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SYMPOSIUM

Increased Temperature and CO_2 Induce Plasticity and Impose Novel Selection on Plant Traits

Derek A. Denney[*,2] and Jill T. Anderson[†,‡,1]

[*]Department of Plant Biology, University of Georgia, Athens, GA 30602, USA; [†]Department of Genetics, University of Georgia, Athens, GA 30602, USA; [‡]Odum School of Ecology, University of Georgia, Athens, GA 30602, USA

[2]Current Address: Plant Resilience Institute and Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA

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[1]E-mail: jta24@uga.edu

Synopsis Climate change is simultaneously increasing atmospheric carbon dioxide concentrations ($[CO_2]$) and temperatures. We conducted a multi-factorial growth chamber experiment to examine how these climate change factors interact to influence the expression of ecologically relevant morphological and phenological traits, clines in these traits, and natural selection on these traits using diverse accessions of *Boechera stricta* (Brassicaceae) sourced from a broad elevational gradient in Colorado, USA. Plastic shifts in a key allocation trait (root mass fraction) in response to temperature accorded with the direction of selection via the probability of flowering, indicating that plasticity in this trait could be adaptive. However, plasticity in a foliar functional trait (leaf dry matter content) in response to temperature and $[CO_2]$ did not align with the direction of selection, indicating that plasticity could reduce fitness . For another ecologically important phenotype, selection favored resource acquisitive trait values (higher specific leaf area) under elevated $[CO_2]$ and resource conservative trait values (lower specific leaf area) at lower $[CO_2]$, despite the lack of plasticity in this trait. This pattern of selection counters published reports that elevated $[CO_2]$ induces low specific leaf area but could enable plants to reproduce across a greater period of the growing season under increasingly warm climates. Indeed, warmer temperatures prolonged the duration of flowering. This plasticity is likely adaptive, as selection favored increased flowering duration in the higher temperature treatment level. Thus, climate change could impose novel and unanticipated patterns of natural selection on plant traits, and plasticity in these traits can be a maladaptive response to stress.

Introduction

Anthropogenic climate change has exposed natural populations to rapidly changing abiotic stresses (Zandalinaset al. 2024). In response, plants and animals have altered their geographic distributions to remain within their historical climatic niche (Lenoir et al. 2020), shifted the timing of key life history events (Stuble et al. 2021; Wolkovich and Donahue 2021), adapted to novel climatic conditions (Franks et al. 2007), altered their phenotypes plastically (Charmantier et al. 2008; Seebacher et al. 2015; Sgro et al. 2016), and undergone population declines and extinctions (Campbell 2019; Soroye et al. 2020). Field and laboratory experiments can evaluate the causal agents underlying biological responses to climate change, yet many such experiments manipulate only one factor at a time despite the complex and multifaceted nature of climate change. Indeed, the combined effects of multiple environmental stressors on trait expression and evolution remain poorly understood, especially in non-crop systems (Zandalinaset al. 2024).

Atmospheric CO_2 concentration ($[CO_2]$) can limit plant growth (Poorter et al. 2022). Thus, it is not surprising that in benign environments or single-factor manipulations, one primary response of plants with C_3 photosynthesis to increasing $[CO_2]$ is heightened photosynthetic rates, which has been documented in numerous systems (Poorter et al. 2022), including our

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focal species, *Boechera stricta* (Brassicaceae) (Denney et al. 2024). In addition to increased photosynthesis, elevated [CO_2] can also alter leaf morphology and chemistry, plant size, root growth and allocation to aboveground vs. belowground biomass, floral traits, fitness, and phenology (Nie et al. 2013; Poorter et al. 2022). However, despite the fertilizing effects of elevated [CO_2] on C_3 plants, environmental stress can reduce fitness and growth benefits that plants receive from increased [CO_2] alone (Poorter and Pérez-Soba 2001; Ainsworth and Long 2021).

In a study of 28 species of C_3 temperate plant species, Temme et al. (2017) found that elevated [CO_2] was associated with resource-conservative strategies along the leaf economic spectrum, such as reduced specific leaf area (thicker leaves). Plants with decreased specific leaf area have higher construction costs, which correspond with decreased water content and higher leaf dry matter content (Simpson et al. 2016; Delpiano et al. 2020). Furthermore, elevated [CO_2] can increase overall root biomass, suggesting that increasing [CO_2] stimulates belowground growth and may alter resource allocation patterns (Nie et al. 2013; De Kauwe et al. 2014). However, some studies indicate elevated [CO_2] has no effect on root biomass allocation (Dusenge et al. 2020) or decreases carbon allocation to the roots (Shan et al. 2023).

Elevated temperatures typically favor trait values associated with tolerance of thermal stress, such as reduced specific leaf area, increased leaf dry matter content, and increased root hair growth (Gray and Brady 2016). Field common gardens corroborate the prediction for specific leaf area, as accessions from hot and arid low elevation sites display lower specific leaf area and greater integrated water-use efficiency than high elevation accessions (Wadgymar et al. 2017). Leaf dry matter content reflects a plant's resource usage (Díaz et al. 2016) and leaf construction costs across environments (Hodgson et al. 2011; Stanisci et al. 2020). For example, plants growing in a subalpine grassland community in arid environments generally adopt a conservative resource usage strategy, with higher leaf dry matter content (Stanisci et al. 2020), as do plants exposed to elevated temperatures (Zhu et al. 2020). Root mass fraction generally declines with mean annual temperature (Mokany et al. 2006) but may also increase with elevational gradients in alpine systems (Li et al. 2008).

Climate change is inducing accelerated reproduction in spring-flowering plant species globally (CaraDonna et al. 2014; Stuble et al. 2021), but it is challenging to identify which agents of selection drive these shifts or whether these shifts confer a fitness advantage (Campbell 2019; Iler et al. 2021). Many plant species flower earlier when exposed to warmer temperatures (Preston and Fjellheim 2022). In addition, elevated [CO_2] either delays or advances flowering time (Springer and Ward 2007), and a recent meta-analysis found low consistency in results across experiments (Poorter et al. 2022). Trade-offs can shape the evolution of integrated suites of traits (Etterson and Shaw 2001; Keith and Mitchell-Olds 2019; Kooyers et al. 2020) and guide our predictions about climate change responses. We hypothesize that three genetically correlated phenological traits—first flowering time, plant size at flowering, and flowering duration—(Bemmels and Anderson 2019) respond in concert to climate change.

