

**The impacts of early snowmelt and summer
precipitation on the physiology and leaf
morphology of *Ipomopsis aggregata***

**Student: Jocelyn Navarro
Mentor: Diane R. Campbell**

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Abstract

Climate change is occurring, and the effects will be visible all over the planet. In a subalpine ecosystem, earlier snowmelt and changes in summer precipitation are predicted to occur due to an increase in air temperature. During the summertime, this variation may lead to changes in traits, a process known as phenotypic plasticity. In plants, these plastic changes could occur in morphological traits or physiological traits. This study aimed to find the impact of an earlier snowmelt as well as variation in summer precipitation on traits in *Ipomopsis aggregata*, a subalpine herb. In this study, we manipulated the snowmelt date and summer precipitation and measured three traits, photosynthetic water-use efficiency, specific leaf area, and trichome density, to determine if the environmental constraints are impacting the plant's physiology and leaf morphology. Plants that had experienced less precipitation had a higher photosynthetic water-use efficiency, but still had lower photosynthetic rates. Water-use efficiency was similar for plants receiving a 50% reduction in summer rainfall and plants forced to bloom one week early but receiving normal rainfall. There was no detectable impact of date of snowmelt or amount of precipitation on the specific leaf area of the plants just 9 days after the onset of the precipitation treatments.

Introduction

Plants are beautiful, an important component of our ecosystems and natural processes, and provide humans with a lot of benefits. Unfortunately, climate change is occurring (Rogora et al., 2018) and the effects of the anthropogenic crisis will be visible all over the planet. These changes will affect several types of organisms across the planet, including plants. Plants may respond in several ways. They could show shifts in range, they could exhibit changes in traits in direct response to the environment (phenotypic plasticity), or they could adapt through natural selection favoring new traits in the new environments that will develop (Jump and Penuelas, 2005). For example, changes in flower color, leaf size, leaf shape, plant height, and stomatal conductance may be favored in a new environment (Jump and Penuelas, 2005 and Conner, 2010). The phenotypic plasticity may, or may not, itself be adaptive. Alpine ecosystems are due to experience a high impact of climate change due to being a high latitude and altitude ecosystem (Wang et al. 2018). In an alpine environment, early snowmelt is predicted to occur due to increased air temperatures because of climate change (Wang et al., 2018 and Klein et al., 2016). In addition, changes in precipitation patterns are also predicted to occur (Gray and Brady, 2016). During the summertime, this variation may lead to changes in traits or phenotypic plasticity. These plastic changes could occur in morphological traits or physiological traits, among others (Gray and Brady, 2016).

A plant's physiology can be measured by various traits such as the water-use efficiency of photosynthesis. Water-use efficiency is defined as the ratio between photosynthetic rate to loss

of water through stomatal conductance. Stomatal conductance may be affected by the type of environment the plants are in (Zheng and Salem, 2016). When there is not enough water and the environment has a high temperature, plants do not open their stomata as much. Climate change could also influence vegetative traits such as specific leaf area (SLA= leaf area/dry mass) and trichome density(number of leaf hairs divided by the area of the leaf). In a drier environment, as that experienced in one with earlier snowmelt, specific leaf area is often low (Poorter et al., 2009). In addition, high trichome density is a plant trait that enhances drought avoidance (Gonzales et al., 2007 and Ehleringer and Mooney, 1978), so it is expected water availability may impact the leaf morphology by altering the specific leaf area and trichome density.

The subalpine plant *Ipomopsis aggregata* spends multiple years in the vegetative stage before flowering and setting seed. This species is an herbaceous perennial plant in the Polemoniaceae family. The leaves in this plant are organized in rosettes (Campbell and Waser, 2007). Previous studies near Rocky Mountain Biological Laboratory in Gunnison County, Colorado have shown that water-use efficiency, specific leaf area and trichome density all differ across populations with different environmental conditions (Campbell et al., 2005; Campbell et al., 2018). In addition, a previous experimental study showed that variation in summer precipitation impacted relative plant growth rate in *Ipomopsis tenuituba*, although not in *I. aggregata* (Campbell and Wendlandt, 2013). Additionally, another study identified the impact of earlier snowmelt on plant growth and reproduction by studying the flowering and fruiting of 10 angiosperms species, *Ipomopsis aggregata* being one of the species (Price and Waser, 1998). However, no study has identified how both earlier snowmelt and variation in summer precipitation will impact the traits of *Ipomopsis aggregata* by looking at the plant's photosynthetic water-use efficiency and leaf morphology. This study aims to fill in the gap and understand the role water availability plays in the phenotypic plasticity of *Ipomopsis aggregata* by implementing water manipulations and earlier snowmelt to *Ipomopsis aggregata*, and measuring the plant's physiology and leaf morphology.

