RESEARCH ARTICLE

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Earlier plant growth helps compensate for reduced carbon fixation after 13 years of warming

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Abstract

1. Drylands play a dominant role in global carbon cycling and are particularly vulnerable to increasing temperatures, but our understanding of how dryland ecosystems will respond to climatic change remains notably poor. Considering that the area of drylands is projected to increase by 11%–23% by 2,100, understanding the impacts of warming on the functions and services furnished by these arid and semi-arid ecosystems has numerous implications.

2. In a unique 13-year ecosystem warming experiment in a south-western U.S. dryland, we investigated the consequences of rising temperature on *Achnatherum hymenoides*, a widespread, keystone grass species on the Colorado Plateau. We tracked individual- and population-level responses to identify optimal strategies that may have been masked if considering only one level of plant response.

3. We found several factors combined to affect the timing and magnitude of plant responses during the 13th year of warming. These included large warming-induced biomass increases for individual plants, an 8.5-day advancement in the growing season and strong reductions in photosynthetic rates and population cover.

4. Importantly, we observed a lack of photosynthetic acclimation and, thus, a warming-induced downregulation of photosynthetic rates. However, these physiological responses were concurrent with warmed-plant increases in growing season length and investment in photosynthetic surfaces, demonstrating the species' ability to balance carbon fixation limitations with warming.

5. These results, which bring together ecophysiological, phenological, reproductive and morphological assessments of plant responses to warming, suggest that the extent of change in *A. hymenoides* populations will be based upon numerous adaptive responses that vary in their direction and magnitude. Plant population responses to climatic warming remain poorly resolved, particularly for Earth's drylands, and our in situ experiment assessing multiple strategies offers a novel look into a warmer world.

KEYWORDS
acclimation, allocation, climate experiment, phenology, plasticity, population dynamics, warming

1 | INTRODUCTION

Dryland systems account for c. 45% of the Earth's terrestrial surface, provide forage for a majority of the world's domestic livestock and play a key role in global carbon cycling (Morgan et al., 2011; Poulter et al., 2014; but see Schlesinger, 2017). Climatic change in these arid and semi-arid systems has critical implications for management and ecosystem services for 38% of the human population that lives in dryland systems world-wide (Reynolds et al., 2007). Further, dryland ecosystems are intrinsically water-limited and, as a result, are particularly vulnerable to increasing temperatures that enhance evapotranspiration, and consequently accelerate drying of terrestrial surfaces (Breshears et al., 2005; Huang, Yu, Guan, Wang, & Guo, 2016). Considering that the area of drylands is projected to increase by 11%–23% by the end of this century (Huang et al., 2016), understanding the impacts of warming on the functions and services furnished by these ecosystems is extremely important.

Rising temperature will force species to either shift ranges to follow ideal conditions, become isolated to refugia within their current ranges, acclimate (via phenotypic plasticity) or adapt (evolutionarily) to changing local conditions (Clark, Salk, Melillo, & Mohan, 2014; McDowell et al., 2019; Moritz & Agudo, 2013). Some of these changes are already occurring in response to recent warming including shifts in elevational ranges (Lenoir, Gégout, Marquet, Ruffray, & Brisse, 2008), physiological acclimation to novel conditions (Aspinwall et al., 2016) and phenological advancement, which allows a species to remain in its current range by tracking shifts in optimal climatic conditions over time (Walther et al., 2002). The ability of species or communities to synchronously shift ranges or timing of key biological activities has varying implications for ecosystem functioning, including carbon cycling and feedbacks to the climate system (Breshears, Huxman, Adams, Zou, & Davison, 2008). Despite these observed responses, an estimated 15%–37% of all species are expected to face extinction due to either their inability to disperse to new, suitable locations or persist given severe habitat fragmentation (Thomas et al., 2004).

Most plant-climate studies in dryland systems are limited to observational data for which linking observed community changes to specific climatic drivers is challenging. Few dryland studies have experimentally tested species responses to warming, especially over the longer term (>5 years; Smith, Monson, & Anderson, 2012; Wertin, Reed, & Belnap, 2015; but see Ryan et al., 2017), limiting our understanding of the underlying processes driving plant responses to higher temperature. Additionally, many of the warming experiments that do exist capture only community-level data and fail to track individual plants in ways that elucidate differing strategies and responses to temperature change for individuals and populations. This includes traits and responses exhibited by new individuals that establish within the community. Thus, divergent patterns can arise between current population dynamics versus a species' persistence and survival. A focus on a single metric and scale can answer many important questions about plant responses to change and is logistically more feasible, as multiple aspects of individual, population and community responses in warming experiments are often difficult to track simultaneously. Nevertheless, concurrent, integrative assessments of the multiple strategies plants use to respond to temperature (e.g. acclimation in phenology vs. in photosynthesis) offer a more holistic and potentially different picture of plant responses that, to date, no dryland studies to our knowledge have explored, and which few have tested in other systems.

