-6400, LiCOR, Inc., Lincoln, NE, USA). Environmental conditions inside the leaf cuvette were kept constant for all measurements. For all steady-state gas exchange measurements, flow rate was 500 μmol s^{-1}, light intensity was 1500 μmol m^{-2} s^{-1}, CO_2 concentration was 400 μmol CO_2 mol^{-1}, and relative humidity was maintained at ambient levels (40–60%). Leaves were allowed to reach steady-state photosynthesis within the cuvette chamber for 5–10 minutes as necessary. Parameters measured included CO_2 assimilation at ambient C_a (A_{max}), stomatal conductance of water vapor (g_s), and transpiration rate (E). Instantaneous water use efficiency (WUE) was calculated by dividing A_{max} by the corresponding E for each individual.

Midday water potential (Ψ_{mid}) was measured with gas exchange, between 1200 hours and 1600 hours. Water potential was measured on a morphologically and developmentally similar leaf of each individual measured for gas exchange using a Scholander-type pressure bomb (PMS Instruments, Albany, OR, USA). Chlorophyll fluorescence was measured using a PAM fluorometer (Li-6400XT, LiCOR Inc., Lincoln, NE, USA). Dark-adapted maximum photochemical efficiency (F_v/F_m) was measured on the same leaves used for gas exchange analysis, at least 1 hour after sunset to allow for complete dark-adaptation.

Biomass

Aboveground biomass was harvested following senescence in the 2011 growing season (October 15). The total number of flowering tillers (FT) and non-flowering tillers (NFT) was counted for each individual immediately upon harvesting. Biomass was dried at 65°C for at least 48 hours and flowering and non-flowering tillers were weighed separately to determine the percent of total biomass allocated to reproductive tissue. Additional parameters calculated include biomass per tiller, biomass per flowering tiller, and biomass per non-flowering tiller.

Genome size analysis

Flow cytometry was used to evaluate the approximate genome size of each individual used in all subsequent analyses. Nuclei suspensions were prepared using the methods described by Arumuganathan and Earle (1991) and Costich et al. (2010), with some modifications. On the day of the experiment, one leaf was harvested per plant, transported on ice, and kept refrigerated until used in sample preparation. Plant tissue was washed with deionized water prior to preparation to remove debris or fluorescent chemical residues. For each sample, approximately 50 mg tissue was finely chopped using a sharp razor blade in 1 ml of a chopping buffer solution over ice. The chopping buffer was prepared fresh on the day of analyses and, for each tissue sample, consisted of 975 μl $MgSO_4$ stock solution (10 mmol L^{-1} $MgSO_4$.7H2O, 50 mmol L^{-1} KCL, 5 mmol L^{-1} Hepes, pH adjusted to 8.0) + 25 μl 10% TritonX-100 solution + 1 mg DTT. The sample was then filtered through a 30 μm nylon mesh (product no. 03–30/18, Sefar Nitex) and centrifuged at 500x g for 7 minutes. The supernatant was discarded and the remaining pellet was re-suspended in a staining solution (2 ml chopping buffer, 2 ml propidium iodide in Vindelov's solution (Vindelov 1977)), with 2 μl triploid trout nuclei (product no. 1012, BioSure, Inc., USA) as an internal standard. Samples were stained for 20 minutes in a light-protected refrigerator immediately prior to flow cytometry analysis.

The relative fluorescence of sample and standard nuclei was measured using a FACS Calibur bench top flow cytometer (BD Biosciences, San Jose, CA). Stained nuclei were excited by an

argon laser operating at a wavelength of 488 nm and propidium iodide fluorescence was measured with a 610/20 nm band pass filter. Doublets and debris were excluded by gating the fluorescent peaks of the plant G_1/G_0 nuclei and the internal standard nuclei, and only samples in which there were >500 nuclei for both the unknown and internal standard (>1000 total nuclei) were included in the analysis. Nuclear DNA content of the unknown sample was determined by comparing mean fluorescent peaks of the plant nuclei with mean peaks of the corresponding internal standard nuclei for each sample. Genome size was calculated using the following formula:

$$\text{Genome size} = \frac{\text{Mean fluorescence}_{\text{sample nuclei}}}{\text{Mean fluorescence}_{\text{standard nuclei}}} \times 7.8 \text{ pg}$$

where 7.8 pg equals the genome size of the triploid trout standard. Because aneuploidy is widespread among *P. virgatum*, ploidy level cannot be designated based on nuclear genome size without cytological confirmation (Costich et al. 2010). Therefore, we used genome size, rather than ploidy, in comparative analyses with plant traits.

Statistical analyses

To examine the effect of population origin on *P. virgatum* responses to precipitation variability, leaf-level physiology and aboveground biomass were analyzed by a model I, two-way analysis of variance (ANOVA, $\alpha = 0.05$), with population and water treatment as main effects. Because *P. virgatum* physiology varies across the growing season, reflecting changes in reproductive development and ambient environmental conditions (Knapp et al. 1998), data were analyzed separately by each measurement period. Differences in RWC between water treatments over the 2011 growing season were also evaluated using a two-way ANOVA, with treatment and sampling date as main effects. Because soil RWC generally increases following the addition of water and we were primarily interested in detecting differences between water treatments over time, RWC measured one day before and one day after watering events in which both ambient and altered treatments received water were analyzed separately. Finally, a one-way ANOVA was used to evaluate differences in nuclear DNA content between populations. Multiple comparison tests between populations were analyzed with Tukey's Honestly Significant Difference test, and data were log transformed when necessary to conform to the assumptions of the ANOVA test.

The relationships between individual parameters and genome size were evaluated using least-squares linear regression analysis. These relationships were evaluated for each water treatment separately, as well as both water treatments combined, for each measurement date. Heterogeneity of slopes for each water treatment was evaluated using ANCOVA, with the measured dependent variable and water treatment as main effects and nuclear DNA content as a covariate. All water treatment × DNA content interactions are presented regardless of significance; however, models were simplified by removing non-significant water treatment × DNA content interactions. Thus, main effects are presented from the simplified model in which non-significant interaction terms were removed, when applicable. All statistical analyses were performed using the open-source statistical package R (R Development Core Team 2011).

Results

Relative water content

Relative water content (RWC) was significantly different between treatment cells before and after watering events in which both treatments received water (Fig. 1). Averaged over the entire experimental period, mean RWC was 19.8% lower in altered cells compared to ambient cells before watering events in which both treatments were watered. However, a significant treatment × date interaction indicated that differences in RWC between water treatments decreased as the growing season progressed ($F_{7,368} = 3.21$, $p < 0.01$). After both treatments were watered, RWC was 7.0% greater in the altered cells compared to the ambient cells ($F_{1,426} = 14.19$, $p < 0.01$). This response did not differ across the growing season.

