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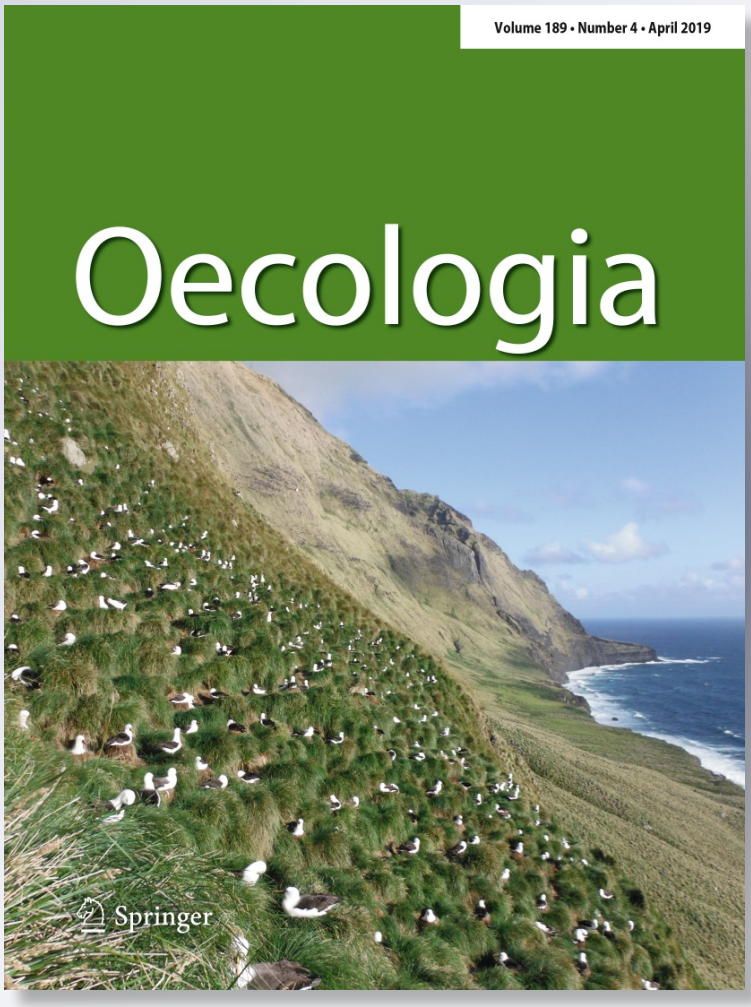

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GLOBAL CHANGE ECOLOGY – ORIGINAL RESEARCH

Experimental drought reduces genetic diversity in the grassland foundation species *Bouteloua eriopoda*

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Abstract

Understanding the resistance and resilience of foundation plant species to climate change is a critical issue because the loss of these species would fundamentally reshape communities and ecosystem processes. High levels of population genetic diversity may buffer foundation species against climate disruptions, but the strong selective pressures associated with climatic shifts may also rapidly reduce such diversity. We characterized genetic diversity and its responsiveness to experimental drought in the foundation plant, black grama grass (*Bouteloua eriopoda*), which dominates many western North American grasslands and shrublands. Previous studies suggested that in arid ecosystems, black grama reproduces largely asexually via stolons, and thus is likely to have low genetic variability, which might limit its potential to respond to climate disruptions. Using genotyping-by-sequencing, we demonstrated unexpectedly high genetic variability among black grama plants in a 1 ha site within the Sevilleta National Wildlife Refuge in central New Mexico, suggesting some level of sexual reproduction. Three years of experimental, growing season drought reduced black grama survival and biomass (the latter by 96%), with clear genetic differentiation (higher F_{ST}) between plants succumbing to drought and those remaining alive. Reduced genetic variability in the surviving plants in drought plots indicated that the experimental drought had forced black grama populations through selection bottlenecks. These results suggest that foundation grass species, such as black grama, may experience rapid evolutionary change if future climates include more severe droughts.

Keywords Climate change · Genotyping-by-sequencing · Natural selection · Experimental evolution · Sevilleta long-term ecological research

Introduction

Climate change can directly reduce plant fitness and population abundance (Parmesan 2006; Anderson 2016). Declines in population abundance under a new climate have the potential to feedback to influence the resistance or resilience of populations to future changes by reducing population genetic diversity (Pauls et al. 2013). Maintaining genetic diversity is important because it can buffer against future climate stress, disturbance, inbreeding depression, and antagonistic species interactions (e.g., Jump et al. 2009; Hart et al. 2016; Isbell et al. 2015). For example, high genetic diversity can buffer populations against unpredictable environmental conditions, such as extreme drought or flooding (e.g. Prati et al. 2016), in part through the portfolio of genotypes that vary in their sensitivity to extremes. The loss of genetic diversity can also influence population-level processes, depressing species-level productivity, population growth, and the ability to

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invade new habitats (Crutsinger et al. 2006; Crawford and Whitney 2010; Cook-Patton et al. 2011; Atwater and Callaway 2015). Mechanisms underlying feedback from genetic diversity to population processes include the loss of specific genotypes that can survive extreme climate events (selection or dominance effect, Fox 2005) and the loss of non-additive effects of diversity, such as niche complementarity or facilitation among genotypes, that can increase population growth and productivity (reviewed by Hughes et al. 2008; Jump et al. 2009; Whitlock 2014).

There is increasing evidence for climate-induced shifts in population-genetic structure (reviewed in Pauls et al. 2013; see also Carroll et al. 2014; Franks et al. 2014) and for the adaptive significance of these shifts in both plants and animals (reviewed by Merilä and Hendry 2014). For example, Rubidge et al. (2012) associated climate warming in Yosemite National Park with a contraction in range size and a reduction in overall genetic diversity for the alpine chipmunk (*Tamias alpinus*). Nevo et al. (2012) reported genetically based changes in flowering time and a significant reduction in simple sequence allele diversity over a 28-year period for wild populations of both wheat (*Triticum dicoccoides*) and barley (*Hordeum spontaneum*). Franks et al. (2007, 2016) described an evolutionary shift to shorter flowering time for *Brassica rapa* that followed a multi-year, naturally occurring drought, with corresponding shifts in allele frequencies in genes related to flowering time and drought stress. Jump et al. (2008) imposed experimental drought and warming on the early successional Mediterranean shrub *Fumana thymifolia* over a 7-year period and found elevated genetic divergence between seedlings in treatment vs. control plots, signaling climate-induced selection. In contrast, other studies have found relatively little evolutionary change in response to climate (e.g., Lau and Lennon 2012; Huber et al.2016). For example, Lau and Lennon (2012) detected only weak evolutionary responses of *B. rapa* populations to greenhouse-imposed drought; instead, plant fitness responded to rapid changes in the composition of soil microbes following drought.

