UC Riverside
UC Riverside Electronic Theses and Dissertations

Title

Effects of Experimentally-Induced Climatic Changes on Plants, Pollinators, and Their Interactions

Permalink

https://escholarship.org/uc/item/6pk3918q

Author

Rose-Person, Annika

Publication Date

2023

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

UNIVERSITY OF CALIFORNIA
RIVERSIDE

Effects of Experimentally-Induced Climatic Changes on Plants, Pollinators, and Their Interactions

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Annika Rose-Person

December 2023

Dissertation Committee:
Dr. Nicole Rafferty, Chairperson
Dr. Marko Spasojevic
Dr. Hollis Woodard

Copyright by
Annika Rose-Person
2023

The Dissertation of Annika Rose-Person is approved:

Committee Chairperson

University of California, Riverside

Acknowledgements

First, I would like to thank my advisor, Dr. Nicole Rafferty. Nicole has provided a shoulder to lean on and her invaluable science and writing expertise throughout this academically and personally rigorous endeavor. Her selflessness and patience have helped me push through the most difficult parts of this program. Throughout, she set a stellar example of how to be a powerful, confident force in science, and I strive to emulate the balance she strikes between power and kindness every day.

I thank my loving partner, Chris Cosma. The full, beautiful life that we have created together during graduate school has filled me with joy every day. I thank Elijah Hall, the best friend I have ever had. Our friendship has given me the strength I need to pursue my goals, and the confidence to rest when I need to. As labmates and roommates, Chris, Elijah, and I created a bond that helped us navigate the intricacies of our unique graduate experience, and that will continue to support us throughout the rest of our lives. I am incredibly fortunate to know them both.

I thank my current and past committee members Marko Spasojevic, Hollis Woodard, Kurt Anderson, Darrel Jenerette, and Jeff Diez for their unending guidance and support. Their guidance throughout the major changes to my dissertation and career path was invaluable. An immense thanks to Andrea Keeler, who has been a terrific labmate, friend, and mentor, especially as I began my graduate studies. Her support helped me get each of my projects, and my goal to become a teacher, off the ground.

I thank Chiara Forrester, William Bowman, Katharine Suding, Clifton Bueno de Mesquita, and Marko Spasojevic for their work in establishing the Extended Growing

Season Experiment at Niwot Ridge (Chapters 1 and 2). I thank Emily Joy, Kenya Gates, Nyika Campbell, Chris Cosma, Elijah Hall, Emily Hu, Justin Dao, and Angela Li for their immense contribution to data collection and insect pinning for Chapters 1 and 2. I also thank Jane G. Smith for her unwavering support in the field and with plant identification at Niwot Ridge. I thank Julian Resasco for his help establishing methodology, Sarah Elmendorf and Meagan Oldfather for their assistance with data interpretation, and Kris Hess for creating a safe workplace. I thank Jennifer Morse, Tyler Lampard, Henry Brandes, and Sander Aplet for their exceptional work in maintaining research at Niwot.

I thank Louis Santiago, Michael Allen, Edith Allen, Sydney Glassman, and Mia Maltz for their assistance in developing our greenhouse experiment (Chapter 3). In addition, I thank Denise Mitchell and Leonie Schonbeck for their help with using lab equipment and analyzing data. Importantly, I thank Mahnur Bharucha, Kim Sevilla, Caryn Iwanaga, Elijah Hall, Mikaela Truong, Ayah Odeh, Alon Barak, and Cathy Kollmorgen for their substantial contribution to data collection. I also thank Hung Lai and the UCR greenhouse technicians for their consistent and patient support.

I thank Loralee Larios, Michael Fugate, Devon Bradley, and Abigail Zoger for their support in my journey toward a career in pedagogy. I thank all of my undergraduate students for providing me with myriad opportunities to learn as I develop as a teacher. I also thank the undergraduates of UCR’s Strategies for Ecology Education, Diversity, and Sustainability (SEEDS) – especially Caryn Iwanaga and Advyth Ramachandran – for reminding me why I love ecology. I thank Dawn Loyola, Kathy Van Horn, Jon Allen, Mi

Kyong, Estella Davalos, Tara Pastucha, Debbie Brown, and Silvana Payne for their unending support of EEOB graduate students.

I am filled with gratitude for my family. Thank you to my mom, Karen Rose; dad, Ron Person; and stepmom, Barbara Morran. Since my childhood, they ceaselessly encouraged my love for science and for the outdoors. Thank you to my grandmother Marjorie Person for teaching me how to bake and how to stand up for myself. Thank you to my Gogo, Anne Morran, for showing me how to remain optimistic in the face of struggle. Thank you to my brother, Rohan Person, the most creative scientist I have ever met. Thank you to my uncles Bruce and David Person, my aunt Lisa, and my cousins Jessica Hoyt and Grace Person. Finally, and with great gravity, I thank all of the pets that have graced my life. Thank you, dearest Jasper, Molly, Sweet Pea, Gatti, and Keeter. We miss you. Thank you to sweet Teev and Theo, who always made coming home a joy.

Lastly, I thank my friends. Thank you to Will and Morgan Ota, Clara Woodie, and Anna Cassady – it was a privilege to spend my first years with you all as we navigated the intricacies of graduate school. Thank you to April Arquilla, Melina Acosta, Catherine Nguyen, and Matt Gabric for giving me something to look forward to on weekends. Thank you to Marisa Grillo for maintaining our tri-state friendship, and to Kayla Rhode and Aston Greico for your ongoing companionship despite our friend group’s broad dispersal. Thank you also to Jared Huxley, Elisa Henderson, Taylor Seals, Ryan Conway, Mari West, and Daniel Pierce for your consistent friendship.

Acknowledgement of previous publications:

The text of Chapter 1 of this dissertation, in full, has been submitted for publication and appears as a preprint under the title *Experimental advancement of snowmelt influences flowering phenology and pollinator visitation in an alpine ecosystem* in the Journal of Alpine Botany as of October 2023. The co-authors Marko J. Spasojevic, Chiara Forrester, William D. Bowman, Katharine N. Suding, and Nicole E. Rafferty listed in that publication developed the research framework in which data collection was performed. Nicole Rafferty and Marko Spasojevic assisted in data analysis. Meagan F. Oldfather helped us to calculate metrics of topography. Chiara Forrester assisted in the collection of data.

The text of Chapter 2 of this dissertation, in full, has been submitted for publication under the title *Drought stress influences foraging behavior of a solitary bee on two wildflowers* to Annals of Botany as of June 2023. The coauthors Louis Santiago and Nicole Rafferty assisted in developing the methodology for this project. Nicole Rafferty also assisted in data analysis for this project. Louis Santiago provided lab and greenhouse equipment and supplies, as well as training to use this equipment.

Acknowledgement of funding sources:

Chapters 1 and 2 received substantial funding from the Niwot Ridge LTER VII program (NWT VII: NSF DEB - 1637686). Chapter 3 received substantial funding from the Shipley-Skinner Reserve—Riverside County Endowment and from UCR's Environmental Dynamics and GeoEcology (EDGE) Institute. Thank you to all these funding sources.

Dedication

I dedicate this dissertation to my three loving parents: mom, dad, and Barb. You taught me to love art, nature, and science. Thank you.

ABSTRACT OF THE DISSERTATION

Effects of Experimentally-Induced Climatic Changes on Plants, Pollinators, and Their Interactions

by

Annika Rose-Person

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, December 2023
Dr. Nicole E. Rafferty, Chairperson

Global anthropogenic climate change presents a threat to species interactions that are the foundation of ecosystems. Mutualistic interactions between plants and pollinators are critical to the maintenance of biodiversity and are threatened directly and indirectly by climate change. Climate change-driven shifts in temperature and precipitation influence both the temporal co-occurrence of flowering and pollinator foraging as well as the cues and rewards by which plants attract pollinators. These processes act at a community level by changing the availability of partners for interaction at a given time and at an organismal level by altering the physiology and behavior of interacting partners. Each of these levels of influence could affect pollination and the critical ecosystem function that it provides. In this dissertation, I use a series of experiments to explore the community- and individual-level impacts of climate change on plant-pollinator interaction. I begin by using a large, manipulative field experiment in an alpine-subalpine system to ask how advanced snowmelt, an outcome of climate change, influences a cascade of abiotic and biotic drivers that affect pollinator visitation rates. Next, I explore how advanced snowmelt in the same system restructures the web of interactions between plants and pollinators. Finally, I use a greenhouse experiment to assess how drought – another possible outcome of climate change – influences floral traits and insect behavior, providing insights into the organism-level responses shaping pollination. Together, this work provides insights into how organism-level responses to climate change may scale up to influence plant-pollinator networks and community structure. This research indicates that climate change has the potential to reshape webs of plant-pollinator interactions by altering the cues and rewards plants offer to pollinators, by restructuring plant-pollinator interactions at a network level, and by diminishing the importance of floral communities to pollinator visitation.

Table of Contents

Chapter 3: Drought stress influences foraging behavior of a solitary bee on two wildflowers

List of Figures

Chapter 1

Figure 1.1: The base piecewise structural equation model (pSEM) used to construct pSEMs for both control and advanced snowmelt plots. The model predicts that topographic position index (TPI) at a radius of 30 m impacts snowmelt timing, that snowmelt timing influences flowering onset, that flowering onset influences flowering duration and the day of peak pollinator visitation rate, that flowering duration influences floral abundance, and that floral abundance influences the pollinator visitation rate across the entire flowering period.

Figure 1.2: Flowering onset in control plots predicts flowering onset in response to advanced snowmelt. A) Segmented regression shown in red, and null expectation of no difference in flowering onset between treatments (1:1 line) shown in black. B) Difference in flowering onset for plants in advanced vs. control subplots, with a regression line shown in black.

Figure 1.3: The final piecewise structural equation models for A) control and B) advanced snowmelt subplots. Both models had an acceptable fit ($p > 0.05$) and each individual regression used site as a random effect. Green arrows indicate a significant, positive effect; yellow arrows indicate a significant, negative effect; and gray arrows indicate a non-significant interaction. Values adjacent to arrows represent the estimated strength of the relationship, and R^2 values indicate the proportion of variance explained by the model for the associated response variable.

Chapter 1 Appendix

Figure S1.1: Effect of black-sand treatment across four sites, shown as day of snowmelt in control plots (dark blue) and plots where black sand was added to the surface of snow (light orange).

Chapter 2

Figure 2.1: Bipartite plant-pollinator networks in A) advanced snowmelt plots and B) unmanipulated snowmelt plots. Interactions were compiled across the flowering season and across all subplots in sites Audubon, Lefty, and Trough. Dark green nodes represent flowering species and light gold nodes represent pollinator species. Width of each interaction is weighted by the number of times that interaction occurred.

Figure 2.2: The effects of advanced snowmelt treatment and week on network indices at the A) network level, B) flower level, and C) pollinator level.

Chapter 2 Appendix

Figure S2.1: The A) number and B) Shannon diversity, H, of plants and the C) number and D) Shannon diversity, H, of insects by treatment and site. Asterisks indicates significance level (*$p < 0.05$, **$p < 0.01$).

Figure S2.2: The number of flowers over time by treatment with linear (A) and quadratic lines of best fit (B), and the number of pollinators per subplot over time by treatment with linear (C) and quadratic (D) lines of bets fit. Significant predictors are listed in text boxes over plots.

Figure S2.3: Redundancy Analysis (RDA)-based ordinations of flower and insect communities across all three sites.

Figure S2.4: Results of Redundancy Analysis (RDA) on pollinator communities after forward model selection in A) advanced snowmelt and B) unmanipulated snowmelt communities. Floral community Principal Coordinate Analysis (PCoA) axes 1-6, floral diversity, floral abundance, day of year, weather PCoA axis 1, and topography PCoA axis1 were used as predictors. Subplot nested within site was used as a random effect in each model.

Chapter 3

Figure 3.1: Number of flowers per plant on a given day for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions. Asterisks indicate significance level (***$p < 0.001$). Outliers were removed from the figure.

Figure 3.2: Corolla length for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions. Asterisks indicate significance level (***$p < 0.001$). Outliers were removed from the figure.

Figure 3.3: Nectar volume per flower by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia.* Outliers were removed from the figure.

Figure 3.4: Nectar sugar concentration by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia*. Outliers were removed from the figure.

Figure 3.5: Nectar calorie content by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia*. Outliers were removed from the figure.

Figure 3.6: Percent foraging time spent by *Osmia lignaria* on non-droughted *Nemophila menziesii* and *Phacelia campanularia* plants when presented with both non-droughted and droughted plants of each species in the morning and afternoon. Asterisks indicate significance level (‘$p < 0.09$, **$p < 0.01$, ***$p < 0.001$).

Chapter 3 Appendix

Figure S3.1: Leaf turgor loss point (π_{tlp}) of *Nemophila menziesii* and *Phacelia campanularia.* More negative values of π_{tlp} indicate higher levels of drought tolerance. Asterisk indicates significance level (*p < 0.05).

Figure S3.2: Leaf water potential by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia.* More negative values of leaf water potential indicate higher levels of drought stress. Outliers were removed from the figure.

Figure S3.3: Flower landing area for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions in 2021 and 2022. Asterisks indicate significance level (***p < 0.001). Outliers were removed from the figure.

Figure S3.4: Flower corolla length for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions in 2021. Asterisks indicate significance level (***p < 0.001). Outliers were removed from the figure.

Figure S3.5: Nectar volume per flower for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions in 2021. Asterisks indicate significance level (**p < 0.01, ***p < 0.001). Outliers were removed from the figure.

Figure S3.6: Nectar concentration for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions in 2021. Asterisks indicate significance level (*p < 0.05, **p < 0.01). Outliers were removed from the figure.

Figure S3.7: Nectar calories for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions in 2021. Asterisks indicate significance level (***p < 0.001). Outliers were removed from the figure.

List of Tables

Chapter 1

Chapter 1 Appendix

Chapter 2 Appendix

shown in parentheses under model index. "*Trt*" refers to snowmelt treatment, and "*wk*" refers to week. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Chapter 3

Table 3.1: Best-fitting model results for plant traits and pollinator preference. "*N.m.*" represents *Nemophila menziesii* and "*P.c.*" represents *Phacelia campanularia.* "Wt" represents watering treatment and "Tod" represents time of day. Plant identity was used as a random effect in all models with plant traits as the responses, and bee identity was used as a random effect in the models with bee preference as the response. Asterisks indicate significance level (*p < 0.05, **p < 0.01, ***p < 0.001).

Chapter 3 Appendix

Table S3.1: Model results for 2021 floral trait measurements. Watering treatment was used as a predictor in each model. Asterisks indicate significance level (*p < 0.05, **p < 0.01, ***p < 0.001).

Introduction

Global anthropogenic climate change is altering the environment that species interactions occur within at an unprecedented rate. Mutualisms are positive interactions between two species that have played a key role in the evolution of life (Bronstein 2015). Two species must co-occur spatially and phenologically for mutualisms to occur and must have traits that enable interaction (Sargent and Ackerly 2008, Descamps et al. 2021). However, climate change is altering precipitation and temperature patterns, resulting in shifts in the ranges, phenologies, and traits of interacting species (Parmesan 2006, IPCC 2007). Plant-pollinator interactions are among the mutualisms threatened by climate change (Vidal et al. 2021). Plant-pollinator mutualisms are critical for the creation and maintenance of global biodiversity: 88% of angiosperms rely on animal pollination for producing seed (Ollerton et al. 2007), and outcrossing will become increasingly important in future, climate change-impacted landscapes (Solbrig 1976, Austin et al. 2022). Further, over a million insect species rely on floral resources for nutrition (Wardaugh 2015). Insect populations are already experiencing precipitous declines due to climate change, deforestation, use of insecticides, introduced species, and other anthropogenic factors (Wagner et al. 2021). Understanding the impact of climate change on plant-insect interactions is critical to predicting the future of plant and insect populations.

Climate change-driven shifts in temperature, the amount and timing of precipitation, and timing of snowmelt can affect the phenology – the timing of life history events – of plants and pollinators (Rafferty et al. 2015, Renner and Zohner 2018). The phenology of mutualists may respond differentially to changes in the same cues, respond to cues in the same way but at different rates, or respond to different cues entirely, potentially leading to a decrease in phenological synchrony (Samplonious et al. 2016, Kudo and Cooper 2019, Gérard et al. 2020). The consequent decreased interaction rates between previously interacting species or interaction turnover can decrease the fitness of the interacting partners, which in turn can cascade to affect the persistence of that population within a community (Memmott et al. 2007). These dynamics have undergone rigorous study in the context of plant-pollinator interactions (Gérard et al. 2020). For example, wild bees and bee-pollinated plants in the northeastern US appear to be advancing in parallel (Bartomeus 2011). However, in another system, there is evidence for a future phenological mismatch between a specialist bee and its four *Vaccinium* host species due to different responses to warming: the host species' phenology advanced across their range, while bee phenology advanced most strongly in its northernmost range (Weaver and Mallinger 2022). Further, in a manipulative experiment on a plant and its pollinator, early snowmelt resulted in a phenological plant-pollinator mismatch in an alpine system and resulted in decreased rates of pollination (Kudo and Cooper 2019). These contrasting results indicate that plant-pollinator interactions may respond differently to phenological advance depending on the availability of pollinators early in the season (Rafferty and Ives 2011), highlighting the importance of further study of these dynamics.

By affecting when species are able to interact with one another, climate change may not only impact rates of interaction but also may alter the biotic context of interactions, with negative consequences for the species involved in the mutualism (Menge et al. 1976, Holt and Lawton 1994, Sargent and Ackerly 2008, Burkle et al. 2016). Considerable turnover in plant communities, pollinator communities, and plant-pollinator interactions occurs in plant-pollinator networks across a single flowering season (Burkle et al. 2016, Caradonna et al. 2017, Ponisio et al. 2019). Therefore, advancements in flowering time or insect emergence, and the extension of floral seasons, can lead to significant changes to the structure of interaction networks that occur among species (Burkle and Alarcón 2011, Caradonna et al. 2017), which may ultimately influence plant fitness (Lázaro et al. 2020) and network stability (Burkle and Alarcón 2011).

Climate change can also impact the traits of mutualists, affecting the ability of flowering plants to attract pollinators (Bronstein et al. 2006). This may occur directly: for example, climate change-induced droughts may alter floral traits – including those that influence pollinator behavior and therefore visitation – by inducing water stress in plants (Rering et al. 2020). Shifts in floral traits can have profound, indirect impacts on the behavior of pollinating insects and thus on plant-pollinator interactions. For example, drought negatively impacted plant size and flower number, which led to a decrease in visitation by both bee and fly pollinators (Glenny et al. 2018). Further, drought has been shown to increase diet breadth among insect pollinators (Endres et al. 2021). Shifts in the abiotic context of mutualisms therefore may lead to a decrease in rates of interaction or a reassembly of interactions among species (Burkle and Alarcón 2011). Individual species of pollinators provide different pollination services (Rering et al. 2020, Page et al. 2021), and different flowering species provide distinct assemblages of nutrients in their nectar and pollen floral resources (Palmer-Young et al. 2018, Treanore et al. 2019), so novel interactions may alter fitness for both mutualists.

In this dissertation, I examine the influence of climate change on plant-pollinator interactions at a community level, explore the patterns of interactions driving network-level shifts, and assess the organism-level responses driving these effects. In chapter one, I begin by using a large, manipulative field experiment to explore the influence of advanced snowmelt and flowering phenology on the processes that shape plant-pollinator interactions in an alpine-subalpine ecosystem. In chapter two, I further explore this alpine-subalpine system by asking how climate change restructures communities of mutualists and their interactions. In chapter three, I use a greenhouse-based experiment to evaluate the influence of drought, a widespread effect of climate change, on the traits of two southern California wildflowers and the behavior of a native solitary bee. This research elucidates how organism- and community-level mechanisms drive the response of plant-pollinator interactions to climate change-induced abiotic shifts by altering pollinator behavior, changing network structure, and removing the links between floral community metrics and pollinator visitation. This work highlights how organism-level processes scale up to influence the structure of plant-pollinator networks.

References

Austin, M. W., Cole, P. O., Olsen, K. M., & Smith, A. B. (2022). Climate change is associated with increased allocation to potential outcrossing in a common mixed mating species. *American Journal of Botany*, 109(7), 1085-1096.

Bartomeus, I., Ascher, J. S., Wagner, D., Danforth, B. N., Colla, S., Kornbluth, S., & Winfree, R. (2011). Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proceedings of the National Academy of Sciences*, 108(51), 20645-20649.

Bronstein, J. L. (Ed.). (2015). Mutualism. *Oxford University Press*, USA.

Bronstein, J. L., Alarcón, R., & Geber, M. (2006). The evolution of plant–insect mutualisms. *New Phytologist*, 172(3), 412-428.

Burkle, L. A., & Alarcón, R. (2011). The future of plant–pollinator diversity: understanding interaction networks across time, space, and global change. *American Journal of Botany*, 98(3), 528-538.

Burkle, L. A., Myers, J. A., & Belote, R. T. (2016). The beta-diversity of species interactions: Untangling the drivers of geographic variation in plant–pollinator diversity and function across scales. *American Journal of Botany*, 103(1), 118-128.

CaraDonna, P. J., Petry, W. K., Brennan, R. M., Cunningham, J. L., Bronstein, J. L., Waser, N. M., & Sanders, N. J. (2017). Interaction rewiring and the rapid turnover of plant–pollinator networks. *Ecology Letters*, 20(3), 385-394.

Descamps, Charlotte, Muriel Quinet, and Anne-Laure Jacquemart. "The effects of drought on plant–pollinator interactions: What to expect?" *Environmental and Experimental Botany*, 182 (2021): 104297.

Endres, K. L., Morozumi, C. N., Loy, X., Briggs, H. M., CaraDonna, P. J., Iler, A. M., ... & Brosi, B. J. (2021). Plant–pollinator interaction niche broadens in response to severe drought perturbations. *Oecologia*, 197, 577-588.

Gérard, M., Vanderplanck, M., Wood, T., & Michez, D. (2020). Global warming and plant–pollinator mismatches. *Emerging Topics in Life Sciences*, 4(1), 77-86.

Glenny, W. R., Runyon, J. B., & Burkle, L. A. (2018). Drought and increased CO2 alter floral visual and olfactory traits with context-dependent effects on pollinator visitation. *New Phytologist*, 220(3), 785-798.

Holt, R. A., & Lawton, J. H. (1994). The ecological consequences of shared natural enemies. *Annual Review of Ecology and Systematics*, 25(1), 495-520. IPCC 2007.

Climate change 2007: Synthesis report. Contrib. Working Grp I, II and III to the 4th Assess. Rep. Intergovernmental Panel on Climate Change. Pachauri, R. K. and Reisinger, A. (eds). – IPCC, Geneva, Switzerland.

Kudo, G., & Cooper, E. J. (2019). When spring ephemerals fail to meet pollinators: mechanism of phenological mismatch and its impact on plant reproduction. *Proceedings of the Royal Society B*, 286(1904), 20190573.

Lázaro, A., Gómez-Martínez, C., Alomar, D., González-Estévez, M. A., & Traveset, A. (2020). Linking species-level network metrics to flower traits and plant fitness. *Journal of Ecology*, 108(4), 1287-1298.

Memmott, J., Craze, P. G., Waser, N. M., & Price, M. V. (2007). Global warming and the disruption of plant–pollinator interactions. *Ecology Letters*, 10(8), 710-717.

Menge, B. A. (1976). Organization of the New England rocky intertidal community: role of predation, competition, and environmental heterogeneity. *Ecological Monographs*, 46(4), 355-393.

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326.

Page, M. L., Nicholson, C. C., Brennan, R. M., Britzman, A. T., Greer, J., Hemberger, J., ... & Williams, N. M. (2021). A meta-analysis of single visit pollination effectiveness comparing honeybees and other floral visitors. *American Journal of Botany*, 108(11), 2196-2207.

Palmer-Young, E. C., Farrell, I. W., Adler, L. S., Milano, N. J., Egan, P. A., Junker, R. R., ... & Stevenson, P. C. (2019). Chemistry of floral rewards: intra-and interspecific variability of nectar and pollen secondary metabolites across taxa. *Ecological Monographs*, 89(1), e01335.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37, 637-669.

Ponisio, L. C., Valdovinos, F. S., Allhoff, K. T., Gaiarsa, M. P., Barner, A., Guimarães Jr, P. R., ... & Gillespie, R. (2019). A network perspective for community assembly. *Frontiers in Ecology and Evolution*, 7, 103.

Rafferty, N. E., & Ives, A. R. (2011). Effects of experimental shifts in flowering phenology on plant–pollinator interactions. *Ecology Letters*, 14(1), 69-74.

Rafferty, N. E., CaraDonna, P. J., & Bronstein, J. L. (2015). Phenological shifts and the fate of mutualisms. *Oikos*, 124(1), 14-21.

Renner, S. S., & Zohner, C. M. (2018). Climate change and phenological mismatch in trophic interactions among plants, insects, and vertebrates. *Annual Review of Ecology, Evolution, and Systematics*, 49, 165-182.

Rering, C. C., Franco, J. G., Yeater, K. M., & Mallinger, R. E. (2020). Drought stress alters floral volatiles and reduces floral rewards, pollinator activity, and seed set in a global plant. *Ecosphere*, 11(9), e03254.

Samplonius, J. M., Kappers, E. F., Brands, S., & Both, C. (2016). Phenological mismatch and ontogenetic diet shifts interactively affect offspring condition in a passerine. *Journal of Animal Ecology*, 85(5), 1255-1264.

Sargent, R. D., & Ackerly, D. D. (2008). Plant–pollinator interactions and the assembly of plant communities. *Trends in Ecology & Evolution*, 23(3), 123-130.

Sargent, Risa D., and David D. Ackerly. "Plant–pollinator interactions and the assembly of plant communities." *Trends in Ecology & Evolution*, 23, no. 3 (2008): 123-130.

Solbrig, O. T. (1976). On the relative advantages of cross-and self-fertilization. *Annals of the Missouri Botanical Garden*, 262-276.

Treanore, E. D., Vaudo, A. D., Grozinger, C. M., & Fleischer, S. J. (2019). Examining the nutritional value and effects of different floral resources in pumpkin agroecosystems on Bombus impatiens worker physiology. *Apidologie*, 50, 542-552.

Vidal, M. C., Anneberg, T. J., Curé, A. E., Althoff, D. M., & Segraves, K. A. (2021). The variable effects of global change on insect mutualisms. *Current Opinion in Insect Science*, 47, 46-52.

Wagner, D. L., Grames, E. M., Forister, M. L., Berenbaum, M. R., & Stopak, D. (2021). Insect decline in the Anthropocene: Death by a thousand cuts. *Proceedings of the National Academy of Sciences*, 118(2), e2023989118.

Wardhaugh, C. W. (2015). How many species of arthropods visit flowers? *Arthropod-Plant Interactions*, 9(6), 547-565.

Weaver, S. A., & Mallinger, R. E. (2022). A specialist bee and its host plants experience phenological shifts at different rates in response to climate change. *Ecology*, 103(5), e3658.

Chapter 1

Experimental advancement of snowmelt influences flowering phenology and pollinator visitation in an alpine ecosystem

Abstract

Climate change is altering interactions among species, including plants and pollinators. In alpine ecosystems, where snowmelt timing is a key driver of phenology, earlier snowmelt may generate shifts in plant and pollinator phenology that vary across the landscape, potentially disrupting interactions. Here we ask how experimentally induced changes in snowmelt timing in a topographically heterogeneous alpine-subalpine landscape impact flowering, insect pollinator visitation, and the pathways connecting key predictors of plant-pollinator interaction. Snowmelt was advanced via the application of black sand on top of snow in manipulated plots, which were paired with control plots. For each forb species, we documented flowering onset and counted flowers throughout the season. We also performed pollinator observations to measure visitation rates. We found that plants flowered earlier in advanced snowmelt plots, with the largest advances in later-flowering species, but flowering duration and visitation rate did not differ between advanced snowmelt and control plots. Using piecewise structural equation models, we assessed the interactive effects of topography on snowmelt timing, flowering phenology, floral abundance, and pollinator visitation. We found that all of these factors interacted to predict visitation rate in control plots. However, in plots with experimentally advanced snowmelt, none of these predictors explained a significant amount of the variation in visitation rate, indicating that different predictors are needed to understand the processes

that shape pollinator visitation to flowers under novel climate conditions. Our findings demonstrate that climate change-induced early snowmelt may fundamentally change the relationships between abiotic and biotic drivers of plant-pollinator interactions in alpine environments.

Funding acknowledgement: *This study received substantial funding from the Niwot Ridge LTER VII program (NWT VII: NSF DEB - 1637686).*

Data availability: *Data associated with this study is available via the Environmental Data Initiative (Rose-Person et al. 2023). DOI:10.6073/pasta/920fe947b69eea415bdca0af3b3e33c9.*

Introduction

Plant-pollinator mutualisms are crucial for the persistence of most flowering plants and many insects. These mutualisms can be disrupted by climate change-driven shifts in the timing of life history events, such as flowering and emergence, that result in phenological mismatches between plants and insect pollinators (Kudo and Ida 2013, Kudo and Cooper 2019). For example, climate change can alter the cues triggering spring flowering and insect emergence differently, and/or plants and insect pollinators can differ in their responses to changing climatic cues (Kudo and Cooper 2019, Stemkovski et al. 2020). Despite the fact that plant-pollinator interactions in alpine ecosystems may be particularly vulnerable to climate change (Inouye 2020), few studies of plant and pollinator phenological responses to climate change have been conducted in these systems. In the alpine, air temperature and the timing of snowmelt are critical cues shaping flowering onset (Totland and Alatalo 2002, Kudo and Ida 2013) and bee emergence (Kudo and Ida 2013). In addition to decreased winter snowpack and increased spring temperatures associated with climate change, earlier snowmelt is driven by the deposition of anthropogenic dust on the surface of snow, which decreases albedo (Hidalgo et al. 2009, Deems et al. 2013, Pederson et al. 2013). Earlier snowmelt timing can cause alpine plant species to emerge from winter dormancy and to flower earlier, influencing pollinator visitation rates (Totland 1993).

