

Photosynthetic responses of a dominant C₄ grass to an experimental heat wave are mediated by soil moisture

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Abstract Extreme heat waves and drought are predicted to increase in frequency and magnitude with climate change. These extreme events often co-occur, making it difficult to separate their direct and indirect effects on important ecophysiological and carbon cycling processes such as photosynthesis. Here, we assessed the independent and interactive effects of experimental heat waves and drought on photosynthesis in *Andropogon gerardii*, a dominant C₄ grass in a native mesic grassland. We experimentally imposed a two-week heat wave at four intensity levels under two contrasting soil moisture regimes: a well-watered control and an extreme drought. There were three main findings from this study. First, the soil moisture regimes had large effects on canopy temperature, leading to extremely high temperatures under drought and low temperatures under well-watered conditions. Second, soil moisture mediated the photosynthetic response to heat; heat reduced photosynthesis under the well-watered control, but not under the extreme drought treatment. Third, the effects of heat on photosynthesis appeared to be driven by a direct thermal effect, not indirectly through other environmental or ecophysiological variables. These results suggest that while photosynthesis in this dominant C₄ grass is

sensitive to heat stress, this sensitivity can be overwhelmed by extreme drought stress.

Keywords Canopy temperature · Climate extremes · Drought · Ecophysiology · Tallgrass prairie

Introduction

In water-limited ecosystems, such as grasslands, soil moisture is the key hydrologic variable integrating climate, soil, and vegetation processes (Rodríguez-Iturbe and Poropato 2004; Seneviratne et al. 2010). Although strong relationships exist between precipitation and plant productivity across grassland ecosystems (Sala et al. 1988), it is soil moisture availability that most directly regulates plant transpiration, carbon assimilation, and other biogeochemical cycles, and thus is the dominant driver of ecosystem structure and function (Noy-Meir 1973; Knapp et al. 2001). The impacts of soil moisture on climate are largely driven by changes in evapotranspiration, a critical flux of water and energy to the atmosphere from the land surface (Seneviratne et al. 2010; Whan et al. 2015). Across the terrestrial landscape, evapotranspiration returns approximately 60 % of the water from precipitation back to the atmosphere (Oki and Kanae 2006), dissipating about half of the incoming solar radiation via latent heat flux (Trenberth et al. 2009). Given this strong coupling between soil moisture and temperature, heat waves and drought often co-occur (Déry and Wood 2005; Trenberth and Shea 2005; De Boeck et al. 2010), and both are predicted to increase in frequency and severity (IPCC 2013). Thus, a critical challenge for ecologists is to understand mechanistically how communities and ecosystems will respond to such extremes, but when two climate drivers co-vary, it can be difficult to separate their independent and interactive effects.

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Experimental approaches can be particularly valuable in this regard (Jentsch et al. 2007; Smith 2011); however, there are unique challenges to experimentally imposing climate extremes. For example, many warming experiments, which have not controlled for heat-induced water losses, have found that the effects of heat on ecological processes have been primarily indirect, through enhanced soil drying (Milbau et al. 2005; Marchand et al. 2006; Arnone et al. 2008; De Boeck et al. 2011). Thus, experiments that alter inputs of heat and water independently can provide the mechanistic understanding needed for predicting ecological responses to such extremes.

In 2011, we subjected a native mesic grassland to a two-week heat wave at four temperature levels under two precipitation treatments that spanned a physiological thresholds: control (non-limiting soil moisture levels) and extreme drought (limiting soil moisture levels). We examined the ecophysiological responses of the dominant C_4 grass, *Andropogon gerardii*, to these different precipitation and heat wave treatment combinations. *A. gerardii* was selected as the focal species, because it can comprise up to 80 % of total aboveground biomass in the tallgrass prairie ecosystem (Smith and Knapp 2003) and its been shown to have strong tolerance to drought but sensitivity to heat (Swemmer et al. 2006; Nippert et al. 2009; Hoover et al. 2014a). It is important to note that it is uncertain whether the sensitivity of *A. gerardii* to heat is from direct thermal effects or indirect through changes in another variable such as vapor pressure deficit or leaf water potential. Here, we focused on leaf-level net photosynthesis because of the important role of the carbon uptake processes in regional and global carbon cycles (Houghton 2007), and the thermal sensitivity of photosynthesis (Wahid et al. 2007).

We had three primary questions for this study. First, what is the magnitude of the impact of contrasting soil moisture regimes (limiting and non-limiting) on canopy temperature? We expected that the plants under the extreme drought treatment would have higher canopy temperature than under the well-watered conditions of the control treatment, and thus, have a strong interaction with the heat wave treatments. Second, how does soil moisture regime impact the effects of heat on photosynthesis? Based on previous work (De Boeck et al. 2016), we predicted that plants under non-limiting soil moisture conditions should tolerate heat better than those under drought. Third, are the effects of heat on photosynthesis direct or indirect? Although we controlled for the indirect effects of soil moisture on photosynthesis, the simulated heat wave could directly affect other environmental or ecophysiological factors that, in turn, impact photosynthesis. To address this final question, we used path analysis to assess the direct and indirect effects of these highly correlated variables.

Materials and methods

Study site

The study was conducted in a native tallgrass prairie grassland at the Konza Prairie Biological station in NE Kansas, USA (39°05'N, 96°35'W). The experiment was located in an annually burned lowland site with deep soil (>1 m) classified as Typic Argiustoll, with a silty clay loam texture (8 % sand; 32 % clay), and a bulk density of 1.5 g m⁻³ (Blecker 2005). The climate is characterized as mid-continental, with cold, dry winters, warm wet summers and high interannual variability in precipitation and temperature (Knapp et al. 1998). In addition to variability, extreme drought and both short- and long-term heat waves are common throughout the history of this region (Weaver 1954; Woodhouse and Overpeck 1998; Burnette et al. 2010; Burnette and Stahle 2012).

