Multidecadal records of intrinsic water-use efficiency in the desert shrub *Encelia farinosa* reveal strong responses to climate change

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While tree rings have enabled interannual examination of the influence of climate on trees, this is not possible for most shrubs. Here, we leverage a multidecadal record of annual foliar carbon isotope ratio collections coupled with 39 y of survey data from two populations of the drought-deciduous desert shrub *Encelia farinosa* to provide insight into water-use dynamics and climate. This carbon isotope record provides a unique opportunity to examine the response of desert shrubs to increasing temperature and water stress in a region where climate is changing rapidly. Population mean carbon isotope ratios fluctuated predictably in response to interannual variations in temperature, vapor pressure deficit, and precipitation, and responses were similar among individuals. We leveraged the well-established relationships between leaf carbon isotope ratios and the ratio of intracellular to ambient CO_2 concentrations to calculate intrinsic water-use efficiency (iWUE) of the plants and to quantify plant responses to long-term environmental change. The population mean iWUE value increased by 53 to 58% over the study period, much more than the 20 to 30% increase that has been measured in forests [J. Peñuelas, J. G. Canadell, R. Ogaya, *Glob. Ecol. Biogeogr.* 20, 597–608 (2011)]. Changes were associated with both increased CO_2 concentration and increased water stress. Individuals whose lifetimes spanned the entire study period exhibited increases in iWUE that were very similar to the population mean, suggesting that there was significant plasticity within individuals rather than selection at the population scale.

Mojave Desert | iWUE | stomatal conductance | carbon isotopes | climate change

Desert and semidesert ecosystems cover a large proportion of global land area (1, 2) and are expected to grow as global temperatures increase and precipitation patterns change (3, 4). Because deserts have sparse vegetation and low primary productivity (1, 5, 6), desert ecosystems have traditionally been studied less intensively in the context of climate change than that of more densely vegetated ecosystems, particularly forests and grasslands (7). However, the response of desert vegetation to climate change has important implications for land management decisions (8, 9), species abundance and biodiversity (10, 11), and future organic and inorganic carbon storage (12, 13). Carbon isotope ratios in tree rings serve as an invaluable record of gas exchange patterns (14) in forested ecosystems and have been extensively used to understand changes in tree water-use dynamics in response to environmental perturbations. In contrast, perennial vegetation in desert ecosystems is characterized by shrubs with a multistem (suffrutescent) growth form instead of a single persistent main stem, limiting study of climate responses. In forests, long-term tree ring studies have found that intrinsic water-use efficiency (iWUE), defined as photosynthetic rate (A) divided by stomatal conductance to CO_2 (g_s) (15–17), increased (18, 19) in response to increasing aridity (20) and rising CO_2

concentrations (21, 22) over the last century. However, it is unclear if similar responses have occurred in desert ecosystems. Increases in intrinsic water-use efficiency associated with rising CO_2 are generally expected to alleviate drought stress as plants could maintain similar rates of carbon assimilation with reduced demand for water.

iWUE serves as an indicator of the trade-off between photosynthetic carbon assimilation and water loss, mediated by both g_s and A (23). Since both the photosynthetic uptake of CO_2 and the loss of water vapor are regulated via stomata, iWUE is related to the intercellular (c_i) and atmospheric (c_a) concentrations of CO_2. Carbon isotope ratios record information about the ratio of c_i to c_a (24, 25) and can therefore be used to calculate iWUE (*Methods*). Increased water limitation, associated with either decreased soil moisture or increased leaf-atmosphere vapor pressure saturation deficit of air (VPD) (26), typically triggers partial stomatal closure and reductions in g_s (20, 23). Assuming leaf photosynthetic capacity does not instantly change, reduced g_s leads to decreased c_i/c_a ratios and therefore increased iWUE and increased carbon isotope ratio values. Studies have found that carbon isotope ratios in desert shrubs increase as water availability decreases across spatial gradients (27, 28), indicating a relationship between plant stress response and local water availability.

In addition to responding to water stress, iWUE can also respond to changes in atmospheric CO_2 concentration via adjustments

Significance

As the proportion of land area covered by arid land vegetation continues to expand and water limitations for plants increase, understanding if and how desert shrubs are responding to environmental change has become more urgent. Among two populations of Mojave Desert shrubs, we found that intrinsic water-use efficiency has increased substantially over the last three decades in response to increasing aridity and CO_2 concentration. While increases in intrinsic water-use efficiency have been widely assumed to mitigate negative effects of decreasing water availability, precise effects on plant productivity, reproduction, and survival remain unknown.

The authors declare no competing interest.

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in both A and g_s. Studies of forests have generally found that increases in CO_2 concentration can lead to increases in iWUE (29–31) and enhanced net primary productivity. Early theoretical predictions suggested that highly water-limited ecosystems like deserts would respond most strongly to CO_2-driven increases in iWUE, and this CO_2 fertilization effect has been cited as one cause of satellite-observed vegetation "greening" in warm, arid regions (32). However, a lack of in situ and long-term studies of desert ecosystems has limited evaluation of those predictions (7, 33). Several free-air CO_2 enrichment studies of shrubs in the Mojave Desert have documented variable increases in A (34–37), increases in iWUE (38), and/or decreases in g_s in response to elevated CO_2. However, these studies were conducted over four or fewer growing seasons, most of the effects have been weaker than expected, and, in many cases, the responses were only observed in particularly wet seasons (33). The relatively short duration and variable findings of these studies make it difficult to forecast long-term responses of desert shrubs to CO_2 and aridity increases.

The carbon isotope ratio of a plant is partially genetically determined and thus heritable (16, 23, 39–41). As water-use strategies can play a role in successful establishment, survival, and reproduction, increased frequency of particular carbon isotope ratios can result from selection on water-use traits under different climatic conditions (42). For instance, plants with high $\delta^{13}C$ values are more likely to survive a drought, but plants with low $\delta^{13}C$ values are able to grow more quickly following competitive release (43–45). Because of this, we may expect population-scale shifts in leaf carbon isotope ratios during periods of high water availability or following drought. In addition to population-scale shifts, individuals do not have fixed iWUE values but rather can exhibit substantial plasticity in iWUE as conditions change (46). While the presence of plasticity suggests that individual plants may be able to acclimate to increasing water stress to a certain degree, plasticity could be the result of either active or passive processes (47), and iWUE acclimation may or may not be advantageous.

So far, very few studies have considered the interactive effects of rising CO_2 and increased aridity in deserts, and none have been conducted over multidecadal timescales. Projected precipitation trends in the United States are spatially variable (48), but vapor pressure deficits and temperature are increasing in the desert southwest more quickly than in many other areas (49, 50). Recently, Williams et al. found that 2000 to 2018 was the second driest 19-y period in the desert southwest in the last 1,200 y, in large part due to anthropogenic warming (51). The Mojave Desert is therefore a particularly useful study location for understanding the impacts of rapid and advanced climate change on vegetation in arid ecosystems.