Advanced snowmelt is likely to have direct impacts on both plants and insect pollinators at the same time it increases the risk of phenological mismatch. In some montane systems, earlier-flowering species have longer flowering periods, which can increase reproductive output (Pardee et al. 2019). However, advanced flowering may have direct, negative impacts on plant reproduction by reducing flowering periods for some functional groups, such as succulents and cushion plants (Prevéy et al. 2019, Jabis et al. 2020). Early flowering can also expose plants to late spring and early summer frost events, causing buds and flowers to be damaged by frost and reducing reproductive success (Pardee et al. 2019). Similarly, harsh, early spring alpine conditions can affect foraging success of insect pollinators. Although some bumble bees and moths have adaptations that permit flying and foraging under low air temperatures and strong winds, such conditions may preclude other species from visiting flowers early in the season (Pyke et al. 2011). Additionally, advanced-flowering plants may experience altered soil moisture patterns, which could affect floral cues and pollinator attraction (Gezon et al. 2016). For example, advanced snowmelt and drought have been shown to decrease flower size and nectar production (Powers et al. 2022). Thus, even if plants and insects overlap spatially and temporally under advanced snowmelt conditions, changes in floral traits and insect behavior may lead to a decoupling of plant-pollinator interactions.

The impacts of snowmelt shifting earlier as a result of anthropogenic change in the alpine will play out across a backdrop of complex topography. Topographic heterogeneity is likely to influence smaller-scale patterns of snowmelt timing across alpine landscapes, contributing to variation in plant and pollinator phenological responses (Bueno de Mesquita et al. 2018, Inouye 2020). Topography modulates soil moisture (Litaor et al. 2008), and topography-driven variation in soil moisture can, in turn, affect plant phenology and floral traits (Suárez et al. 2011). In the context of plant-pollinator interactions, microhabitats that become snow-free at different times confer phenological heterogeneity across the alpine landscape, which could decrease the risk of phenological mismatch between plants and pollinators at the community level (Graae et al. 2018). Topographic complexity, in combination with differential sensitivity to snowmelt timing among plant species, could lead to co-flowering of novel assemblages of plants, altering competition and pollination success (Sargent and Ackerly 2008). Topography is therefore critical to assessing how climate change will affect plant-pollinator interactions in alpine ecosystems.

In this study, we used a large-scale experimental manipulation of snowmelt timing in a topographically complex alpine environment to investigate how advanced snowmelt affected plant-pollinator interactions. In particular, we asked how experimental advancement of snowmelt timing affected (1) flowering onset and duration, (2) floral abundance, and (3) pollinator visitation rate. We also asked how these responses varied with topographic variation that contributes to natural variation in snowmelt timing, using structural equation modeling to examine interacting factors shaping snowmelt timing, flowering phenology, and pollinator visitation rate. We predicted that topography would influence snowmelt timing, which subsequently would influence flowering phenology and floral abundance, and floral abundance would influence pollinator visitation rate. We further predicted that the strength of the relationships between these interacting factors would differ for plots with advanced snowmelt and control plots.

Methods

Study system

This work was performed in 2020 at Niwot Ridge (40.05411, -105.5891), located in the Colorado Rocky Mountains, USA. Four sites (named Audubon, Lefty, East Knoll, Trough) were used within this study to represent subalpine and alpine environments ranging from 3380-3500 m in elevation, dominated by low-growing forbs. Each of our four sites were within an array of five sites that were part of an “early spring” experiment done as part of the Niwot Ridge Long Term Ecological Research Program, wherein 40 m × 10 m control plots were paired with advanced plots of the same size. In advanced plots, inert black sand was spread on the surface of the snow in late spring, prior to snowmelt. Sand was spread in control plots following snowmelt to control for any effects of the sand on the soil surface.

We established five subplots in each of the two plots (control and advanced) at each of three sites (Audubon, East Knoll, Trough) and six subplots in each of the two plots at the fourth site (Lefty), for a total of 21 control subplots and 21 advanced subplots.

These subplots (2 m × 1 m) were paired between control and advanced plots such that each pair had a similar elevation (mean difference ± SD in elevation between pairs = 0.20 ± 0.61 m), and were arranged to capture topographic heterogeneity within each plot. The principal measure of topography used was topographic position index (TPI). TPI compares the elevation of a central point to points around it within a given radius or neighborhood; positive TPI values indicate a peak or ridge occurs at that point in the landscape at a given scale, while negative values indicate that a valley occurs around that point in the landscape at a given scale (Oldfather et al. 2016). The TPI of the subplots at a 15-m radius ranged from -1.11-0.68. To minimize the impacts of any runoff snowmelt into our plots from adjacent areas, which could have impacted soil moisture, we established subplots at least 1 m away from the perimeter of plots.

Data collection

To assess whether the black-sand treatment influenced flowering phenology of forbs, we counted the total number of flowers in subplots twice per week beginning with flowering onset and ending when flowering ceased. A total of 56 forb species were present across all sites (Table S1). Many of the forbs in this system produce flowers that senesce within 3-4 d, and those that have longer floral longevity (e.g. *Geum rossii* (Rosaceae)) occur at similar densities in subplots across treatments. From our flower count data, we created a metric of floral abundance by taking the cumulative sum of flowers across all flowering species at all time points per subplot.

To assess pollinator visitation to flowers, we performed 15-min observations twice per week in subplots where flowers were present. We conducted observations only when cloud cover was less than 50% and winds were under 24 km per h. We recorded the identities of insects and plants upon observing contact of insects with anthers or stigmas and collected the first two insect visitors of each morphospecies per observation period for identification. The total amount of observation time per subplot was the same to ensure equal sampling effort. We therefore used the total cumulative visits to flowers across the entire flowering season at the subplot level as our metric of pollinator visitation rate.

To assess the topography of each subplot, we used a digital elevation model (DEM) for Niwot Ridge from *OpenTopography* (Anderson et al. 2013). Using the R (R Core Team 2022) package *raster* and function *terrain* (Hijmans 2023), we calculated elevation, aspect, and slope. We also calculated TPI at 1.5- and 15-m radii to represent local- and large-scale topography (Oldfather et al. 2016).

Data analysis

To explore the overall impacts of the black-sand treatment on the timing of snowmelt, we fit a linear mixed-effects model (LMM) for each site using snowmelt timing as the response variable, treatment as the predictor, and subplot identity (with subplots paired by elevation) as a random effect.

To explore how snowmelt timing influenced flowering onset, we fit a LMM with flowering onset of plants in advanced snowmelt subplots as our response variable, flowering onset of plants in control subplots as our predictor variable, and plant species identity as a random effect. We tested the normality of residuals using the R package “DHARMa” (Hartig 2022) and found that they were normally distributed. We then determined if this linear fit differed from a null expectation of no difference in flowering onset between treatments (i.e., deviated from a 1:1 line) using the package "emmeans" (Lenth 2023), and tested for breakpoints in the linear fit using the package "segmented" (Muggeo 2017). Finally, we calculated the difference in flowering onset for plants in advanced vs. control subplots, then fitted a LMM with the difference as the response variable, flowering onset in control subplots as the predictor variable, and plant species as a random effect. We used the R package "lme4" (Bates et al. 2015) for all LMMs.

To examine the influence of treatment on floral abundance, flowering duration, and pollinator visitation rate, we fit separate LMMs with each response variable, treatment as the predictor variable, and paired subplots nested in sites as a random effect. We tested the normality of residuals using the R package "DHARMa" (Hartig 2022) and found that they were normally distributed.

Finally, to examine the relationships among topography, snowmelt timing, flowering phenology, floral abundance, and pollinator visitation rate, we constructed piecewise structural equation models (pSEMs) with the R packages *vegan* and *piecewiseSEM* (Lefcheck 2016, Oksanen et al. 2022). The base pSEM model was created using inferences derived from the literature, exploratory analyses, and variation inflation factor (VIF) analyses. Predictor variables were selected based on covariance, which was determined using Pearson correlation with R package *Hmisc*, and then assessed using VIF analyses (Harrell 2023, Zuur et al. 2009). We excluded predictors that led to a VIF > 2.0 to reduce the effect of collinearity (Berglund et al. 2013) and performed model selection by choosing models with the lowest Akaike Information Criterion using the R package “AIC” (Shipley 2013). Variables that did not lead to VIF scores over 2.0 were added based on tests of directed separation (Lefcheck 2016). We tested the normality of residuals of all linear models using the R package “DHARMa” (Hartig 2022).

The base pSEM model (Fig. 1.1) predicted that, at the subplot level, TPI at a 15-m radius impacted the timing of snowmelt, that snowmelt timing influenced flowering onset, that flowering onset influenced flowering duration and the day of peak pollinator visitation rate to flowers, and that floral abundance influenced pollinator visitation rate. Site was used as a random effect in each individual linear model. To compare model structure between treatments, we created two separate models using the same base pSEM model: one with data from control subplots, and one with data from advanced snowmelt subplots. In both models, we used gamma distributions with a log link function for models that had temporal measurements as the response variable, which included models predicting snowmelt timing, flowering onset, and day of peak pollinator visitation rate (Bolker 2008). We found that residuals of three of twelve total models were marginally significantly non-normal. In the control subplot pSEM, models with non-normal residuals were those explaining flowering onset and flowering duration ($p = 0.03$ and $p = 0.02$, respectively). In the advanced subplot pSEM, the only model with non-normal residuals was the model explaining flowering onset ($p = 0.01$). We re-fitted these models with subsets of the data to achieve normality and found that the qualitative results did not change. We report the results from the models with the complete datasets.

Results

Black-sand treatment advanced snowmelt in plots in three sites (Audubon, Lefty, and Trough) by 11.8, 13.0, and 16.8 days, respectively, but there was no effect in the fourth site (East Knoll; Table 1.1, Fig. S1.1). Because snowmelt timing was not affected by the addition of black sand in this fourth site, and because forbs began flowering in this site before we started to monitor subplots for flowering, we excluded this site in all subsequent data analysis. Thus, analyses of flowering onset, floral abundance, flowering duration, and pollinator visitation rate, as well as all pSEMs, use data from three sites (Audubon, Lefy, Trough) comprising 16 paired subplots in six plots.

Observations of flower visitation were conducted twice per week for approximately ten weeks for a total of 7,665 min, during which we observed a total of 2,070 insect-flower interactions and collected 332 flower-visiting insects representing 102 morphospecies and 24 families.

Flowering onset of plants in control subplots was a significant predictor of flowering onset in advanced subplots (Fig 1.2a), with an estimated coefficient that differed significantly from 1 (the null expectation; LMM [estimate ± SE]: 0.73 ± 0.05, $t_{111} = 13.54$, $p < 0.001$; Fig 1.2a). Further, the segmented regression identified a breakpoint at day of year 228 (August 15), where the slope changed from 0.85 to 0.19 (0.74 ± 0.06, $t_{114} = 13.10$, $p<0.001$; Fig 1.2a). Finally, the difference in flowering onset for plants in advanced vs. control subplots was positively correlated with flowering onset (LMM: 0.27 ± 0.05, $t_{111} = 4.89$, $p < 0.001$; Fig. 1.2b).

Black-sand treatment had a significant, positive effect on floral abundance, but did not significantly influence flowering duration or pollinator visitation rate (Table 1.1).

We obtained good fits for pSEMs for both the control (Fisher's C 34.19, AIC = -121.05, p = 0.08, df = 24; Fig. 1.3a, Table S1.2) and advanced subplots (Fisher's C 29.05, AIC = -91.09, p = 0.41, df = 28; Fig. 1.3b, Table S1.2). In both pSEMs, TPI at 15 m was significantly negatively correlated with snowmelt timing, snowmelt timing was significantly positively correlated with flowering onset, and TPI at 15 m was significantly positively correlated with floral abundance, indicating that snow melted later in depressions and that floral abundance was lower on ridges. However, these three paths are the only significant interactions shared by both models. The pSEM for control subplots showed a high degree of agreement with our base model: flowering onset had a significant negative effect on flowering duration, flowering duration had a significant positive effect on floral abundance, and floral abundance had a significant positive effect on pollinator visitation rate. Further, flowering onset and duration had significant positive effects on the day of peak pollinator visitation rate, and the day of peak pollinator visitation rate had a significant positive effect on pollinator visitation rate (Figs. 1.1 and 1.3a, Table S1.2). In contrast, the pSEM for advanced subplots showed few significant paths, and there were no significant predictors of flowering duration, day of peak pollinator visitation rate, or pollinator visitation rate (Fig. 1.3b, Table S1.2).

Discussion

Our large-scale manipulation of snowmelt timing in an alpine ecosystem demonstrated that advanced snowmelt is associated with higher floral abundance and earlier flowering onset, particularly for species that flower later in the season, and that novel predictors are needed to understand the processes that shape pollinator visitation to flowers under climate change. Thus, despite the fact that flowering duration and pollinator visitation rate did not differ between advanced snowmelt and control plots, the relationships between these factors were altered with earlier snowmelt, reducing our ability to identify the mechanisms by which climate change will affect these communities.

As expected, flowering onset was earlier in plots with advanced snowmelt (Fig. 1.2). Indeed, our pSEMs for both control and advanced snowmelt plots showed that flowering onset was positively correlated with snowmelt timing (Fig. 1.3). However, when snowmelt was advanced, earlier-flowering species showed a smaller advance in flowering onset than did later-flowering species (Fig. 1.2a). Thus, the magnitude of advance in flowering onset in advanced snowmelt plots was positively related to flowering onset in control plots (Fig. 1.2b). Experiments in both alpine and temperate ecosystems have tended show the opposite, with early-flowering species experiencing the greatest phenological advancement in response to experimentally advanced snowmelt (Dunne et al. 2003, Petraglia et al. 2014). However, a meta-analysis revealed that the phenologies of late-flowering species in the coldest tundra sites were most strongly impacted by warming (Prevéy et al. 2019). The ability to grow and flower rapidly in response to snowmelt may be an important adaptation to late-melting habitats because of the abbreviated growing season (Totland and Alatalo 2002). Thus, flowering onset in mid- and late-season flowering species may be cued by snowmelt, potentially triggered by associated cues such as light availability and soil moisture. Early-flowering species in alpine systems, on the other hand, may face harsher conditions if they flower early in response to advanced snowmelt, resulting in negative fitness consequences (Prevéy et al. 2019). Thus, early-flowering species may respond to cues such as the amount of solar energy, growing degree days, and number of frost days (Bienau et al. 2015).

Given our finding that plants that flower later in the season showed greater advances in flowering onset, the assemblage of flowering species available to pollinators at a given time may change under climatic conditions that result in earlier snowmelt. For example, Forrest et al. (2010) showed that snowmelt timing in subalpine meadows altered the flowering times and synchrony of historically co-flowering species that share pollinators. Under future climates, the flowering phenologies of species that flower in early- and mid-season may be more synchronous in our study sites, altering competitive and facilitative interactions (Sargent and Ackerly 2008). Indeed, because co-flowering synchrony can affect plant-pollinator interactions (Kraft and Ackerly 2014), shifts in the relative timing of flowering at the community level will likely affect plant reproduction. Further, plots with advanced snowmelt had significantly higher floral abundance than control plots. If increased flower production is distributed unequally among species, thereby changing relative floral abundances, competition among plants for pollination services may increase.

Although our treatment did not generate differences in flowering duration or pollinator visitation rate, advanced snowmelt altered the interrelationships between these and other factors (Fig. 1.3). In control plots, later flowering onset was associated with shorter flowering periods. This relationship between flowering onset and flowering duration was absent in plots with experimentally advanced snowmelt, and studies in other alpine and subalpine ecosystems have similarly found that flowering duration was not affected by treatments combining advanced snowmelt and warming (Semenchuk et al. 2016, Jabis et al. 2020). Similarly, in control plots only, flowering duration was significantly positively correlated with floral abundance in subplots, and later flowering onset was associated with later dates of peak visitation. Finally, the strongest predictor of pollinator visitation rate to control subplots was floral abundance. In contrast, pollinator visitation rate was unpredictable in advanced snowmelt plots. Given a wealth of evidence that floral abundance can influence pollinator visitation (e.g., Eckhart et al. 2006, Shibata and Kudo 2020), the lack of relationship between these factors in advanced snowmelt plots suggests that factors other than floral abundance and phenology may shape plant-pollinator interactions under future climates.

The spatial extent of our study enabled us to ask whether topography interacted with the black-sand treatment to influence snowmelt timing. Topographic position index (TPI) at 15 m was positively correlated with later snowmelt in both control and advanced snowmelt plots (Fig. 1.3), corroborating research showing that topography influences snow depth and melt timing (Bueno de Mesquita et al. 2018). Valleys likely accumulate more snow and may thus have higher soil moisture, influencing flowering phenology (Bueno de Mesquita et al. 2018). Our findings add further evidence that topographic heterogeneity influences the hydrology of alpine ecosystems (Grünewald et al. 2013).

Improving our understanding of how topography influences snow accumulation and plant phenology is critical to predicting how climate change will impact alpine communities.

In isolating the effects of advanced snowmelt across a large area, our study did not attempt to incorporate multiple aspects of climate change. In particular, we note that our study did not address increases in temperature that are predicted to occur with climate change (Diaz et al. 2003). Warming impacts vegetative and reproductive phenology differently (Collins et al. 2021) and shortens flowering periods (Prevéy et al. 2019) in tundra systems, and direct warming would likely have further accelerated snowmelt and altered our findings. In addition, we focused here on community-level visitation rates, rather than examining whether the composition of insect visitors differed between control and advanced snowmelt plots. Given shifts in flowering phenology are known to alter the relative frequency of interaction with different pollinators, affecting reproductive output (Rafferty and Ives 2012), such analyses would be valuable.

This study is the first to demonstrate that experimental advancement of snowmelt can fundamentally change the relationships among the biotic factors that influence plant-pollinator interactions. We provide evidence that under novel climate conditions, models fail to predict the rate of pollinator visitation, indicating that new mechanisms are needed to predict plant-pollinator interaction rates in future climates. Further, our manipulation of snowmelt timing is, to our knowledge, the largest in extent that has been executed in an alpine system (Dunne et al. 2003, Livensperger et al. 2006, Steltzer et al. 2009, Wipf et al. 2009, Cornelius et al. 2013, Petraglia et al. 2014, Sherwood et al. 2017, Blankinship et al. 2018, Wadgymar et al. 2018, Pardee et al. 2019, Frei and Henry 2021, Jerome et al.

2021). By experimentally advancing snowmelt across this landscape, we gained novel insight into how climate change will alter the abiotic and biotic mechanisms driving plant-pollinator interactions. Our findings suggest climate change will fundamentally restructure species interactions and communities, potentially altering the provision of ecosystem services such as pollination.

References

Anderson, R. (2013). Lidar Data, Niwot Ridge Long-Term Ecological Research Site, Colorado. Distributed by *OpenTopography*. https://doi.org/10.5069/G9FJ2DQM. Accessed: 2021-09-18

Bates, D., Maechler, M., Bolker, B., Walker, S. (2015). Ime4: Fitting Linear Mixed-Effects Models Using Lme4.

Berglund, E., Lytsy, P., Westerling, R. (2013). Adherence to and beliefs in lipid-lowering medical treatments: a structural equation modeling approach including the necessity-concern framework. *Patient Education and Counselling, 91*(1):105-112.

Bienau, M. J., Kröncke, M., Eiserhardt, W. L., Otte, A., Graae, B. J., Hagen, D., Eckstein, R. L. (2015). Synchronous flowering despite differences in snowmelt timing among habitats of *Empetrum hermaphroditum*. *Acta Oecologia, 69*:129-136.

Blankinship, J. C., McCorkle, E. P., Meadows, M. W., Hart, S. C. (2018). Quantifying the legacy of snowmelt timing on soil greenhouse gas emissions in a seasonally dry montane forest. *Global Change Biology, 24*(12):5933-5947.

Bolker, B. M. (2008). Ecological models and data in R. *Princeton University Press*, New Jersey

Bueno de Mesquita, C. P., Tillmann, L. S., Bernard, C. D., Rosemond, K. C., Molotch, N. P., & Suding, K. N. (2018). Topographic heterogeneity explains patterns of vegetation response to climate change (1972–2008) across a mountain landscape, Niwot Ridge, Colorado. *Arctic, Antarctic, and Alpine Research*, *50*(1), e1504492.

Collins, C. G., Elmendorf, S. C., Hollister, R. D., Henry, G. H., Clark, K., Bjorkman, A. D., ... & Suding, K. N. (2021). Experimental warming differentially affects vegetative and reproductive phenology of tundra plants. *Nature Communications*, *12*(1), 3442.

Cornelius, C., Leingärtner, A., Hoiss, B., Krauss, J., Steffan-Dewenter, I., & Menzel, A. (2013). Phenological response of grassland species to manipulative snowmelt and drought along an altitudinal gradient. *Journal of experimental botany*, *64*(1), 241-251.

Deems, J. S., Painter, T. H., Barsugli, J. J., Belnap, J., & Udall, B. (2013). Combined impacts of current and future dust deposition and regional warming on Colorado River Basin snow dynamics and hydrology. *Hydrology and Earth System Sciences*, *17*(11), 4401-4413.

Diaz, H. F., Eischeid, J. K., Duncan, C., & Bradley, R. S. (2003). Variability of freezing levels, melting season indicators, and snow cover for selected high-elevation and continental regions in the last 50 years. *Climate Variability and Change in High Elevation Regions: Past, Present & Future*, 33-52.

Dunne, J. A., Harte, J., & Taylor, K. J. (2003). Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. *Ecological monographs*, *73*(1), 69-86.

M. Eckhart, V., S. Rushing, N., M. Hart, G., & D. Hansen, J. (2006). Frequency–dependent pollinator foraging in polymorphic Clarkia xantiana ssp. xantiana populations: implications for flower colour evolution and pollinator interactions. *Oikos*, *112*(2), 412-421.

Forrest, J., Inouye, D. W., & Thomson, J. D. (2010). Flowering phenology in subalpine meadows: Does climate variation influence community co-flowering patterns? *Ecology*, *91*(2), 431-440.

Frei, E. R., & Henry, G. H. (2021). Long-term effects of snowmelt timing and climate warming on phenology, growth, and reproductive effort of Arctic tundra plant species. *Arctic Science*, *8*(3), 700-721.

Gezon, Z. J., Inouye, D. W., & Irwin, R. E. (2016). Phenological change in a spring ephemeral: implications for pollination and plant reproduction. *Global Change Biology*, *22*(5), 1779-1793.

Graae, B. J., Vandvik, V., Armbruster, W. S., Eiserhardt, W. L., Svenning, J. C., Hylander, K., ... & Lenoir, J. (2018). Stay or go–how topographic complexity influences alpine plant population and community responses to climate change. *Perspectives in Plant Ecology, Evolution and Systematics*, *30*, 41-50.

Grünewald, T., Stötter, J., Pomeroy, J. W., Dadic, R., Moreno Baños, I., Marturià, J., ... & Lehning, M. (2013). Statistical modelling of the snow depth distribution in open alpine terrain. *Hydrology and Earth System Sciences*, *17*(8), 3005-3021.

Harrell Jr, F. (2023) Hmisc: Harrell Miscellaneous. R package version 4.8-0. https://CRAN.R-project.org/package=Hmisc

Hartig, F. (2022). _DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models_. R package version 0.4.6, https://CRAN.R-project.org/package=DHARMa

Hidalgo, H. G., Das, T., Dettinger, M. D., Cayan, D. R., Pierce, D. W., Barnett, T. P., ... & Nozawa, T. (2009). Detection and attribution of streamflow timing changes to climate change in the western United States. Journal of Climate, 22(13), 3838-3855.

Hijmans, R. (2023) _raster: Geographic Data Analysis and Modeling_. R package version 3.6-23. https://CRAN.R-project.org/package=raster

Inouye, D. W. (2020). Effects of climate change on alpine plants and their pollinators. *Annals of the New York Academy of Sciences*, *1469*(1), 26-37.

Jabis, M. D., Winkler, D. E., & Kueppers, L. M. (2020). Warming acts through earlier snowmelt to advance but not extend alpine community flowering. *Ecology*, *101*(9), e03108.

Jepsen, S. M., Molotch, N. P., Williams, M. W., Rittger, K. E., & Sickman, J. O. (2012). Interannual variability of snowmelt in the Sierra Nevada and Rocky Mountains, United States: Examples from two alpine watersheds. *Water Resources Research*, *48*(2).

Jerome, D. K., Petry, W. K., Mooney, K. A., & Iler, A. M. (2021). Snow melt timing acts independently and in conjunction with temperature accumulation to drive subalpine plant phenology. *Global Change Biology*, *27*(20), 5054-5069.

Kraft, N. J., & Ackerly, D. D. (2014). Assembly of plant communities. *Ecology and the Environment*, *8*, 67-88.

Kudo, G., & Cooper, E. J. (2019). When spring ephemerals fail to meet pollinators: mechanism of phenological mismatch and its impact on plant reproduction. *Proceedings of the Royal Society B*, *286*(1904), 20190573.

Kudo, G., & Ida, T. Y. (2013). Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology*, *94*(10), 2311-2320.

Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, *7*(5), 573-579.

Lenth, R. (2023) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.8.8. https://CRAN.R-project.org/package=emmeans

Litaor, M. I., Williams, M., & Seastedt, T. R. (2008). Topographic controls on snow distribution, soil moisture, and species diversity of herbaceous alpine vegetation, Niwot Ridge, Colorado. *Journal of Geophysical Research: Biogeosciences*, *113*(G2).

Livensperger, C., Steltzer, H., Darrouzet-Nardi, A., Sullivan, P. F., Wallenstein, M., & Weintraub, M. N. (2016). Earlier snowmelt and warming lead to earlier but not necessarily more plant growth. *AoB Plants*, *8*, plw021.

Muggeo, V. M. (2017). Interval estimation for the breakpoint in segmented regression: A smoothed score-based approach. *Australian & New Zealand Journal of Statistics*, *59*(3), 311-322.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., ... & Weedon, J. (2022) _vegan: Community Ecology Package_. R package version 2.6-4. https://CRAN.R-project.org/package=vegan

Oldfather, M. F., Britton, M. N., Papper, P. D., Koontz, M. J., Halbur, M. M., Dodge, C., ... & Ackerly, D. D. (2016). Effects of topoclimatic complexity on the composition of woody plant communities. *AoB Plants*, *8*, plw049.

Östman, S. A. H. (2018). *Plant-pollinator interactions in the alpine: Landscape heterogeneity acts as a potential buffer against climate-change induced mismatch in the pollinator-generalist Ranunculus acris* (Master's thesis, The University of Bergen).

Pardee, G. L., Jensen, I. O., Inouye, D. W., & Irwin, R. E. (2019). The individual and combined effects of snowmelt timing and frost exposure on the reproductive success of montane forbs. *Journal of Ecology*, *107*(4), 1970-1981.

Pederson, G. T., Betancourt, J. L., & McCabe, G. J. (2013). Regional patterns and proximal causes of the recent snowpack decline in the Rocky Mountains, US. Geophysical Research Letters, 40(9), 1811-1816.

Petanidou, T., Kallimanis, A. S., Sgardelis, S. P., Mazaris, A. D., Pantis, J. D., & Waser, N. M. (2014). Variable flowering phenology and pollinator use in a community suggest future phenological mismatch. *Acta Oecologica*, *59*, 104-111.

Petraglia, A., Tomaselli, M., Petit Bon, M., Delnevo, N., Chiari, G., & Carbognani, M. (2014). Responses of flowering phenology of snowbed plants to an experimentally imposed extreme advanced snowmelt. *Plant Ecology*, *215*, 759-768.

Powers, J. M., Briggs, H. M., Dickson, R. G., Li, X., & Campbell, D. R. (2022). Earlier snow melt and reduced summer precipitation alter floral traits important to pollination. *Global Change Biology*, *28*(1), 323-339.

Prevéy, J. S., Rixen, C., Rüger, N., Høye, T. T., Bjorkman, A. D., Myers-Smith, I. H., ... & Wipf, S. (2019). Warming shortens flowering seasons of tundra plant communities. *Nature ecology & evolution*, *3*(1), 45-52.

Pyke, G. H., Inouye, D. W., & Thomson, J. D. (2011). Activity and abundance of bumble bees near Crested Butte, Colorado: diel, seasonal, and elevation effects. *Ecological Entomology*, *36*(4), 511-521.

R Core Team (2023) _R: A Language and Environment for Statistical Computing_. *R Foundation for Statistical Computing*, Vienna, Austria. https://www.R-project.org/

Rafferty, N. E., & Ives, A. R. (2012). Pollinator effectiveness varies with experimental shifts in flowering time. *Ecology*, *93*(4), 803-814.

Rose-Person, A., Spasojevic, M., Forrester, C., Bowman, W., Suding, K., Oldfather, M., and Rafferty, N. (2023). Pollinator visitation, flower count, and seed set in Black Sand plots, 2020. ver 1. *Environmental Data Initiative*. https://doi.org/10.6073/pasta/920fe947b69eea415bdca0af3b3e33c9 (Accessed 2023-11-08).

Sargent, R. D., & Ackerly, D. D. (2008). Plant–pollinator interactions and the assembly of plant communities. *Trends in Ecology & Evolution*, *23*(3), 123-130.

Semenchuk, P. R., Gillespie, M. A., Rumpf, S. B., Baggesen, N., Elberling, B., & Cooper, E. J. (2016). High Arctic plant phenology is determined by snowmelt patterns but duration of phenological periods is fixed: an example of periodicity. *Environmental Research Letters*, *11*(12), 125006.

Sherwood, J. A., Debinski, D. M., Caragea, P. C., & Germino, M. J. (2017). Effects of experimentally reduced snowpack and passive warming on montane meadow plant phenology and floral resources. *Ecosphere*, *8*(3), e01745.

