

The Effects of Early Snowmelt on Drought Stress in Subalpine Plant Species

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Abstract:

Subalpine plant species rely on water from snowmelt and monsoons to sustain them through the summer. However, climate change is causing the snow to melt earlier which creates a drought period in June prior to the monsoons and may cause plants to become drought stressed. In order to assess the effect of early snowmelt on subalpine plant drought stress, we conducted a snowmelt timing manipulation using shade cloth to accelerate the timing of snowmelt. To assess the degree of drought stress we measured stomatal conductance in *Linum lewisii* and *Geum triflorum*, chlorophyll fluorescence in *Geum triflorum*, and the number of flowers on each plant. We found that early snowmelt had no effect on drought stress in *L. lewisii* and *G. triflorum* as we saw no differences in stomatal conductance, chlorophyll fluorescence nor stomatal conductance over the flowering period. The lack of drought stress, even with earlier snowmelt, may be due to the large amount of June precipitation which gave the plants adequate water to sustain themselves through the drought period previously seen. This suggests that precipitation has a large impact on subalpine plant species, so it is important to monitor how timing and amount of precipitation affects plants in a changing climate.

Introduction:

Snowmelt is crucial for the survival of many subalpine plant species as it serves as one of their main sources of water. Subalpine areas experience a dry period in June and a monsoon season from July through August. Subalpine plants can withstand this dry period because of leftover moisture from snowmelt (Sloat et al. 2015). However, climate change is causing temperatures to warm at an alarming rate globally. The Earth's climate naturally shifts through cycles; but, increased emissions of greenhouse gasses have accelerated this process at a rate that is detrimental to global ecosystems (US EPA 2021). Global warming is causing snow to melt earlier and as a result, plants must withstand a lack of soil moisture for longer before the monsoon season (Stewart et al. 2004). This increased dry period puts plants under drought stress which can cause them to alter their functions to try to survive (Obeso 2002). Alternatively, climate change is causing precipitation to become more variable so while some years may see this drought period, others will experience an abundance of rain during this time (IPCC, 2023). It's possible that a heavy rain year can decrease the dry period in June and allow plants to get an adequate amount of water from precipitation rather than relying on runoff from snowmelt. Drought stress causes many physiological changes in plants like decreased productivity and increased water storage (Powers et al. 2022). When plants are put under drought stress, they can conserve water by closing the stomata, a cell in the epidermis that opens and closes to facilitate gas exchange for photosynthesis (Navarro et al. 2022 and Winkler et al. 2018). When stomata open it releases water vapor; to reduce desiccation plants are closing their stomata at the cost of decreased carbon assimilation, making them less efficient when photosynthesizing (Buckley 2019 and Heschel and Riginos 2005). Decreased efficiency when photosynthesizing over an extended period of time can alter the photosynthetic structure of a plant, eventually leading to less efficient chlorophyll fluorescence (Flexas et al. 2002 and Krause and Weis 1984). If a plant

is under drought stress we would also see a change in stomatal conductance over the flowering period. If a plant has limited resources it's stomatal conductance will be lower at the beginning of the flowering period and then continue to stay low. This is because the plant will favor allocating resources to reproductive structures rather than to leaves and photosynthesis (Bazzaz, Fakhri A., et al. 2024).

Many studies have evaluated plants' response to drought stress, however, there is little understanding of how snowmelt timing affects drought stress. To answer these gaps in knowledge, this study assesses the drought stress of two subalpine species during their flowering period using a snowmelt timing manipulation. For this project I looked at three main questions: Q1.) How does snowmelt timing impact drought stress, measured by stomatal conductance in *Linum lewisii* and *Geum triflorum*, Q2.) How does snowmelt timing affect chlorophyll fluorescence and how does that trait relate to stomatal conductance in *Linum lewisii* and *Geum triflorum*, and Q3.) How does stomatal conductance and chlorophyll fluorescence vary across the flowering period of both species? My hypotheses follow the structure of my questions: H1. Early snowmelt timing will cause decreased stomatal conductance in an effort to decrease water loss. H2. Earlier snowmelt timing will decrease chlorophyll fluorescence due to a decrease in photosynthetic efficiency. H3. Stomatal conductance and chlorophyll fluorescence will decrease over the flowering period as plants allocate more resources for fruit production. The plants *Geum triflorum* and *Linum lewisii* are ideal for this study because of their abundance and flowering times during mid-summer. Additionally, the two species are from different genera which allows us to study effects of snowmelt timing on a more diverse group of species.