We have conducted continuous annual monitoring of *Encelia farinosa*, a common drought deciduous shrub, at two undisturbed, geographically distinct sites in the Mojave Desert over the last 39 y (52). These observational data coupled with an extensive dataset of foliar carbon isotope ratios from the populations provide insight into the water-use dynamics of a widespread woody shrub in a highly resource-limited environment. Just as tree ring records have shed light on iWUE trends in forests, these multidecadal leaf isotope chronologies allow us to evaluate the response of desert shrubs to climate change. Specifically, we address the following hypotheses: 1) carbon isotope ratios vary predictably within a population in response to short-term climate fluctuations, and 2) plants will acclimate to long-term trends of increasing water stress through plastic increases in iWUE.

Results

The Climate Is Changing. Three distinct aspects of climate have changed over the past 39 y at the Mojave Desert study sites.

There have been significant and substantial 1) increases in mean annual temperature, 2) increases in mean daily maximum VPD, and 3) decreases in total annual precipitation (Fig. 1) (for full linear regression results, see *SI Appendix*, Table S1). At both sites, temperature has increased at a rate of ~0.04 °C per year, and VPD has increased at a rate of ~0.1 hPa per year. While total annual precipitation trends have been more variable from year to year, on average, precipitation has decreased by about ~1.7 mm per year at both sites over the study period. In addition to these climatic changes, CO_2 concentration increased by ~58.5 parts per million (ppm) in the region over the 1991 to 2019 period, at a rate of ~2.1 ppm per year. Overall, the study site located near Oatman, AZ was warmer and received slightly more precipitation than the study site located in Death Valley, CA. VPD values were very similar between the two sites. Interannual fluctuations in temperature, VPD, and precipitation at the study sites were tied to the El Niño Southern Oscillation (ENSO). El Niño events were associated with cooler, wetter conditions and La Niña events with warmer, drier conditions. Independent of ENSO-related interannual variation in temperature, VPD, and precipitation, long-term climate trends were very similar between the two sites.

Plants within Populations Exhibited Similar Interannual Fluctuations in Foliar Carbon Isotope Ratios. Twenty-seven years of samples were analyzed from each population for a total of 1,863 unique leaf samples from the Death Valley population and 1,346 samples from the Oatman population. To examine within-population variability, we included annual measurements of 10 individuals with the most continuous leaf sample records available from 1991 to 2019 for each population (Fig. 2). These time series demonstrated that fluctuations in foliar $\delta^{13}C$ values were similar in timing, direction, and magnitude among individuals within each population. Between-year variance in population mean $\delta^{13}C$ values (variance = 1.6 per mil for both Death Valley and Oatman) exceeded the average between-plant variance in $\delta^{13}C$ values within each year (variance = 0.7 per mil in Death Valley and. 0.6 per mil in Oatman), and the $\delta^{13}C$ values of the continuously measured plants did not deviate substantially from the population mean $\delta^{13}C$ values. There was no difference between the annual population mean $\delta^{13}C$ values of plants at the Death Valley and Oatman sites ($P = 0.37$, $t = -0.90$), despite the climatic differences between the two sites, and there were no significant temporal trends in the mean $\delta^{13}C$ value of either population ($P = 0.18$ for Oatman and $P = 0.34$ for Death Valley).

The similarity of interannual fluctuations in $\delta^{13}C$ values among individuals implied that common factors, such as temperature, VPD, or precipitation, were driving a large portion of the interannual variations in $\delta^{13}C$ values. There were significant positive relationships between population mean $\delta^{13}C$ values and mean growing season maximum daily temperature (Fig. 3 A and D) and maximum daily VPD (Fig. 3 B and E), and a significant negative relationship between $\delta^{13}C$ values and total growing season precipitation (Fig. 3 C and F). Multiple regressions of the population mean carbon isotope ratio by precipitation, temperature, and VPD explained 70% of the variation in the mean $\delta^{13}C$ value of the Death Valley population and 77% in the Oatman population (*SI Appendix*, Table S2) ($P < 0.0001$ for both) although VPD was the only significant predictor for either population.

In addition to leaf-level adjustments in stomatal opening in response to water availability, plant leaf cover saturated when moisture availability was high and fell as VPD increased (*SI Appendix*, Fig. S1A). Because $\delta^{13}C$ values and leaf cover both varied with VPD, population mean $\delta^{13}C$ values were negatively related to leaf cover (*SI Appendix*, Fig. S1B), suggesting that leaf-level responses to water availability (via stomatal opening or closing) were coordinated with plant-level responses (via changes in leaf cover).
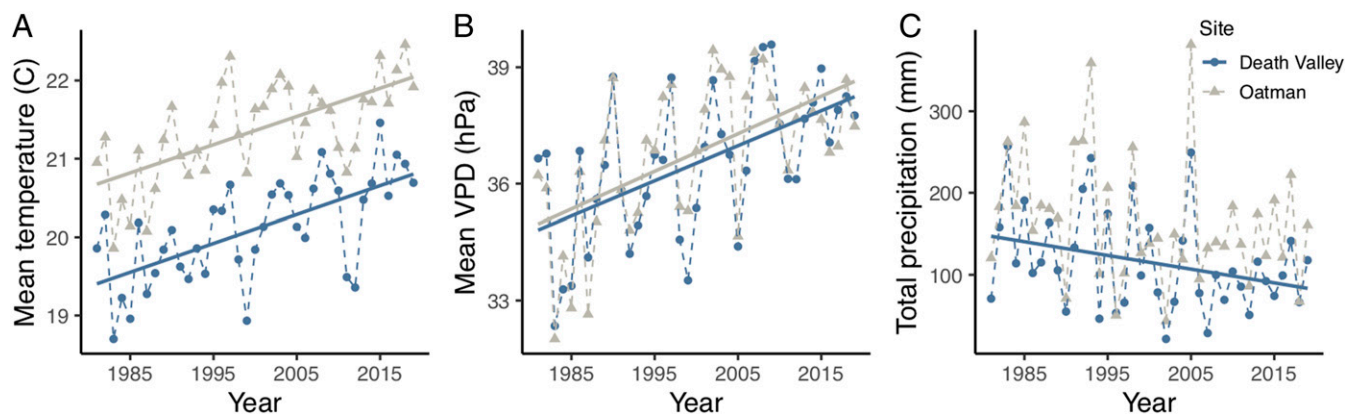
Fig. 1. Annual mean temperature (*A*) and the annual mean of daily maximum VPD (*B*) have significantly increased over the 1981-to-2019 study period at the Oatman site (gray triangles) and Death Valley sites (blue circles; $P < 0.001$). Total annual precipitation (*C*) has significantly decreased over the same period at the Death Valley ($P = 0.049$) site, but the decrease was not statistically significant at the Oatman site ($P = 0.128$). Climate variables were aggregated from April to March in each year to align with the relevant survey period. See *SI Appendix*, Table S1 for full regression results.