Here, we examined the interactions of two key climate change factors, temperature and [CO_2], on the expression of functional and phenological traits and clines in those traits in the perennial plant, *B. stricta* (Rushworth et al. 2011, 2022). We predict that elevated temperatures will induce trait values similar to those expressed by low elevation maternal lines, which have experienced warmer temperatures than their high elevation counterparts across their evolutionary history. Furthermore, we link trait expression to fitness through phenotypic selection analyses to test whether climate change imposes novel selection and if plasticity could be adaptive, in which case, we would expect selection to accord with the direction of plasticity (Ensing and Eckert 2019). The paucity of multifactorial studies increases the challenge of predicting how temperature and [CO_2] interact to shape trait expression and exert natural selection. Taken together, we expect that increased temperatures and [CO_2] both induce decreased specific leaf area, increased leaf dry matter content, and greater root mass allocations, although we do not have concrete predictions about whether these climatic change factors will affect these traits additively or whether they will interact synergistically.

Owing to the high degree of plasticity in reproductive phenology in *B. stricta*, and longitudinal trends in first flowering time (Anderson et al. 2012) , we predict that increased temperatures and [CO_2] will advance the timing of first flowering, reduce the size at flowering, and increase the duration of flowering. If such changes are adaptive, we expect selection to favor this coordinated suite of traits in the high temperature and high [CO_2] treatment combination relative to the other combinations of environments. To the best of our knowledge, the potential interactive effects of increasing temperature and [CO_2] on size at flowering or the duration of flowering have rarely been evaluated, although elevated [CO_2] can augment size at flowering (Song et al. 2009) and overall plant height and stem diameter (Poorter et al. 2022). We note that our prediction runs counter to this observation that increased [CO_2] can increase plant size (Song et al. 2009; Poorter et al. 2022) because we expect that the genetic correlation of flowering time and size at flowering (Bemmels and Anderson 2019) will constrain the evolution of early flowering large plants.

Methods

System

We tested patterns of plasticity and selection to joint manipulations of temperature and $[CO_2]$ using the perennial forb, *B. stricta* (Graham) Al-Shehbaz (Brassicaceae), which is broadly distributed along elevational and latitudinal gradients in montane ecosystems of western North America (Al-Shehbaz and Windham 2010; Rushworth et al. 2011, 2022). Across the landscape, populations of *B. stricta* have adapted to local abiotic and biotic factors, such as temperature, water availability, and herbivore pressure (Lee and Mitchell-Olds 2012, 2013; Lee et al. 2017; Anderson and Wadgymar 2020; Carley et al. 2021; Jameel et al. 2025; Anderson et al. 2025). This species is primarily self-pollinating (Song et al. 2006) and has limited seed dispersal distances. Longitudinal studies conducted over four decades in natural populations of *B. stricta* have documented that flowering phenology has advanced by 3.7 days/decade (Anderson et al. 2012; CaraDonna et al. 2014; Wadgymar et al. 2018).

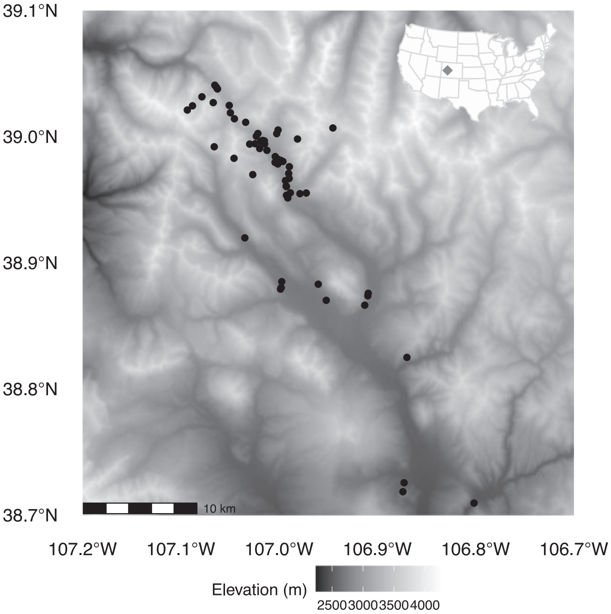

Fig. 1 Source locations for populations used in the growth chamber study. Black circles indicate GPS coordinates of maternal lines. Topographical elevation (m) is depicted in grayscale with white indicating higher elevations. Inset map shows the United States, and the diamond indicates the location of the Rocky Mountain Biological Laboratory, around which these populations were sourced.

Experimental design

We collected seeds from natural populations of *B. stricta* along an elevational gradient near the Rocky Mountain Biological Laboratory in Gothic, Colorado, USA (Fig. 1), where populations at lower elevations experience decreased water availability, longer growing seasons, and elevated temperatures compared to their higher elevation counterparts (Dunne et al. 2003; Anderson 2023; Anderson et al. 2025). To produce the maternal lines for this study and to homogenize maternal effects (Wadgymar et al. 2018), we grew field-collected seeds in the greenhouse for one generation. We exposed 61 maternal lines from disparate populations (source elevation range: 2498–3673 m) to ambient and elevated temperature and $[CO_2]$ (Denney et al. 2024). Our previous analyses of plasticity in physiological variables and fitness components from this experiment revealed that elevated $[CO_2]$ enhanced leaf-level photosynthetic rates by 50%, that both temperature and $[CO_2]$ influenced the expression of intrinsic water-use efficiency, and that $[CO_2]$ interacted with source elevation to shape elevational clines in transpiration (Denney et al. 2024). Furthermore, temperature and $[CO_2]$ interacted with source elevation to shift the fitness landscape, with lower elevation accessions maintaining greater fitness than higher elevation accessions in elevated temperature and $[CO_2]$ (Denney et al. 2024). This temperature pattern was consistent with expectations, as temperature and aridity both decline with elevation in this system (Dunne et al. 2003; Pepin and Lundquist 2008; Anderson and Wadgymar 2020). Here, we extend the initial work of Denney et al. (2024) to examine our hypotheses about plasticity and selection.

Manipulating CO_2 in the field is cost-prohibitive and logistically challenging. As such, we conducted this study in four growth chambers (Conviron BDW40 chambers; Winnipeg, Canada) set for either contemporary or late 21st century temperatures and $[CO_2]$. Growth chambers were configured to a contemporary $[CO_2]$ of 400 ppm and 650 ppm for late century $[CO_2]$. However, airflow within the building constrained the chambers' abilities to scrub CO_2, so plants in the contemporary $[CO_2]$ treatments experienced an average of 465 ppm. The late century concentrations were estimated using the projected concentrations of the Representative Concentration Pathway (RCP) 6.0 (van Vuuren et al. 2011). We calculated monthly average temperatures of the *B. stricta* growing season from May through October 1980 to 2020, as recorded at the Crested Butte, Colorado, USA weather station (USC00051959), which is near several low elevation source populations sampled for this study (Denney et al. 2024). The average maximum and minimum temperatures were used to determine daytime and nighttime growth chamber conditions, respectively. For future conditions, daytime temperatures were set to 4.75°C above the calculated 1980–2020 monthly averages, while nocturnal temperatures were set to 5.5°C above the averages, based on end-of-century projections for the region under the RCP 6.0 climate scenario (Vose et al. 2005; Masson-Delmotte et al. 2021). Limitations to the growth chambers required the minimum nocturnal temperatures be set at 4°C.