Experimental design

The Climate Extremes Experiment was established in 2010 to examine the effects of periods of high temperatures (heat waves) and extreme drought on a tallgrass prairie ecosystem. Treatments were imposed for two consecutive years, with this paper focusing on results from the second year of the experiment (and the most extreme climatically of the two; see Hoover et al. 2014a). We manipulated precipitation inputs using modified greenhouse frames and water additions to create two precipitation treatments (1) well-watered control, resulting from ambient rainfall inputs plus supplemental irrigation, and (2) drought, where ambient rainfall was passively removed using partial roofs attached to greenhouse frames (Yahdjian and Sala 2002). To impose these soil moisture conditions, we used four modified 6 × 24 m greenhouse frames (Stuppy, Inc., Kansas City, MO, USA) constructed over undisturbed native grassland plots. The roofs of the two rainfall reduction shelters were partially covered (75 % of the surface) by 15.2-cm wide strips of Dynaglas Plus[®] clear corrugated polycarbonate plastic (PALRAM Industries LTD., Kutztown, PA, USA), which excluded ~66 % of ambient rainfall during the growing season (April 1–August 30). Shelters over control plots with non-limiting soil moisture were covered with spectrally neutral netting (Cintoflex C, Tenax Corporation, Baltimore, MD, USA) to reduce photosynthetically active radiation by about 10 % (equivalent to rainfall reduction shelter effects), but allowed ambient rainfall to pass through. In addition, control plots were irrigated weekly (if needed) to maintain soil moisture conditions above limiting conditions for the plants. Beneath each shelter, soils and roots were hydrologically isolated by trenching to 1 m

and surrounding the soil columns with plastic and metal flashing to prevent above- and belowground lateral flow of water. Ten 2×2 m plots were established within each shelter and randomly assigned to one of four heat wave treatments (ambient, low, medium, and high). In 2011, the heat wave treatments were imposed mid-summer for two weeks (July 13–26) using passive heat chambers and infrared heat lamps. The transparent heat chambers were built on $\frac{3}{4}$ " PVC frames, with 1-m 6-mil clear polyethylene walls and Dynaglas Plus[®] clear corrugated polycarbonate roofs. Ventilation was achieved by placing chambers 0.5 m aboveground with adjustable gaps between the roofs and walls. To impose four distinct temperature levels, infrared heat lamps (HS/MRM 2420, 2000 W, Kalglo Electronics, Inc., Bethlehem, PA, USA) were placed within the heat chambers as follows: control = no lamp, low heat = one lamp at $\frac{1}{2}$ power ($+250 \text{ W m}^{-2}$), medium heat = one lamp at full power ($+500 \text{ W m}^{-2}$), and high heat = two lamps at full power ($+1000 \text{ W m}^{-2}$). Lamp heights were adjusted to account for different canopy heights due to the effects of precipitation treatments on plant growth (control lamps = 150 cm above the ground; drought = 120 cm) to ensure even heat coverage across the plot, and remained on 24 h a day for the duration of the heat wave.

No supplemental water was added to the control plots during heat wave in the first year of the experiment (2010), mimicking the natural attributes of heat waves (De Boeck et al. 2010; thermal and soil-drying effects). This made it difficult to attribute plant responses to direct vs. indirect effects of heat (Hoover et al. 2014a). Therefore, in the second year of the experiment (2011), the focus of this paper, we added supplemental irrigation to the control plots to maintain soil moisture above limiting levels (target: $>20\%$ volumetric water content).

Environmental measurements

Soil moisture, canopy temperature, air temperature, and relative humidity were monitored in each plot ($n = 40$). Volumetric water content (VWC) was measured with 30-cm time-domain reflectometry probes (Model CS616, Campbell Scientific, Inc., Logan, UT, USA) buried 0–15 cm deep and at a 45° angle. Canopy temperature (CT) was measured with infrared thermometers (Model SI-111, Apogee Instruments, Inc., Logan, UT, USA) mounted in the SE corner of each plot. Within each rainout shelter, air temperature and relative humidity probes (Model HMP50-L, Campbell Scientific, Inc., Logan, UT, USA) were mounted at 1.5 m ($n = 2$ per treatment). Data from VWC, air, and CT sensors were sampled every 30-s and averaged for 30-min periods (CR10X Datalogger, Campbell Scientific, Inc., Logan, UT, USA). During the two-week heat wave, vapor pressure deficit (VPD) was calculated for each plot using air

temperature and relative humidity data sampled at 30-min increments using iButtons (Model DS1923, Maxim Integrated, San Jose, CA, USA) placed in the center of each plot at canopy level and covered with radiation shields.

Ecophysiological measurements

Four intensive sampling campaigns (July 15, 19, 22, 26) were conducted during the simulated two-week heat wave (July 13–26), with each campaign conducted between 11:00 and 15:00 CDT. In each plot, a single tiller of *A. gerardii* was permanently tagged and repeatedly sampled for leaf-level gas exchange. For each individual sampling campaign, another morphologically similar tiller was selected per plot for destructive mid-day leaf water potential (Ψ_{mid}) sampling. Therefore, each precipitation \times heat treatment combination had five replicates, and plot sampling order was randomized prior to each sampling campaign. The youngest fully expanded leaf was measured for both gas exchange and Ψ_{mid} . Gas exchange was measured at 5-s intervals for 2–6 min with a LI-6400 system (LiCOR, Inc., Lincoln, NE, USA) equipped with an LED light source (light intensity was maintained at $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, CO_2 concentration at $400 \mu\text{mol mol}^{-1}$, and relative humidity and temperatures at ambient levels). Net photosynthesis (A_{net}) and stomatal conductance (g_s) were then calculated for each leaf using an objective selection algorithm (Matlab 7.4, The MathWorks, Inc., Natick, MA, USA) to select and average over a 1-min period when variability in A_{net} was minimal. Ψ_{mid} was measured on a single leaf per individual using a Scholander-type pressure chamber (PMS Instruments, Inc., Corvallis, OR, USA).