Ecophysiological Adjustments Have Occurred Over Time: iWUE Has Increased, and c_i/c_a Has Decreased. Over the past three decades, significant and similar decreases in population mean c_i/c_a ratios have occurred in both the Death Valley and Oatman populations (Fig. 4). This trend suggests that limitations due to stomatal control of intracellular CO_2 supply may have increased relative to photosynthetic capacity to assimilate carbon (16, 17). Coincident with decreased c_i/c_a ratios, there have been significant increases in population mean iWUE values among plants at both the Death Valley and Oatman sites (Fig. 4). All plants appeared to become increasingly conservative in their water use relative to photosynthetic gain. A portion of the residual variation in these temporal trends is associated with fluctuations in water availability, parallel to the relationships between climate and $\delta^{13}C$ (Fig. 3). For instance, the unusually high values of c_i/c_a and low values of iWUE observed in 2005 were coincident with exceptionally low VPD (13.8 hPa in Death Valley and Oatman). Similarly, the low values of c_i/c_a and high values of iWUE observed in 2007 were coincident with exceptionally high VPD (20.2 hPa in Death Valley and 21.6 hPa in Oatman).

In theory, the temporal trends in c_i/c_a ratios and iWUE values could be attributable to a population-level shift in ecophysiological characteristics through selection for plants with particular intrinsic water-use efficiencies. Alternatively, the temporal trends in c_i/c_a ratios and iWUE values could be attributed to individual-level acclimation to increased water stress or CO_2 concentrations. To differentiate between the two explanations, we compared temporal trends in c_i/c_a ratios and iWUE values for the entire population versus a subset of plants that survived the duration of the study period because temporal trends among the latter group could only reflect physiological acclimation of individuals, and not population-level selection. Long-term trends in c_i/c_a and iWUE values were nearly identical between the two groups. Over the study period, both the entire population and the subset of long-lived plants underwent a 17% decrease in c_i/c_a at Oatman and a 15% decrease at Death Valley. iWUE values increased by 58% for both the entire population and the subset of long-lived plants at Oatman, by 52% for long-lived plants at Death Valley, and by 53% for the whole Death Valley population. While it is possible that individuals that died had different iWUE acclimation responses, the consistency among trends for long-lived plants and the entire population suggests that plant-level acclimation responses, rather than population-scale shifts due to recruitment, were the primary drivers of the temporal trends in c_i/c_a ratios and iWUE values.
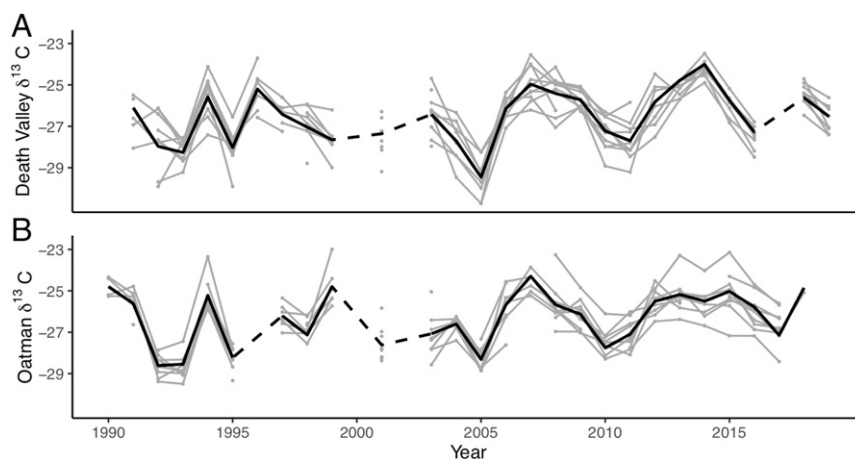
Fig. 2. Leaf $\delta^{13}C$ values (per mil) of 10 long-lived *E. farinosa* plants from the (*A*) Death Valley and (*B*) Oatman populations vary over time and in a similar manner among plants. The black line represents the population mean, and the gray lines and points represent individual plants. Dashed lines connect the population mean across years without isotope data.
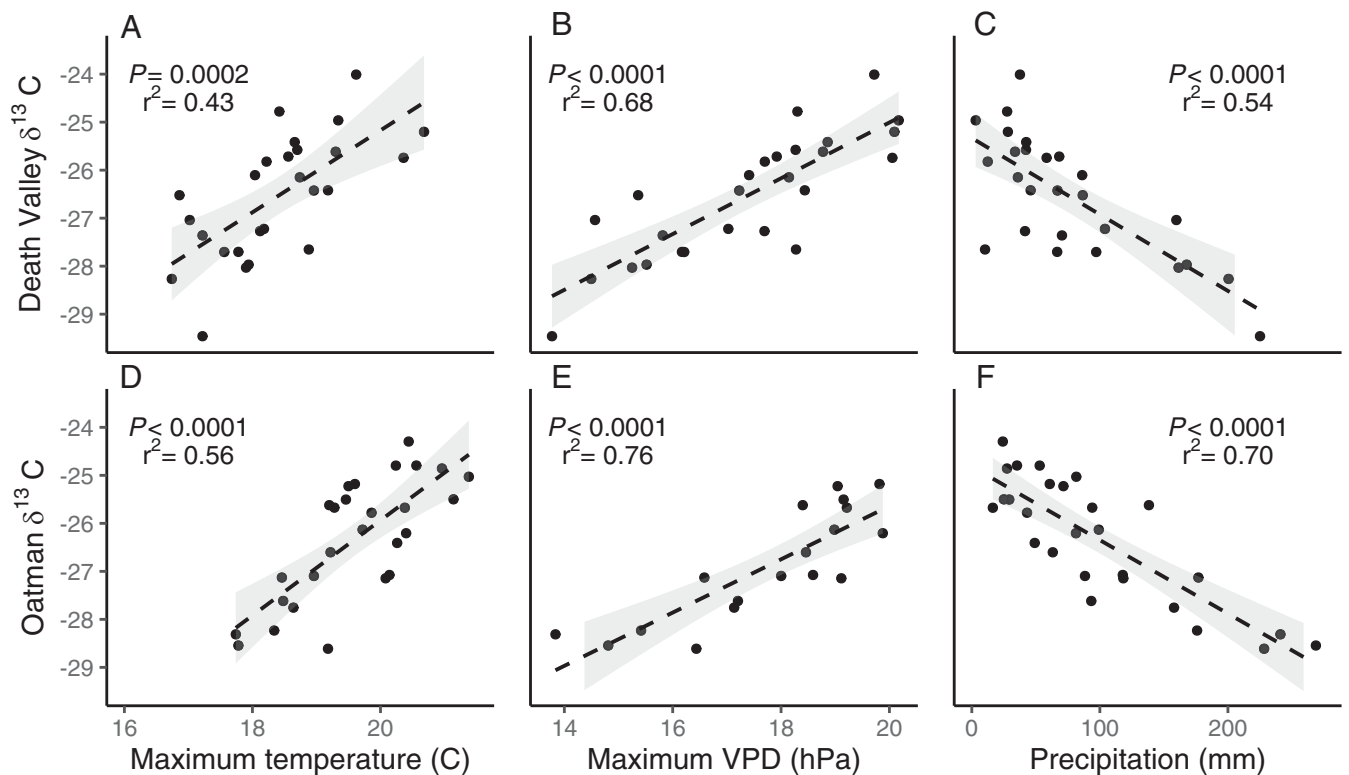
Fig. 3. Leaf $\delta^{13}C$ values (per mil) of *E. farinosa* in the Death Valley population are positively related to (*A*) maximum daily temperature and (*B*) maximum daily VPD, and negatively related to *C* total precipitation over the November-to-March growing season. $\delta^{13}C$ values in the Oatman population follow the same patterns; they are positively related to (*D*) temperature and (*E*) VPD, and negatively related to (*F*) precipitation. Light gray bands represent the 95% CI of the regressions.

