Elevated [CO_2] and temperature augment gas exchange and shift the fitness landscape in a montane forb

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Summary

- Climate change is simultaneously increasing carbon dioxide concentrations ([CO_2]) and temperature. These factors could interact to influence plant physiology and performance. Alternatively, increased [CO_2] may offset costs associated with elevated temperatures. Furthermore, the interaction between elevated temperature and [CO_2] may differentially affect populations from along an elevational gradient and disrupt local adaptation.
- We conducted a multifactorial growth chamber experiment to examine the interactive effects of temperature and [CO_2] on fitness and ecophysiology of diverse accessions of *Boechera stricta* (Brassicaceae) sourced from a broad elevational gradient in Colorado. We tested whether increased [CO_2] would enhance photosynthesis across accessions, and whether warmer conditions would depress the fitness of high-elevation accessions owing to steep reductions in temperature with increasing elevation in this system. Elevational clines in [CO_2] are not as evident, making it challenging to predict how locally adapted ecotypes will respond to elevated [CO_2].
- This experiment revealed that elevated [CO_2] increased photosynthesis and intrinsic water use efficiency across all accessions. However, these instantaneous responses to treatments did not translate to changes in fitness. Instead, increased temperatures reduced the probability of reproduction for all accessions. Elevated [CO_2] and increased temperatures interacted to shift the adaptive landscape, favoring lower elevation accessions for the probability of survival and fecundity.
- Our results suggest that elevated temperatures and [CO_2] associated with climate change could have severe negative consequences, especially for high-elevation populations.

Introduction

Climatic factors strongly influence the expression of traits and the evolution of natural populations (Spence & Tingley, 2020). Industrialization has altered complex suites of climatic conditions, such as atmospheric carbon dioxide concentration (hereafter [CO_2]) and temperature (Tierney *et al.*, 2020; IPCC, 2021), thereby imposing novel patterns of selection on natural populations (Waldvogel *et al.*, 2020), which can decrease fitness (Anderson & Wadgymar, 2020). Elevated [CO_2] alone could enhance plant performance (Mathias & Thomas, 2021); however, intensifying warming could offset these fitness gains (Bogdziewicz *et al.*, 2020; Liu *et al.*, 2022). Additionally, the interactive effects of changing [CO_2] and temperature can vary across the life cycle of a plant (Parmesan & Hanley, 2015; Tietze *et al.*, 2019), which highlights the need to assess plant performance at multiple ontogenetic stages. Furthermore, robust predictions of the effects of climate change on plant performance and fitness require multifactorial manipulations (Zandalinas *et al.*, 2024).

Ice core records indicate that global [CO_2] varied between 175 and 300 ppm over the last 800 000 yr before industrialization (Bereiter *et al.*, 2015; Nehrbass-Ahles *et al.*, 2020), reaching as low as 180–190 ppm during the Last Glacial Maximum (Ahn & Brook, 2008). Thus, plants have evolved in response to fluctuating carbon availability in the past. Today, however, plants are exposed to [CO_2] higher than any atmospheric concentration experienced in the last 3.3 million years (de la Vega *et al.*, 2020), which can have profound ecophysiological (Leakey & Lau, 2012) and evolutionary consequences (Ziska, 2008; Vogan & Sage, 2012; Sage, 2020). For example, elevated [CO_2] can stimulate photosynthesis, especially in C3 plants (Ainsworth & Long, 2005; Ainsworth & Rogers, 2007; Faralli *et al.*, 2017; Zhang *et al.*, 2021), and can augment fitness in some species, particularly when plants have access to ample water and nutrients (Mohan *et al.*, 2004; Wang *et al.*, 2015; Apgaua *et al.*, 2019). Greater photosynthesis in high [CO_2] environments can reduce stomatal conductance (Ainsworth & Rogers, 2007; Saban *et al.*, 2019), and transpiration rates, which can induce stress due to elevated heating (Allakhverdiev *et al.*, 2008), mineral deficiencies (Loladze, 2014), and changes in metabolic signaling (Xu *et al.*, 2015; Baslam *et al.*, 2020). This stress, in turn, can depress photosynthetic rates, growth, and reproductive output (Bernacchi *et al.*, 2006; Maschler *et al.*, 2022; Poorter *et al.*, 2022). Furthermore, elevated [CO_2] can decrease the expression of genes involved in photosynthesis in some species (Moore *et al.*, 1999) or tip the balance between carbon acquisition and carbon usage, potentially resulting in maladaptation to elevated [CO_2] (Sage, 2020). Nevertheless, the effects of high [CO_2] on plant fitness remain largely uncertain, especially in noncrop systems (Menezes-Silva *et al.*, 2019).

Warming temperatures influence plant fitness performance throughout the lifecycle (Zinn *et al.*, 2010; Bewley & Black, 2013; Hatfield & Prueger, 2015). For example, temperature can affect the timing and success of germination (Cochrane *et al.*, 2015; Hoyle *et al.*, 2015), the rate of plant growth (Sage & Kubien, 2007), the timing of flowering (Inouye, 2020), and all aspects of reproduction, from pollen production and size to ovary size, ovule number, floral display size and seed production (Bykova *et al.*, 2012; Tushabe *et al.*, 2023). Photosynthetic responses to temperature vary within a species across geographic ranges, reflecting adaptation to local thermal environments (Fryer & Ledig, 1972; Atkin & Tjoelker, 2003; Gunderson *et al.*, 2010; O'Sullivan *et al.*, 2017; Kumarathunge *et al.*, 2019; Dusenge *et al.*, 2021). Climate change could reduce the fitness of these locally adapted populations, shifting the fitness landscape to favor accessions from lower elevation or latitude populations, which evolved under warmer thermal regimes.