Shibata, A., & Kudo, G. (2020). Floral abundance and bee density affect species-specific foraging patterns of alpine bumble bees. *Arthropod-Plant Interactions*, *14*, 771-783.

Shipley, B. (2013). The AIC model selection method applied to path analytic models compared using ad-separation test. *Ecology*, *94*(3), 560-564.

Steltzer, H., Landry, C., Painter, T. H., Anderson, J., & Ayres, E. (2009). Biological consequences of earlier snowmelt from desert dust deposition in alpine landscapes. *Proceedings of the National Academy of Sciences*, *106*(28), 11629-11634.

Stemkovski, M., Pearse, W. D., Griffin, S. R., Pardee, G. L., Gibbs, J., Griswold, T., ... & Irwin, R. E. (2020). Bee phenology is predicted by climatic variation and functional traits. *Ecology Letters*, *23*(11), 1589-1598.

Suárez, L. H., Pérez, F., & Armesto, J. J. (2011). Strong phenotypic variation in floral design and display traits of an annual tarweed in relation to small-scale topographic heterogeneity in semiarid Chile. *International Journal of Plant Sciences*, *172*(8), 1012-1025.

Suggitt, A. J., Wilson, R. J., Isaac, N. J., Beale, C. M., Auffret, A. G., August, T., ... & Maclean, I. M. (2018). Extinction risk from climate change is reduced by microclimatic buffering. *Nature Climate Change*, *8*(8), 713-717.

Takkis, K., Tscheulin, T., & Petanidou, T. (2018). Differential effects of climate warming on the nectar secretion of early-and late-flowering Mediterranean plants. *Frontiers in Plant Science*, *9*, 874.

Totland, Ø. (1993). Pollination in alpine Norway: flowering phenology, insect visitors, and visitation rates in two plant communities. *Canadian Journal of Botany*, *71*(8), 1072-1079.

Totland, Ø., & Alatalo, J. M. (2002). Effects of temperature and date of snowmelt on growth, reproduction, and flowering phenology in the arctic/alpine herb, Ranunculus glacialis. *Oecologia*, *133*, 168-175.

Wadgymar, S. M., Ogilvie, J. E., Inouye, D. W., Weis, A. E., & Anderson, J. T. (2018). Phenological responses to multiple environmental drivers under climate change: insights from a long-term observational study and a manipulative field experiment. *New Phytologist*, *218*(2), 517-529.

Winkler, D. E., Chapin, K. J., & Kueppers, L. M. (2016). Soil moisture mediates alpine life form and community productivity responses to warming. *Ecology*, *97*(6), 1553-1563.

Wipf, S., Stoeckli, V., & Bebi, P. (2009). Winter climate change in alpine tundra: plant responses to changes in snow depth and snowmelt timing. *Climatic change*, *94*(1-2), 105-121.

Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., Smith, G. M., Zuur, A. F., ... & Walker, N. J. (2009). Negative binomial GAM and GAMM to analyse amphibian roadkills. *Mixed effects models and extensions in ecology with R*, 383-397.

Tables, Images, and Figures

Table 1.1: Model results for influences of treatment on various responses. Asterisks indicate significance level (*p < 0.05, **p < 0.01, ***p < 0.001).

Response	**Predictor**	**Site(s)**	**Random effects**	**Estimate**	**SE**	**F/t †**	**p**	**df**
Snowmelt timing	Treatment	Audubon (A)	Paired subplot	11.80	4.19	7.94	**0.048***	1, 4
		Lefty (L)		13.00	3.08	17.85	**0.0083****	1, 5
		East Knoll (E)		-1.20	0.58	4.24	0.109	1, 4
		Trough (T)		16.80	1.99	71.64	**0.0011****	1, 4
Floral abundance		A, L, T	Paired subplots nested in sites	-183.00	76.04	-2.41	**0.03***	15
Flowering duration		A, L, T		-2.06	4.10	-0.50	0.62	15
Pollinator visitation rate		A, L, T		-6.31	8.83	-0.72	0.49	15

†F statistic reported for models with timing of snowmelt as the response; t value reported for models with floral abundance, flowering duration, and pollinator visitation rate as responses.

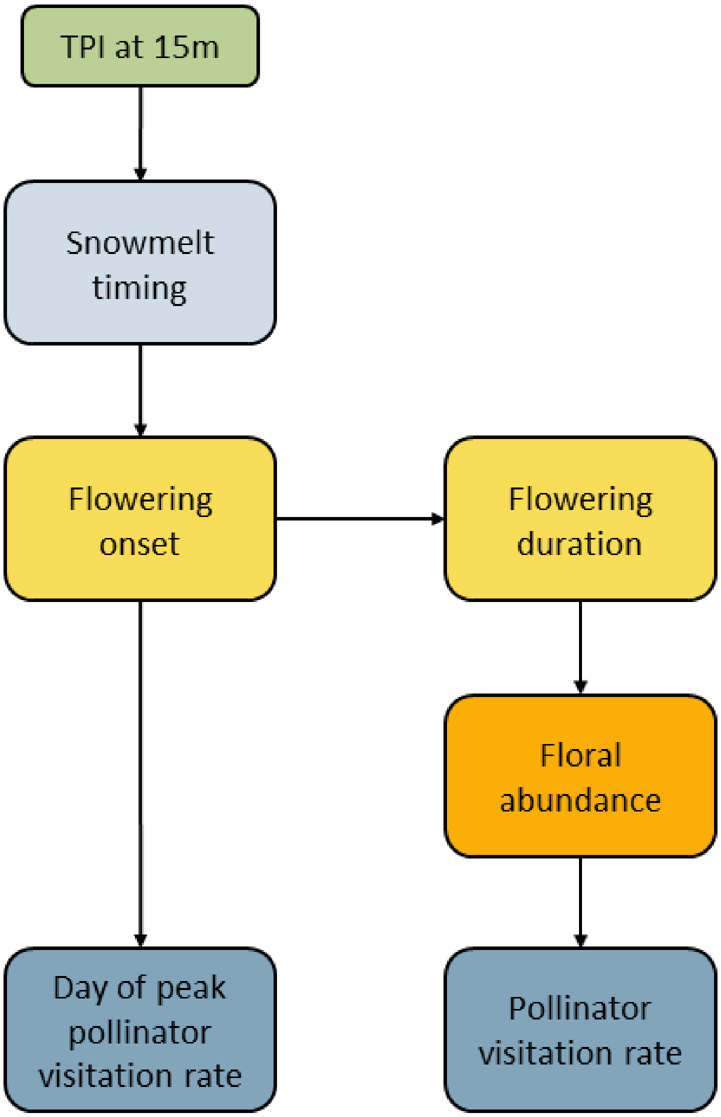


Figure 1.1: The base piecewise structural equation model (pSEM) used to construct pSEMs for both control and advanced snowmelt plots. The model predicts that topographic position index (TPI) at a radius of 15 m impacts snowmelt timing, that snowmelt timing influences flowering onset, that flowering onset influences flowering duration and the day of peak pollinator visitation rate, that flowering duration influences floral abundance, and that floral abundance influences the pollinator visitation rate across the entire flowering period.

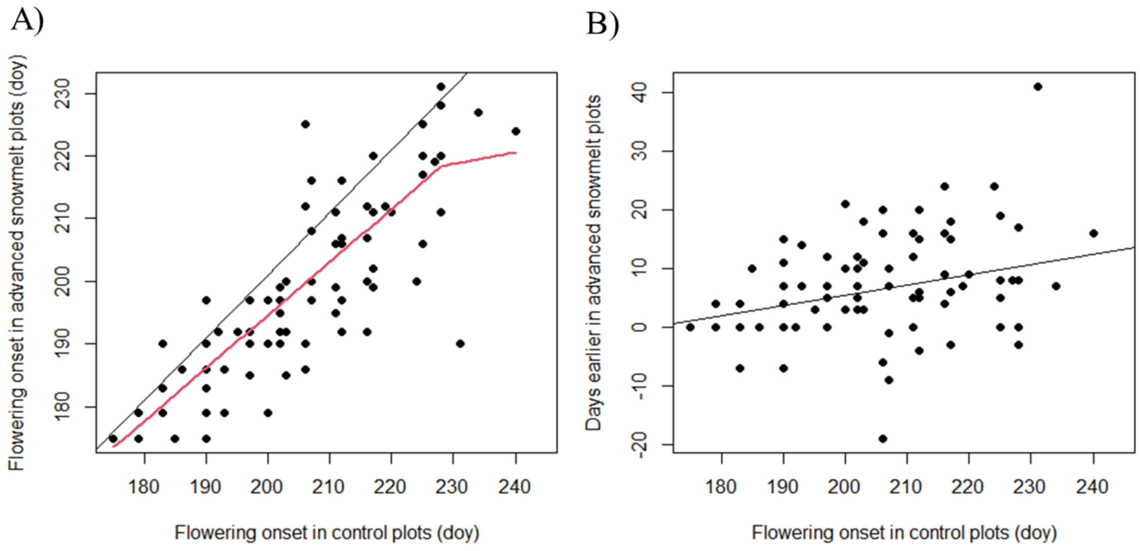


Figure 1.2: Flowering onset in control plots predicts flowering onset in response to advanced snowmelt. A) Segmented regression shown in red, and null expectation of no difference in flowering onset between treatments (1:1 line) shown in black. B) Difference in flowering onset for plants in advanced vs. control subplots, with a regression line shown in black.

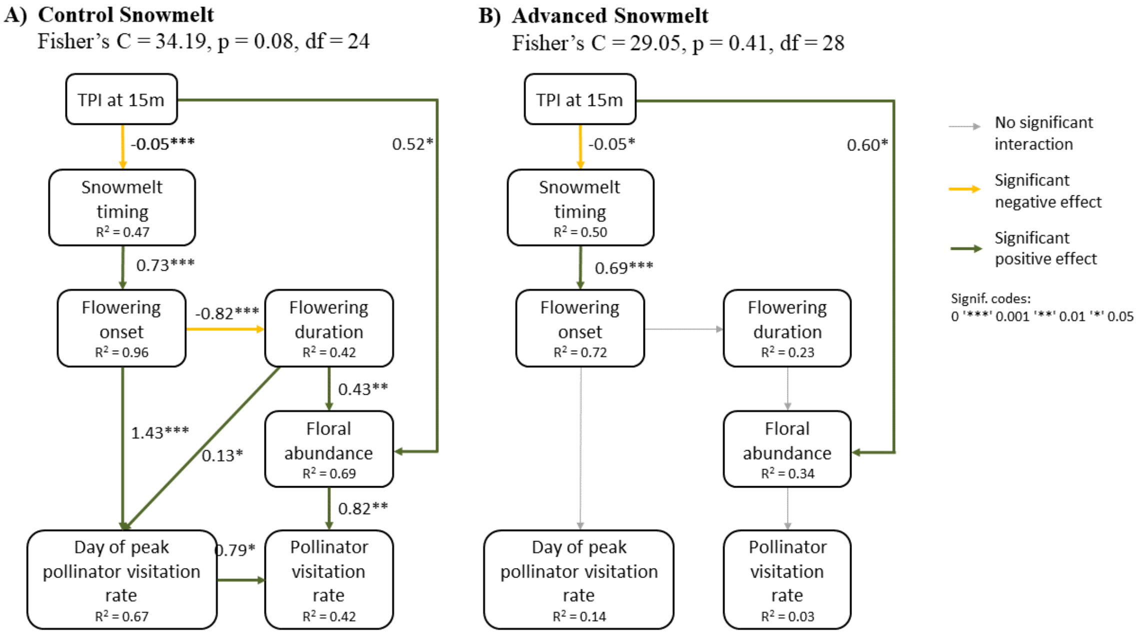


Figure 1.3: The final piecewise structural equation models for A) control and B) advanced snowmelt subplots. Both models had an acceptable fit ($p > 0.05$) and each individual regression used site as a random effect. Green arrows indicate a significant, positive effect; yellow arrows indicate a significant, negative effect; and gray arrows indicate a non-significant interaction. Values adjacent to arrows represent the estimated strength of the relationship, and R^2 values indicate the proportion of variance explained by the model for the associated response variable.

Appendix

Table S1.1: List of plant species found in sites.

Species	**Family**
Achillea millefolium var. *alpicola*	Asteraceae
Agoseris glauca	Asteraceae
Allium geyeri	Liliaceae
Antennaria sp	Asteraceae
Antennaria media	Asteraceae
Arenaria fendleri	Caryophyllaceae
Artemisia sp	Asteraceae
Artemisia scopulorum	Asteraceae
Polygonum bistortoides	Polygonaceae
Polygonum viviparum	Polygonaceae
Caltha leptosepala	Ranunculaceae
Campanula rotundifolia	Campanulaceae
Castilleja occidentalis	Scrophulariaceae
Cerastium arvense ssp. *Strictum*	Caryophyllaceae
Dodecatheon pulchellum	Primulaceae
Draba spp	Brassicaceae
Erigeron	Asteraceae
Erigeron glaucus	Asteraceae
Erigeron melanocephalus	Asteraceae
Eritrichium nanum	Boraginaceae
Erigeron pinnatisectus	Asteraceae
Erigeron simplex	Asteraceae
Gentiana algida	Gentianaceae

Gentianella amarella	Gentianaceae
Gentiana parryi	Gentianaceae
Gentiana tenella	Gentianaceae
Geum rossii var. *turbinatum*	Rosaceae
Lewisia pygmaea	Portulacaceae
Ligusticum tenuifolium	Apiaceae
Lloydia serotina	Liliaceae
Mertensia lanceolata	Boraginaceae
Minuartia obtusiloba	Caryophyllaceae
Oreoxis alpina ssp. *alpina*	Apiaceae
Packera crocata	Asteraceae
Pedicularis groenlandica	Scrophulariaceae
Pedicularis parryi	Scrophulariaceae
Phlox pulvinata	Polemoniaceae
Polemonium viscosum	Polemoniaceae
Potentilla diversifolia	Rosaceae
Ranunculus adoneus	Ranunculaceae
Rhodiola integrifolia	Crassulaceae
Saxifraga rhomboidea	Saxifragaceae
Sedum lanceolatum	Crassulaceae
Sibbaldia procumbens	Rosaceae
Silene acaulis var. *subacaulescens*	Caryophyllaceae
Solidago simplex	Asteraceae
Stellaria longipes ssp. *longipes*	Caryophyllaceae
Tetraneuris acaulis var. *caespitosa*	Asteraceae
Tetraneuris grandiflora	Asteraceae

Tonestus pygmaeus	Asteraceae
Trifolium dasyphyllum	Fabaceae
Trifolium parryi ssp. *parryi*	Fabaceae
Trollius laxus ssp. *albiflorus*	Ranunculaceae
Vaccinium sp	Ericaceae
Veronica wormskjoldii var. *wormskjoldii*	Scrophulariaceae
Viola adunca	Violaceae

Table S1.2: Coefficients for A) control pSEM and B) advanced snowmelt pSEM.

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

A) Control pSEM Coefficients

Response	**Predictor**	**Estimate**	**Std. Error**	**Std. Estimate**	**DF**	**Crit. Value**	**p**	**Sig. Code**
sodoy	tpi15	-0.05	0.01	-0.05	6.0	-3.39	**0.0007**	***
dff	Sodoy	0.73	0.04	0.73	16.00	19.47	**<0.0001**	***
fldur	Dff	-3.36	0.71	-0.82	12.61	-4.75	**0.0004**	***
flabund	Fldur	0.54	0.23	0.43	13.00	2.37	**0.034**	*
flabund	tpi15	0.15	0.05	0.52	13.00	2.88	**0.013**	*
totvis	flabund	1.02	0.29	0.82	12.20	3.45	**0.0047**	**
totvis	vispk	3.47	1.09	0.79	12.71	3.18	**0.0075**	**
vispk	dff	1.43	0.26	1.43	16.00	5.45	**<0.0001**	***
vispk	fldur	0.13	0.06	0.13	16.00	2.08	**0.038**	*

B) Advanced Snowmelt pSEM Coefficients

Response	**Predictor**	**Estimate**	**Std. Error**	**Std. Estimate**	**DF**	**Crit. Value**	**p**	**Sig. Code**
sodoy	tpi15	-0.05	0.01	-0.05	16.00	-3.89	**0.0001**	***
dff	sodoy	0.69	0.08	0.69	16.00	8.59	**<0.0001**	***
fldur	dff	-2.75	1.14	-0.63	13.97	-2.42	**0.03**	*

flabund	fldur	-0.47	0.59	-0.17	13.00	-0.79	0.44	
flabund	tpi15	0.45	0.17	0.60	13.00	2.71	**0.018**	*
totvis	flabund	0.15	0.21	0.18	13.92	0.71	0.49	
vispk	dff	0.41	0.25	0.41	16.00	1.66	0.097	

Key:

Code	**Description**
tpi15	TPI at 15-m radius
sodoy	Snowmelt timing (as day of year)
dff	Flowering onset
fldur	Flowering duration
flabund	Floral abundance
totvis	Pollinator visitation rate
vispk	Day of peak pollinator visitation rate

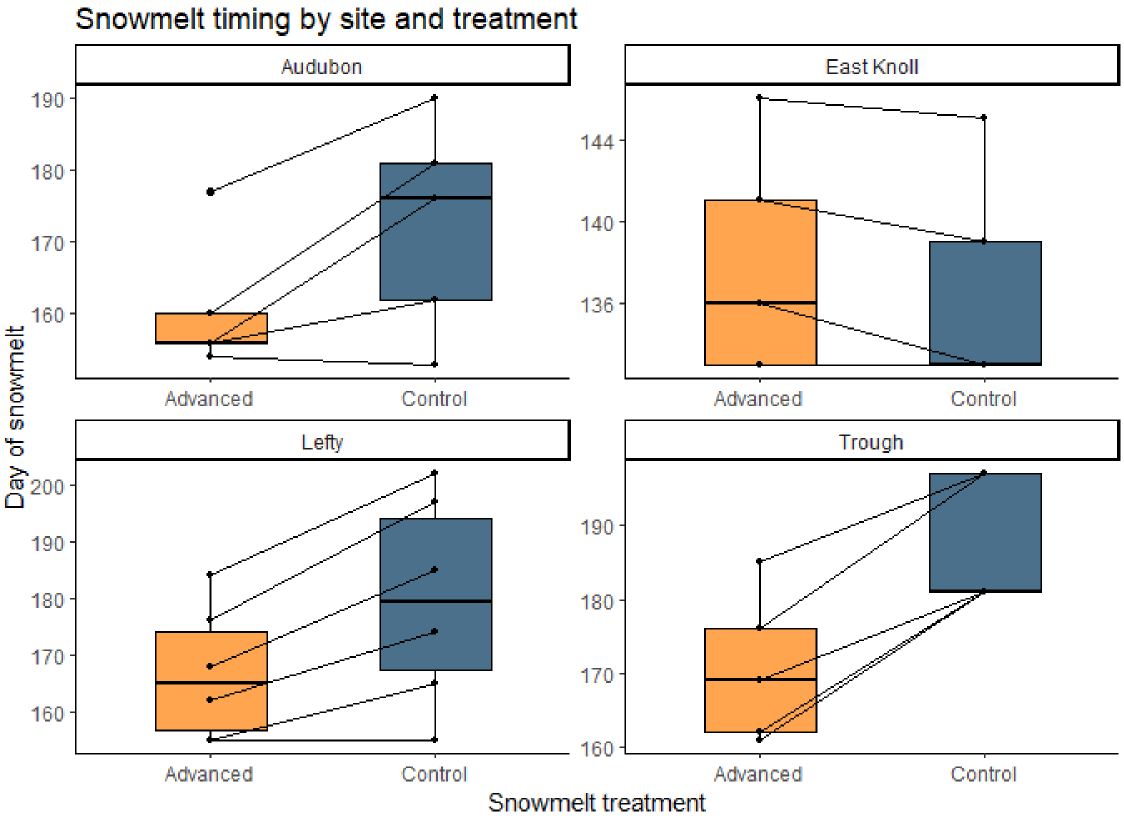


Figure S1.1: Effect of black-sand treatment across four sites, shown as day of snowmelt in control plots (dark blue) and plots where black sand was added to the surface of snow (light orange).

Chapter 2

The structure and stability of plant-pollinator interaction networks are altered by experimentally advanced snowmelt

Abstract

Plant-pollinator mutualisms are critical to the generation and maintenance of plant diversity and the persistence of pollinator communities. These mutualisms are impacted directly and indirectly by climate change in arctic and alpine systems, in part due to the effects of advancing snowmelt. We used large-scale experimental advancement of snowmelt in an alpine-subalpine ecosystem to test how earlier snowmelt influences the assembly of plant-pollinator interaction networks. We performed pollinator observations on forbs throughout the flowering season in unmanipulated plots and paired plots where snowmelt was advanced via the application of black sand. We quantified differences in insect pollinator communities between treatments, asking how earlier snowmelt influenced the abiotic (weather and topography) and biotic (floral community) drivers of pollinator visitation. We found that floral community composition played a larger role in shaping pollinator community composition in advanced plots, while the diversity of plants and weather played a more important role in shaping pollinator community composition in unmanipulated plots. We also explored how the structure of pollination networks changed under advanced snowmelt, examining treatment-induced temporal trends in weekly networks. We found that floral and pollinator functional complementarity decreased over time only in advanced plots but increased over time in unmanipulated plots. Further, interaction evenness increased over time in advanced snowmelt plots but decreased over time in unmanipulated plots. Finally, plant betweenness and degree, as well as pollinator proportional generality, increased over time at a slower rate in unmanipulated plots than in advanced snowmelt plots. Our results indicate that advanced snowmelt treatment altered how these network indices, which have been linked to network stability, change over time. Importantly, we demonstrate that snowmelt timing influences the assembly and structure of plant-pollinator networks, yielding different temporal dynamics that may lead to decreased within-season network stability in a changing climate.

Funding acknowledgement: *This study received substantial funding from the Niwot Ridge LTER VII program (NWT VII: NSF DEB - 1637686).*

Data availability: *Data associated with this study is available via the Environmental Data Initiative (Rose-Person et al. 2023).*

DOI:10.6073/pasta/920fe947b69eea415bdca0af3b3e33c9.

Introduction

Climate change has caused rapid shifts in the environmental context of many species interactions, including those between plants and pollinators (Parmesan 2006, IPCC 2007). Plant-pollinator interactions, mutualistic interactions crucial for the persistence of most flowering plant and many insect species, are threatened by climate change-driven shifts in phenology – the timing of life history events (Bertin 2008, Hegland et al. 2009, Kudo and Ida 2013, Petanidou et al. 2014). Phenological shifts can lead to a phenological mismatch if the phenology of interacting partners responds differentially to climate drivers (Samplonious et al. 2016, Kudo and Cooper 2019, Gérard et al. 2020). Decreased interaction rates that result from phenological mismatches may decrease the fitness of both partners, which in turn may affect population persistence (Memmott et al. 2007). In alpine systems, anthropogenic dust deposition, climate change-driven decreases in snowpack, and increases in late spring temperatures can induce phenological shifts by advancing the timing of snowmelt (Totland 1993, Hidalgo et al. 2009, Deems et al. 2013, Pederson et al. 2013). However, the phenological responses of plant-pollinator interaction networks to advanced snowmelt remain understudied in these threatened ecosystems (Inouye 2020).

Phenological mismatches between plants and pollinators may affect pollination not only by altering floral community composition but also by changing the abiotic context in which the mutualism occurs. These changes may lead to interaction rewiring – shifts in the identities of interacting partners – and alterations to the structure of the network of interacting plants and pollinators (Burkle and Alarcón 2011, CaraDonna et al. 2017). Plants respond differently to advanced snowmelt, some advancing their flowering phenology and others not responding (Rose-Person et al., in review [Ch. 1]). These species-specific shifts in flowering time may alter the dynamics of floral resource availability for pollinators. Indeed, snowmelt timing can influence the assemblage of co-flowering plant species, altering the biotic context in which pollination occurs (Forrest et al. 2010). These shifts in co-flowering synchrony may alter pollinator behavior. For example, specialist bee visitation to *Clarkia xantiana* (Onagraceae) increased by nearly 100% when flowering adjacent to other *Clarkia* species that shared the same pollinator (Moeller 2004, Moeller and Gebre 2005). Ultimately, these changes could modify the costs (heterospecific pollen transfer) and benefits (higher visitation rates) of co-flowering (Sargent and Ackerly 2008). By altering the relative abundances of flowering species, advanced snowmelt may further impact competitive and facilitative dynamics (Groom 1998).

Phenologically advanced plants also experience novel abiotic environments and may be exposed to frost or altered soil moisture conditions, which could impact plant physiology and the floral cues presented to pollinators, leading to shifts in plant-pollinator interactions (Inouye 2008, Gezon et al. 2016, Takkis et al. 2018, Rose-Person et al., in revision [Ch. 3]). Abiotic context can thus influence the assembly of plant-pollinator interactions. For example, a study in the Australian alpine found that wind exposure influenced the foraging behavior of some orders of insects: Hymenoptera were more specialized at more exposed sites and made more visits at higher temperatures, while diptera were the only insects found visiting flowers in inclement weather (Goodwin et al. 2021). Similarly, butterfly and bumblebee foraging activity was constrained by low temperatures (Bergman et al. 1996), and arthropod flower visitors increased in abundance and diversity with increasing temperature in an alpine system (Bonelli et al. 2022).

Within-season changes in the identities of interacting partners, and the frequency of these interactions, are likely to influence the structure of plant-pollinator interaction networks, which could alter their stability. Network structure can be summarized with various network metrics that have been linked to network stability. These include network-level indices such as functional complementarity, interaction evenness, and nestedness (Memmott et al. 2004, Hegland et al. 2009, Kaiser-Bunbury et al. 2015, Wang et al. 2021) as well as species-level indices such as species degree (Landi et al. 2018). Turnover in the identities of plants and insects and interaction rewiring can occur within a single flowering season, leading to variation in network structure and stability over time (CaraDonna et al. 2017). In the context of climate change-driven phenological shifts, it is particularly critical to consider the temporal dynamics of interaction networks (CaraDonna et al. 2021).

In this study, we use a field-based, large-scale experimental manipulation of snowmelt timing to explore the influence of earlier snowmelt on the assembly and structure of plant-pollinator networks in an alpine-subalpine environment. We ask 1) how forb and insect pollinator communities differed between advanced and unmanipulated snowmelt plots, 2) how experimental advancement of snowmelt influenced the drivers of insect pollinator community structure, and 3) how experimental advancement of snowmelt influenced plant-pollinator networks across a flowering season.

Methods

Data collection

We performed pollinator observations at four sites across Niwot Ridge in the summer of 2020. At each of three focal sites, named *Audubon*, *Lefty*, and *Trough*, snowmelt in one 40 x 10-m plot was advanced by placing inert black sand on the surface of the snow before snowmelt occurred, leading to an advance of snowmelt by up to 14 days (Rose-Person et al., in review [Ch. 1]). This 'early' snowmelt plot was paired with an unmanipulated 40 x 10-m adjacent plot. We performed 15-min observations in five 2-m^2 subplots per plot per treatment. We recorded the identities of insects and plants upon observing interactions of insects with anthers or stigmas and collected the first two morphospecies observed of each insect visitor per observation period for identification. Observations were repeated twice weekly. Additionally, we counted and identified flowers in each subplot on all days that we performed pollinator observations (Table S1.1). Fine-scale measurements of snow depth were performed continually at each site. To quantify topography, we measured landscape topographic position index and slope of subplots. Insects were pinned and identified by AR-P and DY to the lowest taxonomic resolution possible (Table S1.2). Voucher specimens of all taxa were deposited in the Entomology Research Museum at the University of California, Riverside, and voucher specimens of most taxa will be deposited in the Entomology Collection at the University of Colorado, Boulder in summer 2024 (Table S1.2).

Data analysis

All analyses were performed using R (R Core Team 2023), and all linear mixed-effects models (LMMs) were fitted using the function *lmer* in the package *lme4* (Bates et al. 2015). To determine whether the total diversity and abundance of pollinators and flowers differed between treatments, we calculated Shannon diversity using the R package *vegan* (Oksanen et al. 2022), then fitted LMMs with treatment as the predictor, site and subplot as random effects, and flower abundance, flower Shannon diversity, visiting insect abundance, and visiting insect Shannon diversity as response variables. We also fitted four LMMs with the interaction between treatment and day of year or day of year^2 as the predictor, site and subplot as random effects, and flower or pollinator abundance as a response variable.

To examine differences in the dispersion of flowering and insect communities between treatments at each site, we first separated the dataset into our focal sites and calculated the mean distance of members of each treatment to the treatment group's centroid in multivariate space using Bray-Curtis distance with *betadisper* in the package *vegan* (Anderson et al. 2011), then performed an analysis of variance in base R to test whether the variances of the groups were different. Finally, we tested the influence of day of year and treatment on insect and floral communities by performing a permutational analysis of variance (PERMANOVA) test using *adonis2* in the package *vegan* using insect or flowering community, respectively; treatment and day of year as predictors; and Bray-Curtis dissimilarity to calculate distance between points.

To identify the factors shaping pollinator communities that visited flowers in our plots, we used redundancy analysis (RDA) with the function *rda* in *vegan.* We first calculated the length of the first axis of a detrended correspondence analysis (DCA) to determine whether an RDA was an appropriate test. Then, we used insect visitor community composition as our response variable; day of year, weather, topographic, and floral community as predictors; and subplot nested within site as a random effect. To create our predictive axes, we performed principal coordinates analysis (PCoA) ordination on predictive matrices. We used the first six axes of a PCoA on floral communities (which explained 39% and 38% of floral community variation in advanced and unmanipulated plots, respectively), the first axis of a PCoA on weather variables (which included maximum and average wind speed, temperature, relative humidity, and percent cloud cover and explained 89% and 86% of the variation in weather in advanced and unmanipulated plots, respectively), and the first axis of a PCoA on topographic variables (which included slope and TPI at 1.5, 5.5, and 15 m, and explained 87% and 95% of the variation in topography in advanced and unmanipulated plots, respectively). To select the best model, we performed forward model selection using the function *ordiR2step* in *vegan.* To examine the species that loaded most heavily onto each PCoA axis of the floral community, we used the function *add.spec.scores* in *Biodiversity2* (Kindt 2005).