On April 4, 2021, we planted 7960 seeds (10–30 seeds/line × 61 maternal lines × 4 treatments) on moistened filter paper in Petri dishes and placed them in growth chambers set for both May treatment temperatures and treatment [CO_2]. Due to low initial germination rates, we conducted a second round of seed planting on 4 September 2021. We accounted for these two planting efforts by including a fixed effect for a cohort in our statistical models. We transplanted 1816 seedlings into containers (Stuewe and Sons, Tangent, OR, USA, RL 10 cu. inch conetainer) with pine bark soil (Sungro Metro-Mix 838; Sun Gro Horticulture, Agawam, MA, USA) when two cotyledons were present.

We simulated a full growing season (May through October) by advancing the treatment temperatures monthly. We set humidity to 55%. Daylength was maintained at 800 μmol s^{-1} for 14 h daily. After 2 weeks of simulated October conditions, we induced a winter vernalization period to promote flowering by decreasing temperatures to a constant 4°C and daylength set to 24 h of 0 μmol s^{-1}, which allowed us to simulate snow cover conditions (Keller and Körner 2003). We placed all blocks into watering troughs filled daily to maintain well-watered conditions. We rotated blocks within each treatment weekly and rotated chambers monthly to avoid block and chamber effects. Prior to vernalization, we applied 20-10-20 fertilizer monthly (JR Peters Jack's Peat Lite; JR Peters Inc., Allentown, PA, USA), and post vernalization, we applied 10–30-20 fertilizer (JR Peters Jack's Blossom Booster; JR Peters Inc.), based on manufacturer recommendations to optimize flowering success. As vernalization is required for flowering, we did not apply this bloom boost fertilizer before vernalization. Additionally, we applied larvicide (Gnatrol WDG; NuFarm, Nufarm Americas Inc., Alsip, IL, USA) monthly to eliminate arthropod herbivores.

We measured phenological and functional traits that influence climatic adaptation in a diversity of systems and mediate responses to temperature, [CO_2], aridity, and other environmental stressors (Wright et al. 2004; Bock et al. 2014; Reich 2014; Iler et al. 2021): the timing of first flowering, plant height at flowering, duration of flowering, specific leaf area, leaf dry matter content, and root mass fraction.

Phenology and fitness

After vernalization, we censused the plants 5–7 days/week to record the timing of first flowering and collect fruits. As each plant flowered, we measured the height of the bolt from the apical meristem to the base of the stem. We continued to census plants daily until the end of the experiment. We considered a plant post-reproductive when all flowers had been (self)pollinated and flowers were no longer present. We calculated the duration of flowering as the number of elapsed days between first reproduction and the first date on which a plant no longer had flowers. To measure fecundity, we counted the total number of mature fruits (siliques) produced, which correlates with the total number of seeds in this system (Wadgymar et al. 2017).

Functional trait measurements

To measure functional traits, we collected leaves from all living plants in June 2022 during July conditions of the second growing season. We collected three recently fully expanded leaves from all plants and recorded fresh weight immediately upon collection. We then scanned the leaves using an Epson Perfection V39 scanner (Epson America Inc., Los Alamitos, CA, USA) with a resolution of 300 dpi, and calculated leaf area using the machine learning software, ilastik, version 1.3.3 (Berg et al. 2019). We trained the model to identify leaf and background and exported binary images distinguishing between the two. We then used a custom Python script to calculate leaf area in cm^2. To calculate leaf dry matter content (LDMC = dry mass/fresh mass) and specific leaf area (SLA = dry mass/leaf area), we dried the leaf collections in a drying oven and weighed them.

At the end of the second growing season (August 2022), we harvested all plants to collect root and shoot tissues. There was no evidence that plants were pot-bound at harvesting. We removed all aboveground tissues from the roots and separated them into organ-specific envelopes to oven dry. Upon drying, we weighed each portion and summed the dry weight. We removed the roots from the soil using a series of wash steps: the first removed bulk soil, the second removed fine particulate matter, and the third removed residual soil. We placed the remaining roots into an envelope, dried them in an oven, weighed biomass, and calculated root mass fraction (dry weight of root biomass/dry weight of total biomass).

Statistical analyses

Phenotypic plasticity and clinal trait variation

To evaluate the magnitude and directionality of plasticity and phenotypic clines, we analyzed trait variation as a function of temperature treatment, [CO_2] treatment, source elevation and all 2- and 3- way interactions, in models that included a covariate for cohort and random effects for maternal line and experimental block. We analyzed each trait separately, owing to their different statistical distributions. We conducted all analyses in R Statistical Software ver. 4.4.1 (R Core Team 2024) using the glmmTMB package ver. 1.1.9 (Brooks et al. 2017) and assessed normality and homoscedasticity of residuals using the DHARMa package ver. 0.4.6 (Hartig 2017). For all models, we assessed the significance of the maternal line and block through likelihood ratio tests comparing models with and without each random effect. To account for inflated type I error rates associated with these multiple tests, we assessed statistical significance using the false discovery rate procedure (FDR) of Benjamini and Hochberg (1995) across all fixed effects (6 traits by 8 fixed effects and interactions), as implemented in the p.adjust function of the R package stats ver. 4.4.1 (R Core Team 2024). We report both the raw and FDR corrected *P*-values. When we found significant interactions of the two treatments, we contrasted estimated marginal means across all four treatment combinations using the emmeans package ver. 1.10.3 (Lenth 2020). This contrast of emmeans was not necessary in cases in which only one treatment significantly influenced trait expression because we only applied two levels of each treatment. We plotted the data with ggplot2 ver. 3.5.1 (Wickham 2016) and visreg 2.7.0 (Breheny and Burchett 2017).

We modeled root mass fraction, specific leaf area, height at flowering, and flowering duration with a gamma distribution and a log link. For flowering time, we used a negative binomial distribution and a log link (nbinom1). Values of leaf dry matter content range from 0 to 1. Such data are often best analyzed using beta regression, which was developed specifically to model proportional data (Douma and Weedon 2019). However, we detected some violations of the assumptions of normality and homoscedasticity of residuals using a beta regression. We then used the model.sel function of the MuMIn package ver. 1.48.4 (Bartón 2022) to contrast models using a Gaussian distribution, a beta distribution, and a gamma distribution for this trait. The top model, as assessed through AICc values, employed a Gaussian distribution. The residuals deviated slightly from homoscedasticity even for this normal distribution, but the deviations were less severe than for the other two models. Furthermore, we highlight that all three models generated qualitatively identical results; therefore, we proceeded with the Gaussian distribution.