Elevated [CO_2] and rising temperatures do not occur in isolation, and these factors can have additive, antagonistic, or synergistic effects on plant ecophysiology and fitness (Zandalinas & Mittler, 2022). In some cases, elevated [CO_2] can counteract the negative fitness effects of increasing temperatures (Clifford *et al.*, 2000). For example, hotter temperatures reduced photosynthesis in perennial ryegrass, but elevated temperature and [CO_2] restored photosynthesis to rates expressed in ambient conditions (AbdElgawad *et al.*, 2015). In other cases, elevated [CO_2] and warming temperatures interact to affect fitness. For example, durum wheat (*Triticum durum*) grown in a combined high [CO_2] and temperature environment displayed lower nitrogen reductase activity and an overall decrease in seed nitrogen content compared with plants exposed to only elevated [CO_2] or elevated temperatures (Jauregui *et al.*, 2015). Comprehensive studies on crop species have illuminated how some plant species will respond to elevated temperatures and [CO_2] at a physiological level (e.g. reviews by van der Kooi *et al.*, 2016; Leakey *et al.*, 2019; Zhang *et al.*, 2021; Eckardt *et al.*, 2023). However, to-date few multifactorial experiments have investigated the interactive effects of increasing temperatures and [CO_2] on the physiology or fitness of noncrop species. The few studies available show that responses to increased temperature and [CO_2] vary by species. Elevated [CO_2] and warmer temperatures do not appear to interact to influence ecophysiology in many tree species (Wang & Wang, 2021) nor in some perennial grassland forb species (Pastore *et al.*, 2020). However, higher temperatures can enhance photosynthesis in the presence of elevated [CO_2] in some grasses (Pastore *et al.*, 2020). Species-specific responses further highlight the uncertainty in predicting performance of native plant species in response to these climate change factors.

Elevational gradients provide exemplary systems for investigating biological responses to climatic variation, as climatic conditions change greatly over short spatial scales (Körner, 2007a, b). In response to divergent selection, natural populations can adapt to local environments across elevations (Halbritter *et al.*, 2015; de Villemereuil *et al.*, 2018; Anderson & Wadgymar, 2020). Globally, one common climatic pattern exists: as elevation increases, temperatures decrease (Pepin & Lundquist, 2008). A logical prediction, therefore, is that increasing temperatures mediated by climate change at mid- or high-elevation locales could favor lower elevation accessions that evolved under hotter climates. Nevertheless, it is challenging to disentangle the contributions of temperature from those of other agents of selection to local adaptation across elevations (Lancaster & Humphreys, 2020). Thus, studies that isolate the effects of temperature on the fitness of locally adapted ecotypes from divergent elevations could illuminate the consequences of novel thermal regimes (Malusare *et al.*, 2023).

Elevational gradients in [CO_2] are not quite as clear (Lin *et al.*, 2017), and we know much less about local adaptation to variation in [CO_2]. The partial pressure of CO_2 declines with elevation (Gale, 1972, 2004; Körner & Diemer, 1987; Smith *et al.*, 2003; Smith & Johnson, 2009; Körner, 2021), but it is not clear whether this change limits photosynthetic rates of high-elevation plants (Wang *et al.*, 2017). In some cases, high-elevation populations have greater rates of photosynthesis than lower elevation populations (Gale, 1972; Körner & Diemer, 1987; Körner *et al.*, 1988; Van de Water *et al.*, 2002; Zhou *et al.*, 2011; Körner, 2021), which could arise due to greater leaf nitrogen content and smaller leaf sizes of the high-elevation accessions (Wieser, 2007). As such, it is difficult to distinguish the effects of [CO_2] vs other factors on photosynthesis along elevational gradients. In some natural plant populations growing near CO_2 springs, individuals can experience [CO_2] as high as 5338 ppm (Onoda *et al.*, 2009; Leakey & Lau, 2012), which is far above the ambient global average of *c.* 417 ppm in current climates (Lan *et al.*, 2023). Common garden experiments using *Plantago asiatica* sourced from these springs have found differentiation in photosynthesis and productivity along this [CO_2] gradient (Onoda *et al.*, 2009), indicating that [CO_2] can act as an agent of selection in plant populations (see also Leakey & Lau, 2012). Nevertheless, the general uncertainty in the extent of local adaptation to [CO_2] reduces our ability to formulate predictions about plant fitness in future [CO_2] climates for species distributed across broad climatic gradients.

We conducted a multifactorial manipulation of temperature and [CO_2] to evaluate the interactive effects of temperature and carbon dioxide on plant growth, physiology, and fecundity in a growth chamber experiment. Additionally, we leveraged an older common garden study to assess clines in photosynthesis under natural conditions in the field. We focus this study on diverse accessions of the ecological model species, *Boechera stricta* (Brassicaceae), sourced from natural populations distributed along an elevation gradient. Natural populations of *B. stricta* have adapted to diverse habitats across climatic gradients, such as elevation (Kiefer *et al.*, 2009; Lee & Mitchell-Olds, 2011; Anderson *et al.*, 2015), making it an excellent model system for investigating plant performance under novel suites of climatic conditions. We assessed plant performance at the leaf level using gas exchange measurements as well as fitness across two simulated growing seasons to assess whether instantaneous measures of performance translate to fitness components. This experiment tested whether enhanced performance from elevated [CO_2] would compensate for depressed performance from increased temperatures. Alternatively, elevated [CO_2] and temperature could have additive or synergistic effects on plant ecophysiology and performance. We examined whether increasing temperatures would favor accessions from low-elevation populations, which evolved in historically warm climates. Finally, we tested whether novel temperature and [CO_2] regimes would interact to further shift the fitness landscape toward accessions best capable of tolerating increasing abiotic stress.

Materials and Methods

Focal species

Boechera stricta (Graham) Al-Shehbaz (Brassicaceae) is a perennial forb native to the Rocky Mountains of North America, where it is distributed broadly over elevational gradients and latitudes ranging from Utah through Alaska (Fig. 1a; Al-Shehbaz & Windham, 2010; Rushworth *et al.*, 2011, 2022). *Boechera stricta* primarily self-pollinates (Song *et al.*, 2006), which allows us to plant closely related siblings in different environments and study genotype by environment interactions. To examine genetic clines in plant physiology and performance in response to variable climatic conditions, we first collected seeds from naturally occurring individuals of *B. stricta* in populations at different elevations near the Rocky Mountain Biological Laboratory in Gothic, Colorado (Fig. 1a,b). We grew these seeds in the glasshouse for one generation to minimize maternal effects and generate full-sibling families via self-fertilization (Wadgymar *et al.*, 2018).

Experimental design

We conducted a multifactorial growth chamber experiment to examine the interactions between elevated [CO_2] and temperature on *B. stricta* ecophysiological traits and fitness. To simulate future [CO_2], we used the projected concentrations of the Representative Concentration Pathway (RCP) 6.0, which considers a medium stabilization effort to mitigate atmospheric greenhouse gases by 2100 (van Vuuren *et al.*, 2011). We used four growth chambers (Conviron BDW40 chambers; Winnipeg, Canada) in the Warnell School of Forestry at the University of Georgia configured for contemporary (400 ppm) and late-century (650 ppm) carbon dioxide concentrations. Owing to airflow in the building, the chambers were unable to maintain [CO_2] as low as 400 ppm; thus, plants in the control treatment experienced an average [CO_2] of 465 ppm (Supporting Information Fig. S1).