Physiology

Leaf-level gas exchange traits (A_{max}, g_s, and E) did not differ between populations or water treatments in July, but differed significantly in September (Fig. 2). In September, A_{max} was

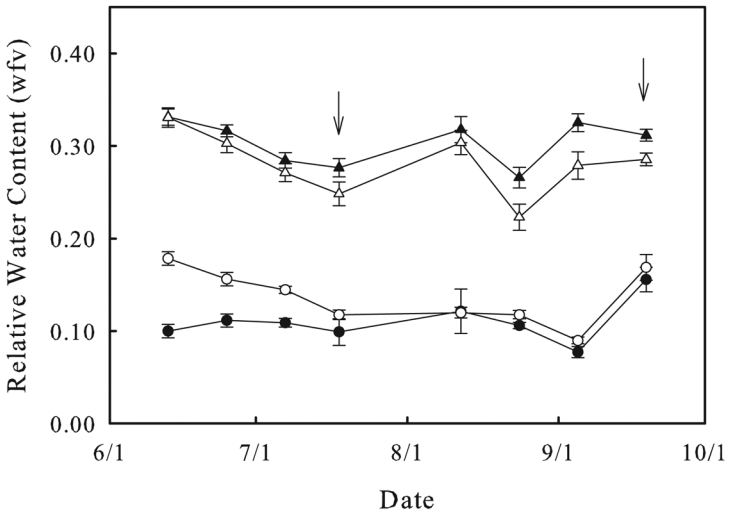

Fig. 1. Mean (±1 SEM) relative water content of soil measured immediately before (circles) and one day after (triangles) watering events in which both treatments received water. Included are data for both ambient (open symbols) and altered (closed symbols) water treatments across the 2011 growing season. Arrows indicate days in which leaf-level physiology was evaluated. For clarity, data collected before and after watering events are presented on the date of the watering event with which they are associated. For each treatment group, n = 26.

83.8% and 48.4% greater in the Texas population than the Kansas and Oklahoma populations, respectively ($F_{2,39} = 8.48$, $p < 0.01$). Similarly, g_s ($F_{2,39} = 8.47$, $p < 0.01$) and E ($F_{2,39} = 5.88$, $p = 0.01$) were significantly greater in the Texas than the Kansas and Oklahoma populations. Water treatment also had significant effects on gas exchange physiology in September. The altered water regime reduced A_{max} by 28.0%, when averaged across all populations ($F_{1,39} = 5.82$, $p = 0.02$). Likewise, g_s was 26.2% lower ($F_{1,39} = 3.88$, $p = 0.06$) and E was 28.9% lower ($F_{1,39} = 4.69$, $p = 0.04$) in plants receiving the altered treatment compared to the ambient treatment. WUE and Ψ_{mid} did not differ between populations or water treatments at either measurement date (Table 1). F_v/F_m differed between populations, but not water treatments, in September (Table 1). F_v/F_m was significantly greater in the Texas population compared to the Kansas and Oklahoma populations ($F_{2,38} = 4.18$, $p = 0.02$). There were no significant population × water treatment interactions for any variable in either measurement period.

Biomass

The total biomass of each individual was significantly different between populations. On average, Texas plants were 77.3% and 63.8% larger than Kansas and Oklahoma plants, respectively ($F_{2,38} = 0.71$, $p < 0.01$). This observation was largely driven by the increase in flowering tiller biomass of Texas plants relative to Kansas and Oklahoma plants ($F_{2,38} = 7.48$, $p < 0.01$). Non-flowering tiller biomass did not differ between populations ($p > 0.05$, Table 2). At the end of the growing season, Texas plants had the fewest number of flowering and non-flowering tillers, although this was not significant ($p > 0.05$, Table 2). However, Texas plants had 139.2% and 102.2% more biomass per tiller than Kansas and Oklahoma plants, respectively ($F_{2,38} = 29.59$, $p < 0.001$), as well as 146.6% and 101.6% more biomass per flowering tiller than Kansas and Oklahoma plants, respectively ($F_{2,38} = 32.66$, $p < 0.01$). There were no significant differences between populations for any other biomass characteristic (Table 2). Water treatment did not affect biomass, nor were there any significant population × water treatment interactions.

Relationships with genome size

Nuclear DNA content was significantly lower in the Texas population compared to the Kansas (46.3% lower) and Oklahoma populations (45.2% lower), but did not differ between the Kansas

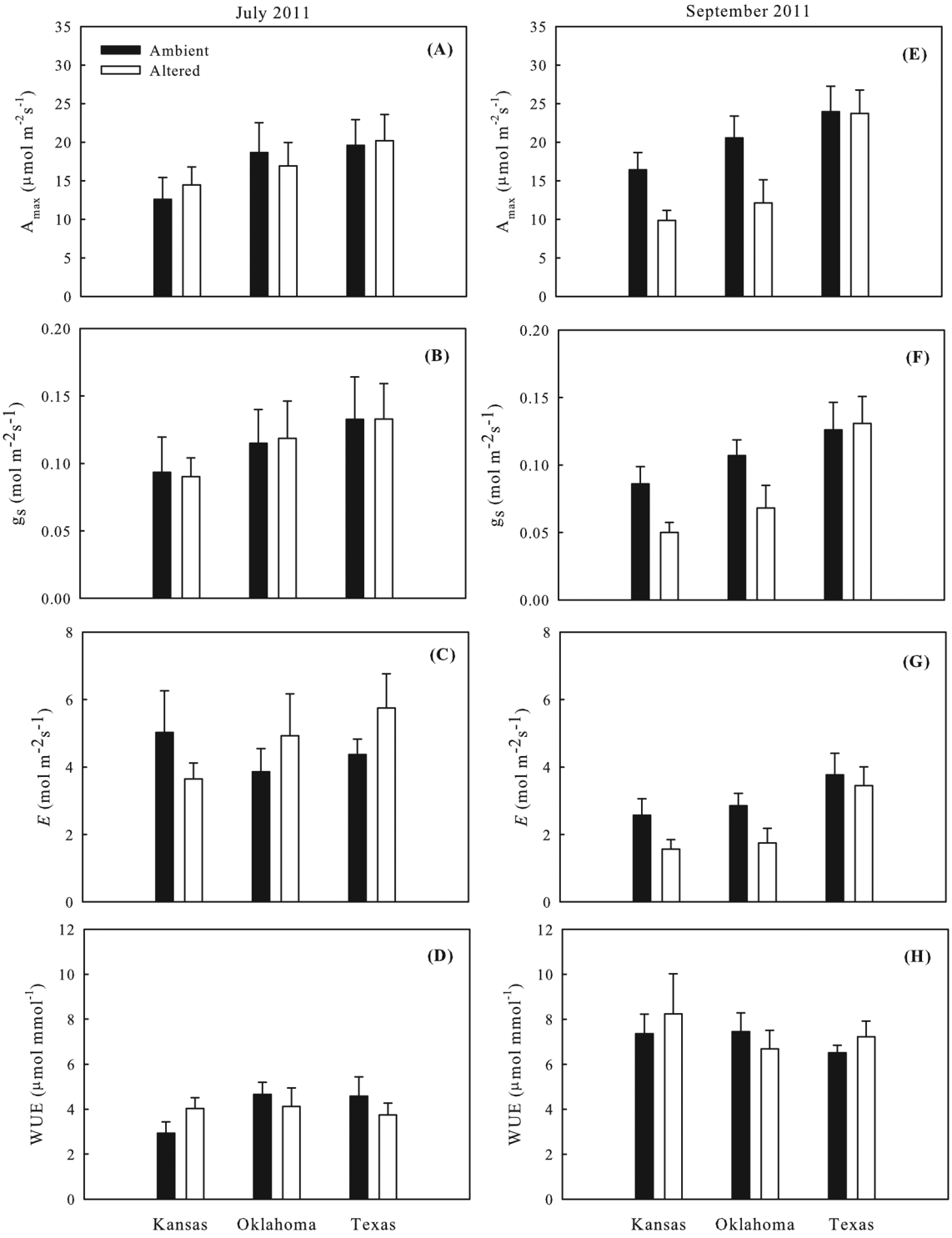

Fig. 2. Mean physiological responses (±1 SEM) of *Panicum virgatum* to ambient and altered precipitation treatments, measured in July and September 2011. (A, E) CO_2 assimilation at ambient C_a (A_{max}), (B, F) stomatal conductance (g_s), (C, G) transpiration rate (E), and (D, H) instantaneous water-use efficiency (WUE). For each population × treatment group, n = 4–10.