In this study, we measured the phenotypic plasticity of *Ipomopsis aggregata* in an experiment that manipulates snowmelt date and summer precipitation in Gunnison County, Colorado. We measured three traits: photosynthetic water-use efficiency, specific leaf area, and trichome density to determine if the environmental constraints are impacting the plant's physiology and leaf morphology. This study is part of a larger study examining whether those phenotypic changes are adaptive, and also how these changes in the environment influence natural selection, as quantified by the relationships between traits like these and fitness measured as seed production.

Methods

This study was conducted in Maxfield Meadow, located 1 km south of Gothic, Gunnison County, Colorado (Fig. 7). In Maxfield Meadow, 6 large plots containing naturally occurring

Ipomopsis aggregata were created with water manipulations implemented. Of the 6 plots, 3 plots experienced normal snowmelt. In the other 3 plots, a black shade cloth laid down during March 29 to April 24, 2018 advanced the date of snowmelt by five days. Additionally, in each of the 6 plots, rain was manipulated in various ways. In one subplot, 50% of the rain was intercepted by clear plastic frames that were placed over the sites so when precipitation occurred, the subplot only received half of the rain that a subplot without this type of treatment would receive. Another subplot received 14 liters of water every 2 days (equivalent to an addition of 50% of average rainfall), and there were 2 controls. The water manipulations began on June 5th, 2018. One control had no treatment, and the other control had a sham rain-out shelter with sides but no top. Each large plot measured 7 x 10 m, and each subplot was 2 m x 2 m (Fig. 8). In each of these subplots, photosynthetic traits and leaf morphology were assessed for multiple plants

Plant Physiology

Leaf gas exchange was measured on one of the leaves on each plant. A Li-Cor 6400 XT portable photosynthesis machine was utilized on July 3rd, July 6th, July 10th, July 13th, July 17th, and July 27th to measure photosynthetic rate and stomatal conductance of each plant during the morning, when the stomata were open. These values were corrected for the area of the leaf inside the leaf chamber, by scanning the leaf and using ImageJ to find the area. Intrinsic water-use efficiency was calculated as photosynthetic rate divided by stomatal conductance. On each of the 6 days, one plant was measured from each of the 24 subplots, with the order chosen at random. Ten measurements could not be used because the plant had a stomatal conductance of zero or a negative c_i value. In total, 134 plants were measured. Soil moisture measurements were taken on 8 days throughout the month of July using a 12 cm probe that measures volumetric water content.

Leaf Morphology

On June 14, 2018, leaf morphology measurements were obtained from a single leaf from 4 randomly chosen vegetative plants in each subplot for a total of 96 leaves measured for specific leaf area and trichome density. The leaves were scanned, and ImageJ was utilized to find the leaf area and count the trichomes present in each leaf at 200% zoom to find the trichome density. The leaves were dried for 2 hours in a drying oven at 70 degrees celsius and weighed to determine dry mass in calculating the specific leaf area as $\text{area}/(\text{dry mass})$. These data provide information on those leaf traits at the onset of manipulations of conditions. A second measurement will be taken in mid-August to determine the impact of the water manipulations later in the study.

Analysis

The experimental design was a split-plot containing 6 large plots, where 3 experienced early snowmelt and 3 had normal snowmelt. Within each plot, there were 4 subplots that contain a water addition, a rainout, a sham shelter, and a control. To analyze each dependent variable, the average values for all the plants in the subplots was found. Then, a split-plot analysis of variance was utilized using R.

Results

Soil moisture

For both the earlier and normal snowmelt plots, the soil moisture was the highest for the plots that were treated with a water addition of 14 liters of water every other day (Table 1). For the earlier snowmelt plots, the water addition plots had 49% more soil moisture than the controls. When compared to plots in the rainout shelters, the water addition plots had 91% more soil moisture (Table 1). For the normal snowmelt plots, the plots that were treated with an addition of water had 68% more soil moisture than the controls (Table 1). The water addition plots had 133% more soil moisture than the plots in the rainout shelters (Table 1).