We based the ambient temperature level on the average daytime temperatures at Crested Butte, CO (elevation 2709 m) from 1980 to 2020 and followed a growing season of May through October, with an 8-wk winter vernalization period at a constant 4°C (Fig. S2). Crested Butte is 9.4 km from the Rocky Mountain Biological Laboratory and is close to several of the lower elevation source populations (Fig. 1b). Nocturnal temperatures in this record sometimes reach as low as −2.2°C early in the growing season, which is below the minimum temperature capability of the growth chambers. Thus, logistical constraints required us to restrict minimum night-time temperatures to 4°C. We set the elevated temperature treatment level to 4.75°C above the 1980–2020 average during the day and 5.5°C during the night (Fig. S1), based on 2080 projections under the RCP 6.0 scenario for the region (Vose *et al.*, 2005; IPCC, 2014).

On 4 August 2021, we planted $n = 7690$ seeds on moist filter paper in Petri dishes, which we placed in growth chambers set for May treatment temperatures and [CO_2] conditions (10–30 seeds/accession × 61 accessions × 4 treatments; with one accession from each of 61 natural populations; source elevation range: 2498–3673 m, Table S1; Fig. 1b). By sampling a single accession from many populations, we maximized genetic diversity in this

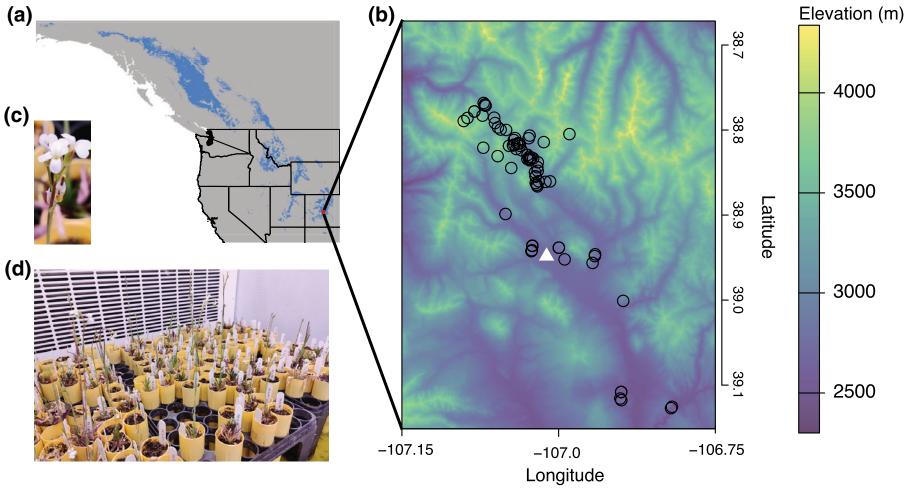

Fig. 1 (a) Range of *Boechera stricta* in blue, with the region near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado highlighted in red (b) Inset depicts a topographic map (elevation, m) of RMBL and the surrounding area. Open circles denote the source populations used in this study, and the white triangle indicates the location of the weather station in Crested Butte, Colorado. (c) A flowering individual of *B. stricta*. (d) An example of the growth chamber blocks with flowering individuals in a bottom-watering trough.

experiment (Goudet & Buchi, 2006; Blanquart *et al.*, 2013; Anderson *et al.*, 2015). Owing to low germination rates (28.9%), we initiated a second round of seed planting on 4 September 2021. We accounted for these two planting efforts by including a fixed effect of cohort in our statistical models. We transplanted $n = 1816$ seedlings from 61 accessions into conetainers (Stuewe and Sons, Tangent, OR, USA, RL 10 cu. inch conetainer; OR, USA) with pine bark soil (Sungro Metro-Mix 838; Sungro Horticulture, Agawam, MA, USA) when two cotyledons were present (see Table S1 for a breakdown of total sample sizes for each cohort, accession, and treatment).

We treated the plants with 20-10-20 fertilizer monthly (JR Peters Jack's peat lite; JR Peters Inc, Allentown, PA, USA) during the juvenile growth phase. In *B. stricta*, exposure to a cold period, or vernalization, is necessary to induce flowering (Anderson *et al.*, 2011); therefore, we reduced growth chamber temperatures to a constant 4°C on 11 February 2022 when plants were 160–190 d old. During the 8-wk vernalization period, we maintained all individuals at their treatment [CO_2] and provided them with ample water, but we did not fertilize them. After we ended vernalization on 11 April 2022, we fertilized adult plants monthly with 10-30-20 fertilizer (JR Peters Jack's blossom booster; JR Peters Inc). Throughout the experiment, plants were treated with larvicide (Gnatrol WDG; NuFarm, Nufarm Americas Inc., Alsip, IL, USA) one to two times per month (except during vernalization) to eliminate arthropod herbivores. We set the daylength to 14 h at 800 μmol s^{-1} throughout the experiment, except during vernalization, when daylength was reduced to 0 h to simulate snow cover conditions (Keller & Körner, 2003). Humidity was set to the May–October average field value of 55%. Every month, we changed the temperature conditions in the growth chambers, allowing us to simulate a full growing season (May through October; Table S2), and we moved plants and their corresponding treatments across chambers to minimize chamber effects. Blocks were rotated weekly within each chamber to reduce positional effects. Due to space limitations, we were unable to vary watering schemes to simulate drought. Therefore, for the duration of the experiment, we placed each conetainer tray into watering troughs, which we filled daily to maintain well-watered conditions.

Ecophysiology

When plants were 100–120 d old, we measured ecophysiological parameters with a LI-6800 portable photosynthesis system (LI-COR, Lincoln, NE, USA) during simulated July conditions of the first growing season (11–16 December 2021). We selected 41 accessions with multiple living siblings across treatments for these measurements ($n = 297$ individuals and 41 accessions spanning the elevational gradient of 2553–3673 m; $n = 2$–6/accessions/treatment). To follow best practices (LI-COR, 2022), we measured gas exchange at 23.1°C for plants in the contemporary temperature treatment and 27.9°C for plants in the elevated temperature treatments, reflecting the temperatures set for that month in the two treatment levels, and a [CO_2] of 400 or 650 ppm for contemporary vs future treatments. We measured photosynthetic active radiation at plant height, and we set the light level in the cuvette to 500 μmol m^{-2} s^{-1} to match this reading. As the leaves of *B. stricta* are smaller than the LI-COR cuvette chamber, after the measurements were completed, we removed all leaves measured for gas exchange and scanned them to determine leaf area using ilastik (Berg *et al.*, 2019). We adjusted the leaf area in LI-COR's gas exchange calculations to obtain assimilation rate (A), evapotranspiration rate (E), stomatal conductance (g_s), ambient CO_2 (C_a) and intracellular CO_2 (C_i) values per leaf area for each sample. We measured plants randomly chosen from each of the four treatments from 10:00 to 14:00 daily over 6 d (11–16 December 2021, average samples per day = 61.2 ± 17.8.)