It is important to understand how a warming climate is going to affect different ecosystems. By testing how key plants such as *Geum* and *Linum* are affected by early snowmelt we can predict how ecosystems are going to respond. These plants play a crucial role in the ecosystem, and it is critical to see if they are going to be able to adapt to these conditions or if we will need to implement conservation strategies to preserve the environment's equilibrium.

Methods:

This study was conducted at the Rocky Mountain Biological Laboratory in a subalpine meadow ecosystem. The Rocky Mountain Biological Laboratory is located in Gothic, Colorado at an approximate elevation of 9,500 ft. The study utilizes a snowmelt timing experimental manipulation which includes eight experimental blocks that each include one early snowmelt plot and control plot that were established in 2020-2022 by Dr. Amy Iler and Elsa Godtfredsen (Figure 1). To simulate early snowmelt timing, we placed black shade cloth on the early snowmelt plots when there was approximately one meter of snow remaining to accelerate the timing of snowmelt using solar irradiation. The shade clothes were removed when all snow had melted across the plot. Early snowmelt and control plots are located as close together within a block as possible to control possible environmental variances across the meadow. Five of the experimental blocks are in the lower research meadow and three are in the upper research meadow. Plots are five meters tall and 1.2 meters across with 0.7-meter walkways and an area of 18 m² total.

I looked at *Geum triflorum* and *Linum lewisii*, which are both subalpine herbs. Both species grow in habitats across North America and are found in mountain meadows, rocky areas, open grasslands, and prairies (Addicott 1977). These flowers are abundant in Gothic, Colorado and are found in substantial numbers in the subalpine meadow where the plots are located. In our plots, we have 60 marked individuals of both *Geum* and *Linum* (30 per treatment) evenly distributed across the blocks.

Once a week I took stomatal conductance measurements using the SC-1 meter porometer. Before measurements were taken, the SC-1 was fully calibrated following instructions at METER to control for humidity and temperature variation. The two species were sampled differently due to differing leaf morphologies. On *Geum* I sampled leaves that are secondly basal, fully green, healthy, fully expanded, and in full or partial sun. On *Linum* I needed to pick 3-5 leaves to make a mat to place on the sensor (following METER protocol for sampling small leafed species). These leaves were fully green and healthy, in full or partial sun, and taken from the middle of the stem. To control variances in stomatal conductance due to light availability from weather and time of day we only sampled from 9:00 to 14:00 on fully clear sunny days. Measuring stomatal conductance is crucial for understanding how plants are responding to different soil moisture conditions. The SC-1 porometer gives us a proxy for stomatal status (open or closed) by measuring the humidity which is created by the leaf. To get more information about how efficiently plants are photosynthesizing (which could be impacted by long term stomatal closure) I used the LI-600 to measure chlorophyll fluorescence on *Geum*. This measures the light emitted when chlorophyll molecules are excited by the sun and allows us to understand the photosynthetic efficiency of the plant. I also counted the number of flowers on each plant every week to understand the timing of the flowering period and compare it to any changes in photosynthetic ability due to resource allocation. I used a soil moisture meter (TDR 100) to measure soil moisture every week to compare differences in water availability between treatment and control plots.

This data was recorded by hand on paper data sheets, transferred to excel, and analyzed using R. Due to the extreme influence of time of day on stomatal conductance measurements, we needed to account for time of day's influence in an inherent way in the data prior to analyses. To do so we regressed the stomatal conductance measurements against time of day and added the residuals from that analysis to the raw measurements to create a modified stomatal conductance measurement that accounted for time of day that was used in all analyses. To analyze the effects of treatment on stomatal conductance, linear mixed effects models were used that incorporated treatment as a fixed effect with plot nested within block, DOY and plant individual as random effects (to account for spatial and temporal non-independence). To understand the relationship between chlorophyll fluorescence and stomatal conductance, a linear mixed effect model was used that incorporated stomatal conductance as a fixed effect with plot nested within block, DOY and plant individual as random effects (to account for spatial and temporal non-independence). Lastly, to analyze the effect of flowering period on stomatal conductance, a linear mixed effect model was used with DOY as a fixed effect with plot nested within block and plant individual as random effects. To incorporate the possible interactive relationship between DOY and treatment, treatment was also included in these models as an interactive fixed effect.