Table 1. Physiological responses of *P. virgatum* populations to water treatments, including chlorophyll fluorescence (F_v/F_m) and midday leaf water potential (Ψ_{mid}).

	Kansas		Oklahoma		Texas	
Trait	Ambient	Altered	Ambient	Altered	Ambient	Altered
F_v/F_m						
July	0.79 ± 0.01	0.79 ± 0.002	0.79 ± 0.004	0.79 ± 0.003	0.80 ± 0.003	0.79 ± 0.002
Sept	0.76^A ± 0.01	0.76^A ± 0.01	0.77^A ± 0.01	0.77^A ± 0.01	0.78^A ± 0.01	0.78^A ± 0.01
Ψ_{mid}						
July	−1.71 ± 0.12	−1.80 ± 0.20	−2.36 ± 0.29	−1.70 ± 0.37	−1.60 ± 0.33	−1.15 ± 0.19
Sept	−1.83 ± 0.20	−2.02 ± 0.19	−1.82 ± 0.16	−1.53 ± 0.34	−1.58 ± 0.15	−1.24 ± 0.29

Notes: Data are sample means (± 1 SEM) of physiological traits measured in July and September from 4–11 individuals per population × treatment group. Significant differences ($\alpha = 0.05$) between populations are indicated by superscript.

and Oklahoma populations ($F_{2,43} = 22.29$, $p < 0.01$). DNA content ranged from 1.61–2.58 pg in the Texas population, 2.15–5.23 pg in the Kansas population, and 1.69–5.27 pg in the Oklahoma population. In addition to having a lower mean, the Texas DNA content had a smaller range (0.97 pg) than the Kansas (3.08 pg) and the Oklahoma (3.58 pg) populations. Finally, the DNA content of the Texas population had a lower variance (0.095) compared to the DNA content of the Kansas (0.702) and Oklahoma (1.07) populations. The low mean, range, and variance of nuclear DNA content in the Texas population suggested that only tetraploid individuals were present, so significant differences in plant traits between the Texas population and the Kansas and Oklahoma populations (Fig. 2, Tables 1 and 2) would likely influence the relationships between plant traits and smaller genome sizes in regression analyses. Therefore, Texas individuals were removed from ANCOVA and linear regression analyses to eliminate the influence of this population-level adaptation when evaluating genome size relationships with plant traits. The Kansas and Oklahoma populations had larger ranges of genomes sizes and did not differ in any

Table 2. Biomass characteristics of *P. virgatum* populations grown under water treatments, including total biomass, flowering tiller (FT) biomass, non-flowering tiller (NFT) biomass, percent reproductive biomass, total tiller number, flowering tiller number, non-flowering tiller number, biomass per tiller, biomass per flowering tiller, and biomass per non-flowering tiller.

	Kansas		Oklahoma		Texas	
Trait	Ambient	Altered	Ambient	Altered	Ambient	Altered
Total biomass (g)	372.95^A ±54.52	430.39^A ±81.92	443.66^A ±419.13	419.13^A ±57.44	678.04^A ±104.04	735.10^B ±164.03
FT biomass (g)	347.94^A ±52.71	418.06^A ±79.59	429.78^A ±54.48	385.96^A ±55.81	653.34^A ±95.08	715.36^B ±162.84
NFT biomass (g)	25.01 ±6.02	12.33 ±4.54	19.86 ±10.86	33.16 ±24.30	24.70 ±11.75	19.74 ±9.96
% Reproductive	92.41 ±2.26	97.08 ±0.75	95.63 ±2.01	92.96 ±4.54	96.90 ±1.20	97.30 ±1.31
Total tiller #	132.50 ±18.96	155.88 ±29.23	121.88 ±22.92	148.00 ±30.40	102.40 ±13.81	93.20 ±15.98
FT #	112.10 ±16.14	142.25 ±27.49	110.25 ±23.26	124.75 ±20.93	89.20 ±9.32	82.60 ±13.68
NFT #	20.40 ±4.56	13.63 ±3.82	11.63 ±5.19	23.25 ±16.58	13.20 ±5.16	10.60 ±3.36
Biomass/tiller (g)	3.02^A ±0.30	2.96^A ±0.40	3.92^A ±0.40	3.16^A ±0.32	6.65^B ±0.58	7.67^B ±1.06
Biomass/FT (g)	3.20^A ±0.19	3.13^A ±0.40	4.45^A ±0.59	3.30^A ±0.32	7.32^B ±0.69	8.31^B ±1.10
Biomass/NFT (g)	2.21 ±1.23	0.91 ±0.21	1.44 ±0.35	1.35 ±0.26	1.61 ±0.22	1.49 ±0.46

Notes: Data are sample means (± 1 SEM) from 5–10 individuals per population × treatment group. Significant differences ($\alpha = 0.05$) between populations are indicated by superscript.

Table 3. ANCOVA results for *P. virgatum* physiology, including CO_2 assimilation at ambient C_a (A_{max}), stomatal conductance of water vapor (g_s), transpiration rate (E), instantaneous water use efficiency (WUE), midday leaf water potential (Ψ_{mid}), and chlorophyll fluorescence (F_v/F_m).

	July			September		
Trait	df	*F*	*p*	df	*F*	*p*
A_{max}						
Treatment	1, 27	<0.01	0.95	1, 29	11.86	<0.01*
DNA	1, 27	0.39	0.54	1, 29	2.03	0.17
T × D	1, 26	4.08	0.05*	1, 28	0.33	0.57
g_s						
Treatment	1, 27	0.10	0.76	1, 29	10.63	<0.01*
DNA	1, 27	1.01	0.32	1, 29	1.95	0.17
T × D	1, 26	1.78	0.19	1, 28	<0.01	0.98
E						
Treatment	1, 27	1.44	0.24	1, 29	12.94	<0.01*
DNA	1, 27	4.23	0.05*	1, 29	6.42	0.02*
T × D	1, 26	1.16	0.29	1, 28	0.11	0.74
WUE						
Treatment	1, 27	1.25	0.27	1, 29	0.15	0.71
DNA	1, 27	0.68	0.42	1, 29	0.52	0.48
T × D	1, 26	0.07	0.79	1, 28	0.32	0.58
Ψ_{mid}						
Treatment	1, 42	1.02	0.32	1, 40	0.01	0.93
DNA	1, 42	2.00	0.17	1, 40	5.90	0.02*
T × D	1, 41	0.29	0.59	1, 39	0.87	0.36
F_v/F_m						
Treatment	1, 32	0.12	0.73	1, 29	13.93	<0.01*
DNA	1, 32	<0.01	0.97	1, 29	1.78	0.19
T × D	1, 31	0.573	0.46	1, 28	14.65	<0.01*

Notes: Results are presented for combined Kansas and Oklahoma populations, in July and September separately. Main effects (Treatment and DNA) are presented from the simplified model in which non-significant interaction terms (T × D) were removed, when applicable. Significance at the $\alpha = 0.05$ level is indicated by an asterisk (*).

measured parameter, including mean nuclear DNA content; therefore, these populations were grouped together when evaluating genome size relationships with plant traits in the initial regression analysis. A subsequent analysis was performed including the Texas population to examine the role of genome size regardless of local adaptation.