Fitness components

We recorded three fitness components: (1) the probability of survival to the end of the experiment, (2) the probability of reproduction, and (3) fecundity among individuals that reproduced. In this study, some individuals reproduced and then died before the end of the experiment, necessitating consideration of both of these binary components of fitness. After transplanting seedlings into conetainers, we monitored survival two to four times a month until we induced vernalization. After vernalization, we censused the plants daily to monitor flowering success. We assessed fecundity by counting the total number of all mature fruits, which is tightly correlated with total seed production (Wadgymar *et al.*, 2017). We completed the experiment at the end of the second simulated September (23 August 2022), when reproductive plants had finished fruiting. As *B. stricta* is a perennial, it can reproduce more than once. However, previous field studies have shown that experimental plantings of *B. stricta* produce most of their lifetime fruit set in their first reproductive year (Wagner & Mitchell-Olds, 2018). For example, in a field experiment in five common gardens, an average of 73% of *B. stricta* seeds produced during 6 yr matured in the first growing season (Hamann *et al.*, 2021). In addition, seed production early in life history contributes more to population growth rate and evolution than later fecundity (Stearns, 1976). Therefore, the fitness components we captured in this experiment likely reflect robust fitness responses to the treatments we imposed.

Field common garden experiment

To test the hypothesis that photosynthetic rate increases with source elevation (Körner & Diemer, 1987; Körner, 2021), we measured assimilation rates on 68 transplanted individuals from 22 accessions (source elevations: 2869–3682 m) in a field common garden at 3133 m near the Rocky Mountain Biological Laboratory (Gothic, Colorado) on 26 July 2013. Only nine accessions from this field experiment were included in our growth chamber experiment while 13 were unique to the field common garden. We used a Li-COR 6400 portable gas analyzer with [CO_2] set to 380 ppm, air temperature in the cuvette set to 20°C, and photosynthetically active radiation set to 1500 μmol m^{-2} s^{-1}. This small ecophysiological dataset was not included in previous publications from this common garden experiment

(Anderson *et al.*, 2015; Anderson & Gezon, 2015; Wadgymar *et al.*, 2017).

Statistical analyses

To examine the interactive effect of elevated [CO_2] and temperature on gas exchange physiology, we analyzed photosynthesis (A), transpiration (E), stomatal conductance (g_s), and intrinsic water use efficiency (A/g_s; iWUE) as a function of source elevation, temperature treatment, CO_2 treatment, and cohort (to account for the two rounds of germination needed to achieve adequate sample sizes), as well as all treatment by elevation interactions, with random effects for accession and experimental block. We modeled these traits using the glmmTMB function of the GLMMTMB package v.1.1.15 (Brooks *et al.*, 2017). We standardized source elevation to a mean of 0 and standard deviation of 1 to enable model convergence. We examined statistical significance of main effects using the Anova function of the CAR R package v.3.0 (Fox & Weisberg, 2019) and random effects through likelihood ratio tests of models with and without accession and block. We visualized the regression results and partial residuals with the VISREG R package v.2.7.0 (Breheny & Burchett, 2017) and using the R package VIOPLOT v.0.4.0 (Adler *et al.*, 2022). To reduce the risk of type I errors, we adjusted our α to 0.0125 (=0.05/4 physiological variables). We square root transformed stomatal conductance, transpiration, and iWUE to meet statistical assumptions of normality and homoscedasticity of residuals. We determined that the residuals were normally distributed and homoscedastic using the simulateResiduals function of the R package DHARMA v.0.4.6 (Hartig, 2022). We present the results of statistical models using these transformed variables, but we plotted raw data in our figures to evaluate our hypotheses using biologically meaningful units.

To examine fitness data, we analyzed survival, the probability of reproduction, and fecundity as a function of cohort, source elevation, temperature, and CO_2 treatment, as well as the two- and three- way interactions of source elevation, temperature, and CO_2, with random effects for block and accession. We standardized source elevation to a mean of 0 and standard deviation of 1 to enable model convergence. As with many studies of fitness, our fecundity data contain an excess of zero values because of individuals that died or simply failed to reproduce. Initial models of composite fitness using approaches such as zero-inflated Gamma regressions in the glmmTMB function of the GLMMTMB package (v.1.1.5; Brooks *et al.*, 2017) generated residuals that did not conform to assumptions of homoscedasticity or normality. Therefore, we modeled fitness components separately and verified normality and homoscedasticity of residuals in all models using the simulateResiduals function of the DHARMA package v.0.4.6 (Hartig, 2022). We analyzed the probability of survival and reproduction using the glmmTMB function of the GLMMTMB package (v.1.1.5; Brooks *et al.*, 2017), with a binomial distribution and logit link. We modeled fecundity (the number of fruits among individuals that reproduced) using the glmmTMB function of the package GLMMTMB as a Gamma distribution with a log link. We visualized results using GGPLOT2 v.3.4.2 (Wickham, 2016) and GGEFFECTS v.1.3.2 (Lüdecke, 2018). We corrected for multiple comparisons using an α of 0.0167 (=0.05/3 fitness components).

To evaluate the effects of source elevation on gas exchange traits in our field common garden data, we analyzed photosynthesis as a function of source elevation with random effects for experimental block and accession using the glmmTMB function of GLMMTMB (Brooks *et al.*, 2017). We followed the same protocols as outlined above to examine the statistical significance.

Results

Gas exchange traits

Owing to the time-intensive nature of the measurements, we measured gas exchange traits on only a subset of the experimental plants ($n = 297$ individuals from 41 accessions). In these plants, elevated [CO_2] augmented photosynthesis rate by *c.* 50% ($\chi^2 = 29.60$, $P < 0.0001$; Fig. 2), but there were no effects of temperature or source elevation on photosynthesis (Table S3). Both elevated temperature and increased [CO_2] significantly heightened iWUE (temperature: $\chi^2 = 8.41$, $P = 0.004$; [CO_2]: $\chi^2 = 34.91$, $P < 0.0001$; Fig. 3) regardless of source elevation

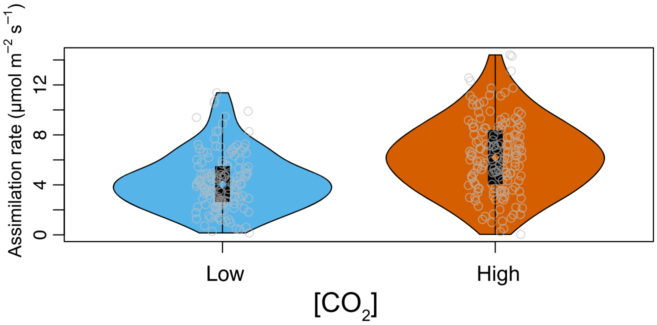

Fig. 2 Violin plots of photosynthesis rates (assimilation) were significantly greater for plants exposed to high [CO_2] in *Boechera stricta*. Gray circles indicate data points. The vertical lines within the violin plots represent the upper and lower quartiles. The center diamond indicates the median. The width of the violin plots depicts the density of data points.

