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Phenotypic plasticity and selection on leaf traits in response to snowmelt timing and summer precipitation

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Summary

- Vegetative traits of plants can respond directly to changes in the environment, such as those occurring under climate change. That phenotypic plasticity could be adaptive, maladaptive, or neutral.
- We manipulated the timing of spring snowmelt and amount of summer precipitation in factorial combination and examined responses of specific leaf area (SLA), trichome density, leaf water content (LWC), photosynthetic rate, stomatal conductance, and intrinsic water-use efficiency (iWUE) in the subalpine herb *Ipomopsis aggregata*. The experiment was repeated in three years differing in natural timing of snowmelt. To examine natural selection, we used survival, relative growth rate, and flowering as fitness indices.

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- A 50% reduction in summer precipitation reduced stomatal conductance and iWUE, and doubled precipitation increased LWC. Combining natural and experimental variation, earlier snowmelt reduced soil moisture, photosynthetic rate and stomatal conductance, and increased trichome density and iWUE. Precipitation reduction reversed the mortality selection favoring high stomatal conductance under normal and doubled precipitation, and higher LWC improved growth.
- Earlier snowmelt is a strong signal of climate change and can change expression of leaf morphology and gas exchange traits, just as reduced precipitation can. Stomatal conductance and SLA showed adaptive plasticity under some conditions.

Key words: adaptive plasticity, *Ipomopsis aggregata*, leaf traits, phenotypic plasticity, precipitation, snowmelt timing, stomatal conductance, water-use efficiency.

Introduction

Anthropogenic climate change has the potential to influence traits of organisms through phenotypic plasticity (Charmantier et al., 2008; Nicotra et al., 2010). Many vegetative and physiological traits of plants correlate with and respond to changes in the environment, including temperature and water stress (review in Poorter et al., 2009). For example, plants tend to reduce stomatal opening under drought conditions, but photosynthetic rate may be less affected, resulting in an increase in intrinsic water-use efficiency (iWUE), the ratio of photosynthetic rate to stomatal conductance (Cowan, 1978; Oren et al., 1999). Plasticity in stomatal conductance and iWUE can improve fitness because high iWUE may limit growth and thus be costly when water is not limiting (Arntz & Delph, 2001). Specific leaf area (SLA), the ratio of leaf area to dry mass, is often low in dry environments, which reduces surface area and conserves water, at the cost of reducing light interception (Poorter et al., 2009). In addition, leaf trichomes that act as reflective hairs can shield leaves from high temperatures that reduce net photosynthesis, at the cost of reducing light absorbance (Ehleringer & Mooney, 1978). As a result, we might expect these traits to show phenotypic plasticity in response to the warming or changes in precipitation associated with climate change (IPCC, 2014).

Phenotypic plasticity occurs when a genotype produces different phenotypes under differing environmental conditions (Bradshaw, 1965). Field manipulations of climatic factors can provide compelling evidence for phenotypic plasticity associated with climate change, and have been used to demonstrate responses of water-use efficiency and SLA to growing season precipitation (e.g., Campbell & Wendlandt, 2013; Pratt & Mooney, 2013; Song et al., 2016; Welker et al., 1993). Field manipulations of snowmelt timing in the spring are by contrast rare, although one study showed advanced snowmelt increased stomatal size and SLA but had no detected effect on leaf water content (LWC) or stomatal density (Anderson & Gezon, 2015), and another study showed a negative effect on SLA (Rosbakh et al., 2017).

Phenotypic plasticity can assist persistence of plant populations *in situ* in the face of climate change if the plasticity is adaptive (Chevin & Lande, 2010). In principle, plant traits can show adaptive, maladaptive, or neutral plasticity, as illustrated for physiological traits in *Lobelia siphilitica* (Caruso et al., 2006). For adaptive plasticity to occur there must be natural selection on the trait (Merilä & Hendry, 2014), and that selection has to be concordant with the plastic change. For example, if a genotype has higher stomatal conductance in a wet vs. dry environment, and fitness increases with higher stomatal conductance in that wet environment, that plasticity would be adaptive (Caruso et al., 2006).

Natural selection that changes with the environment can also allow a population to persist in the face of climate change in the absence of plasticity. Provided there is heritable variation in a trait, it can evolve in response to a change in selection under climate change, and thereby increase absolute fitness, a process termed evolutionary rescue (Gomulkiewicz & Shaw, 2013). Natural selection for some vegetative traits has been demonstrated, including on SLA and water-use efficiency (Agrawal et al., 2008; Dudley, 1996; Kimball et al., 2013), but these selection measurements have rarely been tied to realistic changes in the environment under climate change (Wadgymar et al., 2017). As a result, it is unknown whether the evolutionary response to climate change is sufficiently rapid to outpace direct negative impacts of climate change on survival and reproduction (Gomulkiewicz & Holt, 1995; Jump & Penuelas, 2005).

Here we examined plastic changes in leaf morphological and physiological traits as well as natural selection for plants at high elevation in the Colorado Rocky Mountains. In many mountain ecosystems, including our region, warming temperatures are leading to earlier snowmelt in the spring (Klein et al., 2016; Pederson et al., 2011). Whereas many studies of snowmelt timing focus on shifts in flowering phenology (CaraDonna et al., 2014), snowmelt timing could also influence plant traits through effects on soil moisture. Earlier snowmelt reduces soil moisture during the early part of the summer at high elevation (Blankinship et al., 2014) and lengthens the period before the onset of the summer monsoon rains in the Rockies, the foresummer drought (Sloat et al., 2015). Increasing the strength of that foresummer drought increases water stress and lowers net ecosystem productivity in this area (Sloat et al., 2015). In the region where we worked, global climate change models predict a shift in atmospheric circulation patterns that will alter summer precipitation and lead to a decline in the difference between precipitation and evapotranspiration over the next few decades (Seager et al., 2012). Therefore, plants in subalpine and alpine ecosystems in this region could experience increased drought due to changes in both snowmelt and summer precipitation. Such changes in water availability could lead to plastic changes in vegetative traits as well as changes in the strength and direction of natural selection.

We manipulated snowmelt timing and summer precipitation in factorial combination for the subalpine herb *Ipomopsis aggregata* ssp. *aggregata* (Polemoniaceae) and measured responses of leaf morphology and physiology over time as well as effects of the traits on fitness. We focused on traits that have shown an association with climatic change factors in previous experimental studies (Anderson & Gezon, 2015; Campbell & Wendlandt, 2013; Pratt & Mooney, 2013). In *I. aggregata*, iWUE, SLA, and trichome density vary across an elevational gradient representing different environmental conditions (Campbell et al., 2018; Wu & Campbell, 2007). In addition, experimental addition of summer precipitation decreased iWUE (Campbell & Wendlandt, 2013). However, how earlier snowmelt impacts these traits is unknown, as is the strength of natural selection on those traits. We asked the following questions: 1. How does snowmelt timing in the spring and decreases or increases in summer precipitation influence iWUE, SLA, LWC, and trichome density? 2. Do the effects of manipulating snowmelt timing and summer precipitation interact, and can increased summer precipitation compensate for impacts of early snowmelt on trait values? 3. To what extent are effects on these leaf traits driven by changes in soil moisture? 4. Does natural selection on leaf traits vary with changes to snowmelt and precipitation? We used survival, growth, and flowering as indices of fitness. 5. Are plastic changes to different environmental conditions adaptive, as indicated by concordant changes in the trait and fitness, maladaptive, or neutral?

Materials and Methods

Study system

Ipomopsis aggregata ssp. *aggregata* (Pursh) V. Grant is an herb that is widespread across montane to subalpine regions of the western United States (Grant & Wilken, 1986) where it occurs in dry open meadows. Our study site was at Maxfield Meadow, a dry subalpine meadow 1.0 km south of the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA at 38.9495°N, 106.9908°W and 2880 m above sea level in the West Elk Mountains. In this region, *I. aggregata* plants emerge as a seedling in the spring and spend 2 to 10+ years as a vegetative rosette of leaves, after which they put up a flowering stalk, flower during a single season, and die, with only rare cases of iteroparity (Campbell, 1997).

Previous studies have revealed some impacts of summer water availability on vegetative traits of this plant species. In a field experiment, doubling summer precipitation decreased iWUE by 28% but had little effect on SLA (after 11 - 25 days, Campbell & Wendlandt, 2013). Both photosynthetic rate and stomatal conductance declined with decreasing soil moisture below a volumetric water content of 17% in an experiment with potted plants in a screenhouse (Campbell et al., 2010). Moreover, iWUE was highest in the driest of three field environments tested based on relative humidity (Wu & Campbell, 2007). Across an elevational gradient of 12 populations in a natural hybrid zone with *I. tenuituba*, SLA was higher at low elevations, and in this case the lowest two populations had the highest water availability (Campbell et al., 2018). Leaf trichomes were densest in the high elevation populations, which had relatively low relative humidity during the summer (Campbell et al., 2018). That spatial variation in SLA and trichomes could represent plasticity or genetic differences.

