

Do pollinator and plant diversity vary with microclimate heterogeneity?

Ellen Elizabeth D. Warmerdam
Melanie Kazenel, Postdoctoral Researcher
Rocky Mountain Biological Laboratory, 2023
Full time independent research/REU

ABSTRACT

Montane landscapes are topographically complex and contain a biodiverse community of flora and pollinators. As with many other ecosystems, these subalpine and alpine landscapes are changing with the climate, altering the way these ecosystems regulate. As climates become warmer and drier, microclimates offer pockets of coolness that offset the changing seasons. Microclimates allow for snowpack to last longer in some areas and melt out quicker and others hedging the bets for the survival of plant species. Studying this will give us data as to how plants are adapting to climate change. In order to understand how plant-pollinator diversity is being impacted by the climate we did look at floral community composition and pollinator diversity variation between high and low heterogeneity plots. From this data we hope to see that diversity will increase with higher microclimates. In order to test this we will be sampling for pollinators across different transects as well as doing floral phenology counts. Methods of assessing species richness of pollinators will be the same as they are in the Irwin datasets taking into account their identity and what flowers they visit. From this we can see how pollinator diversity is associated with plants diversity. We learned in our data collection that floral diversity is partial to high heterogeneity transects and that pollinators are responding to floral abundance, not plant diversity. In our efforts to preserve different organisms under climate change, floral abundance is an important metric to keep in mind. Protocols for this data collection came from metadata files for David Inouye's database on flowering phenology and methods developed by Dr. Becky Irwin for Phenology of subalpine bee and plant communities.

1. INTRODUCTION

Despite our efforts to mitigate its impacts, climate change can reduce landscape biodiversity, completely alter historically synchronized events, and ultimately shift ecosystems (Loreau et. al, 2001). We know from the fossil record that species can undergo range shifts in response to climate change (G. Tilman et al., 2019). While these shifts can lead to speciation they can also result in barriers where a species experiences loss in range and biodiversity (Hewitt, 2000). Loss in range can generate a loss in abundance of a species and deflation in genetic diversity. Events of gene flow require larger populations of plants growing across an area, without which the system is less diverse. Changes in ecosystems have cascading effects; as a result, biodiversity loss can alter the environment in multiple ways. Species distribution models show us that we are at risk of extinction to major flora and fauna under climate change (G. Tilman et al., 2019). Evidence suggests that climate change can also alter the phenology of species (Ohler,

2020). Phenology is defined as the timing of cyclical and seasonal life events in organisms. In plants, flowering, leaves unfurling, seed setting, and dispersal are examples of such life events. As our climate warms, early snowmelt can trigger plants to flower earlier, and can extend the growing seasons or cause earlier senescence (Inouye, 2020). These changes in the timing of plant life cycle events could in turn influence the insect pollinators that rely on them for food. On one hand, a longer flowering season could give better chances for pollination, but research suggests otherwise. In a study done by Kehberger and Holzschuh in 2015 on *Pulsatilla vulgaris*, it was found that a factor that limits the reproductive success of flowering plants in early blooming seasons is low pollinator visitation imposed by competition for pollinators for co-flowering plants. As plants begin to flower earlier there may be a lower abundance of pollinators for flowers and thus competition between flora. Additionally, pollinators that are expecting to see flowers bloom in June will encounter low floral resources if the bloom occurs in April. This early blooming can also increase the chances a flower bud will frost over. This means the bud will not survive to produce a flower or seed, which may decrease the number of resources for pollinators and the survival of the pollinator species long-term (Inouye, 2008). Climate change could thus reduce the number of pollinators visiting flowers and the overall diversity of pollinators.

One factor that could influence how plants and pollinators respond to climate change is microclimate heterogeneity in a given location. This heterogeneity is especially relevant in montane systems. Around 15% of the total land surfaces in the world is made of mountain landscapes and they are in general more topographically complex than other ecosystem types, meaning rough and patchy (Inouye, 2019). This gives rise to different microclimate conditions that exist within relatively small spatial scales. Heterogeneity in temperature conditions across a site might in turn shape functional traits and the community structure in plants, which could successively influence pollinator communities. By identifying and studying major pollinator and plant species of subalpine regions, we can understand how small-scale microclimates are impacting plant phenology and plant-pollinator interactions, and gain insight into how ecosystems may adapt to climate change.