In the initial analysis, ANCOVA yielded no significant treatment × DNA content interactions for most physiology, leaf chemistry, and biomass characteristics in both measurement periods (Tables 3 and 4). This indicates that the slopes of the relationships between genome size and individual dependent variables did not differ between water treatments. There were also few significant main effects of water treatment or DNA on most measured characteristics (Tables 3 and 4). Nuclear DNA content had a significant effect on *E* at both measurement dates (Table 3). There was also a significant effect of DNA on total biomass, flowering tiller biomass, total tiller number, and flowering tiller number in September (Table 4). Water treatment had a significant effect on A_{max}, g_s, *E*, F_v/F_m, and flowering tiller number in September (Tables 3 and 4), indicating that the y-intercepts of the relationship between the measured characteristic and DNA content were significantly different for each water treatment. Least-squares linear regression analysis revealed that the y-intercepts of the ambient treatment were greater than that of the altered treatment for A_{max}, g_s, *E*, and flowering tiller number (Tables 5 and 6). However, the y-intercept of the F_v/F_m-DNA content relationship was lower for the ambient treatment than the altered treatment (Table 5). Additionally, only a small fraction of the total variance for each regression relationship could be explained by the corresponding model (Tables 5 and 6).

To confirm that trait differences in the Texas population compared to the Kansas and Oklahoma populations would indeed influence the relationship between genome size and traits, additional ANCOVA and linear regression analyses were conducted using data from all three locations. However, including Texas individuals in the analyses did not result in any significant

relationship between genome size and plant traits (Appendices A and B), suggesting that this relationship is weak regardless of whether or not local adaptation is considered.

Discussion

Although *P. virgatum* physiology varies extensively among ecotypes and geographically separated populations (Stroup et al. 2003, Sanderson et al. 2006), it is unknown if within-population variation in genome size is a more important driver of population responses to environmental change than population-level adaptations to regional climates. The results presented here decouple the influence of population origin from genome size by investigating *P. virgatum* responses to water variability among natural populations, as well as among individuals characterized by different genomes sizes within populations. Leaf-level physiology and growth differed among populations, but not across a range of genome sizes within populations. In addition, physiological responses to water variability did not manifest until late in the growing season, and these responses did not differ with respect to population origin or genome size. These results suggest neither population origin nor genome size may be important predictors of *P. virgatum* responses to future climatic conditions on a broad geographic scale.

We found differences among populations in most traits measured but this did not affect the response of the populations to variation in water timing, as indicated by the lack of a significant population × treatment interaction for all measured variables. For example, leaf physiology (A_{max}, g_s, *E*, and F_v/F_m) was lower in the Kansas and Oklahoma populations, relative to the Texas population, at the end of the growing season (Table 1, Fig. 2). However, altered water timing reduced leaf gas exchange traits (A_{max}, g_s, and *E*) similarly across all populations. This is somewhat surprising because *P. virgatum* physiology is generally more sensitive to changes in water availability than other C_4 grasses (Knapp 1985, Stout et al. 1988, Muir et al. 2001, Barney et al. 2009), and in the current study, extended dry periods between watering events reduced soil RWC for most of the growing season (Fig. 1). However, these changes in physiology did not translate to changes in plant productivity because water variability only impacted leaf physiology after plants had nearly finished seasonal growth. These results are similar to the observations of Craine et al. (2012), who found that drought does not impact grass aboveground net primary productivity (ANPP) late in the growing

Table 4. ANCOVA results for *P. virgatum* biomass, including total biomass, flowering tiller (FT) biomass, non-flowering tiller (NFT) biomass, total tiller number, flowering tiller number, non-flowering tiller number, biomass per tiller, biomass per flowering tiller, biomass per non-flowering tiller, and percent reproductive biomass.

Trait	df	*F*	*p*
Total biomass			
Treatment	1, 29	2.52	0.12
DNA	1, 29	10.55	<0.01*
T × D	1, 28	0.43	0.52
FT biomass			
Treatment	1, 29	2.60	0.12
DNA	1, 29	11.65	<0.01*
T × D	1, 28	0.52	0.48
NFT biomass			
Treatment	1, 29	0.01	0.92
DNA	1, 29	0.03	0.87
T × D	1, 28	0.22	0.64
Total tillers			
Treatment	1, 29	3.67	0.07
DNA	1, 29	5.13	0.03*
T × D	1, 28	0.40	0.53
FT #			
Treatment	1, 29	4.47	0.04*
DNA	1, 29	6.77	0.02*
T × D	1, 28	0.60	0.44
NFT #			
Treatment	1, 29	0.09	0.76
DNA	1, 29	0.02	0.89
T × D	1, 28	<0.01	0.95
Biomass/tiller			
Treatment	1, 29	0.79	0.38
DNA	1, 29	0.18	0.67
T × D	1, 28	1.28	0.27
Biomass/FT			
Treatment	1, 29	1.08	0.31
DNA	1, 29	0.38	0.54
T × D	1, 28	1.71	0.20
Biomass/NFT			
Treatment	1, 29	0.79	0.38
DNA	1, 29	0.03	0.88
T × D	1, 28	0.84	0.37
% Reproductive			
Treatment	1, 29	0.16	0.69
DNA	1, 29	0.06	0.82
T × D	1, 28	0.57	0.46

Notes: Results are presented for combined Kansas and Oklahoma populations. Main effects (Treatment and DNA) are presented from the simplified model in which non-significant interaction terms (T × D) were removed, when applicable. Significance at the $\alpha = 0.05$ level is indicated by an asterisk (*).

Table 5. Least-squares linear regression results for *P. virgatum* physiology, including CO_2 assimilation at ambient C_a (A_{max}), stomatal conductance of water vapor (g_s), transpiration rate (E), instantaneous water use efficiency (WUE), midday leaf water potential (Ψ_{mid}), and chlorophyll fluorescence (F_v/F_m).

	July				September			
Trait	*y*-intercept	Slope	r^2	*p*	*y*-intercept	Slope	r^2	*p*
A_{max}								
Ambient	38.07	−5.71	0.22	0.08	31.74	−3.18	0.11	0.24
Altered	10.03	1.43	0.04	0.49	15.75	−1.43	0.05	0.42
All	18.98	−1.16	0.02	0.48	14.62	−0.08	<0.01	0.96
g_s								
Ambient	0.27	−0.04	0.15	0.15	0.14	−0.01	0.05	0.42
Altered	0.10	<−0.01	<0.01	0.98	0.09	−0.01	0.07	0.29
All	0.15	−0.01	0.03	0.34	0.08	<−0.01	<0.01	0.93
E								
Ambient	12.93	−0.72	0.21	0.09	5.59	−0.68	0.16	0.14
Altered	6.56	−2.01	0.10	0.25	3.45	−0.52	0.22	0.06
All	7.76	−0.91	0.10	0.10	3.11	−0.25	0.03	0.32
WUE								
Ambient	2.82	0.17	0.01	0.80	7.59	−0.04	<0.01	0.97
Altered	2.75	0.38	0.05	0.43	4.38	0.91	0.04	0.47
All	3.25	0.14	0.01	0.68	5.75	0.45	0.01	0.53
Ψ_{mid}								
Ambient	−1.16	−0.16	0.06	0.14	−1.00	−0.21	0.14	0.22
Altered	−1.17	−0.19	0.11	0.60	−1.30	−0.13	0.08	0.05*
All	−1.32	−0.08	0.01	0.10	−0.74	−0.29	0.18	0.02*
F_v/F_m								
Ambient	0.78	<0.01	0.02	0.64	0.68	0.02	0.44	0.01*
Altered	0.79	<−0.01	0.03	0.49	0.80	−0.01	0.23	0.05*
All	0.79	<−0.01	<0.01	0.86	0.76	<0.01	<0.01	0.92

Notes: Results for combined Kansas and Oklahoma populations are presented for water treatments separately (Ambient and Altered), as well as ambient and altered treatments combined (All), for data collected in July and September. Significance at the $\alpha = 0.05$ level is indicated by an asterisk (*).

season at the Konza Prairie. Therefore, water variability associated with climate change may only impact *P. virgatum* physiology early in the growing season, and when it does, geographically separated *P. virgatum* populations will likely respond similarly. Interestingly, these responses do have the potential to vary with local environmental factors. In a long-term irrigation experiment, Collins et al. (2012) found that *P. virgatum* cover increased in abundance relative to other dominant C_4 grass species (e.g., *Andropogon gerardii*). However, this only occurred in irrigated lowlands, suggesting that local environmental factors, such as topoedaphic variation, may be important in determining *P. virgatum* responses to climate change.