Here, we present results from a novel long-term in situ warming experiment in order to determine the whole-plant responses of a widespread, dominant grass species, *Achnatherum hymenoides* (Indian rice grass), to experimental warming (+4°C). We focused on *A. hymenoides* for three key reasons. First, *A. hymenoides* is a common native C_3 bunchgrass of high economic importance that occurs throughout western North America (Jones, 1990). Second, focusing on individual responses within a population allows us to disentangle the potential variable responses of individuals that exist at the start of the experiment from those that establish in warmed conditions. Third, we set out to perform a concurrent assessment of multiple plant strategies in order to bring new insight into the multifaceted mechanisms through which plants are responding to long-term warming, and this level of assessment was not feasible at the community scale.

In an effort to make inferences about the likelihood of this species surviving and how Colorado Plateau dryland ecosystem function may change in the future, we tested for shifts in above-ground biomass, phenology (i.e. advancements and delays in growth initiation and senescence), photosynthesis (i.e. both net photosynthesis and photosynthetic acclimation) and for signs of water stress to increased temperatures after 13 years (2005–2018) of continuous warming. We also evaluated population cover and recruitment in an effort to reveal how changes in population dynamics and individual growth patterns interact. We did this by determining treatment effects on individuals that naturally established during the experiment, effects that would have been masked if only one category of plant response had been observed. We hypothesized that plants would have diverse responses to warming, with some strategies suggesting plants will benefit from warming (e.g. a longer growing season) and others suggesting a negative effect of warming (e.g. reduced photosynthesis until acclimation occurs). In particular, based on previous studies in this system and in other ecosystem types, we predicted that warmed plants would green-up c. 2–5 days earlier, grow larger in response to access to early-season moisture and would show photosynthetic acclimation since more than a decade of treatment has passed and *A. hymenoides* still dominates this grassland.

2 | MATERIALS AND METHODS

2.1 | Study site and experimental design

Our study site is located near the town of Castle Valley in southeastern Utah (36.675 N, −109.416 W) at an elevation of 1,310 m. a.s.l. in the central portion of the Colorado Plateau Desert. The site is on a west-facing slope and has a history of limited to no direct

anthropogenic impacts prior to establishment of the experiment. The soils at the site are classified as sandy loam, calcareous, Rizno series (Grand County Soil Survey) with a bulk density of 1.35 g/cm^3. The plant community is composed of the dominant perennial C$_3$ grass *A. hymenoides*, the perennial C$_4$ grass *Pleuraphis jamesii*, the C$_4$ shrub *Atriplex confertifolia* and the invasive annual grass *Bromus tectorum*. Additional subdominant species include *Descurainia pinnata*, *Eriogonum inflatum*, *Guttierrezia sarothrae*, *Machaeranthera canescens* and *Stanleya pinnata* among others. A full list of species present within the study site is included in Table S1. The site also includes biological soil crust communities dominated by the cyanobacterium *Microcoleus vaginatus*, cyanolichens *Collema tenax* and *C. coccophorum*, and the moss *Syntrichia caninervis*.

Five blocks of warming (+4°C) and ambient (control) treatments were applied to 20 5 m^2 rectangular plots (*n* = 10 per treatment; 2.5 m × 2 m) established in fall 2005. The 20 focal plots were originally made up of four treatments: control, warming, watering and warming + watering. The watering and warming + watering treatments received a set of 1.2 mm precipitation events during the monsoon season from 2005 until 2012, when the treatment was discontinued due to a lack of plant responses (see Reed et al., 2012; Zelikova, Housman, Grote, Neher, & Belnap, 2012 for additional details). Statistical analyses across years following the discontinuation of the watering treatments showed no consistent differences in plant characteristics with the watering treatments, and thus, watered plots were grouped with their unwatered counterpart plots (i.e. warmed + watered plots were grouped with warmed plots and watered plots were grouped with ambient plots) in order to explore the effects of temperature.

All plots had 15 cm deep flashing installed around the border of each plot. Warming was achieved using two 800 W infrared radiant heaters (Kalglo Model MRM-2408) placed 1.3 m above the soil surface of each plot. Lamps were oriented north to south across plots (sensu Harte et al., 1995; Kimball, 2005). Unwarmed plots have identical 'control lamps' placed in the same configuration as warmed plots to account for potential shading effects. Heaters were adjusted until soil sensors achieved the desired elevated temperature relative to controls. Soil temperature was measured with 3-tipped thermopiles constructed from 24-gauge type E thermocouple wire (Omega Engineering, Inc., FF-E-24-TWSH). The tip of each thermocouple was inserted at a 20 cm distance from the edge of the plot with a distance of 40, 60 and 80 cm from the centre of the plot. Thermopiles provide an average temperature across tips, allowing sampling of greater spatial variability while only using one input location on a datalogger. Sensors were wired into multiplexers attached to a Campbell CR 10X datalogger (Campbell Scientific, Inc., Logan, UT). Soil temperature measured at 5 cm depth strongly correlated with air temperature (Wertin, Belnap, & Reed, 2017). Soil moisture was measured using volumetric soil water content sensors (CS616; Campbell Scientific, Inc.) oriented horizontally at 5 cm depth in each plot. Treatment effects on soil temperature varied throughout the duration of the experiment (Reed et al., 2016), but achieved an average +2.5°C daily increase in soil temperatures at 5 cm depth