Leaf gas exchange

The rate of photosynthesis was 51% greater for plants that received additional water when compared to plants that were under the rainout shelters ($F_{3,12}=3.646$, $P=0.0446$; Fig. 3). The stomatal conductance was 135% greater for plants that received additional water when compared to plants that were under the rainout shelter ($F_{3,12}=6.461$, $P=.00751$; Fig. 4). Plants in treatments that had experienced less precipitation had a higher photosynthetic water-use efficiency, even those that had a lower photosynthetic rate. Water-use efficiency was similar for plants receiving a 50% reduction in summer rainfall and plants forced to bloom one week early but receiving normal rainfall (Fig. 2). Overall, plants that were under the rainout shelters had a higher water-use efficiency of 51% when compared to plants that received additional water. Water-use efficiency varied for plants depending on the amount of precipitation the plants received ($F_{3,12}=9.12$, $P=.0020$; Fig. 2). However, there was no statistically significant effect of snowmelt date on water-use efficiency of the plants ($F_{1,4}=1.47$, $P=0.2927$).

Specific Leaf Area

There was no detectable impact of date of snowmelt ($F_{1,4}=1.89, P=.686$) or amount of precipitation on the specific leaf area of the plants just 9 days after the onset of the precipitation treatments ($F_{3,12}=0.005, P=.945$; Fig. 5).

Trichome Density

There was no detectable impact of date of snowmelt ($F_{1,4}=0.12, P=.747$) or amount of precipitation on the trichome density of the plants 9 days after the start of the precipitation treatments ($F_{3,12}=0.029, P=.993$; Fig. 6).

Discussion

Water availability plays a crucial role in a plant's physiological response to the environment. Plants in water-limited environments often have low stomatal conductance and high water-use efficiency (Hetherington and Woodward, 2003). In this study, plants that were in rainout shelters had a lower stomatal conductance when compared to the stomatal conductance of plants in the control and water addition subplots (Fig. 4). A reduction in stomatal conductance increases water-use efficiency (Hetherington and Woodward, 2003), which resonates with the findings in this study (Fig. 2). Plants that were in harsh environmental conditions, such as a reduction in summer precipitation perhaps enhanced their water-use efficiency as a plastic response to the low water availability. Plastic responses are a result of inevitable impacts of environmental constraints on a plant's growth and physiology (Sultan, 1995). A plant may alter its physiology as a response to its environment, and this plasticity may be adaptive in the new environment (Sultan, 2000). A study measuring the water-use efficiency of *Ipomopsis aggregata*, *Ipomopsis tenuituba*, and its hybrids also found increases in water-use efficiency in the dry treatment (Campbell and Wendlandt, 2013). The higher water-use efficiency was a result of a change in stomatal conductance across the various species (Campbell and Wendlandt, 2013). However, in this study, the difference in water-use efficiency across treatments is attributed to both the rate of photosynthesis and stomatal conductance (Figures 3 and 4).

It is not yet clear whether date of snowmelt or amount of summer precipitation influence trichome density or specific leaf area in the experiment. So far these traits have only been measured 9 days after the treatments were fully implemented, and so represent initial conditions. A study that saw an impact on an environmental stress on the trichome density of a plant had implemented the treatments 5 weeks prior to measuring the traits (Kjaer et al., 2012). A second set of morphological traits will soon be taken to see if there is a difference 2 months after the

treatments were implemented. Another study analyzing the physiological and morphological plasticity of *Sesleria nitida*, a perennial herb located in a high latitudinal gradient, indicated a larger plasticity for physiological than for morphological traits (Gratani, 2014). In the study mentioned earlier regarding *I. aggregata*, *I. tenuituba*, and its hybrids, there was no differential effect of the precipitation treatment on the specific leaf area of the plants, although the study measured these traits after 11 to 25 days (Campbell and Wendlandt, 2013). Perhaps it takes longer for morphological plastic responses to occur than for physiological plastic responses to the environment to occur, or plasticity for physiological traits may be larger.