In these models, significant effects of treatment would indicate phenotypic plasticity in response to one or both treatments, and interactions of the two treatments would signify that these factors have non-additive effects on trait expression. Significant effects of source elevation would reveal clinal variation in trait values across the elevational gradient, which could reflect evolutionary responses to divergent selection across the landscape (Kooyers et al. 2015). Interactions of source elevation and treatments reflect genotype-by-environment interactions and indicate that the magnitude or direction of the cline depends on the environmental context (Wadgymar et al. 2017; Jameel et al. 2025).

Natural selection

To examine whether elevated temperature and [CO_2] exert selection on phenological and functional traits, we conducted phenotypic selection analyses linking fitness and trait variation. We modeled fitness as a function of temperature treatment, [CO_2], and traits (described below), along with all 2- and 3-way interactions of treatments and traits, a covariate for cohort, and random effects for maternal line and experimental block. We did not include interactions of traits with each other (e.g., specific leaf area × root mass fraction) because we did not have specific hypotheses about correlational selection. We standardized all traits to a mean of 0 and standard deviation of 1 to compare the strength of selection on traits measured with very different scales.

As is common (Brooks et al. 2019), our metric of fecundity (number of fruits) was zero-inflated because only 571 of 1816 individuals successfully fruited during the experiment. Furthermore, the six traits we measured are expressed at different life history stages. For example, we could not measure reproductive phenology on individuals that did not flower or fruit; however, we were able to measure the vegetative traits (specific leaf area, leaf dry matter content, and root mass fraction) on individuals that failed to flower. A zero-inflated model would not allow us to evaluate selection on all traits, as data on reproductive phenology are not available for plants that failed to flower. To analyze these data effectively, we adopted a hurdle model approach, in which we first evaluated the probability of flowering as a function of the three vegetative traits and their interactions with temperature and [CO_2] treatment using a binomial distribution with a logit link in glmmTMB ver. 1.1.9 (Brooks et al. 2017). We then excluded all individuals that failed to flower and modeled fecundity (fruit count) as a function of all six traits and their 2- and 3-way interactions with temperature and [CO_2] treatments using a negative binomial distribution with a log link in glmmTMB ver. 1.1.9 (Brooks et al. 2017). We checked residuals for normality and homoscedasticity with the DHARMa package ver. 0.4.6 (Hartig 2017). If plots of residuals vs. specific traits suggested non-linear

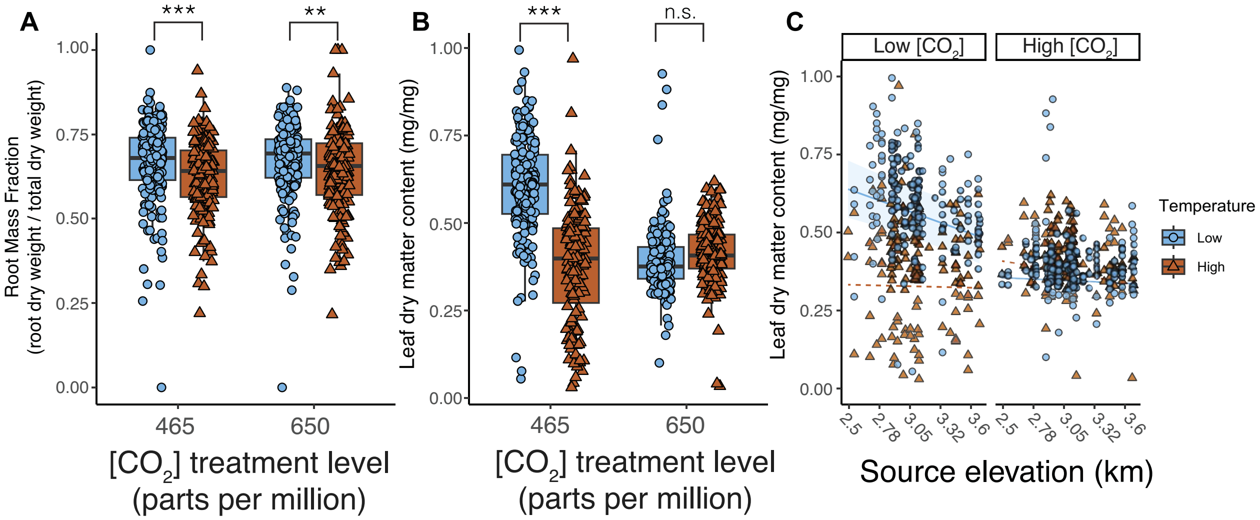

Fig. 2 Plasticity and clines in functional traits. (A) Box plot with jittered data points demonstrating that increased temperatures reduced the root mass fraction across both low and high [CO$_2$]. (B) Box plot displaying the significant interaction of temperature and [CO$_2$] for leaf dry matter content, which was greatest under low temperature and low [CO$_2$] and reduced in all other treatment combinations. (C) A significant cline in leaf dry matter content arose only in the low temperature and low [CO$_2$] treatment combination, in which trait values declined with source elevation. Displayed is the predicted regression line and the 95% confidence interval around that line. We found no evidence of a cline in this trait in any other treatment combination, and we display predicted lines but no 95% confidence intervals solely for comparative purposes. Asterisks indicate significant differences across treatment levels after correction for multiple testing. $***$ $P < .0001$; $**$ $P < .001$; n.s. not significant.

selection, we tested quadratic effects of traits. This second fecundity model includes 11 plants that flowered and died without setting seeds. Our tests revealed no evidence of zero-inflation in this fecundity model.

Since we evaluated selection using two models, we adjusted our alpha to 0.025 (0.05/2 models) to correct for multiple testing. We tested the significance of the random effects of block and maternal line using likelihood ratio tests of analyses with and without each effect. We visualized the regression models using ggeffects ver. 1.7.0 (Lüdecke 2018) and calculated the coefficients of significant trait effects or trait-by-treatment interactions using the emtrends function of the emmeans package ver. 1.10.3 (Lenth 2020).