Impacts of snowmelt timing have not yet been tested experimentally in *I. aggregata*, nor has natural selection on these traits. Long-term observational data, however, suggested the hypothesis that timing of snowmelt might also influence vegetative traits. In a 25-year study, leaves grew more in years with later snowmelt, and plants were more likely to survive to the next growing season (Campbell 2019).

Snowmelt and precipitation manipulations

To simulate two aspects of future climate change that affect water availability and its timing throughout the snow-free growing season, we established a split-plot experiment. Detailed methods for these manipulations and measurements of precipitation and soil moisture are provided in Powers et al. (2021) and summarized in Methods S1.

Snowmelt timing was manipulated at the whole plot level by applying black shade cloth to three 7 m x 7 m plots to accelerate snowmelt, and leaving three other plots unmanipulated (map in Methods

S1). Each whole plot was split into four 2 m x 2 m subplots with different levels of summer precipitation (200% of normal water addition, 50% reduction with a rainout shelter, mock rainout with only the shelter structure, or unmanipulated control). Traits were measured over three years, 2018 - 2020. Snowmelt in control plots occurred on day 119 in 2018, 158 in 2019, and 126 in 2020 (on average, Fig. S1). The snowmelt manipulation accelerated the date of snowmelt, making it earlier by 3 - 11 days (6 days on average).

Trait measurements

Physiological traits

Three physiological traits (photosynthetic rate, stomatal conductance, and iWUE) were obtained from leaf-level gas exchange measurements on vegetative (non-flowering) plants on 5 - 8 days each year (Fig. S1) for a total of 327 measurements of 275 unique plants. Measurements were taken between 33 and 94 days after the average unmanipulated snowmelt plot melted (Fig. S1). Each day we took measurements from subplots in random order and used the longest leaf on one haphazardly selected rosette per subplot that had not been previously measured that year and had a leaf longer than 25 mm. Two consecutive measurements were recorded per leaf and averaged. Leaf gas exchange was measured using a LI-COR 6400 XT Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA). All leaf gas exchange measurements were taken between 08:00 to 12:00 with saturating light conditions (PAR = 1800 μmol m^{-2} s^{-1}), a leaf temperature of 27 °C, and a sample CO_2 concentration of 400 ppm, following previous studies with this species that have used these conditions, although with increases in CO_2 over the years to reflect changes with carbon emissions (Campbell et al., 2005; Wu and Campbell 2007; Campbell et al., 2010; Campbell and Wendlant 2013). With this method, the among-measurement variability in vapor pressure deficit is very small compared to its natural variability among sites (Wu and Campbell 2007). Gas fluxes were calculated by dividing by the leaf area inside the leaf chamber, measured in ImageJ (National Institute of Health, Bethesda, Maryland, USA). Intrinsic water-use efficiency (iWUE, μmol CO_2 mol^{-1} H_2O) was calculated by dividing light-saturated photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}) by light-saturated stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}). Soil moisture as volumetric water content (%VWC) was measured near each plant in 2019 and 2020 using a 12-cm probe (Campbell Scientific, Edmonton, Alberta, Canada).

Morphological traits

Three morphological leaf traits (specific leaf area, leaf water content, and trichome density) were measured during two sampling rounds per year (Fig. S1) for a total of 600 leaves from 264 plants. Each year early in the growing season, we collected one leaf per plant in each subplot from four randomly selected plants that had leaves at least 30 mm in length. These plants were sampled again 39 - 64 days later to separate overwinter plasticity from effects of summer precipitation. If a plant from the first round was not found, another plant from the same subplot was selected. During the first round of sampling in 2019 we also collected leaves from plants that were sampled in 2018 if they survived and could be located. Leaves were weighed for wet mass, dried at 70 °C for 2 hr, and weighed for dry mass. Before drying, leaves were scanned in grayscale at resolution of 236 pixels cm^{-1}. Trichomes (leaf hairs) were counted from the scan at 200% power and leaf area was measured in ImageJ. We calculated trichome density (cm^{-2}) as trichomes / leaf area, specific leaf area (SLA, cm^2 g^{-1}) as leaf area / dry mass and leaf water content (LWC, g H_2O g^{-1}) as (fresh mass - dry mass) / fresh mass. For the second round of leaf measurements in 2019, we measured only trichome density.

Fitness measures

We used three indices of fitness: survival, growth rate, and flowering. Growth rate was included because plant size has a positive effect on reproductive output in this species (Klinkhamer et al., 1992). We censused all tagged plants for survival and flowering in the early summer of each year from 2019 - 2021 (dates in Fig. S1). Survival was measured between two consecutive censuses a year apart. Flowering was measured as whether the plant bloomed the next year, which combines both survival and reproduction. For non-flowering individuals, we counted leaves in the rosette and measured the longest leaf in each rosette in early summer of each year. Size was calculated as the sum of the products of the longest leaf and number of leaves for each vegetative rosette. To convert this size index to a biomass index necessary to calculate growth rate, we used nonlinear least squares to fit a power law between leaf length and leaf dry mass from the second round of leaf collections in 2020: dry mass (g) = 0.0016(leaf length, cm)$^{1.41}$. Relative growth rate (RGR, $year^{-1}$) was calculated as ($\ln B_2 - \ln B_1$) / ($t_2 - t_1$), where B_1 and B_2 are the biomass indices in consecutive years, and t_1 and t_2 are the census dates (Hunt, 1982). Note that RGR was only calculated for plants that survived and did not flower (biomass in flowering individuals is moved into the stalk in addition to the leaves), so analyses involving RGR do not represent the population as a whole.

Statistical analysis

Questions 1 and 2: Plasticity to combinations of snowmelt timing and summer precipitation

Plasticity can be measured either as differences among groups of plants receiving different experimental treatments, or as changes observed within individual plants through time as they respond to shifting environmental conditions (Nussey et al., 2007; Hendry 2016). We emphasized the first approach of measuring “among-plant plasticity”, but also examined “within-plant plasticity” for some traits to demonstrate that they had labile responses. In measuring “among-plant plasticity”, our experimental manipulations of the environment enabled us to measure average plasticity (e.g., Caruso et al., 2006). Although we did not manipulate genotype (Scheiner, 1993), treatments were assigned to plots (and thus also to genotypes) randomly, so there should be no bias in the measurement of average plasticity (see review by Davidson et al., 2011).

For among-plant plasticity, we analyzed responses of each trait to the effects of snowmelt timing and summer precipitation in two ways: first as a response to the experimental manipulations and years coded as discrete levels of the replicated split-plot design, and second as a multiple regression with continuous environmental variables affected by both the treatments and natural variation among years (the date of snowmelt in each plot and summer precipitation estimated for each precipitation treatment). The first set of models tests for causal effects of the treatments, and the second set expands the range of snowmelt timing and summer precipitation to increase statistical power, albeit in a correlational analysis. Detailed methods for these two modeling approaches and testing for compensation of additional precipitation for early snowmelt are given in Powers et al. (2021) and Methods S2. For the morphological traits, we fit both models to traits collected on leaves in the second round after plants were subjected to the precipitation treatments the longest. The second type of model was not fit for SLA or LWC since the available data are both from early snowmelt years (2018 and 2020).

We supplemented these among-plant analyses with analysis of within-plant plasticity for the morphological traits (SLA, LWC, and trichome density) to establish that those traits were labile. Leaf gas exchange traits of photosynthetic rate, stomatal conductance, and iWUE are already known to vary on short timescales within a plant (Wu & Campbell, 2007). For this approach, we tracked morphological traits on the same 92 plants from the beginning to the end of one growing season (2018), and then to the beginning of the next (2019) if they survived (46 plants). The first round of leaf sampling in 2018 followed a long foresummer drought (Methods S1) and 8 days of precipitation treatments. The second round occurred 64 days later, 7 days after precipitation treatments stopped. The first round of sampling in 2019 on the same plants occurred after 4 days of precipitation treatments. A linear mixed model (Methods S2) was fit to vegetative trait data from the subset of plants that were sampled over at least two of these first three rounds. The model included fixed effects of sampling round, the two treatments, and all interactions, as well as a random intercept of plant to account for repeated measures, and random effects of plot and subplot. Since not all plants were sampled in all three rounds, some observed changes may be due to mortality selection rather than plasticity.