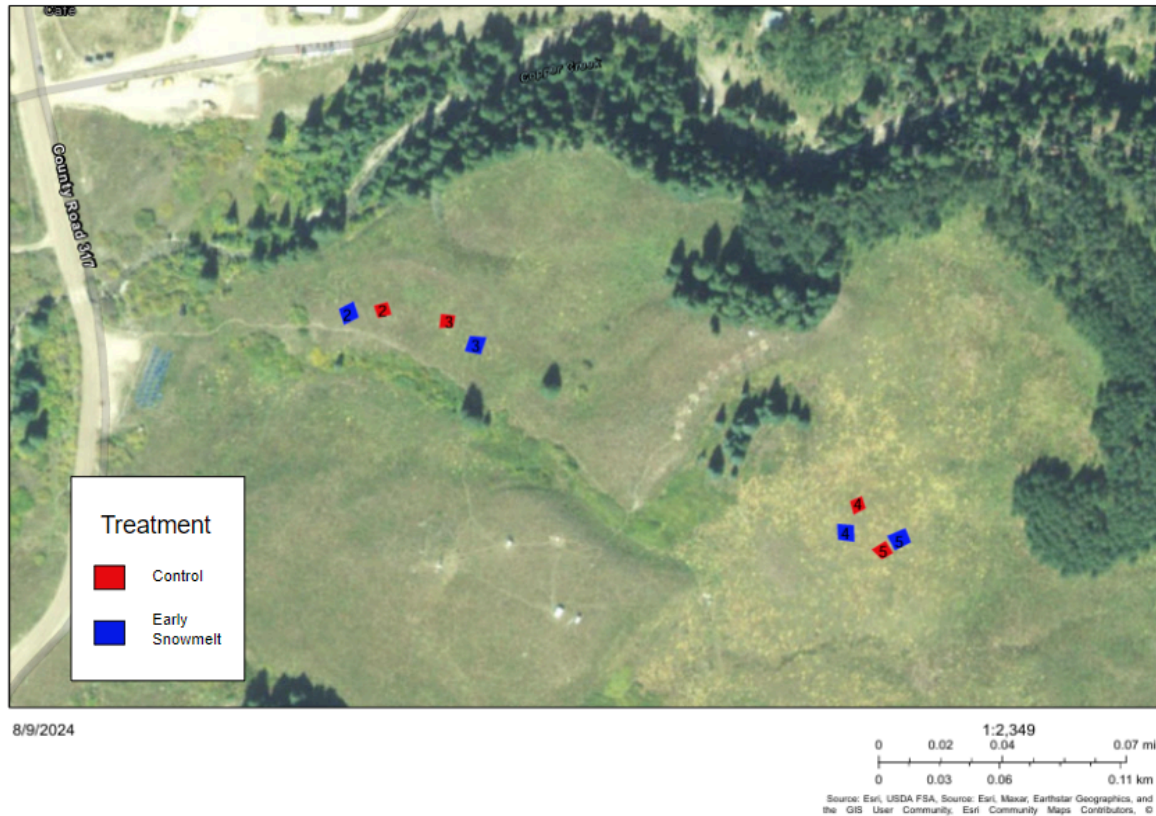


Figure 1: Aerial view of blocks in research meadow with number labels of each block.

Results:

Model Formula	Plant	Instrument	SE	T-value	P-value
SC_Time ~ Clor_Flor + (1 Block/Plot) + (1 DOY) + (1 Plant_Number)	G. triflorum	LICOR	.4754	0.120	0.809
SC_Time ~ Treatment + (1 Block/Plot) + (1 DOY) + (1 Plant_Number)	G. triflorum	METER	23.549	0.248	0.8039
SC_Time ~ Treatment + (1 Block/Plot) + (1 DOY) + (1 Plant_Number)	L. lewisii	METER	17.67	0.152	0.879

Changes in stomatal conductance in response to drought stress.