Significant interactions of treatments with trait values will indicate that selection differs in magnitude or even direction across environments. If selection accords with the direction of plastic trait expression, then we will infer that plasticity may be adaptive (Ensing and Eckert 2019). For example, if increased temperature induces higher values of a given trait, and selection favors individuals with higher values of that trait under elevated temperature, then we can conclude that trait plasticity in response to variation in temperature likely confers a fitness advantage. Similarly, if natural selection aligns with phenotypic clines, then trait values expressed by high elevation maternal lines will have enhanced fitness under cool temperatures, and those expressed by low elevation lines will have a fitness advantage under warm temperatures.

Results

Plasticity and clinal variation in traits

Our analyses revealed evidence for both clines and plasticity in functional and phenological variables (Table S1). Elevated temperatures induced lower root mass fractions ($\chi^2 = 16.08$, d.f. = 1, raw $P < 0.0001$, FDR corrected $P = 0.00048$; Fig. 2A). For leaf dry matter content (LDMC), we found a three-way interaction between source elevation, temperature treatment, and [CO$_2$] treatment ($\chi^2 = 7.68$, d.f. = 1, raw $P = 0.00559$, FDR corrected $P = 0.027$; Fig. 2B and C). This interaction arose from increased LDMC values under low temperatures and low [CO$_2$], compared with the other three treatment combinations (Fig. 2C). Furthermore, in this most benign treatment combination (low temperature and low [CO$_2$]), we found a significantly negative effect of source elevation, such that LDMC declined by 0.035 mg/mg for every one standard deviation increase in source elevation (standard error: 0.0087; 95% CI: −0.052, −0.018). This elevational cline was nonsignificant in the other three treatment combinations, revealing that increasing temperatures and [CO$_2$] alone or in combination could eliminate this cline. We found no evidence for clines or plasticity in specific leaf area (Table S1).

For phenological traits, we found that elevated temperatures prolonged flowering ($\chi^2 = 6.95$, d.f. = 1, raw $P = 0.00837$, FDR corrected $P = 0.0365$; Fig. 3A). Our analyses uncovered a significant geographic cline in

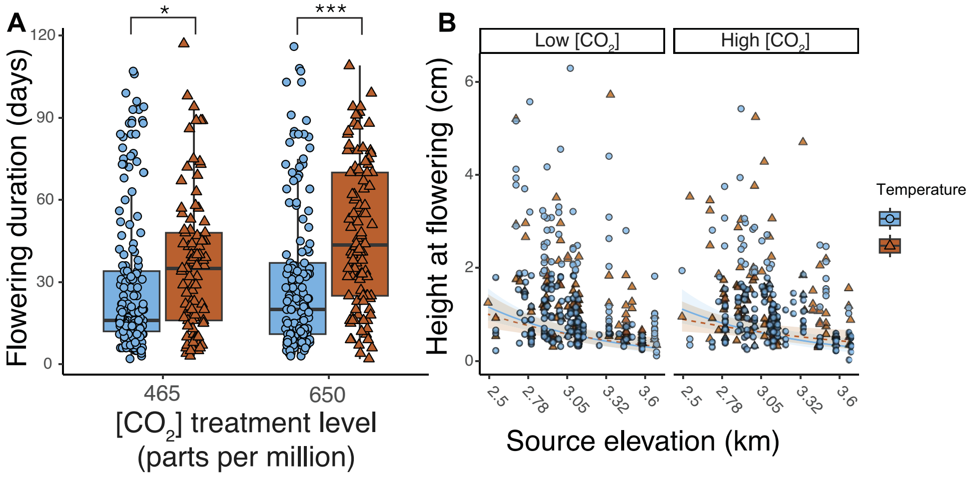

Fig. 3 Plasticity and clines in phenological traits. (A) Box plot with jittered data points showing that elevated temperatures significantly increased the duration of flowering. Asterisks indicate significant differences across treatment levels after correction for multiple testing. $*** P < 0.0001$; $* P < 0.05$; n.s. not significant. (B) Height at flowering declined with source elevation in a similar way across all four treatment combinations.

height at flowering, with height declining significantly with source elevation across all treatment combinations (effect of source elevation: $\chi^2 = 22.3$, d.f. = 1, raw $P < 0.0001$, FDR corrected $P < 0.0001$; Fig. 3B). Finally, plasticity and clines in flowering phenology were only marginal after correction for multiple testing (high [CO$_2$] may delay flowering: $\chi^2 = 5.616$, d.f. = 1, raw $P = 0.0178$, FDR corrected $P = 0.066$; flowering time may decline with source elevation: $\chi^2 = 5.63$, d.f. = 1, raw $P = 0.0176$, FDR corrected $P = 0.066$).

Selection

We found a significant interaction of temperature and root mass fraction on the probability of reproduction ($\chi^2 = 8.42$, d.f. = 1, $P = 0.0037$; Fig 4; Table S2), indicating that selection on this trait varied across temperature treatments. In the high temperature treatment, the odds of reproduction declined by 40.5% for every one standard deviation increase in root mass fraction (odds ratio: 0.595, 95% CI: 0.42, 0.84). We found a non-significant trend toward directional selection for increased root mass fraction under low temperatures (odds ratio: 1.767, 95% CI: 0.098, 3.18).

Additionally, we found evidence for selection on all six reproductive and vegetative traits in our analysis of fecundity amongst individuals that successfully reproduced (Table S3). Furthermore, divergent selection operated on four of the six traits, such that selection favored different trait values in different environments.

Some patterns of selection on vegetative traits did not accord with our analyses of trait expression. For example, despite the lack of plasticity in SLA, we found divergent non-linear selection on SLA in response to [CO$_2$] manipulation (linear SLA × [CO$_2$] treatment: $\chi^2 = 11.03$, d.f. = 1, $P = 0.00090$; quadratic SLA × [CO$_2$] treatment: $\chi^2 = 7.25$, d.f. = 1, $P = 0.007$, Fig. 5A; Table S3). Specifically, stabilizing selection favored high SLA values in the high [CO$_2$] level at both low and high temperatures (optimal SLA under high temperature: 161.8; optimal SLA under low temperature: 163.1; Fig. 5A). Under low [CO$_2$], directional selection favored reduced SLA at low temperature, but we found no relationship between fitness and SLA at high temperature

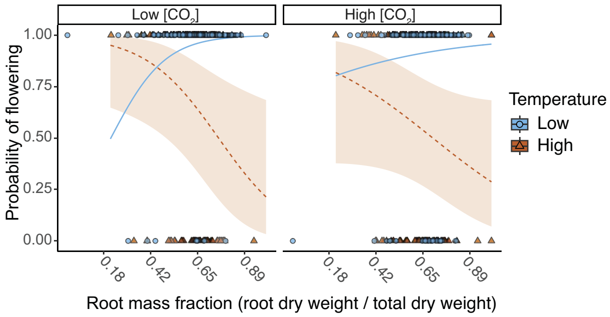

Fig. 4 Selection on root mass fraction for probability of flowering. Directional selection favored lower root mass fractions under elevated temperatures, which is consistent with patterns of plasticity. In the high temperature treatment, the odds of reproduction declined by 40.5% for every one standard deviation increase in root mass fraction. Shown are predicted lines and 95% confidence intervals for statistically significant relationships. We retained the predicted lines for non-significant regression coefficients for ease of comparison, but we do not show the 95% confidence intervals. For analysis, we standardized all traits to a mean of 0 and standard deviation of 1 to facilitate model convergence and comparison of the strength of selection; here, we show the raw data points in the standard units of measurement.