We examined how pollinator visitation and community composition varied with microclimate for floral communities in the Rocky Mountains of Colorado, USA. We hypothesized that plant diversity and pollinator diversity will vary with microclimate, with higher heterogeneity transects having greater overall diversity. Pollinators are responsible for the reproduction and population persistence of many plants so having this data is useful to their conservation.

2. METHODS

We examined how plant-pollinator interactions vary with microclimate by sampling along two 50 meter by 2 meter transects at each of two sites: Judd Falls and Snodgrass. Each transect is divided into 25, 2m x 2m plots. These sites contain a variety of microclimates. At each site one transect has more microclimate heterogeneity than the other. This enables consideration of how heterogeneous environmental conditions will impact plants and pollinators. Using drone imagery, we can capture variables like land surface greenness which are reflective of microclimate. Since 2019, Dr. Ian Breckheimer has been collecting drone imagery at the Judd Falls and Snodgrass sites. Our sampling transects will be in the area of Dr. Breckheimer's drone imagery so that we can link plant-pollinator interactions to microclimate conditions.

Each site was visited once per week on days that are conducive to pollinator activity to collect data on plant-pollinator interactions. On each sampling date at a given site, plant pollinator interactions were recorded once in the morning and once in the afternoon by walking the length of each transect and stopping in each 2-meter x 2-meter plot for 2 person minutes to look for and collect pollinators. The sampling time was recorded alongside weather conditions and further notes on the landscape. In the two sampling minutes for each plot, if an organism was collected, we would pause the timer during capture to keep our time spent observing the plots consistent.

We focused on bees as primary pollinators in our study system. Once a bee was collected it was either be marked and released if it was identified in the field, or it was collected in vials for later identification in the lab. We also noted the visitation of non-bee pollinators (wasps, moths, butterflies, flies). With every collection we wrote down the species identity, collection date, time of day (AM or PM), site, and plant identity. For bumblebees specifically we recorded their caste (queen, worker, drone).

Along each transect, we counted the number of open flowers for all bee-visited plant species within each plot, following protocols developed by Dr. David Inouye for his long-term flowering phenology study at RMBL, but adapted by Dr. Becky Irwin. To answer our research questions, we used data from all the flowers. Analyses examined how abundance of each species changes with microclimate along the transect. We then in turn examined how composition and diversity of pollinators to these plants' changed with microclimate.

In order to answer the research question, we put this data into statistical form. We studied beta diversity between habitats. Calculating transect-level diversity, composition metrics for plants, pollinators, and their interactions too. In order to quantify microsite conditions, we used Dr. Breckheimers drone imagery that gave us information about landscape greenness and snowmelt date.

To look at the interactions between plants and pollinators, matrices of richness and evenness were recorded and pooled across the sampling season. For both plants and pollinators assessing abundance patterns with temporal comparison with the disturbance event being climate based. In order to assess species diversity, we used Shannon Index (H') and species evenness. Using this diversity data and microclimate data we were able to model how pollinator and plant pollinator interaction composition were impacted by the change in critical resources and the diversity within microclimates.

4. RESULTS

4.1 Bee sampling and flowering phenology

In total we collected _____ bees from _____ different families over this two month period. In total we had _____ species of bees and _____ species of *Bombus*. We documented the flowering phenology in our plots and found _____ different species and _____ genus in total. Flowering richness tends to peak in _____ however because of our late season this year the peak of flowers was mostly _____.

4.2 Relationship between floral metrics and microclimate heterogeneity

Using R studio as our main place for statistical analysis we calculated metrics of plant richness and plant Shannon diversity (H'). The statistical analysis resulted in a significant relationship between plant richness and microclimate heterogeneity but not for plant Shannon diversity and microclimate heterogeneity.

In Fig. 1., the boxplot shows that there was a significant correlation between plant richness and high microclimate heterogeneity. Plant richness was higher on the high heterogeneity transects in comparison to the low heterogeneity transects (P -value 0.0167, R -squared 0.51195). As shown in fig. 2., Plant Shannon diversity was higher on high heterogeneity transects but it was only marginally significant (P -value 0.08675, R -squared 0.2163). The graphical data showed a positive relationship between high heterogeneity plots and plant Shannon diversity.