Question 3: Soil moisture as a mechanism for plasticity

To determine the extent to which impacts on traits are mediated by soil moisture, we replaced the treatment levels with a measure of soil moisture in our linear mixed models. Although this analysis is correlational, most variation in summer mean subplot-level soil moisture can be attributed to year and the snowmelt and precipitation treatments ($R^2_m = 0.81$, Methods S1). We allowed each trait averaged by plant to depend on a random effect of subplot (and a random effect of round for the physiology traits) and fixed effects of the year, soil moisture, and their interaction. A quadratic term for soil moisture was included if it had a significant effect ($P < 0.05$, as in Campbell et al., 2010). For morphological traits, we used the average soil moisture in each subplot across the measurement period in each year (Fig. S1). For physiological traits, which respond quickly to new conditions, we used soil moisture next to the plant at the time of measurement. In 2018, we did not measure soil moisture at the time of measuring leaf gas exchange, so we substituted the subplot average on the date closest to the measurement (within 6 days). To compare responses of different plant traits to soil moisture, we plotted curves for each year based on estimated marginal means for the standardized trait values.

Questions 4 and 5: Selection in each environment and adaptive plasticity

To determine whether there is mortality, growth, or reproductive selection on each trait, and whether plastic responses to changes in the environment are adaptive, we analyzed the impact of traits, either the snowmelt or precipitation treatments, year, and their interactions on either survival, RGR, or flowering from one year to the next. The full model included these factors as fixed effects and a random effect of plot. To aid model interpretation, we dropped non-significant three-way interactions ($P > 0.05$). A significant trait × treatment interaction on a fitness component would indicate that selection differed in magnitude or direction among treatments. We fit the generalized linear mixed models using the R package *glmmTMB* (Brooks et al., 2017), and performed Type III Wald χ^2 tests with the *car* package (Fox and Weisberg, 2018). We assumed a binomial distribution for flowering or survival and a gaussian distribution for RGR. We tested for selection in each treatment and year using the *emtrends* function of the R package *emmeans* (Lenth et al., 2021) on the full model. We calculated the standardized selection differential as the difference in phenotypic mean before and after selection (for survival and flowering), or the covariance of relative fitness and the trait (for RGR), each divided by the standard deviation of the trait before selection. We also fit these models without the traits to test whether these fitness measures were affected directly by the treatments in each year. Models fit to the number of flowers produced in 2019 and 2020, using zero if the plant did not survive and flower, gave similar results to those with binary flowering status.

Results

Questions 1 and 2: Plasticity to combinations of snowmelt timing and summer precipitation

We first examined the effects of the year, the summer precipitation and snowmelt treatments, and their interactions using the replicated split-plot models (Table 1, Fig. 1). Whereas the early snowmelt treatment had no detected main effects on traits in these models (all $P > 0.16$), it did reduce SLA more in 2020 compared to 2018 (year × snowmelt interaction, $P = 0.02$). Compared to controls, precipitation reduction decreased stomatal conductance and increased iWUE, and precipitation addition increased LWC and decreased iWUE (post-hoc tests in Table 1). The effects of the precipitation treatments varied across years detectably for LWC, stomatal conductance, and iWUE (interaction $P < 0.01$, Fig. 1). The effect of precipitation on iWUE was stronger in the early snowmelt years of 2018 and 2020 than in the late snowmelt year (2019). All traits, except LWC, varied significantly among years. Photosynthetic rate and stomatal conductance were higher and iWUE lower in 2019 compared to the two early snowmelt years. There were no detected interactions between the snowmelt and precipitation treatments on any trait (all $P > 0.32$). The summer precipitation addition treatment could compensate for the effect of the early snowmelt treatment in years where it occurred; generalized linear hypothesis tests found no differences between subplots with early snowmelt and precipitation addition versus normal snowmelt with control precipitation (all $P > 0.12$), except for LWC, where in 2018 added precipitation overcompensated for earlier snowmelt ($P = 0.02$).

We examined the responses of four traits to the actual snowmelt dates and the total summer precipitation experienced by the plants using our second set of models that incorporated both experimental effects and natural variation in those climatic variables (Table 2, Fig. 2). For snowmelt date, these models detected effects that were not significant in the first models because of low replication of the snowmelt manipulation and the greater statistical power achieved with incorporating the full range of snowmelt dates over a period of 46 days. Earlier snowmelt date increased trichome density and decreased stomatal conductance faster than photosynthetic rate, such that earlier snowmelt was associated with higher iWUE (Fig. 2). Lower total summer precipitation (calculated based on the treatments applied to each subplot) increased trichome density and decreased stomatal conductance ($P < 0.01$) but did not have a significant effect on photosynthetic rate ($P = 0.10$), such that iWUE increased with less precipitation ($P < 0.01$). None of these traits showed a significant interaction between the two climate variables ($0.15 > P > 0.05$), although the trend was for greater effects in early snowmelt years. We estimated the amount of additional total summer precipitation required to compensate for each day that snowmelt occurs earlier, which ranged from 1 - 7 mm (Table 2), or 1 - 8 % of the average total summer precipitation in this area (86 mm, 1990 - 2020, Powers et al., 2021).

While we report effects of the treatments on each trait, traits were correlated across individuals. Notably, photosynthetic rate scaled with stomatal conductance ($r = 0.71$), much of the variation in iWUE was derived from variation in conductance ($r = -0.65$) rather than photosynthesis ($r = -0.19$), and high LWC was associated with high conductance ($r = 0.47$), low iWUE ($r = -0.52$), and high SLA ($r = 0.47$, all associated $P < 0.001$).

All of the morphological traits (SLA, trichome density, and LWC) showed within-plant plasticity, as indicated by effects of round of sampling (all $P < 0.01$) when the same plants were repeatedly sampled (Table S1). Regardless of the snowmelt or precipitation treatments, SLA decreased from the beginning to end of summer 2018, and then rebounded to similar levels by the start of the next summer (Fig. S2). Trichome density declined slightly over the summer. LWC decreased over the summer, with the largest reductions occurring in the control and reduced precipitation treatments (round × precipitation interaction $P < 0.01$), before rebounding to high levels early in the next summer, regardless of treatment.

Question 3: Soil moisture as a mechanism for plasticity

In leaves measured at the end of the summer, decreasing soil moisture was associated with lower LWC, photosynthetic rate, and stomatal conductance, and higher iWUE (all $P < 0.001$), but we did not detect an effect on SLA (Fig. 3, Table 3). For photosynthetic rate and stomatal conductance, there was a significant negative quadratic effect of soil moisture, such that each gas exchange trait increased with soil moisture content until a peak at 10 - 15 %VWC, and then decreased (Fig. 3). The effect of soil moisture varied across years (interaction $P < 0.05$) for trichome density, stomatal conductance, and iWUE (Table 3; slopes in individual years were not tested for significance). Trichome density trended upward with decreasing soil moisture in 2018, but remained constant in 2019 and trended downward in 2020. Soil moisture had a greater effect on stomatal conductance in 2018, and lower soil moisture increased iWUE more rapidly in the early snowmelt years (2018 and 2020) compared to 2019, a late snowmelt year (Fig. 3). Trait averages also varied across years ($P < 0.01$) for SLA, trichome density, stomatal conductance, and iWUE. SLA was higher in 2020 than 2018, trichome density decreased from 2018 to 2019 to 2020, and during the late snowmelt year of 2019 stomatal conductance was higher and iWUE lower compared to the two early snowmelt years.

Questions 4 and 5: Selection in each environment and adaptive plasticity

Examining first overall effects of the year and treatments on average fitness, a higher proportion of plants flowered after the late snowmelt year of 2019 (Table S2, Fig. S3). Survival was more similar between years and decreased the most under early snowmelt and reduced precipitation applied in 2019 (year × snowmelt × precipitation $P = 0.02$). In 2018, RGR was reduced by early snowmelt and increased by precipitation addition, though in 2019 RGR was lower overall and especially so for early snowmelt under precipitation addition (year × snowmelt × precipitation $P = 0.002$).