We found no evidence that earlier snowmelt by five days impacted the plant physiology of *Ipomopsis aggregata* in this study. Maxfield Meadow, the location of the study, received remarkably less snow than it is previously accustomed to (b. barr, unpublished data). Because snowmelt was so early in 2018 (April 30) and coincided with a few warm days, it was difficult to accelerate it by much. In a year with a later date of snowmelt, it would be easier to accelerate snowmelt by a larger amount. It would be interesting to test if a larger difference in snowmelt date has a larger effect on these plant traits.

In the future, we would like to continue this study for another year to determine if the effects take longer to develop. This will allow us to see if there is difference between the treatments and a plant's physiological and morphological response to the earlier snowmelt as well as if the impact of variation in summer precipitation continues to persist. We will also assess whether or not the plastic changes in leaf gas exchange are adaptive, by measuring the fitness of these plants in terms of survival to the next year and eventual seed production.

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Tables and Figures

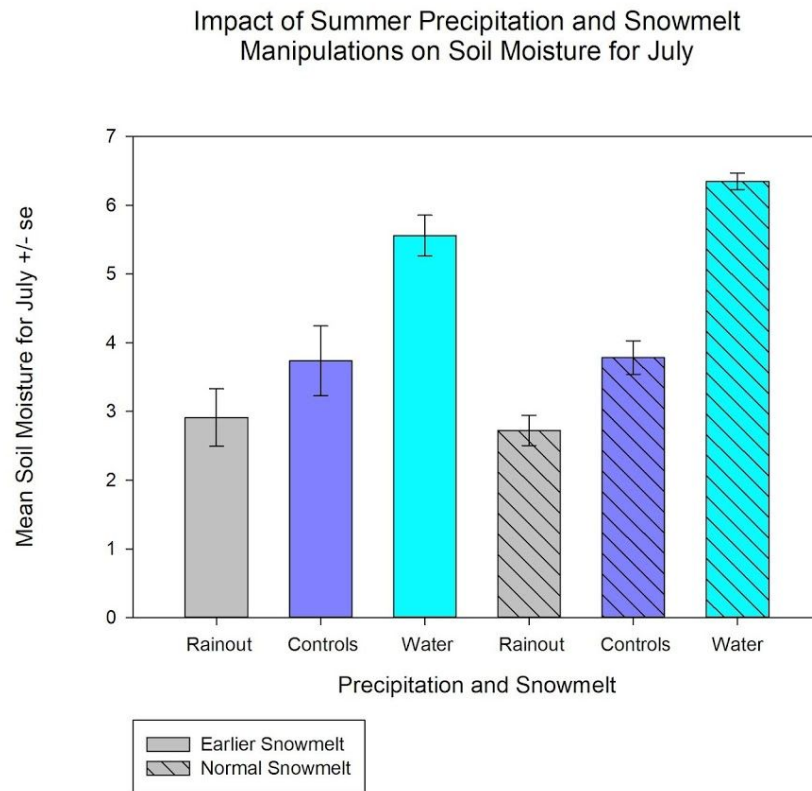


Figure 1. Mean soil moisture with standard error of the 24 subplots in the study for the month of July, when physiological traits were measured. Half of the 24 subplots experienced earlier snowmelt and the other 12 experienced normal snowmelt. Additionally, 6 of the subplots had 50% of the precipitation intercepted, 6 received a water addition of 50%, and 12 received normal precipitation.

Impact of Summer Precipitation and Snowmelt Manipulations on Water-Use Efficiency