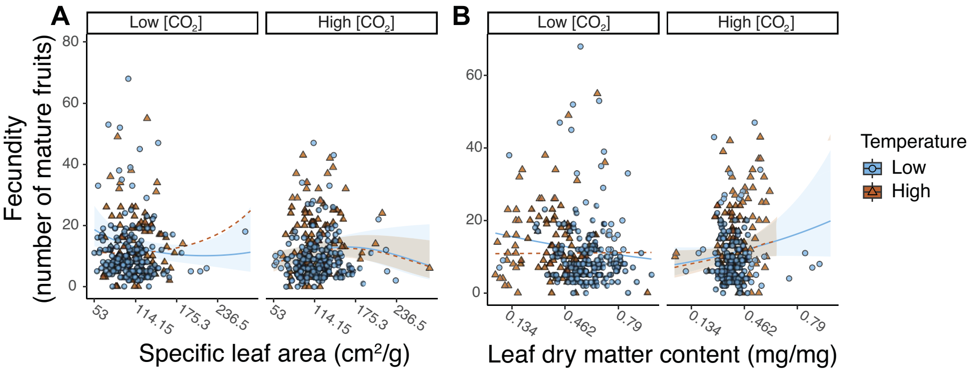

Fig. 5 Divergent selection on foliar traits is not concordant with patterns of plasticity. (A) Stabilizing selection for higher specific leaf area emerged at the high [CO$_2$] level in both temperature treatments. In contrast, directional selection favored reduced specific leaf area in low [CO$_2$] and low temperatures, and no relationship existed between fitness and this trait in the low [CO$_2$] and high temperature environment. Shown are predicted lines and 95% confidence intervals for statistically significant relationships. We retained the predicted lines for non-significant regression coefficients for ease of comparison, but we do not show the 95% confidence intervals. (B) Discordant with patterns of plasticity, directional selection favored increased leaf dry matter content under high [CO$_2$], but there was no relationship between fitness and this trait in low [CO$_2$] environments. For analysis, we standardized all traits to a mean of 0 and standard deviation of 1 to facilitate model convergence and comparison of the strength of selection; here, we show the raw data points in the standard units of measurement.

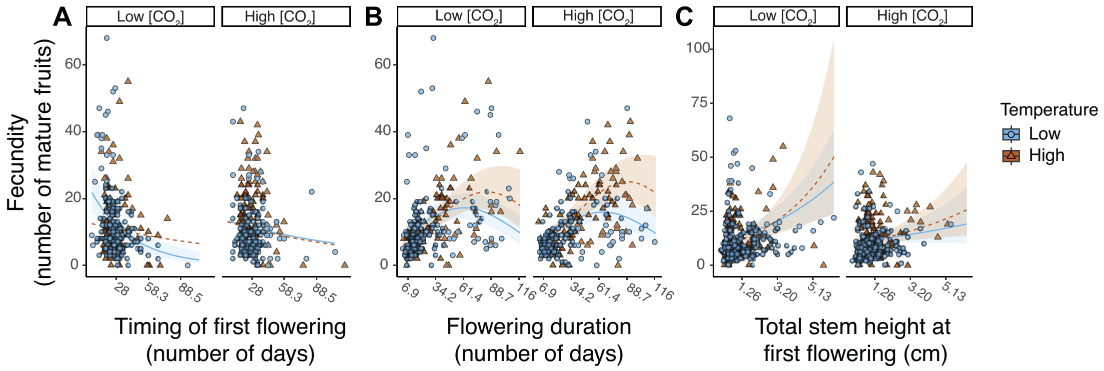

Fig. 6 Selection on the three phenological traits. (A) Directional selection favored earlier flowering in the low temperature and low [CO$_2$] treatment combination, but selection did not operate on flowering phenology in the other treatment combinations. (B) Stabilizing selection favored longer flowering in warmer relative to colder temperatures, irrespective of [CO$_2$] level. (C) Directional selection for increased height at flowering was similar across all treatment combinations. For analysis, we standardized all traits to a mean of 0 and standard deviation of 1 to facilitate model convergence and comparison of the strength of selection; here, we show the raw data points in the standard units of measurement.

(Fig. 5A). Selection operated in the opposite direction as plasticity on LDMC, suggesting that plasticity may be maladaptive. Directional selection favored increased LDMC under high [CO$_2$], but there was no relationship between fitness and this trait in low [CO$_2$] environments (leaf dry matter content × [CO$_2$]: $\chi^2 = 5.72$, d.f. $= 1$, $P = 0.016$, Fig. 5B; Table S3).

Selection operated on all three phenological traits. We found significant interactions of the timing of first flowering and both treatments (flowering time × temperature: $\chi^2 = 5.77$, d.f. $= 1$, $P = 0.016$; flowering time × [CO$_2$] : $\chi^2 = 6.56$, d.f.=1, $P = 0.010$, Fig. 6A; Table S3). These interactions emerged owing to strong directional selection for earlier flowering in the low temperature and low [CO$_2$] treatment combination, and no evidence for selection in any of the other treatment combinations (Fig. 6A). Selection on the duration of flowering differed across temperature treatments (duration × temperature: $\chi^2 = 11.42$, d.f. $= 1$, $P < 0.0001$; Table S3) in a non-linear fashion (quadratic effect of flowering duration: $\chi^2 = 27.64$, d.f. $= 1$, $P < 0.0001$), such that stabilizing selection favored longer flowering in warmer relative to colder temperatures (Fig. 6B). Finally, across all treatment levels, selection favored increased height at flowering ($\chi^2 = 24.0$, d.f. $=$ 1, $P < 0.0001$, Fig. 6C; Table S3).