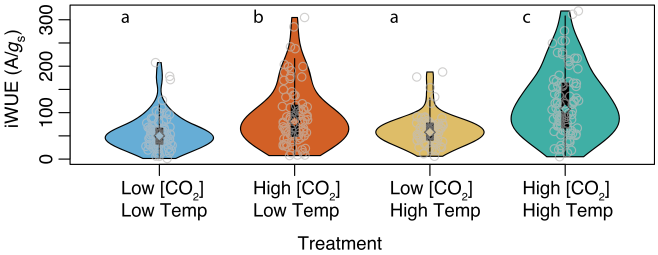

Fig. 3 Violin plots of intrinsic water use efficiency (iWUE), measured as the ratio of assimilation to stomatal conductance (A/g_s), increased under both high temperature and high [CO_2] in *Boechera stricta*. Gray circles indicate data points. Letters denote significance from a *post hoc* pairwise comparison. The vertical lines within the violin plots represent the upper and lower quartiles. The center diamond indicates the median. The width of the violin plots depicts the density of data points.

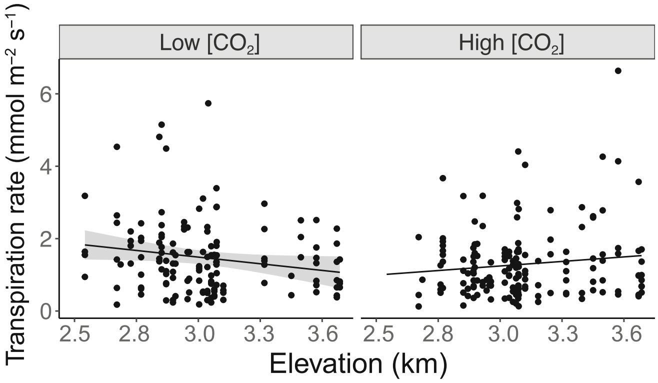

Fig. 4 Transpiration rate (E) declined with increasing source elevation under low (contemporary) [CO$_2$] in *Boechera stricta*. Under high (future) [CO$_2$], we found no significant relationship between transpiration rates and source elevation. Solid black lines indicate the predicted relationship between source elevation and transpiration and gray shading represents 95% confidence intervals for significant relationships only.

(Table S4). Our analysis revealed a significant interaction between CO$_2$ and source elevation for transpiration rates ($\chi^2 = 6.97$, $P = 0.0083$; Table S5). Under low [CO$_2$], transpiration declined by 0.18 mmol m^{-2} s^{-1} for every one standard deviation (280 m) increase in elevation ($\beta = -0.18$, 95% confidence interval: -0.37, $-5.3\text{e-}4$; Fig. 4). Conversely, there was a nonsignificant positive slope under high [CO$_2$] ($\beta = 0.13$, 95% CI: -0.06, 0.31). We recovered a [CO$_2$] by source elevation interaction in stomatal conductance (g_s; $P = 0.019$), which followed the same patterns as transpiration, but this interaction was nonsignificant after correction for multiple testing (Table S6).

Fitness components

The probability of survival varied as a function of a significant three-way interaction among [CO$_2$], temperature and the quadratic effect of source elevation ($\chi^2 = 6.10$, df = 1, $P = 0.014$; Table S7). Under elevated [CO$_2$] and contemporary temperatures, the odds of survival increased by 75% for every standard deviation increase in elevation (odds ratio: 1.75, 95% CI: 1.27, 2.43; Table S8), indicating the highest elevation accessions had the greatest survival in that treatment combination. However, the optimum elevation of origin for survival decreased to 3293 m under projected future environments with high [CO$_2$] and high temperatures (Fig. 5a; odds ratio for quadratic effect of elevation: 0.76, 95% CI: 0.60, 0.97). We found no significant effects of source elevation on survival in either temperature treatment in the low [CO$_2$] environment.

Elevated temperature depressed the probability of reproduction in both [CO$_2$] levels ($\chi^2 = 10.30$, df = 1, $P < 0.01$; Table S7; Fig. 5b). Furthermore, we found a significant effect of source elevation on probability of reproduction, with high-elevation accessions having the highest probability of reproduction in all treatment combinations ($\chi^2 = 5.87$, df = 1, $P = 0.015$, Fig. 5b).

Finally, our fecundity model revealed a significant interaction of [CO$_2$] and source elevation ($\chi^2 = 5.94$, df = 1, $P = 0.015$; Table S7) on the number of mature fruits produced. We found an interaction between source elevation and temperature, but it was only marginal at our adjusted alpha of 0.017 ($\chi^2 = 4.40$, df = 1, $P = 0.04$). Under high [CO$_2$] and high temperatures, mature fruits decreased by 20% for every standard deviation increase in elevation ($\beta = 0.801$, 95% CI: 0.70, 0.91; Table S9; Fig. 5c), but there was no effect of source elevation on fecundity in the other treatment combinations.

Field common garden experiment

Photosynthetic rates increased by 0.28 μmol CO$_2$ m^{-2} s^{-1} for every standard deviation increase in elevation ($\beta = 0.28$, 95% CI: 0.059, 0.51; $\chi^2 = 6.12$, $P = 0.013$; Fig. 6).

Discussion

Our multifactorial growth chamber experiment revealed noteworthy shifts in the fitness landscape under novel climatic factors for several major fitness components. Firstly, elevated temperatures depressed the probability of reproduction across all accessions and both [CO$_2$] levels. Additionally, [CO2] and temperature interacted synergistically to shape the viability and fecundity clines. Specifically, fecundity declined with source elevation only in the high [CO$_2$] and high-temperature treatment combination, indicating that low elevation accessions are uniquely capable of tolerating increased abiotic stress associated with climate change. Furthermore, our analyses demonstrated that increased temperature and elevated [CO$_2$] shifted the viability cline to favor lower elevation accessions compared with what we found under elevated [CO$_2$] alone. These results underscore the importance of considering complex interactions among environmental factors to predict responses to climate change.