Population origin did not influence plant responses to water variability, suggesting that broad geographic responses of *P. virgatum* to future changes in precipitation timing may be similar across the distribution of the species. However within a single population, genetically variable, co-occurring individuals may also exhibit differential responses to environmental change (Jump and Peñuelas 2005, Parmesan 2006), leading to particular phenotypes having a selective advantage (Davis et al. 2005, Skelly et al. 2007, Gienapp et al. 2008, Hoffmann and Willi 2008). In *P. virgatum*, evolutionary changes may be driven by variation in genome size. Genome size varies extensively between and within *P. virgatum* populations and can potentially influence plant traits or plastic responses to environmental change. Therefore, understanding how genome size influences *P. virgatum* traits will be particularly important when forecasting long-term evolutionary changes within and between populations.

Understanding how genome size impacts plant traits and plastic responses to environmental variability is difficult to assess for *P. virgatum* because genome size is typically associated with ecotype and geographic distribution (Parrish and Fike 2005). Based on our flow cytometry analyses, we found a greater range of genome sizes in the Kansas and Oklahoma populations compare to the Texas population, suggesting that both upland and lowland ecotypes were present in

Kansas and Oklahoma but only the lowland ecotype was present in Texas. To our knowledge, this is the first experiment to account for the effects of population origin when investigating the role of genome size in determining trait variation within natural populations of a common grass species. We found that, without bias of population origin, genome size does not influence the *P. virgatum* traits measured or their responses to water variability. Although genome size had a significant effect on some plant traits (*E*, total biomass, flowering tiller biomass, total tiller number, and flowering tiller number), our results indicated that plant traits were only weakly, if at all, associated with genome size. Likewise, genome size did not affect plant responses to water treatments. The altered precipitation treatment reduced some physiological traits, but these responses did not differ according to genome size. These results suggest that genome size may not be an important predictor of physiological differences among *P. virgatum* individuals, and will not likely impact *P. virgatum* responses to altered precipitation patterns in the future. Although these data are limited in that they only include *P. virgatum* from two locations (Kansas and Oklahoma), they do suggest that genome size may not always be expressed in the phenotype of an individual and that increased heterozygosity associated with large genome sizes may not lead to greater plasticity in phenotypic responses to environmental variation. Furthermore, these results did not differ when Texas individuals (all which all were tetraploids) were included in the analysis. The lack of a relationship between genome size and traits that have the potential to improve plant fitness suggest that genome size may not drive adaptive changes that differ between *P. virgatum* populations.

Table 6. Least-squares linear regression results for *P. virgatum* biomass, including total biomass, flowering tiller (FT) biomass, non-flowering tiller (NFT) biomass, total tiller number, flowering tiller number, non-flowering tiller number, biomass per tiller, biomass per flowering tiller, biomass per non-flowering tiller, and percent reproductive biomass.

Trait	*y*-intercept	Slope	r^2	*p*
Total biomass				
Ambient	72.43	80.67	0.13	0.16
Altered	−10.16	126.44	0.39	0.01*
All	88.54	85.63	0.20	0.01*
FT biomass				
Ambient	55.62	79.78	0.13	0.15
Altered	−39.93	128.13	0.43	0.01*
All	61.06	86.99	0.22	0.01*
NFT biomass				
Ambient	−4.89	6.63	0.04	0.46
Altered	29.77	−1.69	<0.01	0.91
All	19.65	1.04	<0.01	0.89
Total tillers				
Ambient	44.71	20.45	0.06	0.34
Altered	20.83	39.18	0.22	0.08
All	65.19	20.1	0.07	0.14
FT #				
Ambient	33.1	16.16	0.06	0.34
Altered	3.02	38.69	0.31	0.03*
All	47.11	20.08	0.09	0.09
NFT #				
Ambient	11.61	1.26	<0.01	0.82
Altered	17.81	0.50	<0.01	0.96
All	18.09	0.03	<0.01	0.99
Biomass/tiller				
Ambient	1.60	0.43	0.07	0.29
Altered	3.31	−0.10	0.01	0.71
All	2.48	0.19	0.03	0.37
Biomass/FT				
Ambient	1.25	0.58	0.10	0.23
Altered	3.47	−0.10	0.01	0.72
All	2.39	0.28	0.05	0.24
Biomass/NFT				
Ambient	−0.89	0.65	0.02	0.55
Altered	1.96	−0.27	0.20	0.10
All	0.45	0.27	0.01	0.54
% Reproductive				
Ambient	0.91	−0.01	<0.01	0.84
Altered	0.85	0.01	0.01	0.69
All	0.89	<−0.01	<0.01	0.95

Note: Results for combined Kansas and Oklahoma populations are presented for water treatments separately (Ambient and Altered), as well as ambient and altered treatments combined (All). Significance at the $\alpha = 0.05$ level is indicated by an asterisk (*).

Although genome size did not influence *P. virgatum* phenotypes or responses to water variability in this study, genome size may have important consequences in other plant species. Therefore, we believe that it is still important to consider the effects of genome size on plant traits independently of genome size. Previous studies have reported conflicting results regarding the relationship between plant traits and genome size, particularly in response to water stress. For example, Chandrasekar et al. (2000) found that tetraploid wheat varieties (genus *Triticum*) were more drought tolerant than hexaploid varieties. Conversely, tetraploid and hexaploid varieties of Russian wildrye (*Psathyrostachys juncea*) (Frank and Berdahl 2001), Japanese honeysuckle (*Lonicera japonica*) (Li et al. 2009), and the genus *Cenchrus* (Chandra and Dubey 2009) have all exhibited higher rates of photosynthesis and

greater tolerance to drought stress compared to diploid varieties. In *P. virgatum*, greater photosynthetic rates have been observed in natural populations of upland octoploids than lowland tetraploids, reflecting variation in biochemistry associated with genome size (Warner et al. 1987). In a similar study, Wullschleger et al. (1996) could not confirm these results, and suggested that *P. virgatum* physiology is controlled primarily by local adaptation to environmental conditions rather than genome size. Although the relationship between genome size and plant traits may be species-specific, the disparities between these findings may actually have resulted from the lack of decoupling genome size from local adaptation effects. Thus, there is a need to control for population-level adaptation in future studies of genome size because genome size may not necessarily be related to traits within populations adapted to site-specific conditions and because within-population variation in genome size may also differ among populations. These results have greater importance in the face of global climate change, as polyploidy is widespread, occurring in up to 80% of all plant species (Otto and Whitton 2000), and may influence the short-term plastic responses of individuals and long-term evolutionary responses of populations to environmental change (Knight et al. 2005, Otto 2007).