Losses of genetic diversity could be particularly consequential in dominant or foundation species, which shape the composition and diversity of associated plant, animal, and microbial communities (Ellison et al. 2005; Bangert et al. 2008; Hughes et al. 2008). Declines in the genetic diversity of foundation plant species could cascade to community-level interactions. For example, genetic diversity in dominant species can enhance resistance to both invasion (Moreira et al. 2014; Yang et al. 2017) and herbivores (Moreira et al. 2014). In addition, high genetic diversity in foundation species can interact with species diversity to produce synergistic gains in ecosystem productivity and functioning (e.g., Crawford and Rudgers 2012; Schoeb et al. 2015). Thus, understanding the genetic consequences of climate change for foundation species could help to refine predictions of future community and ecosystem trajectories (e.g., Ikeda et al. 2017).

We know of just two studies that have examined the effects of experimental climate change on genetic diversity and differentiation in foundation plant species in the field. In a 10-year rainfall manipulation, Avolio et al. (2013) found that genotype richness of big bluestem (*Andropogon gerardii*) decreased under higher within-season variability in precipitation, with a few successful genotypes increasing in abundance. These successful genotypes were both genetically and phenotypically divergent from each other (Avolio et al. 2013; Avolio and Smith 2013), suggesting a role for niche differentiation in population resistance to stress. Ravenscroft et al. (2015) imposed drought, water addition, and warming on grasslands, and after 15 years found elevated genetic divergence between treatments for the forb *Plantago lanceolata* and the foundation grass *Festuca ovina*. We note that both of these studies occurred in mesic grasslands, leaving open the question of climate change-induced evolution in the plants that dominate arid ecosystems. These ecosystems are of global importance: of all the land cover classes, arid and semi-arid ecosystems contribute most to inter-annual variability in global carbon flux due to their high year-to-year variability in primary production (Ahlstrom et al. 2015; Huang et al. 2016a) and large surface area (45% of global land area, Pravalie 2016), which is rapidly expanding (Huang et al. 2016b).

In the current study, we leveraged an established drought experiment in an arid grassland to address the questions: (a) What are standing levels of genetic variation in a foundation plant species? (b) How does drought affect the genetic diversity and composition of a xeric-adapted foundation plant? We focused on black grama grass (*Bouteloua eriopoda*), a dominant of desert grasslands in western North America, which has been reported to reproduce mostly clonally through stolons (Peters 2002). Clonal populations may be highly sensitive to climate disruptions, such as drought, if low standing diversity provides little buffer against change (Jimenez-Alfaro et al. 2016). In our experiment, a 66% reduction of growing season precipitation caused substantial mortality of *B. eriopoda* plants, raising the possibility that differential survival had a genetic basis. We tested this hypothesis using a reduced representation sequencing approach, employing DNA extracted from root tissues from living vs. recently dead plants. To our knowledge, this is the first study to examine whether climate alters population genetic diversity for an arid ecosystem dominant.

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Methods

Study species

Black grama (*Bouteloua eriopoda* (Torr.) Torr. (Poaceae) is a stoloniferous, C_4 perennial grass that dominates desert grasslands of North America. It is a diploid ($2n = 20$) (Streetman and Wright 1960; Gould 1979) with a geographic distribution ranging from Texas to southern California and from Mexico northward to Colorado, Utah, and Wyoming. Black grama is present in diverse dryland ecosystems including desert grasslands, creosote bush shrublands, mesquite scrub, and piñon-juniper woodlands, and it dominates uplands (800–1900 m) with sandy loam soils (Simonin 2000). Historically, black grama covered extensive areas of western Texas, southern New Mexico, southeastern Arizona, and northern Mexico (Nichol 1952; Dick-Peddie 1993), but many desert grasslands have been replaced by encroaching shrublands over the past 150 years (e.g., van Auken 2000; Connin et al. 1997; Báez and Collins 2008; Peters et al. 2010). Prior work has documented the sensitivity of black grama to several abiotic factors, including drought (Báez et al. 2013), grazing (Gosz and Gosz 1996), and fire (Reynolds and Bohning 1956; Parmenter 2008). Monsoon precipitation (July–September) is a more important correlate of black grama production than total annual precipitation (Paulsen and Ares 1962; Thomey et al. 2011; Rudgers et al. 2018).

Study site and drought experiment

The study was conducted at the Sevilleta National Wildlife Refuge (SNWR), a Long-Term Ecological Research (LTER) site in the northern Chihuahuan Desert of central New Mexico, USA. Within the SNWR, the study site was located in a black grama-dominated grassland near a zone where creosote bush (*Larrea tridentata*)-dominated shrubland transitions to grassland. Black grama has been increasing in abundance at this site over the past 3 decades (Collins and Xia 2015). Total annual precipitation is ~ 250 mm, ~ 60% of which falls during the summer monsoon from July through early September (Notaro et al. 2010).

The Extreme Drought in Grassland Experiment (EDGE) was established in Spring 2013 and includes a chronic drought treatment (66% reduction in growing season rainfall) designed to mimic droughts that are predicted to occur in this region by the end of this century (Cook et al. 2015). Drought was imposed by installing roof panels constructed of plastic strips that cover 66% of the surface area (Yahdjian and Sala 2001) to reduce the size of each rain event (Fig. 1a). Roof panels are in place only during the growing season (April through September). These passive drought shelters create a 1-in-100-year drought (Knapp et al. 2015) and effectively mimic rainfall patterns (size, frequency) during natural drought years (Knapp et al. 2017). The shelters were designed to allow substantial airflow, with completely open ends and sides that are open from ground level to 1.2 m above ground (Fig. 1a). Shelters had a minimal effect (~ 0.4 °C) on mean 2013–2017 growing season air