(c. +4°C at the soil surface) in our sampling year 2018 (January–June) with no observable effect on soil moisture (Figure S1). It is important to note that soil moisture was quite low at our site, and it is possible that differences were too small for the resolution of the sensors or that changes occurred in other parts of the soil profile that we did not measure.

2.2 | Measurements

Weekly phenological measurements were carried out throughout the growing season in 2018 (March–July). Thirty-six individual *A. hymenoides* plants were permanently tagged for measurements (15 in warming treatments and 21 in ambient treatments). Each week, individuals were assigned to one or more phenological stages including green-up, or when ≥25% of the above-ground plant biomass was green. This metric included all plant parts and not just leaves. The first sign of flowering was also recorded and included the first sign of a floral bud appearing. Last, the first sign of summer senescence was recorded when individuals showed any signs of browning or yellowing. Plants were observed until all individuals had senesced. These same individuals were measured at peak productivity (mid-spring), and the total number of culms (i.e. above-ground stems of a plant) and flowers was counted. Biomass of *A. hymenoides* is best estimated using the volume of a truncated cone shape determined by measuring the circumference of the plant at the soil surface, average canopy height, and the average width and length of the plant's top canopy (Wertin et al., 2017). Total above-ground biomass for each individual was estimated using allometric relationships between plant volume (cm^3) and dry weights determined from destructive harvests of *A. hymenoides* located outside our experimental plots. Volume of individual plants was measured before plants outside the plot were harvested, dried and weighed to obtain a conversion equation of estimated above-ground biomass = 0.001733 × volume (*p* < .001, R^2 = .948; Wertin et al., 2017).

Plot-level ground cover of *A. hymenoides* was estimated using a survey grid divided into 100 10 cm^2 cells, and plant presence was recorded when a dropped pin intercepted the plant in each cell. Plant mortality occurred throughout the duration of the experiment (six individuals in warmed plots, nine individuals in ambient plots), and as a result, new target individuals were randomly selected as needed. We used repeat photography beginning in 2011 to estimate year of germination for all newly selected individuals. These photographs were also compared to photographs taken in 2009 to determine whether plants established during the warming experiment (post-2009) or prior to 2009 and, as a result, potentially before the experiment began.

In April, May and June 2018, net photosynthesis (A_{net}) measurements were conducted on *A. hymenoides* individuals using a portable photosynthesis system (LI-6400XT; Li-Cor Biosciences, Lincoln, NE). Leaf chamber conditions were set to a photosynthetic photon flux density (PPFD) of 1,500 μmol m^{-2} s^{-1} with a CO$_2$ concentration of 400 μl/mol and a flow speed of 300 μmol/s. Relative humidity was maintained near ambient conditions, and temperature of the heating

block was controlled at ambient air temperature for control plots and ambient +4°C for warming plots. After each measurement was completed, leaf area within the cuvette was calculated by measuring leaf blade width using a mini-graduated hand lens (Peak #2015; GWJ Company, Hacienda Heights, CA) and gas exchange values were adjusted accordingly. Survey measurements were conducted during hours of peak sunlight between 10:00 and 14:00 hr. All 2018 measurements were carried out the same way as in years prior to allow for comparisons. Following gas exchange measurements in May, we revisited plots the next day to measure leaf water potential on detached leaves at predawn (between 05:00 and 06:00 hr local time) and again at midday (between 11:00 and 13:00 hr local time) using a Scholander-type pressure chamber (PMS-1000; PMS Instruments, Corvallis, OR).

Temperature responses of net CO_2 assimilation (A_{net}) were measured in early May around the time of peak productivity using the same portable photosynthesis system settings described above but manipulating temperature settings for multiple temperatures per leaf. We let each leaf stabilize at ambient temperature before cooling leaves to 3°C or the lowest stable temperature achievable. To achieve this, we used the chambers Peltier coolers and flowing ice water through heat exchange plates from an extended temperature control kit (6400-88; Li-Cor Biosciences). Block temperatures were then increased at 5°C steps until we reached 45°C or the highest stable temperature achievable using heated water.