In summary, phenotypic traits varied between *P. virgatum* populations, but not across a range of genome sizes within populations. Additionally, *P. virgatum* responses to water variability did not differ according to population or genome size, indicating that neither are likely to act as a selective force for *P. virgatum* under future precipitation regimes. We can therefore expect precipitation variability to impact *P. virgatum* similarly across geographically separated populations despite differences in genome size. Finally, physiological stress responses did not manifest early enough in the growing season to impact biomass production, suggesting that precipitation variability may not alter *P. virgatum* productivity. Although this experiment only included data from a single growing season, these results have the potential to guide theoretical predictions of how population origin and/or within-population variation in genome size will influence individual and population responses of this ecologically important C_4 grass species to an altered climate, particularly as plant responses to climate change in an evolutionarily relevant context are not yet fully understood.

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Literature Cited

Arumuganathan, K. and E. D. Earle. 1991. Estimation of nuclear DNA content of plants by flow cytometry. Plant Molecular Biology Reporter 9:229–233.

Avolio, M. L., J. M. Beaulieu, and M. D. Smith. 2012. Genetic diversity of a dominant C_4 grass is altered with increased precipitation variability. Oecologia. doi: 10.1007/s00442-012-2427-4

Barney, J. N. and J. M. DiTomaso. 2008. Nonnative species and bioenergy: are we cultivating the next invader? BioScience 58:64–70.

Barney, J. N., J. J. Mann, G. B. Kyser, E. Blumwald, A. Van Deynze, and J. M. DiTomaso. 2009. Tolerance of switchgrass to extreme soil moisture stress: Ecological implications. Plant Science 177:724–732.

Callaway, R. M., S. C. Pennings, and C. L. Richards. 2003. Phenotypic plasticity and interactions among plants. Ecology 84:1115–1128.

Casler, M. D. 2005. Ecotypic variation among switchgrass populations from the northern USA. Crop Science 45:388–398.

Casler, M. D., C. M. Tobias, S. M. Kaeppler, C. R. Buell, Z. Wang, P. Cao, J. Schmutz, and P. Ronald. 2011. The switchgrass genome: tools and strategies. Plant Genome 4:273–282.

Casler, M. D., K. P. Vogel, C. M. Taliaferro, and R. L. Wynia. 2004. Latitudinal adaptation of switchgrass populations. Crop Science 44:293–303.

Chandra, A. and A. Dubey. 2009. Assessment of ploidy level on stress tolerance of *Cenchrus* species based

on leaf photosynthetic characteristics. Acta Physiologia Plantarum 31:1003–1013.

Chandrasekar, V., R. K. Sairam, and G. C. Srivastava. 2000. Physiological and biochemical responses hexaploid and tetraploid wheat to drought stress. Journal of Agronomy & Crop Science 185:219–227.

Christensen, J. H., et al. 2007. Regional climate projections. *In* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Collins, S. L., A. K. Knapp, J. M. Briggs, J. M. Blair, and E. M. Steinauer. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. Science 280:745–747.

Collins, S. L., S. E. Koerner, J. A. Plaut, J. G. Okie, D. Brese, L. B. Calabrese, A. Carvajal, R. J. Evansen, and E. Nonaka. 2012. Stability of tallgrass prairie during a 19-year increase in growing season precipitation. Functional Ecology 26:1450–1459.

Costich, D. E., B. Friebe, M. J. Sheehan, M. D. Casler, and E. S. Buckler. 2010. Genome-size variation in switchgrass (*Panicum virgatum*): flow cytometry and cytology reveal rampant aneuploidy. Plant Genome 3:130–141.

Craine, J. M., J. B. Nippert, A. J. Elmore, A. M. Skibbe, S.L. Hutchinson, and N. A. Brunsell. 2012. The timing of climate variability and grassland productivity. Proceedings of the National Academy of Sciences USA. doi: 10.1073/pnas.1118438109

Davis, M. B., R. G. Shaw, and J. R. Etterson. 2005. Evolutionary responses to changing climate. Ecology 86:1704–1714.

Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Changnon, T. R. Karl, and L. O. Mearns. 2000. Climate extremes: Observations, modeling, and impacts. Science 289:2068–2074.

Fay, P. A., J. D. Carlisle, B. T. Danner, M. S. Lett, J. K. McCarron, C. Stewart, A. K. Knapp, J. M. Blair, and S.L. Collins. 2002. Altered rainfall patterns, gas exchange, and growth in grasses and forbs. International Journal Plant Sciences 163:549–557.

Fay, P. A., D. M. Kaufman, J. B. Nippert, J. D. Carlisle, and C. W. Harper. 2008. Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. Global Change Biology 14:1600–1608.

Frank, A. B. and J. D. Berdahl. 2001. Gas exchange and water relations in diploid and tetraploid Russian Wildrye. Crop Science 41:87–92.

Gienapp, P., C. Teplitsky, J. S. Alho, J. A. Mills, and J. Merilä. 2008. Climate change and evolution: disentangling environmental and genetic responses. Molecular Ecology 17:167–178.

Gordon, H. B., P. H. Whetton, A. B. Pittock, A. M. Fowler, and M. R. Haylock. 1992. Simulated changes in daily rainfall intensity due to the enhanced greenhouse-effect—implications for extreme rainfall events. Climate Dynamics 8:83–102.

Hartman, J. C. and J. B. Nippert. 2012. Physiological and growth responses of switchgrass (Panicum virgatum L.) in native stands under passive air temperature manipulation. Global Change Biology Bioenergy. doi: 10.1111/j.1757-1707.2012.01204.x

Hartman, J. C., J. B. Nippert, and C. J. Springer. 2012. Ecotypic responses of switchgrass to altered precipitation. Functional Plant Biology 39:126–136.

Hoffmann, A. A. and Y. Willi. 2008. Detecting genetic responses to environmental change. Nature Reviews: Genetics 9:421–432.

Hopkins, A. A., C. M. Taliaferro, C. D. Murphy, and D. Christian. 1996. Chromosome number and nuclear DNA content of several switchgrass populations. Crop Science 36:1192–1195.

Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood, and M. Vellend. 2008. Ecological consequences of genetic diversity. Ecology Letters 11:609–623.

Hultquist, S. J., P. Vogel, D. J. Lee, K. Arumuganathan, and S. Kaeppler. 1996. Chloroplast DNA and nuclear DNA content variations among cultivars of switchgrass, *Panicum virgatum* L. Crop Science 36:1049–1052.

Hultquist, S. J., K. P. Vogel, D. J. Lee, K. Arumuganathan, and S. Kaeppler. 1997. DNA content and chloroplast DNA polymorphisms among switchgrasses. Crop Science 37:595–598.

Jump, A. S. and J. Peñuelas. 2005. Running to stand still: adaptation and the response of plant to rapid climate change. Ecology Letters 8:1010–1020.

Knapp, A. K. 1985. Effect of fire and drought on the ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a tallgrass prairie. Ecology 66:1309–1320.

Knapp, A. K., C. Beier, D. D. Briske, A. T. Classen, Y. Luo, M. Reichstein, M. D. Smith, S. D. Smith, J. E. Bell, P. A. Fay, J. L. Heisler, S. W. Leavitt, R. Sherry, B. Smith, and E. Weng. 2008. Consequences of more extreme precipitation regimes for terrestrial ecosystems. BioScience 58:811–821.

Knapp, A. K., J. M. Briggs, D. C. Hartnett, and S. L. Collins. 1998. Grassland dynamics: Long-term ecological research in tallgrass prairie. Oxford University Press, New York, New York, USA.

Knapp, A. K., P. A. Fay, J. M. Blair, S.L. Collins, M. D. Smith, J. D. Carlisle, C. W. Harper, B. T. Danner, M. S. Lett, and J. K. McCarron. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. Science 298:2202–2205.