Our models of the relationships of survival, relative growth rate, and flowering to trait values detected no overall average selection on the six traits across years and treatments, except positive selection on LWC in the models that considered snowmelt (results for precipitation in Table 4, Fig. 4, and Fig. 5, results for snowmelt in Table S3 and Fig. S4). Instead, selection varied across treatments for two traits, as shown by significant trait × treatment interactions. Mortality selection on stomatal conductance depended on precipitation (conductance × precipitation interaction P = 0.01): precipitation reduction favored plants with low conductance (significantly so in the early snowmelt year of 2018), while precipitation addition and controls favored plants with higher conductance (not significantly so for any year, Fig. 4a). We did not see a similar iWUE × precipitation interaction (P = 0.50) as might be expected since iWUE was driven primarily by conductance, but testing selection in each year and treatment, there was significant mortality selection for higher iWUE in the early snowmelt year of 2018 under precipitation reduction (standardized selection differential S' = 0.13, P = 0.044, Fig. 4a). In contrast, under unmanipulated snowmelt in the late snowmelt year of 2019, there was flowering selection for higher stomatal conductance (S' = 0.25, P = 0.049) and lower iWUE (S' = -0.28, P = 0.028, Fig. S4c). Flowering selection on SLA depended on the snowmelt treatment and the year (SLA × snowmelt interaction P = 0.03, SLA × year × snowmelt interaction P = 0.03): in 2018 high SLA tended to be favored under normal snowmelt but disfavored with early snowmelt, while in 2020 there was little selection under the early snowmelt treatment and high SLA tended to be disfavored under normal snowmelt (Fig. S4c). There were no differences in RGR selection by year or treatment (Table 4, Table S3), but in 2018 under unmanipulated snowmelt, both photosynthetic rate (S' = 0.56, P = 0.034) and stomatal conductance (S' = 0.73, P = 0.007) improved RGR, and under early snowmelt higher LWC improved RGR (S' = 0.86, P = 0.031, Fig. S4b).

Synthesizing these selection results with the analyses of trait plasticity, we conclude that stomatal conductance responded plastically to both snowmelt timing (when including annual variation in addition to treatment effects) and summer precipitation (Table 2), and that plastic responses of this trait to summer precipitation were adaptive because the lower conductance induced by reduced precipitation (Fig. 2) improved survival (red lines in Fig. 4a). In the early snowmelt year of 2020 SLA decreased with earlier snowmelt (Fig. 1), and while the benefit to flowering fitness in that year and treatment was small (standardized selection differential S' = -0.05, P = 0.88), the benefit to low SLA was larger in 2018 under early snowmelt (S' = -0.34, Fig. S4c, year × SLA interaction P = 0.03), indicating potential adaptive plasticity of that trait under extreme conditions.

Discussion

Snowmelt timing is a critical event in cold ecosystems that is rapidly advancing with climate change (Pederson et al., 2011; Assmann et al., 2019; Sun et al., 2019). However, the effects of early snowmelt on leaf traits, especially in tandem with changes in summer precipitation, are not well studied. We linked measurements of plasticity and its effects on fitness in different environments to understand how climate change is impacting the expression and natural selection of leaf traits in *Ipomopsis aggregata*. Both earlier snowmelt and reduced summer precipitation restricted water availability and caused plants to modify trait expression. Some, but not all of these plastic changes, were adaptive under the extremely dry conditions anticipated to become more frequent in the future as a result of shrinking snowpack and lengthening drought.

Approaches to studying the effects of snowmelt timing on plant traits include transplants over an elevational gradient (Scheepens et al., 2010), variation in snowmelt timing due to drifting (Winkler et al., 2018), increasing the melt rate (Livensperger et al., 2016), or direct snow removals (Bemmels & Anderson, 2019). We combined experimental manipulation of melt rate (6 days faster on average) with natural variation to examine a 46-day gradient in snowmelt timing. Advancing snowmelt experimentally reduced specific leaf area in one year and reduced RGR on average, but the 6 day change had weak or no effects on other traits. Inability to detect effects on some traits could have resulted from low statistical power due both to low replication of the snowmelt timing treatments and the achieved difference of 6 days. Indeed, examining changes in plant trait expression along the broader gradient of 46 days indicated strong differences in expression of most traits across years that could be explained by snowmelt timing as mediated through changes in soil moisture.

Ipomopsis aggregata increased trichome density on the upper surface of the leaf as snowmelt occurred earlier and as total summer precipitation declined. In principle, this response could have several potential functions for longer and drier growing seasons: preventing lethal leaf temperatures by reflecting more incoming radiation, reducing transpiration through expanding the boundary layer or lowering temperature (Johnson, 1975), or reducing ultraviolet radiation damage (Karabourniotis et al., 1995). While dense trichomes diminish the amount of light available for photosynthesis, their cooling of the leaf can maintain temperatures closer to the optimum for photosynthesis in arid or hot conditions (Ehleringer & Mooney 1972). Despite those potential functions, we saw no evidence for selection on trichome density in our experiment.

Plants of *I. aggregata* showed some responses of SLA to snowmelt timing, but not to summer precipitation. Though SLA did not respond to early snowmelt in the first year of manipulations, in 2020 it was lower for plants in plots receiving three years of early snowmelt. Its plastic response may be slower than for the other traits in this study, as SLA changes require the growth of new leaves, which do not completely turn over each year. Whereas individual *I. aggregata* plants reduced SLA over the course of the summer in 2018 (equally across treatments), over three years plants did not respond to altered precipitation through changes in SLA. This result is surprising given the plasticity of this trait to water availability in other species (Poorter et al., 2009), and the association of high SLA with wetter climates (Westoby & Wright, 2006). However, SLA in *Ipomopsis* did not respond to water addition or reduction in an earlier field manipulation either (Campbell & Wendlandt, 2013). SLA may not have responded in collected leaves if they matured before precipitation treatments were applied, but while this species retains some leaves from the previous year, this lag is less likely after three years of new growth under repeated precipitation treatments. Alternatively, the absence of plasticity in one trait may indicate successful buffering against environmental change, or be maintained by changes in other unmeasured traits (Forsman, 2015).

Experimentally manipulating snowmelt and precipitation during the growing season altered leaf gas exchange for *I. aggregata* in ways supported by prior studies of water stress. Earlier snowmelt and reduced precipitation reduced stomatal conductance more sharply than photosynthetic rate declined, such that plants enhanced iWUE. This pattern of iWUE being driven more strongly by variation in stomatal conductance than photosynthesis has been documented among genetic lines of *Arabidopsis thaliana* (Easlon et al., 2014) and between sites that vary in temperature and humidity for *Ipomopsis* (Campbell et al., 2005).

In addition to serving as an indicator of water stress during leaf development (Zhou et al., 2021), variation in LWC can represent anatomical differences in cell wall thickness (Garnier & Laurent, 1994; Easlon et al., 2014). In *I. aggregata*, reduced precipitation decreased LWC, and by following plants over time, we showed that the responses of LWC to precipitation occurred within each plant over the growing season. However, there was no detected effect of the snowmelt manipulations on LWC.

In the above arguments, we assume that differences in trait means among treatments are due to plastic responses to the imposed conditions. Alternatively, mortality and flowering (which results in mortality the following year) over the three years of the experiment without measurement of a similar number of newly recruited plants mean that trait differences in the second and third years could be partly the result of selection in each environment rather than plasticity. However, because flowering would remove plants with relatively higher fitness, and mortality without flowering would remove those with lower fitness, we expect phenotypic variation in the remaining plants to largely reflect plasticity. In addition, we demonstrated within-plant plasticity and did not detect mortality or flowering selection on SLA or trichome density under any treatment, so can assume most changes in those traits result from plasticity.

Because snowmelt timing and summer precipitation vary independently in a given year, and predictions of changes in precipitation are highly variable (IPCC, 2014), it is important to understand how these environmental drivers interact to affect phenotypic plasticity. In *I. aggregata*, changes in snowmelt date did not significantly modify the effects of total precipitation for trichome density or the three physiological traits (interaction P = 0.06 - 0.15), though the trend for each trait was that early snowmelt exacerbated the effect of reduced precipitation. We found that additional summer precipitation could compensate for earlier snowmelt in those four tested traits, though different amounts of precipitation were required to compensate for the same shift in snowmelt (1 - 8 % additional precipitation per day earlier snowmelt). With current trends in shifting snowpack timing at this site (2.1 ± 1.5 days earlier per decade from 1976 - 2020, data from billy barr, www.gothicwx.org/ground-cover.html), this corresponds to a 2 - 17 % increase in summer precipitation per decade required to maintain current leaf trait values. Such a sustained increase is unlikely given the recent increased variability of summer precipitation and longer, not shorter, droughts observed in the Colorado Plateau and Southern Rocky Mountains (Zhang et al., 2021). Furthermore, other environmental conditions, such as temperature, are also changing with climate change, and plasticity of these leaf traits to high temperature has not yet been described in *I. aggregata*.