Statistical analyses

Statistical analyses were primarily focused on the two-week heat wave period in mid-July. The experimental design was a randomized block split-plot design with block nested within precipitation treatment, heat wave treatments nested within precipitation treatment and the block by heat interaction as a random effect. We used repeated measures mixed model analysis of variance (PROC MIXED, SAS 9.3; SAS Institute, Raleigh, NC, USA) to assess precipitation and heat wave treatment effects over time for environmental (VWC, CT, and VPD) and ecophysiological (A_{net} , g_s , and Ψ_{mid}) variables. Linear regressions were performed using PROC REG (SAS 9.3; SAS Institute, Raleigh, NC, USA).

Path analysis

We used path analysis and the R package 'lavaan' to assess the direct and indirect effects of CT on A_{net} in *A. gerardii*.

Table 1 Repeated measures mixed modal ANOVA results for environmental measurements

Effect	Volumetric water content			Canopy temperature			Vapor pressure deficit		
	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>
Drought	1, 2.0	74.3	0.013	1, 2.0	27.4	0.035	1, 1.9	240.8	0.005
Heat	3, 33.7	1.3	0.288	3, 5.5	95.6	<0.001	3, 120.0	45.3	<0.001
Drought × heat	3, 33.7	0.0	0.996	3, 5.5	5.7	0.039	3, 120.0	9.0	<0.001
Date	3, 94.6	40.1	<0.001	3, 116.0	18.2	<0.001	3, 118.0	7.3	0.000
Drought × date	3, 94.6	27.9	<0.001	3, 116.0	3.4	0.020	3, 118.0	3.9	0.011
Heat × date	9, 94.6	0.8	0.656	9, 116.0	0.3	0.976	9, 118.0	0.4	0.957
Drought × heat × date	9, 94.6	0.5	0.839	9, 116.0	0.5	0.883	9, 118.0	0.6	0.832

Summary of degrees of freedom (*df*; numerator, denominator), *F* and *p* values from a repeated measures mixed model ANOVA for volumetric water content (VWC), canopy temperature (CT), and vapor pressure deficit (VPD) during the four ecophysiology sample dates of the two-week heat wave

Bold values indicate significance ($p < 0.05$)

First, we constructed a conceptual a priori multivariate model with a class of exogenous predictors (CT and VWC), intermediate predictors (VPD, and Ψ_{mid}) with A_{net} as the focal response variable (Fig. S1). Originally, g_s was included in the model, but due to the reciprocal nature of g_s and A_{net} , the model was difficult to interpret; therefore, g_s was removed. The initial a priori model was saturated, including all possible pathways, so we first examined if any pathways should be removed. Weak pathways (large *p* values) were eliminated from the model individually and these new models were tested against the initial model using AIC and Chi-square difference test. The final model was deemed a good fit according to the χ^2 statistic and $p > 0.05$ (Grace 2006).

Results

Environmental responses to extreme treatments and interactions

The magnitude of the precipitation and heat wave treatments imposed in this experiment was near or exceeded records for drought and temperatures for the region. While the growing season precipitation inputs were slightly above average for the control treatment based on this long-term record (control = 607.3 mm; long-term average = 521.6 ± 16.1 mm; Hoover et al. 2014a), experimental rainfall inputs for the drought treatment were the second lowest on record, with the lowest occurring during the driest year of the 1930s US “Dust Bowl” (drought = 236.3 mm; 1934 = 209.9 mm; Hoover et al. 2014a). The heat wave treatments imposed CTs that also ranged from average to extreme, with several heat levels exceeding the 95th percentile for maximum July air temperature (Hoover et al. 2014b).

During the growing season, we successfully established two distinct soil moisture regimes in the control and drought

precipitation treatments (Table 1; Fig. 1a, b). Throughout the heat wave, soil moisture in the drought treatment was half that of the control (Fig. 1b). More importantly, there was no interaction between the precipitation and heat wave treatments on soil moisture (Table 1; Fig. 1c), and thus, we controlled for heat-induced soil drying, a key indirect effect of heat. The two soil moisture regimes resulted in different CT regimes throughout the summer and during the heat wave (Fig. 2a). We first examined the effect of soil moisture on CT by comparing air temperature at 1.5 m to CT from plots within the ambient heat treatment during and after the heat wave, thus removing potential legacy effects or experimental artefacts of the heat chambers. While the relationship between maximum air and CT was similar between control ($p < 0.001$, $r^2 = 0.92$, slope = 0.76; Fig. 2b) and drought treatments ($p < 0.001$, $r^2 = 0.73$, slope = 0.87; Fig. 2b), CTs were substantially warmer in the drought plots as compared to the controls; on average, the controls were $0.86 (\pm 0.2)$ °C cooler than ambient air temperature while drought plots were $5.3 (\pm 0.3)$ °C warmer than ambient air temperature. During the two-week heat wave, CT in the drought varied from 8 to 13 °C warmer than the control plots within a given heat treatment (Fig. 2c). There was a strong non-linear relationship between VWC and CT; under drought, we observed a strong negative relationship between VWC and CT, while a much weaker, but significant, negative relationship when soil moisture was non-limiting in the control (Fig. 3a). There was an apparent threshold around 15 % VWC where the relationship between VWC and CT changed dramatically (Fig. 3a).