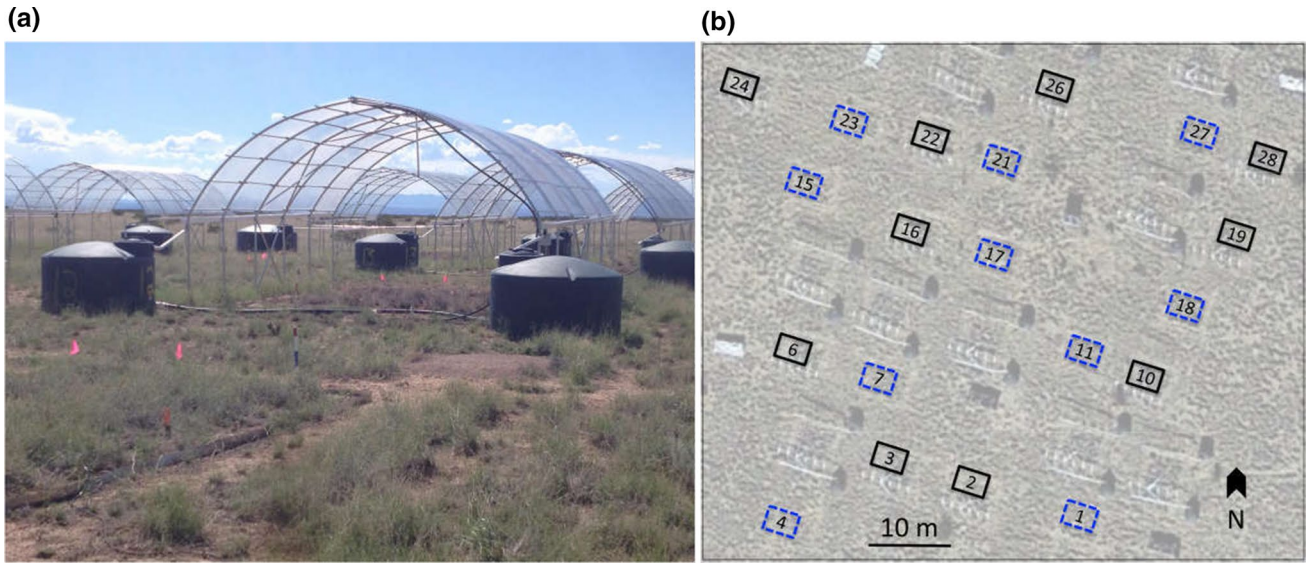

Fig. 1 Extreme Drought in Grasslands Experiment (EDGE) at the Sevilleta National Wildlife Refuge. **a** During summer (monsoon) months, rainout shelters are partially covered with plastic sheeting to remove approximately two-thirds of precipitation from drought plots. **b** Black grama EDGE plot layout. Dashed (control) and solid (drought) rectangles superimposed on a satellite image indicate paired treatments within each block

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temperatures (mean ± SE: shelter 22.84 ± 0.86 °C; control 22.42 ± 0.86 °C). The portion of the experiment used in the current study consists of 20 plots (3 m × 4 m each), with 10 drought plots and 10 controls, paired spatially into blocks (Fig. 1b). Drought treatment was assigned randomly within a block.

Field sampling: biomass

Standing live biomass of black grama was estimated using a non-destructive volumetric method (Huenneke et al. 2001; Muldavin et al. 2008). Volume measurements (% cover × height) of all individual plants were recorded in four permanently located 1-m^2 quadrats in each replicate plot during peak biomass (September) in the 2015–2017 growing seasons, the third through the fifth growing seasons under experimental drought. Volume was then converted to biomass via allometric equations derived from coupled volume measurements and destructive harvests of black grama biomass outside of the experimental plots; volume explained 70% of the variation in biomass in this equation ($R^2 = 0.70$). We analyzed the black grama biomass response with a linear mixed effect model that included the fixed effect of drought treatment, year, and their interaction, as well the random effect of plot to account for the non-independence of replicate quadrats within a plot, using the lmer function in the *lme4* package (Bates et al. 2015; R Core Team 2017). We report estimated marginal means and standard errors from the model (emmeans function).

Field sampling: genetic diversity

We quantified drought effects on the genetic diversity and composition of live (at least one green leaf) versus recently dead (no green tissue present) black grama. At the end of the third growing season (28-Oct-2015), we collected plant leaves and roots from 20 black grama individuals per plot. This sample size was determined by a goal of balanced sampling of live and dead individuals within and across plots, and the fact that some drought plots contained only ten live individuals by the third year of treatment. Collection occurred 1 week after a late 3-day monsoon rain period (which ended 21-Oct-2015), totaling 23 mm. In drought plots, we marked pairs of live and dead individual plants (defined as contiguous patches separated by a minimum of 5 cm, Laurenroth and Adler 2008) to meet the following criteria: (i) at least two pairs were chosen in each of four quadrants of the plot; (ii) no individuals occurred within 50 cm of the plot edge (defined by aluminum flashing); and (iii) members of a live/dead pair were closer to each other than to other marked individuals within a plot. The paired strategy was adopted to ensure that sampling of live vs. dead individuals occurred across equal spatial scales in a plot, in case of within-plot spatial population genetic structure. In control plots, we marked pairs of plants similarly, but both individuals in each control group were live due to the lack of mortality. All plots received equal levels of disturbance and vegetation removal.

From each individual, we removed one to three tillers and three to five root segments (min. ~ 3 cm long) using a soil knife. Tools were cleaned with bleach germicidal wipes (sodium hypochlorite 0.55%, Clorox Healthcare, Oakland, CA, USA) between each sample. Samples were collected into sterile Whirl-Paks (Nasco, Fort Atkinson, Wisconsin) and returned to the lab where roots were washed in DI water, dried with a contaminant-free KimWipe (Kimberly-Clark, Irving, Texas; McManus and Kelley 2005), and stored at – 20 °C within 72 h.

We checked that plants we classified as "dead" were indeed dead (as opposed to temporarily dormant) via two methods. First, we continued monitoring of live biomass in the experimental plots over two more growing seasons to see if there was any evidence of recovery in the drought plots. Second, on 15-Sep-2018 we collected ten apparently dead plants (one from each drought plot) and transplanted them to individual pots containing a native soil/sterilized sand mixture in the greenhouse, which was maintained between 18.3–23.9 °C. We placed them on a drip irrigation system which watered them three times daily. Over a 4-month period, we monitored each plant for signs of new growth.