The plastic responses of leaf traits in *I. aggregata* to earlier snowmelt or reduced summer precipitation were associated with how those environmental changes reduced soil moisture across the growing season. Photosynthetic rate, stomatal conductance, and LWC decreased as soils dried below 10 - 15 %VWC, although the gas exchange traits did not increase with higher soil moisture than that. A dry-down experiment in a screenhouse showed similar peaks in photosynthetic rate and stomatal conductance at intermediate soil moisture (Campbell et al., 2010). Because stomatal conductance decreased faster than photosynthetic rate with reduced soil moisture, iWUE increased with drier soils, in concordance with field measurements of these traits for three sites in an *Ipomopsis* hybrid zone differing in soil moisture (Campbell & Wu, 2013). Furthermore, iWUE plasticity to soil moisture varied among years, with the strongest response in the year with earliest snowmelt (2018). While we present strong correlational evidence that soil moisture mediates plasticity to snowmelt and precipitation changes, untested mechanisms such as temperature buffering of snow cover could also be affected by the treatments and contribute to plastic responses.

We demonstrated that phenotypic plasticity in a key water-saving trait, low stomatal conductance, was adaptive in extremely dry conditions, providing an opportunity to enhance population persistence under climate change. Specifically, under reduced precipitation, mortality selection favored plants with low stomatal conductance in all three years, and so the observed plasticity of stomatal conductance (decreasing with lower precipitation) was adaptive in that environment (S' under reduced precipitation was -0.23 in 2018, -0.08 in 2019, and -0.18 in 2020). On the other hand, additional precipitation caused plants to raise conductance, and this plasticity was adaptive as mortality selection favored higher conductance in those environments. In addition, conductance decreased in years with earlier snowmelt, a response that improves survival under reduced precipitation. Therefore, as climate change accelerates snowmelt timing and reduces summer precipitation, we expect that plasticity in conductance will enhance plant survival (even if survival is lower overall in these harsher conditions). Although we did not detect a significant trait x precipitation interaction for iWUE, the increased iWUE under reduced precipitation in the early year of 2018 also enhanced survival, suggesting adaptive plasticity in iWUE under extreme conditions. SLA showed plasticity that was adaptive under extreme conditions of early snowmelt in 2020. Whereas trichome density and photosynthetic rate had weak or no effects on survival or flowering, LWC did improve RGR, and some plants live for up to 10+ years, so that could influence fitness over a longer term than our three-year experiment.

Ipomopsis aggregata shows reduced population growth rate in years of early snowmelt (Campbell, 2019). In this species, the adaptive plasticity we demonstrated in stomatal conductance, and any further evolution of plasticity, or of conductance in response to the observed selection on it, might assist with population persistence over the long-term in the face of more frequent extreme droughts in this region (Zhang et al., 2021). Ultimately, the likelihood of evolutionary rescue from impacts of early snowmelt or summer drought will depend upon genetic variation in that trait as well as genetic variation in the reaction norm (Chevin et al., 2013), i.e. the relationship of conductance to each environmental driver for a genotype. Heritabilities of stomatal conductance and water-use efficiency have rarely been measured in wild plants, but reported values from non-agricultural systems range from 0.09 to 0.38 for WUE (Agrawal et al., 2008, Ahrens et al., 2019, Culley et al., 2006), indicating that some species could show a strong evolutionary response to selection of the magnitude we observed. Heritability of leaf traits is currently under study in *Ipomopsis*.

Conclusions

Under climate change, *Ipomopsis aggregata* can change traits in response to snowmelt timing and precipitation. This response is driven by how early snowmelt and low summer precipitation reduce soil moisture. In extreme dry conditions, which will become increasingly common in the studied region, plastic decreases in stomatal conductance and SLA are adaptive. This study illustrates the importance of snowmelt timing, in addition to growing season precipitation, for vegetative traits related to water relations.

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Author Contribution

D.R.C. designed the study. A.P., D.R.C., J.M.P., and J.N. authors performed manipulations, collected field data, organized and analyzed the data, wrote the first version, and contributed to revisions.

Data Availability

All data and code used to generate figures and tables are provided at https://jmpowers.github.io/snow-precip-leaf/. Subplot means for physiological traits are deposited in the Dryad Digital Repository at https://doi.org/10.7280/D12H50.

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Supporting Information

Figure S1 Timings of experimental treatments and measurements

Figure S2 Effects of snowmelt and precipitation treatments on vegetative traits

Figure S3 Effects of the treatments on fitness

Figure S4 Effects of the snowmelt treatment on selection

Table S1 Repeated measures of plants in each treatment

Table S2 Effects of the treatments on fitness

Table S3 Effects of the snowmelt treatment on selection

Methods S1 Details of experimental treatments

Methods S2 Statistical models of plasticity

Figures

Figure 1

The effects of snowmelt and precipitation treatments on traits of *Ipomopsis aggregata* across three years. Bars show the estimated marginal mean and standard error, and points show subplot means. Panels on the left show the three morphological traits, and panels on the right show the three physiological traits.

Figure 2

Model results for the effects of the snowmelt date and estimated summer precipitation on traits of *Ipomopsis aggregata* across three years. Lines show the estimated marginal trends for the amounts of summer precipitation listed in the key. Points show the subplot means, colored by the estimated summer precipitation.

Figure 3

Model results for the effects of soil moisture (volumetric water content, VWC) on standardized traits of *Ipomopsis aggregata* across three years. The standardized mean trait values for each plant in each year are shown as points. The estimated marginal trend is shown as a line. Upper panels: morphological traits. Lower panels: leaf gas exchange traits. Photosynthetic rate and stomatal conductance showed significant quadratic relationships with soil moisture, and so second order polynomials are depicted.

Figure 4

Impacts of the precipitation treatments and traits on survival (a) or flowering (b) of *Ipomopsis aggregata*. The year shows the year that the trait was measured; fitness was assessed the following year. Logistic curves are fit within each precipitation treatment and year, with significant slopes ($P < 0.05$) indicated with an asterisk.

Figure 5

Impacts of the precipitation treatments and traits on relative growth rate (RGR) of *Ipomopsis aggregata*. The year shows the year that the trait was measured; RGR was assessed the following year. Logistic curves are fit within each precipitation treatment and year, with no significant slopes detected ($P > 0.05$).

Tables

Table 1

Linear mixed model results for the effects of year and the snowmelt (Snow) and precipitation (Precip) treatments on traits of *Ipomopsis aggregata*.

Trait	**Year**	**Snow**	**Precip**	**Year x Snow**	**Year x Precip**	**Snowx Precip**	**Year x Snow x Precip**	**R^2_m**	**Addition vs. Control**	**Reduction vs. Control**
Specific leaf area	**< 0.001**	0.152	0.666	**0.012**	0.788	0.277	0.154	0.603	0.579	0.914
Trichome density	**< 0.001**	0.489	0.571	0.599	0.115	0.921	0.516	0.209	0.579	0.960
Leaf water content	0.187	0.682	**0.004**	0.076	**0.007**	0.368	0.428	0.217	**0.003**	0.232
Photosynthetic rate	0.062	0.632	0.308	0.458	0.411	0.988	0.914	0.109	0.521	0.598
Stomatal conductance	**< 0.001**	0.467	**0.002**	0.477	**0.002**	0.661	0.689	0.346	0.137	**0.004**
Intrinsic water-use efficiency	**< 0.001**	0.211	**< 0.001**	0.467	**0.003**	0.326	0.851	0.442	**0.005**	**0.001**

P values are listed for each trait under the main effects and interactions, and R^2_m is the marginal R^2 for the proportion of variance explained by the fixed effects. P values for post-hoc tests for the main effects of precipitation (excluding interactions) are listed comparing the precipitation addition and reduction treatments to the controls. Significant effects ($P < 0.05$) are bolded.

Table 2

Linear mixed model results for the effect of the date of snowmelt and estimated summer precipitation on traits of *Ipomopsis aggregata* across three years.

Trait	Snowmelt date	Summer precipitation	Snowmelt date x Summer precipitation	R^2_m	Compensation (mm precipitation / day earlier snowmelt)
Trichome density	**0.002**	**0.007**	0.081	0.189	1.1 ± 0.4
Photosynthetic rate	**0.005**	0.097	0.153	0.082	7.0 ± 5.2
Stomatal conductance	**< 0.001**	**0.025**	0.082	0.230	6.2 ± 2.6
Intrinsic water-use efficiency	**0.002**	**0.010**	0.055	0.205	4.7 ± 2.1

P values are listed for each trait under the main effects, and R^2_m is the marginal R^2 for the proportion of variance explained by the fixed effects. The compensation point is the additional total summer precipitation per day of snowmelt advancement that is required to hold the trait constant (mean ± SE). Significant effects ($P < 0.05$) are bolded.