2.3 | Analyses

We used linear mixed-effects models to evaluate treatment responses for each measured variable including phenological, morphological and physiological measurements. We also tested treatment effects on daily soil temperature and moisture. We included treatment (warming = yes or no) as a fixed effect to test for treatment effects. Models included block, plot and plant ID nested as random effects to account for pseudoreplication across blocks and plots. This also accounted for uneven sample sizes across treatments. All linear mixed-effects models were built using the nlme package in R 3.3.2 (R Core Team, 2014; Pinheiro, Bates, DebRoy, & Sarkar, 2018). Temperature response curves for each individual were calculated by fitting A_{net} rates to corresponding measurements of leaf temperature (sensu Säll & Pettersson, 1994) with the following second-order equation:

$$A_{net}\left(T_{leaf}\right) = A_{opt} - b\left(T_{leaf} - T_{opt}\right)^2,$$

where $A_{net}\left(T_{leaf}\right)$ is the fitted function of T_{leaf}, A_{opt} is the maximum net CO_2 assimilation rate occurring at an individual-based temperature optimum (T_{opt}), and b is a positive factor that determines the spread of the parabola (Battaglia, Beadle, & Loughhead, 1996) and is indicative of the rate of photosynthetic decline as T_{leaf} deviates from T_{opt} (Säll & Pettersson, 1994). Only individuals with measurements that were adequately described ($p \le .05$) by the above equation were included in analyses ($n = 11$). Fitted lines had R^2 values that ranged from .78 to .97 (mean 0.89 ± 0.02 SEM).

3 | RESULTS

Warming affected plant growth through large enhancements in above-ground production (Figure 1) with individual plants doubling the number of culms (i.e. photosynthetic surfaces) they produced compared with ambient plants ($p = .04$). The number of inflorescences produced also increased in warmed plots, though this change
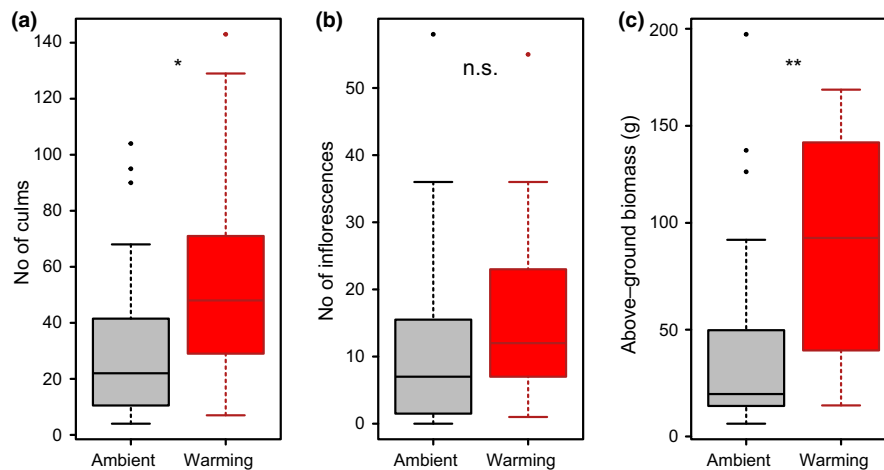
FIGURE 1 Morphological responses to warming. Above-ground *Achnatherum hymenoides* growth responses to warming reveal that (a) the number of culms (i.e. photosynthetic surfaces) produced by individual plants more than doubled in response to warming in 2018 (*$p = .04$). (b) On average, individuals produced c. 32% more inflorescences in response to warming though this change was not significant ($p = .25$). (c) Overall, measured plants produced twice as much above-ground biomass in warming treatments (**$p < .01$). Boxes denote the upper and lower quartiles, and the median is marked by a vertical line within the box. Whiskers (or circles when present) denote highest and lowest observations. Significance values are for treatment effects in linear mixed-effects models. Colours indicate treatments (grey = ambient, red = warming)

was not significant ($p = .25$). Overall, measured plants produced twice as much above-ground biomass in the warming treatment relative to controls ($p < .01$; Figure 1). However, there were far fewer *A. hymenoides* individuals in warmed plots due to lower survival and recruitment over the course of the experiment compared with unwarmed plots (Table 1), resulting in an overall warming-induced reduction in *A. hymenoides* cover ($p = .05$; Figure S2). Further, repeat photography revealed that 60% of extant plants in the warmed plots germinated under the treatment since 2012, while only 21% of the plants from the ambient plots germinated during the experiment (Table 1).

Individual *A. hymenoides* plants also responded to warming through large changes in phenological cycles (Figure 2). Spring green-up advanced 8.5 ± 2.9 days (mean $\pm$ *SEM*; $p = .01$) in response to warming. Date of first flower also advanced 10.8 ± 1.3 days (mean $\pm$ *SEM*; $p < .0001$) in warmed plots relative to controls. Last, the onset of senescence in individual plants advanced 2.2 ± 1.1 days (mean $\pm$ *SEM*; $p = .05$; Figure 2), leading to a longer growing season overall in warmed plots (+6.3 days).