To compare networks of interacting species, we first visualized bipartite plant-pollinator networks using weighted data from all interactions in each treatment in each site using the functions *frame2webs* and *plotweb* in the package *bipartite* (Dormann et al. 2008). To calculate network-level and species-level indices, we compiled species interaction data across subplots and by week. We used the function *networklevel* in *bipartite* to calculate network-level indices and the function *specieslevel* in *bipartite* to calculate species-level network indices for pollinators and plants, respectively (Dormann et al. 2009, Dormann 2011). Finally, we fit LMMs with each network-level and species-level index as a response variable; treatment, week, and their interaction as predictors; and site and species as random effects.

Results

There were significantly more flowers in advanced snowmelt plots than unmanipulated plots (Fig. S2.1, Table S2.3). Floral abundance decreased significantly over time in both treatments, and peaked mid-season (Fig. S2.2, Table S2.3). Communities had even dispersion between treatments across sites with the exception of floral communities in sites Lefty and Trough (Fig. S2.3, Table S2.4). Floral community composition differed significantly by treatment, day of year, and their interaction across all three sites (Fig. S2.3, Table S2.4). The Shannon diversity of insects was significantly lower in advanced snowmelt plots (Fig. S2.1, Table S2.3). Pollinator abundance decreased over time in advanced snowmelt plots, but increased over time in unmanipulated plots, and was highest at the beginning and end of the season (Fig. S2.2, Table S2.3). Pollinator community composition differed by day of year and its interaction with treatment in sites Lefty and Trough, but only differed by treatment in site Audubon (Fig. S2.3, Table S2.4).

The length of the first DCA axes for insect communities in plots with unmanipulated and advanced snowmelt were 1.01 and 0.65, respectively, indicating that an RDA was an appropriate test for these data. RDA ordinations for both advanced and unmanipulated plots fit the data ($R^2 = 0.13$, $p<0.001$ and $R^2= 0.09$, $p<0.001$, respectively; Fig. S2.4, Table S2.5). In both treatments, day of year, site, and floral community PCoA axes 2, 4, and 6 significantly predicted pollinator communities (Fig. S2.4, Table S2.5). However, floral community PcoA axes 1 and 3 significantly predicted pollinator community in advanced plots only, while weather significantly predicted pollinator community composition in unmanipulated plots only (Fig. S2.4, Table S2.5). In both treatments, *Geum rossii* and *Solidago simplex* loaded heavily onto floral PcoA axis 1, *Ligusticum tenuifolium* onto PcoA 2, *Artemisia scopulorum* onto PcoA 3, *Artemisia scopulorum* and *Minuartia obtusiloba* onto PcoA 4, and *Potentilla diversifolia* and *Arenaria fendleri* onto PcoA 6 (Table S2.6).

Several network-level indices (described in Table S2.7) differed significantly by the interaction of week and treatment (Table S2.8a, Fig. 2.1, Fig. 2.2a). Functional complementarity of both plants and pollinators decreased over time in advanced snowmelt plots but increased over time in unmanipulated plots (Table S2.8a, Fig. 2.2a). Interaction evenness increased over time in advanced snowmelt plots but decreased over time in unmanipulated plots (Table S2.8a, Fig. 2.2a). Nestedness, as measured by weighted Nestedness based on Overlap and Decreasing Fill (NODF), decreased over time in both treatments (Table S2.8a, Fig. 2.2a).

Plant species-level indices were also influenced by the interaction of treatment and week (Table S2.8b, Fig. 2.2b). Betweenness of plants decreased over time in advanced snowmelt plots, but increased over time in unmanipulated plots (Table S2.8b, Fig. 2.2b). Plant species degree increased at a slower rate in advanced plots than in unmanipulated plots (Table S2.8b, Fig. 2.2b). Proportional generality of plants decreased over time in both treatments (Table S2.8b, Fig. 2.2b). Finally, proportional generality of pollinators increased over time in advanced plots at a slower rate than in unmanipulated plots (Table S2.8c, Fig. 2.2c).

Discussion

Our results demonstrate that snowmelt timing influenced the functional complementarity and interaction evenness of plant-pollinator networks, and altered how these network indices changed over the season. We also found evidence that advanced snowmelt altered the factors structuring pollinator communities. Altogether, the impacts of our experimental manipulation of snowmelt timing on the seasonal dynamics of network structure suggest that plant-pollinator network stability and assembly may be altered under future climates, with potential implications for species persistence.

Many of the patterns we found suggest that advanced snowmelt alters how network stability changes over the season. At the network level, functional complementarity (FC) of both plants and pollinators decreased over time in advanced snowmelt plots, but increased over time in control plots. Higher FC, a metric of ecological niche differentiation, may result from higher interspecific competition (Blüthgen and Klein 2011). The trend in FC therefore suggests that competition among pollinators for floral resources starts low and then increases through time in unmanipulated plots, but starts high and decreases over time in advanced snowmelt plots. This higher competition over time between pollinators for floral resources in unmanipulated plots may have been driven by the overall lower floral abundance in unmanipulated plots combined with lower floral abundance and higher pollinator visitation at the end of the season in unmanipulated plots (Prendergrast and Ollerton 2021). By conferring resistance to disturbance and species extinctions, FC has been hypothesized to increase network stability (Memmott et al. 2004, Hegland et al. 2009, Song et al. 2017, Landi et al. 2018, Wang et al. 2021). Therefore, the observed temporal trends in FC indicate that while stability decreased over time in advanced plots, it increased over time in unmanipulated plots.

Additionally, interaction evenness (IE) increased over time in advanced snowmelt plots, but decreased over time in unmanipulated plots. This indicates that interactions became more homogeneous over time in advanced snowmelt plots, but became more heterogeneous over time in unmanipulated plots. While higher IE may in some cases support greater ecosystem functions, lower IE indicates that interactions between subsets of individuals are relatively stronger than those between other groups, which could ultimately yield higher network stability (Kaiser-Bunbury et al. 2015). Thus, our findings indicate that the increase in IE over time in advanced snowmelt plots and decrease over time in unmanipulated plots may confer lower stability over time in advanced plots and greater stability over time in unmanipulated plots.

Treatment also influenced how species-level metrics changed over the season. Plant betweenness decreased over time in advanced snowmelt plots, but increased over time in unmanipulated plots (Chakraborty et al. 2021). This finding indicates that fewer species acted as network connectors in advanced snowmelt plots by the end of the season. Since network connectors are important for network stability, this provides further evidence that advanced snowmelt may result in less stable late-season networks (Newman 2004, González et al. 2010, Chakraborty et al. 2021). In addition, we observed a slightly lower rate of increase in plant degree in advanced snowmelt plots (Jordano et al. 2003, Novella-Fernandez et al. 2019). Degree represents the number of interaction partners and thus provides a simple measure of generality (Jordano et al. 2003). We also found that in advanced plots, pollinator generality increased at a slower rate. Because generality is predicted to increase network resilience to climate change-driven shifts, these results corroborate our results for FC, IE, and betweenness, further demonstrating that advanced snowmelt plots will be less stable and have lower resilience to climate change at the end of the season (Waser et al. 1996, Jordano et al. 2003, Hegland et al. 2009, Landi et al. 2018).

This study demonstrates that treatment influenced the direction of change over time of network indices that are important drivers of stability – such as FC, IE, and betweenness – and resilience to change – such as generality. Nestedness is also associated with higher network stability, and decreased over time in both treatments. Nevertheless, our findings demonstrate that advanced snowmelt altered network stability and the processes, such as competition, which structure communities. This could influence population persistence in our study system by putting networks and the pollination services they provide at a greater risk of impacts from invasive species and climate change-driven abiotic shifts (Bascompte and Scheffer 2023). Seedling recruitment is an important driver of plant establishment at Niwot Ridge (Forbis 2003), so reduced pollination success could negatively influence plant demography in this system. Importantly, many alpine tundra habitats – including Niwot Ridge – experience mid-summer drought (Billings and Mooney 1968, Bowman and Fisk 2001, Knowles et al. 2015, Sloat et al. 2015). Drought may influence plant-pollinator network structure by either increasing or decreasing specialization (Endres et al. 2021, Morozumi et al. 2022). If networks in plots with advanced snowmelt are less stable than those with unmanipulated snowmelt, they may be more susceptible to drought-induced shifts in network structure during mid-summer periods of drought.

Network indices such as nestedness, specialization, and connectance generally have high rates of change within a single season (CaraDonna and Waser 2020, CaraDonna et al. 2021), likely reflecting the dynamic roles of individual plants and pollinators (Dupont et al. 2009, Simanonok and Burkle 2014, CaraDonna and Waser 2020). This variability is critical to the coexistence of species and other community dynamics (Saavedra et al. 2016, 2017; Valdovinos et al. 2016). Our finding that IE decreased over time in unmanipulated plots but increased over time in advanced snowmelt plots suggests that in unmanipulated plots only, plant-pollinator interactions became more strongly sorted by the end of the season, indicating that the assembly processes that shaped unmanipulated plots were absent in advanced snowmelt plots (Albrecht et al. 2010). In contrast with our findings, IE increased as plant-pollinator networks assembled in the 140-year period following glacial retreat (Albrecht et al. 2010). The authors attribute this pattern to the observed increase in generality over time (Albrecht et al. 2010). In our study, generality increased more over time in unmanipulated plots than in advanced snowmelt plots, but did not confer the same increase in IE over time. Further, our result that FC increased over time in unmanipulated plots but decreased over time in advanced plots may indicate that the competitive processes shaping FC play a different role in shaping the assembly of communities under advanced snowmelt (Blüthgen and Klein 2011, Wagg et al. 2017).

Across three major network-level indices, we observed that advanced snowmelt likely led to a decrease in stability over time, while stability tended to increase over time in unmanipulated plots. The species-level metric betweenness, also positively correlated with stability, showed a similar pattern. Reduced network stability under climate change may make plant-pollinator networks more susceptible to invasion, or reduced rates of pollination, ultimately eroding biodiversity and ecosystem function.

References

Albrecht, M., Riesen, M., & Schmid, B. (2010). Plant–pollinator network assembly along the chronosequence of a glacier foreland. *Oikos*, 119(10), 1610-1624.

Anderson, M. J., Crist, T. O., Chase, J. M., Vellend, M., Inouye, B. D., Freestone, A. L., ... & Swenson, N. G. (2011). Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. *Ecology Letters*, 14(1), 19-28.

Bascompte, J., & Scheffer, M. (2023). The resilience of plant–pollinator networks. *Annual Review of Entomology*, 68, 363-380.

Bates, D., Maechler, M., Bolker, B., Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1-48. doi:10.18637/jss.v067.i01.

Bergman, P., Molau, U., & Holmgren, B. (1996). Micrometeorological impacts on insect activity and plant reproductive success in an alpine environment, Swedish Lapland. *Arctic and Alpine Research*, 28(2), 196-202.

Bertin, R. I. (2008). Plant phenology and distribution in relation to recent climate change. *The Journal of the Torrey Botanical Society*, 135(1), 126-147.

Billings, W. D., & Mooney, H. A. (1968). The ecology of arctic and alpine plants. *Biological reviews*, 43(4), 481-529.

Blüthgen, N., & Klein, A. M. (2011). Functional complementarity and specialisation: the role of biodiversity in plant–pollinator interactions. *Basic and Applied Ecology*, 12(4), 282-291.

Bonelli, M., Eustacchio, E., Avesani, D., Michelsen, V., Falaschi, M., Caccianiga, M., ... & Casartelli, M. (2022). The early season community of flower-visiting arthropods in a high-altitude alpine environment. *Insects*, 13(4), 393.

Bowman, W. D., & Fisk, M. C. (2001). Primary production. *Structure and function of an alpine ecosystem: Niwot Ridge, Colorado*, 177-197.

Burkle, L. A., & Alarcón, R. (2011). The future of plant–pollinator diversity: understanding interaction networks across time, space, and global change. *American Journal of Botany*, 98(3), 528-538.

CaraDonna, P. J., & Waser, N. M. (2020). Temporal flexibility in the structure of plant–pollinator interaction networks. *Oikos*, 129(9), 1369-1380.

CaraDonna, P. J., Burkle, L. A., Schwarz, B., Resasco, J., Knight, T. M., Benadi, G., ... & Vazquez, D. P. (2021). Seeing through the static: the temporal dimension of plant–animal mutualistic interactions. *Ecology Letters*, 24(1), 149-161.

CaraDonna, P. J., Petry, W. K., Brennan, R. M., Cunningham, J. L., Bronstein, J. L., Waser, N. M., & Sanders, N. J. (2017). Interaction rewiring and the rapid turnover of plant–pollinator networks. *Ecology Letters*, 20(3), 385-394.

Chakraborty, P., Chatterjee, S., Smith, B. M., & Basu, P. (2021). Seasonal dynamics of plant pollinator networks in agricultural landscapes: how important is connector species identity in the network? *Oecologia*, 196(3), 825-837.

Deems, J. S., Painter, T. H., Barsugli, J. J., Belnap, J., Udall, B. (2013). Combined impacts of current and future dust deposition and regional warming on Colorado River Basin snow dynamics and hydrology. *Hydrology and Earth System Sciences*, 17(11):4401-4413. https://doi.org/10.5194/hess-17-4401

Dormann, C. F., Gruber, B., & Fründ, J. (2008). Introducing the bipartite package: analysing ecological networks. *Interaction*, 1(0.2413793), 8-11.

Dormann, C. F. (2011). How to be a specialist? Quantifying specialisation in pollination networks. *Network Biology 1*, 1 - 20.

Dormann, C. F., Fruend, J., Bluethgen, N. & Gruber B. (2009). Indices, graphs and null models: analyzing bipartite ecological networks. *The Open Ecology Journal*, 2, 7-24.

Dupont, Y. L., Padrón, B., Olesen, J. M., & Petanidou, T. (2009). Spatio-temporal variation in the structure of pollination networks. *Oikos*, 118(8), 1261-1269.

Endres, K. L., Morozumi, C. N., Loy, X., Briggs, H. M., CaraDonna, P. J., Iler, A. M., ... & Brosi, B. J. (2021). Plant–pollinator interaction niche broadens in response to severe drought perturbations. *Oecologia*, 197, 577-588.

Forbis, T. A. (2003). Seedling demography in an alpine ecosystem. *American Journal of Botany*, 90(8), 1197-1206.

Forrest, J., Inouye, D. W., & Thomson, J. D. (2010). Flowering phenology in subalpine meadows: Does climate variation influence community co-flowering patterns? *Ecology*, 91(2), 431-440.

Gérard, M., Vanderplanck, M., Wood, T., & Michez, D. (2020). Global warming and plant–pollinator mismatches. *Emerging Topics in Life Sciences*, 4(1), 77-86.

Gezon, Z. J., Inouye, D. W., & Irwin, R. E. (2016). Phenological change in a spring ephemeral: implications for pollination and plant reproduction. *Global Change Biology*, 22(5), 1779-1793.

González, A. M. M., Dalsgaard, B., & Olesen, J. M. (2010). Centrality measures and the importance of generalist species in pollination networks. *Ecological Complexity*, 7(1), 36-43.

Goodwin, E. K., Rader, R., Encinas-Viso, F., & Saunders, M. E. (2021). Weather conditions affect the visitation frequency, richness and detectability of insect flower visitors in the Australian alpine zone. *Environmental Entomology*, 50(2), 348-358.

Groom, M. J. (1998). Allee effects limit population viability of an annual plant. *The American Naturalist*, 151(6), 487-496.

Hegland, S. J., Nielsen, A., Lázaro, A., Bjerknes, A. L., & Totland, Ø. (2009). How does climate warming affect plant-pollinator interactions? *Ecology Letters*, 12(2), 184-195.

Hidalgo, H. G., Das, T., Dettinger, M. D., Cayan, D. R., Pierce, D. W., Barnett, T. P., ... & Nozawa, T. (2009). Detection and attribution of streamflow timing changes to climate change in the western United States. Journal of Climate, 22(13), 3838-3855.

Inouye, D. W. (2008). Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology*, 89(2), 353-362.

Inouye, D. W. (2020). Effects of climate change on alpine plants and their pollinators. *Annals of the New York Academy of Sciences*, 1469(1), 26-37.

IPCC 2007. Climate change 2007: Synthesis report. Contrib. Working Grp I, II and III to the 4th Assess. Rep. Intergovernmental Panel on Climate Change. Pachauri, R. K. and Reisinger, A. (eds). – IPCC, Geneva, Switzerland.

Jordano, P., Bascompte, J., & Olesen, J. M. (2003). Invariant properties in coevolutionary networks of plant–animal interactions. *Ecology Letters*, 6(1), 69-81.

Kaiser-Bunbury, C. N., & Blüthgen, N. (2015). Integrating network ecology with applied conservation: a synthesis and guide to implementation. *Annals of Botany Plants*, 7, plv076.

Kindt, R., & Coe, R. (2005). _Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies_. World Agroforestry Centre (ICRAF). ISBN 92-9059-179-X, <http://www.worldagroforestry.org/output/tree-diversity-analysis>.

Knowles, J. F., Burns, S. P., Blanken, P. D., & Monson, R. K. (2015). Fluxes of energy, water, and carbon dioxide from mountain ecosystems at Niwot Ridge, Colorado. *Plant Ecology & Diversity*, 8(5-6), 663-676.

Kudo, G., & Cooper, E. J. (2019). When spring ephemerals fail to meet pollinators: mechanism of phenological mismatch and its impact on plant reproduction. *Proceedings of the Royal Society B*, 286(1904), 20190573.

Kudo, G., & Ida, T. Y. (2013). Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology*, 94(10), 2311-2320.

Landi, P., Minoarivelo, H. O., Brännström, Å., Hui, C., & Dieckmann, U. (2018). Complexity and stability of ecological networks: a review of the theory. *Population Ecology*, 60, 319-345.

Memmott, J., Craze, P. G., Waser, N. M., & Price, M. V. (2007). Global warming and the disruption of plant–pollinator interactions. *Ecology Letters*, 10(8), 710-717.

Memmott, J., Waser, N. M., & Price, M. V. (2004). Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society B*, 271(1557), 2605-2611.

Metelmann, S., Sakai, S., Kondoh, M., & Telschow, A. (2020). Evolutionary stability of plant–pollinator networks: efficient communities and a pollination dilemma. *Ecology Letters*, 23(12), 1747-1755.

Moeller, D. A. (2004). Facilitative interactions among plants via shared pollinators. *Ecology*, 85(12), 3289-3301.

Moeller, D. A., & Gebre, M. A. (2005). Ecological context of the evolution of self-pollination in *Clarkia xantiana*: Population size, plant communities, and reproductive assurance. *Evolution*, 59(4), 786-799.

Morozumi, C., Loy, X., Reynolds, V., Schiffer, A., Morrison, B., Savage, J., & Brosi, B. (2022). Simultaneous niche expansion and contraction in plant–pollinator networks under drought. *Oikos*, 2022(11), e09265.

Newman, M. E. (2004). Detecting community structure in networks. *The European Physical Journal B*, 38, 321-330.

Novella-Fernandez, R., Rodrigo, A., Arnan, X., & Bosch, J. (2019). Interaction strength in plant-pollinator networks: Are we using the right measure? *PloS One*, 14(12), e0225930.

Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2022). _vegan: Community Ecology Package_. R package version 2.6-4, <https://CRAN.R-project.org/package=vegan>.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37, 637-669.

Pederson, G. T., Betancourt, J. L., & McCabe, G. J. (2013). Regional patterns and proximal causes of the recent snowpack decline in the Rocky Mountains, US. Geophysical Research Letters, 40(9), 1811-1816.

Petanidou, T., Kallimanis, A. S., Sgardelis, S. P., Mazaris, A. D., Pantis, J. D., & Waser, N. M. (2014). Variable flowering phenology and pollinator use in a community suggest future phenological mismatch. *Acta Oecologica*, 59, 104-111.

Prendergast, K. S., & Ollerton, J. (2022). Impacts of the introduced European honeybee on Australian bee-flower network properties in urban bushland remnants and residential gardens. *Austral Ecology*, 47(1), 35-53.

Resasco, J., Chacoff, N. P., & Vázquez, D. P. (2021). Plant–pollinator interactions between generalists persist over time and space. *Ecology*, 102(6), e03359.

Rose-Person, A., Spasojevic, M., Forrester, C., Bowman, W., Suding, K., Oldfather, M., and Rafferty, N. (2023). Pollinator visitation, flower count, and seed set in Black Sand plots, 2020. ver 1. *Environmental Data Initiative*. https://doi.org/10.6073/pasta/920fe947b69eea415bdca0af3b3e33c9 (Accessed 2023-11-08).

Saavedra, S., Cenci, S., del-Val, E., Boege, K., & Rohr, R. P. (2017). Reorganization of interaction networks modulates the persistence of species in late successional stages. *Journal of Animal Ecology*, 86(5), 1136-1146.

Saavedra, S., Rohr, R. P., Olesen, J. M., & Bascompte, J. (2016). Nested species interactions promote feasibility over stability during the assembly of a pollinator community. *Ecology and Evolution*, 6(4), 997-1007.

Samplonius, J. M., Kappers, E. F., Brands, S., & Both, C. (2016). Phenological mismatch and ontogenetic diet shifts interactively affect offspring condition in a passerine. *Journal of Animal Ecology*, 85(5), 1255-1264.

Sargent, R. D., & Ackerly, D. D. (2008). Plant–pollinator interactions and the assembly of plant communities. *Trends in Ecology & Evolution*, 23(3), 123-130.

Simanonok, M. P., & Burkle, L. A. (2014). Partitioning interaction turnover among alpine pollination networks: spatial, temporal, and environmental patterns. *Ecosphere*, 5(11), 1-17.

Sloat, L. L., Henderson, A. N., Lamanna, C., & Enquist, B. J. (2015). The effect of the foresummer drought on carbon exchange in subalpine meadows. *Ecosystems*, 18, 533-545.

Song, C., Rohr, R. P., & Saavedra, S. (2017). Why are some plant–pollinator networks more nested than others? *Journal of Animal Ecology*, 86(6), 1417-1424.

Takkis, K., Tscheulin, T., & Petanidou, T. (2018). Differential effects of climate warming on the nectar secretion of early-and late-flowering Mediterranean plants. *Frontiers in Plant Science*, 9.

Totland, Ø. (1993) Pollination in alpine Norway: flowering phenology, insect visitors, and visitation rates in two plant communities. *Canadian Journal of Botany*, 71(8):1072-1079. https://doi.org/10.1139/b93-124

Valdovinos, F. S., Brosi, B. J., Briggs, H. M., Moisset de Espanés, P., Ramos-Jiliberto, R., & Martinez, N. D. (2016). Niche partitioning due to adaptive foraging reverses effects of nestedness and connectance on pollination network stability. *Ecology Letters*, 19(10), 1277-1286.

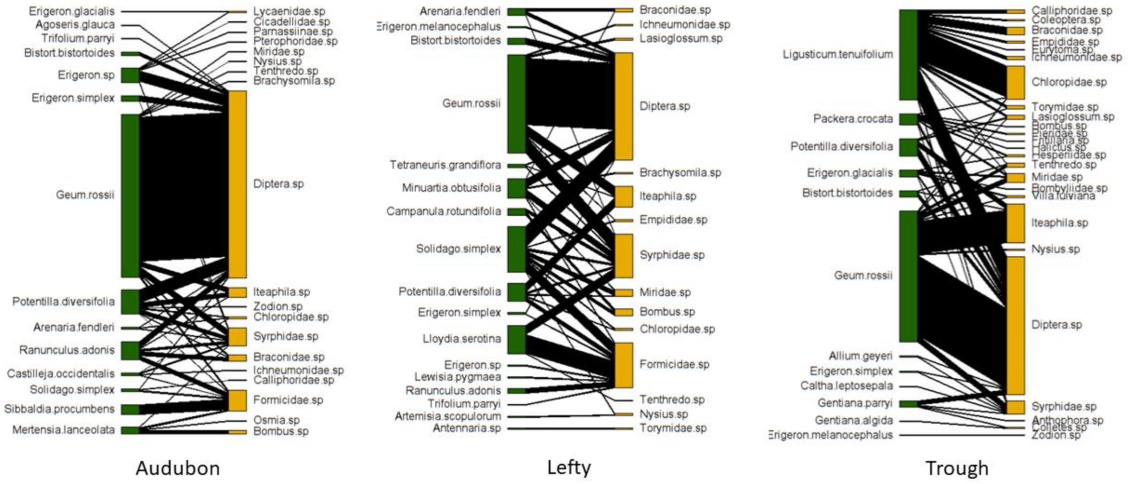
Wagg, C., Ebeling, A., Roscher, C., Ravenek, J., Bachmann, D., Eisenhauer, N., ... & Weisser, W. W. (2017). Functional trait dissimilarity drives both species complementarity and competitive disparity. *Functional Ecology*, 31(12), 2320-2329.

Wang, S., Isbell, F., Deng, W., Hong, P., Dee, L. E., Thompson, P., & Loreau, M. (2021). How complementarity and selection affect the relationship between ecosystem functioning and stability. *Ecology*, 102(6), e03347.

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, 77(4), 1043-1060.

Tables, Images, and Figures

A) Advanced snowmelt plots



B) Unmanipulated snowmelt plots

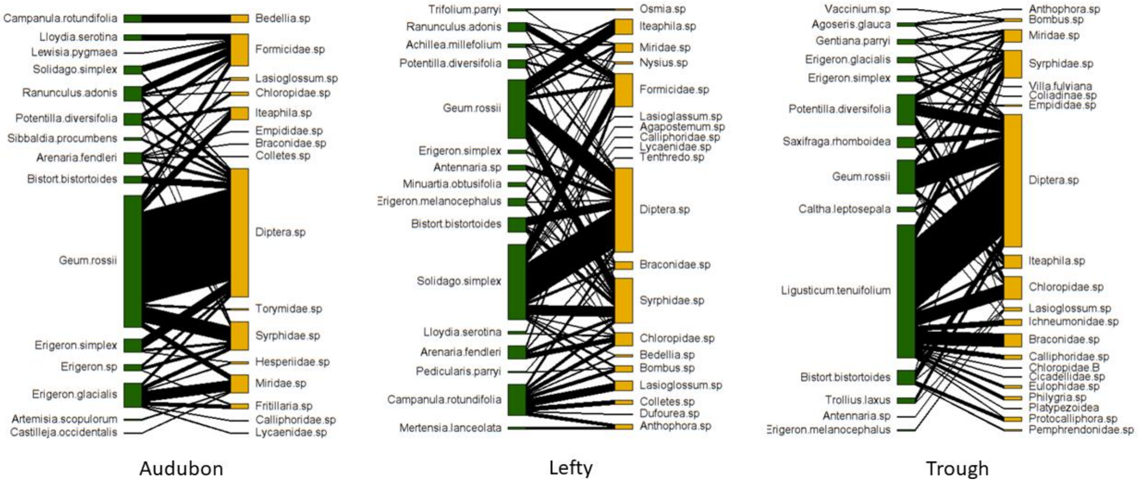
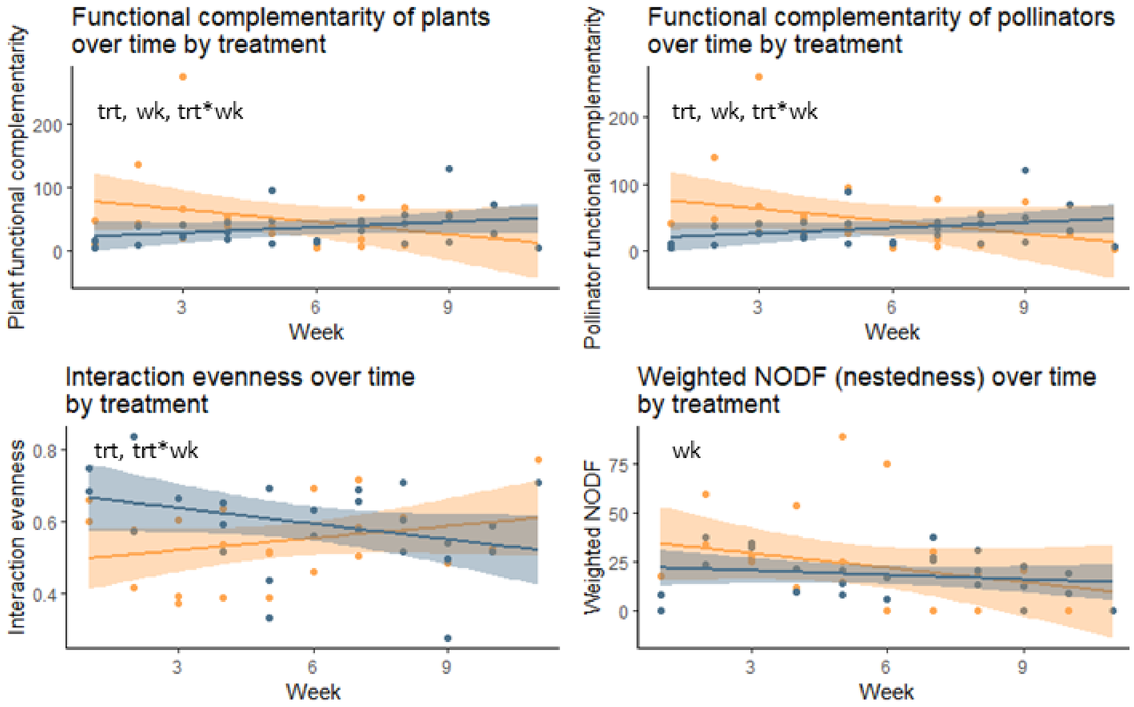
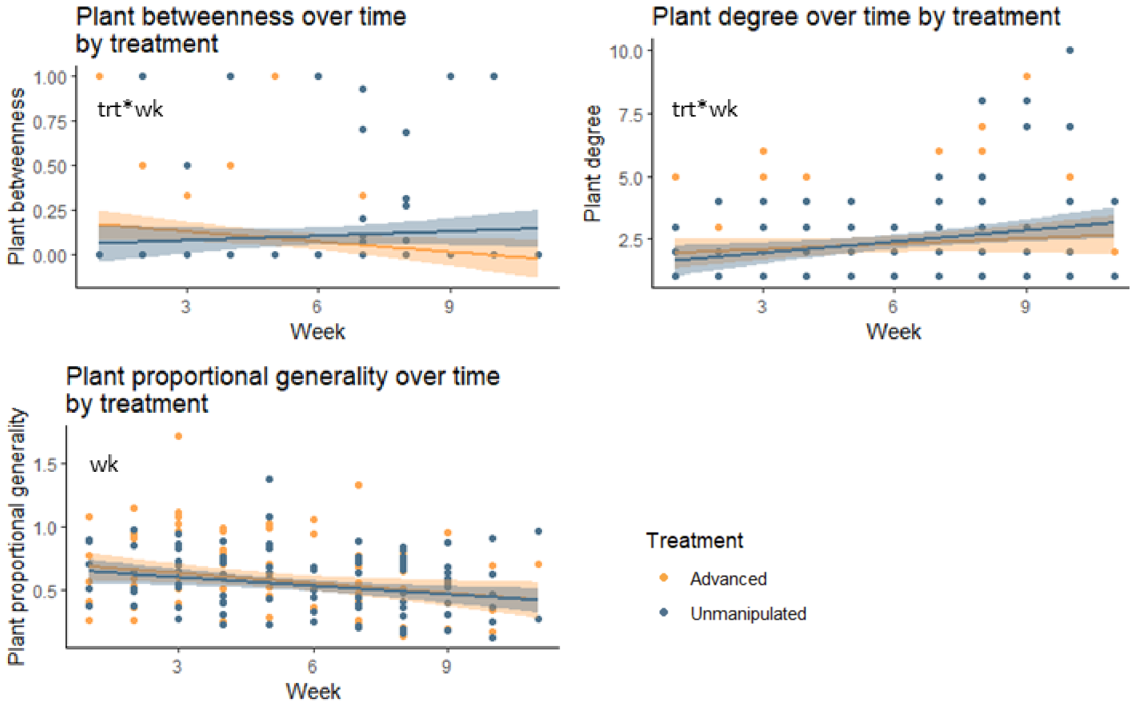


Figure 2.1: Bipartite plant-pollinator networks in A) advanced snowmelt plots and B) unmanipulated snowmelt plots. Interactions were compiled across the flowering season and across all subplots in sites Audubon, Lefty, and Trough. Dark green nodes represent flowering species and light gold nodes represent pollinator species. Width of each interaction is weighted by the number of times that interaction occurred.