To test variances in stomatal conductance between the two treatments we ran a linear mixed model and checked its fit through a qq plot and residual plot. We found that treatment plots had similar average stomatal conductance for both *L. lewisii* and *G. triflorum* compared to control plots. Thus, we found that the size of the difference in stomatal conductance relative to the variation in treatment versus control plots was not significant (see figure 2). When graphed as a boxplot we see that there is minimal variation in both *G. triflorum* and *L. lewisii* in the stomatal conductance of snow and treatment plots (figures 3 and 4). Average soil moisture for both plots over the course of 3 months was recorded at 11.8 VWC.

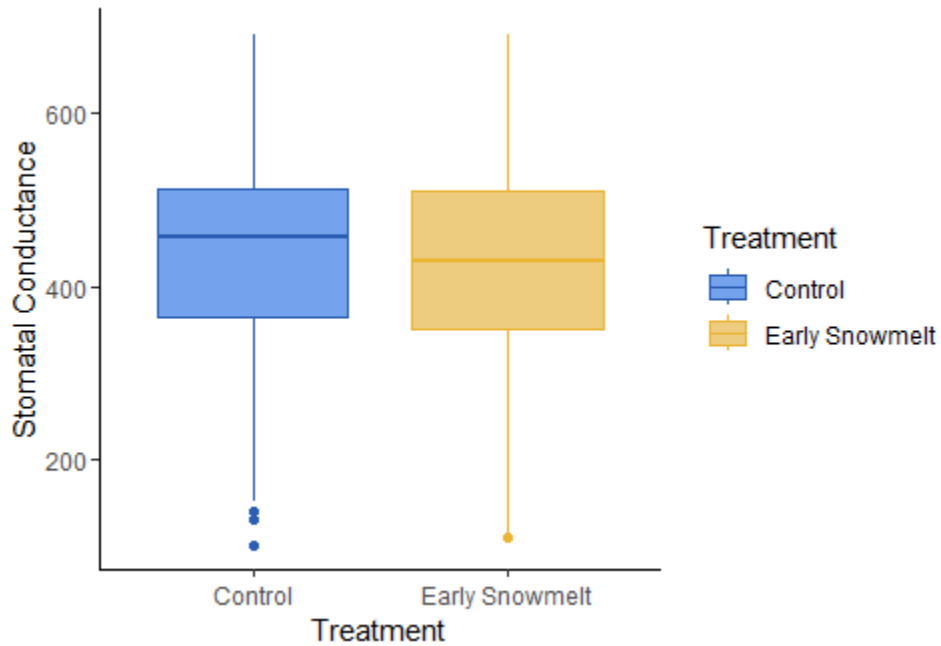


Figure 3: Stomatal conductance of *Geum* for early snowmelt plots and control plots