We hypothesized that warming would result in photosynthetic acclimation for *A. hymenoides*, with plants in warmed plots increasing their temperature optima, thereby lessening the warming-induced reductions in photosynthesis seen earlier in the experiment. In contrast to our predictions, no treatment effects were found on photosynthetic optima (A_{opt}; $p = .49$; Figure 3) or temperature optima (T_{opt}; $p = .88$; Figure 3). Net photosynthesis in the warmed plots was reduced by 30% on average relative to the ambient plants when soil moisture was not limiting early in the growing season (A_{net}; $p = .001$; Figure 4). Overall differences in A_{net} between treatments declined as the season progressed and were largely constrained by stomatal conductance (g_s), which had a strong correlation to A_{net} values over the survey period ($R^2 = .88$, $p < .0001$; Figure 4). Finally, both pre-dawn and midday leaf water potential measurements did not differ between treatments (Figure S3), indicating that water stress is not directly driving reductions in A_{net} rates.

TABLE 1 Population-level recruitment

Year	# of new plants established	
	Warming	Ambient
Pre–2009	10	23
2011	1	2
2012	0	0
2013	0	0
2014	21	47
2015	4	4
2016	1	0
2017	0	0
2018	0	2
Total	37	78

Note: Total recruitment by treatment and by year based on repeat photography.

4 | DISCUSSION

Drylands represent our largest terrestrial biome and are predicted to expand in response to warming (Huang et al., 2016). Some studies suggest grasses will gain an advantage in a warmer, drier world given their relatively high drought tolerance (Craine et al., 2013). However, this likely only applies to ecosystems that are currently not grass-dominated and those in which water is not already limiting (Moritz & Agudo, 2013). Instead, dryland systems like those in the western United States are expected to see grasses replaced by woody shrub species (Gremer, Bradford, Munson, & Duniway, 2015; Munson, Belnap, Schelz, Moran, & Carolin, 2011; Winkler, Belnap, Hoover, Reed, & Duniway, 2019) given that water-limited systems are not expected to respond as strongly to warming as they will to drought (Smith et al., 2012). However, results from our in situ warming experiment revealed a strong temperature response in *A. hymenoides*, with distinct responses at the population and individual plant levels. Overall, our study suggests that the Colorado Plateau may see declines in *A. hymenoides* cover but that individuals that establish under warmer conditions may be more productive and can adjust phenological and growth strategies to tolerate a warmer world.

After 13 years of continuous warming, we found fewer but much larger individuals occupying warmed plots; a multifaceted response with important implications for the services these plants provide (e.g. forage) that would have been missed had multiple metrics of assessment not been used simultaneously (Grady et al., 2013). In particular, we observed substantial, positive long-term growth response to increased temperatures after 13 years of warming that counter shorter-term declines in productivity observed earlier in the experiment (Wertin et al., 2015). Plants in warmed plots were much larger and were primarily individuals that established during the warming experiment. While plant age/germination date was not a significant determinant of response for any of our measured variables, assessment across additional years could help determine how warming interacts with germination, selection and lower average plant cover to result in *A. hymenoides* individuals that are almost double in size relative to their ambient plot counterparts.