Molecular methods and genetic analyzes

Root tissue from each individual was ground in liquid nitrogen and 0.25 g of ground material was extracted using PowerSoil kits (MoBio, Carslbad, California). DNA extracts were cleaned and concentrated using ZR-96 DNA Clean and Concentrator (Zymo Research, Irvine, California). DNA concentration was determined using Nanodrop 2000c (Thermo Scientific, Waltham, Massachusetts). We obtained high-molecular weight DNA from the roots of all samples. Due to the limited financial resources for sequencing and because our questions about genetic diversity do not require tracking individual genotypes, we then pooled DNA into two pools per plot (each containing DNA from ten individuals). For each individual plant, the amount of DNA added to the pool was normalized to the sample in the pool with the lowest DNA concentration. Thus, there were a total of 40 DNA pools corresponding to 10 pools in each of 4 cohorts: "control live 1", "control live 2", "drought live" and "drought dead".

Potential clonality in black grama (Peters 2002) could have limited the effective number of genotypes contributing to each pool, affecting the absolute values of estimated population genetic parameters such as allele frequency and F_{ST}. A related issue is that the small pool sizes

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of ten individuals (necessitated by the small number of living individuals remaining in drought plots post-treatment) could have resulted in some imprecision in allele frequency estimates. Allele frequency estimates using pooled sequencing are cost effective and robust, but can be undermined by small pool sizes (Kofler et al. 2016; Gautier et al. 2013; Anand et al. 2016; Fracassetti et al. 2015; Schlotterer et al. 2014; Lynch et al. 2014). Nevertheless, Anand et al. (2016) found robust reproduction of allele frequencies ($R^2 > 0.98$) in pools with only 12 individuals. Using the PIFs software (Gautier et al. 2013), we compared our pool-based sequencing to expectations for individual sequencing. Using our minimum pool coverage of 5× and ten individuals (either sequenced as a pool or individually), along with an Illumina error rate of 0.24% (Pfeiffer et al. 2018), we estimate that the standard deviation of the allele frequency increased only 1.265× by sequencing pools instead of individuals. Furthermore, though our pool size is small, it represents a substantial portion of the population from which it is derived.

We further note, as described above, that we applied treatments randomly to plots in space, and further balanced our sampling by making sure that each pool had the same number of individuals and that samples collected were spaced similarly within each plot (see "Field sampling: genetic diversity"). Thus, any potential effects of clonality and small pool size should apply equally to parameters estimated for the drought and control treatments, and are not expected to bias the results. At worst, they should create noise, making it harder to detect significant differences between the treatments and resulting in a conservative test for the presence of a genetic response to drought. While we do not expect the data to be biased between drought and control plots, we have avoided generating locus-specific genetic parameters because they are more susceptible to the noise in the data. We expect that locus-specific genetic parameters would have lower power and sensitivity and inflated false-positive rates (Luikart et al. 2003).

Sequencing

We constructed standard Illumina libraries for DNA samples digested with *Pst*I and *Msp*I, except that TruSeq Universal Adapters were changed to include an inline barcode, as described in Elshire et al. (2011). The 40 samples were multiplexed together and sequenced in two lanes on an Illumina HiSeq2000 machine using Illumina TruSeq v3 chemistry. Samples were filtered and base called using the Illumina RTA 1.13.48.0 and CASAVA 1.8.2 pipelines and were demultiplexed with an in-house script. A total of 331,701,293 sequence pairs and 66,340,258,600 nts of filtered sequence were generated.

Genotyping

Genotyping of pooled samples aimed to identify the allele(s) present within the pool rather than to obtain individual plant genotypes. We used the UNEAK program (Lu et al. 2013), which uses a network-based approach to identify alleles present in sequencing tags. This approach removes alleles that appear to be sequencing errors and focuses on loci with two alleles (Lu et al. 2013). In this way, we could differentiate between loci that were fixed in a given pool from those that had at least two alleles. We did not include putative triallelic and quadrallelic loci in our analyses, because loci with > 2 alleles are expected to be quite rare, and thus these putative loci are much more likely to be false positives. UNEAK identified a total of 559,023 polymorphic loci (SNPs), with genotypes called on pooled DNA samples. Only alleles with at least 5% allele frequency were converted into genotypes to reduce sequencing error. With ten samples in each pool, alleles present in only one sample should have a frequency of approximately 10% (assuming samples are equally represented) and, therefore, alleles with < 5% frequency likely represented sequencing error. Sites with only one allele with a frequency ≥ 5% were called as homozygous and those with two called as heterozygous. Since these genotypes were called on pools, a heterozygous call indicated simply that both alleles were present in the pool, but did not provide information on the frequencies of homozygous and heterozygous individuals within the pool. The R package *SeqArray* was used to import the VCF file into Genomic Data Structure (GDS) format in R (Zheng and Gogarten 2015). A genotype matrix was generated with the R package *SeqVarTools* (Gogarten and Zheng 2017).

Analyses of genetic similarity and F_{ST}

We first evaluated spatial heterogeneity of genetic diversity within the study area by assessing genetic versus physical distance. Since each sequenced DNA sample represented pooled DNA from ten individual plants, we assessed pairwise genetic distances between pools rather than plants. Pairwise genetic distances were calculated using genetic loci that were fixed for each pool (i.e., only one allele was called) and that were supported by at least 5× coverage. We focused on fixed loci to clearly identify those that differed between pools, and to reduce noise arising from the fact that our limited sample size may have missed alleles in populations, especially those present at low frequency. Our focus on fixed loci avoided comparisons of a pool with two alleles to a pool with only one, which could falsely be declared different despite the fact that the second allele might be present (but unsampled) in the latter population. We focused on sites that were fixed across all samples given that differences seen between fixed sites

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are more likely to be real. Under our approach, false differences would arise only if both pools were missing an allele present in the population. The same set of loci was used for all samples, and we, therefore, expect that any potential bias that resulted from using only fixed loci was applied equally to all samples, and that the relative genetic distances between pairs of pools was preserved. Genetic similarity was defined as the percentage of loci that were identical between two pools, divided by the total number of loci shared by both. Physical distances were determined to the nearest 0.1 m, by measuring distances between the centers of plots in a pairwise fashion. Graphics were generated in R with the ggplot2 package (Wickham 2009) unless otherwise noted.