4.3 Relationship between pollinator metrics and microclimate heterogeneity

Pollinator metrics of richness and Shannon diversity were compared to the microclimate heterogeneity. We expected to see that along with high heterogeneity there would be higher pollinator diversity. The data in fig. 3. showed that pollinator richness was not higher on the high heterogeneity transects (P -value 0.7838, R -squared 0.00468). There was no statistical significance. The graph also showed that there was not much variation between the two plots.

In fig. 4. there is a box plot for pollinator Shannon diversity variation with microclimate heterogeneity.

We saw a similar trend in that pollinator Shannon diversity was not higher on high heterogeneity transects (*P-value 0.6424, R-squared 0.01330*). They had the same Shannon diversity for pollinators.

4.4 Pollinator richness and floral diversity

In order to understand how floral abundance dynamics impact pollinator diversity we compared the pollinator richness of each transect alongside total flower count as shown in fig. 7. From this we saw a positive trend line suggesting that as the total floral count in a transect went up so did the diversity of pollinators. The transects with higher floral abundance had higher pollinator diversity (*P-value 0.08226, r-squared 0.1587699*). If the data is split so that we can see the data from each site, the same trend is shown.

4.5 Floral abundance and microclimate heterogeneity

Interestingly, there was no difference between floral count of low and high heterogeneity plots shown in fig. 6. (*P-value 0.1677, R-squared 0.465*). We can assume from the data that the total abundance of flowers was the same.

5. DISCUSSION

Mountain environments range topographically and foster a diverse ecosystem of plants and pollinators making it an ideal place to carry out research on microclimates. Studies have been looking at the range of microclimates, yet there has not been research that connects microclimate environments to organismal diversity. In this research we decided connecting these aspects would give us better insight into how montane and subalpine environments are responding to climate change. In our analysis we used matrices of richness and evenness pooled across a sampling season and then assessed species diversity using the Shannon diversity Index (H'). We learned that while plant diversity does correlate to microclimate heterogeneity, pollinator diversity does not. That said, floral abundance does impact floral diversity, and we see that with more total flowers the diversity of pollinators increases. There were external factors having to do with the delayed phenology and issues with sample sizes that we hypothesize had some impact in making the data look this way.

The low and high heterogeneity microclimate plots were determined using topography, landscape greenness, and observations on the landscape diversity. In figures 7 and 8, the plots demarcated with yellow coloring show high heterogeneity and the red plots are low heterogeneity. In the field, the low heterogeneity plots span a horizontal axis and are relatively flat in aspect. The high heterogeneity plots span an elevation gradient and look to have a broader range of environmental conditions. Studies have shown that among floral species such as *Helianthella quinquenervis*, *Delphinium barbeyi*, and *Erigeron speciosus* these plants are especially sensitive to frosts and typically show strong a relationship to

snowmelt timing and phenology (Inyoue, 2008). More specifically, these mountain flowers are good indicators to how the changes in climate are impacting plant diversity.

In our data analysis for plant diversity, we found that plant richness and the Shannon diversity index was significantly higher on the plots with high microclimate heterogeneity. This would indicate that the diversity of temporal conditions positively impacted floral diversity. One way to understand what this means is through the lens of climate change and the phenology patterns of flowers. In our high heterogeneity transects we see a greater diversity in landscape topography, differences in snowmelt timing, and therefore it experiences a more heterogeneous microclimate. These different conditions then have buffering effects for the plants, specifically offering different niches for a diversity of plants to habitat. The microclimates offset the timing of floral phenology leading us to believe that is why diversity is greater at the higher heterogeneity plots. In a study by Ohler et al. in 2020 they reveal that these niches created in the microclimates support floral establishment and reproduction. What is unknown to them is how diversity is impacted however from our studies we see an increase in diversity.