On the other hand, although individual-level biomass increased in response to warming, the overall cover of *A. hymenoides* was lower in the warmed plots because there were fewer individuals. So, while the warmed plants were larger, they were also lower in abundance, which could help explain their larger size (e.g. reduced competition; Reich et al., 2015). The plot-level ground cover decline was explained by changes in natural recruitment through time, as 47% fewer new individuals established in warmed plots (Table 1). Furthermore, variance in the number of culms, inflorescences and above-ground biomass generally increased in measured plants of the warmed plots relative to ambient (Figure 1). This increased variation in anatomical and morphological traits is the opposite of what would be expected if the observed responses were due to natural selection favouring a subset of adaptive phenotypes in response to warming (Jump, Marchant, & Peñuelas, 2009). Given the increase in phenotypic variation we observed, establishing and persisting in a
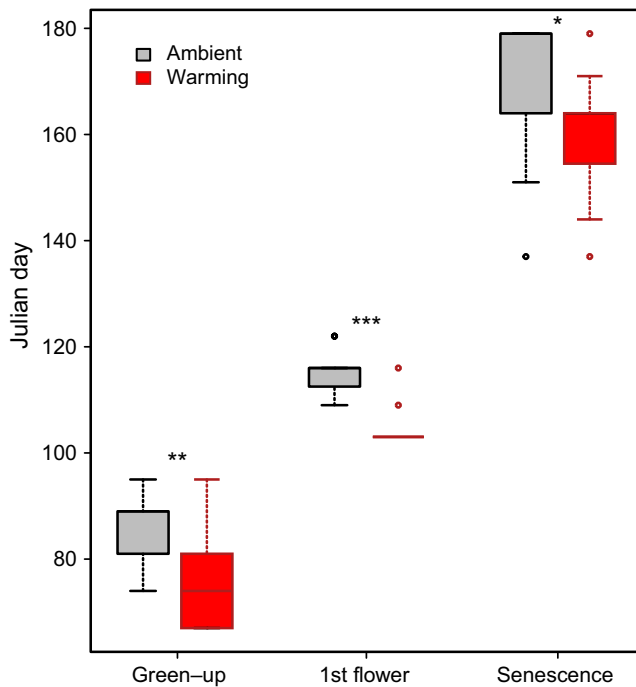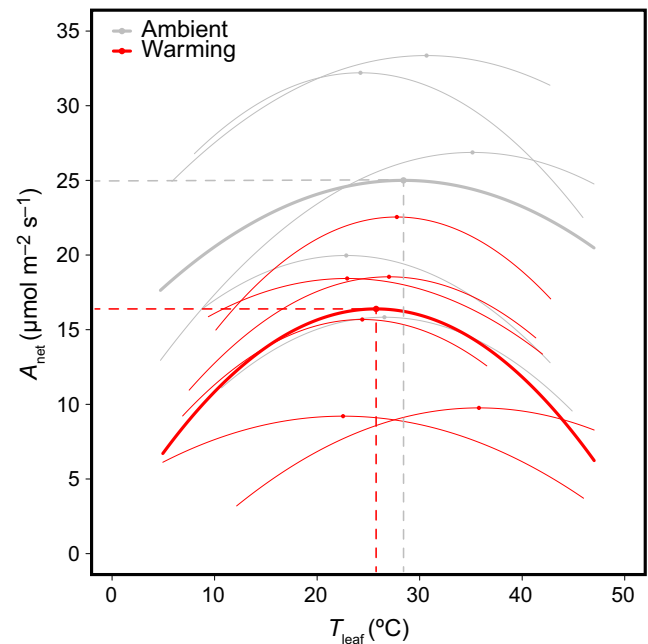
FIGURE 2 Phenological responses to warming. Spring green-up, or when ≥ 25% of an individual *Achnatherum hymenoides* plant first turned green in 2018, advanced 8.5 ± 2.9 days (*SEM*; **p = .01), the date of first flower advanced 10.8 ± 1.3 days (*SEM*; ***p < .0001), and the first sign of senescence advanced 2.2 ± 1.1 days (*SEM*; *p = .05) in response to warming. Boxes denote the upper and lower quartiles, and the median is marked by a vertical line within the box. Whiskers (or circles when present) denote highest and lowest observations. Significance values are for treatment effects in linear mixed-effects models. Colours indicate treatments (grey = ambient, red = warming)

FIGURE 3 Temperature response curves. Net CO_2 assimilation (A_{net}; μmol m^{-2} s^{-1}) responses of individual plants to varied temperature by treatment (thin grey lines = ambient, thin red lines = warming). Fitted lines had R^2 values that ranged from .78 to .97 (mean 0.89 ± 0.02 *SEM*). Thick lines indicate treatment means, and dashed lines and circles show photosynthetic optima (A_{opt}) at temperature optima (T_{opt}). There were no detectable treatment effects on A_{opt} (p = .49) or T_{opt} (p = .88) based on linear mixed-effects models, but warming resulted in lower net CO_2 assimilation rates

warmer world is likely the result of phenotypic plasticity (Nicotra et al., 2010), but whether this plasticity is conferred by a heritable trait enhanced by natural selection or a result of acclimation is not yet clear. Additional studies are needed to test potential genetic differences between newly established individuals in warmed and control plots (sensu Avolio, Beaulieu, & Smith, 2013) as well as potential responses below-ground. To our knowledge, this is the first study to utilize a natural population to demonstrate divergent yet counteracting responses at the population (i.e. plot) and individual levels, suggesting *A. hymenoides* will likely experience population declines with future warming, yet the species is likely to persist via significant establishment and growth compensation when conditions are favourable. This includes not only favourable climatic conditions tracked via phenological adjustments, but also likely results in part from reduced competition for resources (Reich et al., 2015) since fewer individuals were present in warmed plots.