The F_{ST} (fixation index) was calculated in R using the snpgdsFST function in the *SNPRelate* package (Zheng et al. 2012) based on all genotypes from the UNEAK program, including those that were not fixed. To test for drought treatment effects on F_{ST}, we ran two analyses. First, we determined the pairwise F_{ST} for the two pools of ten individuals sampled within each plot. In control plots, both pools consisted of randomly selected live individuals and should have low pairwise F_{ST}. In drought plots, if drought-induced mortality were selective among genotypes, it should amplify the within-plot pairwise F_{ST} relative to two randomly selected living pools in control plots. Thus, our a priori hypothesis was that the within-plot pairwise F_{ST} would be larger for dead-live pairs within the drought treatment than for the live–live pairs within control plots. We evaluated this hypothesis using a one-tailed, paired *t* test (pairing the control and drought plots within each spatial block), given that the distribution of residuals was Gaussian. Pairing by block and separately analyzing within-plot differences from between-plot differences was motivated by the finding of effects of physical distance on genetic distance (see "Results").

Second, we compared pairwise F_{ST} values among cohorts between plots. For both the live and dead drought cohorts, we expected their mean pairwise F_{ST} against live plants in control plots to be larger than the mean pairwise F_{ST} between any two control plots. Thus, we used a general linear model to compare among these three sets of between-plot pairwise F_{ST}s: drought dead-control live, drought live-control live, and control live-control live (lm function in *R*). Since the analysis was of pairwise distances among all pairs of plots, it was not meaningful to include spatial block in the analysis. If the pairwise F_{ST} between drought dead-control live was larger than that between drought live-control live, it could indicate that drought selectively killed a unique set of genotypes, whereas the reverse could indicate the drought selectively favored unique genotypes. Data were plotted using SigmaPlot version 12 (Systat Software, San Jose, California, USA).

Analyses of allelic diversity and composition

We determined a measure of within-locus allelic diversity for each pool by calculating the percentage of polymorphic loci. A minimum of five sequence reads was required to improve the chances of sampling multiple alleles from loci where multiple alleles were present in a pool. A minimum of 40% of the reads had to call each allele to minimize sequencing error. We then used a general linear mixed effects model to test the random effects of plot and block and the fixed effect of treatment cohort on allelic diversity, implemented in the *lme4* package. Pairwise contrasts using FDR correction for multiple comparisons tested whether drought live plants had significantly lower allelic diversity than drought dead or control live plants; data were plotted in SigmaPlot v. 12. Next, we determined a within-sample measure of allelic richness by calculating the total number of alleles present across 3614 loci that had genotype calls in all samples. Paired *t* tests were used to compare between cohorts.

To examine drought effects on allelic composition, we used the matrix of allele frequencies across all alleles identified by UNEAK to conduct a permutational MANOVA using the adonis function in the *vegan* package in R (Oksanen et al. 2016). We used the Bray–Curtis distance metric in all allelic composition analyses, with 9999 permutations that were stratified by the random effect of spatial block. The global analysis compared allelic compositional differences among all four cohorts (control live 1, control live 2, drought live, or drought dead), and we followed with planned contrasts between drought live and drought dead, and between drought dead and all controls. To examine drought treatment effects on allelic dispersion, we used the betadisper function in the *vegan* package with 9999 random permutations. To visualize differences among treatment cohorts, we conducted nonmetric multidimensional scaling (NMDS) analysis using default settings in metaMDS in the *vegan* package. In addition, heatmaps were generated in R v 3.2.3 (R Core Team 2017) using the *SNPrelate* package (Zheng et al. 2012). The genotypes from UNEAK were fed into the snpgdsIBS function and the heatmap plotted with R's core image function. Finally, we conducted a principal component analysis (PCA) by feeding the genotype matrix into the pca function of the *SeqVarTools* package. Plots used the ggrepel function to separate labels (Slowikowski 2016).

Results

Experimental drought caused black grama mortality and reduced biomass

After three growing seasons, extreme drought (66% reduction in growing season rainfall) resulted in substantial

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mortality, with numerous ramets of recently dead black grama grass noted in drought plots but none found in control plots. At the time of genetic sampling (September 2015), average (±SE) above-ground live (green) biomass of black grama was only 4.6 (±5.3) g m^{-2} in drought plots compared to 119.0 (±5.3) g m^{-2} in control plots ($X^2 = 245.48$, $P < 0.0001$), a reduction of 96.2%. This represented actual mortality (vs. temporary dormancy) as live biomass continued to decline in the drought plots, to 3.0 (±5.3) and 1.6 (±5.3) g m^{-2} in September 2016 and September 2017, respectively. Further, we saw no evidence of new growth in ten dead plants transplanted from the drought plots to the greenhouse and watered daily over a 4-month period.

Substantial genetic variation in black grama populations

We observed substantial genetic variation among black grama plants across the study site. Sequencing analysis produced a total of 559,023 SNPs, of which 46.90% of called SNPs on average (range 43.89–51.38%) showed variation between control samples from different plots, at a minimum rejecting the idea that the study site comprises one large black grama clone. The two pools of DNA collected from within the same plot were more similar to each other than to pools from other plots (*t* test on pairwise F_{ST} within-plot vs. between-plot, two-tailed with unequal variances, $P < 0.0001$). In addition, the physical distance between plots was negatively correlated with the genetic similarity between pools ($r = -0.29$ (control and drought treatments) and -0.43 (control treatment only), both $P < 0.0001$, Fig. 2), supporting an isolation-by-distance model. The apparent continuous distribution of genetic similarities along the *y*-axes of the similarity vs. distance plots (Fig. 2) is further evidence against widespread clonality, as a clone large enough to encompass two or more of our plots would have instead generated clumps of identical pairwise similarities. The data thus suggest a minimum of 20 distinct black grama clones in our study (one per plot), with the potential for a much larger number of clones.

Drought altered the population genetic structure of black grama

Within plots, pairwise F_{ST} values were ~25% larger between drought dead and drought live cohorts than between the two randomly selected cohorts of live plants from control plots (Fig. 3a, paired *t* test, $P = 0.0494$). In addition, between-plot pairwise F_{ST} values for drought dead versus controls were ~20% larger than any F_{ST} comparing cohorts of live plants (control live vs. control live, or drought live vs. control live; Fig. 3b, $F_{2,577} = 75.0$, $P < 0.0001$). Thus, the plants that died in the drought treatment were genetically differentiated from the remaining live plants. This differentiation was apparent in a heatmap analysis of genetic similarity, in which the plants that died under drought were dissimilar to the control plants and to the drought live plants (Fig. 4).