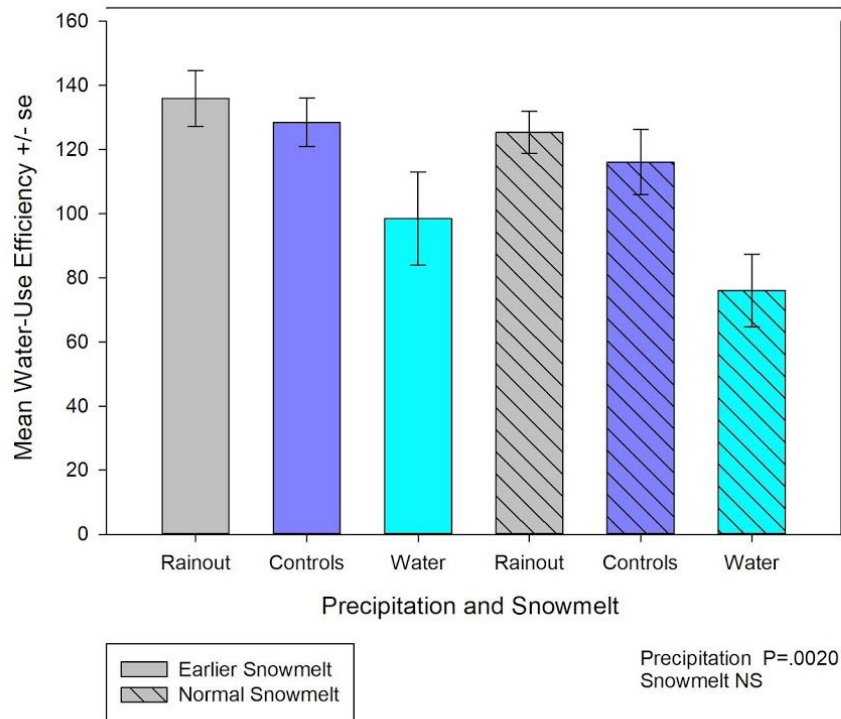


Figure 2. Mean water-use efficiency with standard error of the the 24 subplots in the study. Half of the 24 subplots experienced earlier snowmelt and the other 12 experienced normal snowmelt. Additionally, 6 of the subplots had 50% of the precipitation intercepted, 6 received a water addition of 50%, and 12 received normal precipitation.

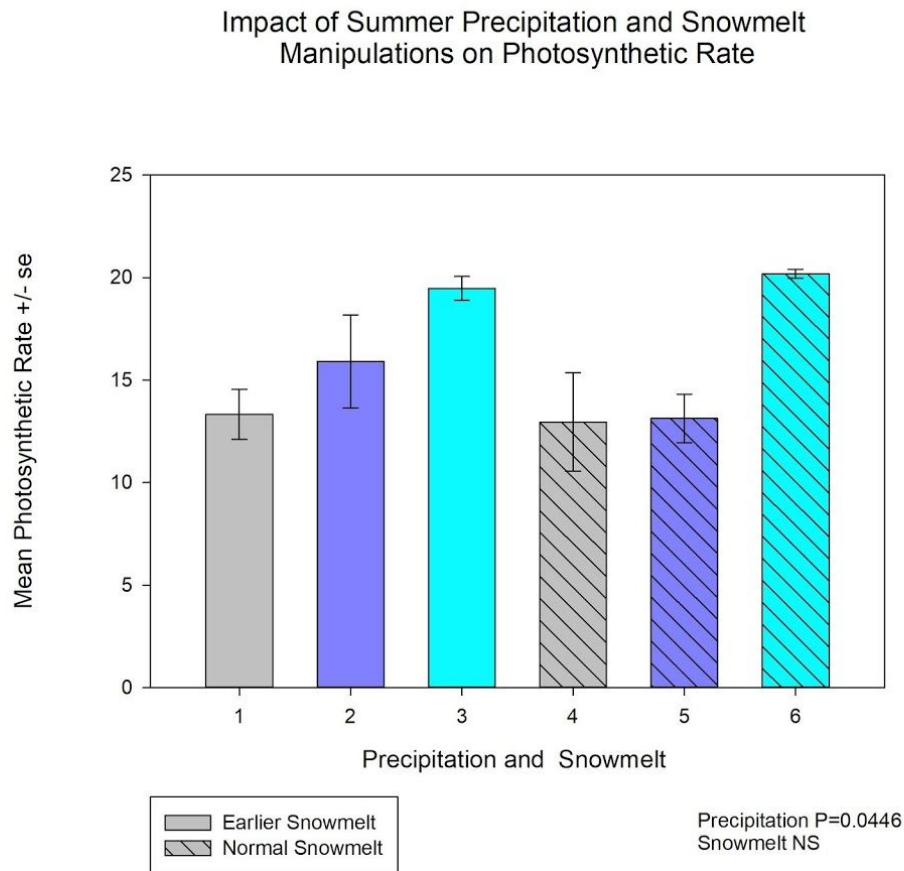


Figure 3. Mean photosynthetic rate with standard error of the *Ipomopsis aggregata* leaves measured from the 24 subplots in the study. Half of the 24 subplots experienced earlier snowmelt and the other 12 experienced normal snowmelt. Additionally, 6 of the subplots had 50% of the precipitation intercepted, 6 received a water addition of 50%, and 12 received normal precipitation.