Based on this information and our understanding of the mutualisms between pollinators and flowers, we hypothesized that because of the greater diversity of flowers in the high heterogeneity transects, pollinators would also experience greater diversity. After comparing pollinator richness and Shannon diversity in the transects we found no difference between the two. Resource availability is crucial in determining the population dynamics of insect pollinators like the ones we looked at. Studies in the past have also shown that composition and structure of floral communities do in fact have an impact on bee abundance (Williams et al., 2012) and an increase alongside greater floral abundance (Ogilvie et al., 2017). Perhaps because of the now shifting seasons that are impacting phenology, the data we have is somewhat skewed as opposed to the normal season. Not only this but while a majority of bees are generalists, diversity could have been altered by the fact that some have preferences to certain floral taxa altering pollinator diversity. Flowers are strongly responsive to climate which could impact how pollinators are foraging without regular seasonal patterns to guide them (Iler et al., 2013). Diversity could also be less important during these changing seasons making general abundance more imperative. The high heterogeneity plots are more florally diverse based on our data but that does not speak to their nectar levels or pollen amounts which could give us insight as to why pollinators prefer these sites. We expected that increased landscape heterogeneity would allow increased resource diversity as suggested by other studies (Boscolo, 2017). We don't see this in our data for reasons that could have to do with experimental error or the fact that there really is correlation between the two.

In order to further understand why our statistical analysis turned out this way we decided to look at different metrics that could have impacted pollinator diversity. We compared pollinator richness to total flower count and found a positive linear trend indicating that with more floral abundance there is greater species diversity. Data in the past has shown that floral resources do determine bee

abundances and diversity (Gerner and Sargent, 2022). Diverse plant communities sustain more diverse communities because pollinators can be specialists (Spasojevic et al. 2014). The flowering phenology of plants provides floral resources through the season and supports a wider pollinator community. We did see a late phenology season especially for the bees which could have caused the low numbers in our data but we can also say that bees are more concerned with the abundance of floral resources rather than diversity. Not all pollinators are specialists which in that case means an area with an abundance of flowers is just as beneficial as one with many kinds of flowers. Thinking from a survival perspective, as the climate changes and there is late timing to certain flowers, relying on one species to bloom is not the safest way to go.

In order to further see if microclimate is impacting these results we measured total floral abundance in the low and high heterogeneity plots. Data showed that the total number of flowers did not differ in high and low heterogeneity transects. So, floral abundance, the indicator of bee richness, does not correlate to microclimate. Aforementioned there are many reasons for this to be. Microclimate effects on flora abundance will be more impatient than floral diversity because pollinators are responding to abundance.

For our research there were aspects that could be improved upon for future sampling. Not only did we end up getting a small sample size of pollinators but because of the late season, shown by the fact that workers are coming out in early August and lack of rain, the sites we were at were very dry and lacked in pollinators. At our Judd Falls site we had a much earlier snowmelt whereas at Snodgrass, a south facing plot, we experienced late snowmelt at around days 130-135. Total number of flowers does not necessarily vary with snowmelt across all plots but it does vary between sites. It's interesting to consider this keeping considering that we had hypothesized our results without these factors in mind.

Microclimates do buffer the effects of climate change but not completely in the way that we had initially hypothesized. While pollinators seemingly have no connection to microclimate heterogeneity, plant abundance and diversity do. The more heterogeneous an area is for microclimates, its predicted that plants will do better. While this study was completed to the extent of which it was planned to, had we had more time and days to sample there is a chance the data would have shown different results. The zealousness of summer plans and conflicting field days made it so sampling was only feasible once per weekend only sampled over a two month period. It could be interesting to see this data set over a longer period of time considering snowmelt timing. A caveat to a lot of ecology is lack of time. One of the reasons long term data sets are so valuable is because they have a bout of information to offer. The same idea applies to this project. Hopefully this research can develop into something longer term so we can prove the impacts climate change is having on plants and pollinators but also so that we can show the interesting ways the natural world is buffering against these negative things.

Xerces Society for Invertebrate Conservation has the mission statement of "...being committed to

establishing and maintaining a diverse and inclusive community that collectively supports our mission: to protect the natural world by conserving invertebrates and their habitat.” (Xerces, year). They would be a good conservation group to reach out to with this data to give further evidence to the effect of climate change and maybe inspire efforts of preservation.