Plants that survived drought had a lower within-locus allelic diversity, as measured by an 11% drop in the percentage of polymorphic loci compared to plants that died in the drought treatment (Fig. 5, Wald $X^2 = 24.5$, $P < 0.0001$). Although not statistically significant, plants that survived drought had a 3% drop in within-locus allelic diversity compared to the pre-drought population, as represented by the control plots.

Allelic composition significantly diverged among the four cohort types (perMANOVA: $R^2 = 0.30$, pseudo-$F_{3,36} = 5.1$, $P < 0.0001$; visualized with NMDS in Fig. 6). Composition

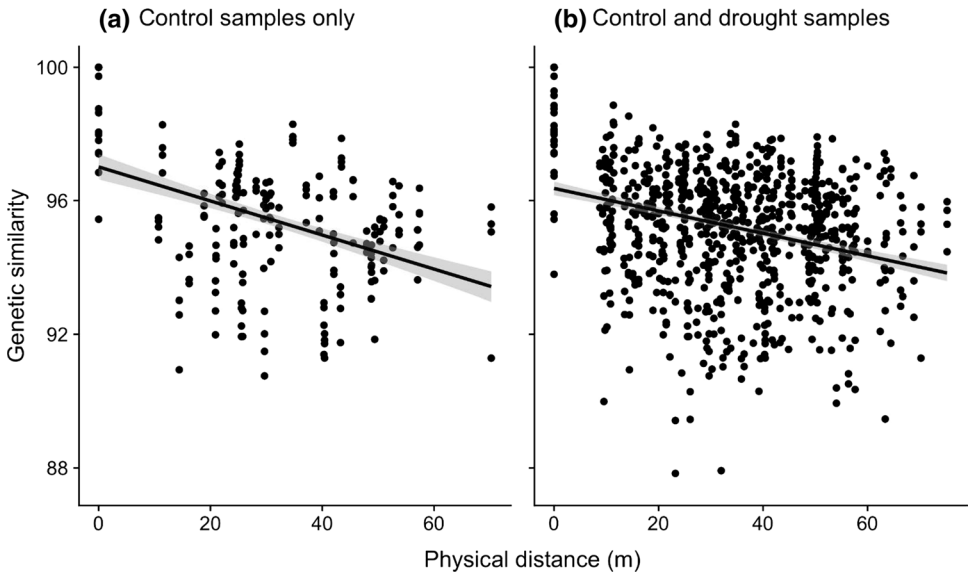

Fig. 2 Relationships between genetic similarity and physical distance. Points on the graphs represent pairwise comparisons of genetic similarity among pools of ten black grama individuals. Points at zero physical distance represent comparisons among pools obtained from the same plot. Each point at 100% genetic similarity represents a pool compared with itself. Linear regression lines with 95% confidence level shading are shown. **a** Comparisons among pools in the control treatment only; $r = -0.43$, $P < 0.0001$. **b** Comparisons among all pools in both treatments (drought and control); $r = -0.29$, $P < 0.0001$

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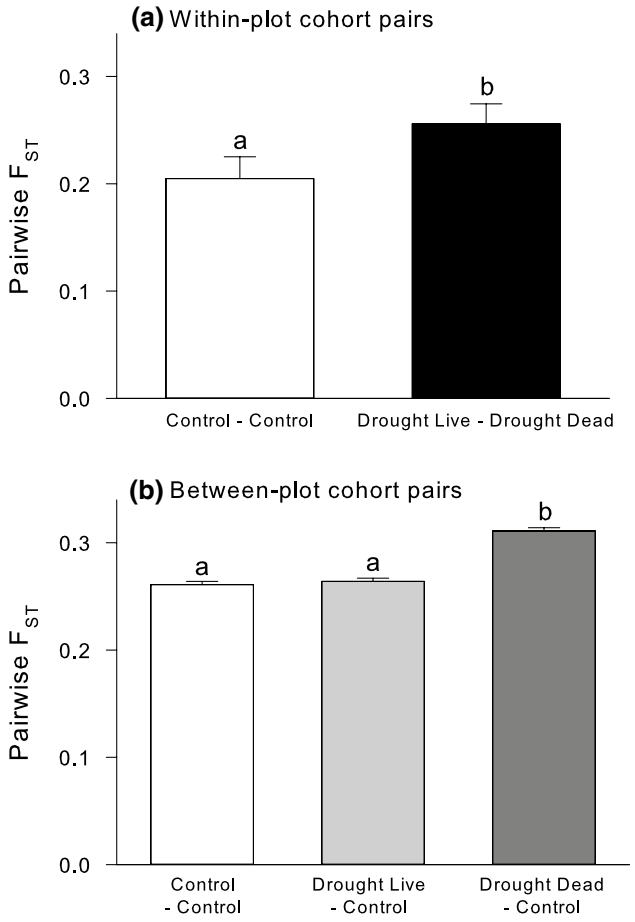

Fig. 3 Mean (±SE) pairwise F_{ST} values among pairs of pools of ten black grama individuals. **a** Mean F_{ST} among pools within plots. **b** Mean F_{ST} among pools between different plots. Letters show statistically significant differences among the means within each graph

differed both between the dead and living plants exposed to drought ($R^2 = 0.31$, pseudo-$F_{1,18} = 9.1$, $P < 0.0001$) and between the dead plants in drought treatments and living plants in control plots ($R^2 = 0.30$, pseudo-$F_{1,28} = 11.8$, $P < 0.0001$). Multivariate dispersion in allelic composition was not significantly different between live and dead pools within drought treatments (permutation test, $F = 2.0$, $P = 0.18$) or between pools of dead plants in drought treatments and controls (permutation test, $F = 2.7$, $P = 0.11$), indicating similar among-plot variability. PCA analysis employing genetic distances derived from sequence variants also supported results of these other analyses. Pools of plants that survived drought clustered more closely with each other than did control pools or drought dead pools. In fact, none of the outliers in this analysis were from plants that survived drought treatments. In contrast, plants that died under drought were more unique than those that survived drought, or those in adjacent control plots (Online Resource 1). These patterns are further supported by measures of within-sample allelic diversity. Drought dead samples had, on average, a higher number of alleles than any other cohort: 6206 alleles in drought dead vs. 5641 in drought live ($P = 0.0036$), 5852 in control live 1 ($P = 0.0684$), and 5665 in control live 2 ($P = 0.0022$), although the difference was not always significant.