A) Network indices at the network level



B) Network indices at the species level for plants only



C) Network indices at the species level for pollinators only

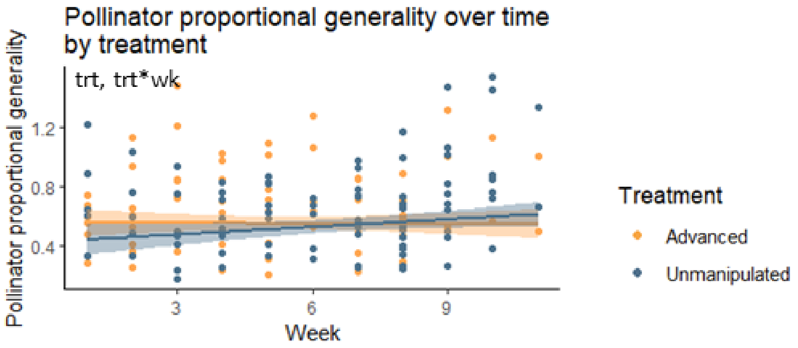


Figure 2.2: The effects of advanced snowmelt treatment and week on network indices at the A) network level, B) flower level, and C) pollinator level. Significant predictors are listed in text boxes over plots.

Appendix

Table S2.1: List of plant species found in sites.

Species	**Family**
Achillea millefolium var. *alpicola*	Asteraceae
Agoseris glauca	Asteraceae
Allium geyeri	Liliaceae
Antennaria sp	Asteraceae
Antennaria media	Asteraceae
Arenaria fendleri	Caryophyllaceae
Artemisia sp	Asteraceae
Artemisia scopulorum	Asteraceae
Polygonum bistortoides	Polygonaceae
Polygonum viviparum	Polygonaceae
Caltha leptosepala	Ranunculaceae
Campanula rotundifolia	Campanulaceae
Castilleja occidentalis	Scrophulariaceae
Cerastium arvense ssp. *Strictum*	Caryophyllaceae
Dodecatheon pulchellum	Primulaceae
Draba spp	Brassicaceae
Erigeron spp	Asteraceae
Erigeron glaucus	Asteraceae
Erigeron melanocephalus	Asteraceae
Eritrichium nanum	Boraginaceae
Erigeron pinnatisectus	Asteraceae

Erigeron simplex	Asteraceae
Gentiana algida	Gentianaceae
Gentianella amarella	Gentianaceae
Gentiana parryi	Gentianaceae
Gentiana tenella	Gentianaceae
Geum rossii var. *turbinatum*	Rosaceae
Lewisia pygmaea	Portulacaceae
Ligusticum tenuifolium	Apiaceae
Lloydia serotina	Liliaceae
Mertensia lanceolata	Boraginaceae
Minuartia obtusiloba	Caryophyllaceae
Oreoxis alpina ssp. *alpina*	Apiaceae
Packera crocata	Asteraceae
Pedicularis groenlandica	Scrophulariaceae
Pedicularis parryi	Scrophulariaceae
Phlox pulvinata	Polemoniaceae
Polemonium viscosum	Polemoniaceae
Potentilla diversifolia	Rosaceae
Ranunculus adoneus	Ranunculaceae
Rhodiola integrifolia	Crassulaceae
Saxifraga rhomboidea	Saxifragaceae
Sedum lanceolatum	Crassulaceae
Sibbaldia procumbens	Rosaceae
Silene acaulis var. *subacaulescens*	Caryophyllaceae

Solidago simplex	Asteraceae
Stellaria longipes ssp. *longipes*	Caryophyllaceae
Tetraneuris acaulis var. *caespitosa*	Asteraceae
Tetraneuris grandiflora	Asteraceae
Tonestus pygmaeus	Asteraceae
Trifolium dasyphyllum	Fabaceae
Trifolium parryi ssp. *parryi*	Fabaceae
Trollius laxus ssp. *albiflorus*	Ranunculaceae
Vaccinium sp	Ericaceae
Veronica wormskjoldii var. *wormskjoldii*	Scrophulariaceae
Viola adunca	Violaceae

Table S2.2: List of insect species collected and identified. *1 = University of California, Riverside Entomological Museum; 2 = University of Colorado, Boulder Entomological Museum.

Visitor Nomenclature Used in Study	**Visitor Order**	**Visitor Family**	**Visitor ID**	**Specimen Voucher Location***
Agapostemum.sp	Hymenoptera	Halictidae	*Agopostemum angelicus*	1
Anthophora.sp	Hymenoptera	Apidae	*Anthophora montana*	1 and 2
Anthophora.sp	Hymenoptera	Apidae	*Anthophora urbana*	1 and 2
Bedellia.sp	Lepidoptera	Bedelliidae	Bedellia sp.	1
Bombus.sp	Hymenoptera	Apidae	*Bombus flavifrons*	1
Bombus.sp	Hymenoptera	Apidae	*Bombus mixtus*	1 and 2
Bombus.sp	Hymenoptera	Apidae	*Bombus sylvicola*	1 and 2
Bombyliidae.sp	Diptera	Bombyliidae	*Villa fulviana*	1 and 2
Brachysomila.atra	Coleoptera	Cerambycidae	*Brachysomila atra*	1
Braconidae.sp	Hymenoptera	Braconidae	*Bracomidae* sp. A	1
Braconidae.sp	Hymenoptera	Braconidae	*Bracomidae* sp. B	1
Braconidae.sp	Hymenoptera	Braconidae	*Bracomidae* sp. C	1
Braconidae.sp	Hymenoptera	Braconidae	*Bracomidae* sp. D	1
Braconidae.sp	Hymenoptera	Braconidae	*Bracomidae* sp. E	1
Chloropidae.sp	Diptera	Chloropidae	Chloropidae sp. A	1 and 2
Chloropidae.sp	Diptera	Chloropidae	Chloropidae sp. B	1
Chloropidae.sp	Diptera	Chloropidae	Chloropidae sp. C	1 and 2
Chloropidae.sp	Diptera	Chloropidae	Chloropidae sp. D	1 and 2
Chloropidae.sp	Diptera	Chloropidae	Chloropidae sp. E	1
Chloropidae.sp	Diptera	Chloropidae	Chloropidae sp. F	1

Colletes.sp	Hymenoptera	Colletidae	*Colletes* sp. A	1
Colletes.sp	Hymenoptera	Colletidae	*Colletes* sp. B	1
Diptera.sp	Diptera	Anthomyiidae	Anthomyiidae sp. A	1 and 2
Diptera.sp	Diptera	Anthomyiidae	Anthomyiidae sp. B	1 and 2
Diptera.sp	Diptera	Anthomyiidae	Anthomyiidae sp. C	1 and 2
Diptera.sp	Diptera	Anthomyiidae	Anthomyiidae sp. D	1
Diptera.sp	Diptera	Anthomyiidae	Anthomyiidae sp. E	1
Diptera.sp	Diptera	Anthomyiidae	Anthomyiidae sp. F	1
Diptera.sp	Diptera	Anthomyiidae	Anthomyiidae sp. G	1
Diptera.sp	Diptera	Anthomyiidae	Anthomyiidae sp. H	1 and 2
Diptera.sp	Diptera	Calliphoridae	*Calliphora coloradensis*	1 and 2
Diptera.sp	Diptera	Muscidae	*Helina* sp.	1 and 2
Diptera.sp	Diptera	Muscidae	Muscid sp. A	1 and 2
Diptera.sp	Diptera	Muscidae	Muscid sp. B	1 and 2
Diptera.sp	Diptera	Muscidae	Muscid sp. C	1
Diptera.sp	Diptera	Muscidae	Muscid sp. D	1 and 2
Diptera.sp	Diptera	Muscidae	*Pagonomyia* sp.	1
Diptera.sp	Diptera	Muscidae	*Stanoxys calcitrans*	1 and 2
Diptera.sp	Diptera	Muscidae	*Thricops* sp. A	1 and 2
Diptera.sp	Diptera	Muscidae	*Thricops* sp. B	1
Diptera.sp	Diptera	Sarcophagidae	Sarcophagidae sp. A	1
Diptera.sp	Diptera	Sarcophagidae	Sarcophagidae sp. B	1 and 2
Diptera.sp	Diptera	Sarcophagidae	Sarcophagidae sp. C	1 and 2
Diptera.sp	Diptera	Sarcophagidae	Sarcophagidae sp. D	1 and 2
Diptera.sp	Diptera	Tachinidae	*Estheria cinerea*	1 and 2

Diptera.sp	Diptera	Tachinidae	*Phasia* sp.	1
Diptera.sp	Diptera	Tachinidae	Tachinidae sp. A	1
Diptera.sp	Diptera	Tachinidae	Tachinidae sp. B	1 and 2
Diptera.sp	Diptera	Tachinidae	Tachinidae sp. C	1 and 2
Diptera.sp	Diptera	Tachinidae	Tachinidae sp. D	1
Diptera.sp	Diptera	Tachinidae	Tachinidae sp. E	1
Diptera.sp	Diptera	Tachinidae	Tachinidae sp. F	1
Dufourea.sp	Hymenoptera	Halictidae	*Dufourea* sp.	1
Eulophidae.sp	Hymenoptera	Eulophidae	Eulophidae sp. A	1
Eulophidae.sp	Hymenoptera	Eulophidae	Eulophidae sp. B	1
Eulophidae.sp	Hymenoptera	Eulophidae	Eulophidae sp. C	1
Eurytoma.sp	Hymenoptera	Eurytomidae	*Eurytoma* sp.	1
Formicidae.sp	Hymenoptera	Formicidae	Formicidae sp.	1 and 2
Halictus.sp	Hymenoptera	Halictidae	*Halictus virgatellus*	1 and 2
Hesperiidae.sp	Lepidoptera	Hesperiidae	Hesperiidae sp.	1
Ichneumonidae.sp	Hymenoptera	Ichneumonidae	Ichneumonidae sp. A	1
Ichneumonidae.sp	Hymenoptera	Ichneumonidae	Ichneumonidae sp. B	1
Ichneumonidae.sp	Hymenoptera	Ichneumonidae	Ichneumonidae sp. C	1
Ichneumonidae.sp	Hymenoptera	Ichneumonidae	Ichneumonidae sp. D	1
Iteaphila.sp	Diptera	Empididae	*Iteaphila* sp. A	1
Iteaphila.sp	Diptera	Empididae	*Iteaphila* sp. B	1 and 2
Lasioglossum.sp	Hymenoptera	Halictidae	*Lasioglossum* sp. B	1 and 2
Lasioglossum.sp	Hymenoptera	Halictidae	*Lasioglossum* sp. A	1 and 2
Lycaenidae.sp	Lepidoptera	Lycaenidae	Lycaenidae sp.	1
Miridae.sp	Hemiptera	Miridae	Miridae sp. A	1
Nysius.sp	Hemiptera	Lygaeidae	*Nysius* sp.	1

Osmia.sp	Hymenoptera	Megachilidae	*Osmia tarsata*	1
Pemphrendonidae.sp	Diptera	Pemphredoninae	Pemphrendonidae sp.	1
Philygria.sp	Diptera	Ephydridae	*Philygria* sp.	1
Platypezoidea.sp	Diptera	Phoridae	Phoridae sp.	1
Protocalliphora.sp	Diptera	Calliphoridae	*Protocalliphora* sp.	1 and 2
Pterophoridae.sp	Lepidoptera	Pterophoridae	Pterophoridae sp.	1
Syrphidae.sp	Diptera	Syrphidae	*Asemosyrphus polygrammus*	1 and 2
Syrphidae.sp	Diptera	Syrphidae	*Cheilosia* sp. A	1 and 2
Syrphidae.sp	Diptera	Syrphidae	*Chrysotoxium* sp.	1 and 2
Syrphidae.sp	Diptera	Syrphidae	*Dasysyrphus* sp.	1 and 2
Syrphidae.sp	Diptera	Syrphidae	*Didea fuscipes*	1
Syrphidae.sp	Diptera	Syrphidae	*Eupodes* sp. A	1
Syrphidae.sp	Diptera	Syrphidae	*Eupodes* sp. B	1
Syrphidae.sp	Diptera	Syrphidae	*Eupodes* sp. C	1
Syrphidae.sp	Diptera	Syrphidae	*Eupodes* volucris	1 and 2
Syrphidae.sp	Diptera	Syrphidae	*Lapposyrphus lappocus*	1
Syrphidae.sp	Diptera	Syrphidae	*Melanogyna* sp.	1
Syrphidae.sp	Diptera	Syrphidae	*Paragus* sp.	1 and 2
Syrphidae.sp	Diptera	Syrphidae	*Parasyrphus* sp. A	1 and 2
Syrphidae.sp	Diptera	Syrphidae	*Parasyrphus* sp. B	1 and 2
Syrphidae.sp	Diptera	Syrphidae	*Platycheirus* sp. A	1 and 2
Syrphidae.sp	Diptera	Syrphidae	*Platycheirus* sp. B	1
Syrphidae.sp	Diptera	Syrphidae	*Platycheirus* sp. C	1 and 2
Syrphidae.sp	Diptera	Syrphidae	*Platycheirus* sp. D	1 and 2
Syrphidae.sp	Diptera	Syrphidae	*Scaeva pyrastri*	1

Syrphidae.sp	Diptera	Syrphidae	*Sericomyia flagrans*	1
Syrphidae.sp	Diptera	Syrphidae	*Syrphus* sp.	1 and 2
Tenthredo.sp	Hymenoptera	Tenthredinidae	*Tenthredo* sp. A	1
Tenthredo.sp	Hymenoptera	Tenthredinidae	*Tenthredo* sp. B	1
Torymidae.sp	Hymenoptera	Torymidae	Torymidae sp.	1 and 2
Zodion.sp	Hymenoptera	Conopidae	*Zodion* sp.	1

Table S2.3: Results of linear models comparing flower and pollinator abundance and diversity between treatments. In all models, site was used as a random effect and treatment was used as the predictor. “DOY” = day of year, and “trt” = treatment. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

Response	Predictor	Estimate	SE	t-value	p	Sig. code	DF
Sum of flowers	trt	-11.24	3.87	-2.90	**0.0039**	**	497.33
Flower H		-0.01	0.04	-0.16	0.87		497.73
Sum of visitors		-0.36	0.77	-0.47	0.64		290.20
Visitor H		0.11	0.06	2.02	**0.045**	*	292.16
Sum of flowers (AIC = 3,937.13)	trt	-111.20	45.78	-2.43	**0.016**	*	371.15
	DOY	-1.05	0.16	-6.57	**<0.0001**	***	258.88
	DOY*trt	0.48	0.22	2.21	**0.028**	*	371.05
Sum of flowers, quadratic fit (AIC = 3,965.32)	trt	-63.37	23.78	-2.67	**0.008**	**	381.90
	DOY^2	0.00	0.00	-7.03	**<0.0001**	***	180.20
	DOY^2*trt	0.00	0.00	2.28	**0.023**	*	380.80
Sum of pollinators (AIC = 1,974.77)	Trt	-20.89	9.57	-2.18	**0.029**	*	283.10
	DOY	-0.09	0.03	-2.86	**0.0078**	**	282.81
	DOY*trt	0.10	0.05	2.20	**0.029**	*	283.22
Sum of pollinators, quadratic fit (AIC = 1,998.94)	trt	-10.80	4.86	-2.22	**0.027**	*	282.90
	DOY^2	-2.21e-4	0.00	-2.67	**0.0081**	**	282.70
	DOY^2*trt	2.43e-4	0.00	2.26	**0.025**	*	283.10

Table S2.4: Results of Permutational Analysis of Variance (PERMANOVA) tests and tests of dispersion on communities of flowers and pollinators by site. DOY = day of year and trt = treatment. Asterisks indicates significance level (‘p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001).

Site	**Taxon**	**Testing**	**Predictor**	**F-value**	**p**	**Group DF**	**Residuals DF**	**R2**
Audubon	Pollinators	Dispersion	treatment	0.03	0.87	1	80	
		Community composition	treatment	1.63	**0.08’**	1	78	0.03
			DOY	1.88	0.11			0.02
			treatment*DOY	0.82	0.54			0.01
	Flowers	Dispersion	treatment	1.59	0.21	1	110	
		Community composition	treatment	3.06	**0.007****	1	93	0.03
			DOY	3.34	**0.015***			0.03
			treatment*DOY	2.92	**0.015***			0.03
Lefty	Pollinators	Dispersion	treatment	0.12	0.73	1	114	
		Community composition	treatment	0.81	0.53	1	112	0.01
			DOY	2.93	**0.019***			0.03
			treatment*DOY	2.34	**0.038***			0.02
	Flowers	Dispersion	treatment	2.98	**0.086’**	1	161	
		Community composition	treatment	5.83	**0.001****	1	141	0.03
			DOY	33.52	**0.001****			0.18
			treatment*DOY	4.08	**0.002****			0.02
Trough	Pollinators	Dispersion	treatment	0.00	0.97	1	96	
		Community composition	treatment	1.57	0.16	1	94	0.015
			DOY	10.11	**0.001*****			0.094
			treatment*DOY	2.14	**0.048***			0.020
	Flowers	Dispersion	treatment	9.66	**0.002***	1	130	
		Community composition	treatment	8.21	**0.001****	1	113	0.05
			DOY	40.10	**0.001****			0.25
			treatment*DOY	1.96	**0.071'**			0.01

Table S2.5: Results of Redundancy Analysis (RDA) model selection using day of year, floral community, weather, and topography as predictors of pollinator community in advanced and unmanipulated plots. Italicized predictors are predictors that significantly explain variation in pollinator communities between treatments. “flPCoA” represent Principal Coordinate Analysis (PCoA) axes for floral communities, and “wPCoA” represents the weather PCoA axis.

Treatment	**Predictor**	**Variance**	**F-value**	**p**	**Sig. code**
Unmanipulated (p<0.001, R^2 = 0.087, DF = 1, Resid. DF = 140)	*doy*	0.02	5.42	**0.001**	***
	sitefact	0.01	2.23	**0.037**	*
	flPCoA 2	0.02	4.24	**0.001**	***
	flPCoA 4	0.01	2.92	**0.005**	**
	flPCoA 6	0.01	2.21	**0.038**	*
	wPCoA 1	0.01	2.84	**0.002**	**
Advanced (p<0.001, R^2 = 0.132, DF = 1, Resid. DF = 141)	*doy*	0.02	6.84	**0.001**	***
	sitefact	0.01	2.51	**0.028**	*
	flPCoA 1	0.03	8.45	**0.001**	***
	flPCoA 2	0.01	3.40	**0.006**	**
	flPCoA 3	0.01	3.13	**0.007**	**
	flPCoA 4	0.01	2.54	**0.024**	*
	flPCoA 6	0.01	2.60	**0.02**	*

Table S2.6: Loadings of species on Principal Coordinate Analysis (PCoA) axes of floral communities. “U” = unmanipulated plots, “A” = advanced snowmelt plots. Stars indicate where axes significantly predicted pollinator community composition.

PCoA 1		PCoA 2		PCoA 3	
U	A*	U*	A*	U	A*
Geum rossii -0.95	*Geum rossii* -0.95	*Solidago simplex* -0.64	*Ligusticum tenuifolium* -0.73	*Potentilla diversifolia* -0.38	*Ligusticum tenuifolium* -0.49
Lloydia serotina -0.25	*Potentilla diversifolia* -0.36	*Arenaria fendleri* -0.61	*Artemisia scopulorum* -0.31	*Mertensia lanceolata* -0.33	*Minuartia obtusifolia* -0.45
Ligusticum tenuifolium 0.47	*Arenaria fendleri* 0.51	*Erigeron glaucus* 0.39	*Arenarai fendleri* 0.36	*Solidago simplex* 0.25	*Solidago simplex* 0.37
Solidago simplex 0.48	*Solidago simplex* 0.53	*Ligusticum tenuifolium* 0.73	*Minuartia obtusiloba* 0.72	*Artemisia scopulorum* 0.74	*Artemisia scopulorum* 0.46

PCoA 4		PCoA 6	
U*	A*	U*	A*
Minuartia obtusiloba -0.61	*Artemisia scopulorum* -0.59	*Campanularia rotundifolia* -0.35	*Potentilla diversifolia* -0.33
Artemisia scopulorum -0.41	*Minuartia obtusiloba* -0.40	*Potentilla diversifolia* -0.33	*Arenaria fendleri* -0.28
Solidago simplex 0.27	*Polygonum bistortoides* 0.31	*Ligusticum tenuifolium* 0.41	*Erigeron glaucus* 0.47
Achillea millefolium 0.38	*Arenaria fendleri* 0.40	*Arenaria fendleri* 0.54	*Polygonum bistortoides* 0.61

Table S2.7: Descriptions of relevant network indices.

Level of Index	**Index**	**Description from *bipartite* functions** (Dormann et al. 2009, Dormann et al. 2011)
Network	Functional Complementarity- HL	Functional complementarity of pollinators
	Functional Complementarity- LL	Functional complementarity of plants
	Interaction Evenness	Homogeneity of interaction strengths of links between networks
	Weighted NODF	Weighted Nestedness based on Overlap and Decreasing Fill: A weighted index for nestedness. Higher values indicate greater nestedness
Species	Betweenness	The number of shortest paths between nodes that pass through a particular node
	Degree	The total number of links per species
	Proportional Generality	The number of partner species in relation to the potential number of partner species

Table S2.8: Network indices calculated at the A) network level, B) species level for plants, and C) species level for pollinators. All models included treatment, week, and their interaction as predictors and site and species as random effects. Adjusted R^2 values are shown in parentheses under model index. “*Trt*” refers to snowmelt treatment, and “*wk*” refers to week. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1.

A) Model results for network level indices.

Index	**Predictor**	**Sum DF**	**F-val**	**t-val**	**Estimate**	**p**	**Sig. code**
Functional Complementarity-Pollinator Level (R^2 = 0.16)	trt	44.57	6.09	-2.47	-64.96	**0.0175**	*
	wk	44.62	0.87	-2.06	-6.65	**0.0454**	*
	trt*wk	45.35	4.72	2.17	9.22	**0.035**	*
Functional Complementarity-Flower Level (R^2 = 0.17)	trt	44.49	6.02	-2.45	-66.86	**0.0182**	*
	wk	45.3	0.98	-2.13	-7.18	**0.0383**	*
	trt*wk	45.2	5.02	2.24	9.86	**0.03**	*
Interaction Evenness (R^2 = 0.19)	trt	44.55	7.98	2.83	0.2	**0.007**	**
	wk	45.01	0.04	1.38	0.01	0.1746	
	trt*wk	45.29	5.37	-2.32	-0.03	**0.0251**	*
Weighted NODF (R^2 = 0.27)	trt	44.1	1.96	-1.4	-15.62	0.1683	
	wk	45.96	5.83	-2.47	-3.46	**0.0172**	*
	trt*wk	44.59	1.68	1.3	2.34	0.202	

B) Model results for species-level indices performed on plants.

Index	Predictor	Sum.DF	F-val	t-val	Estimate	p	Sig. code
Betweenness (R^2 = 0.04)	trt	216.87	3.08	-1.75	-0.14	0.0808	"
	wk	199.6	0.44	-1.95	-0.02	0.0526	"
	trt*wk	217.92	4.9	2.21	0.03	**0.0279**	*
Degree (R^2 = 0.38)	trt	205.41	2.7	-1.64	-0.75	0.1022	
	wk	211.82	2.7	-0.21	-0.01	0.8311	
	trt*wk	210.34	5.71	2.39	0.18	**0.0178**	*
Proportional Generality (R^2 = 0.23)	trt	210.45	0.89	-0.95	-0.07	0.3454	
	wk	161.7	10.39	-3.03	-0.03	**0.0029**	**
	trt*wk	213.66	1.22	1.1	0.01	0.2716	

C) Model results for species-level indices performed on pollinators

Index	Predictor	Sum.DF	F-val	t-val	Estimate	p	Sig. code
Proportional Generality (R^2 = 0.30)	trt	294.46	8.33	-2.89	-0.2	**0.0042**	**
	wk	287.7	2.34	-0.75	-0.01	0.4534	
	trt*wk	297.04	8.84	2.97	0.03	**0.0032**	**

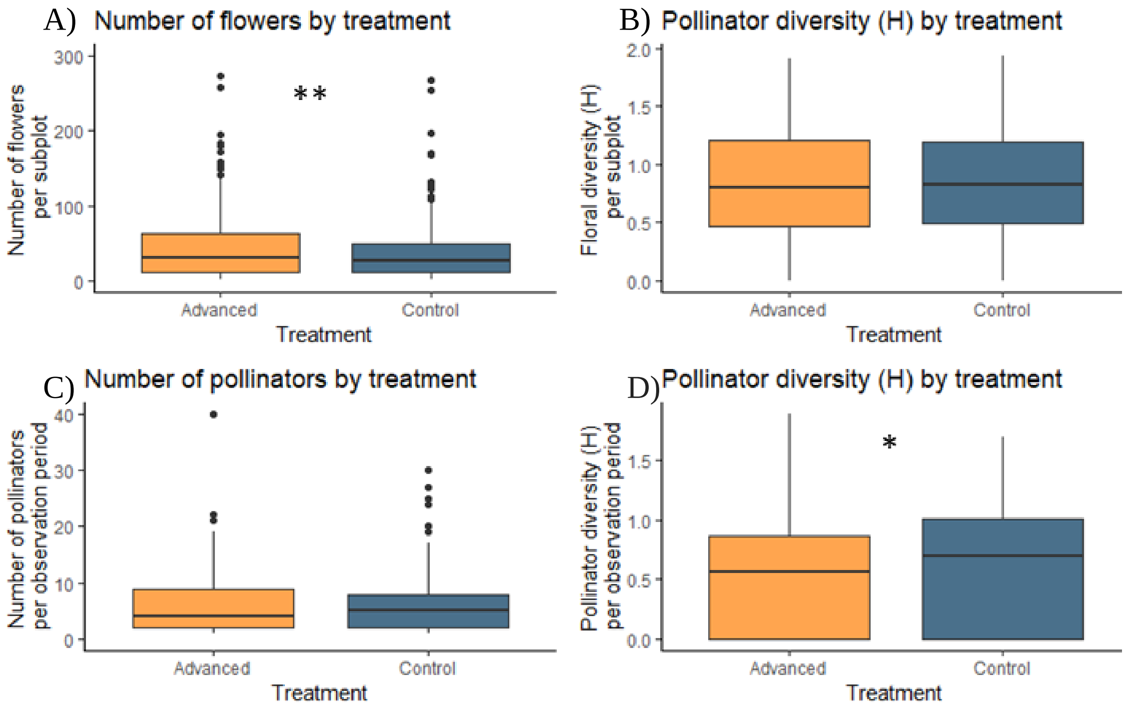


Figure S2.1: The A) number and B) Shannon diversity, H, of plants and the C) number and D) Shannon diversity, H, of insects by treatment and site. Asterisks indicates significance level (*$p < 0.05$, **$p < 0.01$).