Ecophysiological responses to extreme treatments and interactions

The soil moisture regimes resulting from the two precipitation treatments were clearly above and below a physiological threshold for *A. gerardii*. During the heat wave,

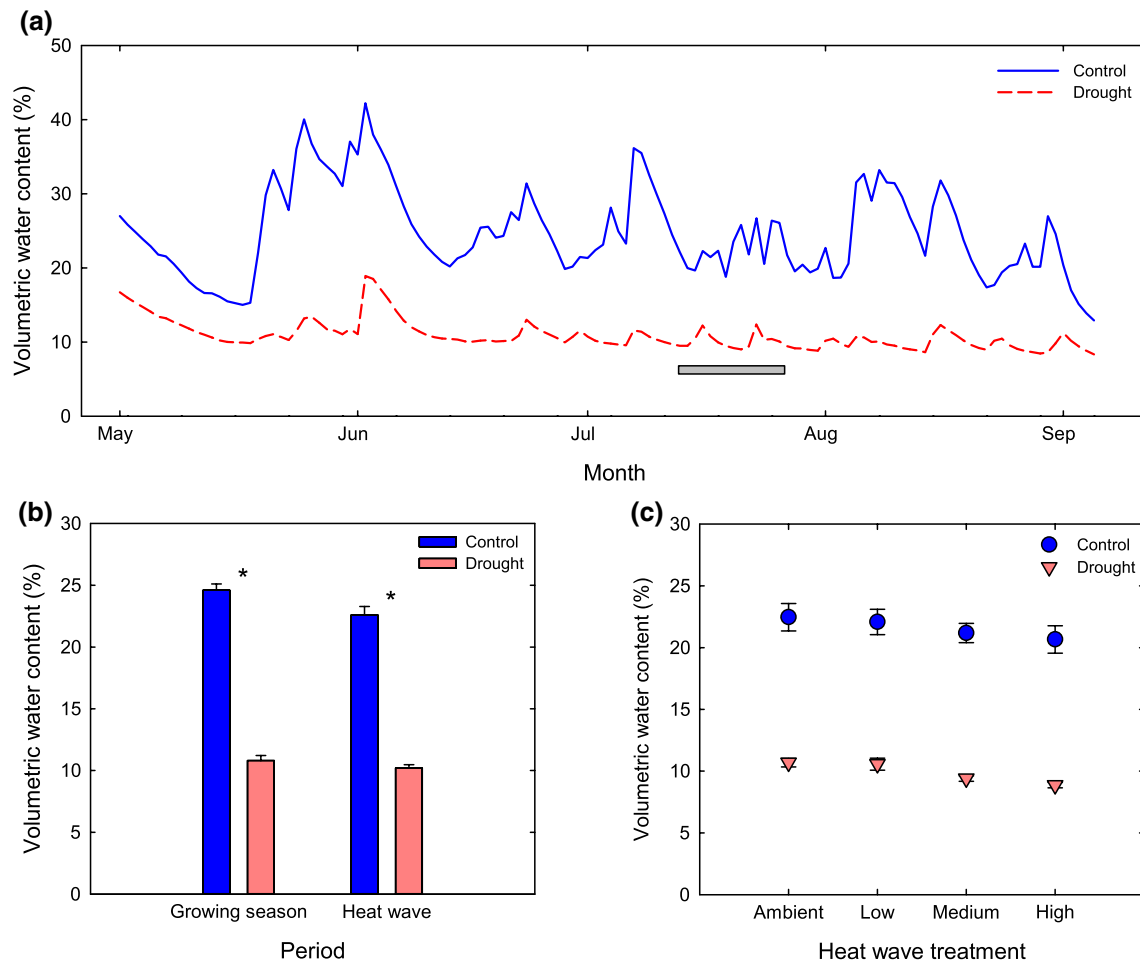


Fig. 1 Volumetric water content (VWC) during the 2011 growing season for the control (ambient rainfall plus supplemental irrigation) and drought (66 % reduction in ambient rainfall) precipitation treatments. **a** Daily mean volumetric water content for the control and drought treatment, the *gray horizontal bar* indicates when the two-week heat wave treatments were imposed (July 13–26). **b** Mean volu-

metric content (± 1 SE) during the growing season and two-week heat wave for the two precipitation treatments. *Asterisks* indicate significant difference between the treatments ($p < 0.05$). **c** Mean volumetric water content (± 1 SE) during the two-week heat wave for each precipitation (control = *circles*, drought = *triangles*) and heat wave treatment combination

the precipitation treatments resulted in on average 22.6 % VWC in the control and 10.2 % VWC in the drought treatment (Fig. 1b). These soil moisture levels straddle what appears to be a critical threshold for water limitation; below 15 % VWC, we observed a rapid decline in Ψ_{mid} , while above this threshold, there was little effect of VWC on Ψ_{mid} (Fig. 3b). This is similar to the non-linear relationship observed for VWC and CT (Fig. 3a). Therefore, the heat wave treatments were imposed under physiologically relevant limiting and non-limiting soil moisture conditions.

The precipitation and heat wave treatments impacted the ecophysiology of *A. gerardii*, but the responses were not consistent across all three measured variables (A_{net} , g_s , and Ψ_{mid}). The drought treatment significantly reduced all three ecophysiological variables relative to the control including a 60 % decrease in A_{net} , and a 62 % decrease in

g_s (Table 2), but the responses to heat varied. There were significant main effects of heat (Table 2) for both A_{net} ($F = 3.1$, $p < 0.037$) and g_s ($F = 6.2$, $p < 0.018$), but not Ψ_{mid} ($F = 0.4$, $p = 0.733$). On the other hand, there were significant interactions between heat and drought (Table 2) for A_{net} ($F = 3.7$, $p = 0.021$) and Ψ_{mid} ($F = 3.1$, $p = 0.039$) but not g_s ($F = 1.8$, $p = 0.228$). Given that photosynthesis was the focal variable of this study, we explored these responses further (Fig. 4). Although there is a main effect of heat on A_{net} (Table 2), the drought \times heat interaction shows that this is driven by the response to heat in the control precipitation treatment (Fig. 4). Hence, the effects of heat were only apparent under the well-watered control treatments, while A_{net} in the drought treatment has no response to heat irrespective of level of extremity.