Previous warming studies in semi-arid grasslands and other terrestrial systems, including those in the tropics and temperate forests, have resulted in reductions, increases or no changes in productivity (Morgan et al., 2011; Scheffers et al., 2016; Slot & Winter, 2018). These divergent changes are often attributed to local environmental factors including interannual climate

variability (Shaver et al., 2000), but may also result from differing approaches to measuring responses, including only measuring established individuals (Penuelas et al., 2004) or focusing on community-level changes (Walker et al., 2006). In fact, productivity changes (positive or otherwise) at the community level can be explained by individual responses of functional groups or species (Cowles, Wragg, Wright, Powers, & Tilman, 2016; Winkler, Chapin, & Kueppers, 2016). Thus, the warming-induced changes to germination patterns observed in our experiment could help explain these varied results. Further, our data suggest that instead of a forecast for complete loss of this critical plant species from the south-western United States (Munson et al., 2011), we may instead expect fewer, larger plants as evidenced by reduced recruitment but increases in individual-level biomass. This variation would be dependent upon not only a given year's weather (e.g. growing season precipitation), but also the more rapidly changing population dynamics. If experienced across numerous species and drylands, such dynamics would have substantial effects on the strength of the carbon–climate feedback (Poulter et al., 2014; Wu, Dijkstra, Koch, & Hungate, 2012). Overall, an outcome with *A. hymenoides* remaining on the landscape would have more positive implications for land managers and ranchers as well, who depend on the plant as a critical forage source for wildlife and livestock across the western United States (Jones, 1990; Winkler, Backer, et al., 2018).
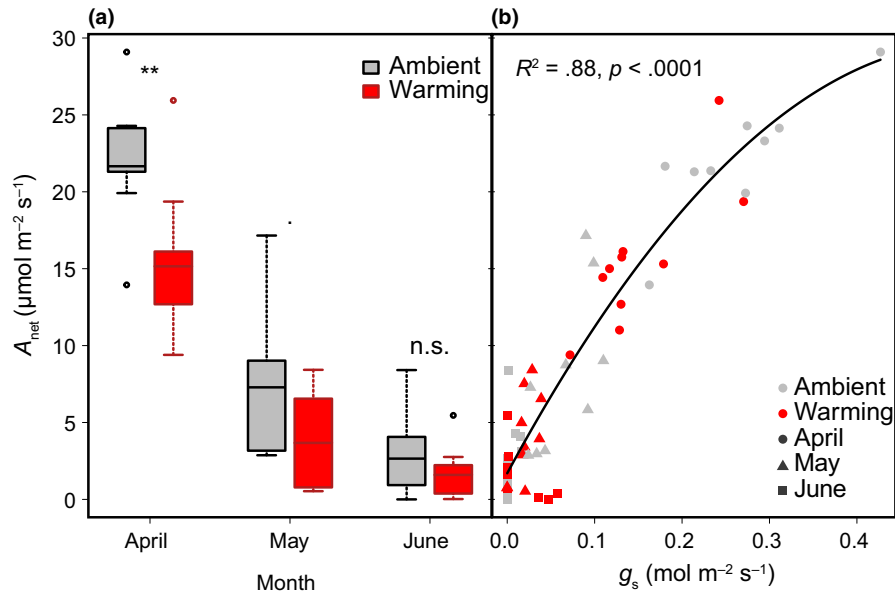
FIGURE 4 Photosynthetic responses to warming. (a) Monthly net CO_2 assimilation (A_{net}; μmol m^{-2} s^{-1}) by treatment (grey = ambient, red = warming). A_{net} rates for warmed *Achnatherum hymenoides* were 30% lower early in the season (April; **p = .01), marginally different in the middle of the growing season (May; p = .07) and had no difference at the end of the spring growing season (June). Significance values are for treatment effects in linear mixed-effects models. (b) A_{net} rates were constrained by stomatal conductance (g_s; mol m^{-2} s^{-1}) over the survey period, with the fitted second-order polynomial $A_{net} = 1.70 + 104.99 \times g_s - 98.42 \times g_s^2$ (R^2 = .88, p < .0001, residual SE = 2.91). Boxes denote the upper and lower quartiles, and the median is marked by a vertical line within the box. Whiskers (or circles when present) denote highest and lowest observations. Colours indicate treatments (grey = ambient, red = warming), and polygons indicate sampling month (circles = April, triangles = May, squares = June)

Our observed phenological shifts in spring green-up are a likely underlying mechanism for the observed individual plant increases in biomass in warmed plots, as individuals may have been able to take advantage of wetter soils earlier in the season (Figure S1). Further, these phenological shifts are some of the largest seen to date in any warming experiment (Wolkovich et al., 2012) – which have regularly under-predicted plant phenological responses to higher temperature – and are similar to the average shifts in phenology in response to recent warming measured in observational studies across >1,500 plant species over the past 30+ years (Wolkovich et al., 2012). The greater response to warming relative to other experimental studies is perhaps due in part to the longer duration of our experiment, which captured phenological shifts in response to long-term, chronic warming versus short-term responses that can dissipate with time (Parmesan & Yohe, 2003; Wolkovich et al., 2012). These dramatic phenological shifts in growth initiation, flowering and senescence highlight the strong and likely less flexible response to temperature cues that initiate *A. hymenoides* growth, but also indirectly allow individuals to take advantage of wetter soils for growth earlier in the season. Further assessment of phenological shifts across years could provide additional insight into phenological controls and limits.