Changes in VPD and CO$_2$ Concentration Are Driving Trends in c_i/c_a Ratios and iWUE. Both VPD and atmospheric CO$_2$ concentration have increased over the study period, and both are likely to drive changes in c_i/c_a ratios and iWUE values. We evaluated the influence of VPD and CO$_2$ concentration on c_i/c_a and iWUE by comparing the marginal effect of each independent variable on c_i/c_a and iWUE while holding the other constant (Fig. 5). There was some covariation between CO$_2$ concentration and VPD across the study period in Oatman ($r^2 = 0.25$, $P = 0.006$) and Death Valley ($r^2 = 0.05$, $P = 0.14$), but covariation was minimal enough to enable analysis of the respective effects of VPD and CO$_2$ on c_i/c_a and iWUE using partial correlation (variable inflation factors <1.5).

After controlling for CO$_2$ concentration, there was a strong, negative relationship between VPD and c_i/c_a ratios (Fig. 5*A*) for the Death Valley ($r^2 = 0.67$, $P < 0.0001$) and Oatman populations ($r^2 = 0.77$, $P < 0.0001$). After controlling for VPD, there was no significant relationship between CO$_2$ concentrations and c_i/c_a ratios in the Oatman population (Fig. 5*B*) (partial correlation, $P = 0.47$). In contrast to the Oatman data, there was a significant relationship between CO$_2$ concentrations and c_i/c_a ratios in the Death Valley population after controlling for VPD (Fig. 5*B*) (partial correlation, $r^2 = 0.21$ and $P = 0.022$), indicating that a constant c_i/c_a ratio was not maintained as c_a increased. These results indicate that CO$_2$ concentration and VPD synergistically drove the decrease in c_i/c_a ratios in the Death Valley population, but that VPD exclusively drove the decrease in c_i/c_a ratios in Oatman.

CO$_2$ concentration played a larger role in influencing iWUE values than c_i/c_a ratios. Both VPD and CO$_2$ concentration contributed substantially to increases in iWUE values (Fig. 5 *C* and *D*) although VPD drove a larger proportion of the increase than

did CO$_2$ (partial correlations, $r^2 = 0.66$ for VPD and 0.52 for CO$_2$, $P < 0.0001$ in Death Valley; and $r^2 = 0.77$, $P < 0.0001$ for VPD and $r^2 = 0.39$, $P = 0.0012$ for CO$_2$ in Oatman). A multiple regression including VPD and CO$_2$ concentration explained 80% of the variability in iWUE in Death Valley and 87% in Oatman (*SI Appendix*, Table S3). Therefore, while other variables associated with the environment (such as soil moisture availability) or characteristics of the population (such as plant age) may also influence iWUE, we found that two environmental parameters accounted for a large majority of iWUE variability in these populations.

Discussion

There is general consensus that aridity will continue to increase in the North American Southwest due to both decreasing precipitation and increasing evaporative demand (49, 51, 53–55), but current understanding of how desert shrubs will respond to climate change lags behind understanding of forest responses. Using a multidecadal record of foliar $\delta^{13}C$ values, we identified similarities in carbon isotope ratio fluctuations among individuals, strong relationships between carbon isotope ratios and growing season climate, and substantial increases in iWUE associated with increasing water stress and CO$_2$ concentration.

Time series of the $\delta^{13}C$ values of individuals measured repeatedly throughout the study period supported the hypothesis that interannual variations in leaf carbon isotopes are similar among individuals within a population and that these year-to-year fluctuations can be largely explained by climate variability. The observed patterns were very similar in two geographically distinct populations. Specifically, $\delta^{13}C$ values increased as water stress increased, driven by either evaporative demand (VPD and temperature) or water supply (precipitation). While VPD was a
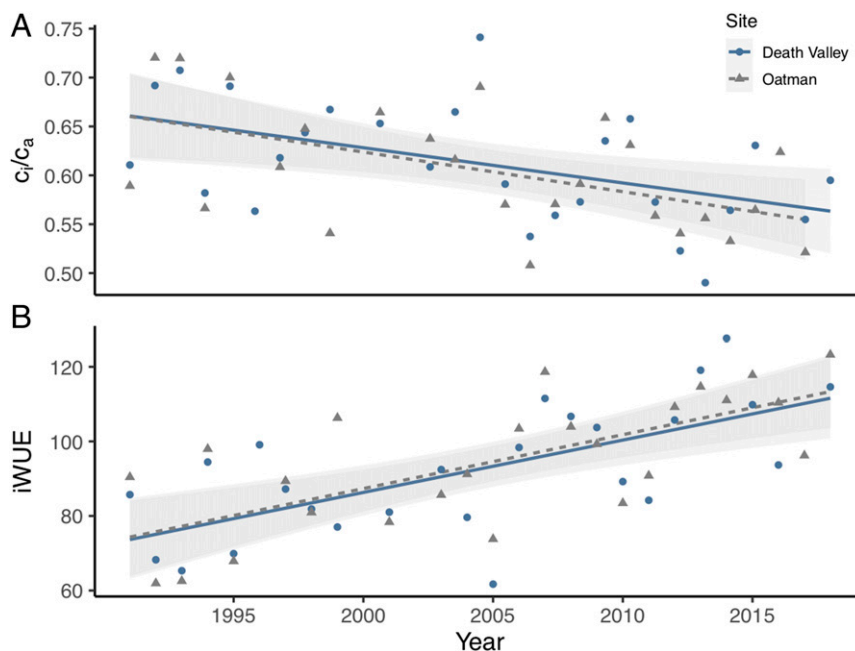
Fig. 4. Ecophysiological changes in leaves of *E. farinosa* were detected between 1991 and 2018. (*A*) c_i/c_a ratios have decreased significantly over time in both the Death Valley (blue circles; $r^2 = 0.21$, slope = −0.003, $P = 0.011$) and the Oatman (gray triangles; $r^2 = 0.25$, slope = −0.004, $P = 0.007$) populations. (*B*) iWUE values have increased significantly in both populations ($r^2 = 0.42$, slope = 1.3, $P = 0.0002$ for Death Valley; and $r^2 = 0.46$, slope = 1.4, $P = 0.0001$ for Oatman). Light gray bands represent the 95% CI of the regressions.

stronger predictor of $\delta^{13}C$ values than either temperature or precipitation, precipitation is an imperfect proxy for soil moisture, precluding the conclusion that $\delta^{13}C$ values in *E. farinosa* are more strongly influenced by evaporative demand than soil moisture. However, we found that VPD successfully captured the impacts of both precipitation and temperature on shrub intrinsic water-use efficiencies over the growing season in this interior desert ecosystem and would expect similar relationships in other arid regions

in which VPD is closely related to soil moisture, especially over annual or longer timescales.