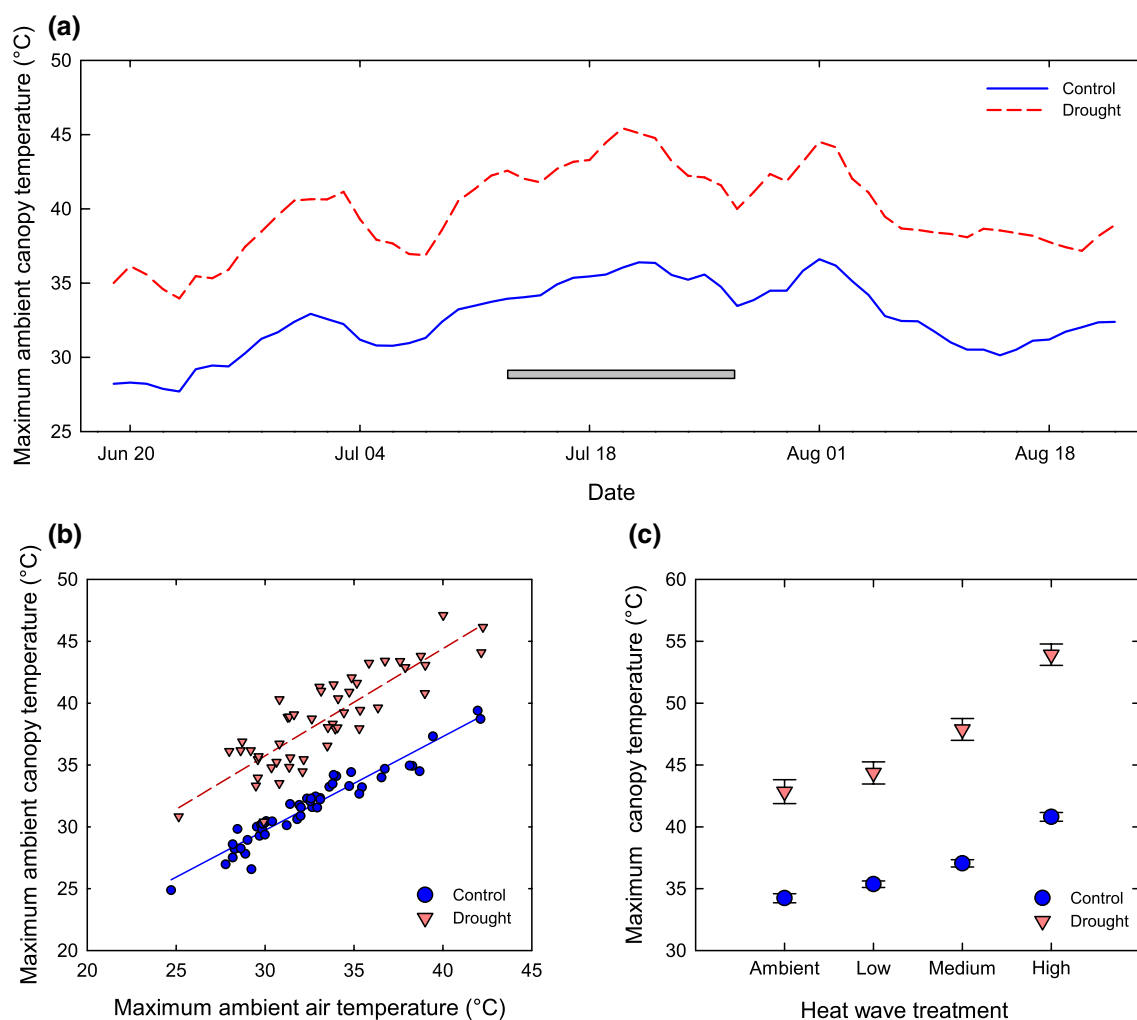


Fig. 2 Canopy temperature (CT) during the summer of 2011 for the control (ambient rainfall plus supplemental irrigation) and drought (66 % reduction in ambient rainfall) precipitation treatments. **a** Mean maximum temperature for the ambient heat wave treatment (no heat imposed) under the control and drought precipitation treatments. The data were smoothed using a 5-day running average. The gray horizontal bar indicates when the two-week heat wave treatments were imposed (July 13–26). **b** Maximum air temperature versus canopy

temperature for the ambient heat wave treatment during the summer (excluding heat wave period). Linear regressions were performed separately for the control (circles, solid line; $p < 0.001$, $r^2 = 0.92$, intercept = 7.0, slope = 0.76) and drought (triangles, dashed line; $p < 0.001$, $r^2 = 0.73$, intercept = 9.8, slope = 0.87) precipitation treatments. **c** Mean maximum canopy temperature (± 1 SE) during the two-week heat wave for the each precipitation (control = circles, drought = triangles) and heat wave treatment combination

Next, we examined the sensitivity of A_{net} in *A. gerardii* to CT over time for each precipitation treatment using a regression-based approach. For each sample date, we examined the relationship between CT and A_{net} , defining sensitivity by slope magnitude and the significance of the relationship. As with the ANOVA results (Table 2), CT had no effect on A_{net} under drought on any of the sample days during the heat wave (Fig. 5). On the other hand, A_{net} was sensitive to CT under the well-watered control treatment (Fig. 5). This sensitivity attenuated with time, with the highest sensitivity early in the heat wave (day 3) and the lowest sensitivity on the last day of the heat wave (day 14; Fig. 5).