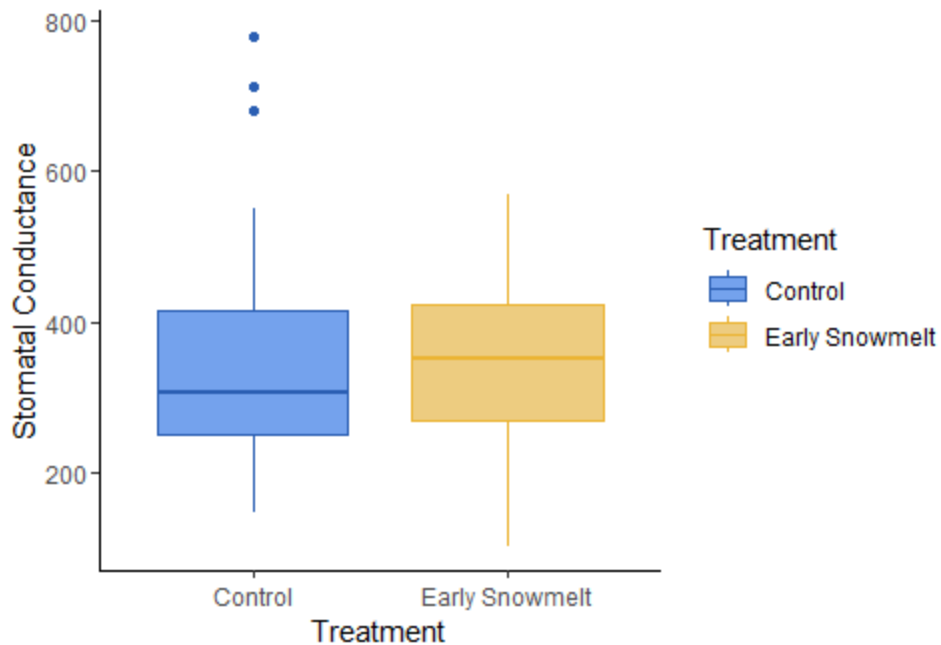


Figure 4: Stomatal conductance of *Linum* for early snowmelt plots and control plots

Changes in chlorophyll fluorescence in response to drought stress

After running a linear mixed model we found no significant differences in chlorophyll fluorescence in *G. triflorum* for control and treatment plots (see figure 2). We also see that there was minimal correlation between chlorophyll fluorescence and stomatal conductance (figure 5).

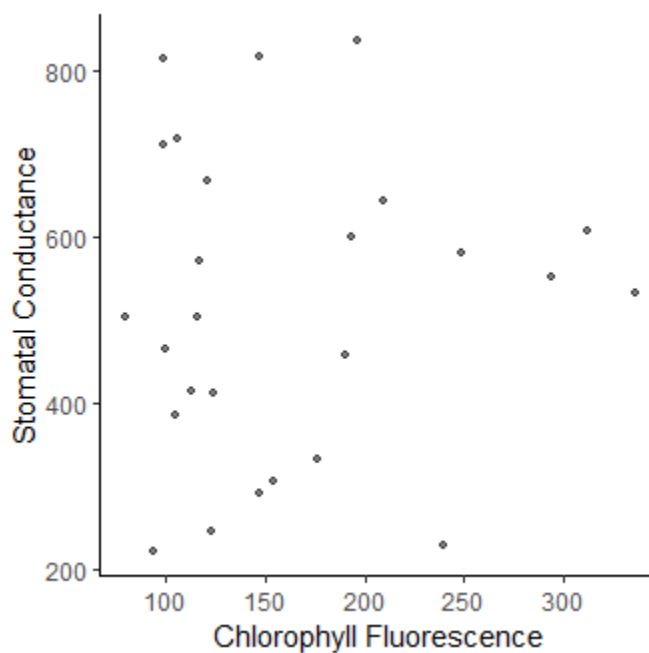


Figure 5: Scatter Plot of chlorophyll fluorescence compared to stomatal conductance.

Changes in stomatal conductance over the flowering period

We found that DOY accounted for the largest variation in stomatal conductance between plants. In *G. triflorum* we found that stomatal conductance in both the treatment and control peaked during the flowering period and dropped off at the end of the flowering period (see figure 6). In *G. triflorum* we found that stomatal conductance also peaked at the start of the flowering period but showed more variation in stomatal conductance towards the end of the flowering period (figure 7).