We predicted that elevated [CO$_2$] would increase leaf-level ecophysiology and fitness. In line with our prediction, we found that high [CO$_2$] induced greater photosynthesis and water use efficiency. Nevertheless, this instantaneous increase in performance did not translate to higher fitness for all accessions. Rather, the effect of heightened temperature and [CO$_2$] on fitness after two seasons of growth depended upon the source elevation of the accession, indicating that the evolutionary history of the accession drives the fitness responses to changing climatic factors. Elevated [CO$_2$] may offset reductions in fecundity for low-elevation accessions, but we found no evidence that greater [CO$_2$] compensates for thermal stress for other accessions or components of fitness. In fact, higher temperature reduced the probability of reproduction for all accessions under both low and high [CO$_2$] regimes.

Plant performance through gas exchange traits

Concordant with other work (Ainsworth & Rogers, 2007; Busch & Sage, 2017; Dusenge *et al.*, 2019), we found that elevated [CO$_2$] augmented photosynthesis and water use efficiency via phenotypic plasticity. Increased photosynthesis and water use efficiency are the most common responses to elevated [CO$_2$] in C3 plants (Drake *et al.*, 1997; Leakey *et al.*, 2019). This increase

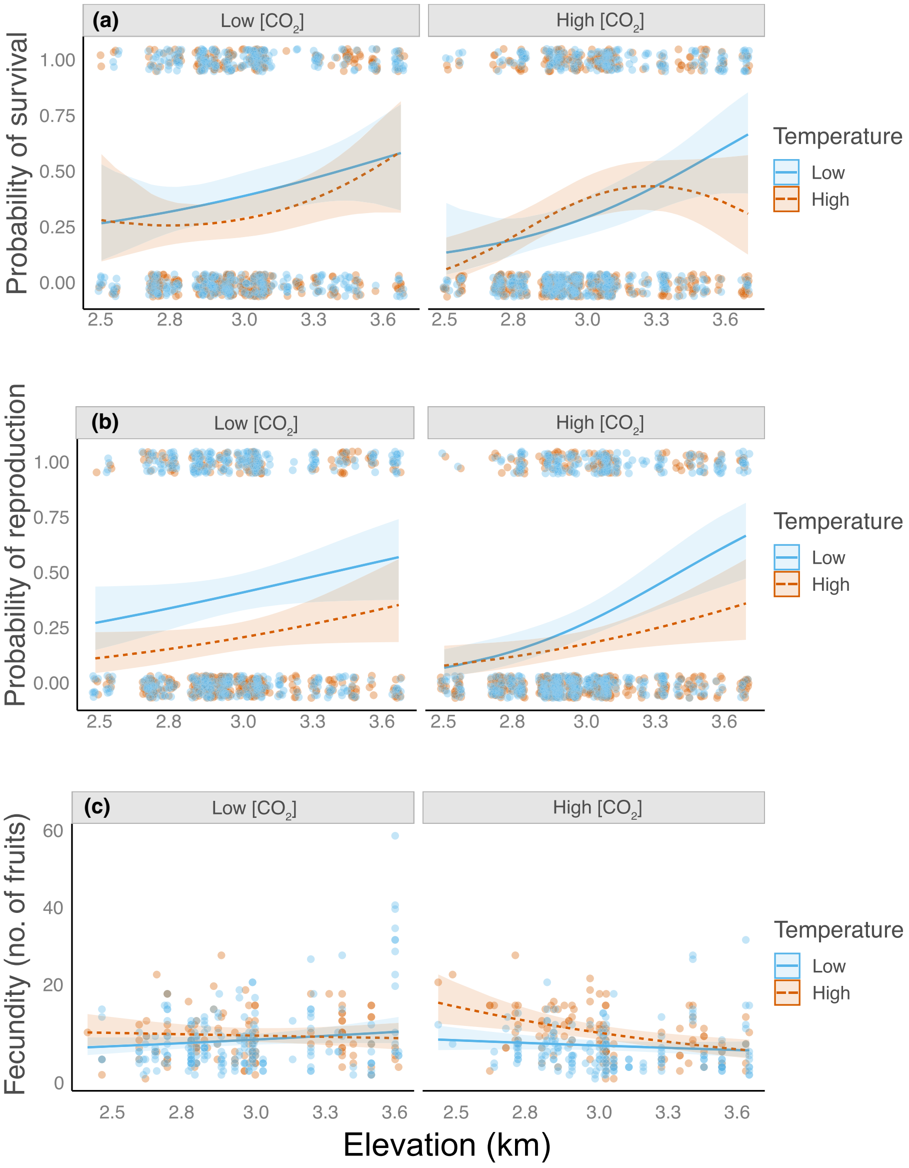

Fig. 5 Fitness varied with treatment in *Boechera stricta* accessions source from across a broad elevational gradient. (a) The probability of survival increased with source elevation (km) under contemporary (low) temperatures in the high [CO$_2$] treatment level. Elevated [CO$_2$] and high temperatures induced a shift towards a lower optimal elevation of 3.3 km. (b) Elevated temperatures depressed the probability of reproduction for all accessions at both [CO$_2$] levels. (c) Low-elevation accessions had elevated fecundity in the combined [CO$_2$] and temperature treatment level, but fecundity did not vary with source elevation in the other treatment combinations. In all panels, high-temperature treatments are indicated in red with dashed line prediction regression relationships and low-temperature treatments are in blue with solid lines. Circles indicate data points. Shading represents the 95% confidence intervals for low (blue) and high (red) temperatures.