6. REFERENCES

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7. TABLES AND FIGURES

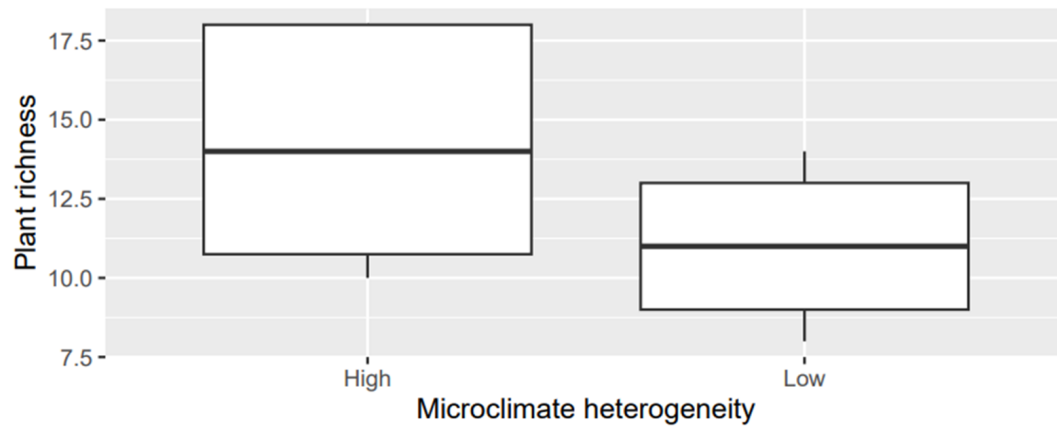


Fig. 1. Plant richness variation with Microclimate Heterogeneity This box plot shows the correlation between plant richness and microclimate heterogeneity divided into low and high. This data accounted for all flowering individuals in a plot (p -value is 0.0167 and r -squared 0.5119).

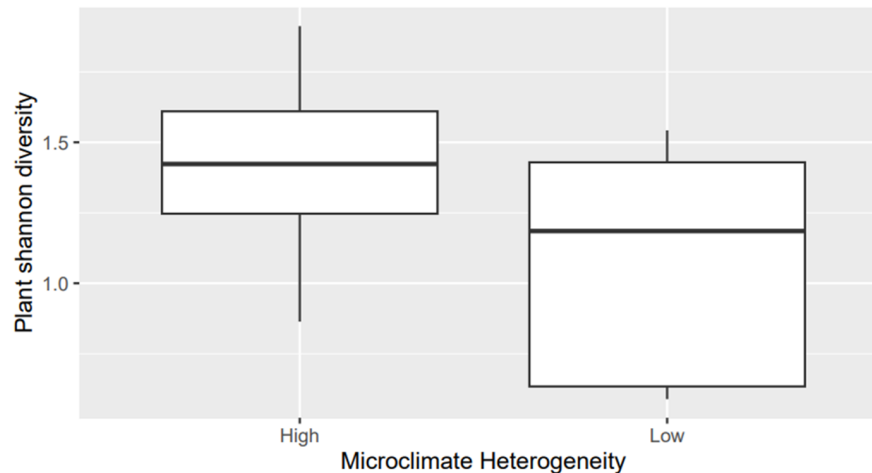


Figure 2. Plant Shannon diversity variance with Microclimate Heterogeneity This boxplot shows the relationship of calculated plant Shannon diversity and microclimate heterogeneity divided into low and high.

The data accounted for all the individuals flowering in each plot (*P-value is 0.08675 and r-squared is 0.5119*).