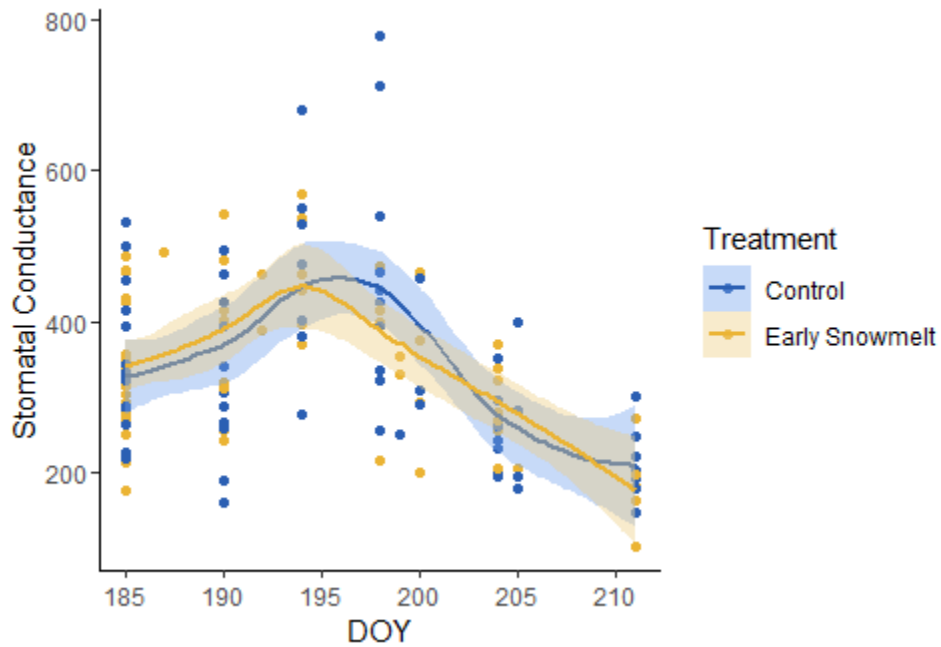


Figure 6: Stomatal conductance of *Linum* compared to day of year

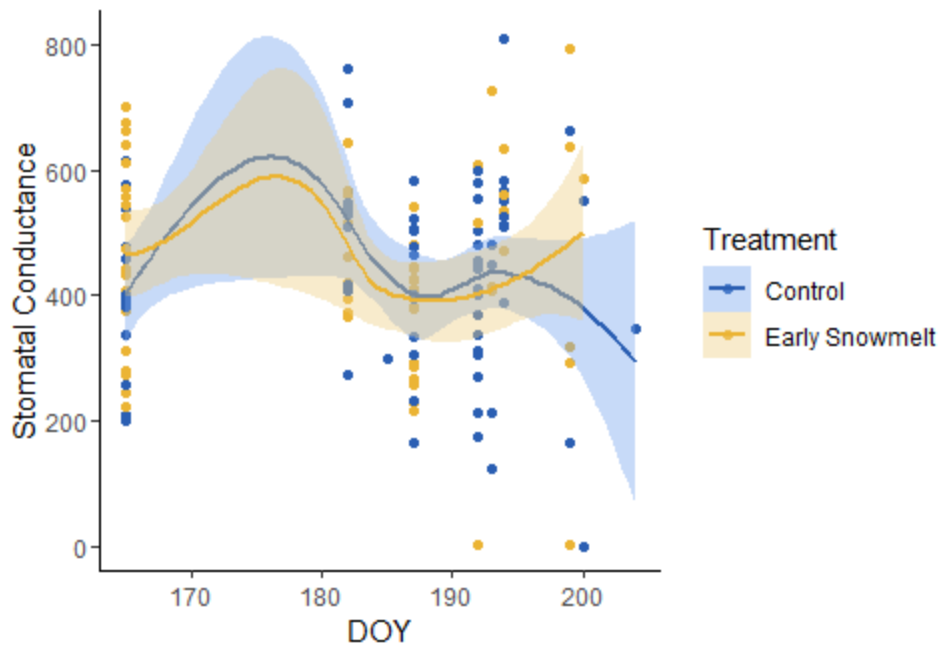


Figure 7: Stomatal conductance of *Geum* compared to day of year

Discussion:

While climate change is undoubtedly causing earlier snowmelt, it is also affecting the average precipitation. The amount of water availability in June is the deciding factor of whether plants are going to get enough moisture until the monsoon season in July, however if June gets more precipitation in one year it may close the gap between moisture from snowmelt and moisture from monsoons. Climate change is causing more variation in precipitation (IPCC, 2023). So, some years the plants will be getting more than enough water while other years they will have to make it through a longer period of drought. The amount of rain in June essentially erased the dry period we expected to see in so precipitation may have a larger impact on drought stress in plants compared to snowmelt.

Plant responses to drought stress

It is most likely that we are not seeing any changes in stomatal conductance in the treatment plots compared to control plots because precipitation is the main deciding factor of how much water plants are receiving. Since 2024 was a heavy rain year, with 2.56" in June (Billy Barr, figure 8), both plots received a sufficient amount of water in June which closed the gap for the drought period previously seen. This is evidenced by the fact that both plots had the same soil moisture levels throughout the observation period. Since both plots were receiving an adequate amount of water the plants did not need to conserve water by closing their stomata so we did not see a decrease in stomatal conductance. No differences in chlorophyll fluorescence were seen because plants were photosynthesizing at the same level of efficiency because they were not experiencing drought stress due to the increase in precipitation.