Counter to our predictions, we found no difference in photosynthetic and temperature and optima between warmed and control plots. This suggests that *A. hymenoides* did not acclimate to changes in temperature, likely due to low photosynthetic plasticity, resulting in warming which negatively affected *A. hymenoides'* capacity to fix CO_2. Reduced photosynthesis in the warmed plants has now

persisted across numerous years throughout more than a decade of warming (Wertin et al., 2017, 2015). Thus, a shift in T_{opt} may not be necessary for local persistence in Colorado Plateau ecosystems given that an increase of +4°C is likely already within the species temperature range for successful carbon gain.

Reduced A_{net} rates as a consequence of stomatal closure were likely driven by higher evaporative demand rather than by changes in soil moisture (Figures S1 and S3). This finding supports emerging evidence that evaporative demand may become a major driver of plant responses to climatic change (Williams et al., 2013) and highlights that, instead of temperature alone, more focus may need to be placed on the role of vapour pressure deficit in future studies. Our results from this perennial grass corroborate previous findings from warming studies focused on woody plants in different dryland and warm-temperate systems (Aspinwall et al., 2016; Hamerlynck, Huxman, Loik, & Smith, 2000). They also suggest that *A. hymenoides* may be shifting towards a greater reliance on early-season carbon fixation when evaporative demand is lower (Wu et al., 2012) followed by downregulation of A_{net} rates that likely reflects individuals shifting allocation towards reproduction (Gremer, Kimball, Angert, Venable, & Huxman, 2012). This supposition is supported by both predawn and midday leaf water potential measurements, which did not differ between treatments (Figure S3), indicating that water stress is not directly driving reductions in A_{net} rates.

Our study evidences a plastic response of *A. hymenoides* to tolerate 13 years of warming by shifting growth and above-ground allocation strategies and downregulating CO_2 fixation to prevent plant

damage. Specifically, given that A_{net} rates were largely constrained by g_s, *A. hymenoides* is likely preventing water loss that would ultimately lead to irreversible photosystem damage (Berry & Bjorkman, 1980; Sage, Way, & Kubien, 2008). Also, increased carbon investment to photosynthetic structures may be an attempt to correct for lower A_{net} rates per unit of leaf area in response to warming. Our study is the first to show warming-induced increases in production in spite of consistent reductions in leaf-level photosynthesis. This result highlights this species' ability to balance fixation limitations with increased growing season length – as seen in the extended time from first green-up to first brown-down in the warmed plots – and increased investment in additional photosynthetic structures. These responses likely reduce the need for plants to acclimate their photosynthetic apparatus in response to warmer temperatures and suggest a scenario through which some species of plants may be capable of facing increased temperatures (McDowell et al., 2008). This does not mean *A. hymenoides* will be as abundant or will represent as much of the systems' total plant cover under future climate scenarios, but it does suggest that at least some individuals can persist under future warming.

Carbon balance in pulse-driven systems such as the Colorado Plateau is largely determined by seasonal precipitation (Huxman et al., 2004). If precipitation variability is maintained at current levels, *A. hymenoides* may be able to tolerate increased temperatures and reduced soil moisture by changing particular strategies for success. Current plant populations may not be able to acclimate to climatic change if stressors lead to mortality of individuals and/or prevent regeneration and establishment of new individuals (Granda, Escudero, & Valladares, 2014; Winkler, Conver, Huxman, & Swann, 2018). Long-lived, perennial species such as *A. hymenoides* have evolved to tolerate substantial variance in temperature and precipitation given their life spans (Smith et al., 2012). This combined with the inertia of phenotypic or phenological changes in response to warming presents a challenge to studying perennial species. That said, our data show it is possible for individuals within a population to positively respond to warming by altering growth and allocation strategies (Jump & Peñuelas, 2005), although the ability of these changes to be maintained long-term is poorly known. Our study demonstrates multiple individuals within a population tolerating warmer temperatures by shifting their phenology, likely to track ideal growing conditions and compensate for lower productivity as spring advances to summer and conditions warm. This 'mixed-bag' strategy may represent one of the ways species can withstand some of the negative impacts of climatic change. These results are valuable given this species' ecological and economic importance as a food source both for native wildlife and for domestic livestock across the western United States.

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AUTHORS' CONTRIBUTIONS

D.E.W., C.G., J.B. and S.C.R. conceived the study. D.E.W., C.G, A.H. and H.S. collected the data. D.E.W. analysed the data with contributions from A.H. D.E.W. wrote the manuscript with all co-authors contributing to the final version.

DATA AVAILABILITY STATEMENT

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