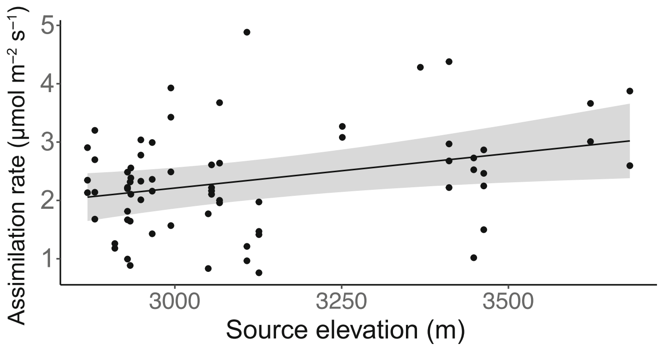

Fig. 6 Photosynthesis (assimilation rate) increased with source elevation (m) in 68 individuals from 22 *Boechera stricta* accessions growing in a common garden at 3133 m. Circles indicate data points. The gray band indicates the 95% confidence interval.

occurs due to higher carbon availability and reduced photorespiration – the carbon-costly process in which Rubisco fixes O_2 rather than CO_2 (Busch & Sage, 2017; Ainsworth & Long, 2021). We found a significant interaction between [CO$_2$] and source elevation on transpiration, which indicates that accessions from across this climatic gradient differentially respond to [CO$_2$] under contemporary conditions. Under elevated [CO$_2$], low-elevation accessions may experience reduced transpiration rates compared with high-elevation accessions. Low transpiration can decrease nutrient uptake (Loladze, 2014) and reduce photosynthesis (Dusenge *et al.*, 2019; Ainsworth & Long, 2021; Gojon *et al.*, 2023). As *B. stricta* is locally adapted to nutrient and water availability (MacTavish & Anderson, 2020), decreased transpiration rates could disproportionately affect nutrient uptake in low-elevation accessions under future [CO$_2$] environments.

The genotype by environment (G × E) interaction for transpiration indicates that *B. stricta*'s ecophysiological response to elevated [CO_2] associated with climate change depends upon the elevation in which the accession evolved. Detecting genotype by environment interactions in gas exchange traits can be difficult, as it requires exposing accessions collected from multiple populations to a variety of environmental conditions. In *Arabidopsis thaliana*, elevated [CO_2] induced greater stomatal density in some accessions from low-elevation sites but reduced stomatal density in some high-elevation accessions (Caldera *et al.*, 2017). Additionally, Premoli & Brewer (2007) found substantial differences in assimilation rates and water use efficiency in low- vs high-elevation accessions of southern beech (*Nothofagus pumillo*) in a common garden experiment and natural field populations. Similarly, assimilation rate and stomatal conductance increased with elevation in a species of European oak (*Quercus petraea*) and beech (*Fagus sylvatica*) in natural settings (Bresson *et al.*, 2011). However, when grown in a common garden, source elevation did not influence ecophysiology, indicating that plasticity likely underlies phenotypic variation in gas exchange across populations of these trees (Bresson *et al.*, 2011). Exposing accessions sourced from multiple locations to a variety of environmental factors will increase realism and enhance our predictive abilities to understand how climate change may disrupt local adaptation. This can provide valuable insight into a species' response to environmental changes, especially as we bridge the gap from genomic and cellular realms to ecosystems and earth modeling (Lasky *et al.*, 2023; Verslues *et al.*, 2023).

Many environmental factors such as temperature, water availability, solar radiation, vapor pressure deficit, and atmospheric pressure change rapidly along elevational gradients and can influence gas exchange (Gale, 1972; Gale, 2004; Körner, 2007a, b, 2021; Smith & Johnson, 2009). Additionally, high-elevation plants often have thicker mesophyll layers and higher nitrogen content than low-elevation populations, which can facilitate greater photosynthesis at higher elevations (Körner & Diemer, 1987; Körner, 2021). In our common garden field experiment, *B. stricta* photosynthetic rates increased with source elevation, concordant with Körner & Diemer's (1987) hypothesis. Nonetheless, our growth chamber experiment did not uncover any effect of source elevation on photosynthesis in any combination of [CO_2] and temperature, indicating environmental variables beyond [CO_2] and temperature contribute to the evolution of photosynthesis rates in nature. Taken together, the field and growth chamber data suggest that high-elevation accessions may have a photosynthetic advantage under specific contemporary conditions, which may not translate to future environments expected under climate change. These results highlight the need to exercise caution in using gas exchange data from contemporary environments to scale to future scenarios. For example, a common practice in modeling the impacts of climate change is to use leaf-level data and scale up to ecosystems (Smith *et al.*, 2019; Denney *et al.*, 2020; Fisher & Koven, 2020). However, neglecting the complexity of accessions from along environmental gradients and their varying responses to future conditions will decrease our accuracy in model climate change in natural systems (Funk *et al.*, 2017).

Fitness components

We found elevated [CO_2] enhanced photosynthesis and water use efficiency, but this increase did not translate to greater fitness in all accessions, as high-elevation accessions showed decreased probability of survival, reproduction, and fecundity. Thus, instantaneous measurements of ecophysiology may not reflect fitness components that manifest over longer periods of time (Kimball *et al.*, 2012; Laughlin *et al.*, 2020). For instance, enhanced photosynthesis and growth under elevated [CO_2] did not increase the reproductive output of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*; Wagner *et al.*, 2008). Indeed, an analysis of 30 yr of free-air CO_2 enrichment studies indicated that elevated [CO_2] and temperature often elicit enhanced photosynthesis but this does not frequently result in greater seed production in crop plants (Ainsworth & Long, 2021). A reduction in fecundity can have dire consequences for local populations, especially those facing contractions due to climate change (Pearson *et al.*, 2014; Valladares *et al.*, 2014).

Spatial constraints in the growth chambers prevented us from including soil moisture as an additional factor in our experiment. In the Rocky Mountains, elevated temperatures have reduced soil moisture availability through declining snowpack and early snowmelt (Rangwala *et al.*, 2012; Pepin *et al.*, 2015; Fyfe *et al.*, 2017). The timing of snowmelt will continue to accelerate under projected climate scenarios (Lute *et al.*, 2022), exposing *B. stricta* and other montane species to heightened drought stress. A glasshouse experiment demonstrated that drought stress depresses fecundity in high-elevation accessions of *B. stricta* (MacTavish & Anderson, 2020). In our growth chamber study, low-elevation accessions had greater fecundity when exposed to increased temperature and [CO_2] than the higher elevation accessions. Under drought conditions, this fitness advantage of low-elevation ecotypes may be further exacerbated. This hypothesis awaits testing in future experiments.