Changes in stomatal conductance over flowering period

Linum produces new leaves before the flowering period and retains those same leaves throughout its lifecycle. New leaves have the highest photosynthetic capacity and as they age their capacity is decreased. However, *Geum* has a differing flowering period with gaps, and continues to produce new leaves throughout its flowering period. This explains why we do not see as steep of a drop off in stomatal conductance since the new leaves continue to photosynthesize. *Geum* and *Linum*'s photosynthetic capacity is highest in the early stages of the flowering period to produce and store energy to create fruit. Once the plant begins to produce fruit its photosynthetic capacity is reduced because it is focusing more energy on fruit production rather than photosynthesizing. So, over the flowering period its stomatal conductance decreases as it is allocating more resources for fruit production as the leaves age and become less efficient.

Discrepancies

This study was conducted over one season and with varying precipitation it is difficult to get an accurate representation of how early snowmelt may affect plants with such limited data. To get a more clear picture of the effects of early snowmelt it is best if this study is repeated over several years. This accounts for changes that might be seen with an increase or decrease in rainfall and allows us to see the general trend of precipitation and its effects. We used a Meter Porometer to measure both *G. triflorum* and *L. lewisii* and were only able to use a LICOR to get 30 samples of *G. triflorum* as *L. lewisii*'s leaves were too small for the LICOR sensor. The measurements of the two devices varied too much so we only analyzed stomatal conductance using the meter porometer. However, if this study were to be reproduced it would be best to use a LICOR for its updated technology and additional sensor. We used plants in plots across a research meadow but controlled for variation in location by placing control and treatment plants close together. We also tested random effects to look at variation and found that plot and block had no effect on the variance of our results.

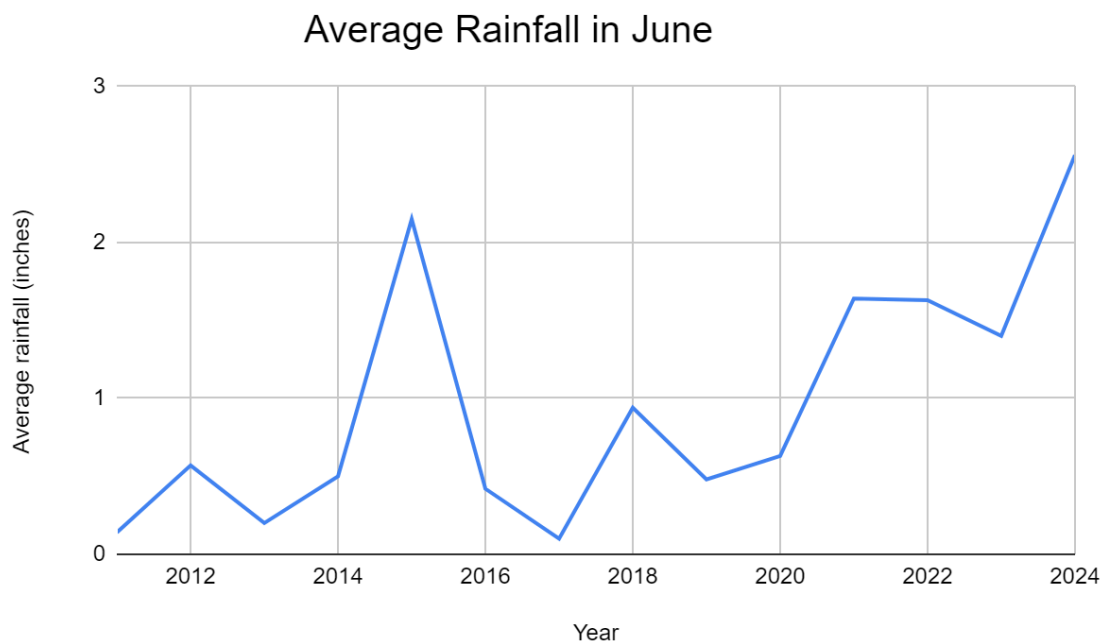


Figure 8 : visualization of Billy Barr's data on average precipitation in June from 2011-2024