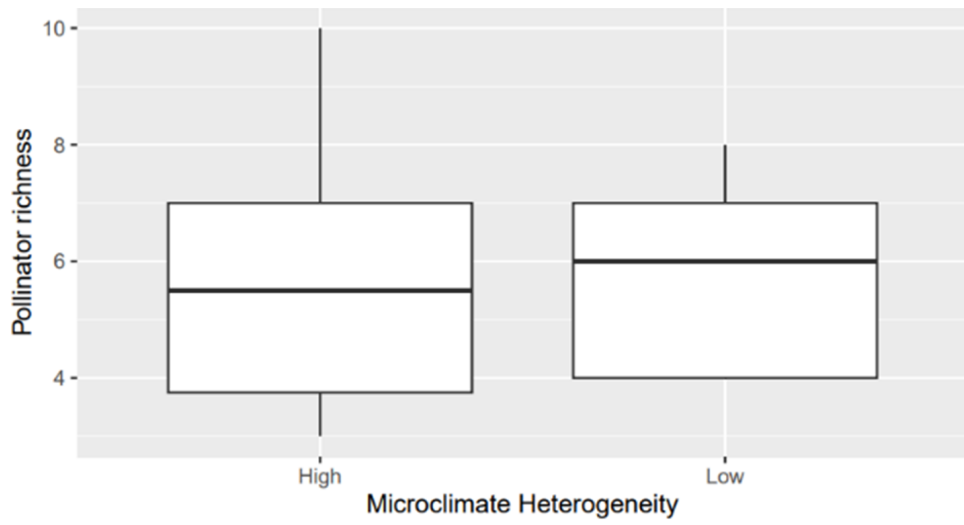


Figure 3. Pollinator richness variation with microclimate heterogeneity This shows the metric of pollination richness alongside microclimate heterogeneity divided into low and high. This was measured by netting bees and marking others (*p-value is 0.7838 and r-squared is 0.0047*).

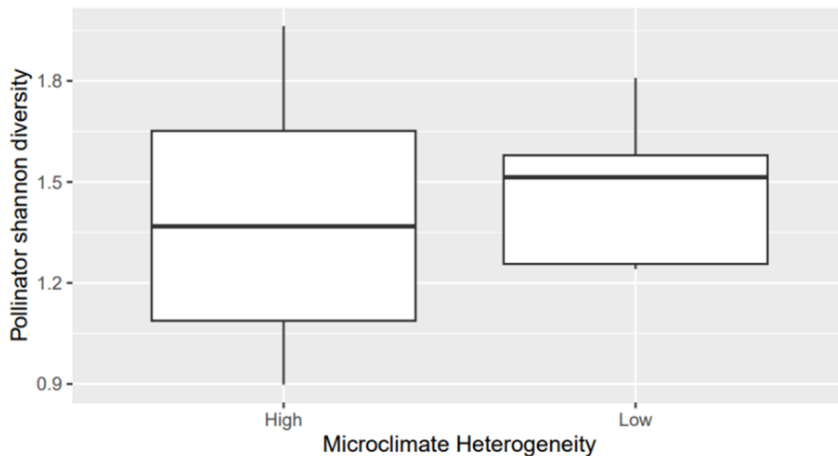


Fig. 5. Pollinator Shannon Diversity variation with Microclimate Heterogeneity This shows pollinator Shannon diversity metrics and how they differed among the different microclimate heterogeneity transects. In higher transects we see higher pollinator diversity. The p-value for this boxplot was 0.08675 and the r-squared was 0.2163.

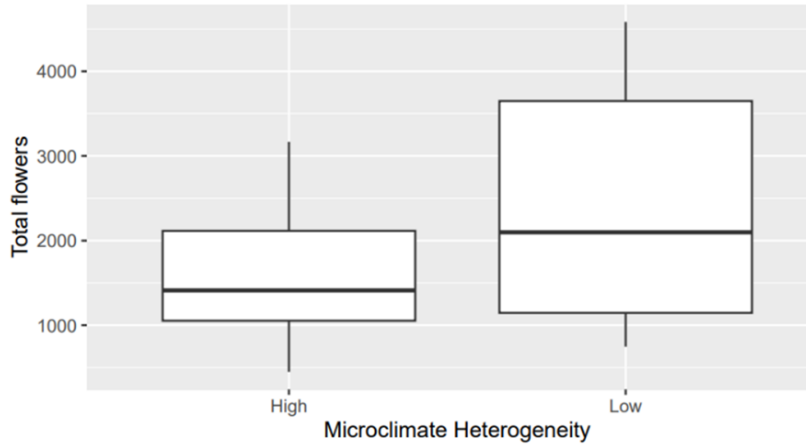


Fig. 6. Total Flower variation with Microclimate Heterogeneity This figure shows pollinator richness alongside total flowers found in each transect on a given day. This accounted for flowers that were open, with reproductive parts showing. Pollinators were counted in netting some and marking others (*p-value* 0.8226 and *r-squared* 0.1588).