Path analysis

Given that the heat effects were only apparent in the control precipitation treatment (Table 2; Figs. 4, 5) and there was a strong interaction between heat and drought treatments on CT (Table 1; Fig. 3a), we restricted our path analysis to the control treatment. While these previous analyses show a relationship between A_{net} and heat, we could not determine if A_{net} was directly responding to CT or indirectly through another environmental variable (e.g., VPD) or an ecophysiological process (e.g., Ψ_{mid}). The goal of this analysis was to identify direct and indirect effects of heat on A_{net} .

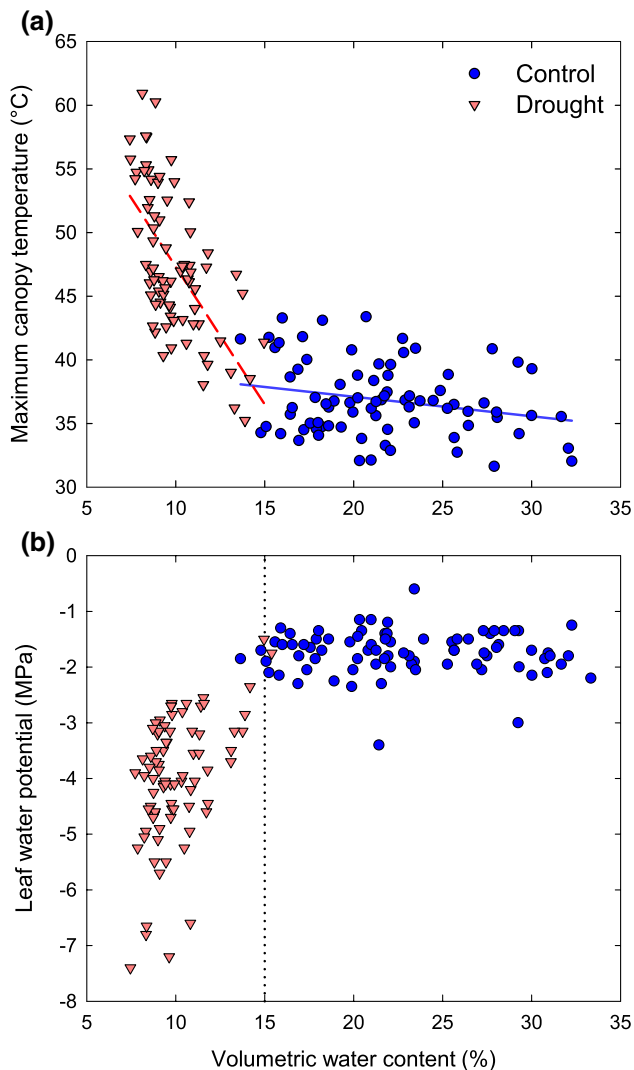


Fig. 3 Volumetric water content (VWC) versus canopy temperature (CT) and leaf water potential (Ψ_{mid}) for the control (*circles*) and drought (*triangles*) precipitation treatments during the two-week heat wave (includes all heat wave treatments). **a** Volumetric water content versus maximum canopy temperature. Linear regressions for volumetric water content and maximum canopy temperature were performed separately for the control (*solid line*; $p < 0.001$, $r^2 = 0.41$) and the drought (*dashed line*; $p = 0.032$, $r^2 = 0.06$) precipitation treatments. **b** Volumetric water content versus mid-day leaf water potential (Ψ_{mid}). *Dotted line* highlights 15 % volumetric water content, which appears to be a critical soil moisture threshold

We first constructed the a priori conceptual multivariate model based on our theoretical knowledge of the relationship between the different environmental and ecophysiological variables (Fig. S1). From this original model, we tested for weak pathways, and only the pathway between VPD and Ψ_{mid} was removed ($p = 0.774$). We chose this as our final model, because it has one less parameter and it is not an inferior model than the saturated a priori conceptual multivariate model based on AIC (saturated = 1329.9; link

removed = 1328.0) and Chi-squared differences (0.082). The final path analysis model showed a good fit between the model and data ($\chi^2 = 0.083$; $df = 1$; $p = 0.774$; Fig. 6).

Overall, the path analysis results suggest that CT had a strong direct negative effect on A_{net} (-0.59), but there was no evidence for any indirect effects (Fig. 6). Soil moisture did not have a significant effect on A_{net} (Fig. 6), which was not surprising given the plants were well-watered in the control and above a critical physiological threshold (Fig. 3). However, neither VPD nor Ψ_{mid} had significant effects on A_{net} , despite the significant effects of CT on those two variables ($+0.82$ and -0.61 , respectively; Fig. 6). This indicates that CT was not affecting A_{net} indirectly through increases in VPD or decreases in Ψ_{mid} . Overall, the model was able to explain 0.31 of the variance in A_{net} .

Discussion

In this study, we examined the ecophysiological responses of a dominant C_4 grass to an experimentally imposed two-week mid-summer heat wave under two contrasting precipitation regimes and soil moisture conditions. By establishing limiting and non-limiting soil moisture levels, we were able to control one of the main indirect effects of heat waves—heat-induced soil drying—to better assess the direct effects of heat waves of differing intensities on photosynthesis. There were three main results from this study. First, the imposed soil moisture regimes had large effects on CT, which interacted with the heat wave treatments leading to extremely high temperatures under drought while transpiration kept CTs cooler under the well-watered control. Second, soil moisture mediated the photosynthetic response to heat; heat waves did not affect photosynthesis under the extreme drought treatment (limiting soil moisture), but there were declines in photosynthesis with increasing CTs under control conditions (non-limiting soil moisture). Third, these effects of heat under high soil moisture conditions appeared to have been driven by direct heat effects on photosynthesis rather than indirectly through other environmental or ecophysiological variables.