Conclusion:

Linum lewisii and *Geum triflorum* are part of an interconnected network of plants, insects and animals that inhabit the subalpine ecosystem. Climate change is causing alterations to these ecosystems and it is important that we work to understand how a changing climate is going to impact and create a changing ecosystem. If we do more work to understand how plants are going to react to earlier snowmelt and more variable precipitation then we can predict what changes we might see in the ecosystem and in the future

In this study we found that early snowmelt did not have an impact on drought stress because heavy precipitation eliminated the period of drought in June. Overall, when adequate water is present from precipitation in the month of June plants are not reliant on water from snowmelt and therefore do not become drought stressed when the snow melts early. To get a more clear understanding of how plants will respond to early snowmelt and changing weather patterns it is best if this study is conducted over multiple years to account for variation in precipitation. Future studies should include precipitation as a studied and controlled variable to adequately assess its impacts. Future directions might also include comparing whether precipitation is more important than snowmelt timing for maintaining healthy plants.

Acknowledgements:

Thank you to Elsa Godtfreksen for mentoring me and making this project possible. Additionally, thank you to Rocky Mountain Biological Laboratory for making this project possible by providing the space and resources to do research.

MAJOR PROFESSIONAL OBJECTIVES:

My objectives are to develop the skills to do field work. I want to learn to work in a hands-on environment and develop complex research based on my observations of the natural world. By completing this program I hope to further the knowledge of how plants are reacting in response to climate change and contribute to a growing understanding of how various ecosystems function.

PROJECTED OUTCOMES.

I plan to complete a research project this summer that I will present at the RMBL symposium in August and will be added to the RMBL publications database. My goal is to contribute to the growing knowledge and data on plant responses to climate change. I hope to procure data that can be used in future research to better our understanding of the natural world.

DATA ARCHIVING:

As part of the RMBL Data Archiving, Curation, and Vouchers, I will participate in workshops and training for spatial and non-spatial metadata and archiving. My Data Management Plan includes a description of the datasets being archived, the dissemination methods, and the timeline for data sharing. I will use the RMBL template metadata form. I will use the default data

repository Environmental Data Initiative (EDI), <https://environmentaldatainitiative.org/> unless my mentors request a different repository.

ENVIRONMENTAL IMPACTS

The short-term impacts will be the trampling of plants around the field sites, the removal of leaves on Linum, and change in water availability. To mitigate the effects of pulling leaves from Linum we will only be pulling 3-5 leaves every week and we will vary which stems we choose from. The trampling will not have a long-lasting effect as it will have a chance to regrow once the experiment is concluded. This project should have no long-term impact because once the experiment is concluded plants will no longer be in drought stress and can resume their usual functioning.

RELATIONSHIP TO EXISTING RESEARCH

The plots for this research project were established in 2020 by Amy Iler and additional plots have been added by Elsa Godtfredsen in 2021 and 2022. This work relates to Elsa Godtfredsen's PhD work in the same climate manipulation which started in 2021 and is in its final year in 2024 and Dr. Amy Iler's snowmelt timing demography project which began in 2023 and will run until 2028. Both research plans and all methods have been approved by RMBL.

IMPORTANCE OF RMBL TO RESEARCH

RMBL is important to this research because it provides a unique ecosystem because subalpine areas are seeing greater effects of climate change and earlier snowmelt. Its short flowering period is ideal for analyzing effects in a condensed period. It also contains conditions to research early snowmelt because the species here rely heavily on snowmelt as one of their water sources. This study is important for understanding how climate change is going to be affecting these communities and working at RMBL will help connect me with other field scientists to help build on a network of growing information. The more we understand about these ecosystems, the more we will be able to protect them.

TIMELINE

July 1, 2024	Begin collecting physiology and flower phenology measurements
July 8, 2024	Collect physiology and flower phenology measurements
July 15, 2024	Collect physiology and flower phenology measurements
July 22, 2024	Collect physiology and flower phenology measurements
July 29, 2024	Analyze data in R
August 5, 2024	Complete draft of research paper

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