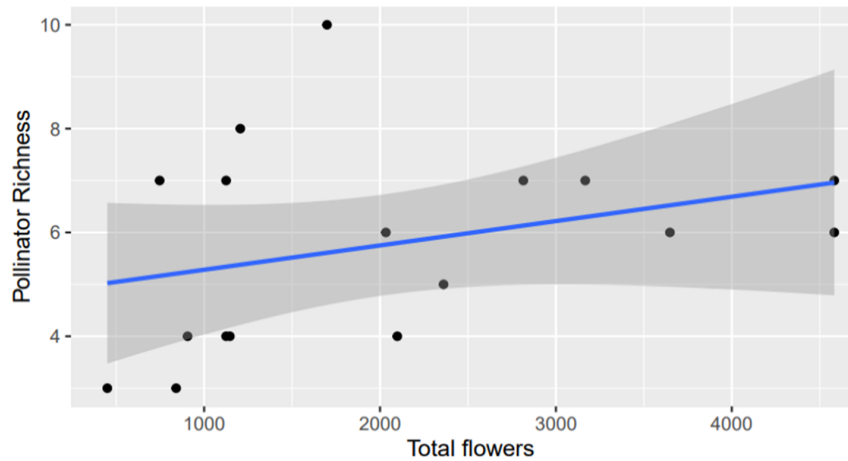


Fig. 7. Pollinator Richness variation with Floral Abundance This figure shows total flowers in each transect alongside microclimate heterogeneity plots. High heterogeneous plots had lower total flower count and the opposite for low heterogeneous plots (*P-value* 0.1677 and *r-squared* was 0.456).



Fig. 7.1. Pollinator richness compared with total flower count at Snodgrass and Judd Falls This scatterplot with a linear regression point shows how pollinator richness increased as the total flower count increased. The data is split so that we can see how this was measured at each site (*P-value: 0.08226* and *r squared: 0.1587699*).

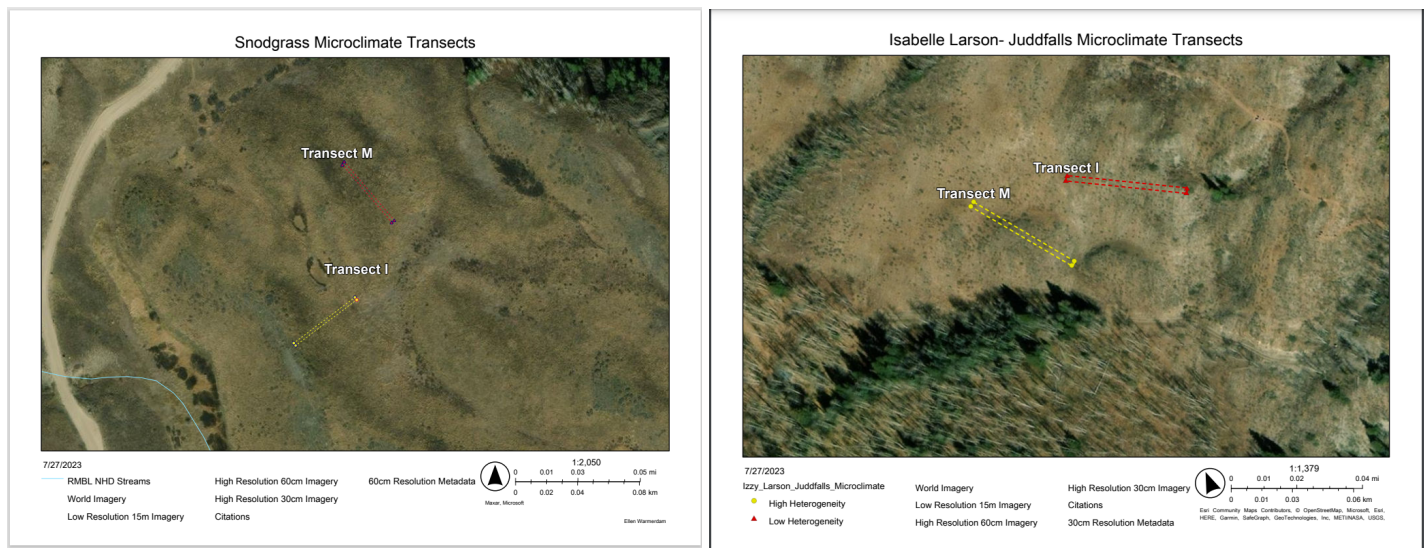


Fig. 8. Snodgrass Microclimate Transects In this we see the microclimate transects at Judd Falls. Yellow is high heterogeneity and red is low heterogeneity.

Fig. 8.1 Judd Falls Microclimate Transects In this we see the microclimate transects at Snodgrass. Yellow is high heterogeneity and red is low heterogeneity.