Knight, C. A., N. A. Molinari, and D. A. Petrov. 2005. The large genome constraint hypothesis: evolution,

ecology and phenotype. Annals of Botany 95:177–190.

Li, W., D. K. Biswas, H. Xu, C. Xu, X. Wang, J. Liu, and G. Jiang. 2009. Photosynthetic responses to chromosome doubling in relation to leaf anatomy in *Lonicera japonica* subjected to water stress. Functional Plant Biology 36:1–10.

Liu, H., E. J. Edwards, R. P. Freckleton, and C. P. Osborne. 2012. Phylogenetic niche conservatism in C_4 grasses. Oecologia 170:835–845.

MacGillivray, C. W. and J. P. Grime. 1995. Genome size predicts frost resistance in British herbaceous plants: Implications for rates of vegetation response to global warming. Functional Ecology 9:320–325.

McLaughlin, S. B. and L. A. Kszos. 2005. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. Biomass & Bioenergy 28:515–535.

McNaughton, S. J. and L. L. Wolf. 1970. Dominance and the niche in ecological systems. Science 167:131–139.

Meehl, G. A., J. M. Arblaster, and C. Tebaldi. 2005. Understanding future patterns of increased precipitation intensity in climate model simulations. Geophysical Research Letters 32:L18719.

Muir, J. P., M. A. Sanderson, W. R. Ocumpaugh, R. M. Jones, and R. L. Reed. 2001. Biomass production of 'Alamo' switchgrass in response to nitrogen, phosphorus, and row spacing. Agronomy Journal 93:896–901.

Nielson, E. L. 1944. Analysis of variation in *Panicum virgatum*. Journal of Agricultural Research 69:327–53.

Nippert, J. G., P. A. Fay, and A. K. Knapp. 2007. Photosynthetic traits in C_3 and C_4 grassland species in mesocosm and field environments. Environmental and Experimental Botany 60:412–420.

Nippert, J. B., A. K. Knapp, and J. M. Briggs. 2006. Intra-annual rainfall variability and grassland productivity: can the past predict the future? Plant Ecology 184:65–74.

Ohri, D. 2005. Climate and growth form: the consequences for genome size in plants. Plant Biology 7:449–458.

Otto, S. P. 2007. The evolutionary consequences of polyploidy. Cell 131:452–462.

Otto, S. and J. Whitton. 2000. Polyploid incidence and evolution. Annual Review Genetics 34:401–437.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics 37:637–669.

Parrish, D. J. and J. H. Fike. 2005. The biology and agronomy of switchgrass for biofuels. Critical Reviews in Plant Sciences 24:423–459.

Petrie, M. D. and N. A. Brunsell. 2012. The role of precipitation variability on the ecohydrology of grasslands. Ecohydrology 5:337–345.

Porter, C. L. 1966. An analysis of variation between upland and lowland switchgrass *Panicum virgatum* L. in central Oklahoma. Ecology 47:980–992.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sanderson, M. A. 1992. Morphological development of switchgrass and kleingrass. Agronomy Journal 84:415–419.

Sanderson, M. A., P. R. Adler, A. A. Boateng, M. D. Casler, and G. Sarath. 2006. Switchgrass as a biofuels feedstock in the USA. Canadian Journal of Plant Science 86:1315–1325.

Sanderson, M. A., and R. L. Reed. 2000. Switchgrass growth and development: Water, nitrogen, and plant density effects. Journal of Range Management 53:221–227.

Skelly, D. K., L. N. Joseph, H. P. Possingham, L. K. Freidenburg, T. J. Farrugia, M. T. Kinnison, and A. P. Hendry. 2007. Evolutionary responses to climate change. Conservation Biology 21:1353–1355.

Smith, M. D. and A. K. Knapp. 2003. Dominant species maintain ecosystem function with non- random species loss. Ecology Letters 6:509–517.

Stout, W. L., G. A. Jung, and J. Shaffer. 1988. Effects of soil and nitrogen on water use efficiency of tall fescue and switchgrass under humid conditions. Soil Science Society of America Journal 52:429–434.

Stroup, J. A., M. A. Sanderson, J. P. Muir, J. M. McFarland, and R. L. Reed. 2003. Comparison of growth and performance in upland and lowland switchgrass types to water and nitrogen stress. Bioresource Technology 86:65–72.

Travers, S. E., Z. Tang, D. Caragea, K. A. Garrett, S. H. Hulbert, J. E. Leach, J. Bai, A. Sahel, A. K. Knapp, P. A. Fay, J. Nippert, P. S. Schnable, and M. D. Smith. 2010. Climate change induced dynamics in gene expression of *Andropogon gerardii*. Journal of Ecology 98:374–383.

Tucker, S. S., J. M. Craine, and J. B. Nippert. 2011. Physiological drought tolerance and the structuring of tallgrass prairie assemblages. Ecosphere 2:1–19.

Vindelov, L. L. 1977. Flow Microfluorometric Analysis of nuclear DNA in cells from solid tumors and cell suspensions: A new method for rapid isolation and staining of nuclei. Virchows Arch.B Cell Pathology 24:227–242.

Warner, D., M. Ku, and G. Edwards. 1987. Photosynthesis, leaf anatomy, and cellular constituents in the polyploid C_4 grass *Panicum virgatum*. Plant Physiology 84:461–466.

Wright, L. and A. Turhollow. 2010. Switchgrass selection as a "model" bioenergy crop: A history of the process. Biomass and Bioenergy 34:851–868.

Wullschleger, S. D., M. A. Sanderson, S. B. McLaughlin,

D. P. Biradar, and A. L. Rayburn. 1996. Photosynthetic rates and ploidy levels among populations of switchgrass. Crop Science 36:306–312.

Xu, B. C., F. M. Li, L. Shan, Y. Q. Ma, N. Ichizen, and J. Huang. 2006. Gas exchange, biomass partition, and water relationships of three grass seedlings under water stress. Weed Biology and Management 6:79–88.

Supplemental Material

Appendix A

Table A1. ANCOVA results for *P. virgatum* physiology, including CO_2 assimilation at ambient C_a (A_{max}), stomatal conductance of water vapor (g_s), transpiration rate (E), instantaneous water use efficiency (WUE), midday leaf water potential (Ψ_{mid}), and chlorophyll fluorescence (F_v/F_m).

	July			September		
Trait	df	*F*	*p*	df	*F*	*p*
A_{max}						
Treatment	1, 37	<0.01	0.97	1, 40	9.55	<0.01*
DNA	1, 37	2.60	0.12	1, 40	9.02	0.01*
T × D	1, 36	1.80	0.19	1, 39	0.55	0.46
g_s						
Treatment	1, 37	0.06	0.81	1, 40	7.11	0.01*
DNA	1, 37	2.34	0.13	1, 40	8.12	0.01*
T × D	1, 36	0.72	0.40	1, 39	1.11	0.30
E						
Treatment	1, 37	0.23	0.64	1, 40	10.13	<0.01*
DNA	1, 37	4.15	0.05*	1, 40	13.22	<0.01*
T × D	1, 36	0.16	0.69	1, 39	0.59	0.45
WUE						
Treatment	1, 37	0.23	0.63	1, 40	0.26	0.61
DNA	1, 37	0.05	0.82	1, 40	0.95	0.34
T × D	1, 36	1.47	0.23	1, 39	0.15	0.70
Ψ_{mid}						
Treatment	1, 42	1.02	0.32	1, 40	0.01	0.93
DNA	1, 42	2.00	0.17	1, 40	5.90	0.02*
T × D	1, 41	0.29	0.59	1, 39	0.87	0.36
F_v/F_m						
Treatment	1, 43	0.35	0.56	1, 38	3.74	0.06*
DNA	1, 43	0.27	0.60	1, 38	1.51	0.23
T × D	1, 42	0.33	0.57	1, 38	4.66	0.04*

Notes: Results are presented for all populations combined (Kansas, Oklahoma, and Texas), in July and September separately. Main effects (Treatment and DNA) are presented from the simplified model in which non-significant interaction terms (T × D) were removed, when applicable. Significance at the $\alpha = 0.05$ level is indicated by an asterisk (*).