Table 3

Linear mixed model results for the effects of year and soil moisture (volumetric water content, VWC) on traits of *Ipomopsis aggregata*.

Trait	Year	VWC	Year x VWC	VWC^2	R^2_m
Specific leaf area	**0.012**	0.166	0.395		0.585
Trichome density	**< 0.001**	0.906	**0.033**		0.195
Leaf water content	0.178	**< 0.001**	0.083		0.184

Photosynthetic rate	0.449	**< 0.001**	0.669	**0.006**	0.150
Stomatal conductance	**< 0.001**	**< 0.001**	**0.022**	**0.039**	0.386
Intrinsic water-use efficiency	**< 0.001**	**< 0.001**	**0.003**		0.435

The quadratic term (VWC^2) was dropped from the model if not significant. P values are listed for each trait under the main effects, and R^2_m is the marginal R^2 for the proportion of variance explained by the fixed effects. Significant effects ($P < 0.05$) are bolded.

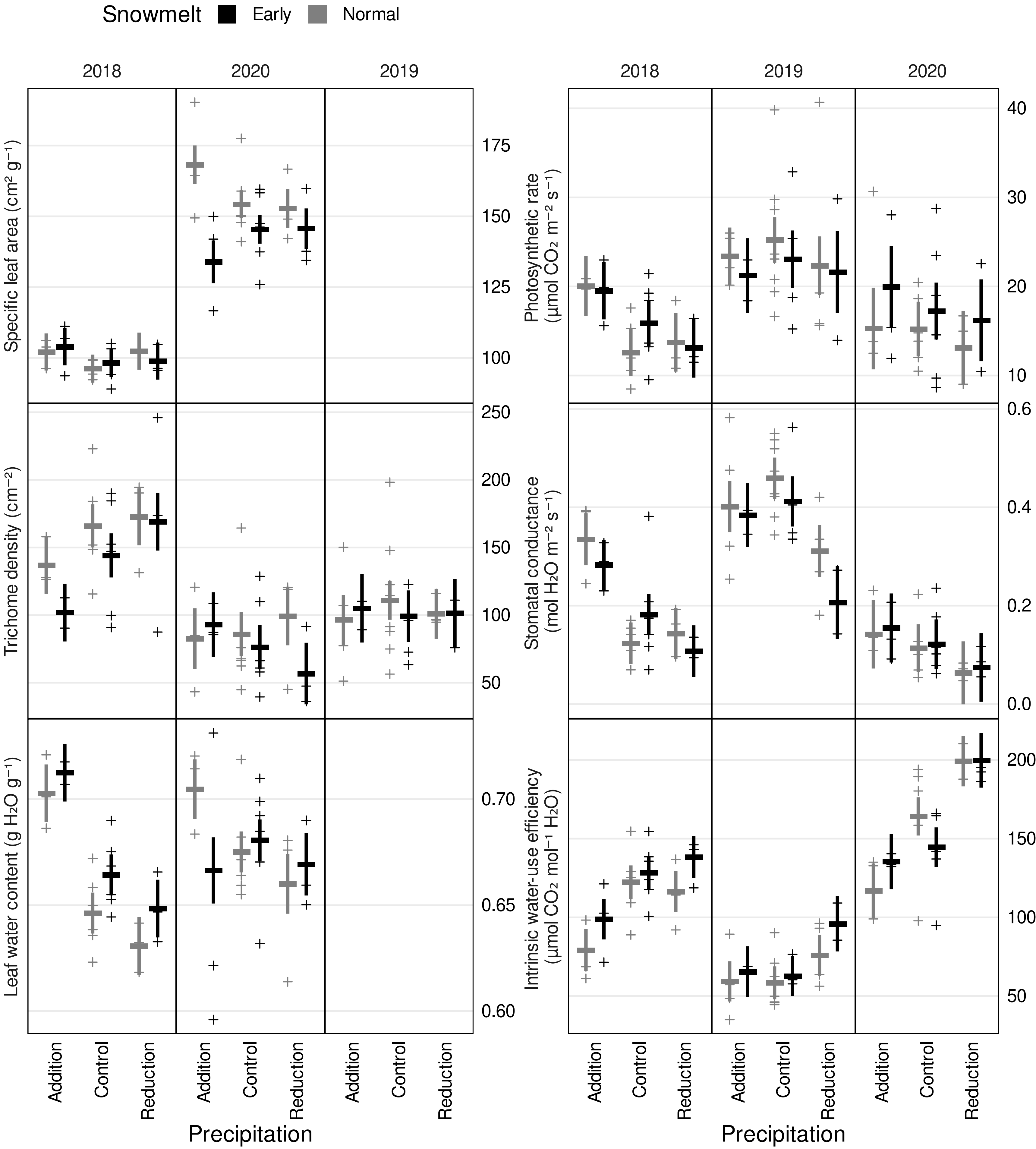
Table 4

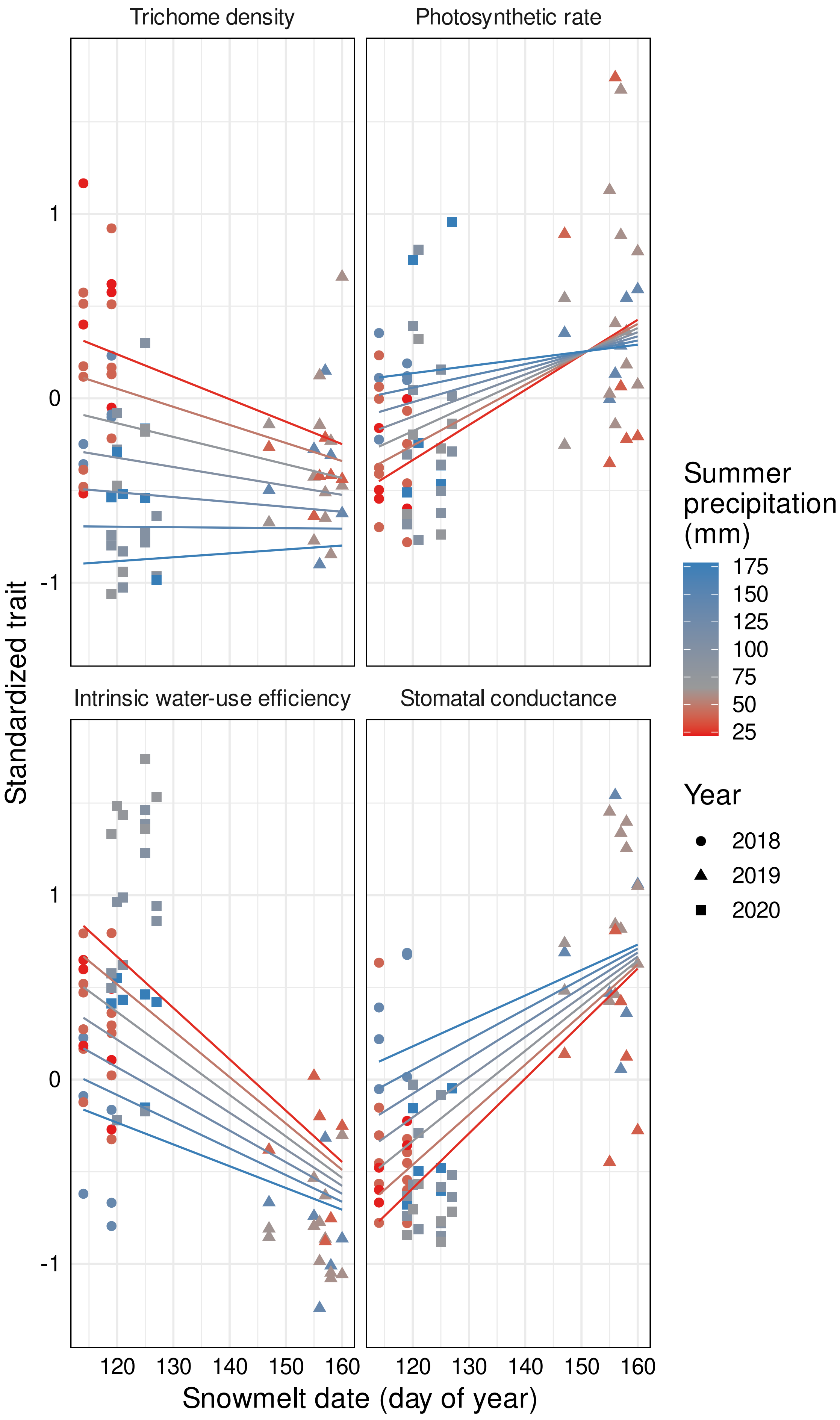
Generalized linear mixed model results for the effects of precipitation (Precip) treatments and traits on survival, relative growth rate, and flowering of *Ipomopsis aggregata*.