Discussion

Our joint manipulation of temperature and [CO_2] generated results that supported some of our predictions while refuting others. Temperature and [CO_2] either interacted or operated singly to impose selection on five of six traits. Furthermore, we found significant plasticity in response to these manipulations. Surprisingly, we found no evidence that these factors altered first flowering time or height at flowering plastically. Additionally, selection only favored early flowering under the most benign treatment combination, and selection favored larger size at flowering across all treatment combinations. Collectively, these results suggest that temperature and [CO_2] could play a limited role in the expression of the timing of flowering and height at flowering and that other environmental factors that vary across the landscape likely underlie existing population-level divergence in these traits. In contrast, higher temperatures induced longer flowering duration. This plasticity was concordant with patterns of selection, suggesting that adaptive plasticity could enable diverse accessions to respond to increasing temperatures. We expected temperature and [CO_2] to decrease specific leaf area. Instead, we found no evidence for plasticity in this trait, and divergent selection opposed predictions, favoring higher SLA values in high [CO_2] treatment levels. Selection via the probability of reproduction revealed evidence consistent with adaptive plasticity in root mass fraction. Increased temperatures decreased root mass fraction, which accords with the direction of selection for lower root mass fraction via probability of reproduction. Finally, we found no evidence of adaptive plasticity in leaf dry matter content in either of our fitness metrics. Ultimately, these results indicate that temperature and [CO_2] shape the expression of some phenological and functional traits and exert strong selection, and that climate change could alter the evolutionary dynamics of coordinated suites of traits in ways we have not been able to identify in field studies.

Functional traits

Despite strong predictions that elevated temperature and [CO_2] would reduce specific leaf area (SLA) (Dong et al. 2020; Poorter et al. 2022), we found no evidence for plasticity in this trait in response to these climatic factors. Furthermore, our study did not reveal the elevational cline in SLA that has been documented in field common gardens (Wadgymar et al. 2017). Nevertheless, divergent selection operated on the trait (Fig. 5A), suggesting that climate change factors can impose novel selection even without immediate plastic changes in trait values. However, discordant with predictions, directional selection favored reduced SLA only in the most benign treatment combination (low temperature and low [CO_2]), and stabilizing selection favored higher trait values in both temperature treatments under high [CO_2]. This surprising result suggests that climate change shifts fitness landscapes in unexpected directions, imposing novel selection on natural populations. Greater SLA is often associated with a resource-acquisitive strategy, as these leaves typically have heightened photosynthetic rates (Wright et al. 2004; Shipley 2006; Reich 2014; Onoda et al. 2017). Indeed, in a previous analysis of physiological traits from this study, we found that elevated [CO_2] increased photosynthesis on a per leaf area basis (Denney et al. 2024). Selection for increased SLA could potentially enable more rapid development and even longer flowering under elevated [CO_2] conditions. However, selection on SLA could also be explained by selection favoring altered allocation of carbon toward non-structural carbohydrates, which may play a role in buffering against the negative effects of increased temperatures expected from climate change (Du et al. 2020). Intriguingly, in a field experiment in a natural *B. stricta* population, early snowmelt achieved via snow removal induced higher SLA, which countered predictions for drought-induced reductions in this trait under climate change (Anderson and Gezon 2015). However, that result aligns with pronounced accelerations in the timing of first flowering under snow removal (Anderson and Gezon 2015). We suggest that climate change could favor changes in coordinate]\ suites of traits, such as higher specific leaf area and earlier flowering, altering eco-evolutionary dynamics in ways that could be challenging to predict if we consider trait expression and evolution separately.

We predicted that temperature and [CO_2] would both induce greater leaf dry matter content (LDMC) (Gray and Brady 2016). Instead, we found the highest LDMC levels in the most benign treatment combination (low temperature and low [CO_2]). Furthermore, an elevational cline emerged only in this benign environment; this cline accorded with predictions (Stanisci et al. 2020) that LDMC would decline with source elevation, such that accessions from hot and dry, low elevation locations would have the highest values. This cline disappeared entirely under all other treatment combinations, revealing significant genotype-by-environment interactions for elevational clines. Our result suggests that climate change factors could alter conditions so extensively that they eliminate clines that may have evolved in response to environmental variation across the landscape. Interestingly, the direction of selection accords with expectations that elevated [CO_2] could augment LDMC (Poorter et al. 2022), as directional selection in this experiment favored increased LDMC in elevated [CO_2]. It is clear that the direction of plasticity is incongruent with the direction of selection. Thus, we infer that plasticity in LDMC in our experiment is maladaptive and could reduce fitness under elevated [CO_2].

Contrary to expectations but in line with some studies (Li et al. 2008; Dusenge et al. 2020), root mass fraction declined at hotter temperatures, and we found no effect of [CO_2] on this trait. Selection via the probability of reproduction favored decreased root mass fraction under high temperatures, which is consistent with the direction of plasticity. However, we found no evidence for selection on this trait across any [CO_2] or temperature treatment for fecundity. We postulate that the shift to lower root mass fractions under hotter temperatures is an adaptive response to temperature stress in this system.

Reproductive phenology

We found marginal evidence that elevated [CO_2] may delay flowering in this system, which could accord with delays in other species (Springer and Ward 2007), including *Arabidopsis thaliana* (Springer et al. 2008). However, this marginal delay is inconsistent with significant accelerations in the timing of reproduction in natural *B. stricta* populations in Colorado since the early 1970s (Anderson et al. 2012; CaraDonna et al. 2014; Wadgymar et al. 2018), and in other spring-flowering species globally (Dorji et al. 2013; Inouye and Wielgolaski 2024; Ma et al. 2025). Our results suggest that neither [CO_2] nor temperature drives the longitudinal pattern for advancing flowering time, even though increased temperatures can hasten flowering in other plant systems (Dorji et al. 2020; Preston and Fjellheim 2022). Instead, we hypothesize that reductions in snowpack and concomitant early snowmelt (Fyfe et al. 2017) are the primary environmental drivers of this longitudinal pattern in *B. stricta* (Anderson and Gezon 2015). Indeed, Bjorkman et al. (2015) found snowmelt timing influenced flowering phenology to a greater extent than temperature in four common species in the Alexandra Fiord lowlands of Canada. As such, snowpack availability in high elevation herbaceous systems may be a stronger driver of phenological shifts than temperature (Inouye and Wielgolaski 2024).

We found a marginally significant geographic cline in flowering phenology consistent with genetic clines documented in field common garden experiments, wherein high-elevation accessions flower early compared with their lower-elevation counterparts (Wadgymar et al. 2017; Jameel et al. 2025), suggesting that this experiment replicated key findings from the field. We expected selection for early reproduction to be strongest in conditions reflecting climate change projections, owing to long-term trends toward earlier flowering in recent decades (Anderson et al. 2012; CaraDonna et al. 2014; Wadgymar et al. 2018). Contrary to this prediction, however, directional selection favored early flowering only in the low temperature and low [CO_2] treatment combination, indicating that increasing temperatures and [CO_2] could weaken selection on flowering phenology.