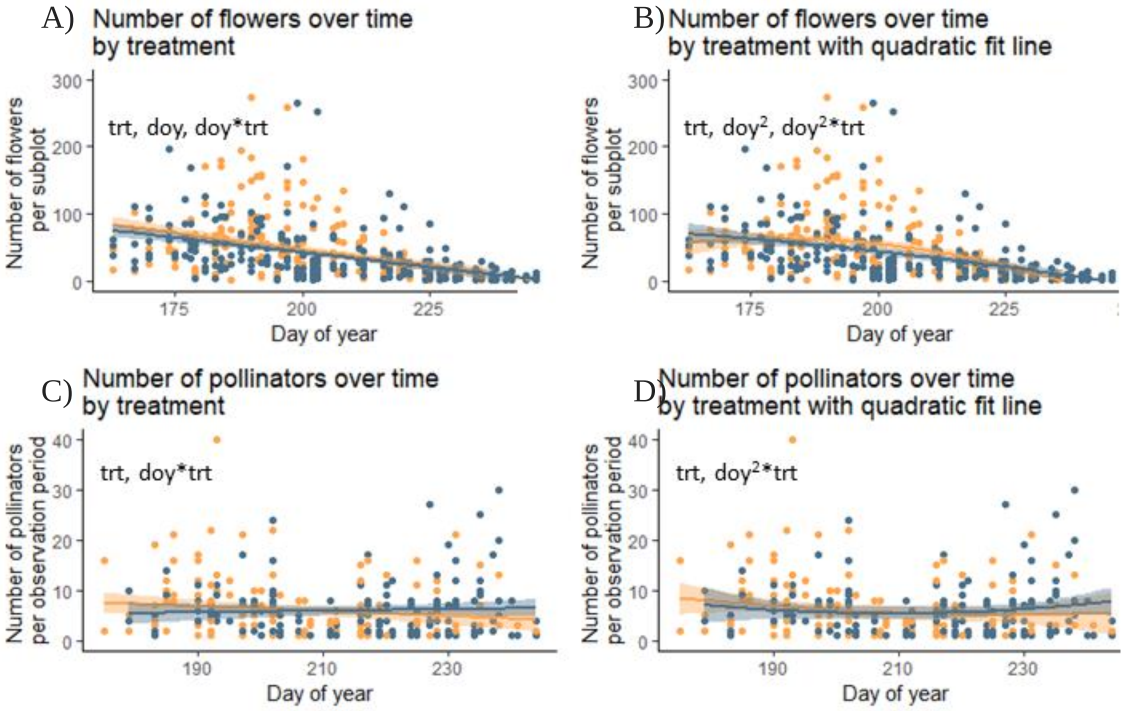


Figure S2.2: The number of flowers over time by treatment with linear (A) and quadratic lines of best fit (B), and the number of pollinators per subplot over time by treatment with linear (C) and quadratic (D) lines of best fit. Significant predictors are listed in text boxes over plots.

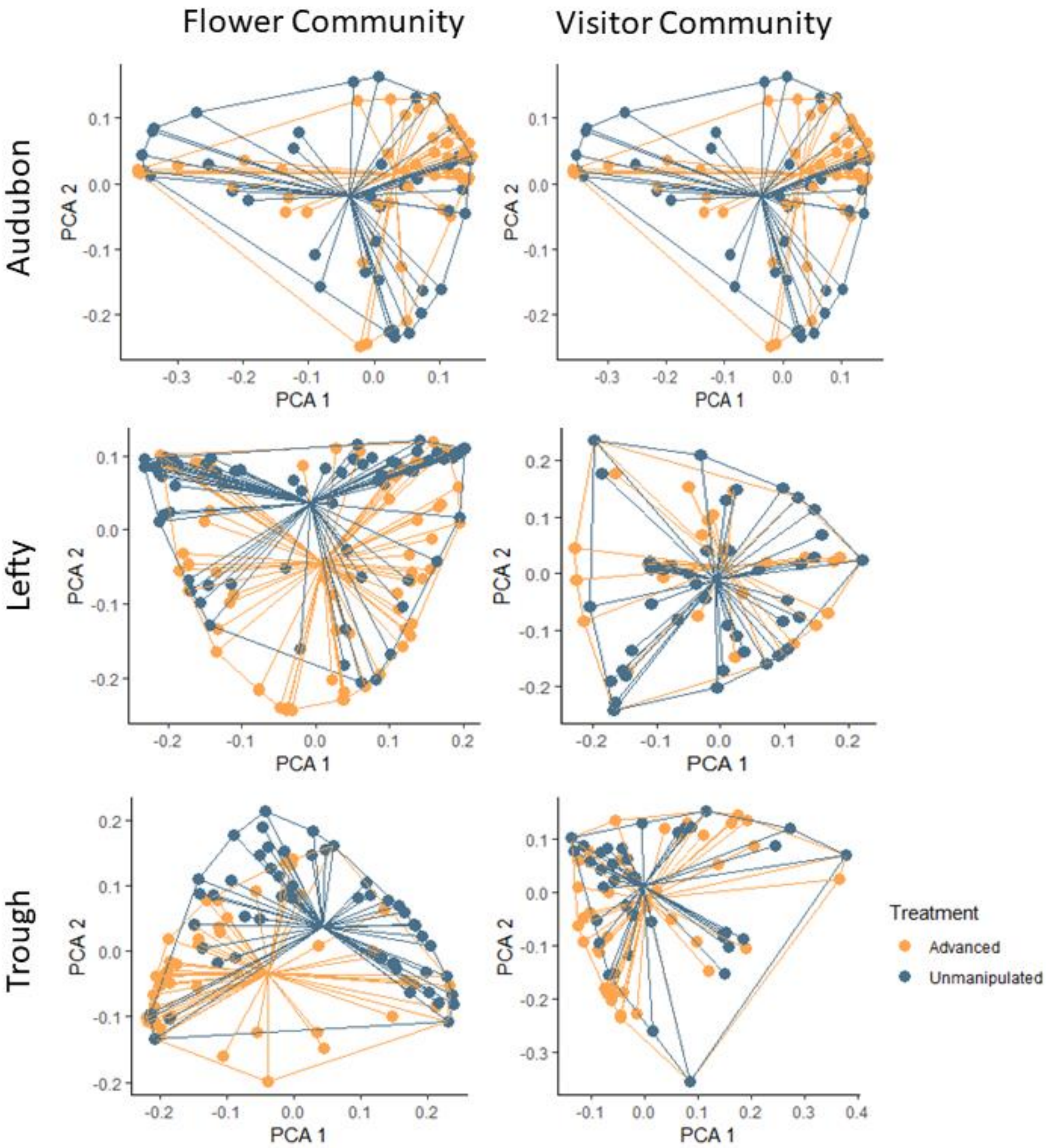


Figure S2.3: Redundancy Analysis (RDA)-based ordinations of flower and insect communities across all three sites.

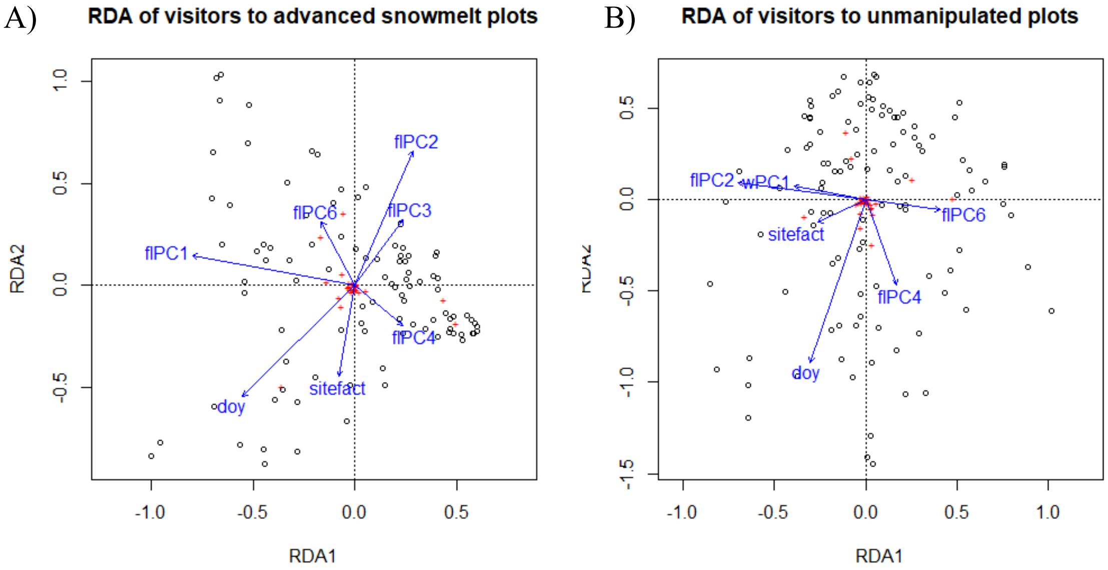


Figure S2.4: Results of Redundancy Analysis (RDA) on pollinator communities after forward model selection in A) advanced snowmelt and B) unmanipulated snowmelt communities. Floral community Principal Coordinate Analysis (PCoA) axes 1-6, floral diversity, floral abundance, day of year, weather PCoA axis 1, and topography PCoA axis1 were used as predictors. Subplot nested within site was used as a random effect in each model.

Chapter 3

Drought stress influences foraging behavior of a solitary bee on two wildflowers

Abstract

Plant-pollinator interactions provide critical ecosystem services, maintaining biodiversity and benefiting global food production. However, plants, pollinators, and their mutualistic interactions may be affected by drought, which has increased in severity and frequency under climate change. Using two annual, insect-pollinated wildflowers (*Phacelia campanularia* and *Nemophila menziesii*), we asked how drought impacts floral traits and foraging preferences of a solitary bee (*Osmia lignaria*). In greenhouses, we experimentally subjected plants to drought conditions, as verified by measures of the water potential at which leaves lose turgor. To assess the impact of drought on floral traits, we measured flower size, floral display, nectar volume, and nectar sugar concentration. To explore how drought-induced effects on floral traits affected bee foraging preferences, we performed choice trials. Individual female bees were placed into foraging arenas with two conspecific plants, one droughted and one non-droughted, and were allowed to forage freely. We determined that *P. campanularia* is more drought-tolerant than *N. menziesii* and confirmed that plants in our drought treatment were more drought-stressed than non-droughted plants. For droughted plants of both species, floral display size was reduced, and flowers were smaller and produced less, more-concentrated nectar. We found that bees preferred non-droughted flowers of *N. menziesii*. However, bee preference for non-droughted *P. campanularia* flowers depended on the time of day and was detected only in the afternoon. The lack of preference for non-droughted *P.*

campanularia flowers in the morning may reflect the higher drought tolerance of this species. Our findings indicate bees prefer to visit non-droughted flowers, which will likely reduce pollination success for drought-stressed plants. This work highlights the potentially intersecting, short-term plant physiological and pollinator behavioral responses to drought and suggests such responses may reshape plant-pollinator interactions, ultimately reducing reproductive output for less drought-tolerant wildflowers.

Funding acknowledgement: *This study received substantial funding from the Shipley-Skinner Reserve—Riverside County Endowment and from UCR's Environmental Dynamics and GeoEcology (EDGE) Institute.*

Introduction

To predict the ecological consequences of global climate change, it is critical to understand how changes in abiotic context affect interactions between species, particularly those interactions that provide essential ecosystem services (Kattenberg 1996, Cubasch et al. 2001, Gilman et al. 2010). In many terrestrial environments, climatic change is linked to reduced precipitation, or meteorological drought, and reduced water availability in soils, or soil moisture drought (Orlowsky and Seneviratne 2013). Some regions may experience a 20% increase in drought frequency by the year 2100 (Prudhomme et al. 2013, Spinoni et al. 2014), and the duration of droughts is expected to increase with global warming, due in part to increased evaporation of soil moisture with higher atmospheric temperatures (IPCC 2014, Naumann et al. 2018). Frequent, prolonged droughts can influence the incidence, strength, and direction of species interactions by altering abundances, phenologies, and traits of populations (Kuppler et al. 2021, Forrest 2015, Phillips et al. 2018). Among species interactions impacted by drought, plant-pollinator mutualisms are important for the reproduction of most flowering plant species and for the human food supply (Klein et al. 2007, Ollerton et al. 2011). Most immediately, drought can impact interactions between plants and pollinating insects by altering floral traits (Carroll et al. 2001, Phillips et al. 2018, Kuppler and Kotowska 2021).

Drought can lead to both short-term structural and physiological changes that impact the ability of plants to attract pollinating insects (Bartlett et al. 2014). Structurally, plants exposed to drought tend to produce fewer and smaller flowers (Carroll et al. 2001, Phillips et al. 2018, Rering et al. 2020). These structural changes, in combination with decreased production of volatile organic compounds (Glenny et al. 2018), may in turn reduce attractiveness of drought-stressed plants to pollinators (Conner and Rush 1996, Descamps et al. 2018). Drought has also consistently been shown to decrease the volume of nectar produced by flowers (Carroll et al. 2001, Phillips et al. 2018, Rering et al. 2020). Changes in floral resource availability and quality have important implications for pollinator visitation and pollination success. For example, flowers of *Polemonium viscosum* (Polemoniaceae) that were 4-6 mm smaller in diameter were visited about 30% less frequently by their primary pollinator, queen bumble bees, resulting in a 50% reduction in seed set compared to larger flowers (Galen and Newport 1987).

Populations of insect pollinators may also be negatively affected by the effects of drought on flowering plants. For example, lower availability of resources such as pollen and nectar may diminish reproductive output of insect pollinators, especially bees that rely on these floral resources to provision their offspring (Vaudo et al. 2015, Wilson Rankin et al. 2020). For example, bumble bee foragers fed nectar reflecting the sugar content of optimally-watered flowers of *Trifolium willdenovii* (Fabaceae) lived 60% longer than those fed nectar reflecting the sugar content of droughted flowers (Wilson Rankin et al. 2020). Further, drought-driven changes to floral traits may cause changes in insect behavior that can ultimately affect the persistence of both plants and pollinators (Filazzola et al. 2021). Animal pollination facilitates outcrossing, yielding genetic variation critical to plant adaptation in response to global climate change (Leimu et al. 2010). Decreased rates of pollinator visitation to drought-stressed plants may reduce outcrossing, resulting in lower seed set and reduced fitness of offspring (Hodges 1995, Huang and D'Odorico 2020). Changes in floral traits may also alter pollinator preferences for coflowering species, potentially favoring drought-tolerant species (Sargent and Ackerly 2008). Drought-driven changes in floral traits may therefore lead to rewiring of interactions within a community of plants and pollinators (Burkle and Alarcón 2011, CaraDonna et al. 2017), which could increase heterospecific pollen transfer, further reducing seed set (Descamps et al. 2021 b, Kuppler and Kotowska 2021).

Although the influence of drought on plant-pollinator interactions is a topic of growing concern (Waser and Price 2016, Gallagher and Campbell 2017, Descamps et al. 2021 b), no studies have focused on the foraging responses of solitary bees to drought-induced changes in the floral traits of annual plants. Yet, climate change may have pronounced effects on the population dynamics and persistence of annual plants and univoltine insect pollinators (Fitter and Fitter 2002, Biesmeijer et al. 2006), making it important to understand their physiological and behavioral responses to drought. Here, we used two annual wildflowers and a solitary bee that pollinates them to test how drought stress affects floral traits and, in turn, pollinator preference and visitation. We experimentally subjected plants to water limitation in greenhouses and asked: (1) how does drought stress affect floral traits, such as flower size and nectar volume?; (2) do bees exhibit preferences for the flowers of droughted vs. non-droughted plants?; and (3) are pollinator preferences mediated by plant drought tolerance or time of day?

Methods

Study species

Our focal plant species were *Phacelia campanularia* (Boraginaceae) and *Nemophila menziesii* (Boraginaceae). These annual, spring-blooming species are native to Southern California: *P. campanularia* occurs in deserts of California and Arizona, and *N. menziesii* occurs throughout the western United States (USDA Plants Database, 2023). Both are self-compatible, but their floral morphologies and developmental timing promote outcrossing, and autogamy results in low seed set (Cruden 1972, Gillett 1961). Flowers of both species are actinomorphic, typically have five anthers and five petals, and produce nectar and pollen, though *N. menziesii* is gynodioecious, and female plants produce sterile pollen (Cruden 1972, McCall 2006, McCall 2008, Wróblewska 2010). We chose these species in part because we expected they would differ in drought tolerance, as the range of *P. campanularia* includes the Mojave and Sonoran deserts.

The focal insect species used in choice trials was *Osmia lignaria* (Megachilidae), a solitary, cavity-nesting bee that is native to western North America (Williams 2003, Haider et al. 2014) and is known to visit flowers of both of our focal plant species in natural communities (Boyle et al. 2020) and to effectively pollinate them in experimental settings (de Manincor et al. 2023). Further, *O. lignaria* is an important commercial pollinator of spring-blooming orchard crops (Torchio and Asensio 1985, Peterson and Artz 2014, Pitts-Singer et al. 2018).

Plant preparation

To prepare plants for the experiment, we germinated seeds of *P. campanularia* and *N. menziesii* in peat pellets in greenhouses in Riverside, California, USA in 2021 and 2022. In 2021, our goals were to measure the drought tolerances of our focal species and to test whether drought affected their floral traits; in 2022, we again quantified the effects of drought on floral traits, but our main goal was to determine whether bees prefer to visit non-droughted flowers. Seeds were purchased from a local nursery (Theodore Payne Foundation) and were sourced from San Diego County, California, USA. Once seedlings had two true leaves we planted them in 1-L pots filled with a 1:1 mix of potting soil (SunGro Sunshine Professional Mix 1) and coarse sand. Plants were watered with 125 ml every two days for four weeks, then randomly assigned to droughted and non-droughted treatments. In 2021, non-droughted plants were watered with 125 ml every two days and droughted plants were watered with 125 ml only when the soil moisture of a randomly selected subset of droughted plants was <10% volumetric water content. In 2022, non-droughted plants were watered with 125 ml daily and droughted plants were watered with

125 ml every two days. Throughout the experiment, both droughted and non-droughted plants were watered at the same time of day.

Plant traits

With our 2021 plant cohort, we quantified drought tolerance as leaf turgor loss point (π_{tlp}), for which more negative values indicate greater drought tolerance. Turgor loss point is the value of water potential at which leaves lose turgor, a metric useful for comparing drought tolerance among species (Bartlett et al. 2012). To determine the turgor loss point of each species, we collected three leaf samples from three plants and used a vapor pressure osmometer (Wescor Vapro Model 5600) set on "delay" mode until measurements equilibrated for at least five steps (Bartlett et al. 2012). To quantify plant water status, at 7:00 AM and 1:00 PM we measured leaf water potential from five randomly-selected plants of each species per treatment. We used a pressure chamber (Model 1000, PMS Instrument Company, Albany, Oregon, USA) to determine the leaf water potential of these leaves as balancing pressure, for which more negative values indicate reduced tissue hydration (Rodriguez-Dominguez et al. 2022).

For both our 2021 and 2022 cohorts, we recorded the date of flowering onset and monitored plants every two days until flowering ceased. We measured floral traits daily from a subset of droughted and non-droughted plants of both species. We counted the flowers on these plants and measured floral traits for 1-2 flowers per plant, which were chosen randomly and marked with paper tags on strings to prevent repeated measurement. We measured flower size (widest diameter, diameter at a right angle to this measurement, and corolla length measured parallel to the petiole from the base of the corolla to the longest petal), nectar volume, and nectar sugar concentration. We used the flower size measurements to calculate the landing area of flowers as the product of the two diameter measures (Knauer and Schiestl 2015). Nectar volume was measured using microcapillary tubes (Drummond Microcaps), and nectar concentration was measured using handheld optical refractometers (Bellingham + Stanley). In 2022 we measured nectar volume and concentration twice per day (morning and afternoon) for the same flowers, to quantify the nectar that was refilled in flowers prior to their use in afternoon choice trials. To calculate the caloric value of nectar on a per-flower basis, we used the equation: $y = 0.00226 + 0.00937x + 0.0000585x^2$, where x = % sugar concentration and y = g of sugars in 1 µL of nectar (Bolten et al. 1979, Lange et al. 2017, Dafni et al. 2005). We then multiplied y by the total volume of nectar (µL), then by 4 to convert to total calories (Dafni et al. 2005).

Pollinator preference

To measure pollinator preference via choice trials, we used female *O. lignaria* (Mountain West Mason Bees, Utah, USA). In April 2022, diapausing adult bees in cocoons were stored at 4 °C, then warmed to 20 °C over 24 hours to trigger emergence. All bees were used in choice trials within 14 hours post-emergence to attempt to minimize starvation time. For each choice trial, we placed one emerged bee inside each mesh foraging arena (5.8 m^3; EVEN Naturals or 4.6 m^3; BugDorm) at 8:00 AM. The number of trials performed per day ranged from 4-14, depending on the number of bees that emerged. We selected plants from our 2022 cohorts that had been flowering for 14-21 days, measured traits, and enclosed all but five flowers per plant in mesh drawstring bags to standardize the number of flowers accessible to bees and to control for differences in floral display size. For morning choice trials, we placed one droughted and one non-droughted plant of the same species into a foraging arena and then observed the bee for 10 min, recording time spent foraging for nectar or pollen. Prior to the 10 min observation period, bees were moved to the floor of the foraging arena to standardize their starting position. Plants remained in the arenas except for a 10 min interval in the afternoon when they were removed so that a second set of floral trait measurements could be made. We then performed a second afternoon 10 min observation of the same bee in a foraging arena with the same plants. We performed a total of 155 successful choice trials (wherein bees foraged on flowers at some time during the observation period) using 90 naive female *O. lignaria*. We performed 28 morning and 27 afternoon trials on *N. menziesii* and 53 morning and 47 afternoon trials on *P. campanularia.*

Data analysis

All analyses were performed in R (R Core Team, 2022). To compare turgor loss point between species, we used a linear model, with log-transformed turgor loss point values as the response variable and plant species as the predictor variable. Aside from the analysis of turgor loss point, all analyses were conducted separately for each plant species. Baseline models for all other plant traits included watering treatment and, when applicable, time of day (morning vs. afternoon) and the interaction between treatment and time of day as predictors. We performed model selection using likelihood ratio tests (R package lmtest; Zeileis and Hothorn 2002). When the best-fitting model included the interaction between treatment and time of day, we used estimated marginal means to compute the contrasts among predictors (R package emmeans; Lenth 2023).

To compare water potential between droughted and non-droughted plants, we used linear mixed-effects models (LMMs) with water potential as the response variable and plant identity as a random effect. Any water potential measurements that were below the turgor loss point were interpreted as measurements on wilted leaves for that species, and indicate severe, potentially life-threatening drought stress (Tyree and Hammel 1972).

To test for differences in the number of flowers, we used generalized linear mixed-effects models (GLMMs) with flower counts, modeled with Poisson error distribution, as the response (R package glmmTMB; Brooks et al. 2017), and plant identity as a random effect. For flower size, we fitted LMMs using flower landing area and corolla length as response variables, and plant identity as a random effect. For nectar volume and calories, we fitted LMMs using log+1 transformed nectar volume and log+1 transformed nectar calories as response variables, whereas for nectar concentration, we used beta regressions with logit-link. Across all nectar analyses, we used plant identity as a random effect.

To determine whether bees exhibited a preference for non-droughted flowers, we first calculated the percentage of time that bees spent on non-droughted flowers out of their total time spent foraging within one observation period. We then fitted LMMs with insect preference centered around 0 as the response variable (such that no preference was equal to 0, preference for non-droughted plants was positive, and preference for droughted plants was negative), time of day as the predictor variable, and bee identity as a random effect, and the intercept forced through 0.

Results

Plant traits

Turgor loss point differed between our study species ($F_{1,4} = 20.07$, $p < 0.05$), with *N. menziesii* showing less-negative and relatively drought-intolerant values (turgor loss point: -0.68 ± 0.10 MPa [mean ± SD]) and *P. campanularia* showing more negative and relatively drought tolerant values (-1.20 ± 0.13 MPa; Supplementary data Fig. S3.1). For all analyses for which baseline models included multiple predictors, we report best-fitting models in Table 1. Droughted *N. menziesii* and *P. campanularia* plants had significantly lower leaf water potential in morning measurements than non-droughted plants (Table 3.1, Fig. S3.2). Water potential was not significantly different between droughted and non-droughted plants of either species in midday measurements, but was significantly higher in non-droughted *P. campanularia* plants in morning measurements ($t_{160} = -3.36$, $p < 0.001$, Supplementary data Fig. S3.2). In *P. campanularia,* 33.8% and 16.4% of water potential measurements on droughted and non-droughted plants, respectively, were below the turgor loss point. In *N. menziesii,* 63.8% and 45.0% of water potential measurements on droughted and non-droughted plants, respectively, were below the turgor loss point.

Droughted plants of both *N. menziesii* and *P. campanularia* had 61% and 75% fewer flowers, respectively, than non-droughted plants (Table 3.1, Fig. 3.1). Droughted *N. menziesii* and *P. campanularia* produced flowers with 26% and 30% smaller corolla lengths (Table 1, Fig. 2) and 55% and 39% smaller landing areas, respectively (Table 3.1, Supplementary data Fig. S3.3). Similar patterns were found in 2021 (Supplementary data Table S3.1, Fig. S3.3, S3.4).

Watering treatment, time of day, and their interaction had significant effects on the nectar volume of *N. menziesii* flowers (Table 3.1, Fig. 3.3). In. *P. campanularia*, only the interaction of watering treatment and time of day had a significant effect on nectar volume (Table 3.1, Fig. 3.3). In *N. menziesii* only, droughted plants produced 75% less morning nectar than non-droughted plants ($t_{152} = -8.46$, $p < 0.0001$; Fig. 3.3). Droughted *N. menziesii* and *P. campanularia* produced 89% and 61% less refill (afternoon) nectar, respectively, than non-droughted plants ($t_{152} = -2.79$, $p < 0.01$ and $t_{279} = -4.37$, $p < 0.001$, respectively; Fig. 3.3).

Time of day and its interaction with watering treatment had a significant effect on nectar concentration in *N. menziesii* (Table 3.1, Fig. 3.4). In *P. campanularia*, watering treatment, time of day, and their interaction had a significant effect on nectar concentration (Table 3.1, Fig. 3.4). In *P. campanularia*, morning and afternoon nectar sugar concentration was 14% and 118% higher, respectively, in droughted plants ($t_{181} = 2.86$, $p < 0.01$ and $t_{181} = 4.67$, $p < 0.001$; Fig. 3.4). In *N. menziesii*, refill nectar was 38% more concentrated in droughted than in non-droughted plants ($t_{89} = 2.86$, $p < 0.01$; Fig. 3.4).

In both *N. menziesii* and *P. campanularia*, watering treatment, time of day, and their interaction significantly impacted the calories available in nectar (Table 3.1, Fig. 3.5). In *N. menziesii*, droughted plants produced 71% and 82% fewer nectar calories in their morning and afternoon nectar, respectively ($t_{140} = -9.66$, $p < 0.001$ and $t_{140} = -2.44$, p

< 0.05, respectively; Fig. 3.5). In *P. campanularia*, droughted plants produced 119% more nectar calories in their morning nectar, but 57% fewer calories in their afternoon nectar ($t_{250} = 2.35$, $p < 0.05$ and $t_{250} = -2.73$, $p < 0.01$, respectively; Fig. 3.5).

Pollinator preference

When presented with droughted and non-droughted flowers of *N. menziesii,* bees spent significantly more of their total time foraging on flowers of non-droughted plants in both the morning and afternoon, spending 75% and 77% of their time on non-droughted flowers in the morning and afternoon, respectively (Table 3.1, Fig. 3.6). This preference was not significantly different between morning and afternoon observations ($t_{51} = 0.27$, $p = 0.79$; Fig. 3.6). However, for *P. campanularia*, preference differed between morning and afternoon: time spent foraging on non-droughted plants increased from 57% in morning foraging bouts to 77% in afternoon foraging bouts ($t_{96} = 3.37$, $p < 0.01$; Fig. 3.6). While there was a marginally significant preference for non-droughted flowers in the morning, afternoon foraging preference for non-droughted flowers was significantly greater than 50% (Table 3.1, Fig. 3.6).

Discussion

Our results demonstrate that drought can alter floral traits and thereby shape pollinator visitation, as bees preferred to visit flowers on non-droughted plants of both wildflower species. Because even slight changes in floral attractive cues and rewards can lead to changes in the community of pollinators visiting a plant species (Bradshaw and Schemske 2003), these results indicate that the increased prevalence of droughts associated with climate change will likely alter the pollination success of drought-stressed plants and, ultimately, both the ecology and evolution of plant-pollinator interactions.

Plant traits

The differences in the turgor loss point of our focal species likely reflect adaptations to different environments (Bartlett et al. 2014). The relatively drought vulnerable *N. menziesii* is found in regions with higher soil moisture and average annual precipitation, whereas *P. campanularia* is found in more-arid regions (Calflora 2023). For both of our focal wildflower species, our drought treatment effectively decreased leaf water potential. Lower water potential in afternoon versus morning was likely driven by higher transpiration rates under warmer conditions (Chapin 1995, Carrol 2000, Carrol et al. 2001).

Drought stress resulted in the production of fewer and smaller flowers in both of our study species, an effect that has been documented in field and greenhouse studies (Gallagher and Campbell 2017, Kuppler et al. 2021, Descamps et al. 2018). Reduced flower production and size under drought are likely driven by reduced photosynthetic capacity (Descamps et al. 2021 a), which leads to lower availability of photosynthetically-derived sugars that can be fixed into reproductive tissues (De Souza et al. 1997). Because flowers have high water costs, producing fewer flowers under drought stress may be adaptive (De la Barrera and Nobel 2004). In addition, more water is needed to retain turgor pressure in flowers with larger corollas due to lower cell wall density (Galen et al. 1999). Flowering can therefore further decrease rates of photosynthesis by inducing stomatal closure in leaves, incurring carbon costs to water-stressed plants

(Galen et al. 1999). Thus, a reduction in flower size and number likely confers benefits to plants that are water-stressed (De la Barrera and Nobel 2004).