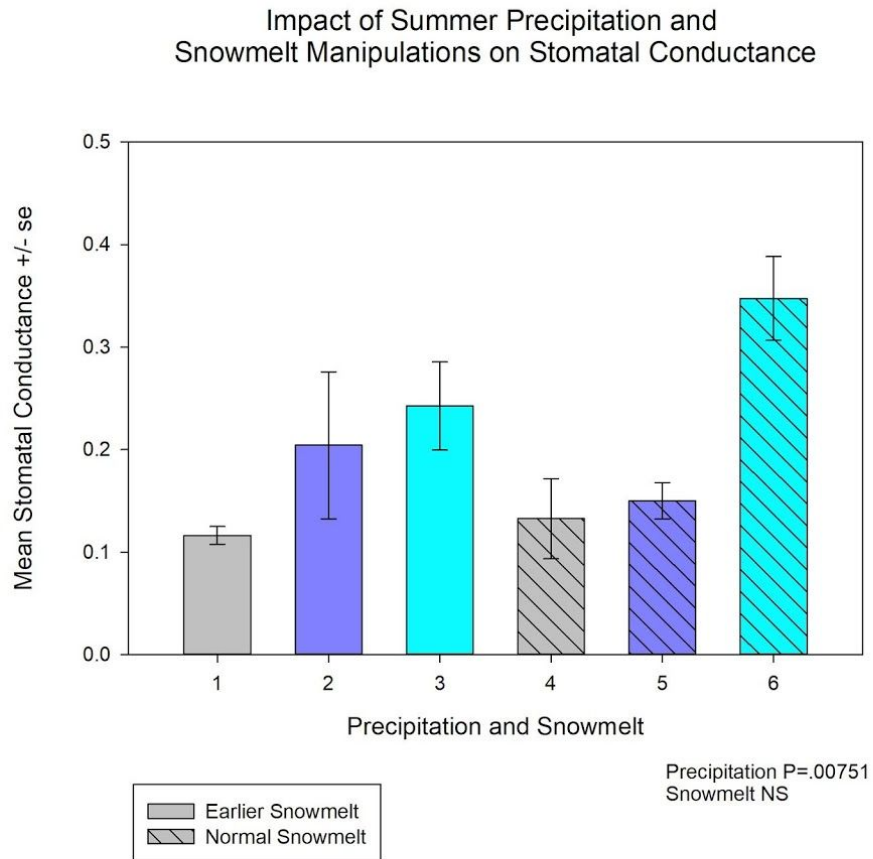


Figure 4. Mean stomatal conductance with standard error of the *Ipomopsis aggregata* leaves measured from the 24 subplots in the study. Half of the 24 subplots experienced earlier snowmelt and the other 12 experienced normal snowmelt. Additionally, 6 of the subplots had 50% of the precipitation intercepted, 6 received a water addition of 50%, and 12 received normal precipitation.