Height at flowering declined with source elevation, consistent with findings from field experiments (Wadgymar et al. 2017). Owing to the strong genetic correlation of the timing of flowering and size at flowering (Bemmels and Anderson 2019), we anticipated that any temperature or [CO_2]-mediated advancements in phenology would be accompanied by a reduction in the height at flowering. Since we found that elevated [CO_2] could delay flowering, that initial prediction would be reversed. Instead, we found no effect of either treatment on height at flowering. We note that this result runs counter to the finding that elevated [CO_2] often stimulates plant growth (Song et al. 2009; Poorter et al. 2022). We found no evidence for divergent selection on height at flowering. Instead, directional selection favored taller plants at flowering across all treatment combinations. Thus, temperature and [CO_2] are likely not the primary agents of selection operating on plant size at flowering in this system. We postulate that the elevational cline evolved in response to variable growing season length across the elevational gradient that was not captured by the manipulations we performed here.

Despite intensive investigations into climate-change-mediated shifts in the timing of first flowering, few studies have evaluated the consequences of climate change for the duration of reproduction (Bock et al. 2014) even though this trait can be subject to strong selection (Jameel et al. 2025). In 232 plant species from the island of Guernsey in the English Channel, climate change shortened the duration of flowering at nearly twice the rate that it accelerated flowering from 1985 to 2011, averaged across species (Bock et al. 2014). In contrast, for 68 herbaceous and woody species from a diversity of ecosystems in the Ozarks (Missouri, USA), climate change over a 168 year period increased the duration of flowering (Austin et al. 2024). These impressive long-term studies focused on species-level variation in flowering duration and were unable to examine individual-level responses to climate change. Thus, much remains to be resolved about the effects of climate change on flowering duration.

In our experiment, elevated temperatures increased the duration of flowering. We infer that this plasticity is adaptive (Ensing and Eckert 2019), as natural selection favored longer flowering under higher temperatures. Based on genetic correlations amongst reproductive phenology traits in field common gardens

(Bemmels and Anderson 2019), along with longitudinal trends in first flowering time (Anderson et al. 2012; CaraDonna et al. 2014; Wadgymar et al. 2018), we predicted that elevated temperature and [CO_2] would induce a coordinated shift toward earlier reproduction, reduced size at flowering, and prolonged flowering. As only the prediction for flowering duration held, we suspect that genetic trade-offs might not constrain the potential responses of *B. stricta* to increasing temperature. Furthermore, our results are concordant with genetic clines in common gardens, where accessions from hot, dry, low-elevation locations flower for the longest period of time (Anderson and Gezon 2015).

Conclusions and future directions

Our study exposed high elevation accessions to temperatures that exceed what they likely experience in their home sites and challenged all genotypes to [CO_2] levels that are higher than current values. These conditions reflect conditions projected for the region under various climate change scenarios (Masson-Delmotte et al. 2021; Anderson et al. 2025) and enabled us to isolate the effects of temperature and [CO_2] on trait expression and selection, which is challenging to accomplish in the field. Phenotypic plasticity could enable populations to persist through environmental change (Nicotra et al. 2010; Walter et al. 2023). Indeed, we found extensive plasticity in response to our manipulated conditions. Previous efforts have found similar levels of plasticity as we document in this study in specific leaf area, flowering phenology, size at flowering, and flowering duration in response to spatiotemporal dynamics across natural populations and common garden experiments; however, plant height at flowering is typically larger in the field than it was in this experiment (Anderson and Gezon 2015; 2025; Wadgymar et al. 2017, 2018). Additionally, field experiments have detected strong signatures of selection on these traits in common gardens at different elevations (Wadgymar et al. 2017; Bemmels and Anderson 2019).

Our current experiment demonstrates that adaptive plasticity in response to temperature variation can shape the evolution of this trait and might contribute to rapid responses to climate change as natural populations are exposed to increasing temperatures. Nevertheless, natural populations simultaneously experience a complex agents of selection, such as snow dynamics, nutrient availability, herbivory and drought stress. A greenhouse experiment manipulated nutrient and water availability in *B. stricta* and revealed low nutrient availability did not influence either the probability of reproduction or fecundity, whereas drought stress played a larger role in shaping adaptation in these populations (MacTavish and Anderson 2020, 2022). Additionally, a field common garden experiment revealed an interactive effect of drought stress and herbivory on patterns of selection (Jameel et al. 2025), while water availability through snowpack dynamics has shaped local adaptation in this system (Anderson and Wadgymar 2020). Thus, elevated temperatures and [CO_2] may interact with other agents of selection, such as nutrient availability, herbivory, and snowpack, to further drive selection in *B. stricta*. Additionally, studies on other montane forbs have found temperature and water availability elicit species-specific plastic responses for functional traits such as root growth and specific leaf area (Visakorpi et al. 2023; Vollenweider et al. 2023), but these responses may be insufficient for adaptation under climate change (Visakorpi et al. 2023), further highlighting our need to study selection mediated by elevated [CO_2] and temperature in alpine plant systems.

Our study revealed that climate change could alter multi-trait selection in surprising and unexpected ways and that plastic trait shifts under novel climates could reflect maladaptive responses to stress that could depress fitness instead of enhancing it. Our work highlights that clinal variation that has evolved in response to existing environmental gradients can be used to generate robust predictions about trait expression and selection, but that multitrait plasticity in response to elevated temperatures and [CO_2] does not always align with these expectations. Furthermore, climate change factors can alter patterns of natural selection even if trait expression does not immediately respond via plasticity, as we saw for specific leaf area. Multifactorial manipulations provide valuable insights into realistic biological responses to climate change, which is inherently multifaceted (Poorter and Pérez-Soba 2001; Masson-Delmotte et al. 2021; Powers et al. 2022; Renziehausen et al. 2024; Zandalinaset al. 2024). We encourage future work manipulating temperature and [CO_2] variables in tandem with other factors that are changing rapidly, such as drought stress, snowmelt timing, herbivore interactions, and nutrient deposition, among others, to examine the extent to which climate change could disrupt plant form and function, whether local populations have sufficient genetic variation to adapt to these changes, and how to best conserve biodiversity on a changing planet.

Author contributions

J.T.A. and D.A.D. acquired funding for the project and designed the experiment. D.A.D. executed the experiment. J.T.A. and D.A.D. analyzed data, produced figures, and wrote and edited the manuscript.

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Supplementary data

Supplementary data available at *ICB* online.

Data availability

Data and code are available at https://github.com/ddenney1/CO2_plasticity_selection.

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