Discussion

Several lines of evidence support the conclusion that black grama populations experienced substantial genetic changes caused by the harsh 1-in-100-year experimental drought we imposed. Drought caused substantial mortality and reduced live black grama biomass by 96% relative to control plots. Allelic diversity of survivors, as measured by the percentage of polymorphic loci, was on average 11% lower than the plants killed by drought. In addition, plants that died during drought were differentiated from both drought survivors and living plants in control plots (20–25% larger F_{ST}, and altered allelic composition). These results are consistent with previous studies on both plants and animals that have correlated specific climatic events with reduced genetic variability (e.g., Nevo et al. 2012; Rubidge et al. 2012). However, like Jump et al. (2008), Avolio et al. (2013), and Ravenscroft et al. (2015), our experimental approach allowed us to assign causality to drought, side-stepping the correlation-not-causation issue inherent in tracking changes in genetic structure following a natural event.

The observed genetic differentiation between drought survivors and non-survivors is strong evidence of the action of natural selection. A key future direction would be to functionally characterize the genes differing between these groups, which may provide insight into the basis of drought adaptation in this species and allow comparisons to studies on other plant species (e.g., Franks et al. 2016; Yoder et al. 2014). However, the general reduction in allelic diversity in the drought-stressed populations is also consistent with (but does not prove) a population bottleneck, which would involve genome-wide loss of diversity at neutral loci via genetic drift. Again, characterization of genes as neutral vs. non-neutral could resolve the bottleneck question by determining both the degree to which neutral loci have been affected.

Potential community-level consequences of genetic diversity loss

Our experiment simulated the effects of a short term (3-year), but extreme, drought. A corresponding event in the wild might have varied genetic effects, depending for instance, on whether the drought encompassed only a restricted geographic area or the entire range of the species.

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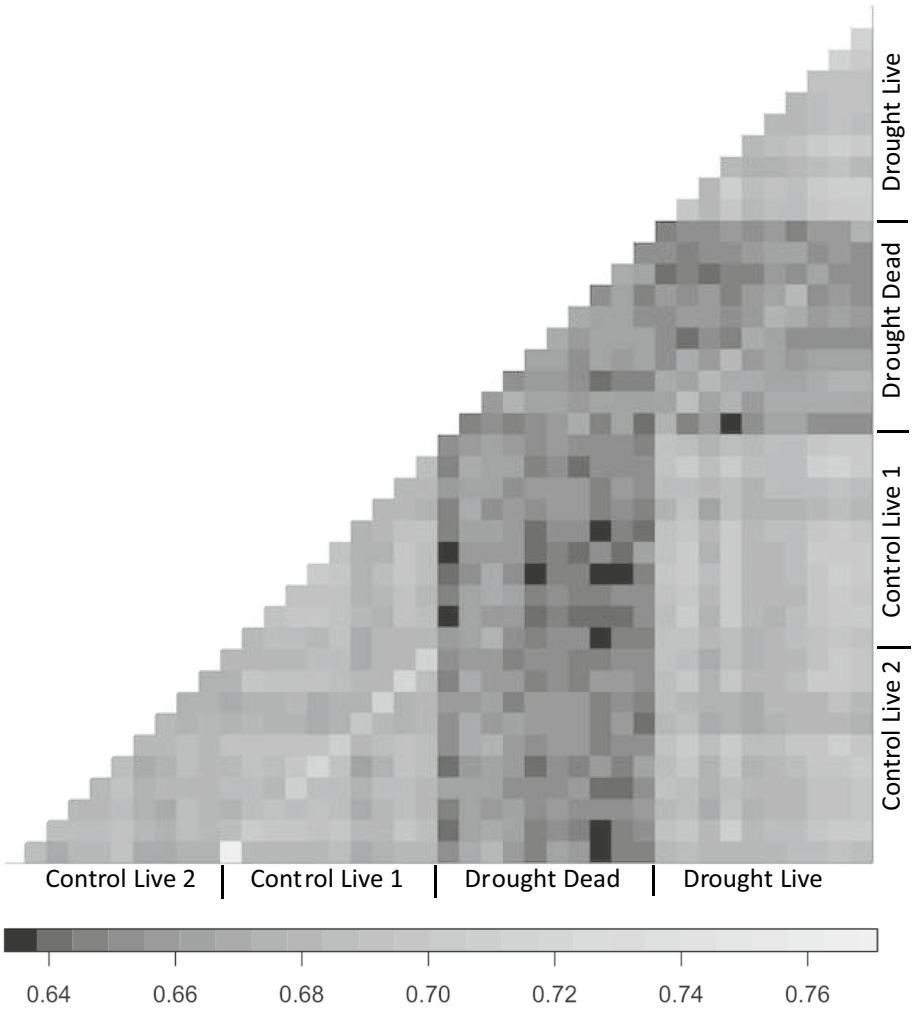

Fig. 4 Heatmap of genetic similarity among black grama subpopulations. Each square represents a comparison between two pools, with each containing pooled DNA from ten individuals collected from a single plot. Darker colors reflect lower proportions of SNP loci that are identical by state (i.e., greater genetic distances between pools)

In the former case, gene flow from unaffected populations might reduce or erase the genetic signature of the drought over time. In the latter, genetic diversity loss across the species range might limit future adaptation, reduce population-level resilience to future climatic extremes, and/or increase inbreeding depression (Potvin and Tousignant 1996).

As in most other plant species (see Pauls et al. 2013), we currently lack resolution on what the functional consequences of drought-induced losses of genetic diversity in black grama would be for the communities and ecosystems where it dominates. A recent meta-analysis demonstrated that adaptive genetic diversity within plant populations had more significant ecological consequences (e.g., for productivity, or diversity of food webs) than neutral genetic diversity (Whitlock 2014). Thus, it will be important to determine what traits, if any, are affected by the loss of allelic diversity in black grama. While trait-based studies remain dominated by interspecific trait variation, new work has been documenting significant within-species trait variability (reviewed by Siefert et al. 2015); in some cases, this variability is as ecologically important as the functional differences among species (Cook-Patton et al. 2011). A trait-based approach (e.g., McGill et al. 2006) could provide a mechanism to connect intraspecific phenotypic variation with genetic variation in dryland plant populations. Future experiments that manipulate genetic diversity in black grama populations by planting monocultures and genetic mixtures (e.g., Crawford and Whitney 2010; Atwater and Callaway 2015) could help to resolve the long-term ecological consequences of drought-induced changes to population genetic structure (Milla et al. 2009).