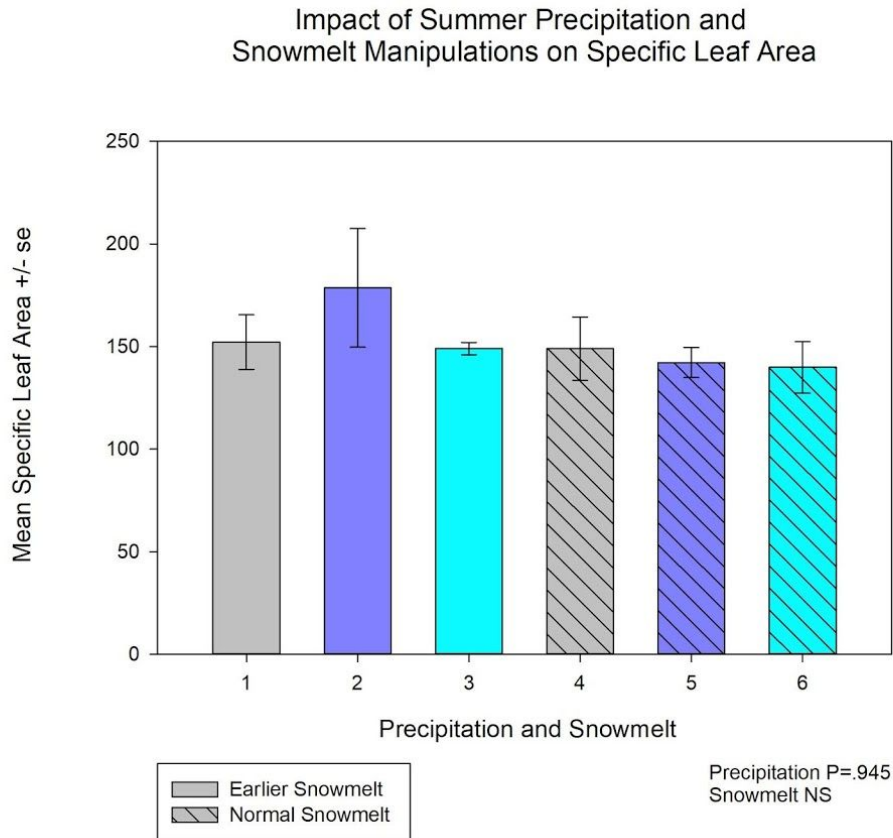


Figure 5. Mean specific leaf area with standard error of the *Ipomopsis aggregata* leaves measured from the 24 subplots in the study. Half of the 24 subplots experienced earlier snowmelt and the other 12 experienced normal snowmelt. Additionally, 6 of the subplots had 50% of the precipitation intercepted, 6 received a water addition of 50%, and 12 received normal precipitation.

Impact of Summer Precipitation and Snowmelt Manipulations on Trichome Density

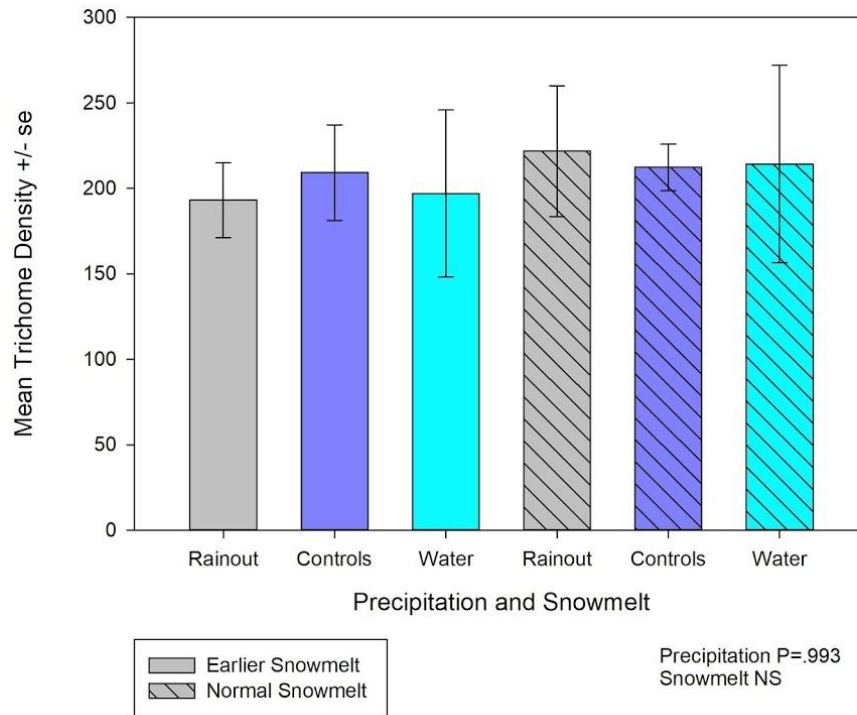


Figure 6. Mean trichome density with standard error of the *Ipomopsis aggregata* leaves measured from the 24 subplots in the study. Half of the 24 subplots experienced earlier snowmelt and the other 12 experienced normal snowmelt. Additionally, 6 of the subplots had 50% of the precipitation intercepted, 6 received a water addition of 50%, and 12 received normal precipitation.

Table 1. Mean soil moisture (% volumetric water content) of the 24 subplots in the study for the month of July, when physiological traits were measured. Half of the 24 subplots experienced earlier snowmelt and the other 12 experienced normal snowmelt. Additionally, 6 of the subplots had 50% of the precipitation intercepted, 6 received a water addition of 50%, and 12 received normal precipitation.

Snowmelt Date	Rainout	Controls	Water
Early	2.91	3.73	5.56
Normal	2.72	3.78	6.35



Figure 7. This study was conducted in Macfield Meadow, located 1 km south of Gothic.