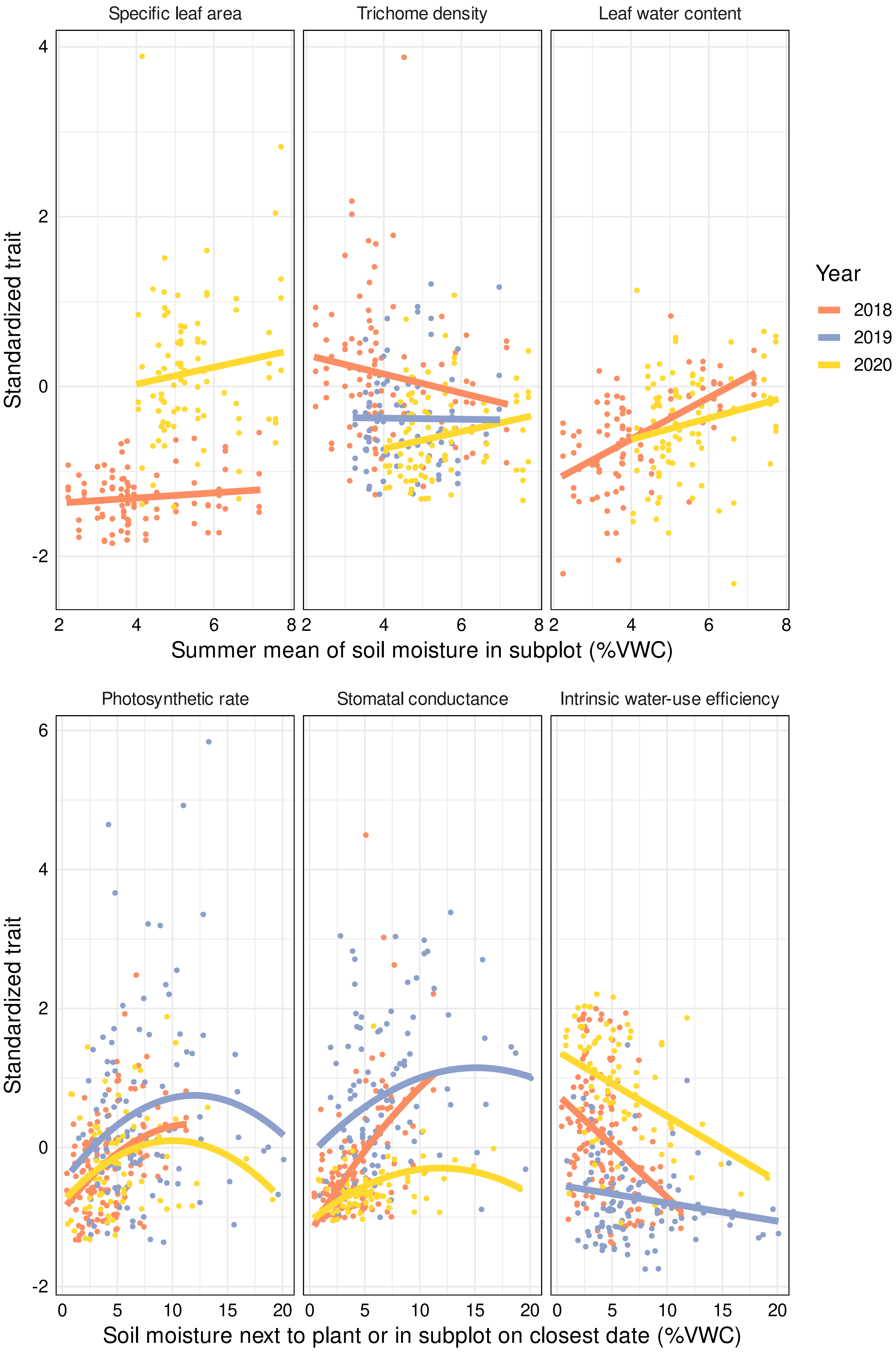
		Trait	**Precip**	**Year**	**Year x Precip**	**Trait x Year**	**Trait x Precip**
Survival	Specific leaf area	0.21	0.69	0.98	0.56	0.85	0.72
	Trichome density	0.37	0.63	0.87	0.26	0.94	0.83
	Leaf water content	0.41	0.98	0.89	0.21	0.92	1.00
	Photosynthetic rate	0.13	0.52	0.42	0.93	0.43	0.39
	Stomatal conductance	0.60	0.07	0.96	0.45	0.93	**0.01**
	Intrinsic water-use efficiency	0.31	0.62	0.19	0.85	0.13	0.50
Relative growth rate	Specific leaf area	0.97	0.71	0.74	0.41	0.96	0.70
	Trichome density	0.41	0.57	0.15	**< 0.01**	0.27	0.92
	Leaf water content	0.65	0.81	0.48	0.11	0.61	0.91
	Photosynthetic rate	0.51	**< 0.01**	0.12	**0.04**	0.61	0.32
	Stomatal conductance	0.34	**0.01**	0.31	**0.03**	0.63	0.46
	Intrinsic water-use efficiency	0.30	0.07	0.51	**< 0.01**	0.91	0.21
Flowering	Specific leaf area	0.17	0.25	0.99	0.06	0.94	0.32
	Trichome density	0.54	0.79	0.17	0.33	0.91	0.93
	Leaf water content	0.50	0.63	0.18	0.37	0.23	0.70
	Photosynthetic rate	0.68	0.19	**0.03**	0.82	0.88	0.36
	Stomatal conductance	0.58	0.23	0.07	0.80	0.43	0.15

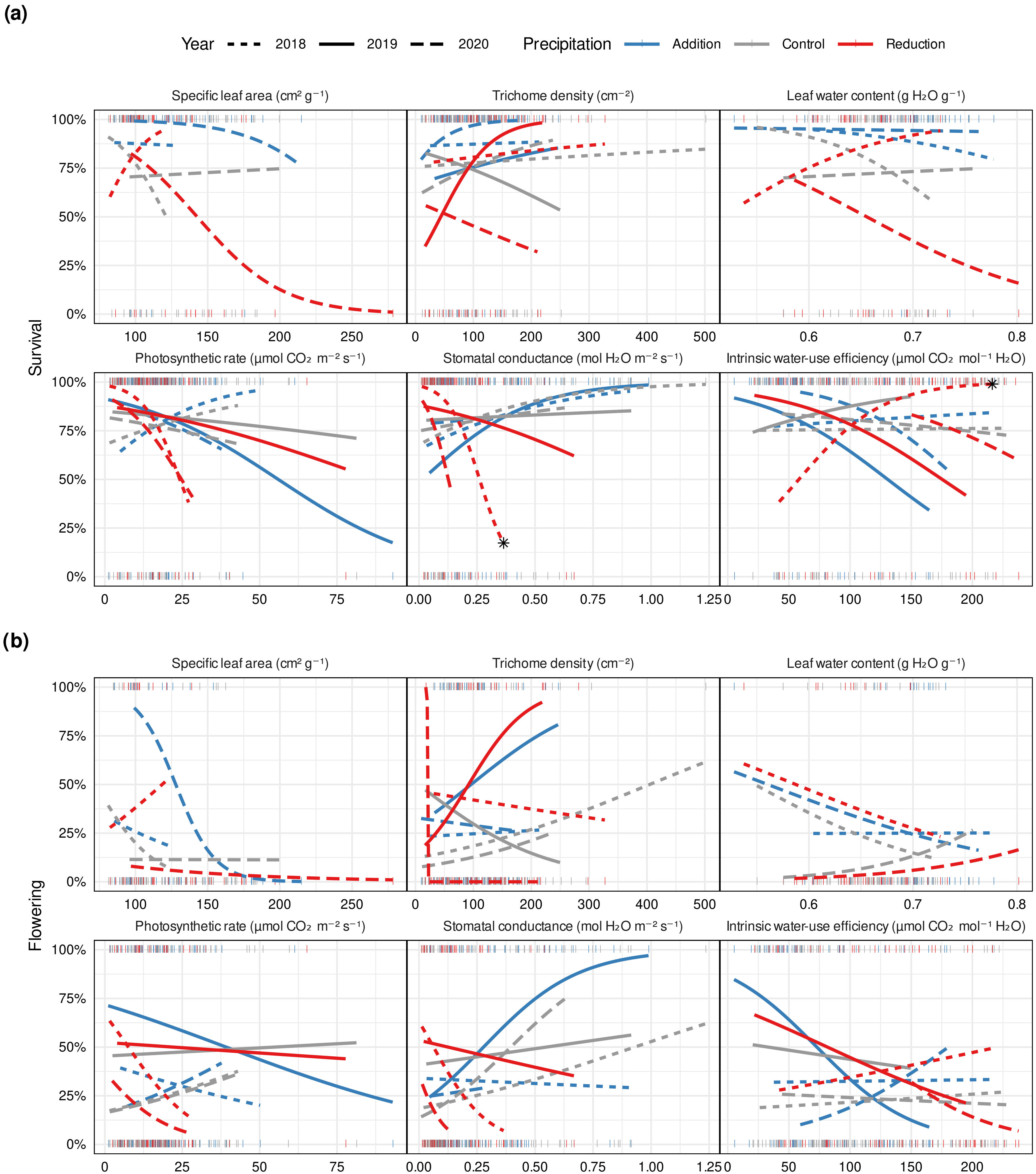
	Intrinsic water-use efficiency	0.28	0.59	**< 0.01**	0.86	0.13	0.87