Interactive effects of drought and heat waves on canopy temperature and ecophysiology

There was clear evidence for coupling between soil moisture, canopy temperature, and plant physiology. The limiting and non-limiting soil moisture regimes imposed by the precipitation treatments spanned a physiological threshold, which had large impacts on CT. Above a critical soil moisture threshold (~ 15 % VWC), we saw little response in Ψ_{mid} to changing soil moisture (Fig. 3b) and consequently, little change in CT (Fig. 3a). It appears the

Table 2 Repeated measures mixed modal ANOVA results for ecophysiological measurements

Effect	Net photosynthesis			Stomatal conductance			Leaf water potential		
	<i>df</i>	<i>F</i>	<i>p</i> value	<i>df</i>	<i>F</i>	<i>p</i> value	<i>df</i>	<i>F</i>	<i>p</i> value
Drought	1, 37.5	42.4	<0.001	1, 7.9	124.5	<0.001	1, 35.9	78.9	<0.001
Heat	3, 37.5	3.1	0.037	3, 7.9	6.2	0.018	3, 35.9	0.4	0.733
Drought × heat	3, 37.5	3.7	0.021	3, 7.9	1.8	0.228	3, 35.9	3.1	0.039
Date	3, 81.8	4.2	0.008	3, 86.9	9.5	<0.001	3, 90.3	2.4	0.076
Drought × date	3, 81.8	2.8	0.047	3, 86.9	7.9	<0.001	3, 90.3	2.9	0.037
Heat × date	9, 81.4	1.2	0.316	9, 86.9	1.1	0.357	9, 90.3	0.7	0.680
Drought × heat × date	9, 81.4	1.2	0.316	9, 86.9	1.0	0.490	9, 90.3	0.3	0.965

Summary of degrees of freedom (*df*; numerator, denominator), *F* and *p* values for net photosynthesis (A_{net}), stomatal conductance (g_s), and mid-day leaf water potential (Ψ_{mid}) during the four ecophysiology sample dates of the two-week heat wave

Bold values indicate significance ($p < 0.05$)

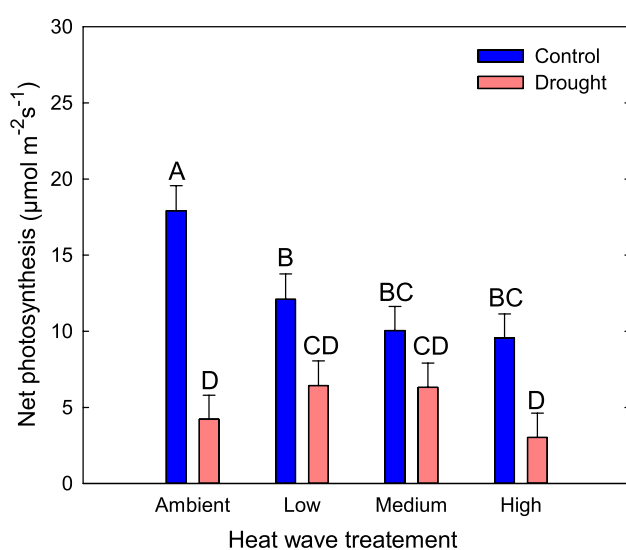


Fig. 4 The effects of the heat wave treatments on net photosynthesis (A_{net}). Precipitation × heat wave treatment interaction on net photosynthesis. Letters denote significant differences ($p < 0.05$) from pairwise comparisons in mixed model ANOVA (see Table 2)

high soil moisture availability in the control prevented CTs from reaching extreme levels (as seen under drought) by enhanced evaporative cooling via transpiration. Below this threshold, as soil moisture levels approached the wilting point, there were large decreases in Ψ_{mid} and increases in CT (Fig. 3). These results suggest that below this threshold, water stress decreased stomatal conductance, which reduced transpiration and evaporative cooling, leading to higher CTs, as seen in other studies (De Boeck et al. 2010) and as expected from the leaf energy budget (Gates 1965).

There was strong evidence for interactive effects between drought and heat wave treatments. Under drought, CTs in the ambient heat wave treatment (no added heat) exceeded the CTs in highest heat treatment under the control (Fig. 2). Furthermore, average CTs in the drought

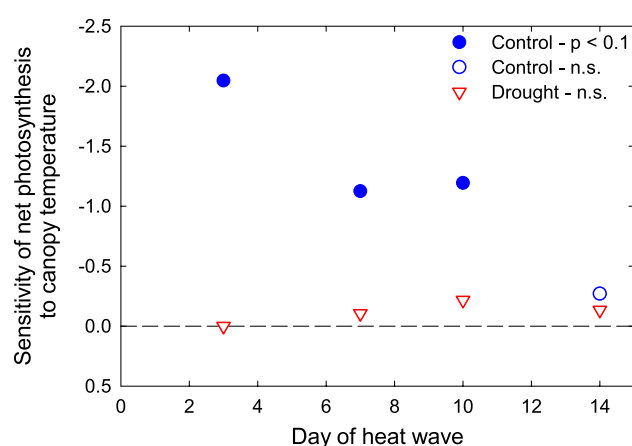


Fig. 5 Sensitivity of net photosynthesis (A_{net}) to canopy temperature (CT) during the two-week heat wave. Linear regressions were run for each date and precipitation treatment combination (control = circles, drought = triangles), and sensitivity was based on the slope (*y*-axis) and significance was determined at a p value < 0.01 where closed = significant ($p < 0.1$) and open = non-significant (ns; $p > 0.1$). Negative values indicate a negative relationship between canopy temperature and net photosynthesis. Dashed line at zero is for reference

treatment was almost 9 °C warmer than the control during the heat wave (Fig. 2). These results are similar to the global temperature differences between station-based air temperature and satellite-based land surface temperature; in non-forested areas including grasslands, land surface temperatures can be 10–20 °C warmer than air temperature during dry periods (Mildrexler et al. 2011). The CT differences also had strong interactions with the heat treatments. Despite control and drought treatments receiving the same infrared heat inputs within a given heat treatment (e.g., medium heat), these treatments resulted in dramatically different CTs dependent upon soil moisture. The effects of drought on CT should be considered when assessing extremes using historic records, which are generally limited