In general, droughted plants also produced flowers with less nectar. The often-observed negative effect of drought on nectar volume (Descamps et al. 2018, Gallagher and Campbell 2017, Rering et al. 2020) is likely linked to reduced water availability within the plant (Grant 2012). Although we found that nectar in droughted flowers tended to be more concentrated in sugars, flowers produced by non-droughted plants generally provided more total calories from nectar. These results are consistent with many studies (Descamps et al. 2018, 2020 a; but see Clearwater et al. 2018), and were likely driven by simultaneously decreased amounts of water diverted to nectar and decreased sugars produced through photosynthesis in our droughted plants (De Souza et al. 1997, Carroll et al. 2001). Interestingly, morning nectar volume and sugar concentration were not significantly different between droughted and non-droughted flowers of *P. campanularia*, a result that probably reflects high drought tolerance. Although droughted *P. campanularia* plants had significantly lower leaf water potential, drought-adapted plants can prioritize water transport to reproductive structures (Harrison Day et al. 2022, Suni et al. 2020). Altogether, the short-term responses we detected in nectar traits indicate our study plants adjusted floral resource allocation under drought conditions, which is likely to modify pollinator attraction and visitation patterns (Boose 1977).

Pollinator preference

Bees preferred non-droughted plants, spending significantly more time in the morning and afternoon foraging on non-droughted flowers of our less drought-tolerant study species, *N. menziesii*. In our more drought-tolerant species, *P. campanularia*, bees showed a significant preference for non-droughted flowers only in afternoon foraging bouts. These findings indicate that female *O. lignaria* tend to preferentially visit larger, more nectar-rich flowers, as has been found for various other pollinators (Best and Bierzychudek 1982, Cresswell 1990, Höfer et al. 2021). Decreases in floral rewards such as nectar can result in abandonment of a patch of flowers by pollinators, as demonstrated by bumble bees leaving flowering patches after receiving nectar rewards that are lower than average (Chittka et al. 1997).

Nectar is the main source of sugars for bees (Nicolson 2007), and female *O. lignaria* rely heavily on nectar for their own metabolic needs and provisioning their offspring (Williams 1999, Bosch and Kemp 2004). Though foraging preference by *O. lignaria* has not to our knowledge previously been linked to nectar volume and sugar concentration, higher nectar volumes and sugar content are associated with more visits by bumble bees (Blarer et al. 2002, Roldan-Serrano and Guerra-Sanz 2005) as well as honey bees (Silva and Dean 2000, Mallinger and Prasifka 2017). By visiting more-rewarding flowers, bees increase their foraging efficiency (Dreisig 2012). We did not measure sugar composition, but drought can also decrease the ratio of sucrose to other sugars in nectar (Rering et al. 2020), an effect that may have further influenced bee foraging preferences (Abrahamczyk et al. 2017). Similarly, we did not quantify emissions of floral volatile organic compounds, but these can shape the responses of some pollinators to droughted flowers (Rering et al. 2020), and *O. lignaria* females are known to use olfactory cues to locate their nesting sites (Guédot et al. 2006).

Flower size also shapes visitation by insect pollinators (Kuppler et al. 2021), and the effects of our drought treatment on flower size may also explain the preference of *O. lignaria* for non-droughted flowers. For example, in flight cage experiments, bumble bees made fewer visits to flowers of an annual wildflower grown under low soil moisture conditions, likely because the bees were less attracted by, and less able to handle, small flowers (Kuppler et al. 2021).

Learning may have influenced the strength of preference for non-droughted flowers in our bees, particularly in the afternoon foraging bouts when bees were no longer naive. *O. lignaria* can remember the color of flowers that provide a greater volume of nectar reward for up to three hours (Amaya-Márquez et al. 2008). Likewise, bumble bees can remember the location of the most profitable flower patches (Cartar 2004) and can learn to visit plants with fewer flowers in exchange for more nectar (Makino and Sakai 2007). As they forage, pollinators tend to learn to prefer flowers with 'honest signals' that accurately reflect their resource availability (Knauer and Schiestl 2015). Therefore, the bees in our experiment may have learned to associate larger flowers with larger floral rewards, as these traits covaried in our experiment.

Consequences for pollination

Though both of our focal plant species are capable of autogamy, their floral traits promote outcrossing, and autogamous fruits yield few seeds (Gillett 1961, Cruden 1972). Thus, population persistence is reliant on pollination by animals, which may be disrupted if pollinators are less likely to visit the flowers of droughted populations. Similarly, drought-induced changes in flower size and floral display may lead to visitation by different assemblages of pollinators (Thompson 2001, Gambel and Holway 2023), which, if visitation shifts from more- to less-efficient pollinators, could reduce the reproductive output of droughted plants. Furthermore, drought can directly limit plant reproduction by reducing flower display, as documented herein, as well as by reducing pollen viability (M Bharucha and A Rose-Person, UCR, Riverside, California, unpubl. res.) and increasing flower and fruit abortions (Akhalkatsi and Lösch 2005, Descamps et al. 2018, Gallagher and Campbell 2017). Together, these direct and pollinator-mediated negative effects could reduce the reproductive success of drought-stressed plants. As entire communities of plants and pollinating insects are exposed to drought, coflowering species with different drought tolerances may become relatively more or less attractive to their pollinators. This may alter competitive dynamics across the floral landscape by favoring plants with higher drought tolerance (Sargent and Ackerly 2008, Mesgaran et al. 2017, Faust and Iler 2022).

Conclusions

Our findings demonstrate that drought can structure plant-pollinator interactions. Drought-induced reductions in flower size and nectar volume were associated with preferential visitation by bees to larger, non-droughted flowers that contained more nectar, with the strength of these preferences shaped by the drought tolerance of the plants. These experimental results therefore point to the potential for more-frequent and sustained drought to alter the attractiveness of annual wildflowers in the short-term, thereby reshaping interactions with pollinators, which may in turn alter the population dynamics of both partners.

Drought is likely to co-occur with climate-change driven increases in temperature (Barnabás et al. 2008), and their combined effects will likely modify plant traits and plant-pollinator interactions in complex ways (Descamps et al. 2018, 2021 b). Indeed, in a similar greenhouse setting, experimental warming of our focal plant species resulted in reduced visitation by *O. lignaria* and reduced seed set (de Manincor et al. 2023). Work that assesses the interactive effects of multiple abiotic factors on plant-pollinator interactions should lead to more accurate predictions of how climate change will affect pollination services and plant reproduction.

References

Abrahamczyk, S., Kessler, M., Hanley, D., Karger, D. N., Müller, M. P., Knauer, A. C., ... & Humphreys, A. M. (2017). Pollinator adaptation and the evolution of floral nectar sugar composition. *Journal of Evolutionary Biology*, *30*(1), 112-127.

Akhalkatsi, M., & Lösch, R. (2005). Water limitation effect on seed development and germination in Trigonella coerulea (Fabaceae). *Flora-Morphology, Distribution, Functional Ecology of Plants*, *200*(6), 493-501.

Amaya-Márquez, M., Hill, P. S., Barthell, J. F., Pham, L. L., Doty, D. R., & Wells, H. (2008). Learning and memory during foraging of the blue orchard bee, Osmia lignaria Say (Hymenoptera: Megachilidae). *Journal of the Kansas Entomological Society*, *81*(4), 315-327.

Barnabás, B., Jäger, K., & Fehér, A. (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant, cell & environment*, *31*(1), 11-38.

Bartlett, M. K., Scoffoni, C., & Sack, L. (2012). The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecology letters*, *15*(5), 393-405.

Bartlett, M. K., Zhang, Y., Kreidler, N., Sun, S., Ardy, R., Cao, K., & Sack, L. (2014). Global analysis of plasticity in turgor loss point, a key drought tolerance trait. *Ecology letters*, *17*(12), 1580-1590.

Best, L. S., & Bierzychudek, P. (1982). Pollinator foraging on foxglove (Digitalis purpurea): a test of a new model. *Evolution*, 70-79.

Bharucha, M., Rose-Person., A. (2023). Effects of drought stress on the pollen quality and quantity of the insect-pollinated species *Phacelia campanularia*. Unpublished Results.

Biesmeijer, J. C., Roberts, S. P., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., ... & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, *313*(5785), 351-354.

Blarer, A., Keasar, T., & Shmida, A. (2002). Possible mechanisms for the formation of flower size preferences by foraging bumblebees. *Ethology*, *108*(4), 341-351.

Bolten, A. B., Feinsinger, P., Baker, H. G., & Baker, I. (1979). On the calculation of sugar concentration in flower nectar. *Oecologia*, *41*, 301-304.

Boose, D. L. (1997). Sources of variation in floral nectar production rate in Epilobium canum (Onagraceae): implications for natural selection. *Oecologia*, *110*, 493-500.

Bosch, J., & Kemp, W. P. (2004). The life cycle of Osmia lignaria: implications for rearing populations. *Solitary bees. Conservation, rearing and management for pollination. Imprensa Universitária, Fortaleza*, 153-160.

Boyle, N. K., Artz, D. R., Lundin, O., Ward, K., Picklum, D., Wardell, G. I., ... & Pitts-Singer, T. L. (2020). Wildflower plantings promote blue orchard bee, Osmia lignaria (Hymenoptera: Megachilidae), reproduction in California almond orchards. *Ecology and Evolution*, *10*(7), 3189-3199.

Bradshaw Jr, H. D., & Schemske, D. W. (2003). Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature*, *426*(6963), 176-178.

Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., ... & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R journal*, *9*(2), 378-400.

Burkle, L. A., & Alarcón, R. (2011). The future of plant–pollinator diversity: understanding interaction networks across time, space, and global change. *American journal of botany*, *98*(3), 528-538.

CalFlora Database [A Non-Profit Organization]. (2016). CalFlora, information on California plants for education, research and conservation, with data contributed by public and private institutions and individuals, including the Consortium of California Herbaria.

CaraDonna, P. J., Petry, W. K., Brennan, R. M., Cunningham, J. L., Bronstein, J. L., Waser, N. M., & Sanders, N. J. (2017). Interaction rewiring and the rapid turnover of plant–pollinator networks. *Ecology letters*, *20*(3), 385-394.

Carroll, A. B. (2000). *The relationship between plant water status and floral trait expression in fireweed, Epilobium angustifolium* (Doctoral dissertation, University of Missouri-Columbia).

Carroll, A. B., Pallardy, S. G., & Galen, C. (2001). Drought stress, plant water status, and floral trait expression in fireweed, Epilobium angustifolium (Onagraceae). *American Journal of Botany*, *88*(3), 438-446.

Cartar, R. V. (2004). Resource tracking by bumble bees: responses to plant-level differences in quality. *Ecology*, *85*(10), 2764-2771.

Chapin, D. M. (1995). Physiological and morphological attributes of two colonizing plant species on Mount St. Helens. *American Midland Naturalist*, 76-87.

Chittka, L., Gumbert, A., & Kunze, J. (1997). Foraging dynamics of bumble bees: correlates of movements within and between plant species. *Behavioral Ecology*, *8*(3), 239-249.

Clearwater, M. J., Revell, M., Noe, S., & Manley-Harris, M. (2018). Influence of genotype, floral stage, and water stress on floral nectar yield and composition of mānuka (Leptospermum scoparium). *Annals of botany*, *121*(3), 501-512.

Conner, J. K., & Rush, S. (1996). Effects of flower size and number on pollinator visitation to wild radish, Raphanus raphanistrum. *Oecologia*, *105*, 509-516.

Cresswell, J. E. (1990). How and why do nectar-foraging bumblebees initiate movements between inflorescences of wild bergamot Monarda fistulosa (Lamiaceae)?. *Oecologia*, *82*, 450-460.

Cruden, R. W. (1972). Pollination biology of Nemophila menziesii (Hydrophyllaceae) with comments on the evolution of oligolectic bees. *Evolution*, 373-389.

Cubasch, U., Meehl, G. A., Boer, G. J., Stouffer, R. J., Dix, M., Noda, A., ... & Yap, K. S. (2001). Projections of future climate change. In *Climate Change 2001: The scientific basis. Contribution of WG1 to the Third Assessment Report of the IPCC (TAR)* (pp. 525-582). Cambridge University Press.

Dafni, A., Kevan, P. G., & Husband, B. C. (2005). Practical pollination biology. *Practical pollination biology.*

De la Barrera, E., & Nobel, P. S. (2004). Nectar: properties, floral aspects, and speculations on origin. *Trends in plant science*, *9*(2), 65-69.

de Manincor, N., Fisogni, A., & Rafferty, N. E. (2023). Warming of experimental plant–pollinator communities advances phenologies, alters traits, reduces interactions and depresses reproduction. *Ecology Letters*, *26*(2), 323-334.

De Souza, P. I., Egli, D. B., & Bruening, W. P. (1997). Water stress during seed filling and leaf senescence in soybean. *Agronomy Journal*, *89*(5), 807-812.

Descamps, C., Quinet, M., Baijot, A., & Jacquemart, A. L. (2018). Temperature and water stress affect plant–pollinator interactions in Borago officinalis (Boraginaceae). *Ecology and evolution*, *8*(6), 3443-3456.

Descamps, C., Boubnan, N., Jacquemart, A. L., & Quinet, M. (2021). Growing and flowering in a changing climate: effects of higher temperatures and drought stress on the bee-pollinated species Impatiens glandulifera royle. *Plants*, *10*(5), 988.

Descamps, C., Quinet, M., & Jacquemart, A. L. (2021). The effects of drought on plant–pollinator interactions: What to expect?. *Environmental and Experimental Botany*, *182*, 104297.

Dreisig, H. (2012). How long to stay on a plant: the response of bumblebees to encountered nectar levels. *Arthropod-Plant Interactions*, *6*(2), 315-325.

Faust, M. N., & Iler, A. M. (2022). Pollinator-mediated reproductive consequences of altered co-flowering under climate change conditions depend on abiotic context. *Climate Change Ecology*, *3*, 100043.

Filazzola, A., Matter, S. F., & MacIvor, J. S. (2021). The direct and indirect effects of extreme climate events on insects. *Science of the Total Environment*, *769*, 145161.

Fitter, A. H., & Fitter, R. S. R. (2002). Rapid changes in flowering time in British plants. *Science*, *296*(5573), 1689-1691.

Forrest, J. R. (2015). Plant–pollinator interactions and phenological change: what can we learn about climate impacts from experiments and observations?. *Oikos*, *124*(1), 4-13.

Galen, C., & Newport, M. E. A. (1987). Bumble bee behavior and selection on flower size in the sky pilot, Polemonium viscosum. *Oecologia*, *74*, 20-23.

Galen, C., Sherry, R. A., & Carroll, A. B. (1999). Are flowers physiological sinks or faucets? Costs and correlates of water use by flowers of Polemonium viscosum. *Oecologia*, *118*, 461-470.

Gallagher, M. K., & Campbell, D. R. (2017). Shifts in water availability mediate plant–pollinator interactions. *New Phytologist*, *215*(2), 792-802.

Gambel, J., & Holway, D. A. (2023). Divergent responses of generalist and specialist pollinators to experimental drought: Outcomes for plant reproduction. *Ecology*, e4111.

Gillett, G. W. (1961). An experimental study of variation in the Phacelia sericea complex. *American Journal of Botany*, *48*(1), 1-7.

Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W., & Holt, R. D. (2010). A framework for community interactions under climate change. *Trends in ecology & evolution*, *25*(6), 325-331.

Glenny, W. R., Runyon, J. B., & Burkle, L. A. (2018). Drought and increased CO 2 alter floral visual and olfactory traits with context-dependent effects on pollinator visitation. *New Phytologist*, *220*(3), 785-798.

Grant, O. M. (2012). Understanding and exploiting the impact of drought stress on plant physiology. *Abiotic stress responses in plants: metabolism, productivity and sustainability*, 89-104.

Guédot, C., Pitts-Singer, T. L., Buckner, J. S., Bosch, J., & Kemp, W. P. (2006). Olfactory cues and nest recognition in the solitary bee Osmia lignaria. *Physiological Entomology*, *31*(2), 110-119.

Haider, M., Dorn, S., Sedivy, C., & Müller, A. (2014). Phylogeny and floral hosts of a predominantly pollen generalist group of mason bees (Megachilidae: Osmiini). *Biological Journal of the Linnean Society*, *111*(1), 78-91.

Harrison Day, B. L., Carins-Murphy, M. R., & Brodribb, T. J. (2022). Reproductive water supply is prioritized during drought in tomato. *Plant, Cell & Environment*, *45*(1), 69-79.

Hodges, S. A. (1995). The influence of nectar production on hawkmoth behavior, self pollination, and seed production in Mirabilis multiflora (Nyctaginaceae). *American Journal of Botany*, *82*(2), 197-204.

Höfer, R. J., Ayasse, M., & Kuppler, J. (2021). Bumblebee behavior on flowers, but not initial attraction, is altered by short-term drought stress. *Frontiers in Plant Science*, *11*, 564802.

Huang, H., & D'Odorico, P. (2020). Critical transitions in plant-pollinator systems induced by positive inbreeding-reward-pollinator feedbacks. *Iscience*, *23*(2).

Intergovernmental Panel on Climate Change (IPCC). (2014). *Climate Change 2014: Synthesis Report*.

Kattenberg, A., Giorgi, F., Grassl, H., Meehl, G. A., Mitchell, J. F. B., Stouffer, R. J., ... & Wigley, T. M. L. (1996). Climate models: projections of future climate. In *Climate Change 1995: the science of climate change. Contribution of WG1 to the Second Assessment Report of the IPCC* (pp. 299-357). Cambridge University Press.

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the royal society B: biological sciences*, *274*(1608), 303-313.

Knauer, A. C., & Schiestl, F. P. (2015). Bees use honest floral signals as indicators of reward when visiting flowers. *Ecology letters*, *18*(2), 135-143.

Kuppler, J., & Kotowska, M. M. (2021). A meta-analysis of responses in floral traits and flower–visitor interactions to water deficit. *Global Change Biology*, *27*(13), 3095-3108.

Kuppler, J., Wieland, J., Junker, R. R., & Ayasse, M. (2021). Drought-induced reduction in flower size and abundance correlates with reduced flower visits by bumble bees. *AoB Plants*, *13*(1), plab001.

Lange, D., Calixto, E. S., & Del-Claro, K. (2017). Variation in extrafloral nectary productivity influences the ant foraging. *PloS one*, *12*(1), e0169492.

Leimu, R., Vergeer, P., Angeloni, F., & Ouborg, N. J. (2010). Habitat fragmentation, climate change, and inbreeding in plants. *Annals of the New York Academy of Sciences*, *1195*(1), 84-98.

Lenth, R. (2023). _emmeans: Estimated Marginal Means, aka Least-Squares Means_. *R package version 1.8.6* https://CRAN.R-project.org/package=emmeans.

Makino, T. T., & Sakai, S. (2007). Experience changes pollinator responses to floral display size: from size-based to reward-based foraging. *Functional Ecology*, *21*(5), 854-863.

Mallinger, R. E., & Prasifka, J. R. (2017). Bee visitation rates to cultivated sunflowers increase with the amount and accessibility of nectar sugars. *Journal of Applied Entomology*, *141*(7), 561-573.

McCall, A. C. (2006). Natural and artificial floral damage induces resistance in Nemophila menziesii (Hydrophyllaceae) flowers. *Oikos*, *112*(3), 660-666.

McCall, A. C. (2008). Florivory affects pollinator visitation and female fitness in Nemophila menziesii. *Oecologia*, *155*(4), 729-737.

Mesgaran, M. B., Bouhours, J., Lewis, M. A., & Cousens, R. D. (2017). How to be a good neighbour: facilitation and competition between two co-flowering species. *Journal of Theoretical Biology*, *422*, 72-83.

Naumann, G., Alfieri, L., Wyser, K., Mentaschi, L., Betts, R. A., Carrao, H., ... & Feyen, L. (2018). Global changes in drought conditions under different levels of warming. *Geophysical Research Letters*, *45*(7), 3285-3296.

Nicolson, S. W. (2007). Nectar consumers. *In*: Nicolson SW, Nepi M, Pacini E, eds. *Nectaries and Nectar*. Dordrecht: Springer, 289-342

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, *120*(3), 321-326.

Orlowsky, B., & Seneviratne, S. I. (2013). Elusive drought: uncertainty in observed trends and short-and long-term CMIP5 projections. *Hydrology and Earth System Sciences*, *17*(5), 1765-1781.

Peterson, S. S., & Artz, D. R. (2014). Production of solitary bees for pollination in the United States. In *Mass production of beneficial organisms* (pp. 541-558). Academic Press.

Phillips, B. B., Shaw, R. F., Holland, M. J., Fry, E. L., Bardgett, R. D., Bullock, J. M., & Osborne, J. L. (2018). Drought reduces floral resources for pollinators. *Global change biology*, *24*(7), 3226-3235.

Pitts-Singer, T. L., Artz, D. R., Peterson, S. S., Boyle, N. K., & Wardell, G. I. (2018). Examination of a managed pollinator strategy for almond production using Apis mellifera (Hymenoptera: Apidae) and Osmia lignaria (Hymenoptera: Megachilidae). *Environmental Entomology*, *47*(2), 364-377.

Prudhomme, C., Giuntoli, I., Robinson, E. L., Clark, D. B., Arnell, N. W., Dankers, R., ... & Wisser, D. (2014). Hydrological droughts in the 21st century, hotspots and uncertainties from a global multimodel ensemble experiment. *Proceedings of the National Academy of Sciences*, *111*(9), 3262-3267.

R Core Team. (2023). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. Vienna, Austria. https://www.R-project.org/.

Rering, C. C., Franco, J. G., Yeater, K. M., & Mallinger, R. E. (2020). Drought stress alters floral volatiles and reduces floral rewards, pollinator activity, and seed set in a global plant. *Ecosphere*, *11*(9), e03254.

Rodriguez-Dominguez, C. M., Forner, A., Martorell, S., Choat, B., Lopez, R., Peters, J. M., ... & Sack, L. (2022). Leaf water potential measurements using the pressure chamber: Synthetic testing of assumptions towards best practices for precision and accuracy. *Plant, Cell & Environment*, *45*(7), 2037-2061.

Roldan-Serrano, A. S., & Guerra-Sanz, J. M. (2005). Reward attractions of zucchini flowers (Cucurbita pepo L.) to bumblebees (Bombus terrestris L.). *European Journal of Horticultural Science*, *70*(1), 23-28.

Sargent, R. D., & Ackerly, D. D. (2008). Plant–pollinator interactions and the assembly of plant communities. *Trends in Ecology & Evolution*, *23*(3), 123-130.

Silva, E. M., & Dean, B. B. (2000). Effect of nectar composition and nectar concentration on honey bee (Hymenoptera: Apidae) visitations to hybrid onion flowers. *Journal of Economic Entomology*, *93*(4), 1216-1221.

Spinoni, J., Naumann, G., Carrao, H., Barbosa, P., & Vogt, J. (2014). World drought frequency, duration, and severity for 1951–2010. *International Journal of Climatology*, *34*(8), 2792-2804.

Suni, S. S., Ainsworth, B., & Hopkins, R. (2020). Local adaptation mediates floral responses to water limitation in an annual wildflower. *American Journal of Botany*, *107*(2), 209-218.

Thompson, J. D. (2001). How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system?. *Oecologia*, *126*, 386-394.

Torchio, P. F., & Asensio, E. (1985). The introduction of the European bee, Osmia cornuta Latr., into the US as a potential pollinator of orchard crops, and a comparison of its manageability with Osmia lignaria propinqua Cresson (Hymenoptera: Megachilidae). *Journal of the Kansas Entomological Society*, 42-52.

Tyree, M. T., & Hammel, H. T. (1972). The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *Journal of experimental Botany*, *23*(1), 267-282.

United States Department of Agriculture, Natural Resources Conservation Service. Web application. (2023). *The PLANTS Database*. http://plants.usda.gov. Accessed: Mar 20, 2023.

Vaudo, A. D., Tooker, J. F., Grozinger, C. M., & Patch, H. M. (2015). Bee nutrition and floral resource restoration. *Current opinion in insect science*, *10*, 133-141.

Waser, N. M., & Price, M. V. (2016). Drought, pollen and nectar availability, and pollination success. *Ecology*, *97*(6), 1400-1409.

Williams, N. M. (1999). *The evolution and ecology of diet specialization in two osmiine bees*. State University of New York at Stony Brook.

Williams, N. M., & Tepedino, V. J. (2003). Consistent mixing of near and distant resources in foraging bouts by the solitary mason bee Osmia lignaria. *Behavioral Ecology*, *14*(1), 141-149.

Wilson Rankin, E. E., Barney, S. K., & Lozano, G. E. (2020). Reduced water negatively impacts social bee survival and productivity via shifts in floral nutrition. *Journal of Insect Science*, *20*(5), 15.

Wróblewska, A. (2010). Flowering dynamics, nectar secretion and insect visitation of Phacelia campanularia A. Gray. *Acta Agrobotanica*, *63*(1).

Zeileis, A., & Hothorn, T. (2002). *Diagnostic checking in regression relationships* (Vol. 2, No. 3, pp. 7-10). na.

Tables, Images, and Figures

Table 3.1: Best-fitting model results for plant traits and pollinator preference. "*N.m.*" represents *Nemophila menziesii* and "*P.c.*" represents *Phacelia campanularia.* "Wt" represents watering treatment and "Tod" represents time of day. Plant identity was used as a random effect in all models with plant traits as the responses, and bee identity was used as a random effect in the models with bee preference as the response. Asterisks indicate significance level (*p < 0.05, **p < 0.01, ***p < 0.001).

Response	**Distribution**	**Species**	**Predictor**	**Estimate**	**SE**	**z**	**p**	**n**
Water potential	Gaussian	*N.m.*	Wt	-0.08	0.03	-3.23	__0.001**__	106
		P.c.	Wt	-0.23	0.07	-3.36	__0.0008***__	166
			Tod	-0.16	0.07	-2.41	__0.02*__	
			Wt * Tod	0.19	0.09	2.07	__0.04*__	
Number of flowers	Poisson	*N.m.*	Wt	-0.91	0.06	-14.87	__<0.00001***__	68
		P.c.	Wt	-1.38	0.04	-32.45	__<0.00001***__	153
Flower landing area	Gaussian	*N.m.*	Wt	-379.15	36.41	-10.41	__<0.00001***__	80
		P.c.	Wt	-179.48	16.66	-10.77	__<0.00001***__	151
Corolla length		*N.m.*	Wt	-1.62	0.45	-3.59	__0.0003***__	80
		P.c.	Wt	-4.26	0.51	-8.41	__<0.00001***__	151
Nectar volume		*N.m.*	Wt	-0.21	0.03	-8.46	__<0.00001***__	158
			Tod	-0.21	0.02	-8.63	__<0.00001***__	
			Wt * Tod	0.14	0.03	4.15	__0.00003***__	
		P.c.	Wt	0.13	0.11	1.16	0.25	285
			Tod	-0.02	0.11	-0.15	0.89	
			Wt * Tod	-0.62	0.15	-4.10	__0.00004***__	

Nectar concentration	Beta with logit link	*N.m.*	Wt	0.14	0.15	0.97	0.33	95
			Tod	-0.57	0.17	-3.38	**0.0007*****	
			Wt * Tod	0.57	0.29	1.99	**0.047***	
		P.c.	Wt	0.31	0.12	2.67	**0.008****	187
			time of day	-1.40	0.13	-10.69	**<0.00001*****	
			Wt * Tod	0.75	0.25	2.97	**0.003****	
Nectar calories	Gaussian	*N.m.*	Wt	-0.53	0.05	-11.59	**<0.00001*****	146
			Tod	-0.47	0.05	-9.66	**<0.00001*****	
			Wt * Tod	0.34	0.06	5.28	**<0.00001*****	
		P.c.	Wt	-0.48	0.14	-3.49	**0.0005*****	256
			Tod	0.33	0.14	2.35	**0.02***	
			Wt * Tod	-0.76	0.20	-3.70	**0.0002*****	
Bee preference		*N.m.*	AM	0.25	0.05	4.69	**<0.00001*****	28
			PM	0.27	0.06	4.92	**<0.00001*****	27
		P.c.	AM	0.07	0.04	1.71	0.09	53
			PM	0.27	0.05	5.79	**<0.00001*****	47

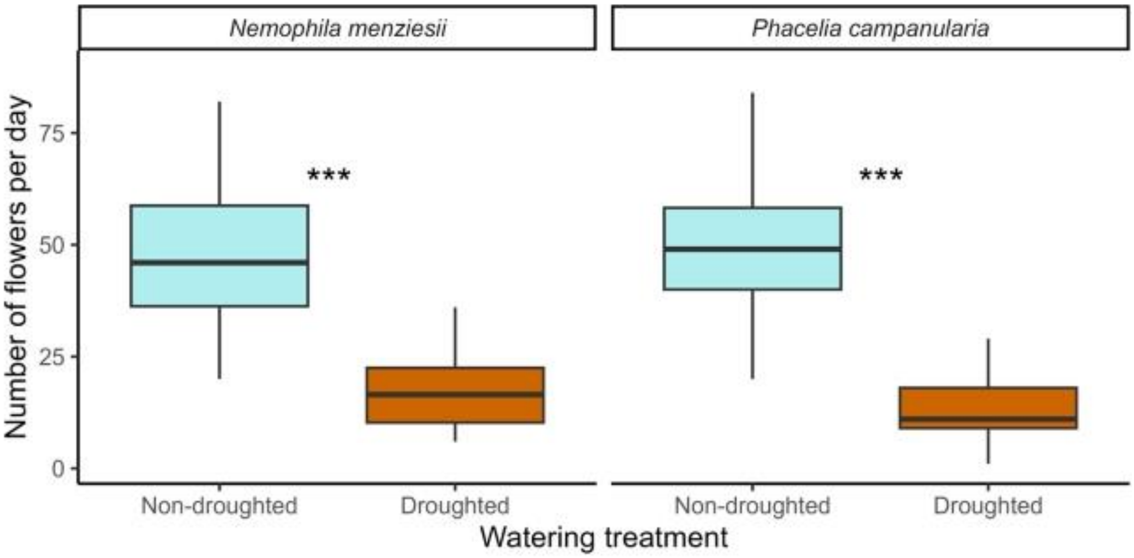


Figure 3.1: Number of flowers per plant on a given day for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions. Asterisks indicate significance level (***$p < 0.001$). Outliers were removed from the figure.