The decrease in c_i/c_a ratios over time was strongly associated with increasing water stress in both populations, but the response of c_i/c_a ratios to increasing CO_2 concentration varied between populations. Most previous studies of the response of carbon isotope ratios to CO_2 enrichment find that plants regulate stomata so as to maintain an approximately constant c_i/c_a ratio after
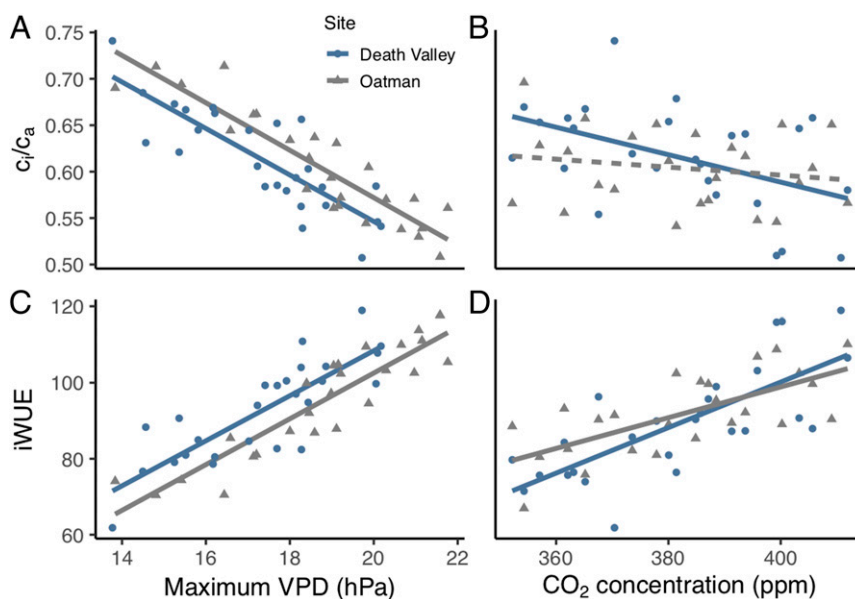
Fig. 5. Effects plots showing the relative impact of VPD (*A* and *C*) and CO_2 concentration (*B* and *D*) on the population mean c_i/c_a ratio (*A* and *B*) and iWUE (*C* and *D*) of *E. farinosa* leaves for the Death Valley population (blue circles) and the Oatman population (gray triangles) when the other variable is held constant at the mean value. Significant relationships ($P < 0.05$) are denoted by solid lines, and insignificant relationships are denoted with dashed lines. See *Results* for regression statistics.

controlling for fluctuations in water availability (24, 56, 57), leading to increased iWUE but relatively constant carbon isotope ratios. Data from the Oatman population agreed with those findings: CO_2 concentration was not significantly related to c_i/c_a ratios after controlling for VPD, indicating that a constant c_i/c_a ratio would be maintained in the absence of increasing atmospheric water demand. In contrast, other studies have found inconsistent responses (58) or decreases in c_i/c_a (59) in response to rising CO_2 concentration, which we observed in the Death Valley population. There was a significant but weak negative relationship between CO_2 concentration and c_i/c_a ratios in the Death Valley population after controlling for VPD ($r^2 = 0.21$ and $P = 0.022$), indicating that there is potential for CO_2 concentration to drive changes in c_i/c_a ratios under the CO_2 increases that occurred over the study period (about 60 ppm). If future c_i/c_a ratios were to decrease in response to increasing c_a, as occasionally observed in other studies (59, 60), larger global increases in iWUE would be expected (61) than if c_i/c_a ratios remained constant. While the possibility of CO_2-driven changes in c_i/c_a ratios merits additional study, VPD accounted for the majority of the temporal trend in c_i/c_a ratios in both populations over this time period.

As a result of changes in c_i and c_a, iWUE has increased by 53 and 58% over the study period in the Death Valley and Oatman populations, respectively. The iWUE increase is related to both VPD and CO_2 concentration although the increase in VPD explains a larger proportion of the iWUE trend than the increase in CO_2 concentration. Many tree ring studies have identified an increase in iWUE associated with rising CO_2 concentration over the last century (18, 21, 22, 31, 59, 62, 63), but most estimates of the change in iWUE have been substantially lower than those measured in this study. A metaanalysis of studies using tree ring $\delta^{13}C$ values to examine iWUE increases across a variety of biomes from the 1960s to the early 2000s found that estimates of iWUE increases ranged from 5.6 to 36.2%, with an average increase of 20.5% (18). A subsequent review, also considering studies of tree ring $\delta^{13}C$ values across biomes, reported an average iWUE increase of about 26% between 1960 and 2010 (21). iWUE changes among desert shrubs have not previously been quantified over long timescales or under natural conditions, but a CO_2 enrichment study found that three Mojave shrubs increased iWUE by an average of 48% in elevated (~550 ppm) versus ambient (~360 ppm) conditions (38). The magnitude of that increase is comparable to the iWUE increases measured in this study under a much smaller increase in CO_2. Our data indicate that the increase in iWUE among desert shrubs may be considerably larger than the average response of forests, compounded by simultaneous increases in both CO_2 and water stress. Although the two *E. farinosa* populations had slightly different responses to increasing CO_2 concentration, most of the trends in, and drivers of, $\delta^{13}C$ values, c_i/c_a ratios, and iWUE were nearly identical despite the fact that they are located over 200 km apart from one another. This consistency suggests that the observed patterns are likely widespread rather than an anomalous result restricted to a single microsite.

Selection for particular $\delta^{13}C$ values in response to variations in water availability has been identified in *E. farinosa* previously (42, 46), and we expected genetic changes to play a role in driving long-term population-scale trends in iWUE. However, temporal changes in the iWUE of individual plants were not different from those of the population, suggesting that selection did not significantly contribute to the population-level changes in iWUE values over the study period. Earlier analysis found that 90% of *E. farinosa* die by age 24 (52), and the vast majority of the plants present in the 2019 populations were recruited after 1991. However, substantial recruitment events (>50 individuals) have only occurred in 3 y since 1991 in Death Valley (1992, 2005, and 2016) and in 5 y in Oatman (1992, 1998, 2003, 2005, and 2008),

providing relatively little opportunity for selection. The infrequency of establishment events likely means that the 39-y study period was too short to capture evolutionary changes in iWUE values.