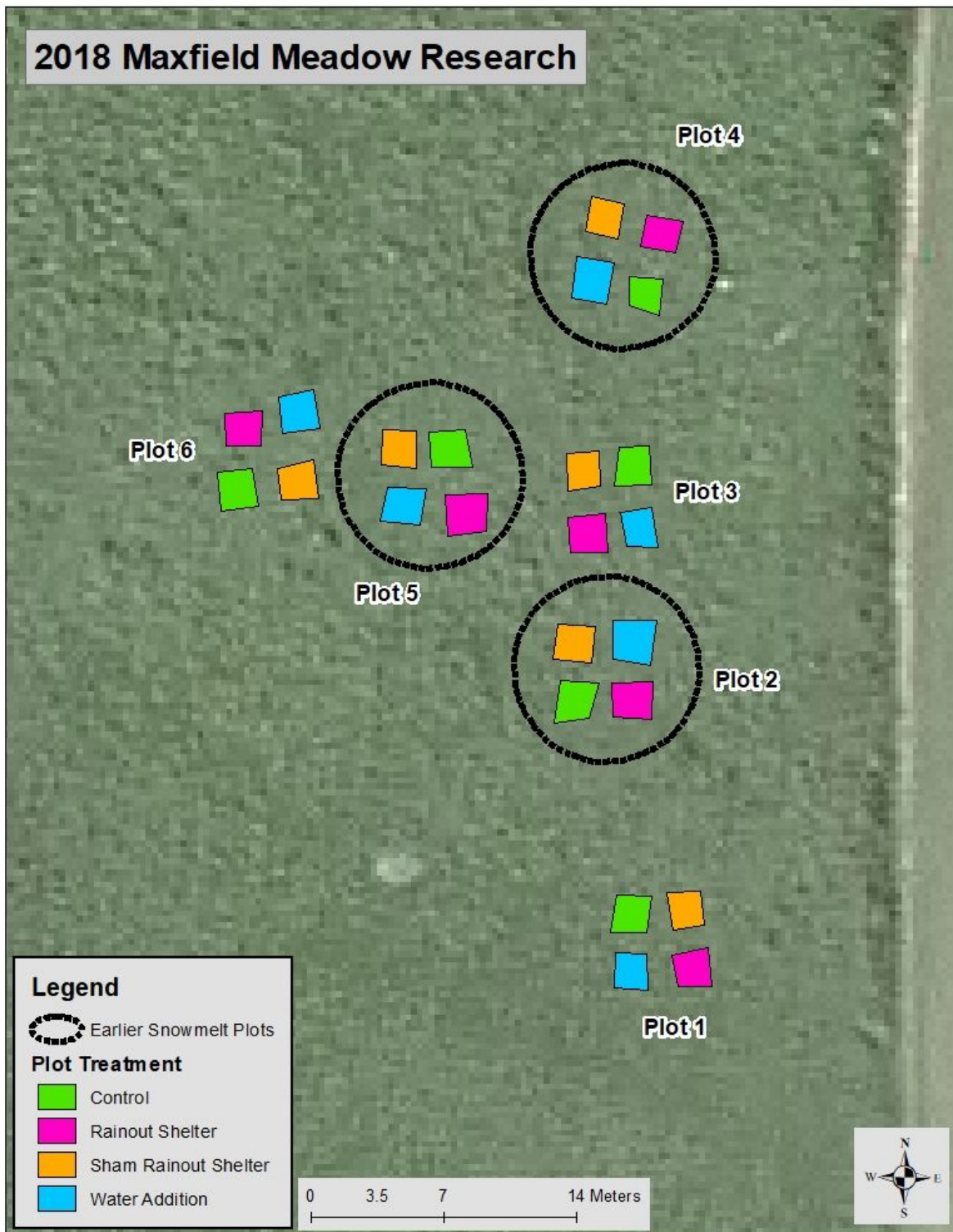


Figure 8. This study was a split-plot design. There were 6 large plots with 4 subplots for a total of 24 subplots. Half of the 24 subplots experienced earlier snowmelt and the other 12

experienced normal snowmelt. Additionally, 6 of the subplots had 50% of the precipitation intercepted, 6 received a water addition of 50%, and 12 received normal precipitation.