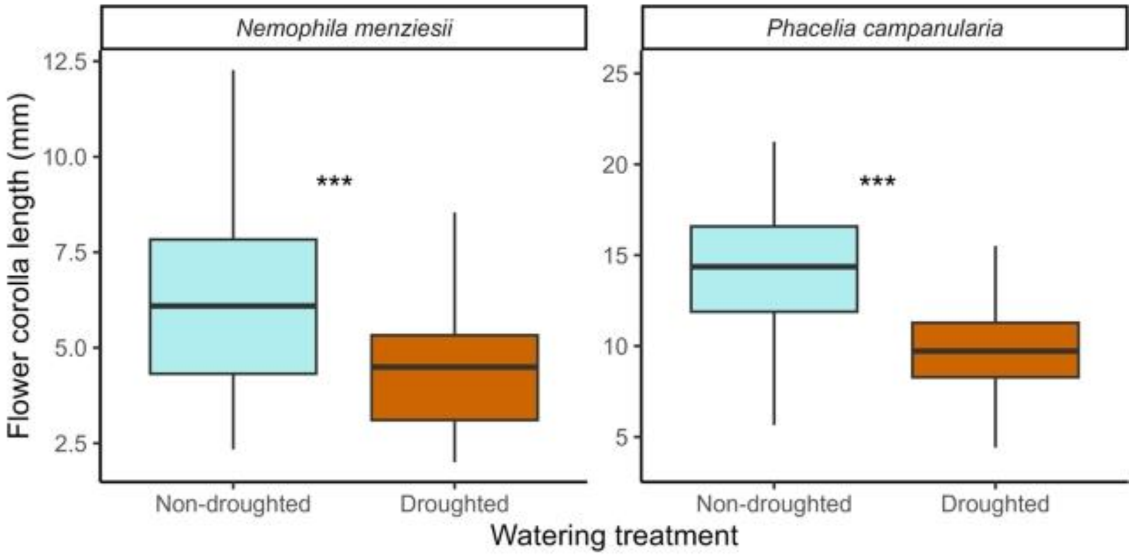


Figure 3.2: Corolla length for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions. Asterisks indicate significance level (***$p < 0.001$). Outliers were removed from the figure.

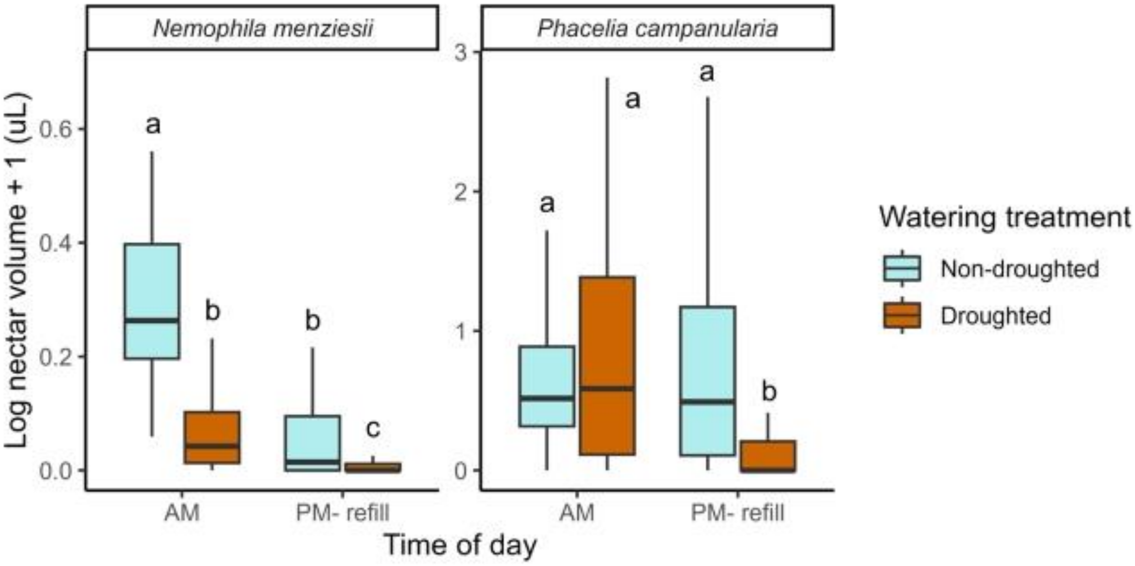


Figure 3.3: Nectar volume per flower by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia.* Outliers were removed from the figure.

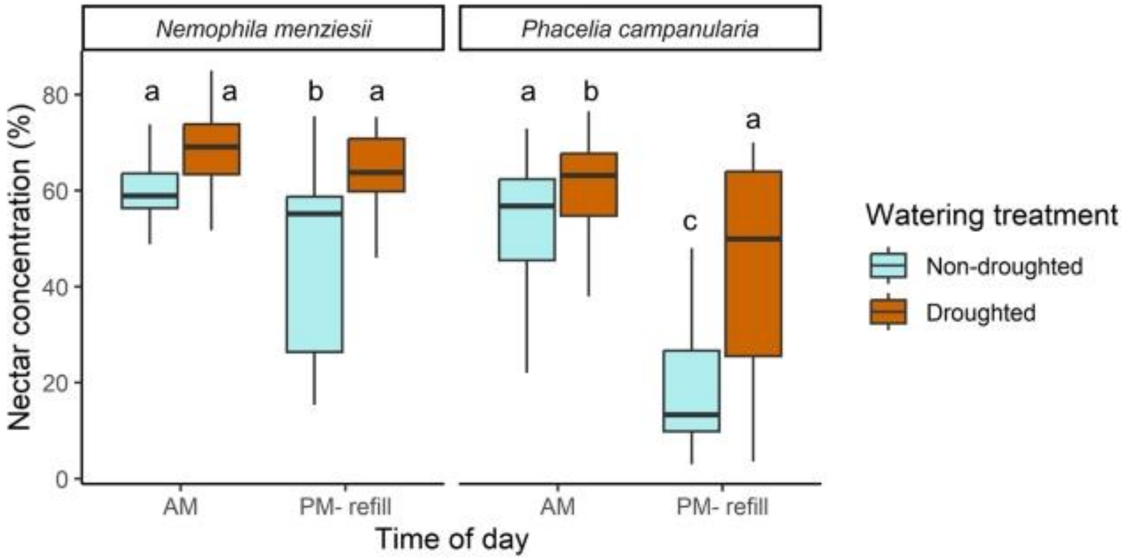


Figure 3.4: Nectar sugar concentration by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia*. Outliers were removed from the figure.

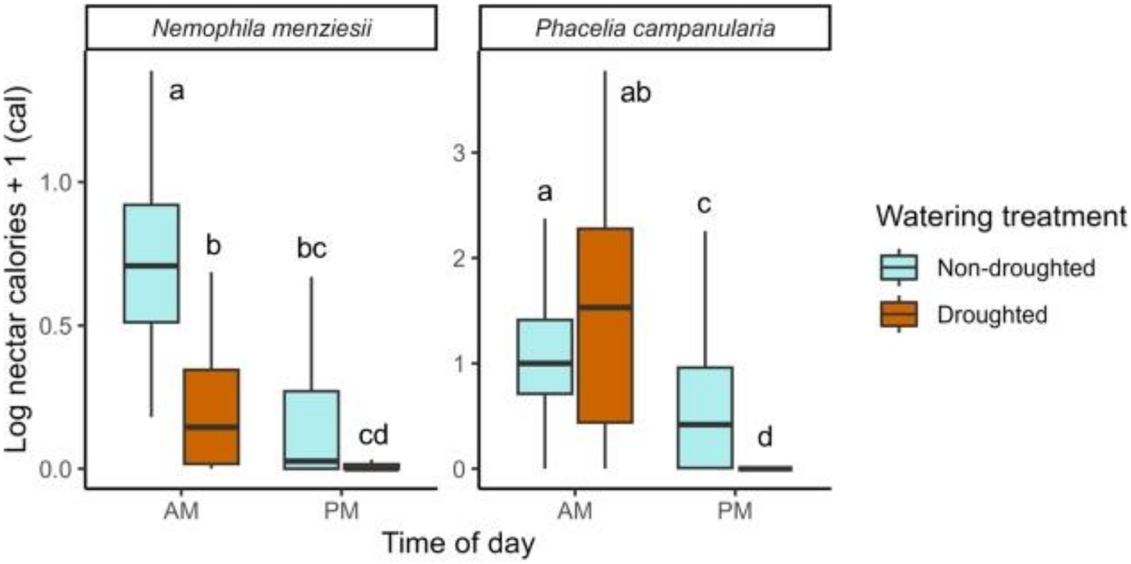


Figure 3.5: Nectar calorie content by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia*. Outliers were removed from the figure.

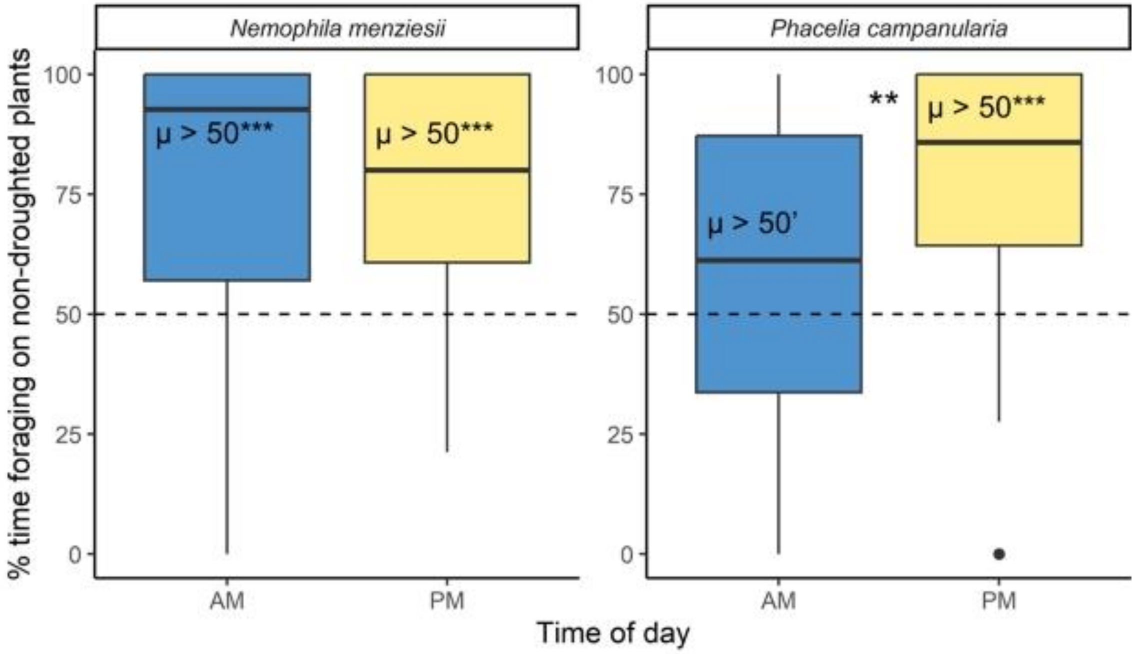


Figure 3.6: Percent foraging time spent by *Osmia lignaria* on non-droughted *Nemophila menziesii* and *Phacelia campanularia* plants when presented with both non-droughted and droughted plants of each species in the morning and afternoon. Asterisks indicate significance level ('p < 0.09, **p < 0.01, ***p < 0.001).

Appendix

Table S3.1: Model results for 2021 floral trait measurements. Watering treatment was used as a predictor in each model. Asterisks indicate significance level (*p < 0.05, **p < 0.01, ***p < 0.001).

Response	**Species**	**Estimate**	**SE**	**z**	**p**	**n**
Corolla length	*N. menziesii*	-0.76	0.10	-7.86	**3.98e-15*** **	1104
	P. campanularia	-1.52	0.19	-7.95	**1.93e-15*** **	1129
Flower landing area	*N. menziesii*	-44.74	7.83	-5.71	**1.11e-08*** **	1104
	P. campanularia	-31.00	7.18	-4.32	**1.59e-05*** **	1129
Nectar volume	*N. menziesii*	-0.02	0.01	-3.20	**0.0014** **	1109
	P. campanularia	-0.33	0.08	-4.03	**5.59e-05*** **	1134
Nectar concentration	*N. menziesii*	0.17	0.06	2.92	**0.0035** **	470
	P. campanularia	0.08	0.04	2.14	**0.032* **	899
Nectar calories	*N. menziesii*	-0.53	0.05	-10.33	**<2e-16*** **	966
	P. campanularia	-0.48	0.12	-3.90	**9.60e-05*** **	1051

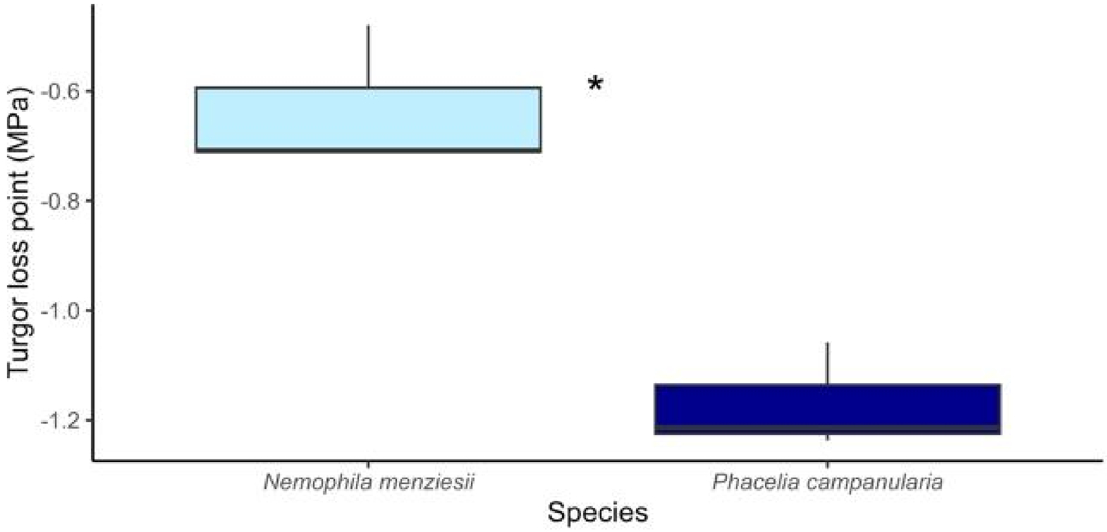


Figure S3.1: Leaf turgor loss point (π_{tlp}) of *Nemophila menziesii* and *Phacelia campanularia.* More negative values of π_{tlp} indicate higher levels of drought tolerance. Asterisk indicates significance level (*p < 0.05).

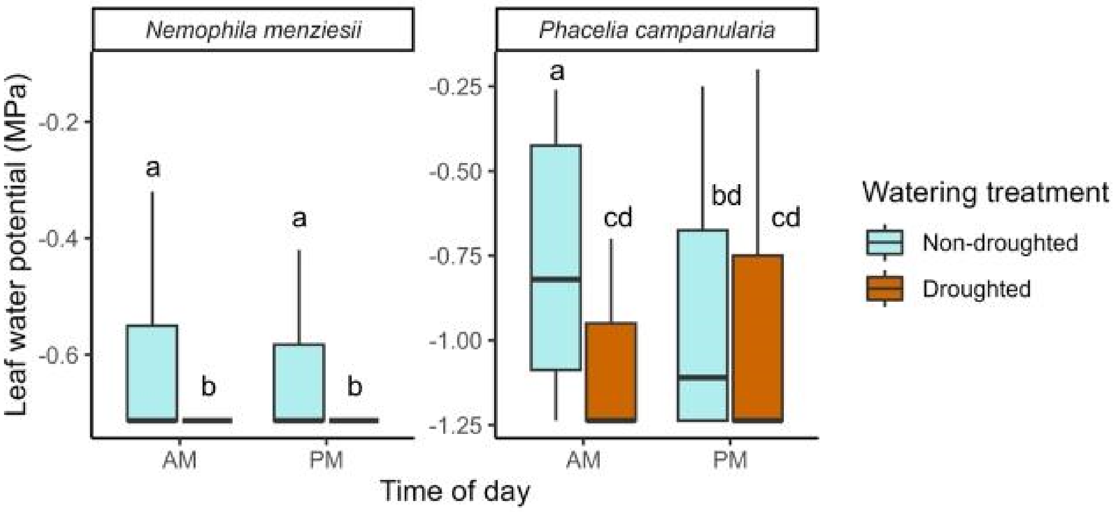


Figure S3.2: Leaf water potential by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia.* More negative values of leaf water potential indicate higher levels of drought stress. Outliers were removed from the figure.

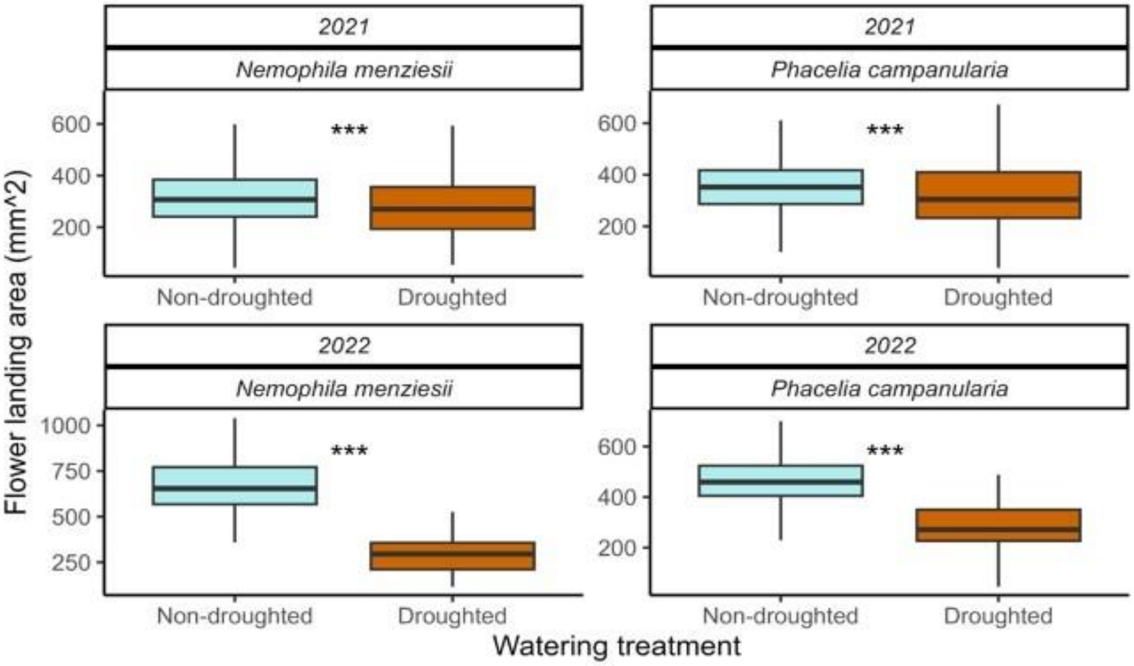


Figure S3.3: Flower landing area for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions in 2021 and 2022. Asterisks indicate significance level (***$p < 0.001$). Outliers were removed from the figure.

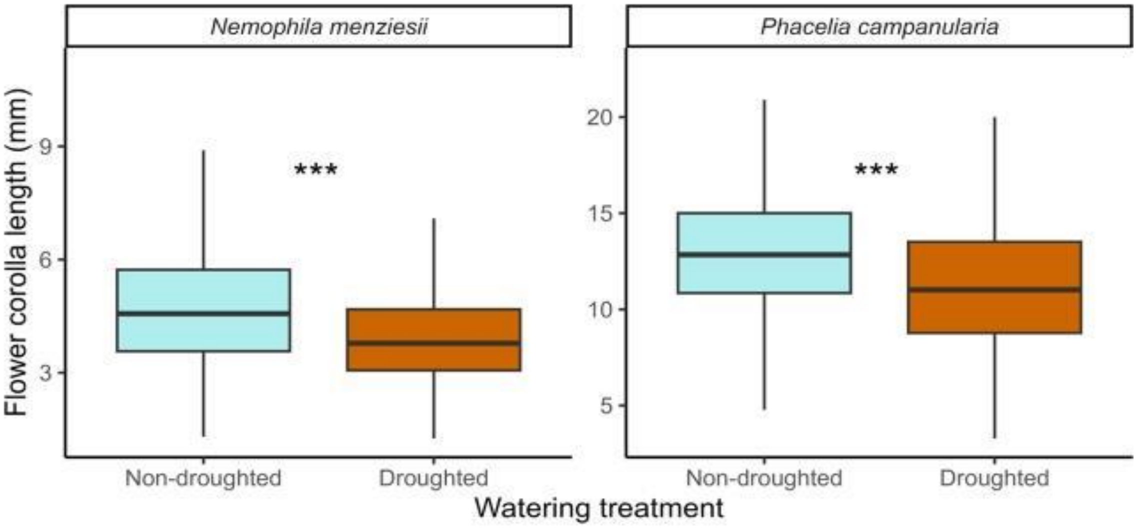


Figure S3.4: Flower corolla length for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions in 2021. Asterisks indicate significance level (***p < 0.001). Outliers were removed from the figure.

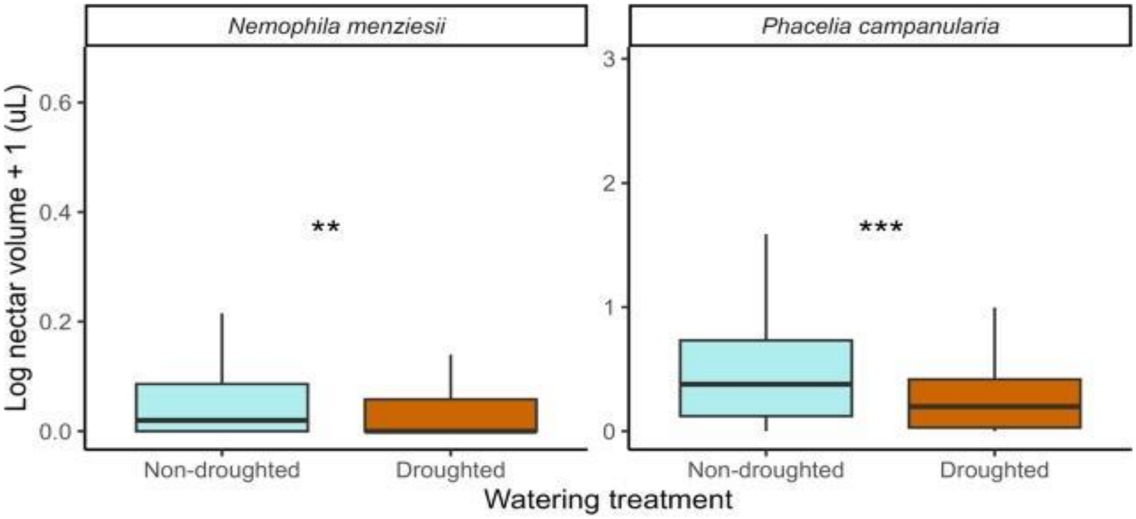


Figure S3.5: Nectar volume per flower for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions in 2021. Asterisks indicate significance level (**p < 0.01, ***p < 0.001). Outliers were removed from the figure.

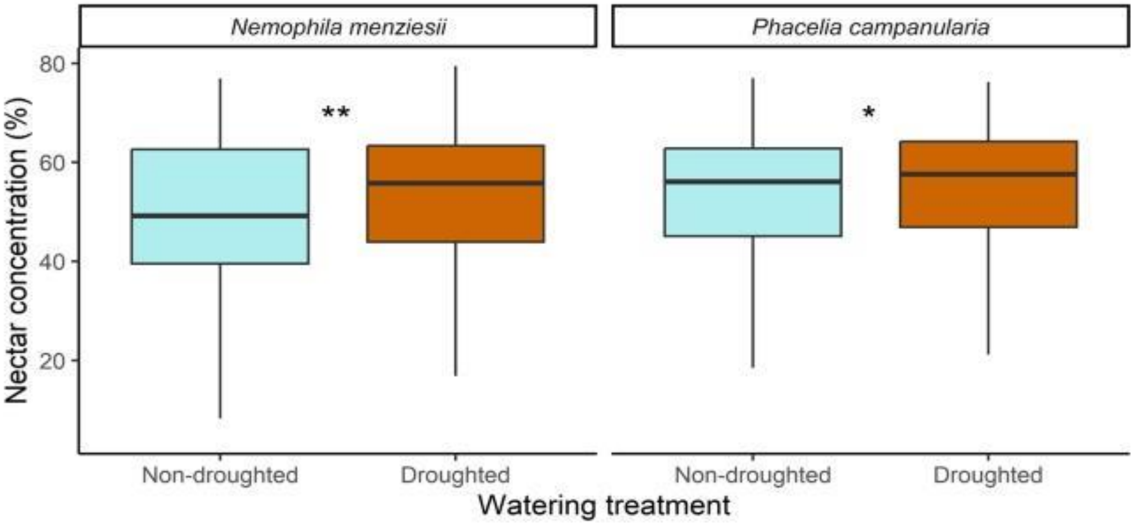


Figure S3.6: Nectar concentration for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions in 2021. Asterisks indicate significance level (*$p < 0.05$, **$p < 0.01$). Outliers were removed from the figure.

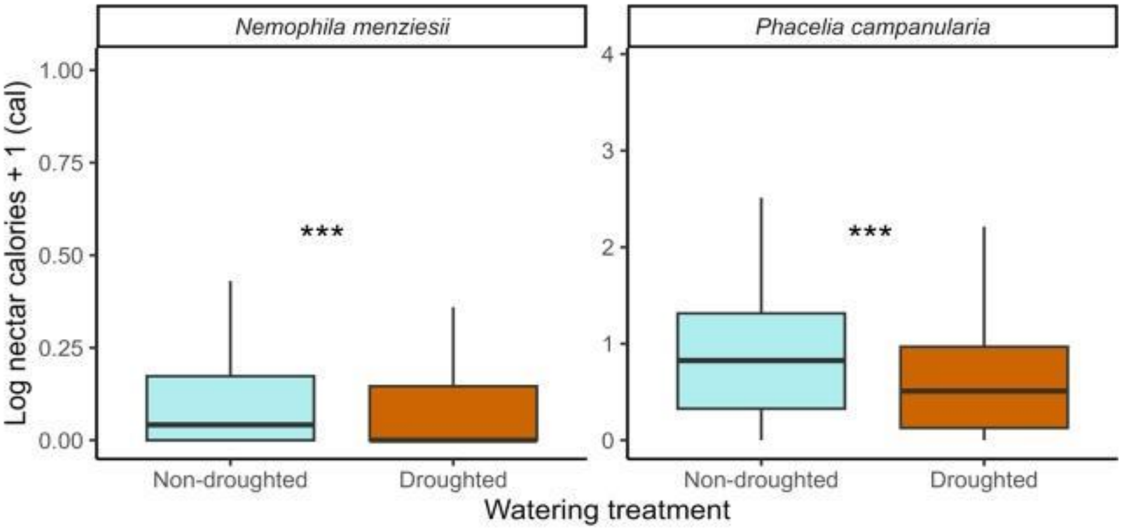


Figure S3.7: Nectar calories for *Nemophila menziesii* and *Phacelia campanularia* grown under non-droughted and droughted conditions in 2021. Asterisks indicate significance level (***$p < 0.001$). Outliers were removed from the figure.

Conclusion

Global anthropogenic climate change is altering the organism-level mechanisms that shape community-level plant-pollinator interactions. This dissertation explored the effects of climate change on plant-pollinator interactions as they scale up from an organismal to a community level. Using a large, manipulative field experiment, we found that experimentally-advanced snowmelt reduced our ability to predict the drivers of pollinator visitation rate (Chapter 1). Further, we demonstrated that forbs that flower later in the summer displayed advanced flowering in response to advanced snowmelt, while early-flowering species did not flower earlier in response to advanced snowmelt. To determine what processes may drive the lack of predictors of pollinator visitation found in Chapter 1, we used network analyses (Chapter 2). We found that network- and species-level indices linked to network stability change over the flowering season, but in distinct ways in advanced and unmanipulated snowmelt plots. This suggests that advanced snowmelt influences plant-pollinator communities by affecting the assembly of plant-pollinator interactions, which is likely to influence network stability in complex ways. Finally, we explored how organism-level climate change-induced trait shifts could influence community-level processes (Chapter 3). We used a greenhouse experiment, two California wildflowers, and a native solitary bee to ask how drought stress altered the traits of flowering species and pollinator behavior. We found that drought-driven alterations to floral traits decreased pollinator visitation to droughted flowers, but that plant drought tolerance may play a role in mediating the effects of drought on pollination.

In sum, we demonstrated that climate change can alter pollinator behavior by influencing plant physiology and traits, which may scale up to influence the structure of pollination networks, which could ultimately change the drivers of plant-pollinator interactions as climate change reshapes ecosystems across the world. We demonstrated that drought-induced shifts in floral rewards and cues decrease pollinator visitation, and that advanced snowmelt both decreases the predictability of pollinator visitation rate and alters the structure of plant-pollinator networks. This research highlights the importance of organism- and community-level effects of climate change on plant-pollinator interactions.