Shifts in the fitness landscape

In our experiment, elevated temperature and [CO_2] shifted the fitness landscape for the probability of survival and fecundity, highlighting the importance of multifactorial climate manipulations. Specifically, the probability of survival increased with source elevation under high [CO_2] and contemporaary temperature, but not in elevated [CO_2] and higher temperatures, in which the optimal survival occurred for accessions from a full 380 m lower in elevation (3293 m) than our highest elevation ecotype (3673 m). Furthermore, the shift in fitness landscape varied across fitness components, with the lowest elevation accessions (2499 m) expressing the highest fecundity in the multifactor climate change scenario (high [CO_2] + high temperature). Thus, it is critical to evaluate fitness components across life history stages (Acasuso-Rivero *et al.*, 2019), especially as plants experience variation in environmental conditions during the course of their life (Kulbaba *et al.*, 2023). Multiple abiotic and biotic agents of selection interact to influence a plant's fitness. (Greenbury *et al.*, 2022). Water (Lee & Mitchell-Olds,

2013) and nutrient availability (MacTavish & Anderson, 2022) as well as snowmelt (Anderson & Wadgymar, 2020) are important drivers of fitness and local adaptation in *B. stricta*. For example, low-elevation accessions have elevated fitness under drought stress in the glasshouse (MacTavish & Anderson, 2022) and advancing snowmelt projected under climate change in the field (Anderson & Wadgymar, 2020). A meta-analysis of locally adapted species along elevation gradients revealed temperature and precipitation are strong drivers of local adaptation (Midolo & Wellstein, 2020), indicating that many systems may experience adaptational lag or local maladaptation as climate change progresses (Wilczek *et al.*, 2014; Kooyers *et al.*, 2019; Anderson & Wadgymar, 2020; Leites & Benito, 2023). Here, elevated [CO_2] and temperature interacted to reduce the fitness of high-elevation accessions, demonstrating that climate change could further disrupt local adaptation. In many systems, increasing temperatures are causing maladaptation, including *T. repens* (Wright *et al.*, 2022), *Clarkia pulchella* (Bontrager & Angert, 2019), and *A. thaliana* (Exposito-Alonso *et al.*, 2019).

Atmospheric [CO_2] is a strong selective agent on plant traits (Ward & Kelly, 2004; Steinger *et al.*, 2007; Leakey & Lau, 2012; Jonas & Cioce, 2019), and increasing levels will differentially affect species based on their carbon capture mechanism (i.e. C3 vs C4 and CAM photosynthesis, Sage, 2020; Verslues *et al.*, 2023). Earlier flowering time in *A. thaliana* may be driven by the interaction of rising temperatures and elevated [CO_2] (Walker 2nd & Ward, 2018), which altered fitness landscapes across life history stages of *B. stricta* in our study. Elevated [CO_2] could also reduce drought tolerance (Medeiros & Ward, 2013; Temme *et al.*, 2019), which can be exacerbated by warming temperatures (Duan *et al.*, 2014; Grossiord *et al.*, 2020). We may be able to improve our predictions for the future by examining plant resposes to lower [CO_2] in historical environments (Gerhart & Ward, 2010). For example, C3 plants evolving during the glacial–interglacial cycles experienced drastic fluctuations in [CO_2] and may already contain variation that can buffer against negative effects of rapidly changing [CO_2] (Beerling, 2005) or facilitate adaptation through plasticity (Gunderson *et al.*, 2010; Andresen *et al.*, 2016). For example, *B. stricta* demonstrates substantial plasticity in stomatal traits (Anderson & Gezon, 2015), which may facilitate adaptation to elevated [CO_2] under drought stress and rising temperatures. Conversely, adaptation to lower [CO_2] in the geological past could constrain adaptations to increasing [CO_2] in contemporary environments (Etterson & Shaw, 2001; Temme *et al.*, 2019).

In this study, low-elevation accessions experienced higher fecundity under elevated [CO_2] and temperatures. Similarly, in five common gardens in field conditions, low-elevation ecotypes outperformed local ecotypes in current climates and under snow removal manipulations, which simulate advancing snowmelt under climate change (Anderson & Wadgymar, 2020). Together, these results highlight that low-elevation ecotypes are locally adapted to hot arid environments with high [CO_2]. This pattern is congruent with the elevational gradient in climate in this system, with hot and arid low-elevation environments becoming cooler and moister at higher elevations (Anderson & Wadgymar, 2020). Furthermore, this result suggests that these low-elevation ecotypes may be the most capable of tolerating warming and aridifying conditions. Nevertheless, a demographic model using data from a reciprocal transplant experiment in *B. stricta* indicates that low- and mid-elevation populations risk rapid decline under decreased snowpack driven by climate change (Anderson & Wadgymar, 2020), likely because climate change is pushing these trailing edge populations outside of their climatic niche. Furthermore, increasing [CO_2] is occurring in conjunction with shifts in aridity, snow dynamics and temperature, all of which influence local adaptation and fitness in *B. stricta* (MacTavish & Anderson, 2020; Hamann *et al.*, 2021). Thus, the interaction of elevated [CO_2], temperature, drought stress, and other environmental stressors may have profound effects on fitness under future climate scenarios even for low-elevation ecotypes that are locally adapted to hot and dry conditions. As such, local adaptation to historical climates may not be sufficient to buffer even these low-elevation ecotypes from reductions in fitness due to climate change.

Conclusions

In this multifactorial experiment, we demonstrate temperature and [CO_2] interact to shape the fitness landscape of *B stricta*. Additionally, instantaneous measurements of ecophysiological performance did not translate to more integrated proxies for fitness, as we found greater [CO_2] did not offset thermal stress in most accessions as expected (Zandalinas & Mittler, 2022; Eckardt *et al.*, 2023). However, in the lowest elevation accessions, the combination of elevated [CO_2] and temperature increased fecundity. By exposing accessions sourced from along an elevational gradient to temperatures and [CO_2] levels relevant to contemporary landscapes, we found the evolutionary history of this species drives the fitness response to changing climates. Our results suggest that climate change will have severe fitness effects on this locally adapted species.

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Competing interests

None declared.

Author contributions

JTA acquired the funding. DAD and JTA designed the experiment. DAD and PP conducted the study. DAD and JTA analyzed the data and wrote the manuscript.

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Data availability

Data and R scripts used in this study are available on GitHub (https://github.com/ddenney1/co2_gc).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Mean recorded $[CO_2]$ and temperatures by treatment across the duration of the growth chamber experiment.

Fig. S2 Monthly mean temperatures from the Crested Butte, CO weather station (May – Oct, 1980–2020).

Table S1 Source populations, elevation, and GPS coordinates for all accessions used in the experiment.

Table S2 Mean recorded temperatures and $[CO_2]$ over the course of the growth chamber experiment.

Table S3 ANOVA tables for photosynthesis from the growth chamber experiment.

Table S4 ANOVA tables for water use efficiency from the growth chamber experiment.

Table S5 ANOVA tables for transpiration from the growth chamber experiment.

Table S6 ANOVA tables for stomatal conductance from the growth chamber experiment.

Table S7 ANOVA tables for fitness components (probability of survival, probability of reproduction, and fecundity).

Table S8 Odds ratios for the probability of survival from the growth chamber experiment.

Table S9 Beta coefficients and 95% confidence intervals for fecundity under different treatment combinations in the growth chamber experiment.