Notably, iWUE acclimation could either reflect an active and advantageous adjustment of gas exchange dynamics to optimize carbon–water trade-offs or simply a passive and nonadaptive stomatal response. While it is intuitive that iWUE increases may improve plant tolerance of increased aridity, increased iWUE in other ecosystems has not been universally associated with increased plant growth, increased reproduction, or reduced mortality (18, 21). Moreover, the transitions in species composition and biotic interactions that are accompanying abiotic changes in many desert regions may mediate the effects of iWUE increases. Further studies will be required to identify any potential benefits of observed increases in iWUE and to determine if phenotypic iWUE increases are sufficient for long-term survival of the population as water stress increases. Despite remaining uncertainty regarding the implications of increased iWUE on plant growth and survival, this study fills a major gap in the understanding of long-term patterns in the water use of desert shrubs and reveals that *E. farinosa* leaves have undergone substantial acclimation in response to changing climate.

Methods

Site Descriptions, Sample Collection, and Data Collection. Two near-monospecific stands of *E. farinosa* were identified and marked with cairns in the early 1980s for continuous, long-term monitoring. The first site is located ~21 km southwest of Shoshone, CA, covering about 450 m^2 of a south-facing slope in Death Valley National Park (referred to as the "Death Valley" site). The second site is about 315 m^2 and is located on a slope ~8 km southwest of Oatman, AZ (referred to as the "Oatman" site). A map of the approximate locations of the study sites is provided as SI Appendix, Fig. S2. There were no visual indications that human impacts affected the populations over the survey period.

The sites were surveyed annually during the last 2 wk of March beginning in 1981 at the Death Valley site and 1982 at the Oatman site. In the first survey year, all mature plants (1 y or older) were tagged and their coordinates on an x,y grid were recorded. Each year, new individuals were tagged and added to the record if they had a woody basal stem, indicating that they were at least 1 y old. Tags were removed, and no further data were recorded following an individual's death. During each annual survey, data were collected for plant size, flowering status, leaf cover, canopy dieback, and parasite presence (52). Additionally, five to 10 sun leaves from the most recent mature leaf flush were collected annually from each plant for carbon and nitrogen isotope analysis beginning in 1991 at the Death Valley site and in 1989 at the Oatman site. Because *E. farinosa* plants are drought-deciduous and drop their leaves every summer, leaf carbon isotope ratios reflect only photosynthate produced within the current season. Leaves were not collected from plants that would be damaged by leaf collection, and not all plants had leaves in every year. Annually summarized data that are reported in this manuscript are provided in SI Appendix, Table S4, including data on climate, CO_2 concentration and $\delta^{13}C$, population and sample sizes, and population mean $\delta^{13}C$ values, c_i/c_a ratios, and iWUE values.

Isotope Analysis. Leaf samples were dried upon returning from the field and stored in a cool, dark, dry place until analysis was conducted. Selected samples were ground to <40 mesh and loaded into tin capsules for analysis of carbon isotope ratios. Isotope analyses were conducted using a Carlo-Erba EA-1110 elemental analyzer coupled to a Finnigan Mat Delta+ isotope-ratio mass spectrometer (IRMS) via a continuous flow interface (ThermoFinnigan Conflo III, Bremen, Germany). Laboratory reference materials were calibrated using international standards USGS40 ($\delta^{13}C = -26.24$‰) and USGS41 ($\delta^{13}C = 37.76$‰), and all results are reported in delta notation on the Vienna Pee Dee Belemnite (VPDB) scale. Long-term measurement uncertainty for quality control materials is 0.2‰ for $\delta^{13}C$.

Climate Data. We acquired monthly data on total precipitation, mean daily maximum VPD, and mean daily minimum, mean, and maximum temperature for each site from the Parameter-Elevation Regressions on Independent Slopes Model (PRISM) Climate Group datasets (4 km resolution, https://prism.oregonstate.edu/, accessed 15 January 2020). Mean temperature in the PRISM dataset is calculated as the average of the minimum and maximum

daily temperatures. For the Death Valley population, we used climate data from the grid cell containing Shoshone, CA because the presence of a weather station in Shoshone improved the reliability of the data. For the Oatman population, we used the grid cell containing the site coordinates. Because *E. farinosa* at these sites typically leaf out in late fall and annual surveys were conducted in late March, we considered the November-to-March period preceding each survey to be the climatic period relevant for leaf δ^{13}C values. Climate data included throughout are averages over this November-to-March growing season, unless otherwise specified.

Calculation of c_i/c_a and iWUE. The carbon isotope ratio of a leaf is a function of the δ^{13}C value of atmospheric CO_2 ($\delta^{13}C_{atm}$), the c_i/c_a ratio, and the fractionations associated with CO_2 diffusion ($a = 4.4‰$) and net carboxylase discrimination ($b = 27‰$) (Eq. **1**) (23, 25):

$$\delta^{13}C_{plant} = \delta^{13}C_{atm} - a - (b-a)\left(\frac{c_i}{c_a}\right) \qquad [1]$$

The relationship between iWUE and c_i and c_a is given in Eq. **2**, in which 1.6 is the ratio of the diffusivities of CO_2 in air to that of water vapor in air.

$$iWUE_{leaf} = \frac{A}{g_s} = \frac{c_a\left(1-\frac{c_i}{c_a}\right)}{1.6} \qquad [2]$$

There has been a decrease in the δ^{13}C value of atmospheric CO_2 over the study period due to fossil fuel combustion, known as the Suess effect (64, 65), which confounds interpretation of long-term trends in plant δ^{13}C. However, the c_i/c_a ratio and iWUE are unaffected by changes in the δ^{13}C value of the atmosphere and reflect only physiologically relevant trends in plant gas exchange.

δ^{13}C Value and Concentration of Atmospheric CO_2. Publicly available data on the δ^{13}C (66) and concentration (67) of atmospheric CO_2 in Wendover, UT were obtained from the National Oceanic and Atmospheric Administration (NOAA) Earth System Research Laboratory Global Monitoring Division (ESRL). Although other NOAA ESRL sites have longer term datasets, we chose to use data from the Wendover site as it is similar to the study sites in terms of latitude, aridity, vegetation, and proximity to urbanized areas. Data

on the δ^{13}C values of CO_2 were available for 1993 to 2014, and data on CO_2 concentrations were available for 1993 to 2018. Average $\delta^{13}CO_2$ values were strongly linearly correlated with year ($r^2 = 0.965$) so we extrapolated values for 1991, 1992, and 2015 to 2019 based on the linear regression between $\delta^{13}CO_2$ and year (y = −0.028597x + 49.031911). We also extrapolated CO_2 concentration for 1991, 1992, and 2019 based on the linear regression between CO_2 and year ($r^2 = 0.995$; y = 2.091x − 3,811).

Statistical Analysis. All data analyses were conducted in R version 3.6.1 (R Core Team). Unless otherwise specified, statistical tests including δ^{13}C, c_i/c_a, and iWUE values were applied to annual population means using all collected data (provided in *SI Appendix*, Table S4) (n = 26 y for Death Valley and 25 y for Oatman). Single linear regressions of mean annual temperature, mean daily maximum VPD, total annual precipitation, CO_2 concentration, and population mean δ^{13}C, c_i/c_a, and iWUE values by year were applied for each population to identify temporal trends. For comparison, we also conducted linear regression of c_i/c_a, and iWUE values by year for a subset of plants that survived the duration of the study period (n = 322 observations from 10 plants in Death Valley and n = 302 observations from nine plants in Oatman). We compared annual population mean δ^{13}C values between the Death Valley and Oatman populations using a t test. The effects of temperature, VPD, and precipitation on population mean δ^{13}C values were assessed using single and multiple linear regression. Partial correlation and multiple regression were used to identify the independent and cumulative effects of VPD and CO_2 concentration on population mean c_i/c_a, and iWUE values.