Table A2. ANCOVA results for *P. virgatum* biomass, including total biomass, flowering tiller (FT) biomass, non-flowering tiller (NFT) biomass, total tiller number, flowering tiller number, non-flowering tiller number, biomass per tiller, biomass per flowering tiller, biomass per non-flowering tiller, and percent reproductive biomass.

Trait	df	*F*	*p*
Total biomass			
Treatment	1, 39	0.02	0.88
DNA	1, 39	0.44	0.51
T × D	1, 38	0.45	0.51
FT biomass			
Treatment	1, 39	0.02	0.89
DNA	1, 39	0.45	0.51
T × D	1, 38	0.47	0.50
NFT biomass			
Treatment	1, 39	<0.01	0.99
DNA	1, 39	0.06	0.81
T × D	1, 38	0.05	0.83
Total Tillers			
Treatment	1, 39	2.69	0.11
DNA	1, 39	9.14	0.01
T × D	1, 38	2.00	0.17
FT #			
Treatment	1, 39	3.16	0.08
DNA	1, 39	10.76	<0.01*
T × D	1, 38	2.71	0.11
NFT #			
Treatment	1, 39	0.11	0.74
DNA	1, 39	0.38	0.54
T × D	1, 38	<0.01	0.95
Biomass/tiller			
Treatment	1, 39	1.13	0.30
DNA	1, 39	12.95	<0.01*
T × D	1, 38	0.59	0.45
Biomass/FT			
Treatment	1, 39	1.45	0.24
DNA	1, 39	12.07	<0.01*
T × D	1, 38	0.70	0.41
Biomass/NFT			
Treatment	1, 39	1.00	0.32
DNA	1, 39	0.03	0.86
T × D	1, 38	0.95	0.34
% Reproductive			
Treatment	1, 39	0.04	0.84
DNA	1, 39	0.37	0.55
T × D	1, 38	0.69	0.41

Notes: Results are presented for all populations combined (Kansas, Oklahoma, and Texas). Main effects (Treatment and DNA) are presented from the simplified model in which non-significant interaction terms (T × D) were removed, when applicable. Significance at the $\alpha = 0.05$ level is indicated by an asterisk (*).

APPENDIX B

Table B1. Least-squares linear regression results for *P. virgatum* physiology, including CO_2 assimilation at ambient C_a (A_{max}), stomatal conductance of water vapor (g_s), transpiration rate (E), instantaneous water use efficiency (WUE), midday leaf water potential (Ψ_{mid}), and chlorophyll fluorescence (F_v/F_m).

	July				September			
Trait	*y*-intercept	Slope	r^2	*p*	*y*-intercept	Slope	r^2	*p*
A_{max}								
Ambient	28.06	−3.44	0.21	0.05*	28.90	−2.49	0.14	0.10
Altered	17.34	−0.27		0.88	27.05	−4.10	0.23	0.02*
All	22.48	−1.94	0.07	0.09	24.96	−2.42	0.10	0.04
g_s								
Ambient	0.19	−0.02	0.13	0.14	0.15	−0.01	0.11	0.16
Altered	0.13	−0.01	0.01	0.64	0.15	−0.03	0.24	0.02*
All	0.16	−0.01	0.06	0.13	0.14	−0.01	0.10	0.04*
E								
Ambient	6.78	−0.62	0.06	0.31	4.83	−0.50	0.18	0.06
Altered	7.41	−0.93	0.17	0.07	4.53	−0.77	0.33	<0.01*
All	6.93	−0.72	0.10	0.05	4.19	−0.49	0.15	0.01*
WUE								
Ambient	4.61	−0.23	0.02	0.53	6.14	0.29	0.02	0.52
Altered	2.85	0.36	0.06	0.29	5.41	0.65	0.03	0.45
All	3.80	0.02		0.93	5.98	0.40	0.02	0.38
Ψ_{mid}								
Ambient	−1.11	−0.19	0.11	0.14	−1.30	−0.13	0.08	0.22
Altered	−1.32	−0.08	0.01	0.60	−0.74	−0.29	0.18	0.05*
All	−1.16	−0.16	0.06	0.10	−1.00	−0.21	0.14	0.02*
F_v/F_m								
Ambient	0.80	<−0.01	0.02	0.55	0.76	<0.01	0.02	0.59
Altered	0.79	<0.01	<0.01	0.95	0.80	−0.01	0.29	0.01*
All	0.79	<−0.01	<0.01	0.70	0.78	<−0.01	0.04	0.21

Notes: Results for all populations combined (Kansas, Oklahoma, and Texas) are presented for water treatments separately (Ambient and Altered), as well as ambient and altered treatments combined (All), for data collected in July and September. Significance at the $\alpha = 0.05$ level is indicated by an asterisk (*).

Table B2. Least-squares linear regression results for *P. virgatum* biomass, including total biomass, flowering tiller (FT) biomass, non-flowering tiller (NFT) biomass, total tiller number, flowering tiller number, non-flowering tiller number, biomass per tiller, biomass per flowering tiller, biomass per non-flowering tiller, and percent reproductive biomass.

Trait	*y*-intercept	Slope	r^2	*p*
Total biomass				
Ambient	643.35	−44.07	0.06	0.28
Altered	488.97	4.71	<0.01	0.94
All	576.65	−25.05	0.01	0.46
FT biomass				
Ambient	620.42	−43.71	0.06	0.26
Altered	465.84	4.79		0.94
All	553.10	−24.66	0.01	0.46
NFT biomass				
Ambient	15.29	2.24	0.01	0.64
Altered	23.13	−0.08	<0.01	0.99
All	18.93	1.27	0.07	0.80
Total Tillers				
Ambient	64.03	16.23	0.11	0.13
Altered	10.92	41.27	0.30	0.01*
All	54.06	22.59	0.15	0.01*
FT #				
Ambient	55.00	14.35	0.11	0.14
Altered	6.94	38.94	0.38	<0.01*
All	43.44	20.85	0.17	0.01*
NFT #				
Ambient	9.03	1.88	0.02	0.50
Altered	9.98	2.33	0.01	0.74
All	10.62	1.75	0.01	0.59
Biomass/tiller				
Ambient	7.20	−0.81	0.26	0.02*
Altered	8.02	−1.24	0.26	0.02*
All	7.29	−0.90	0.23	<0.01*
Biomass/FT				
Ambient	7.74	−0.84	0.22	0.03*
Altered	8.70	−1.36	0.27	0.02*
All	7.81	−0.96	0.21	<0.01*
Biomass/NFT				
Ambient	0.71	0.30	0.02	0.57
Altered	2.03	−0.28	0.16	0.08
All	1.03	0.14	0.01	0.62
% Reproductive				
Ambient	99.80	−1.40	0.07	0.24
Altered	94.18	0.39	<0.01	0.84
All	97.39	−0.71	0.01	0.48

Notes: Results for all populations combined (Kansas, Oklahoma, and Texas) are presented for water treatments separately (Ambient and Altered), as well as ambient and altered treatments combined (All). Significance at the $\alpha = 0.05$ level is indicated by an asterisk (*).