Unexpectedly high levels of genetic variation in black grama

To our knowledge, this is the first study to examine the population genetics of black grama. The high level of variation observed among plants on a local scale (< 1 ha) was unexpected because field studies on levels of vegetative reproduction vs. recruitment from seed have suggested that black grama reproduces primarily vegetatively (Valentine 1970; Peters and Yao 2012). Our results are more consistent

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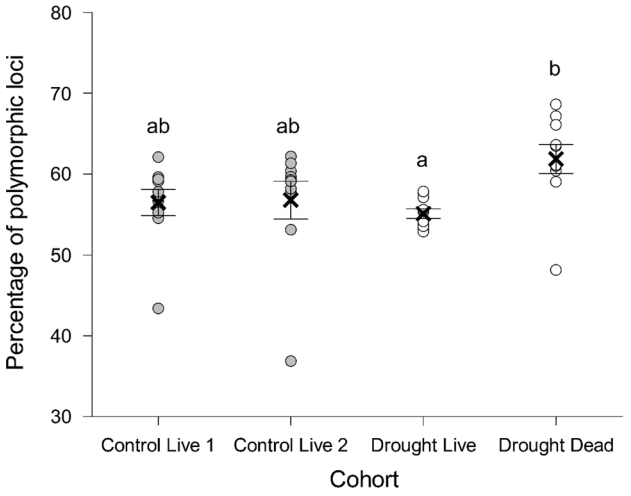

Fig. 5 Loss of genetic diversity among black grama plants that survived drought. Cohort is shown on the *x*-axis and indicates treatment (control or drought) and plant status (live or dead). The percentage of polymorphic loci was calculated for sample pools using data for loci for which there was fivefold or greater sequence coverage in GBS analyses. Each pool is represented by a circle. The "×" shows the treatment mean ± SE. Letters indicate significant differences between means following FDR correction for pairwise contrasts

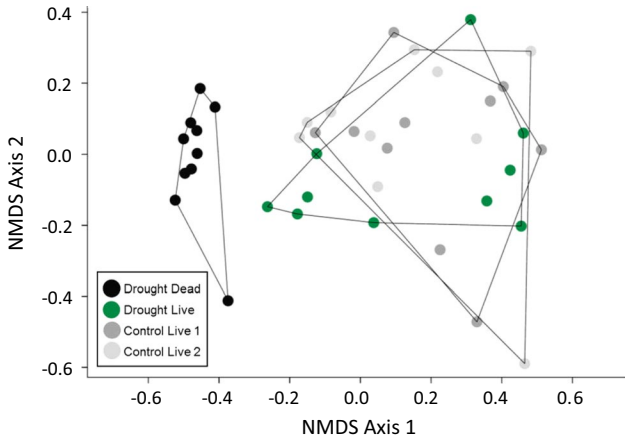

Fig. 6 Non-metric multidimensional scaling (NMDS) analysis showing two-dimensional plot of all pools examined for allelic composition; each pool is represented by a circle. 2D stress = 0.15. Lines delineate the allelic space occupied by each cohort: two randomly selected groups of live plants from control plots (control live 1, control live 2), dead plants in drought plots, or live plants in drought plots

with recent seed addition experiments that suggest high soil moisture levels favor episodic recruitment of black grama over relatively short time intervals (5–8 years) relative to the lifespan of this perennial grass (Moreno-de las Heras et al. 2016); episodic recruitment of sexually produced seeds could, therefore, underlie the relatively high standing genetic variation observed at small spatial scales. The existence of substantial local variation was critical to our ability to detect the effects of short-term abiotic stress in shaping population-genetic structure, and it suggests that black grama is an excellent candidate for future studies of evolutionary change in response to climate events in arid grasslands.

Although we captured substantial genetic variation using DNA-based methods, there may yet be additional epigenetic variation in black grama that we have missed with our approach. For example, Rico et al. (2014) compared control versus experimentally drought-stressed Holm oak (*Quercus ilex*) and found significant differences in methylation patterns. Such epigenetic modification could have important population- and community-level effects. For example, using similar genetic lines of *Arabidopsis thaliana*, Latzel et al. (2013) showed that an increase in epigenetic diversity alone boosted plant productivity by 20%. Epigenetic variation may be an important component of intraspecific functional diversity, and in some plant species, epigenetic diversity exceeds genetic diversity (Medrano et al. 2014). Transcriptomic approaches applied to drought stressed versus unstressed field plants (e.g., Travers et al. 2010) could help to resolve possible epigenetic consequences of drought as well as to identify changes in plant gene expression that may explain the drought-tolerance mechanism in surviving plants.

Implications for management and the future of dryland ecosystems

Our results have applied significance for the management of black grama-dominated grasslands, for which restoration is desirable (Cox et al. 1986; Peters et al. 2006). Higher than expected genetic diversity in natural populations suggests that restoration efforts may benefit from maintaining naturally high levels of genetic diversity in plantings, rather than using clonal transplants (Lucero et al. 2010) or seed stock derived from few individuals. Since drought-induced suppression of genetic diversity could constrain the resilience of desert grasslands to future abiotic stress, efforts to augment diversity following extreme drought events could benefit long-term grassland stability.

Understanding the evolutionary consequences of climate change may be important for predicting the future of dryland ecosystems. Drylands constitute 45% of global land area (Pravalie 2016) and are expanding globally (Huang et al. 2016b). In many drylands, primary productivity is fueled by long-lived, perennial plants; changes in their population genetic structure could thus have long-lasting effects on the resistance and resilience of primary production to future stressors and contribute to the ecological memory of response to drought (Ogle et al. 2015). Drought is increasingly likely to be a significant stressor in drylands (IPCC 2013; Williams et al. 2013; Garfin et al. 2014; Shi et al. 2014), although the consequences of more frequent, longer,

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or more severe droughts for the population genetics of most dryland primary producers remain unresolved.

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Author contribution statement KDW and JAR conceived the genetic diversity study; SLC and WTP designed and established the EDGE drought experiment; JAR, DON and JB conducted field sampling; JB extracted the DNA; JM and AS performed the genotyping; JM, JAR, DON and KDW analyzed the data; KDW, JM, DON, SLC and JAR wrote the manuscript; KDW, JM, JAR, SLC, and WTP contributed funding for the study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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