Data Availability. All data that are reported in this manuscript are provided in *SI Appendix*, Table S4, including data on climate, CO_2 concentration and δ^{13}C, population and sample sizes, and population mean δ^{13}C values, c_i/c_a ratios, and iWUE values.

1. R. Lal, Carbon sequestration in dryland ecosystems. *Environ. Manage.* **33**, 528–544 (2004).
2. Intergovernmental Panel on Climate Change (IPCC), Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems, P. R. Shukla et al., Eds., in press. https://www.ipcc.ch/site/assets/uploads/2019/11/SRCCL-Full-Report-Compiled-191128.pdf. Accessed 11 July 2020.
3. J. Huang, H. Yu, X. Guan, G. Wang, R. Guo, Accelerated dryland expansion under climate change. *Nat. Clim. Chang.* **6**, 166–171 (2016).
4. W. H. Schlesinger et al., Biological feedbacks in global desertification. *Science* **247**, 1043–1048 (1990).
5. J. Ehleringer, H. A. Mooney, "Productivity of desert and mediterranean-climate plants" in *Physiological Plant Ecology IV: Ecosystem Processes: Mineral Cycling, Productivity and Man's Influence*, O. L. Lange, P. S. Nobel, C. B. Osmond, H. Ziegler, Eds. (Encyclopedia of Plant Physiology, Springer, 1983), pp. 205–231.
6. J. Grace, Understanding and managing the global carbon cycle. *J. Ecol.* **92**, 189–202 (2004).
7. E. Lioubimtseva, J. M. Adams, Possible implications of increased carbon dioxide levels and climate change for desert ecosystems. *Environ. Manage.* **33**, S388–S404 (2004).
8. C. G. Curtin, N. F. Sayre, B. D. Lane, Transformations of the Chihuahuan borderlands: Grazing, fragmentation, and biodiversity conservation in desert grasslands. *Environ. Sci. Policy* **5**, 55–68 (2002).
9. T. Svejcar et al., Carbon fluxes on North American rangelands. *Rangeland Ecol. Manag.* **61**, 465–474 (2008).
10. D. Bachelet, K. Ferschweiler, T. Sheehan, J. Strittholt, Climate change effects on southern California deserts. *J. Arid Environ.* **127**, 17–29 (2016).
11. C. W. Barrows, Sensitivity to climate change for two reptiles at the Mojave–Sonoran Desert interface. *J. Arid Environ.* **75**, 629–635 (2011).
12. N. N. Barger et al., Woody plant proliferation in North American drylands: A synthesis of impacts on ecosystem carbon balance. *J. Geophys. Res. Biogeosci.* **116**, G00K07 (2011).
13. R. L. Jasoni, S. D. Smith, J. A. Arnone, Net ecosystem CO_2 exchange in Mojave Desert shrublands during the eighth year of exposure to elevated CO_2. *Glob. Change Biol.* **11**, 749–756 (2005).
14. D. McCarroll, N. J. Loader, Stable isotopes in tree rings. *Quat. Sci. Rev.* **23**, 771–801 (2004).
15. J. Comstock, J. Ehleringer, Photosynthetic responses to slowly decreasing leaf water potentials in *Encelia frutescens*. *Oecologia* **61**, 241–248 (1984).
16. J. R. Ehleringer, A. E. Hall, G. D. Farquhar, Eds., *Stable Isotopes and Plant Carbon-Water Relations* (Elsevier, 1993).
17. G. D. Farquhar, K. T. Hubick, A. G. Condon, R. A. Richards, "Carbon isotope fractionation and plant water-use efficiency" in *Stable Isotopes in Ecological Research*, P. W. Rundel, J. R. Ehleringer, K. A. Nagy, Eds. (Ecological Studies, Springer, 1989), pp. 21–40.
18. P. Peñuelas, J. G. Canadell, R. Ogaya, Increased water-use efficiency during the 20th century did not translate into enhanced tree growth. *Glob. Ecol. Biogeogr.* **20**, 597–608 (2011).
19. M. Lévesque, R. Siegwolf, M. Saurer, B. Eilmann, A. Rigling, Increased water-use efficiency does not lead to enhanced tree growth under xeric and mesic conditions. *New Phytol.* **203**, 94–109 (2014).
20. J. -L. Dupouey, S. Leavitt, E. Choisnel, S. Jourdain, Modelling carbon isotope fractionation in tree rings based on effective evapotranspiration and soil water status. *Plant Cell Environ.* **16**, 939–947 (1993).
21. L. C. R. Silva, M. Anand, Probing for the influence of atmospheric CO_2 and climate change on forest ecosystems across biomes. *Glob. Ecol. Biogeogr.* **22**, 83–92 (2013).
22. P. van der Sleen et al., No growth stimulation of tropical trees by 150 years of CO_2 fertilization but water-use efficiency increased. *Nat. Geosci.* **8**, 24–28 (2015).
23. G. D. Farquhar, J. R. Ehleringer, K. T. Hubick, Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40**, 503–537 (1989).
24. J. R. Ehleringer, T. E. Cerling, Atmospheric CO_2) and the ratio of intercellular to ambient CO_2 concentrations in plants. *Tree Physiol.* **15**, 105–111 (1995).
25. G. D. Farquhar, M. H. O'Leary, J. A. Berry, On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Funct. Plant Biol.* **9**, 121–137 (1982).
26. K. A. Novick et al., The increasing importance of atmospheric demand for ecosystem water and carbon fluxes. *Nat. Clim. Chang.* **6**, 1023–1027 (2016).
27. J. P. Comstock, J. R. Ehleringer, Correlating genetic variation in carbon isotopic composition with complex climatic gradients. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7747–7751 (1992).
28. J. R. Ehleringer, T. A. Cooper, Correlations between carbon isotope ratio and microhabitat in desert plants. *Oecologia* **76**, 562–566 (1988).
29. D. C. Frank et al., Water-use efficiency and transpiration across European forests during the Anthropocene. *Nat. Clim. Chang.* **5**, 579–583 (2015).
30. K. Yi et al., Linking variation in intrinsic water-use efficiency to isohydricity: A comparison at multiple spatiotemporal scales. *New Phytol.* **221**, 195–208 (2019).
31. D. Li et al., Climate, intrinsic water-use efficiency and tree growth over the past 150 years in humid subtropical China. *PLoS One* **12**, e0172045 (2017).
32. R. J. Donohue, M. L. Roderick, T. R. McVicar, G. D. Farquhar, Impact of CO_2 fertilization on maximum foliage cover across the globe's warm, arid environments. *Geophys. Res. Lett.* **40**, 3031–3035 (2013).