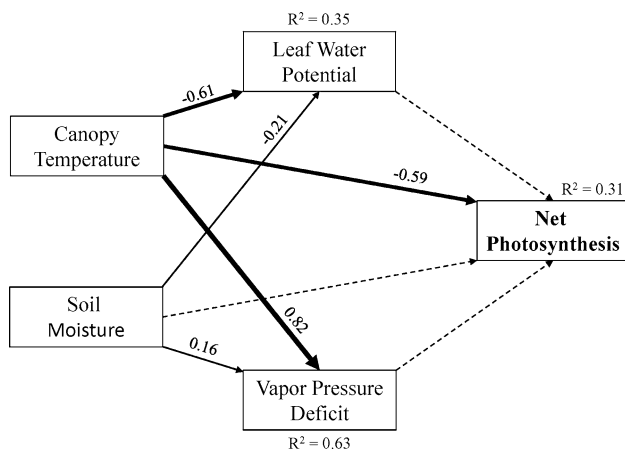


Fig. 6 Final path analysis model results for non-limiting soil moisture conditions (control precipitation treatment; $\chi^2 = 0.083$; $df = 1$; $p = 0.774$). Dotted lines indicate non-significant pathways ($\alpha > 0.05$), while solid lines are significant pathways ($\alpha < 0.05$) with associated path coefficients indicating the magnitude and direction of the relationship. For each endogenous response variable, R^2 is displayed indicating the proportion of variation explained by the model

to air temperature, as it is likely that the actual temperature long-lived perennial plants have experienced in historic extremes exceed that of air temperature records.

Soil moisture mediates the photosynthetic response to drought

Contrary to our hypothesis and previous work (De Boeck et al. 2016), the effects of the heat wave on photosynthesis were not apparent under the drought treatment despite experiencing historically low precipitation levels, and extremely dry soils (Ψ_{mid} approaching -8 MPa; Fig. 3b). We had expected that the added stress of the simulated heat waves would result in large responses. Instead, it appears that drought stress overwhelmed any response to heat, as already low A_{net} rates in the drought treatment did not vary across the different heat treatments and all were lower any A_{net} rates in the control (Fig. 4). This lack of response to heat was also surprising, given that the CTs in every heat wave treatment under drought exceeded every heat wave treatment under the control (Fig. 2). However, it is important to consider that photosynthetic rates were low and highly variable under drought, which may have reduced our ability to detect an effect of heat.

Although we observed no response of A_{net} to heat under drought, there were significant heat effects under the well-watered control precipitation treatment. We eliminated the indirect effect of heat on the soil moisture through irrigation as evidenced by the lack of significant main effects of the heat wave treatments or interaction between the heat wave and precipitation treatments on VWC (Table 1). The

question that remained was: How did heat impact photosynthesis without the indirect effect of soil drying?

Direct versus indirect effects of heat on photosynthesis

We used path analysis to assess whether photosynthesis was impacted by heat through other key indirect eco-physiological or environmental factors or if there was evidence for direct thermal effects. Water stress in a plant is driven by an imbalance between supply (soil moisture) and demand (VPD) as indicated by Ψ_{mid} , but while both VPD and Ψ_{mid} were significantly affected by heat, neither had significant effects on A_{net} (Fig. 6). This result, combined with the strong direct effects of CT on A_{net} in the model (Fig. 6), suggests direct thermal effects were driving reductions in photosynthesis.

There are several possible direct effects of heat related to non-stomatal limitations that could have resulted in declines in A_{net} under the control precipitation treatment. First, heat can directly damage photosystems II in the photosynthetic apparatus, which is particularly heat sensitive (Wahid et al. 2007). Second, heat can affect enzymatic kinetics and thereby alter metabolic processes. Enzymatic activities increase with temperature, and therefore, respiration should increase with temperature and cause a decrease in A_{net} if gross assimilation rates do not have a corresponding increase (Salvucci and Crafts-Brandner 2004). In addition, reductions in A_{net} due to heat stress have been correlated with a decrease in activation state of Rubisco (Salvucci and Crafts-Brandner 2004). It is also important to note that the sensitivity of A_{net} to the heat wave declined over two-week period (Fig. 5), suggesting photosynthetic acclimation to heat. The observed acclimation may have been caused by several factors including increasing the heat stability of the photosynthetic apparatus (e.g., thylakoid membranes or photosynthetic enzymes; Yamori et al. 2014) and/or reallocating nitrogen resources between photosynthetic components (Dwyer et al. 2007).

Implications

In this study, we determined that soil moisture governed the photosynthetic response of a dominant C_4 grass species to experimental heat waves of increasing magnitude, although the results contradicted our original hypothesis. When soil moisture was low, severe water stress reduced photosynthetic rates to low levels, thereby precluding the heat waves from reducing these rates any further. However, when we eliminated soil drying as a co-varying factor during the heat wave, photosynthesis was sensitive to increasing CTs. Soil moisture also governed the maximum temperatures the leaves experienced during the simulated heat wave; cooling from transpiration prevented CTs under non-limiting

soil moisture conditions from reaching the extreme levels experienced under drought. Overall, these results suggest that with extreme drought in this ecosystem, the main impacts of photosynthesis will be through severe water deficits rather than combined effects of heat and water stress. While under historical climate, extreme drought and heat waves often co-occur, with climate change the likelihood for novel and no-analog mismatches between these climate extremes may increase, with potentially large direct effects of heat waves on plant performance.

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