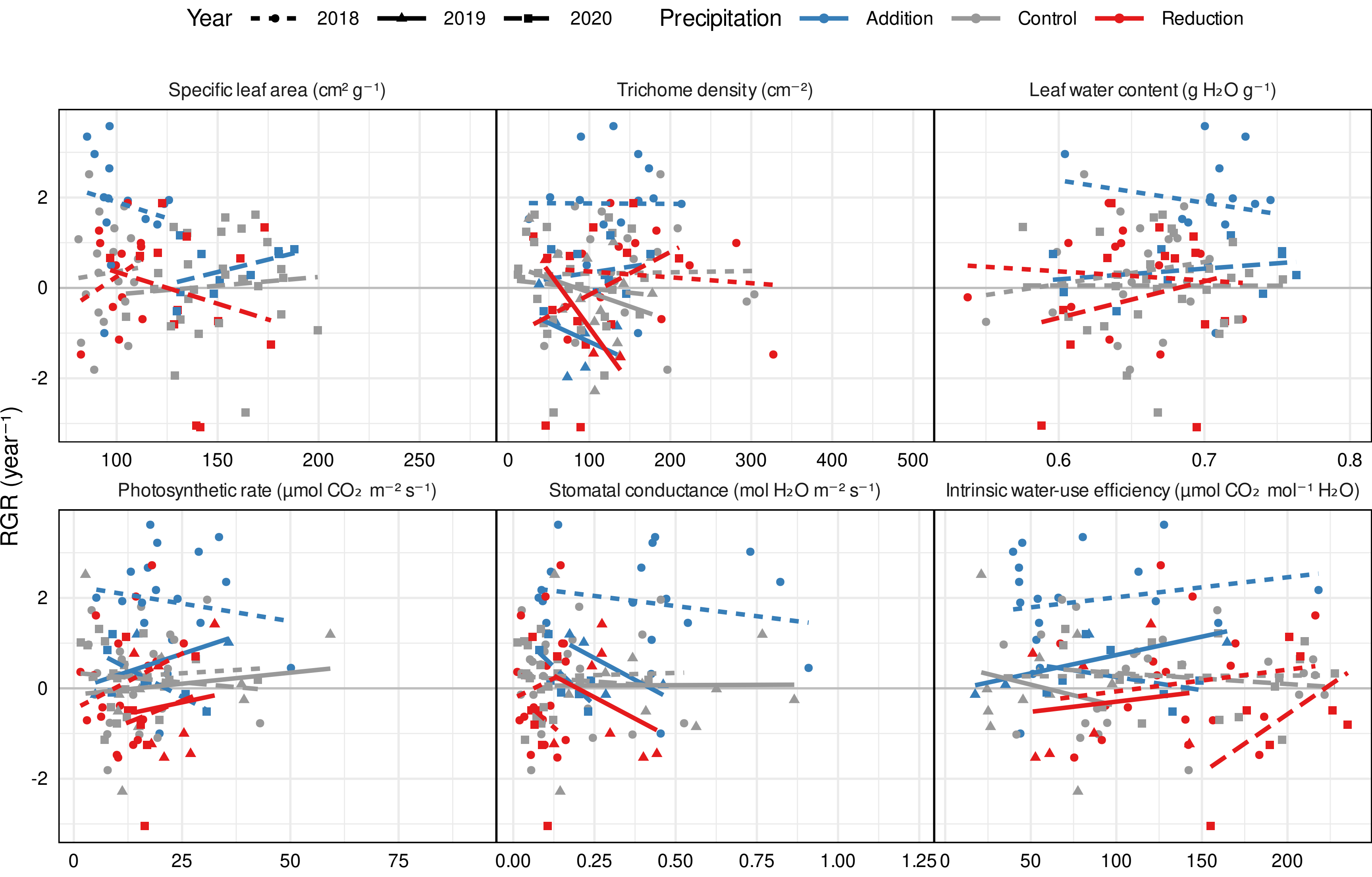
The three-way interaction was dropped from the model as it was not significant for any trait (P > 0.05). Significant effects (P < 0.05) are bolded.


Snowmelt
Early
Normal
2018
2020
2019
2018
2019
2020
Specific leaf area (cm^2 g^{-1})
175
150
125
100
Trichome density (cm^{-2})
250
200
150
100
50
Leaf water content (g H_2O g^{-1})
0.70
0.65
0.60
Photosynthetic rate
(µmol CO_2 m^{-2} s^{-1})
40
30
20
10
Stomatal conductance
(mol H_2O m^{-2} s^{-1})
0.6
0.4
0.2
0.0
Intrinsic water-use efficiency
(µmol CO_2 mol^{-1} H_2O)
200
150
100
50
Addition
Control
Reduction
Addition
Control
Reduction
Addition
Control
Reduction
Precipitation
Addition
Control
Reduction
Addition
Control
Reduction
Addition
Control
Reduction
Precipitation


Trichome density
Photosynthetic rate
Intrinsic water-use efficiency
Stomatal conductance
Standardized trait
1
0
-1
120
130
140
150
160
Snowmelt date (day of year)
Summer precipitation (mm)
175
150
125
100
75
50
25
Year
2018
2019
2020


Specific leaf area
Trichome density
Leaf water content
Standardized trait
4
2
0
-2
2
4
6
8
Summer mean of soil moisture in subplot (%VWC)
Year
2018
2019
2020
Photosynthetic rate
Stomatal conductance
Intrinsic water-use efficiency
Standardized trait
6
4
2
0
-2
0
5
10
15
20
Soil moisture next to plant or in subplot on closest date (%VWC)


(a)
Year
2018
2019
2020
Precipitation
Addition
Control
Reduction
Specific leaf area (cm² g⁻¹)
Trichome density (cm⁻²)
Leaf water content (g H₂O g⁻¹)
Photosynthetic rate (µmol CO₂ m⁻² s⁻¹)
Stomatal conductance (mol H₂O m⁻² s⁻¹)
Intrinsic water-use efficiency (µmol CO₂ mol⁻¹ H₂O)
Survival
100%
75%
50%
25%
0%
100 150 200 250
0 100 200 300 400 500
0.6 0.7 0.8
0 25 50 75
0.00 0.25 0.50 0.75 1.00 1.25
0 50 100 150 200
(b)
Specific leaf area (cm² g⁻¹)
Trichome density (cm⁻²)
Leaf water content (g H₂O g⁻¹)
Photosynthetic rate (µmol CO₂ m⁻² s⁻¹)
Stomatal conductance (mol H₂O m⁻² s⁻¹)
Intrinsic water-use efficiency (µmol CO₂ mol⁻¹ H₂O)
Flowering
100%
75%
50%
25%
0%
100 150 200 250
0 100 200 300 400 500
0.6 0.7 0.8
0 25 50 75
0.00 0.25 0.50 0.75 1.00 1.25
0 50 100 150 200


Year
2018
2019
2020
Precipitation
Addition
Control
Reduction
Specific leaf area (cm² g⁻¹)
Trichome density (cm⁻²)
Leaf water content (g H₂O g⁻¹)
Photosynthetic rate (µmol CO₂ m⁻² s⁻¹)
Stomatal conductance (mol H₂O m⁻² s⁻¹)
Intrinsic water-use efficiency (µmol CO₂ mol⁻¹ H₂O)
RGR (year⁻¹)
2
0
-2
100
150
200
250
0
100
200
300
400
500
0.6
0.7
0.8
0
25
50
75
0.00
0.25
0.50
0.75
1.00
1.25
0
50
100
150
200