33. R. S. Nowak, D. S. Ellsworth, S. D. Smith, Functional responses of plants to elevated atmospheric CO_2: Do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytol.* **162**, 253–280 (2004).

34. S. D. Smith et al., Elevated CO_2 increases productivity and invasive species success in an arid ecosystem. *Nature* **408**, 79–82 (2000).

35. E. Naumburg et al., Photosynthetic responses of Mojave Desert shrubs to free air CO_2 enrichment are greatest during wet years. *Glob. Change Biol.* **9**, 276–285 (2003).

36. E. P. Hamerlynck et al., Photosynthetic responses of *Larrea tridentata* to a step-increase in atmospheric CO_2 at the Nevada Desert FACE Facility. *J. Arid Environ.* **44**, 425–436 (2000).

37. D. S. Ellsworth et al., Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. *Glob. Change Biol.* **10**, 2121–2138 (2004).

38. D. C. Housman et al., Increases in desert shrub productivity under elevated carbon dioxide vary with water availability. *Ecosystems (N. Y.)* **9**, 374–385 (2006).

39. A. G. Condon, R. A. Richards, Broad sense heritability and genotype × environment interaction for carbon isotope discrimination in field-grown wheat. *Aust. J. Agric. Res.* **43**, 921–934 (1992).

40. W. S. F. Schuster, S. L. Phillips, D. R. Sandquist, J. R. Ehleringer, Heritability of carbon isotope discrimination in *Gutierrezia microcephala* (Asteraceae). *Am. J. Bot.* **79**, 216–221 (1992).

41. J. Zhang, J. D. Marshall, B. C. Jaquish, Genetic differentiation in carbon isotope discrimination and gas exchange in Pseudotsuga menziesii: A common-garden experiment. *Oecologia* **93**, 80–87 (1993).

42. D. R. Sandquist, J. R. Ehleringer, Carbon isotope discrimination differences within and between contrasting populations of *Encelia farinosa* raised under common-environment conditions. *Oecologia* **134**, 463–470 (2003).

43. J. R. Ehleringer, Variation in leaf carbon isotope discrimination in *Encelia farinosa*: Implications for growth, competition, and drought survival. *Oecologia* **95**, 340–346 (1993).

44. J. R. Ehleringer, Intraspecific competitive effects on water relations, growth and reproduction in *Encelia farinosa*. *Oecologia* **63**, 153–158 (1984).

45. W. S. F. Schuster, D. R. Sandquist, S. L. Phillips, J. R. Ehleringer, Comparisons of carbon isotope discrimination in populations of aridland plant species differing in lifespan. *Oecologia* **91**, 332–337 (1992).

46. L. A. Donovan, J. R. Ehleringer, Potential for selection on plants for water-use efficiency as estimated by carbon isotope discrimination. *Am. J. Bot.* **81**, 927–935 (1994).

47. S. A. M. McAdam, T. J. Brodribb, The evolution of mechanisms driving the stomatal response to vapor pressure deficit. *Plant Physiol.* **167**, 833–843 (2015).

48. W. R. Anderegg, N. S. Diffenbaugh, Observed and projected climate trends and hotspots across the National Ecological Observatory Network regions. *Front. Ecol. Environ.* **13**, 547–552 (2015).

49. R. Seager, G. A. Vecchi, Greenhouse warming and the 21st century hydroclimate of southwestern North America. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 21277–21282 (2010).

50. Z. R. Stahlschmidt, D. F. DeNardo, J. N. Holland, B. P. Kotler, M. Kruse-Peeples, Tolerance mechanisms in North American deserts: Biological and societal approaches to climate change. *J. Arid Environ.* **75**, 681–687 (2011).

51. A. P. Williams et al., Large contribution from anthropogenic warming to an emerging North American megadrought. *Science* **368**, 314–318 (2020).

52. J. R. Ehleringer, D. R. Sandquist, A tale of ENSO, PDO, and increasing aridity impacts on drought-deciduous shrubs in the Death Valley region. *Oecologia* **187**, 879–895 (2018).

53. D. R. Cayan et al., Future dryness in the southwest US and the hydrology of the early 21st century drought. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 21271–21276 (2010).

54. A. P. Williams et al., Forest responses to increasing aridity and warmth in the southwestern United States. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 21289–21294 (2010).

55. B. Udall, J. Overpeck, The twenty-first century Colorado River hot drought and implications for the future. *Water Resour. Res.* **53**, 2404–2418 (2017).

56. R. F. Keeling et al., Atmospheric evidence for a global secular increase in carbon isotopic discrimination of land photosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 10361–10366 (2017).

57. J. D. Marshall, R. A. Monserud, Homeostatic gas-exchange parameters inferred from $^{13}C/^{12}C$ in tree rings of conifers. *Oecologia* **105**, 13–21 (1996).

58. S. L. Voelker et al., A dynamic leaf gas-exchange strategy is conserved in woody plants under changing ambient CO_2 : Evidence from carbon isotope discrimination in paleo and CO_2 enrichment studies. *Glob. Change Biol.* **22**, 889–902 (2016).

59. T. F. Keenan et al., Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature* **499**, 324–327 (2013).

60. G. Battipaglia et al., Elevated CO_2 increases tree-level intrinsic water use efficiency: Insights from carbon and oxygen isotope analyses in tree rings across three forest FACE sites. *New Phytol.* **197**, 544–554 (2013).

61. M. Saurer, R. T. W. Siegwolf, F. H. Schweingruber, Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. *Glob. Change Biol.* **10**, 2109–2120 (2004).

62. F. Giammarchi, P. Cherubini, H. Pretzsch, G. Tonon, The increase of atmospheric CO_2 affects growth potential and intrinsic water-use efficiency of Norway spruce forests: Insights from a multi-stable isotope analysis in tree rings of two alpine chronosequences. *Trees (Berl.)* **31**, 503–515 (2017).

63. Z. Gedalof, A. A. Berg, Tree ring evidence for limited direct CO_2 fertilization of forests over the 20th century. *Global Biogeochem. Cycles* **24**, GB3027 (2010).

64. H. E. Suess, Radiocarbon concentration in modern wood. *Science* **122**, 415–417 (1955).

65. C. D. Keeling, The suess effect: ^{13}carbon-^{14}carbon interrelations. *Environ. Int.* **2**, 229–300 (1979).

66. J. W. C. White, B. H. Vaughn, S. E. Michel, Data from "Stable isotopic composition of atmospheric carbon dioxide (^{13}C and ^{18}O), 1990–2014." NOAA ESRL. ftp://aftp.cmdl.noaa.gov/data/trace_gases/co2c13/flask/. Accessed 15 January 2020.

67. E. J. Dlugokencky, J. W. Mund, A. M. Crotwell, M. J. Crotwell, K. W. Thoning, Data from "Atmospheric carbon dioxide dry air mole fractions, 1968–018." NOAA ESRL. https://www.esrl.noaa.gov/gmd/ccgg/arc/?id=132. Accessed 15 January 2020.